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FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE [X] Postdoctoral Researcher [ ] Ph.D. Graduate Student

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Proposed Research

Role of Exotics as Ecosystem Engineers Affecting Estuarine Food Webs in Suisun Marsh

Introduction

Vascular plants have major structuring roles in marine ecosystems (Bruno and Bertness 2001). For coastal wetlands, it is known that the presence of plants affects ecosystem-level processes such as hydrology, sedimentation rate, and nutrient cycling (Bertness 1988, Leonard and Luther 1995, Levin and Talley 2000). Plant shoots and detrital material partially fuel the salt marsh food web (Peterson et al. 1985, Levin and Talley 2000, Levin et al. 2006). In addition, vascular marsh plants modify the amount and quality of light reaching the sediment, thus affecting temperature (Gallagher 1971, Bertness and Hacker 1994) and algal growth (Lüning 1980, Seliskar et al. 2002). On a larger scale, critical salt marsh functions, such as nursery habitat provision, coastal stabilization, runoff filtration, and trophic support are directly and indirectly tied to the presence of vascular plants (Gleason et al. 1979, Warren and Niering 1993). Thus, shifts in vegetation composition through invasion can cause large-scale ecosystem changes due to differences in how they influence soil characteristics, geomorphology, biogeochemistry, regional climate, and activity and distribution of other organisms relative to native plants (Eviner and Chapin 2003).

Invasive plants that cause large-scale ecosystem changes by altering the physical structure of the ecosystem itself have been termed "ecosystem engineers" by Jones et al. (1994). The impacts of these plants can be indirect or direct control of resource availability mediated by an organism's ability to cause physical state changes in abiotic or biotic materials (Crooks 2002). In short, ecosystem engineering is the creation, destruction, or modification of habitats. The physical resources that may be affected by ecosystem engineers include light, humidity, sediment properties, temperature, water content, and salinity. Understanding the effect of ecosystem engineers involves thorough identification and quantification of the resources affected. Once effects on these resources are quantified, it is possible 1) to more directly relate engineering to impacts on other species, 2) to predict the response of invasives to future conditions, and 3) to develop effective management and eradication strategies.

We propose to examine the hypothesis that invasive plants alter the physical environment thus affecting productivity and biodiversity of microalgae and invertebrate communities, with significant consequences for the trophic function of estuarine ecosystems. The overall goals are (1) to identify key functional differences among invaded and natural areas within the marsh, (2) to document if these differences are pervasive across different invader species, disturbance scales, and patch sizes, and (3) to develop the best methods of eradication in a setting where there are numerous constraints (permits; access; competing interest groups; presence of several listed species, esp. CA clapper rail, salt-marsh harvest mouse, soft bird's beak, Suisun thistle).

Study site

We will focus our research on Suisun Bay and Marsh because, as the largest contiguous brackish water marsh remaining on the west coast of North America, it is a critical part of the San Francisco Bay-Delta estuary ecosystem. In California, this marsh encompasses more than 10% of the state's remaining natural wetlands thus serving as an important refuge along the Pacific Flyway (www.iep.ca.gov) and provides critical habitat for a number of endangered and threatened fishes, mammals, birds, and plants (Moyle 2002, Matern et al. 2002).

General approach

Investigations will document changes in hydrogeographic parameters, biogeochemical parameters (porewater nutrients, oxygen, sulfides), algal, plant and faunal diversity and productivity, and trophic pathways in areas invaded by several species of plant and in non-invaded areas (referred to hereafter as "natural"). In addition, several of the invasive species in the marsh have become targets of eradication programs, such as *Lepidium latifolium* at Rush Ranch Preserve (Solano Land Trust). In collaboration with these programs (namely the Solano Land Trust and the San Francisco Bay National Estuarine research Reserve, of which Rush Ranch is a part), we plan to develop an integrated approach to eradicating several invaders in the high marsh and marsh-grassland transition zone, thus developing a science-based, adaptive restoration design.

