Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

Project Information

1. **Proposal Title:**

   Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

2. **Proposal applicants:**

   Steven Obrebski, San Francisco State University
   George Irwin, San Francisco Satete University

3. **Corresponding Contact Person:**

   Bruce Macher
   San Francisco State Univeristy
   1600 Holloway Ave, HSS 204 San Francisco State University San Francisco, CA 94132
   415 338-7091
   macher@sfsu.edu

4. **Project Keywords:**

   Monitoring
   Restoration Ecology
   Wetlands Ecology

5. **Type of project:**

   Research

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

   No

7. **Topic Area**:

   Shallow Water, Tidal and Marsh Habitat

8. **Type of applicant:**

   University

9. **Location - GIS coordinates:**

Latitude: 37.58

Longitude: -122.24

Datum:

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

Proposed research work will be done in marshes on Petaluma river, tolay creek reserve and muzzi marsh in corte madera - location in boxes above is for tolay creek seems there is no place for the other locations

10. **Location - Ecozone:**

    2.4 Petaluma River, 2.5 San Pablo Bay

11. **Location - County:**

    Marin, Sonoma

12. **Location - City:**

    Does your project fall within a city jurisdiction?

    Yes

    If yes, please list the city: Corte Madera

13. **Location - Tribal Lands:**

    Does your project fall on or adjacent to tribal lands?

    No

14. **Location - Congressional District:**

    6

15. **Location:**

    California State Senate District Number: 6

    California Assembly District Number: 3

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: 50%

Total Requested Funds: 297864

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)

21. **Comments:**

Reviewers please take note: The proposal document submitted has 30 pages of which 17 are narrative and 13 pages are figures. Owing to a bug in Acrobat5 Fig. 10 and 11 was lost from the PDF file, and only the figure legends remain.

Environmental Compliance Checklist

<u>Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.</u>

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.

      The proposal describes a research project that will only involve sampling non-endangered animals, non-endangered plants, soil and water samples.

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

   <u>CEQA Lead Agency:</u>
   <u>NEPA Lead Agency (or co-lead:)</u>
   <u>NEPA Co-Lead Agency (if applicable):</u>

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?

      None

   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:

Permission to access federal land. Required
Agency Name:

Permission to access private land.
Landowner Name:

6. **Comments.**

Land Use Checklist

<u>Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.</u>

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).

   Proposal involves research only.

4. **Comments.**

Conflict of Interest Checklist

<u>Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.</u>

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):

Steven Obrebski, San Francisco State University
George Irwin, San Francisco Satete University

Subcontractor(s):

Are specific subcontractors identified in this proposal? No

Helped with proposal development:

Are there persons who helped with proposal development?

No

Comments:

Budget Summary

Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

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.

Federal Funds

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 studies	1664	33503	7174	2000	1500		2000	500	46677.0	23339	70016.00
2	nutrients	1165	23452	5022	1000	4000		6000	500	39974.0	16337	56311.00
3	feeding biology	499	10051	2152	500	1500		3000	500	17703.0	7002	24705.00
		3328	67006.00	14348.00	3500.00	7000.00	0.00	11000.00	1500.00	104354.00	46678.00	151032.00

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 studies	1165	24625	5273	1500	500			600	32498.0	16489	48987.00
2	nutrients	1165	24625	5273	1000	2400			600	33898.0	16489	50387.00
3	feeding biology	998	21107	4519	500	1100			600	27826.0	14133	41959.00
		3328	70357.00	15065.00	3000.00	4000.00	0.00	0.00	1800.00	94222.00	47111.00	141333.00

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**
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Grand Total=<u>**292365.00**</u>

Comments.
no comment

Budget Justification

Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

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

Obrebski - 1248 Irwin - 1248 Technician - 2080 Grad student - 2080

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

Obrebski - Year 1 - 30% $15173 Year2 - 30% $15931 Irwin - Year 1 - 30% $16474 Year2 - 30% $17297 Tech Year 1 - 50% $ $20800 Year2 - 50% $21840 Grad Student Year 1 - 1040 hrs $14560 Year2 - 1040hrs $15288

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

Obrebski 28% Irwin 12% Tech - 38% Grad Student 1.5%

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

no non-local travel intended

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

Amounts for both years entered Office $200, Laboratory $7000 Computer $300 Field $3500

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.

no service or consultant costs

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.

no equipment to be purchased fits this category

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 presentatons, reponse to project specific questions and necessary costs directly associated with specific project oversight.

A research project is being proposed - this requests seems to fit a construction project which we are not proposing. Cannot imagine how to calculate all this.

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

Total direct costs of project - excluding overhead is $104354 in first year $9422 in second year.

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.

Total indirect costs are $46677 in first year and $47111 in second year based on 50% rate.

Executive Summary

Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

We provide evidence of a mutualistic interaction between an abundant amphipod, Traskorchestia traskiana, and pickleweed, Salicornia virginica, a dominant salt marsh plant in San Francisco Bay. Experimental increases in amphipod density increase growth and flowering of pickleweed, probably by increasing nitrogen, which is shown to limit pickleweed growth. Amphipods living in picklweed stands are protected from predation and exposure. Further studies of this functional interaction are proposed. These include: (a)estimation of amphipod population density, structure and movement in pickleweed marshes (b) studies of fluctuations and variation in nutrient and other chemical and physical factors along elevation gradients in pickleweed marshes, in conjunction with estimates of pickleweed growth rate and field experiments designed to detect increases in plant tissue nitrogen concentrations in response to increases in amphipod densities, and (c) experimental and field studies of the feeding ecology of the amphipod. The proposed work will elucidate the structural features of a newly discovered functional relationship in North Pacific salt marshes and provide quantitative ecological data for evaluating the status of historical and restored marshes in the San Francisco Bay area. The Traskorchestia-Salicornia mutualism was discovered and described, and appropriate study methods were developed, in studies between 1996 - 2001 on Muzzi Marsh, Corte Madera, in which tidal action was restored in 1976. To explore the generality of findings in Muzzi Marsh, more experimentation has just been completed in the Tolay Creek area. The proposed work would occur in Muzzi Marsh and various sites in the Tolay Creek area and also on sites along the Petaluma River. This will provide a variety of Salicornia virginica habitat types, differing in growth form, exposure, and age for the study of the ecology of the Traskorchestia-Salicornia interaction.

Proposal

San Francisco State University

Amphipod (Traskorchestia traskiana) - pickleweed (Salicornia virginica) interactions in San Francisco Bay area salt marshes.

Steven Obrebski, San Francisco State University
George Irwin, San Francisco Satete University

Research Proposal – CALFED Bay-Delta Program

Amphipod (*Talorchestia traskiana*) – Pickleweed (*Salicornia virginica*) Interactions in San Francisco Bay Salt Marshes

Applicants:

Dr. Steven Obrebski and Dr. George H. Irwin

Romberg Tiburon Center for Environmental Studies
San Francisco State University
3152 Paradise Dr.
Tiburon, CA. 94920

October 4, 2001

INTRODUCTION

In marshes bordering San Francisco Bay, California, dense stands of the perennial pickleweed, *Salicornia virginica*, are separated from the lower intertidal cord grass; *Spartina foliosa*, by a sharp ecotone characterized by higher salinities and densely packed soils (Mahall & Park, 1976a,b and c)] *Salicornia virginica* is a dominant stem-succulent halophyte occupying higher tidal elevations of San Francisco Bay marshes, which is more tolerant of high salinities and than Spartina foliosa, the native cordgrass, both in its growth and photosynthetic performance (Pearcy & Ustin, 1986). We have observed very abundant populations of the North Pacific Talitrid amphipod (beach hopper) *Traskorchestia traskiana* living in the pickleweed, foraging apparently mostly on plant detritus, although its feeding habits in marshes are poorly known. It is known to live and feed on beach wrack elsewhere (Koch, 1990) and we have seen it on beaches in South San Francisco Bay and Tomales Bay. The amphipods are sometimes especially active at night during spring tide periods (personal observations), and burrow among the plant roots, sometimes for several weeks, during neap tides when the surface dries out (see data below).

In exploratory field experiments and surveys conducted between 1996-2000, which are described in detail -below, we discovered that increases in the density of the amphipod *Traskorchestia traskiana* increased the above ground biomass and flowering rate of *Salicornia virginica* (Pickleweed), on a marsh where growth of the plant was found to be nitrogen limited (Obrebski& Irwin,to be submitted). We hypothesize that in this newly discovered facultative mutualism, amphipods obtain cover from daytime predation and exposure underneath the *Salicornia* (henceforth pickleweed) canopy, and burrow among the plant roots during neap tide periods when the substrate surface dries out. We believe that the amphipod supplies nitrogen to the plant by defecation at the surface and ammonia release when buried among pickleweed roots. We speculate that the amphipod may also benefit from oxygen release from the plant at this time. Mutualism between species is well known in marshes, and more generally under physiologically stressful environmental conditions (Bertness, 1993, 1994a, 1994b, 1997; Menge, 2000; Stachowicz, 2001). We believe that we have discovered a functional relationship between the two species and ultimately it will be interesting to determine how much variation in pickleweed production can be attributed to amphipod influences and how variation in the pickleweed habitats influences the size and distribution of amphipod populations. Ultimately we would seek to develop a quantitative description of the influence of amphipod populations of the flux of nitrogen to the plant, but, owing to the many physical and biological processes that influence the nitrogen cycle in marsh sediments and waters, this would require a quite broad inter-disciplinary approach. In this proposal, we seek support to develop a better description of the structural features of the *Traskorchestia-Salicornia* functional interaction which would, in any event, be needed to achieve the latter broad goal.

