

LIFE HISTORY OF EGERIA DENSA IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

Project Information

1. Proposal Title:

LIFE HISTORY OF EGERIA DENSA IN THE DELTA: FACTORS CONTROLLING
PRODUCTION & FRAGMENT VIABILITY

2. Proposal applicants:

Mark Sytsma, Portland State University
Toni Pennington, Portland State University

3. Corresponding Contact Person:

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4. Project Keywords:

Aquatic Plants
Limnology
Nonnative Invasive Species

5. Type of project:

Research

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

No

7. Topic Area:

Non-Native Invasive Species

8. Type of applicant:

University

9. Location - GIS coordinates:

Latitude: 38.0413284

Longitude: -121.6026611

Datum:

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

Franks Tract and its associated tributaries are located in the Sacramento-San Joaquin Delta in California approximately 15 miles West of Stockton, California and immediately borderd on the Sw by Bethel Island. It is approximately 3300 acres.

10. Location - Ecozone:

1.4 Central and West Delta

11. Location - County:

Contra Costa

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:

10

15. Location:

California State Senate District Number: 7

California Assembly District Number: 15

16. How many years of funding are you requesting?

2

17. Requested Funds:

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

No

If no, list single overhead rate and total requested funds:

Single Overhead Rate: 41

Total Requested Funds: \$327937

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?

No

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)

David Spencer	USDA-ARS Aquatic Weed Research Laboratory	530-752-1096	dfspencer@ucdavis.edu
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Lars Anderson	USDA-ARS Aquatic Weed Research Laboratory	530-752-7870	lwanderson@ucdavis.edu
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**Pat
Thalken**

**CA Dept. of Boating &
Waterways**

916-263-8141 pthalken@dbw.ca.gov

Kim Webb

US Fish & Wildlife

209-946-6400 x311

kim_webb@r1.fws.gov

21. Comments:

Environmental Compliance Checklist

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

1. CEQA or NEPA Compliance

a) Will this project require compliance with CEQA?

No

b) Will this project require compliance with NEPA?

No

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

Compliance is not required because activities proposed will not cause direct physical change or a reasonably foreseeable indirect physical change in the environment. The proposed work does not require public works construction activities, clearing or grading of land, improvements to existing public structures, enactment and amendment of zoning ordinances, or adoption and amendment of local general plans.

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

CEQA Lead Agency: None

NEPA Lead Agency (or co-lead:)

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

Xnone

NEPA

-Categorical Exclusion

-Environmental Assessment/FONSI

-EIS

Xnone

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.

4. **CEQA/NEPA Process**

a) Is the CEQA/NEPA process complete?

Not Applicable

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	Required
Variance	Required
Subdivision Map Act	Required
Grading Permit	Required
General Plan Amendment	Required
Specific Plan Approval	Required
Rezone	Required
Williamson Act Contract Cancellation	Required
Other	Required

STATE PERMITS AND APPROVALS

Scientific Collecting Permit	Required
CESA Compliance: 2081	Required
CESA Compliance: NCCP	Required
1601/03	Required
CWA 401 certification	Required
Coastal Development Permit	Required
Reclamation Board Approval	Required
Notification of DPC or BCDC	Required
Other	Required

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation	Required
ESA Compliance Section 10 Permit	Required
Rivers and Harbors Act	Required
CWA 404	Required
Other	Required

PERMISSION TO ACCESS PROPERTY

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

Agency Name:

Permission to access state land.

Agency Name:

Permission to access federal land.

Agency Name:

Permission to access private land.

Landowner Name:

6. Comments.

Land Use Checklist

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

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?**

Yes

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 only

4. **Comments.**

Conflict of Interest Checklist

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

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):

Mark Sytsma, Portland State University
Toni Pennington, Portland State University

Subcontractor(s):

Are specific subcontractors identified in this proposal? No

Helped with proposal development:

Are there persons who helped with proposal development?

Yes

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

Lars Anderson USDA-ARS Aquatic Weed Research Laboratory

Comments:

Grand Total=327983.00

Comments.

Budget Justification

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

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

Hours are for the two year duration of the proposal PI (930 hrs) Grad Student (2440) Lab Tech (1550) Field Tech (540)

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

Estimated rate per hour PI \$34.62 year -1 and \$36.35 year - 2 Grad Student \$16.28 year-1 and \$17.09 year-2 Lab Tech \$7.50 year-1 and \$7.88 year-2 Field Tech \$25.00 year-1 and \$26.25 year-2 Field Tech

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

PI (\$11564) Grad Student(\$17210) Lab Tech (\$1283) Field Tech (\$1662)

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

Monthly travel for field work is based on 1260 miles/trip x 12 trips x \$0.36/mile, Lodging (12 nights @ \$60/night), and per diem at \$35/day. Travel mileage is based on round-trip between Portland, Oregon and the Sacramento area. The PI and graduate student will attend two meetings with CALFED and attend one scientific conference. Total travel = \$17121.40

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

Office (\$2700) Laboratory (\$27300) Computing (\$1900) Field supplies (\$6426)

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.

N/A

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.

Carbon-Hydrogen-Nitrogen (CHN) Elemental Analyzer - Approximately \$60000. Greenhouse equipment, including lighting, temperature control, aeration, and aquaria - Approximately \$10000.

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.

Project management, provided by the PI, includes time for preparing quarterly reports to CALFED, providing one presentation per year to CALFED, attending one scientific meeting, preparing documents for peer reviewed journal(s), overseeing performance measure evaluation protocol as outlined in Section 5: Performance measures by meeting regularly with graduate student and laboratory

and field technicians.

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

Publication costs = \$1160

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.

All indirect costs are based on federal rate of 41% and includes rent, furniture, general office staff, greenhouse, water, accounting, and electricity.