Experimental Design

There are numerous invasive plants within the Suisun Marsh, as documented in different agency surveys. In 2004, eight non-native vascular plant species (*Arundo donax, Carpobrotus edulis, Centaurea solstitialis, Conium maculatum, Eucalyptus species, Foeniculum vulgare, Lepidium latifolium*, and *Phragmites australis*) were present in the Suisun marsh (Brown 2004). Based on preliminary data, we have chosen to work on three plants that we think constitute the greatest threats to the ecosystem: *Arundo donax* (giant reed), *Lepidium latifolium* (perennial pepperweed), and *Phragmites australis* (common reed).

In other North American salt marshes, several of these are invaders are known aggressive, ecosystem engineers with changes in the physical environment, decreases in associated invertebrate diversity (i.e. Herrera and Dudley 2003, Bedford and Powell 2005), and changes to the food web structure (i.e. Currin et al. 2003). Yet, their potential to alter the benthic community and its trophic functioning is relatively unknown for this specific marsh. Knowing the particulars for a particular ecosystem is essential before an effective management and eradication plan can be developed.

This research will be conducted using a randomized complete block design. Before any eradication efforts begin, we will establish replicate plots in the salt marsh that will include: a) areas with native vegetation, b) areas with invasive vegetation that will be eradicated, and c) areas with invasive vegetation that will remain as controls for the course of the experiment. Native and invasive plots will be of comparable size, and each size plot (within 4 size classes) and each species will be replicated at least 8 times. All invaded plots will be removed at the end of the experiment.

Within each plot, we will characterize a variety of physical and biological properties (discussed below in detail). We will collect data in spring and fall before eradication and on multiple dates after eradication. Monitoring will occur at least monthly for one year and more frequently for some response variables. The pre-eradication samples will allow us to compare the physical and biological attributes of invaded and un-invaded sites. Post-eradication examination of plots with native vegetation and control plots with remaining tamarisk will allow further temporal comparisons of differences in plots with the native and exotic vegetation types. while also allowing us to determine how biotic communities respond to the removal of the invasive plant. The results of these studies will be analyzed using a variety of univariate (e.g. Randomized Complete Block ANOVA's) and multivariate (e.g. non-metric Multi-Dimensional Scaling and Canonical Correspondence Analysis) statistics.

Hydrogeographic parameters

SFNERR has two existing hydrogeographic monitoring stations, located at Rush Ranch and China Camp that can provide continuous data. In addition, the Department of Water Resources (DWR) maintains an extensive network of compliance, monitoring and reporting stations throughout the marsh and this data could be incorporated into the experimental design as well as provide valuable data about changes over time. (http://wwwiep.water.ca.gov/)

Measurements of pore water soil salinity, redox, humidity, and temperature will be made in the field using a refractometer, digital redox meter, digital humidity meter, and a digital thermometer, respectively. Samples of sediment will be collected at each sampling for subsequent laboratory measurement of grain size and percent organic matter using methods in Talley and Levin

(1999). These properties often reflect flow regime and typically influence the diversity of flora and fauna.

Biogeochemical parameters

Porewater nutrients will be sampled using peepers (porewater equilibrators) to collect water samples devoid of sediment. Once back in the laboratory, these water samples will be acidified to pH = 2 using 6 N HC1, and sparged with argon to drive off H_2S. Dissolved inorganic nutrient concentrations of these sparged samples were determined colorimetrically using the ascorbate method for soluble reactive phosphate (SRP) and the indo-phenol blue method for ammonium (NH +) given in Parsons et al. (1984). Porewater sulfides will be determined using spectrophotometric determination (Cline 1969).

Algal diversity and productivity

Diversity of microalgae will be assessed through High Performance Liquid Chromatography (HPLC) pigment analyses to indicate high-level taxonomic (functional) diversity (richness and evenness based on quantification of diatoms, cyanobacteria, green algae, yellow-green algae and other photosynthetic bacteria). HPLC has been successfully applied to quantify the major taxon composition of mixed microalgal assemblages in benthic sediments (Pinckney et al. 1995). Subsamples of sediment material will be analyzed by spectrophotometric methods and HPLC to estimate microalgal biomass (chlorophyll *a* and bacteriochlorophyll *a*).