A general outline of the proposed research follows with commentary on how it addresses CalFed study goals. A detailed description of research results obtained in 1996 – 2001 follows in the BACKGROUND – REVIEW OF PREVIOUS RESEARCH section. More detailed descriptions of the methods to be used in carrying out the tasks are listed in the subsequent PROPOSED RESEARCH section.

The research will be done in several historic (Petaluma River) and restored (Muzzi Marsh, Tolay Creek marsh sites, San Pablo Bay). Three research tasks are proposed.

(1) Description of seasonal changes in intertidal distribution and population ecology of *Traskorchestia traskiana* in *Salicornia virginica* stands and the calibration of trapping methods developed for sampling the amphipod populations and determining their abundance. Data will be obtained in several historic

and restored marshes and this will be usable as a base for evaluating structural features of other marshes. Biomass estimates of the amphipods will be needed in future studies of their contribution to pickleweed production.

(2) Monitoring of growth rates of pickleweed along with measurements of seasonal changes in interstitial water and sediment nutrient (principally nitrogen) concentrations and measurements of other environmental variables.. These studies will occur along tidal elevation gradients in the same sampling areas and in conjunction with the amphipod studies described above. This work will also involve an experimental field manipulation of amphipod densities in conjunction with measurements of changes nitrogen concentrations in picklweed.

(3) Field and laboratory studies of the feeding ecology of the amphipod, with emphasis on descriptions of what they eat and determination of growth and survival rates under different diet regimens.

The proposed studies address several CalFed and general core areas pertaining to marsh restoration efforts in San Francisco bay and elsewhere on the Pacific coast. According to the CALFED implementation plan, more information is needed about the influence of water depth and salinity regimes on key native and non-native species in tidal wetlands (CALFED, 2000, p.35). Information on limiting factors influencing the distribution, abundance and population dynamics of selected species under varying hydrological conditions can improve evaluations of restoration efforts. Our project will provide basic structural data on a newly discovered functional relationship among species. Understanding of the structural features of natural communities that increase estuarine productivity and protect and restore functional habitat types is a major CalFed task for improving natural communities in the San Francisco Bay region.

In their critical review of approaches to habitat restoration, the linkage between structure and function has been questioned by Zedler & Lindig-Cisneros (2000) who suggest that indices of functional equivalency and biotic integrity are needed to evaluate restoration efforts, and, while comparisons of restored and natural sites based on structure are attractive, measures of structure may reflect different functional relationships among the sites being compared. Their review indicates that assessment of structural-functional relationships in marshes is embedded in a complex network of physiographic (complexity, order and density of tidal creek networks, elevation gradients), ecological (bird use, population fish abundance and size distribution, benthic invertebrate abundance, feeding relationships, vegetation structure, etc.), and physical environmental (soil attributes, nutrients and organic matter concentrations, etc.) variables. They argue that structural measures cannot be implicitly assumed to indicate ecosystem function, and while “structural equivalency” and “biotic integrity” are acceptable relative site comparisons, in the absence of adequate proof, references to function should be omitted to reduce misunderstanding.

However, we believe that our field experimental results (reported below) strongly suggest that a mutualistic functional relationship exists between *Traskorchestia traskiana* and *Salicornia virginica* in a restored marsh in San Francisco Bay. The ultimate goals of future research on this system is to (a) determine the generality of the phenomenon, and (b) obtain quantitative assessment of the degree to which the amphipod contributes to the growth and reproduction of the plant, and the plant contributes to the growth, survival and reproduction of the amphipod. Progress in goal (b) will depend upon acquisition of baseline data on the amphipod population biology and natural history, and development of techniques for measuring the flux on nitrogen from the amphipod to the plant. It is our contention, that the findings presented herein provide sufficient evidence for a functional relationship that merits the gathering of "structural" data on the amphipod populations and on the distribution of nutrients in the interstitial waters and sediments of marshes in San Francisco Bay. These data will provide a necessary structural template within which future studies of functional relationships will occur. For example, future measurements of flux of nitrogen from amphipods to pickleweed will depend on the development and estimation of amphipod population (and biomass) fluctuations and distributions in pickleweed marshes.

The proposed studies will be conducted in marshes on the Petaluma River, and along the western and northern shore of San Pablo Bay. Considerable attention will be given to the placement of study transects owing to recent studies by Sanderson et. al.(1999) who found that the composition of plant species assemblages in Petaluma March, Sonoma County, CA varies with channel size and distance from channel banks. They argued that the geographic disposition of tidal channels influences the environmental conditions within which ecological processes such as competition, mutualism and responses to disturbance interact to determine observed species distributions. Since sediment salinity, nutrient content, and sulfide toxicity are influenced by proximity to tidal channels, channel geography establishes the basic conditions under which biological functions develop. According to this view, distance from tidal channels and channel size are dominant physiographic features of marshes that influence the magnitude of interactions between other species. Therefore our placement of study transects will take special account of local physiographic features including tidal elevation and the proximity and size of tidal channels. We believe that our studies will provide information that will be useful in future research on the influence of the foregoing factors on biological function in marshes.

The proposed studies may inform another aspect of marsh ecology. According to Kneib (1997), the role of permanent resident nekton in marshes has been neglected owing to emphasis on commercially harvested species. As a consequence, even though the intertidal vegetal component of marshes is dominant in areal extent, the transfer of production from this region to other trophic components in estuaries is poorly known. Kneib argues that juveniles of various permanent marsh residents of these areas obtain partial refuge from predators while obtaining access to food resources in shallow puddles and channels on the marsh surface. Adults emerge from intertidal pools and subtidal habitats in drainage areas to feed on benthic invertebrates and the young resident nekton. These in turn are consumed by transient predators from open water areas and coastal areas. This predatory chain (or trophic relay) from young resident nekton to adult resident nekton to juvenile transient predators moves marsh production toward the open estuary. Our work may expand the trophic relay concept to incorporate the large semi-terrestrial population of *Traskorchestia traskiana* that occur in *Salicornia* marshes. The amphipod is food for marsh fishes (notably the long-jawed mudsucker, personal observations), and possibly various rodent and bird populations (numerous personal communications). Thus, these animals may relay marsh production both into lower as well as into higher intertidal regions and beyond.

We should stipulate at this point that the proposed research is largely descriptive, not hypothesis testing, although some field and laboratory experiments will be done.

BACKGROUND - REVIEW OF PREVIOUS RESEARCH

Introduction

In field experiments and surveys and laboratory studies conducted between 1996 - 2001 in Muzzi marsh, Corte Madera, in San Pablo Bay, we have developed methods for studying the interaction between the intertidal Talitrid amphipod *Talorchestia traskiana* and pickleweed, *Salicornia virginica*. *T. traskiana* is very abundant in *S. virginica* stands, and we have been able to show in field experiments that pickleweed growth and reproduction is positively correlated with amphipod abundance, probably because the animal contributes nitrogen, a limiting nutrient for plant growth in salt marshes. Indeed, the two species form a mutualistic pair, the plant obtaining nutrients from the animal, and the animal obtaining shelter and food from the plant. Owing to the dominance of *S. virginica* in salt marshes in San Francisco Bay, our results suggest that this functional association needs to be studied in more detail to develop basal criteria for evaluating marsh restoration projects. A summary of our findings to date is presented below.

Effects of Amphipods on Pickleweed Biomass Growth – Field Experiments

In the spring of 1996 we observed large populations of the Amphipod *Traskorchestia traskiana* in stands of Pickleweed, *Salicornia virginica* in Muzzi Marsh, Corte Madera. In laboratory experiments the amphipods were found to feed on fresh and dry pickleweed, plant detritus and coffee filter paper. This suggested field experiments to determine the degree to which amphipod abundance influences pickleweed growth. We developed field methods for investigating the interactions between these two species. Amphipod abundance was varied in 1 m square enclosures during the spring and summer growth phase of the plant in 1996 and 1997 on Muzzi Marsh. The enclosures were made of plastic window screen (1mm square) supported by 0.5 inch PVC frames buried 10 cm below the surface and they projected 60-70 cm above the marsh surface.

Amphipod abundances estimates and collections were made with traps consisting of open plastic vials (66 mm long by 25 mm diameter) attached to the substrate by wire. Pieces of coffee filter paper were placed in the vials to attract the amphipods. When moistened by tidal inundation these traps attracted amphipods which could be counted and transferred between experimental enclosures.

In1996 field trials, nine 1 m sq. enclosures were established in groups of three at various locations. Three treatments in each group of three enclosures. In “Depleted” enclosures amphipods were regularly trapped in 40 vials and released into “Enhancement” enclosures. No amphipods were added or removed in “Not Changed” enclosures. Each group of three enclosures was placed in an area that appeared visually homogeneous with respect to pickleweed height and density. Transfers were made 3 times per week between May and July 1996, the primary growth phase of the pickleweed but the amphipods were not counted. Pickleweed was harvested by clipping the plants at the base, and determining their wet and dry weights. As no drying oven was available, dry weights were obtained for plants dried to constant weight in the sun. Based on our feeding observations we hypothesized that relative to unmodified cages, pickleweed growth would be significantly higher in the depleted cages and significantly lower in the enhanced cages. The results were exactly opposite. As shown in Fig 1., relative to unmodified (Not Changed) cages, pickleweed biomass was significantly lower in the Depleted and higher in the Enhanced cages (ANOVA, $F_{(2,6)} = 20.26$, $p = 0.002$).