Executive Summary

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

The life history of Brazilian elodea (*Egeria densa*), a non-native invasive aquatic plant, will be examined in Franks Tract and its tributaries in the Sacramento-San Joaquin Delta, California and in greenhouse experiments. Life history information for *E. densa* in the Delta is lacking. Life history information on non-native invasive species is critical to management efforts. The objective of the proposed research is to inform *E. densa* management decisions through description of seasonal, morphological, and ecophysiological responses of *E. densa* to important environmental variables. The proposed research will evaluate the following hypotheses: 1) seasonal and environmental (light and temperature) influences on *E. densa* photosynthesis and dissolved organic carbon loss are important determinants of *E. densa* success and can be exploited to enhance efficacy of management actions, 2) seasonal changes in morphology and allocation of nutrients and energy influence ramet demography and growth and can be exploited to enhance efficacy of management actions, and 3) dispersal and establishment success are related to energy and nutrient content and double-node frequency of plant fragments. Seasonal changes in photosynthetic response to light will be examined monthly in the field. Light and temperature response curves for photosynthesis will also be examined in the laboratory for comparison to similar studies on *E. densa* from Florida. Morphological measurements and determination of nutrient allocation to various plant organs (leaves, stems, roots, root crowns, and double nodes) will be measured monthly on field-collected plants. Effect of season and fragment size on fragment viability and establishment success will be examined in greenhouse experiments. Information from the field and laboratory studies will be used to model *E. densa* growth and determine optimal timing of management actions. The mechanistic understanding of how *E. densa* functions in the Bay-Delta system that will be developed in this research is a multi-regional priority (MR-1) and will help meet Objective 7 of Goal 5 of the CALFED Ecosystem Restoration Program.

Proposal

Portland State University

LIFE HISTORY OF EGERIA Densa IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

Mark Sytsma, Portland State University
Toni Pennington, Portland State University

LIFE HISTORY OF *EGERIA DENSA* IN THE DELTA: FACTORS CONTROLLING PRODUCTION & FRAGMENT VIABILITY

A. Project Description: Project Goals and Scope of Work

1. Problem

Invasive weeds are known to disrupt navigation, compromise flood control, reduce recreational activities, displace native vegetation, and impair fisheries (Mullin et al. 2000, Myers et al. 2000). The occupation of non-native invasive species (NIS) in the Sacramento-San Joaquin Delta poses a serious threat to healthy ecosystem structure and function. Successful restoration efforts will continue to be hampered until life history patterns and competitive strategies of such species are understood. Only then will a deeper understanding of the invasion process be gleaned.

E. densa Planchon (Hydrocharitaceae), which is native to Brazil and Argentina (Cook and Urmi-König 1984), has invaded New Zealand (Coffey and Clayton 1986), Japan (Haramoto and Ikusima 1988), Chile (Cook and Urmi-König 1984) and Australia (Roberts et al. 1999). In the U.S., *E. densa* has invaded lakes and ponds from Washington to California, across the South, and as far North as Vermont and New Hampshire on the East Coast (Cook and Urmi-König 1984). In California *E. densa* covers about 3,900 acres in the Sacramento-San Joaquin Delta, is spreading at about 100 acres/year, and is the subject of an intensive chemical control program (California Department of Boating and Waterways 2000) that has spawned lawsuits aimed at reducing or eliminating the use of herbicides. Grimaldo and Hymanson (1999) found *E. densa* growing as deep as 3.5 m in some areas of the Delta. In Frank's Tract, the increased growth is likely responsible for inhibiting water movement which reduces suspension of particles, increases water clarity, and promotes further plant growth. Additionally, stands of *E. densa* in the Delta support more introduced fishes than native (Grimaldo et al. 2000) and provides habitat for another NIS, the Chinese Mitten Crab.

Controversy over the use of aquatic herbicides to control invasive aquatic plants has been exacerbated by recent NPDES permit requirements by the Ninth Circuit Court of Appeals. Additionally, the expense associated with mechanical harvesting can limit effective control efforts against invasive aquatic plants. To date, no viable biocontrol other than grass carp is available to control *E. densa*. Ultimately, the limited resources available to prevent and control NIS emphasizes the need for an improved mechanistic understanding of NIS success and eradication options.

Description of *E. densa*

E. densa is a dioecious, submersed perennial found in lentic and lotic freshwater systems. It has leaves in whorls of 4 (although 3-5 are sometimes observed) and branches irregularly along the stem in areas referred to as "double nodes" (Jacobs 1946). These double node regions are important in carbohydrate storage and vegetative reproduction of lateral buds, adventitious roots, and root crowns (Getsinger 1982). Male flowers are borne on pedicels with 3 white petals, 3 green sepals, 9 stamen and open above the water surface. Only male plants are

found in the U.S. and, even in its native range of Brazil and Argentina, male plants outnumber female plants by as much as 6:1. Under optimal conditions (15-25 C with sufficient light and nutrients) the plant flowers almost continually (Cook and Urmi-König 1984), however since only males are present outside the indigenous region, dispersal and perennation of *E.densa* is only by vegetative fragmentation.

Although *E. densa* does not produce typical perennating structures such as tubers or turions, as is common in many other invasive aquatic plants, it stores carbohydrates in root crowns and stems with double nodes (Getsinger 1982). Double nodes occur every 10 –12 internodes (Cook and Urmi-König 1984). Thus, the frequency of double nodes is important in plant architecture, canopy formation, biomass production, and overwintering success.

Lack of sexual reproduction has resulted in an extremely homogenous *E. densa* genotype in the Pacific Northwest. Carter and Sytsma (in press) found that *E. densa* collected throughout western Oregon was essentially one genotype. Remarkably, a limited number of samples from Chile exhibited the same genotype as the Oregon population. In Brazil, *E. densa* (as well as *E. najas*, *Ceratophyllum demersum*, and *Eichhornia crassipes*) have severely infested hydropower reservoirs. It was estimated that 48,000 m³ of aquatic weeds were removed from water intake structures in Jupia Reservoir (Marcondes et al. 2000). Currently, biological, chemical, mechanical, and nutrient limitation methods to control the infestations are being examined by Companhia Energetica de Sao Paulo, one of the largest power generating companies in Brazil, and Sao Paulo State. Additionally, surveys to map infestations and evaluations of genetic variability are being undertaken to document the genetic spread of invasive plants in Brazilian reservoirs.

A detailed phenological assessment of *E. densa* has been made in the Southeastern U.S. and Japan, however basic phenological information has not been evaluated for *E. densa* on the West Coast. *E. densa* exhibited two peaks in biomass in the Santee-Cooper system in South Carolina and Japan: July/August and December/January (Getsinger and Dillon 1984, Haramoto and Ikusima 1988, respectively). Differences in summer and winter plants were observed. Summer plants were characterized by profuse branching of long stems. Winter plants had fewer branches and numerous new roots and stems per root crown.

Little is known about *E. densa* biology on the West Coast. In a small, coastal Oregon lake, the plant did not senesce during the winter of 2001; it remained in the water column, with spring growth originating from regularly positioned double node regions on the overwintering stems. (Pennington unpublished data). Data collected bi-weekly during the summer of 2001 in this reservoir indicate subtle seasonal changes in the partitioning of double nodes: flowers, undifferentiated double nodes, branches, and buds (Figure 1). Per cent undifferentiated double nodes did not change greatly throughout the summer, however per cent differentiation to branches suggests a bimodal growth pattern as observed in South Carolina.