Microphytobenthic (microalgal and bacterial) productivity rates will be estimated in live sediment cores transported to the laboratory. Oxygen microelectrodes will be used to obtain high-resolution productivity measurements at different depths within surface sediments (Revsbech & Jørgensen 1986). This technique allows for repeated, non-destructive, and relatively rapid rate measurements in sedimentary communities.

Plant diversity

Plant cover and biomass estimates for each species will be made using $0.25m^2$ quadrats. Heights and stem density of the structurally dominant plants will be recorded in the field to estimate total stem length, a proxy for biomass. Vascular plant productivities will be estimated from biomass (TSL) change and for selected areas by harvesting above-ground and below-ground plant material in $0.125m^2$ sections of the larger sampling quadrats. The biomass of halophytes will be separated into dead and live material (Zedler et al. 2001).

Extensive aerial surveys of Rush Ranch (Suisun Bay), being conducted by SFNERR, will be used to assess patch size and geographic extent of invader with the marsh. Diversity will be measured for plants at the species level using percent cover as indicated by the number of species present in the lagoon, the number of species per unit area, dominance (J', Berger-Parker index), H' (information index), and through rarefaction (Hurlbert 1984).

In addition, samples of geographically distinct plants will be collected and preserved in silica for genetic analysis. These samples will be sent to Jun Bando at UC Davis for analysis. Dr. Bando has studied the genetics of hybridization and invasion in *Spartina* on the Pacific coast and will assist in the interpretation and analysis of the genetic data, working closely with the fellow and research mentor. Using her lab and skills will allow us to analyze these samples using well-tested methodology and will improve the utility of these data by making them comparable to a large existing database of over samples from the entire San Francisco Bay as well as North America (where possible).

Faunal diversity

Macrofaunal sediment cores will be collected using a cylindrical push core (4.8 cm diameter) inserted to a depth of 6 cm (Levin & Talley 2002). These cores will be subdivided in the field into 0-2 cm and 2-6 cm sections and preserved (unsieved) in 8% buffered formalin with Rose Bengal dye. For macroinfaunal quantification, cores will be washed through 0.3 mm mesh. The animals that are retained will be sorted from the sediments using a dissecting microscope, identified to the lowest taxonomic level possible, weighed for biomass, and stored in 70% ethanol.

For more mobile species, a modified leaf blower and/or sweep net will be used to "suck" insects from invasive plant and surrounding native areas. The animals that are retained will be sorted, identified to the lowest taxonomic level possible, weighed for biomass, and stored in 70% ethanol.

Diversity will be measured for animals at the species level as indicated by the number of species present in the lagoon, the number of species per unit area, dominance (J', Berger-Parker index), H' (information index), and through rarefaction (Hurlbert 1984). We will use counts of individuals and will also assess functional diversity and feeding mode for the animal groups.

Trophic pathways

Trophic structure, the base and consumers within the food chain, and the importance of different primary producers (i.e. invasive plant species versus native food sources) will be examined using natural abundance and enrichment isotopic techniques. Samples of suspended particulate organic carbon (POC), sediment organic matter (SOM), vascular plants, microalgae, macroalgae, infauna, epifauna, nekton, and bird feathers and droppings will be examined for $\delta^{13}C$, $\delta^{15}N$ natural abundance stable isotopic composition. Methods are reviewed in Currin et al. (1995), Sullivan and Currin (2000), and Whitcraft and Levin (submitted).

Microalgae will be collected using both vertical migration through nylon mesh screen (Currin et al. 1995) and by density centrifugation with colloidal Si (Blanchard 1988). Both methods provide a pure algal sample (devoid of sediment) that can be collected and frozen for subsequent isotope analyses. Epifauna (gastropods, decapods) will be collected by hand. Infaunal invertebrate taxa will be sorted live, with methanol-cleaned forceps, from sediments (after sieving on a 0.3 mm mesh), separated by species, and dried within tin boats for isotopic analyses. To examine higher trophic levels, we will assay fish species collected by enclosure traps, and bird guano and feathers (shed feathers from ground) will be collected by hand.