The foregoing experiment was repeated on a larger scale in the spring and early summer of 1997 with counts of amphipods included in the design. The underlying premise of the experiment was that if *Salicornia* growth is somehow stimulated by the presence of *Traskorchestia*, then, in homogeneous stands of *Salicornia*, experimental increases in amphipod abundance ought to be correlated with increases in measures of growth performance of pickleweed such as above ground wet and dry biomass, and, corresponding decreases in amphipod density should result in decreases in those measures. Homogenous *Salicornia* stands were picked visually because no non-destructive method of estimating pre-treatment biomass was available. We did not consider other measures of pickleweed response to amphipod abundance at the beginning of the experiment. Subsequently, a device for measuring plant height was developed, and prior to harvesting at the end of the experiment, the flowering rate was determined by counting flowering stem tips. In addition, a measure of the water content of the plants was obtained from the wet to dry biomass ratio.

Treatments included Depleted, Not Changed and Enhanced enclosures as before, as well as Open quadrats in which abundances of free living amphipods were monitored. Twelve one square meter enclosures and four one square meter open areas were located in an area appearing to have a homogeneous stand of pickleweed and arranged in rows and columns of 4 treatments according to a Latin Square design. Each treatment area contained 30 vial traps filled with coffee filter paper. Amphipod counts in the vials and transfers were made two or three times per week, between March 1 and June 6, 1997. Enhanced enclosures were counted first and the amphipods placed back into the enclosures, as was the case for the Not-Changed enclosures. Amphipods in the Open quadrats were released within the Open quadrats. Amphipods counted in the Depleted cages were the put into the Enhanced cages. The number of amphipods affecting each quadrat was determined from the cumulative sum of the amphipods counted in each treatment area during the survey period. Biomass harvesting occurred on August 12, 1997. Five pickleweed response variables were measured. Wet and dry weights, plant height, wet/dry weight ratio, and flower number .(average of five counts of numbers of flowering buds per quadrat between July 17 and August 11, 1997), plant height (average of 10 random measurements per quadrat), and wet/dry biomass ratio. Wet and dry weight biomass and plant height are measures of plant growth response to variations in amphipod density. Flower number is a measure of reproductive response. The wet/dry weight ratio roughly measures the ratio of green photosynthesizing tissue to stems and water content of the plants.

Results for this experiment are summarized in Figures 2 – 5. Correlations between *Salicornia* wet weight biomass and log-transformed flower numbers and amphipod abundance were 0.573 ($p = 0.02$) and 0.692 ($p = 0.003$), respectively. The other measures of *Salicornia* response, showed positive, but less significant, correlations with amphipod abundance: dry biomass, 0.410 ($p = 0.115$); average height, 0.475 ($p = 0.063$); wet/dry weight ratio, 0.482 ($p = 0.059$). To investigate the effects of amphipod density on all five measures simultaneously, the five measures were standardized by subtracting their respective means and dividing them by their respective standard deviations. This normalized each measure on the same scale, and the five responses were combined by averaging them to calculate a “mean response deviation” that produced a correlation of 0.733 ($p = 0.001$) with amphipod abundance (Fig. 5). Similar results can be obtained using principal component analysis (Obrebski, Irwin, Mazotta, submitted).

Assuming that the response measures (wet and dry weights, plant height, wet/dry weight ratio, and flower number) are correlated with the “Darwinian fitness” of the plant, our results suggest that increases in the abundance of the amphipod *Traskorchestia traskiana*, increase the fitness of *Salicornia virginica*.

On the basis of the foregoing results, the generality of our findings has recently been addressed in the spring and summer of 2001 in the Tolay Creek Unit of the San Pablo Bay Wildlife Refuge under the direction and with the support of Dr. John Takekawa, and with the collaboration of Giselle Downard, Wildlife Biologists with the USGS Biological Resource Division at the San Francisco Estuary Field Station, Vallejo, CA. Amphipod depletions and enhancements have been carried out at eight stations, along with measurements of soil chemistry, *Salicornia* growth, and using more efficient method (weighing) to measure amphipod biomass. Analysis of this data is pending.

Amphipod Behavior and Migration

Information on the intertidal migrations and tide related behavior of *Traskorchestia traskiana* was obtained in 1996 and 1997. Intertidal migrations were made at 40 stations at different tidal elevations in Muzzi Marsh. Two vial traps with filter paper were placed at each station and the numbers of amphipods in the traps were counted one to several days later and the animals were released. Between 8/10 to 10/10, 1996 1120 such paired vial samples were taken. The numbers in the two vials at each station were averaged to produce 1200 estimated of abundance at different states of the tide between the dates mentioned. Tidal elevations at each station were determined by a method described in a following section (page).placing a clean white 0.5 inch PVC at each station and marking it with colored water soluble dry erase marker ink. The ink was removed as tidal waters removed up to the highest tide level obtained just prior to the time of measurement of the height to which the ink had been removed. The data were sorted according to increasing tidal elevation and accumulated into 112 sequential data sets for which the average amphipod abundance and average elevation were computed. Tidal elevations were recalculated as deviations from the highest high tide just previous to the time of collection. The results are summarized in Fig. 6 and show that amphipod abundances are highest at the tidal elevation corresponding to the highest high tide in the 24 hours previous to the date of collection. This suggests that the amphipods tend to follow the wrack zone deposited by the higher high tide as it progresses up and down within pickleweed stands. If *Traskorchestia* is principally a detritus feeder, this behavior would be consistent with this specialization.

Additional information was obtained from summaries of counts of amphipods obtained in the experimental enclosures and open areas described above. Summaries of counts of amphipods along with the elevation of the highest high during the census period are shown in Fig. 7. Decreases in amphipod abundance in the enclosures corresponded to declines in the height of the higher high tide and increases occurred as tidal levels rose. Enclosed amphipods returned twice to abundances observed at the beginning of the census period. Abundances in open areas followed a similar pattern until April, 1997, when abundances remained low even as tidal levels remained high. These results indicate that amphipods in the enclosures burrowed in the substrate during the lower (neap) tide periods while free animals either burrowed, migrated elsewhere or died out.

Discussion - Amphipod Effects on Pickleweed – The Fertilization Hypothesis

The major hypotheses concerning the mechanisms by which amphipods influence pickleweed growth are:

1. Fertilization Hypothesis: Amphipods add nitrogen to the system by defecating on the substrate or by releasing ammonia when buried amongst the roots of the plant during lower tide periods.
2. Oxygenation Hypothesis: By burrowing into the substrate they increase oxygen levels in the anoxic layer. The implications of this possibility have not been addressed.

Only the first hypothesis was addressed by performing field experiments. We performed a fertilization experiment in the spring and summer of 1999 in two regions, choosing visually homogeneous stands of pickleweed in each. Urea nitrogen was scattered by hand on the surface of replicate one square meter test plots in concentrations of 0, 20, 40 and 80 grams nitrogen per square meter six times during the growing season between May 1 and August 30, 1999 after which the plant was harvested. The concentrations used were based on amounts used is a study of fertilization effects of pickleweed and cord grass by Covin & Zedler (1988). Production of pickleweed was determined from estimates of grams wet weight per square meter. The results (Fig. 8) show that there were significant differences in the response to fertilization between regions and average pickleweed production increased substantially at high fertilization levels. Another fertilization experiment was done in 1 square meter plots along a transect in Muzzi Marsh in 2000 at stations varying in elevation from 5.4 to 6.2 ft. above MLLW. Eighty grams of urea nitrogen per square meter was added to each plot between 1 April and July 15 2000 at intervals of two weeks. The experimental design was flawed because the scattered fertilizer was rapidly dissolved and washed away at lower elevations and remained on the surface for long periods of time at higher elevations. A sub-surface injection method mat have been more appropriate. However, analysis of the results for stations pooled for elevations between 5.7 and 5.9 feet above MLLW showed that fertilized plot standing crops were significantly higher than unfertilized standing crops ($F_{(1, 32)} = 7.63$, $p = 0.009$)(Fig. 9). These fertilization studies suggest that growth of pickleweed on Muzzi marsh is limited by insufficient availability of nitrogen.

Increased biomass production by the addition of nitrogen has been demonstrated in many marshes (see Rozema et al. (2000, p.484 ff. for brief review) . Some studies have shown that higher nitrogen levels occur in salt marsh soils bordering channels (Valiela et al., 1978, and others), while others suggest the opposite (Mitch & Gosselink, 1993, p. 236 ff.). To our knowledge, the influence of marsh channel structure on nutrient distribution in San Francisico Bay marshes is unknown.

Our emerging conceptual picture from the foregoing studies is that the amphipod obtains physiological shelter (lower temperatures, higher humidity, escape from desiccation during neap tides) and protection from predators such as birds within the pickleweed canopy. The animals may be able to climb up into the canopy to avoid fish predation. The presence of amphipods might enhance pickleweed biomass and seed production by increasing nitrogen levels in a chronically nitrogen depleted habitat. The enhancement of seed production, incidentally, increases the potential for seeding new areas with *Salicornia*. Indeed, in a recent study, Coffman (1998) showed that species of *Salicornia* were the major component of seed banks in old and new marshes in San

Francisco Bay and pickleweed seeds were most abundant in the wrack zone. The fertilization effect of amphipods may depend on variation in the tidal regime during the year as shown in Fig. 13. The higher high tides that inundate Muzzi marsh follow recurrent fluctuations with a minimum in the early spring, at which time the animals may tend to escape desiccation by burrowing and thus fertilizing the plants during this early growth phase. This proposition could be tested in the field experimentally by appropriate manipulations of amphipod density – that is by using the amphipods as living fertilizer. This notion is addressed in the proposed research below.