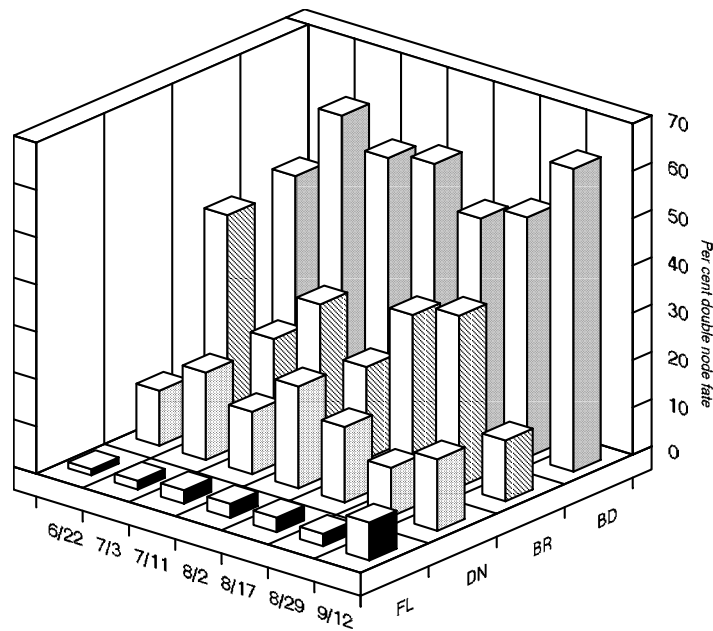


Figure 1. Fate of double nodes (%) on *E. densa* stems collected bi-weekly from Big Creek Reservoir, Oregon in 2001. FL = flowers, DN = undifferentiated double nodes, BR = branches ≥ 0.5 cm), and BD = buds < 0.5 cm.

Aquatic plant control

Timing of management efforts could be improved by the identification of “weak points” in the lifecycle of a plant, permitting optimum management based on the plant’s biology. For example, management of *Eichhornia crassipes* (Luu and Getsinger 1990), *Hydrilla verticillata* (Madsen and Owens 1998), and *Myriophyllum spicatum* (Perkins and Sytsma 1987) have been enhanced by targeting management to periods when carbohydrate reserves and the ability of the plant to recover from the management are lowest. Figure 2 illustrates seasonal low points in total nonstructural carbohydrates (TNC) in Eurasian watermilfoil. Treating aquatic plants when carbohydrate reserves are lowest has been suggested for *E. densa* in Lake Marion, South Carolina. Carbohydrate reserves were lowest in late May-April, therefore considered the recommended time of treatment in that study (Getsinger 1982). To date, no similar research has been published on seasonal nutrient and energy allocation patterns and morphology of *E. densa* growing on the West Coast. This information is critical in determining the life history of *E. densa* for improved management efforts and curtail further spreading of this non-native invasive weed.

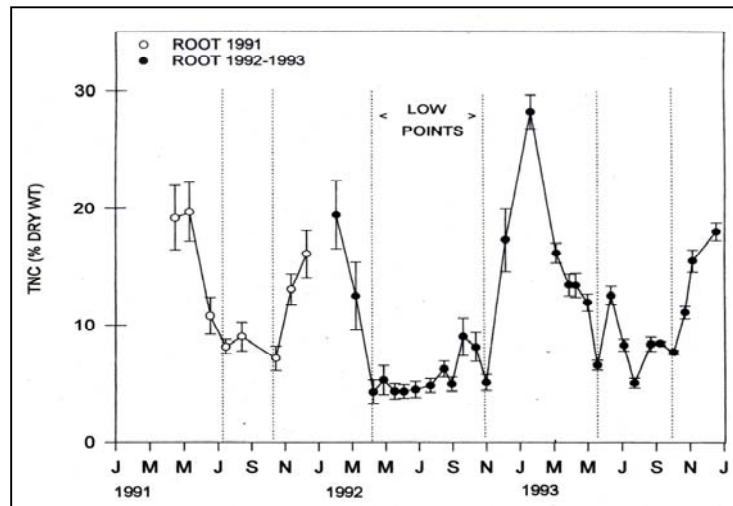


Figure 2. Total nonstructural carbohydrates (TNC) over time in Eurasian watermilfoil. Vertical lines indicate areas of low TNC in root material. From Madsen 1997.

Several different *E. densa* control methods were investigated by Jacoby et al. (in press) in Long Lake, Washington, and California Department of Boating and Waterways has supported extensive evaluations of herbicide efficacy, fragment viability, and fauna associated with *E. densa*. A four-month evaluation of *E. densa* in portions of the Sacramento-San Joaquin Delta indicate maximum density in June (~70 g dry wt./0.25 m²), however the focus of the research was not long-term biomass determination (Obrebski et al. 2000). Anderson (2000) indicated greater viability from stem fragments harvested in the fall than those harvested in spring. The greater viability may have been due to increased nodal frequency, although that was not evaluated in the study.

Photosynthesis and Organic Carbon Loss

Submersed macrophytes excrete organic carbon during active metabolism (Hough and Wetzel 1975). Excreted organic carbon reduces carbon available for growth, but presumably provides some benefit to the plant. For example, the excreted carbon compounds may be allelopathic and reduce competition with phytoplankton and other macrophytes. The relationship between photosynthetic rate and organic carbon release has important implications for plant growth and competitiveness. Understanding growth and productivity of *E. densa* in the Delta requires information on how photosynthetic rate varies with irradiance and temperature. Photosynthesis versus Irradiance (P vs. I) curves are commonly used to determine photosynthetic rates under varying light levels in algae and aquatic plant communities (Van der Bijl et al. 1989, MacIntyre et al. 1996, Kirk 1994). In addition to the potential allelopathic properties, dissolved organic carbon release by aquatic plants may contribute to trihalomethane precursors in the drinking water treatment process (Cooke and Carlson 1989).

Some P vs. I characterization has been conducted on *E. densa*, however none of this work has been done on California populations. Carbon (determined by ¹⁴C uptake) assimilation was highest in April through July in apical tissues of *E. densa* collected from Lake Marion, South Carolina when temperatures ranged from 21 C to 29 C (Getsinger 1982). In a detailed study by Barko and Smart (1981), maximum photosynthetic rate

(determined by CO₂ release) occurred at 24 C, but temperature had a greater influence on photosynthetic rate than light.