Various herbivores are known to impact salt-marsh plant succession by affecting nitrogen mineralization rates. Owing to the accumulation of litter and woody shoots in ungrazed plots, net mineralization rates were much higher there in comparison to areas grazed by geese, hares and rabbits in Dutch marshes studied by (van Wijnen et al. , 1999). They suggested that selective grazing affects litter quality, by removal of palatable plants, and retention of slowly decomposing unpalatable species. Grazing will also affect the litter pool size which can affect mineralization rates, partly by increasing soil dryness which would inhibit mineralization. Nevertheless, deposition of fecal material by grazers can stimulate plant production, but in the areas studied N – mineralization dominated nitrogen release.

Clearly the effect of amphipods is embedded in the complexities of numerous factors affecting the nitrogen cycle in marshes. For example, can litter removal by feeding *Traskorchestia traskiana* influence N-mineralization rates in pickleweed habitats, and if so, how much is that compensated for by amphipod defecation and release of ammonia.? There is data in a food web analysis of some southern California wetlands that an amphipod, Orchestia traskiana may consume various macroalgae as well as species of Salicornia and Spartina. (Kwak & Zedler, 1997) . Creach et al. (1997) found diatoms to be the major source of organic material assimilated by macro-consumers in a middle elevation marsh areas inhabited by species of *Salicornia* and other plants. marsh The amphipod has d13C values between C3 halophytic plants and micro-algae. While carbon nitrogen and gut analysis indicated the O. gamarellus was an omnivore , the isotopic analysis showed that although over 50% of its food was of higher plant origin, on one occasion diatoms were the basic trophic source..

It is by no means certain what *Traskorchestia traskiana* eats. We have been able to maintain them on moistened coffee filter (bleached and unbleached) papers for several months in laboratory cultures,. This suggests that cellulose can be a major component of their diet. But under these conditions it is not clear where they might obtain their nitrogen, unless they harbor intestinal nitrogen fixing microorganisms (as suggested by Dr. Tim Hollibaugh), although they have been observed also to feed on dead amphipod carcasses. A crude laboratory experiment involving the manipulation of numbers of amphipods on fixed amounts of food, showed that the amphipod tended to consume filter paper and dry pickleweed stems at a faster rate than green pickleweed stems. More qualitative and quantitative information on the feeding biology of this amphipod is needed.

PROPOSED RESEARCH

Proposed field work described below will be carried out in various transects at sites on the Petaluma River, in Tolay Creek and on Muzzi Marsh. The exact localities, numbers of

transects, arrangement of stations within transects, and other field sampling locality details will be determined in the first few months of the project. Some of these details will have to be determined after completion of preliminary surveys. Attempts will be made to pick areas differing in the influence of major physiographic features such as channel order, slope and intertidal elevation. These details are therefore not referred to in the work description below. Three categories of research, or tasks are enumerated and described below.

TASK 1: Studies Of Abundance, Intertidal Migration and Distribution of *T. traskiana.*

More accurate methods of estimating amphipod population size , structure and redistribution are needed. The degree to which the amphipod Talorchestia traskiana influences pickleweed growth under natural conditions in San Francisco Bay Marshes is very likely to depend on its abundance, or, more specifically, the distribution of its biomass during the main growing period of the plant (spring to mid summer).. Ultimate determinations of the flux of nitrogen from the animal to the plant population will depend on obtaining accurate estimates of seasonal and intertidal variations in its abundance and distribution. We will develop methods for calibrating our vial trapping methods against quantitative methods for estimating population size.

Standard trapping methods to describe changes in abundance and distribution of amphipods and use the specimens obtained to determine changes in population structure (size, sex ratio, fecundity, mating frequency). Animals will be trapped using vials filled with coffee filters. Replicate vials will be placed at stations in pickleweed stands along transects varying in tidal distribution. Elevations will be determined by the methods described above. Collection frequencies will be sufficient to detect seasonal spring and neap tide changes. An example of the kind of data that we will obtain is described in Fig. 10. At collection time vials will be injected with CO_2 to narcotize and kill the animals. This will insure that the females will not void their eggs and mating pairs will remain intact. Specimens will be preserved in 70% ethylene alcohol. Animals will be measured using digital photographic and image analysis techniques recently developed in our laboratory (Brown, Zahari & Obrebski, in preparation). The techniques and kind of data that can be obtained are described in Fig. 11 – 12. While developing initial sampling methods, we used core samples extracted from the substrate in pickleweed stands. This procedure proved to be impractical owing to the extreme difficulty encountered in breaking apart the very solidly packed sediment and extracting the amphipods so the sediment has to be broken up by hand, and this took several hours or more per core. .Nevertheless, extraction of animals from 9 cores yielded ranges of abundance between 300 and 2500 animals per square meter. In nighttime observations, hundreds thousands of amphipods could be observed foraging, especially in areas that had been cleared in the experiments reviewed above. When using the previously described vial traps, as many as 2500 amphipods were collected in 30 traps! Flooding a 20 cm diameter, 15 cm high metal core on the surface of the substrate and flooded it with water caused the amphipods to emerge and they could be collected with a portable vacuum when the water receded after 5 – 10 minutes. Repeated flooding would yield successive diminishing numbers of the animals. Estimates of abundance could also be obtained by successively removing animals out of the 1 square meter enclosures.

The following methods will be used to estimate abundance and population structure. The level of effort, replication and timing of each will depend upon initial trials and cannot be specified at this time.

A. Depletion of enclosed areas. Sampling areas of different sizes will be enclosed with enclosures such as those used in the experiments previously described. Vial traps will be used to collect and remove the enclosed animals. Appropriate statistical methods for estimating population size from removal data will be used to obtain estimated of abundance per unit area. In particular, Wang & Lonergan (1996) developed an improved method for estimating confidence intervals of population size from removal data.

B. Depletion of flooded core cylinder: Flooding and repeated collection of emergent amphipods from cores placed on the surface of the substrate as described above.

C. Mark and recapture experiments. Various non-toxic dyes and fluorescent powders (including Neon-Nits a powder for detecting head louse eggs) will be used in mark and recapture methods to estimate population size measure amphipod population redistributions (Turchin, 1998).

TASK2: (a) Nutrient Fluctuations and Physical Environment (b) *Salicornia* growth studies. (c) Amphipod Fertilization Experiments

Nutrient Fluctuations and Physical Environment The environmental variables that will be measured over a seasonal cycle will include NH_4^+, $(NO_3^- + NO_2^-) – N$, Kjeldahl N, Redox, Sulfate, Salinity, Temperature, O^2, pore water levels, and humidity measured in pore water and sediments as is pertinent. Several 24 to 48 hour intensive studies will be made to characterize tidal fluctuations in the variables. We pattern our nitrogen assessment on a comparison of soil nitrogen pools in a constructed and natural salt marsh in San Diego Bay Langis et al. (1991). We will use their sampling and other methods for determining pore water and sediment NH_4^+ and $(NO_3^- + NO_2^-) - N$, soil water content, Kjeldahl nitrogen, and "foliar" nitrogen and phosphorus in Salicornia, soil water content, and redox potential. Orion selective ion electrodes will also be used for NH_4^+, NO_3^- and NO_2^- measurements. Pore water salinity will be measured with a temperature compensating refractometer. Information on tidal fluctuations in pore water levels will be obtained using a piezometer and a HOBO pressure transducer as described by Tyler (1997). Long temperature measurements will be done with HOBO continuous recording devices and mini-max thermometers. Redox potential (Eh) will be measured using the platinum electrode method (Tyler, 1997).

The Nitrogen/Phosphorus (N:P) ratio is a new tool for detecting nutrient limitation in wetland ecosystems (Koerselman & Meulman, 1996). A review of 40 fertilization studies showed that ratios exceeding 16 indicate P limitation, while ratios below 14 indicate N limitation. Measurement of this ratio is a cheap alternative to fertilization studies of the type summarized in Fig. 4. Small samples of pickleweed collected for estimation of pickleweed production will be used for determination of N:P ratios. The results will be correlated with estimated of soil nitrogen concentrations, pickleweed growth and amphipod abundance.

***Salicornia* growth studies.** Individual *Salicornia* plants will be tagged at different nodes along the plant and their growth will be measured according to a technique acquired by Giselle Downard, Wildlife Biologist with the USGS Biological Resource Division at the San Francisco Estuary Field Station, Vallejo, CA. Flower production will also be monitored using methods described above.

Amphipod Fertilization Experiments. *Salicornia virginica* may favor uptake of NH4-N over NO3-N and grows in soils where is the most concentrated inorganic form of nitrogen (Page (1995). Amphipods will be used experimentally as fertilizer during the growth seasonal phase of *Salicornia*. Amphipod populations will be added to 0.5 to 1 m^2 enclosures and "foliar" nitrogen in S*alicornia* will be monitored over a period of time after amphipod addition. When scarce, nitrogen is rapidly taken up pickleweed (). Experiments will be run during different tidal phases such as the approach of neap tides when the animals will burrow in the substrate or on approaching spring tides, when they will remain at the surface. If substantial nitrogen transfer from amphipods to pickleweed occurs, increases in foliar nitrogen over periods of days should be detectible, and the rate of foliar nitrogen acquisition should increase with the biomass of amphipods added, after correction for the resident plant biomass in the enclosure. These experiments will by accompanied by and embedded within the soil and pore water nitrogen monitoring described above. The location of the study areas will be determined after initial estimates of available soil and pore water nitrogen concentrations are available. Amphipod fertilization treatments will be controlled as amphipod biomass and vary from none to substantial amphipod additions. We have developed methods for collecting large numbers of amphipods in the field (with many traps) and weighing them using a small battery operated digital balance. Additional treatments involving injection of known amounts of nitrogen (in a form to be determined) will be used to calibrate the amphipod effect, if any against actual nitrogen inputs. The timing of the experiment will be planned in conjunction with some recurrent annual features in tidal fluctuations in this areas (Fig. 13).

Task 3: Amphipod Feeding Studies

According to Kneib et al. (1997) intertidal vegetation has proved to be an important base for salt marsh food chains. However, detritus is thought to be a low quality food scource that cannot be eaten in the quantity required support the growth and reproduction detritus feeding species unless their diets are supplemented by other sources. The study of detrital food chains has been advanced by S. Y,. Newell of the University of Georgia, in particular, with the emphasis on the role of eukaryotic decomposers in the decay of marsh plants (see extensive review by Newell & Porter, 2000). Notably fungal biomass can exceed prokaryotic masses hundereds of times, and fungal productivity can greatly exceed prokaryotic productivity in winter and consumers such as snails and amphipods can obtain much of their nutrition from fungal decomposers,. The basic life histories of these marsh decomposers such as fungi are poorly known and the role of shredding invertebrates such as snails or crustaceans in this system is not well studied. Kneib et al (1997) studied the feeding biology of the Talitrid amphipod *Uhlorchestia spartinophila* which lives on cord grass shoots. They could not reproduce on senescent leaf sheaths, but grew and reproduced, apparently at natural rates, on decayed leaf parts. Removal of clay films from leaf parts, the highest reproductive, survivorship and male/female sex ratio of 1:1 were obtained on diets of decayed leaf blades having the highest content of living fungi. Our proposed amphipod feeding studies are patterned after this work.. It may be asked why we are not interested in pursuing stable isotope ratio studies at this time. As emphasized by Kreeger and Newell (2000), such studies are important in discerning the relative trophic importance of allochthonous as against autochthonous producers in marsh consumer diets, but they lack the precision for elucidating trophic links between consumers and autochthonous producers, or how direct or indirect the links may be. Isotopic studies will be more informative once various food resources for *Traskorchestia traskiana* are identified and their flux through the system is quantified.

Food and Feeding Studies

Plant litter and detritus will be collected and described. Digital photography and image analysis will be used for qualitative and quantitative description and record keeping. Different types of litter and detrital particles will be fed to amphipods and appropriate behavior and quantitative consumption observations will be used to determine food preferences. Once these are identified, laboratory cultures will be established to determine growth and reproductive rates of amphipods on different food types. The observations and experiments to be done are described in general terms below.

Laboratory Cultures

Amphipods will be cultured individually or in groups in plastic cages equipped with covers having screen mesh windows to permit aeration. Cage bottoms will be perforated with small holes and will contain fine sand to a depth of one to two centimeter. The cages will be maintained in trays filled with one cm of water which will rise through the perforations in the bottom and keep the sand moist. The cultures will be kept under constant temperature conditions (to be determined) with an appropriate day-night illumination cycle (to be determined). Temperature and humidity in the culture chambers will be monitored with HOBO continuous monitoring units. Amphipod growth will be measured by knocking them out with CO2, obtaining their wet weight on a Sartorius balance, and photographing them using the previously described digital photographic methods. Measurements of individual amphipods will yield data on variation in growth rates (molting frequency, weight and size increase, etc.) with respect to sex, age, locality and other factors. Quantitative methods for measuring food consumption rates will be used.

Allocation of Effort and Expected results

Support is sought for a two year study. The principal investigators, Drs Steven Obrebski and Georhe H. Irwin will active on the project at 30% for both years and have the assistance of a 50% time technician and an 50% time graduate student. The student will be encouraged to develop thesis research within on an area complementary to, or embedded in, the proposed research. The technician shall be principally responsible for maintaining and organizing the laboratory and field materials and equipment for Task 2, the chemical analysis. The graduate student shall have primary responsibility for maintaining the field operations associated with Task 1. In the first year the total time allotted will be 50% to Task 1, 35% to Task2, and 15% to Task 3. In the second year Task 1 and 2 will be allotted 35% of the time each, and Task 3, 30% of the time.

We are assuming that several, up to three months, will be required to establish and maintain the field and laboratory operations described. At the end of two years we expect to have:

1: A data base describing fluctuations in distribution, structure, reproduction and quantitative estimates of the abundance of *Traskorchestia traskiana* populations in several localities on the Petaluma river and Tolay Creek, and in Muzzi Marsh. These will be accompanied by associated measurements of pore water and sediment nitrogen levels and other associated chemical and physical variables and estimates of growth and flowering rates of *Salicornia virginica*. These data will for a base that can be used to evaluate structural features in other marshes. Sampling will be designed to produce estimates of seasonal variations with some overlap over one or two seasons to produce estimates of inter-annual variation. The results will also include detailed descriptions of the population biology of the amphipod.

2: Results of experimental field manipulations of amphipods designed to measure the influence of their burrowing activities on local distributions of pore water and sediment nitrogen and effects on plant tissue nitrogen concentrations. Associated treatments involving injection of nitrogen into the sediments will be used to calibrate the amphipod fertilization effect, if any. This study bears on our hypotheses concerning the influence of the amphipod on pickleweed.

3: Information on the feeding biology of *Traskorchestia traskiana* and some estimates of effects of variations in diet on amphipod survival and reproduction. It will provide the basis for more detailed studies of trophic relations and structure in *Salicornia virginica* habitats.

Qualifications of the Principal Investigators

The co-principal investigators, Drs. George H. Irwin and Steven Obrebski initiated, developed and supported the research summarized in this proposal between 1996 and 2001. Support for the development of digital photographic methods developed in the spring and summer of 2001 was received from the Romberg Tiburon Center.

Dr. Obrebski received his PhD in Paleozoology in 1968 at the University of Chicago. He has taught marine biology and ecology, and currently teaches courses in experimental design and multivariate statistics. He is a Senior Research Scientist at the Romberg Tiburon Center and has been associated with the laboratory since 1985. He has participated as a co-pi with Dr. George H. Irwin in developing the work on the amphipod pickleweed interactions. He and Dr. Irwin are currently participating in development of field methods for studying the growth of *Egeria densa* for the Dept. of Boating and waterways.

Other projects in which Dr. Obrebski participated are summarized below.

Research Associate – Experimental studies of the effect of aquatic weed control agents and environmental factors on the growth of *Egeria densa*, an introduced aquatic weed in the Sacramento/San Joaquin Delta. California Department of Boats and Waterways. Research Associate – Development of methods for verifying remote sensing studies of the distribution of Egeria densa, an introduced aquatic weed in the Sacramento/San Joaquin Delta. California Department of Boats and Waterways. Principal Investigator – Evaluation of the biological effects of control methods of *Egeria densa*, an introduced aquatic weed in the Sacramento/San Joaquin Delta. California Department of Boats and Waterways. Co-Principal Investigator – Long term monitoring studies of Bolinas Lagoon. CALTRANS. Principal Investigator - Benthic Studies In San Pablo Bay to provide information for locating the outfall of a desalination plant. Marin Water District..Co-Principal Investigator - Statistical analysis of long term zooplankton data in the Sacramento/San Joaquin Delta. California Department of Boats and Waterways. Principal Investigator – Statistical analysis of Delta Smelt abundance changes. California Department Water Resources..

Dr. George H. Irwin an Associate Scientist at the Romberg Tiburon center, San Francisco State University since 1995, received his PhD in Biology at Emory University in 1971. He has expertise in physiological, pharmacological and chemical techniques having expanded methodology for measuring diffusibility and volumetric cerebral blood flow, and designed microcomputer system for measurement of cerebral blood flow by ^{133}Xe clearance. He also designed, installed, operated a human gamete and embryo culture laboratory at Alameda Hospital In 1982 – 84 he was director of the Human In Vitro Fertilization Laboratory at the Foundation for Obstetrics & Gynecology at the Kansas University Medical Center. Since 1997 he has participated as co-principal investigator with Dr. Steven Obrebski in developing and completing the studies of the *Traskorchestia-Salicornia* interaction described above. In 1997 – 1998, and in 1999 he participated as a co-pi and designer of field equipment in field studies to obtain data on the abundance and response to control measures of the aquatic weed, *Egeria densa*, in the Sacramento/San Joaquin Delta for the California Department of Boats and Waterways. He and Dr. Obrebski are currently participating in development of field methods for studying the growth of *Egeria densa* for the Dept. of Boating and waterways.

Literature Cited

Bertness, M. D. (1991). "Interspecific interactions among high marsh perennials in a New England salt marsh." Ecology 72: 125-137.

Bertness, M. D. and S. W. Shumway (1993). "Competition and facilitation in marsh plants." American Naturalist 142: 718-724.

Bertness, M. D. and R. Callaway (1994a). "Positive interactions in communities." Trends in Ecology and Evolution 9(3): 191 - 193.

Bertness, M. D. and S. D. Hacker (1994b). "Physical stress and positive associations among marsh plants." American Naturalist 144: 363 - 372.

Bertness, M. D. and G. H. Leonard (1997). "The role of positive interactions in communities: lessons from intertidal habitats." Ecology 78(8): 1976 - 1989.

Brown, D., Zahari, N. & Obrebski, S. (in preparation). Digital photography and measurement of size distributions of amphipods.

Cline, J. D. (1969). "Spectrophotometric determination of hydrogen sulfide in natural waters." Limnol. Oceanogr. 14(: 454-458.).

Coffman, G. C. (1998). "Natural and restored salt marsh soil seed banks in San Francisco Bay." MS Thesis, San Francisco State University: 59 pp.

Covin, J. and J. B. Zedler (1988). "Nitrogen effects on Spartina foliosa and Salicornia virginica in the salt marsh at Tijuana Estuary, California." Wetlands 8: 51-65.