Similar relationships have not been established for *E. densa* on the West Coast. Identifying the relationship between maximum photosynthetic rate and DOC excretion is key in understanding seasonal growth dynamics.

Goals and Hypotheses

The goal of the proposed research is to develop a mechanistic understanding of the life history of *E. densa* that will improve management of this highly invasive species and improve restoration efforts in the Delta. This goal will be accomplished by evaluating the following hypotheses:

Hypothesis 1: There are seasonal and environmental influences on *E. densa* photosynthesis and dissolved organic carbon loss rates that are important for plant growth, which can be exploited to enhance efficacy of management actions.

Hypothesis 2: Seasonal changes in allocation of nutrients, energy, and morphology influence ramet demography and growth, which can be exploited to enhance efficacy of management actions.

Hypothesis 3: Plant fragment viability and establishment success are related to energy and nutrient content and double-node frequency of the fragments.

These hypotheses will be tested in field and greenhouse experiments outlined in Section 3 (Approach). Field work will be conducted in Frank's Tract and adjacent tributaries in the Sacramento-San Joaquin Delta between Sacramento and Stockton, in Contra Costa County, California. The area of Frank's Tract is approximately 1335 ha. An estimated 26% of the surface area of Frank's Tract is infested with *E. densa* (California Department of Boating and Waterways 2000). Greenhouse experiments will be conducted on the Portland State University campus in Portland, Oregon.

2. Justification

Conceptual Model

Growth of aquatic plants is determined, among other things, by sediment nutrients, light, temperature, and chemistry of the water column (Barko and Smart 1981, Beer and Wetzel 1981, Barko and Smart 1986, Van der Bijl et al. 1989) (Figure 3). Under optimal conditions, maximum growth rate and biomass may be achieved. At any point in time, the growth rate will trigger physiological responses such as reproduction and competition, thus plant demography can be provided by plant growth studies (Chiarello et al. 1989). Understanding how environmental factors such as light, temperature and chemistry influence primary production is fundamental to developing predictive plant growth models that can be used to evaluate management approaches. This understanding is needed to effectively manage this NIS and improve restoration efforts in that region.

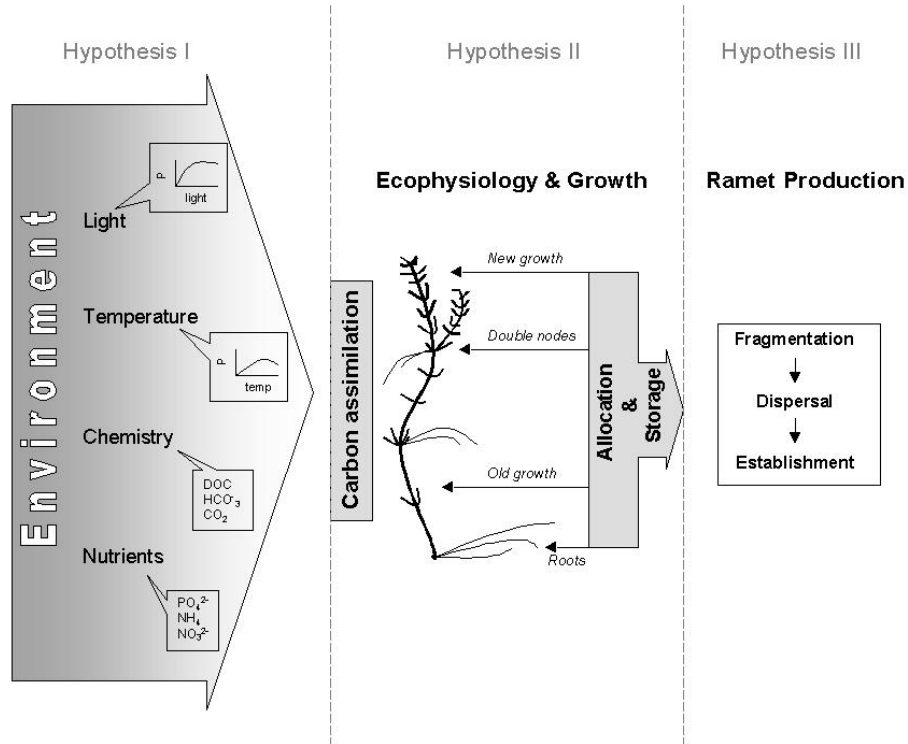


Figure 3. Conceptual model of *E. densa* growth and ramet production as a function of its environment and subsequent carbon allocation. Elements of each hypothesis are partitioned by a dotted line.

In *E. densa*, new plant growth originates from double node regions located along the stem (Jacobs 1946, Cooke and Urmi-König 1984, Getsinger and Dillon 1984). The occurrence of double nodes is not limited to old or new stems, however the frequency (double nodes \cdot cm⁻¹) appears to vary seasonally (Pennington and Sytsma unpublished data, Figure 4). *E. densa* does not reproduce sexually and the viability of vegetative fragments is critical to its survival. *E. densa* success is determined by seasonal sequestering of sufficient carbohydrate reserves to establish spring growth and produce double nodes imperative for branching, root formation, and flowering (Jacobs 1946). Ramets of *E. densa* from South Carolina and Japan have demonstrated carbohydrate allocation patterns that change seasonally and spatially within the plant (Getsinger and Dillon 1984; Haramoto and Ikusima 1988). Similar observations have been made for other invasive aquatic plants (Luu and Getsinger 1990; Madsen 1997; Perkins and Sytsma 1987).

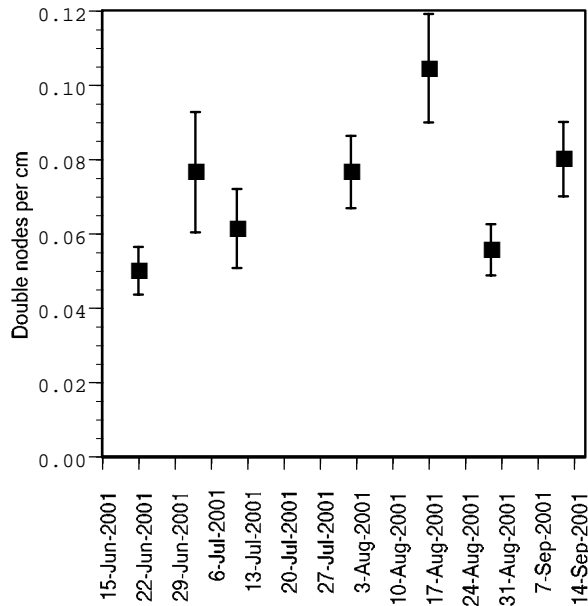


Figure 4. Double nodes per cm of stem from *E. densa* plants collected from Big Creek Reservoir, Oregon. Values indicate means \pm 1 SE.