Creach, V., M. T. Schricke, et al. (1997). "Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers." Estuarine, Coastal, and Shelf Science 44: 599 - 611.

Kneib, R. T., S. Y. Newell, et al. (1997). "Survival, growth and reproduction of the salt-marsh amphipod Uhlorchestia spartinophilareared on natural diets of senescent and dead Spartina alterniflora leaves." Marine Biology 128: 423 - 431.

Kneib, R. T. (1997). "The role of tidal marshes in the ecology of estuarine nekton." Annual Review of Oceanography and Marine Biology 35: 163 - 220.

Koch, H. (1990). "Aspects of the population biology of Traskorchestia traskiana (Stimpson, 1857)(AMPHIPODA, TALITRIDAE) in the Pacific Northwest, U.S.A." Crustaceana 59(1): 35 - 52.

Koerselman, W. and A. F. M. Meuleman (1996). "The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation." Journal of Applied Ecology 33: 1441 - 1450.

Kreeger, D. A. and R. I. E. Newell (2000). "Trophic complexity between producers and invertebrate consumers in salt marshes." M. P. Winstein & D. A. Kreeger, Eds. Concepts and Controversies in Tidal Marsh Ecology, Kluwer Academic Publishers: 187 - 220.

Kwak, T. J. and J. B. Zedler (1997). "Food web analysis of southern California coastal wetlands using multiple stable isotopes." Oecologia 110: 262 - 277.

Langis, R., M. Zalejko, et al. (1991). "Nitrogen assessments in a constructed and a natural salt marsh of San Diego Bay." Ecological Applications 1(1): 40 - 51.

Mahall, B. E. and R. B. Park (1976a). "The ecotone between Spartina foliosa Trin. and Salicornia virginica L. in salt marshes of northern San Francisco Bay. I. Biomass and Production." Journal of Ecology 64: 421 - 433.

Mahall, B. E. and R. B. Park (1976b). "The ecotone between Spartina foliosa Trin. and Salicornia virginica L. in salt marshes of northern San Francisco Bay. II. Soil water and salinity." Journal of Ecology 64: 793 - 809.

Mahall, B. E. and R. B. Park (1976c). "The ecotone between Spartina foliosa Trin. and Salicornia virginica L. in salt marshes of northern San Francisco Bay. III. Soil aeration and tidal immersion." Journal of Ecology 64: 811 - 819.

Martindale, M. (1987). "Salicornia auropaea L. and Salicornia virginica L. on a San Francisco Bay salt marsh: a study of factors contributing to their zonation pattern." MS Thesis, San Francsisco State university: 52 pp.

Menge, B. A. (2000). "Testing the relative importance of positive and negative effects on community structure." TREE 15: 46 - 47.

Mitsch, W. J. and J. G. Gosselink (1993). "Wetlands." Van Nostrand Reinhold, New York: 722 pp.

Newell, S. Y. and F. Barlocher (1993). "Removal of fungal and total organic matter from decaying cordgrass leaves by shredder snails." Journal of Experimental Marine Biology and Ecology 171: 39 - 49.

Newell, S. Y. and D. Porter (2000). "Microbial secondary production from salt marsh-grass shoots, and its known and potential fates." M. P. Winstein & D. A. Kreeger, Eds. Concepts and Controversies in Tidal Marsh Ecology, Kluwer Academic Publishers: 159 - 185.

Obrebski, S., & Irwin, G. H. , 2001 The *Talorchestia traskiana – Salicornia virginica* interaction: experimental evidence for a mutualistic interaction in a San Francisco Bay salt marsh. (To be submitted).

Page, H. M. (1995). "Variation in the natural abundance of 15N in the halophyte, Salicornia virginica, associated with groundwater subsidies of nitrogen in a southern California salt marsh." Oecologia 104: 181-188.

Pearcy, R. and S. L. Ustin (1986). "Effectos of salinity on growth and photosynthesis of three California tidal marsh species." Oecologica 62: 68 - 73.

Sanderson, E. W., S. L. Ustin, et al. (2000). "The influence of tidal channels on the distribution of salt marsh plant species in Petaluma marsh, USA." Plant Ecology 146: 29 - 41.

Stachowicz, J. J. (2001). "Mutualism, facilitation and the structure of ecological communities." Bioscience 15(3): 235 - 246.

Turchin, P. (1998). " Quantitative Analysis of Movement: measuring and modeling population redistribution in plants and animals." Sinauer Associates, Sunderland, MA.

Tyler, C. (1997). Geomorphological and hydrological controls of pattern and process in a developing barrier island salt marsh. MS Thesis, University of Virginia.

Valiela, I., J. Teal, et al. (1978). "Nutrient and particulate fluxes in a salt marsh ecosystem: tidal changes and inoputs by precipitation and ground water." Limnol. Oceanogr. 23: 798 - 812.

Wijnen, H. J. v., R. v. d. Wal, et al. (1999). "The impact of herbivores on nitrogen mineralization rate: consequences for salt marsh succession." Oecologia 118: 225-231.

Zedler, J. and R. Lindig-Cisneros (2000). "Functional equivalency of restored and natural salt marshes." M. P. Winstein & D. A. Kreeger, Eds. Concepts and Controversies in Tidal Marsh Ecology, Kluwer Academic Publishers: 565 - 582.

FIGURES

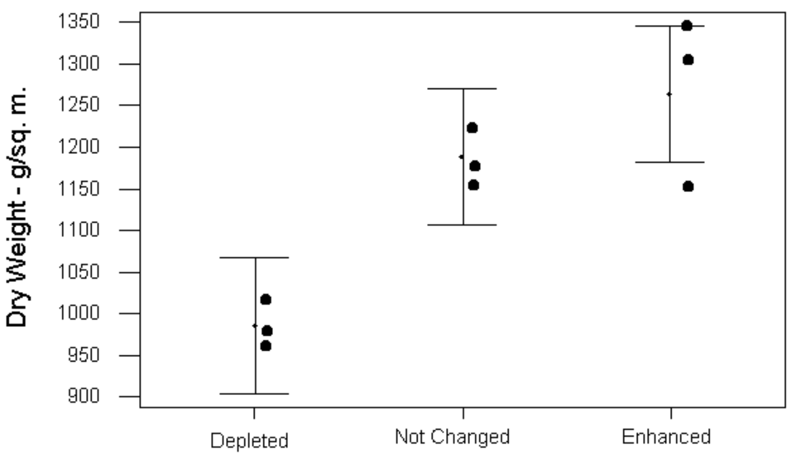

Fig. 1. Effects of changes in amphipod abundance on pickleweed dry weight biomass. Squares are means, circles are data, vertical lines are 95% confidence intervals. Treatments: Depleted – amphipods removed, Not Changed – no changes in enclosed amphipod numbers, Enhanced – amphipods added. Differences among means were found to be significant by analysis of variance ($F_{(2,6)} = 20.26$, $p = 0.002$). Relative to unmodified (Not Changed) cages, pickleweed biomass was significantly lower in the Depleted and higher in the Enhanced cages.

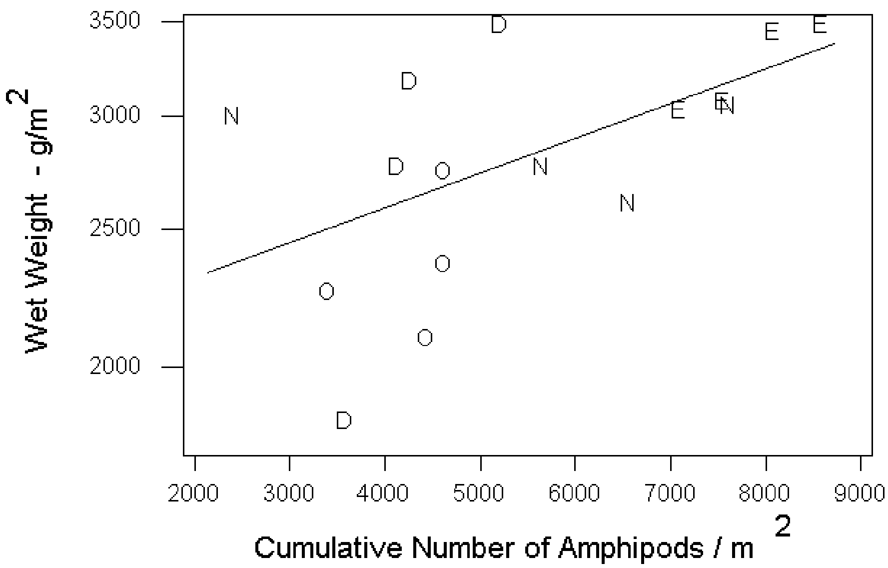

Fig. 2. Relationship between of pickleweed biomass (grams Wet Weight/$m^{2)}$ and cumulative amphipod abundance. The linear regression line shown , Y = 1957 + 0.15 X, where Y is pickleweed wet weight biomass and X is cumulative sum of amphipods, is significant (R^2 = 32.9%, p = 0.02). Fit of the data to a non-linear asymptotic regression (in the form $Y = K + e^{BX}$) resulted in only a slight increase in significance (R^2 = 34%, p = 0.018). Treatments were E = Enhanced, N = Not Changed, D = Depleted and O = Open Areas. The cumulative number of amphipods was obtained by summing counts of amphipods obtained in 30 traps in each treatment area during the period of study between 3/10/97 and 6/6/97.