E. densa establishment in the Sacramento-San Joaquin Delta may be better understood by identifying factors that influence carbohydrate allocation patterns and stem viability. Additionally, identifying points in the life history of *E. densa* where carbohydrate reserves are lowest may provide for improved management efficacy. Figure 3 illustrates how the examination of various key environmental factors can be related carbohydrate allocation patterns that lead to double node production, branching, fragmentation, dispersal, and eventually establishment.

Hypotheses

The hypothesis that environmental influences on *E. densa* photosynthesis and dissolved organic carbon loss can be used to enhance efficacy of management actions (hypothesis #1), will be tested by describing P v. I curves in the field and greenhouse. These data will be coupled with that of the second hypothesis to improve management strategies.

The second hypothesis is subtitled in the model as ecophysiology and growth. Based on this hypothesis, seasonal changes in nutrient allocation and morphology can be used to increase efficacy of management alternatives. This component will be evaluated by determining the seasonal and spatial allocation of carbohydrates and nitrogen in varying parts of *E. densa* plants obtained from the Delta and by assessing seasonal morphological changes.

The third hypothesis is illustrated in the model as ramet production where plant fragment viability and establishment success are related to energy and nutrient content and double-node frequency of the fragments.

Adaptive management

E. densa biology will be assessed in situ and in greenhouse investigations as a targeted research project. Specific objectives will be addressed as information is gathered on *E. densa* plants growing in Frank's Tract of the Delta and alterations will be made to subsequent greenhouse investigations. Through the Adaptive Management Process, information will be assessed using statistical analysis and the hypotheses evaluated for appropriateness. From there, research efforts will be continued as planned or the model and hypotheses will be adapted to reflect new information. Given the invasive nature of *E. densa* in the Delta, it is unlikely the problem will need to be re-addressed, however we may find negative impacts from *E. densa* are more pervasive than previously thought.

Efforts to control *E. densa* have been met with expense and controversy, *e.g.* harvesting and herbicide applications, respectively. A full-scale *E. densa* eradication project would be inadequate considering basic questions about *E. densa* in the Delta have not been addressed. For example, stem viability was greater after a fall harvest compared to a spring harvest (Anderson 2000). It may have been that double node frequency was greater during the fall or that carbohydrate reserves were greater – providing greater nutrient reserves for sprouting. This information indicates how timing of control measures needs to be investigated further before pilot and full-scale projects can be effectively implemented. Without this basic information available to managers, indiscriminate chemical applications and harvesting techniques will continue and may actually exasperate the problem by dispersing viable fragments and reducing the competitive influence of native plants.

3. Approach

This study will examine the three research hypotheses through field and greenhouse studies. The research will be conducted under five tasks, as described below. Unless otherwise stated in the text, all methods are detailed in Table 1. A timeline of all collections and analysis are provided in Table 2 of Section 8: Work Schedule.

Task 1: Field studies of productivity

For clonal plants, like *E. densa*, that perennate and are dispersed as plant fragments, production rate is a fundamental determinant of success. Under this task, seasonal variation in *E. densa* photosynthetic rate and physical growth will be measured monthly over a two-year period.

P vs. I curves will be developed for *E. densa* in the Delta. Two, 8-cm stem apices collected from the top of the plant canopy will be placed into 300 ml biological oxygen demand (BOD) bottles containing filtered (Gelman GF-F) Delta water. Neutral density shade cloth covers will be placed over individual bottles to produce 0, 25, 50, 75, and 100% surface irradiance. Bottles containing filtered Delta water but no plants (controls) will be incubated at each light intensity. All bottles will be incubated in triplicate at approximately 10 cm from the water surface.

Photosynthetic rate will be calculated from change in dissolved oxygen concentration in the bottles. Dissolved oxygen concentration will be measured each hour over a four-hour incubation period (roughly 10 AM till 2 PM) with a self-stirring dissolved oxygen probe (YSI 5000). Following the incubation period plants will be

removed from the BOD bottles and preserved DMSO and stored on ice in the dark for transport to the laboratory for chlorophyll *a* and *b* measurement. Following DMSO extraction of pigments, plant dry mass (65 C to constant weight) will be measured. Net photosynthetic rate will be expressed as mg oxygen/mg dry mass/hour and as mg oxygen/mg chlorophyll/hour and will be illustrated similar to the P v. I curves in Figure 3.

Initial and final pH, dissolved oxygen, alkalinity, dissolved organic carbon, and chlorophyll *a* (algal) in the incubation bottles will be measured using the methods outlined in Table 1. Dissolved inorganic carbon concentration will be calculated from pH and alkalinity measurements. Surface light and temperature during the incubation period will be measured with a Datasonde 4a (Hydrolab, Inc., Austin, Texas). Light extinction will be calculated from light measurements at 50 cm increments in the water column at the midpoint of the incubation period.

Seasonal changes in growth of *E. densa* in the Delta will be determined by measuring stem elongation rates in the spring, summer, and fall. Thirty stem apices will be flagged with plastic tape tied to the stem 10 cm from the apex. After one month of growth, the stem apices will be clipped at the tape, rinsed in Delta water to remove attached periphyton and debris, and placed on dry ice for transport to the laboratory. In the laboratory, stem length will be measured and number of double nodes will be counted (see Task 2) then the plants will be lyophilized and analyzed for N, P, and K.

Seasonal changes in light response of photosynthesis and stem elongation will be used to develop a model of *E. densa* growth in the Delta. The model will permit testing the hypothesis that seasonal variation in growth of *E. densa* in the Delta can be used to inform and optimize management actions.

Task 2: Morphology, allocation, and phenology

Demography of double nodes, which control morphology and growth, and resource allocation will be measured on *E. densa* in the Delta. *E. densa* plants will be collected monthly at Frank's Tract or its tributaries for morphological measurement. Plants will be collected at random (determined by selecting a random number between 1 and 360 to determine compass coordinate and a random number between 1 and 10 to determine distance (m) from a given point). Whole plants (root crown and attached stems) will be collected at 15 points. Plants will be rinsed with Delta water and transported to the laboratory on ice. Total stem length will be measured and the number of double nodes counted on each plant. Data will be presented as frequency of double nodes per cm (similar to Figure 4) and coupled with nutrient allocation patterns and results from Task 1 to determine periods of maximum/minimum growth.

Additionally, 15 entire plants will be collected similarly to assess seasonal nutrient and carbohydrate allocation patterns. Samples will be rinsed with lake water to remove epiphyton and sediments, separated into apical meristems, new and old leaves, new and old stems, roots, root crowns, double nodes, and flowers (when available). Sections will be immediately placed on dry ice then freeze-dried in the laboratory for at least 48 hours at approximately 40 mTorr. Freeze-dried samples will be analyzed for carbohydrates, nitrogen, and carbon

as described in Table 1. Changes in nonstructural carbohydrate and plant nutrient concentration will be analyzed using repeated-measures ANOVA with plant part and season as factors in the analysis. .

This work will be conducted in collaboration with researchers from the USDA-ARS Aquatic Weed Research Laboratory at the University of California at Davis. Results will compliment their ongoing investigations on *E. densa* nutrient allocation studies in the Delta. When possible, data will be compared with those of Getsinger and Dillon (1984) in South Carolina, Haramoto and Ikusima (1988) in Japan, and concurrent investigations in Oregon (Pennington and Sytsma unpublished data) and California (Anderson unpublished data).

Task 3: Fragment viability

Viability of harvested fragments will be determined in the spring, summer, and fall. Twenty plants will be randomly collected as described in Task 2 and clipped to mimic harvested fragments. Effects of season and fragment length (10 and 20 cm stem apices) will be tested. Prior to planting, fragments will be measured for double node frequency, adventitious root length, and flowering frequency. Fragments will be planted at ambient temperature using methods similar to those described by Anderson (2000) to simulate lodged shoreline fragments. Initial nutrient and carbohydrate concentrations will be estimated from plants collected from the Delta at the same time in Task 2. Plants will be grown for four weeks in a greenhouse (described in Task 4). Measurements of lateral shoots, stem lengths, double node frequency, adventitious roots, flowers, nutrients, and carbohydrates will be determined. Significant differences between seasons, stem length, double node frequency, and nutrient content will be determined as in Task 2. This information will be coupled with seasonal nutrient allocation patterns obtained in Task 2 and used to identify periods when harvesting would be most and least effective.

Task 4: Photosynthetic response to light and temperature

These studies will be conducted to develop photosynthetic light and temperature response curves for *E. densa* and allow comparison of the ecophysiology of California plants with plants from Florida studied by Barko and Smart (1981). Plants will be grown in 40 L aquaria by planting two-8 cm apical meristems in 0.5 L containers of sediment obtained from Frank's Tract in the Delta and covered in washed silica sand to reduce algae growth. Sediment particle size, nutrients, and organic matter will be determined according to methods identified in Table 1. Each aquarium will contain three pots and artificial water, prepared according to Smart and Barko (1985). Aquaria will be continuously aerated with filtered air. Three light levels will be provided using neutral shade cloth and fluorescent lights and three temperature regimes will be maintained, low (10 C), medium (15 C), and high (20 C). Plants will be allowed to equilibrate for two weeks prior to the investigation. Each treatment will be replicated three times, totaling 27 aquaria for this experiment. Additionally, 27 aquaria containing only artificial water will be used to examine the release of DOC by algae as part of a concurrent investigation on the impact of *E. densa* on THM formation potential in chlorinated drinking water.

Growth rate will be determined by tagging plants with plastic tape 10 cm from the tip and measuring stem elongation weekly for four weeks. Water quality parameters including pH, DO, alkalinity, Chlorophyll *a*, DOC, and DIC will be determined weekly. Photosynthetic rates (as O₂ evolution) will be determined on two-8 cm apices randomly removed from each treatment aquarium and incubated as in Task 1 (in artificial water) under previously described light and temperature regimes and DO determined every four hours. Following incubation, plant tissues will be analyzed for chlorophyll *a* and *b* and DOC, DIC, alkalinity, chlorophyll *a*, and pH will be determined from the water.

To measure morphological changes, one pot from each treatment will be removed, the plants harvested and double node frequency, stem length, and root length measured. Dry weight-wet weight correlations will be determined by analysis of 20 plants prior to the experiment and will be used to determine biomass after six weeks of growth. Plants not used in the growth rate experiment or morphological measurements will be freeze-dried for nutrient and carbohydrate allocation as described in Task 2. Sediment, water, and plant tissues will be analyzed according to methods described in Table 1.

The greenhouse to be used in this study, located on the Portland State University campus, was recently completed (September 2001). Essential equipment requirements for the greenhouse portion of this study (Task 4) include aquaria and temperature, light, and aeration systems.

Table 1. Analytical Methods.

Parameter	Method (Citation)
Water Quality Analysis	
Field - DO, temperature, pH, conductivity, light	Datasonde 4a (Hydrolab, Inc., Austin, Texas)
Laboratory - pH	Orion 290A pH Meter
Chlorophyll <i>a</i> - algae	Acetone extraction/Fluorometry (Parsons et al. 1984)
Chlorophyll <i>a</i> and <i>b</i> - plants	DMSO extraction/Spectrophotometry (Barnes 1992)
Nitrogen	TKN (APHA 1998)
Ammonia-Nitrogen (NH ₄ -N)	Phenate/Spectrophotometry (APHA 1998)
Nitrate-Nitrogen (NO ₃ + NO ₂ -N)	Cadmium Reduction (APHA 1998)
Soluble Reactive Phosphorus	Ascorbic Acid (APHA 1998)
Total Phosphorus	Persulfate digestion/Ascorbic Acid (APHA 1998)
Alkalinity	Wetzel and Likens (1991)
Dissolved organic carbon	Persulfate-UV Oxidation (APHA 1998)
Plant Tissue Analysis	
Carbon and Nitrogen	Combustion-reduction (CHN Elemental Analyzer)
Potassium and Phosphorus	Chapman and Pratt (1982)
Total Nonstructural Carbohydrates (TNC)	Benzoic Acid Digestion (Modified Swank 1982)
Photosynthesis	O ₂ evolution (YSI 5000 dissolved oxygen/BOD probe)
Sediment	
Organic matter, N, P, Ca and K	Chapman and Pratt (1982)
Per cent composition	Chapman and Pratt (1982)

Task 5: Project management

Quarterly reports will be provided to CALFED. Reports will outline the status of the budget, preliminary data analysis, assess the appropriateness of the methods in testing the hypotheses, and make recommendations as needed. Objectives outlined in Figure 5 of Section 5: Performance Measures will be addressed. A year-1 report

will also be generated and contain similar, but more detailed information. Presentations will be made to CALFED staff following year-1 and year-2. Two scientific meetings will be attended during year-2, the Ecological Society of America and the California Weed Science Society meetings.