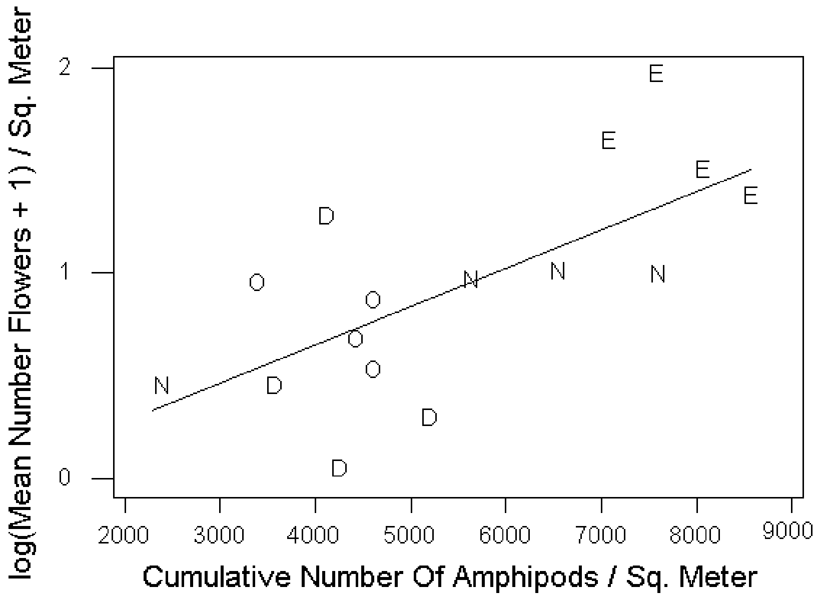

Fig. 3. Relationship between mean number of flowers and cumulative number of amphipods. The mean number of flowers is the mean of five successive counts between 7/17/97 and 8/11/97, which was converted to a logarithmic scale after adding 1 to all the data. The linear regression shown, Y = -0.18 + 0.0002 X, is significant (R^2 = 47*8%, p = 0.003). Treatments were E = Enhanced, N = Not Changed, D = Depleted and O = Open Areas.

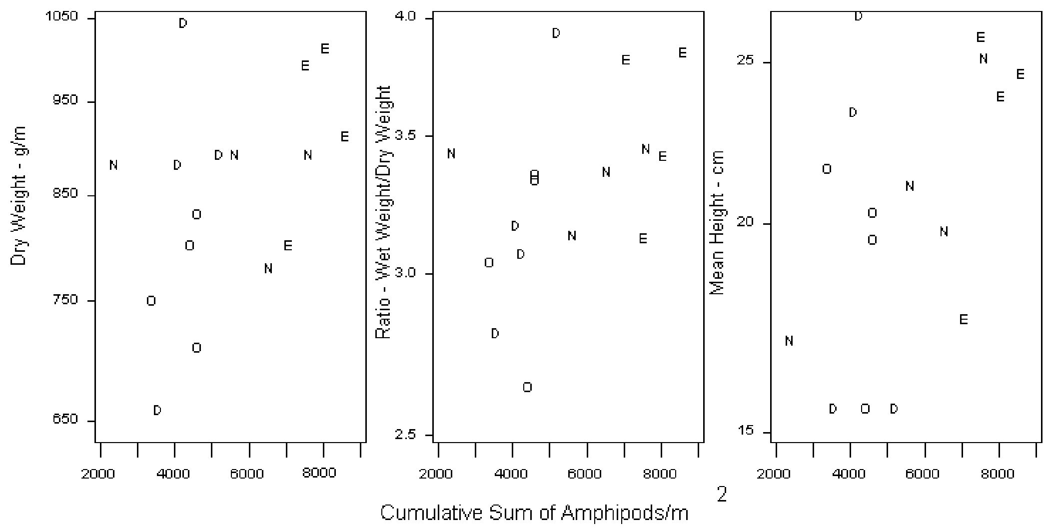

Fig. 4. Plots of Dry weight biomass, Wet/Dry weight ratio and Average heights of *Salicornia* vs. cumulative number of amphipods. Correlations (and their significance levels) of these variables with cumulative amphipod sums were 0.410(p = 0.115), 0.482(p = 0.059) and 0. 475(p = 0.063), respectively. Treatments were E = Enhanced, N = Not Changed, D = Depleted and O = Open Areas.

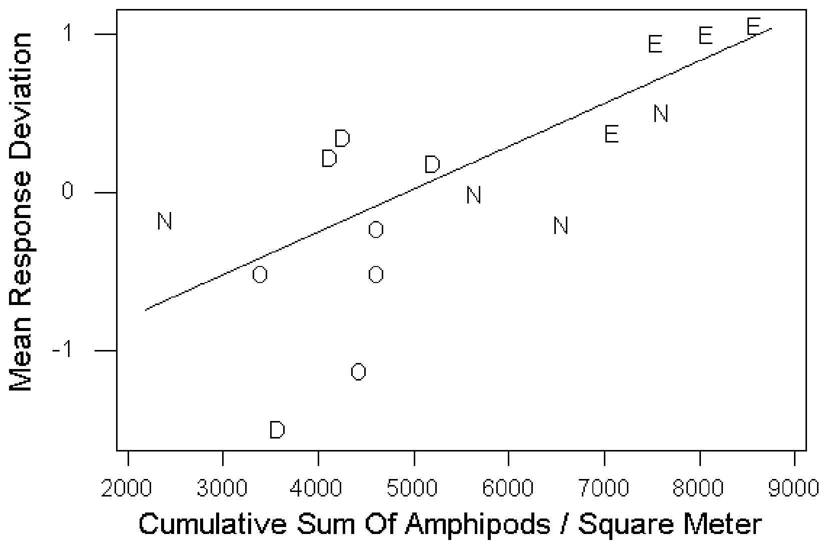

Fig 5. Relationship of mean response deviations to cumulative sum of amphipods.
Mean response deviations were calculated by averaging standardized values of wet weight, dry weight, flower number, height and wet/dry biomass ratio. The linear regression shown, Y = -1.55 + 0.0003 X, is significant (R^2 = 53.7%, p = 0.001). Treatments were E = Enhanced, N = Not Changed, D = Depleted and O = Open Areas.

.

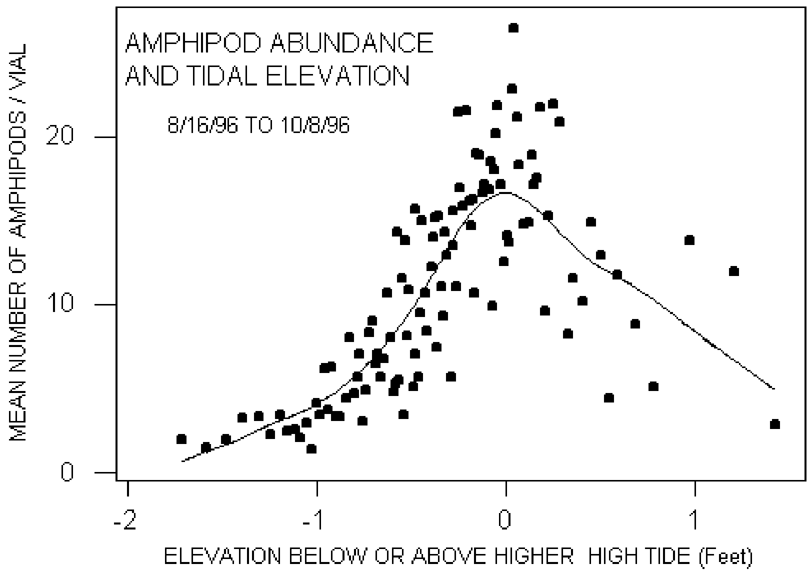

Fig. 6. Relationship between amphipod abundance and tidal elevation. The points are averages of ten neighboring sample abundances and tidal elevations obtained from the raw data sorted according to tidal elevation. Tidal elevation are deviations, in feet, from the highest high tide just previous to the time of collection. The line was calculated using a data smoothing technique. Amphipod abundances are highest at the tidal elevation corresponding to the highest high tide in the 24 hours previous to the date of collection.

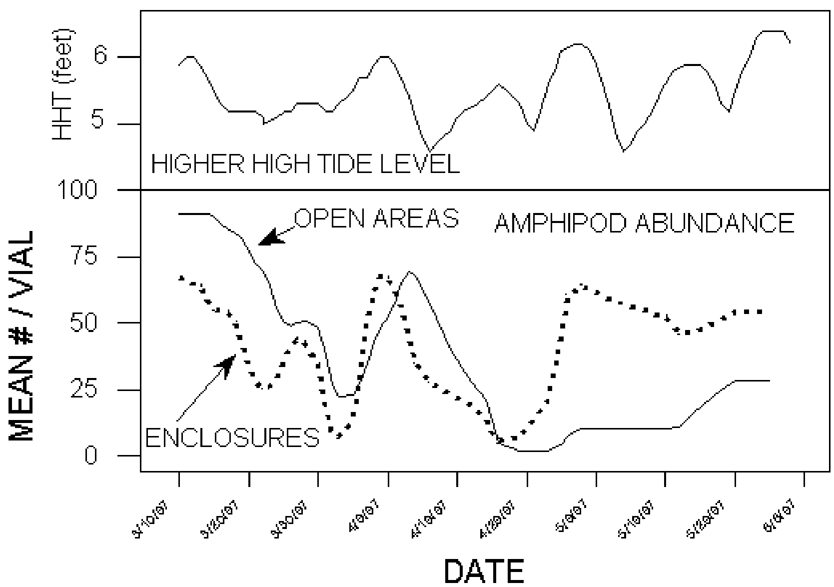

Fig. 7. Lower Panel: Trends in amphipod abundance in enclosures and open areas between 3/10/97 – 6/6/97. Lines were fit to the data using a smoothing technique. Decreases in amphipod abundance in the enclosures corresponded to declines in the height of the higher high tide and increased occurred as tidal levels rose. Abundances in open areas followed a similar pattern until April, 1997, when abundances remained low even as tidal levels remained high. These results indicate that amphipods in the enclosures burrowed in the substrate during the lower (neap) tide periods while free animals either burrowed or migrated elsewhere.. Note that abundances in enclosures returned to similar levels during higher tidal periods.