4. Feasibility

The hypotheses being tested for this research will provide critical information on the life history of *E. densa* growing in the Delta and improve control of this NIS and subsequent restoration efforts. The specific questions being addressed in this research will provide information readily usable by Delta managers. The timing of herbicide applications or harvesting techniques can be improved by targeting plants when energy reserves are lowest and recovery would be least likely to occur. Additionally, determining periods of maximum photosynthetic rate may increase efficacy of systemic herbicides.

Data collection will be completed in two years. This includes gathering productivity data in the field and completing greenhouse trials. The potential for inclement weather, especially high winds, always exists with respect to field activities, however ample time will be allotted. It is anticipated that the final report will be provided to CALFED within two months of the last sampling occasion and procedures to submit publications to peer reviewed journals will follow.

Permits for sampling *E. densa* are not required as it is not a federal or state listed noxious weed. None of the above tasks will be conducted on private land or waters that require owner permission for access. Permission is not required to extract water samples and in situ activities do not incorporate regulated methodologies.

5. Performance Measures

The performance of the project will be evaluated based on three main parameters: the budget, timeline outlined in Section 8, and the quality of data collection, preservation, analysis (Figure 5). A timeline of the project is provided in Table 2 of Section 8 and includes data collection, analysis, experiment set-up, travel, presentations, and report generation. Quality of data collection and analysis will be maintained by adhering to appropriate methodologies as cited in Table 1, replicated as outlined in Section 3 (Approach), and by following calibration standards provided by manufacturer of equipment. Quarterly meetings will be held with the Primary Investigator to review the objectives and assess the validity of data, appropriate sampling protocol, statistical analysis, and compile reports for CALFED.

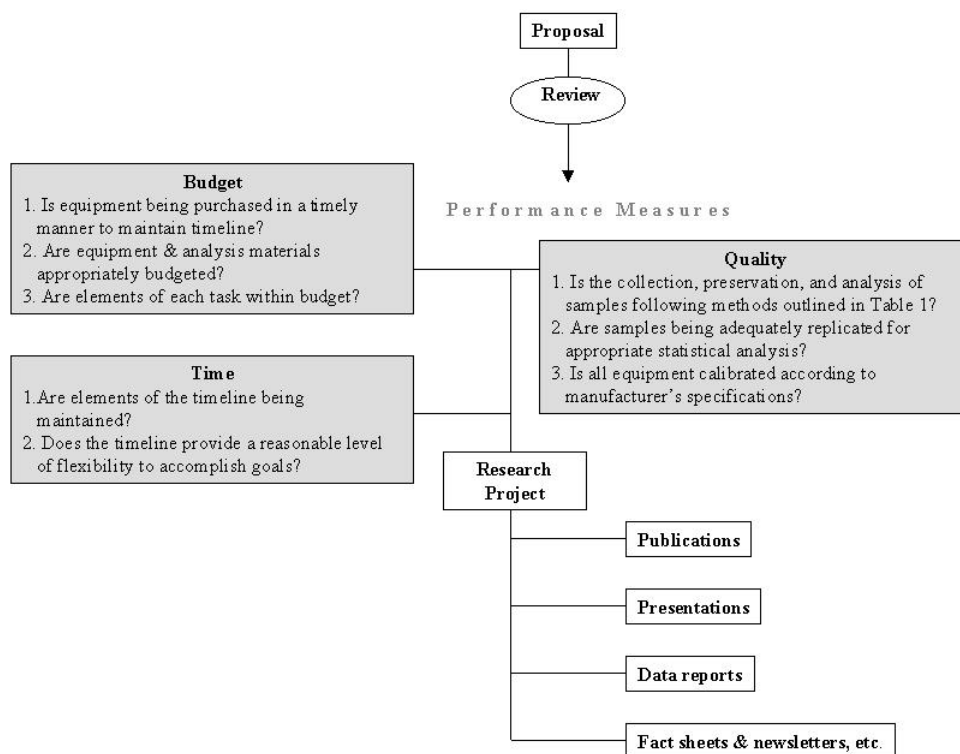


Figure 5. Project performance evaluation components.

6. Data Handling and Storage

All data will be will be logged in a journal upon collection and entered into EXCEL spreadsheets within a week and stored on discs. Copies of the electronic and paper versions will be maintained in a geographically dissimilar location. Raw data will be made available to CALFED staff upon request, quarterly reports will be provided to CALFED as well as yearly presentations and a final report. Information on this study will also be available on the internet via a webpage maintained by the graduate student.

7. Expected Products/Outcomes

Again, quarterly reports and end of year reports will be provided to CALFED. Yearly presentations will also be made to CALFED staff. Abstracts for annual conferences will be submitted to the California Weed Science Society and the Ecological Society of America. Presentations will also be made to regional managers upon request by CALFED. This research is in partial fulfillment of a doctoral dissertation, therefore results will be incorporated into associated documents and presentations, including academic seminars and posters and peer reviewed journals.

The information provided by this research will compliment research currently being conducted by the authors at Portland State University in Oregon examining the effects of *E. densa* on drinking water quality and the life history of *E. densa* in Oregon. Additionally, this information will build on research conducted at the University of California at Davis on stem fragment viability and herbicide efficacy. These data will be

compared with those previously obtained from South Carolina and Japan and the information used to improve important management decisions that will lead to enhanced habitat for native fish, wildlife, and aquatic plants.

8. Work Schedule

Table 2. Timeline

Year 1	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Task 1: Field studies of productivity												
Determine specific study sites	x											
Purchase materials	x											
Productivity		x	x	x	x	x	x	x	x	x	x	x
Diurnal water quality	x	x	x	x	x	x	x	x	x	x	x	x
Pre-incubation water quality analysis		x	x	x	x	x	x	x	x	x	x	x
Plant chlorophyll determination		x	x	x	x	x	x	x	x	x	x	x
Flag plants for growth rate determination		x				x						x
Analyze morphology & nutrients from flagged plants			x				x					
Task 2: Morphology, allocation & phenology												
Collect plants for morphology		x	x	x	x	x	x	x	x	x	x	x
Collect plants & lyophilize for carbohydrates & nutrients		x	x	x	x	x	x	x	x	x	x	x
Analyze for carbohydrates & nutrients				x			x			x		
Task 3: Fragment viability												
Prepare greenhouse	x	x										
Collect "summer" plants, det. morphology & est. in greenhouse			x									
Carbohydrate, nutrient & morphological analysis of "summer" plants				x								
Collect "fall" plants, det. morphology & est. in greenhouse						x						
Carbohydrate, nutrient, & morphological analysis of "fall" plants							x					
Collect "spring" plants, det. morphology & est. in greenhouse												x
Task 5: Project management												
Quarterly report to CALFED				x				x				x
Prepare Year 1 report to CALFED											x	x
Presentation to CALFED												x
Tasks 1 and 2 are considered inseparable as the data compliment one another and form the basis of this life history study												
Funding for the CHN Analyzer is necessary for nutrient analysis included in Task 2												
Greenhouse equipment is necessary for Task 3 in year-1; however, this task is not linked to Tasks 1 or 2												