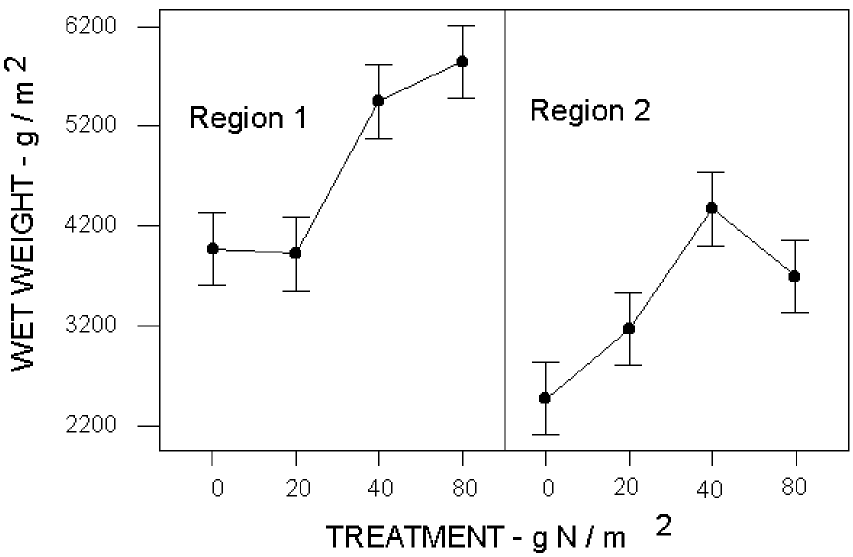

Fig. 8. Response of *Salicornia virginica* to fertilization, Muzzi Marsh, 1999. Circles are means of two replicates, vertical lines are pooled 95% confidence intervals. The results were analyzed by 2-way analysis of variance. Interactions were significant ($p = 0.009$). Differences between nitrogen additions were significant ($p < 0.001$) and differences between regions were highly significant ($p < 0.001$).

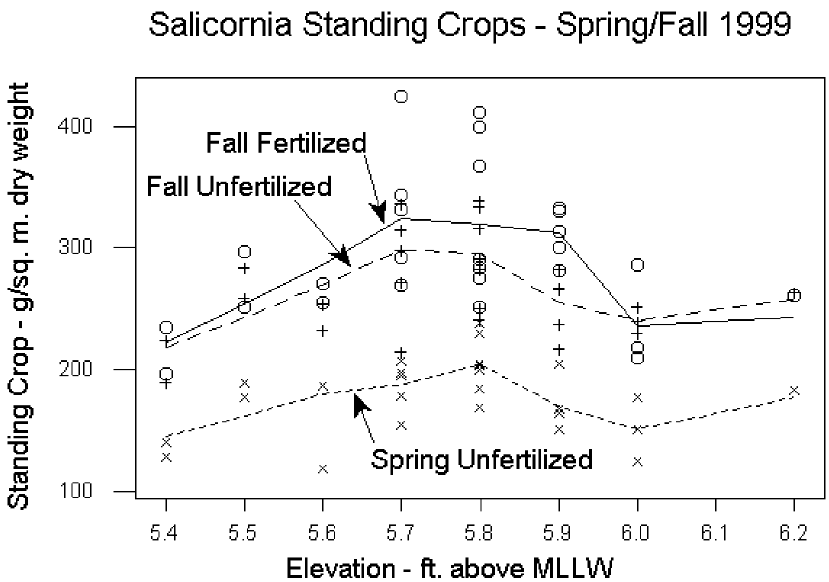

Fig. 9. *Salicornia* standing crops sampled along a 28 station transect on Muzzi Marsh. Each data point represents the dry weight biomass obtained in a one square meter quadrat. Legend: x - spring (March 23, 1999) standing crops, o - fertilized plot fall (August 1999) standing crops, + - unfertilized plot fall standing crops. Curves are lines fitted to the data with a smoothing technique. Analysis of variance of differences in fertilized and unfertilized standing crops were significant (P = 0.009) for data pooled for elevations from 5.7 to 5.9 feet above MLLW. Fertilized mean standing crop was 324 (N = 17, SD = 40), unfertilized standing crop was 278 (N = 17, SD = 51.7) grams per square meter.

Fig. 10. Distribution of abundance of *Talorchestia traskiana* on Muzzi Marsh between 1 March and 14 July, 2001. Dots are means, vertical bars are 95% confidence intervals.   Three replicate samples from 28 stations differing in tidal elevation were pooled over stations with similar elevation and over sampling dates to produce the means. The results summarize the general pattern that is usually observed. Amphipod distribution with respect to tidal elevation shows an abrupt step like decrease in abundance between 5.7 and 5.8 ft above MLLW.

Fig. 11. Variation in size frequency distributions of *Talorchestia traskiana* with elevation at Muzzi on Feb. 21, 2001. Numbers of individuals measured from top to bottom panel were 298, 289, and 248 specimens, respectively. Smaller amphipods tend to occur at lower intertidal levels. Size is measured by the square root of the area of amphipods obtained from digital photographs processed by Sigma Scan image analysis software. The animals were placed on a light table on their sides and photographed with a darkened cover slip of known dimensions to permit calibration of the areas determined by image analysis using Sigma Scan.

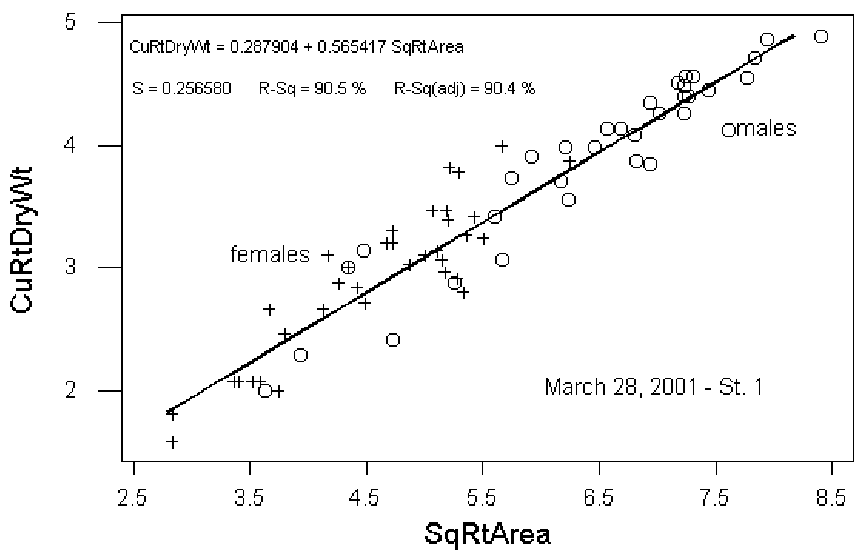

Fig. 12. Results of calibration of image analysis of amphipod sizes. Males and females were photographs and the square root of the area of each specimen was determined. Then, each specimen was individually dried and weighted on a Sartorius balance. Plots of the cube root of the weight (on the Y – axis) against the square root of the area, yield the linear relationships shown. This is a typical result. The regression obtained can be used to determine the weight of a sample from the photographs of the area. The methods makes it possible to measure large numbers of individuals. Comparison of this sample with samples from other stations yielded regressions that were extremely similar and not significantly different when analyzed by analysis of covariance.

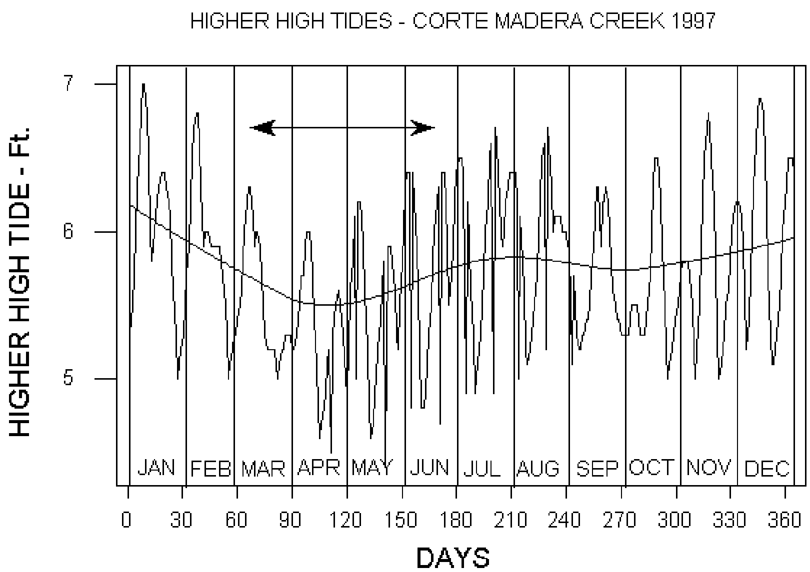

Fig. 13. Seasonal changes in elevation of the higher high tide at Corte Madera Creek, January to Dec, 1997. Data were obtained from a tide simulation program. Arrow indicated the period during which experiments were done in 1997. Note that this is a period during which the average higher high tides decrease in elevation and periods between high tides approaching 6 feet are longer. This is a recurrent seasonal feature of tidal fluctuation that could induce amphipods to spend longer periods of time buried in the substrate and would influence the timing of amphipod fertilization experiments.