Year 2	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Task 1: Field studies of productivity												
Productivity	x	x	x	x	x	x	x	x	x	x	x	x
Diurnal water quality	x	x	x	x	x	x	x	x	x	x	x	x
Pre-incubation water quality analysis	x	x	x	x	x	x	x	x	x	x	x	x
Plant chlorophyll determination	x	x	x	x	x	x	x	x	x	x	x	x
Flag plants for growth rate determination			x			x						
Analyze morphology & nutrients from flagged plants	x			x			x					
Task 2: Morphology, allocation & phenology												
Collect plants for morphology	x	x	x	x	x	x	x	x	x	x	x	x
Collect plants & lyophilize for carbohydrates & nutrients	x	x	x	x	x	x	x	x	x	x	x	x
Analyze for carbohydrates & nutrients	x			x			x			x		x
Task 3: Fragment viability												
Carbohydrate, nutrient, & morphological analysis of "spring" plants	x											
Collect "summer" plants, det. morphology & est. in greenhouse			x									
Carbohydrate, nutrient, & morphological analysis of "summer" plants				x								
Collect "fall" plants, det. morphology & est. in greenhouse						x						
Carbohydrate, nutrient, & morphological analysis of "fall" plants							x					
Task 4: Greenhouse P v I												
Collect plants & establish greenhouse cultures in aquaria	x	x										
Tag plants for 4 wk growth rate		x										
Weekly water quality analysis		x										
Productivity measurement (incubation for 3 hours)		x										
Morphological assessment		x										
Plant tissue analysis (chl a & b, carbohydrate & nutrients)		x										
Water analysis (DOC, alkalinity, chl a, pH)		x										
Sediment analysis			x									
Task 5: Project management												
Quarterly report to CALFED				x				x				
Presentation to CALFED												x
Conference - Ecological Society of America			x									
Conference - California Weed Science Society								x				
Peer reviewed journal article preparation												x

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

1. ERP, Science Program and CVPIA Priorities

This proposal addresses Goal 5: Non-native invasive species outlined in Section 3 of the Draft Stage 1 PSP. Specifically, hypotheses outlined in this proposal will be tested in an effort to describe the life history of *E. densa* found in the Sacramento-San Joaquin Delta Region. Understanding life history characteristics such as maximum growth rate, nutrient and carbohydrate allocation patterns, and phenological events will improve management efforts of this NIS that will contribute to restoration efforts of the Delta and its tributaries.

2. Relationship to Other Ecosystem Restoration Projects

This research will compliment ongoing studies conducted by researchers at the USDA-ARS Aquatic Weed Research Laboratory at the University of California at Davis on nutrient allocation and herbicide efficacy on *E. densa* plants growing in the Delta. Funding for those projects is primarily being provided by California Department of Boating and Waterways.

3. Requests for Next-Phase Funding

N/A

4. Previous Recipients of CALFED Program or CVPIA Funding

No CALFED or CVPIA funds have been previously distributed to the primary investigator.

5. System-Wide Ecosystem Benefits

Results of this study will improve management of *E. densa* and enhance habitat in the Sacramento-San Joaquin Delta. Improvements will be made in cooperation with previous and on-going studies by researchers from UC at Davis and California Department of Boating and Waterways. Improved management that leads to a reduction in *E. densa* biomass will have expansive benefits to the Delta. Foreseeable restoration improvements include enhanced water quality by increased mixing of dissolved oxygen, reduced siltation from trapped particles, enhanced native plant establishment, and improved habitat for native fishes.

6. Additional Information for Proposals Containing Land Acquisition

No land acquisition is required for this research.

C. Qualifications

Dr. Mark Sytsma will be responsible for project administration and will supervise the graduate student and technicians on the project. Dr. Sytsma has over 20 years experience in submersed aquatic vegetation management. He received his Ph.D. in Ecology from UC Davis in 1992. His doctoral work was on biology and management of *Myriophyllum aquaticum* and included research on management of the plant in the Delta. Since 1994, he has been on the faculty at Portland State University where he is an Associate Professor of Environmental Sciences and Resources and director of the Center for Lakes and Reservoirs, which was established by the Oregon legislature to address aquatic weed and invasive aquatic species issues. Dr. Sytsma is a past-president of the Western Aquatic Plant Management Society, is a founding member and coordinator of the Columbia Aquatic Nuisance Species Initiative, and is on the executive committee of the Western Regional Panel on Aquatic Nuisance Species. Dr. Sytsma has received over \$1.5 million in grants and contracts in the past three years for work on lakes and aquatic invasive species problems.

Toni Pennington is beginning the second year of a doctoral program at Portland State University in Portland, Oregon under the direction of Dr. Mark D. Sytsma. At PSU she has been working on the life history of *E. densa* on the West Coast and its impacts on drinking water quality as it relates to the formation of carcinogenic trihalomethanes in chlorinated drinking water. She holds a M. S. in Aquatic Biology and has four years experience working with aquatic macrophytes including endangered species and other NIS. She also has experience working in Texas, Louisiana, Minnesota, and Oregon on invasive aquatic plants such as *Hydrilla verticillata*, *Potamogeton crispus*, and *E. densa*. Toni will conduct the field and greenhouse experiments with the assistance of (to be named) a technician and will oversee the planning, analysis, dissemination of information, and day to day activities of the project. This research is in partial fulfillment of

D. Costs

Budget (submitted electronically)

1. Cost-Sharing

N/A

E. Local Involvement

Activities of this research should not impact or disrupt activities in the Delta, however efforts will be made to personally communicate with local businesses and applicable watershed groups. Research will be conducted with in-kind support from the USDA-ARS Aquatic Weed Research Laboratory located on the University of California at Davis campus.

F. Compliance with Standard Terms and Conditions

The applicant understands and agrees to the contract terms and conditions outlined in Attachments D and E of the Proposal Solicitation Package for federal and state funds.

G. Literature Cited

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