In addition to using naturally-occurring stable isotopes, we will also conduct isotope enrichment experiments to track a particular invasive plant through the food web. These enrichment experiments create an unambiguous marker that can be tracked through the food chain. Microalgae labeling will be carried out in the field by spraying $H1_3CO_3$ (purchased from Sigma-Aldrich) on the sediment surface in replicate 1 m^2 plots at low tide (Middelburg et al. 2000; Levin et al. 2006). Invasive plants and macroalgae (such as *Ulva sp*.) will be labeled with tracer-level concentrations of ^{15}N (vascular plants) and ^{13}C (algae), respectively, and the spiked material will be redeployed in the field as surface and subsurface detritus. Experiments will be initiated within replicate plots (1 m^2) within several habitats (invaded, non-invaded) within the marsh. Incorporation of the isotopic labels occurs rapidly (within hours), and sampling of microalgae to assess tracer uptake will take place 24 hours after labeling. ^{15}N-spiked invasive plants will be generated by growing plants over several weeks in pots placed in a field location remote from the subsequent sampling area. ^{15}N will be added in the form of ammonium sulfate (Sigma-Aldrich) to the root zone via lysimeters (White & Howes 1994). Three to four additions will be made at daily intervals early in the growing season, and the plants will be either transplanted to experimental plots or the blades will be harvested for placement as detritus. Following placement, the ^{15}N and ^{13}C signals will be measured in fauna and sediment organic matter pools at 4 days, 1 week, 1 month, and 6 month intervals. Plants, macroalgae, microalgae, and consumers will be sampled and processed as described above for natural abundance stable isotope analysis (SIA) to evaluate the significance of these invaders in the food web. These isotopic labels are not radioactive, they cycle through the system quickly (<1 y), and they will produce no long-term isotopic contamination of the wetland habitat (L. Levin, pers. observ.).

Animals destined for natural abundance and tracer -SIA are kept alive in seawater and allowed to evacuate guts for up to 24 h. Fish are filleted for removal of muscle tissue and separation of gut contents. Plant and animal material is washed in distilled water and frozen in pre-combusted vials or tin boats until analysis. Muscle tissue from larger organisms is removed from the shell or carapace, dried at 65ºC and then ground with a mortar and pestle. All samples are treated with Pt Cl_2 to eliminate inorganic C. Isotopic composition of both tracer and natural abundance samples will be analyzed by D. Harris (Stable Isotope Facility, UC Davis) using a PDZ Europa 20-20 mass spectrometer connected to an elemental analyzer (PDZ Europa ANCA-GS, PDZ Europa Northwich, UK) . Stable isotope abundance is expressed in part per thousand with internal instrument precision of 0.014 ‰. Typical sample precision is better than 0.1%. The contribution of primary producers to consumer food webs will be estimated using mixing models (Koch & Phillips 2002) and data from both natural abundance and tracer experiments.

Output / Anticipated Benefits

This project will generate important information about the consequences of invasion in altering ecosystem diversity and trophic function. Studies that consider how the impact of invaders on a species level are crucial for making informed decisions regarding estuarine modification, invader eradication, and marsh restoration. California wetlands, especially San Francisco Bay ones, are subject to continual pressure from invasion, so the lessons learned from one system may be applied to the other ecosystems in the future.

The beneficiaries of this research will be all agencies and authorities involved in wetland reserve management or restoration of salt marsh ecosystems in California. Examples include regional port and transit authorities, the California Coastal Commission, the California Dept. of Fish and Game, the US Fish and Wildlife Service, NOAA NERRS, coastal cities within the delta, consulting companies, local residents, fishing groups (e.g. California Sportfishing Protection

Alliance), and NGOs (e.g., Solano Land Trust). Together these groups spend large amounts of tax dollars, mitigation funds, human time and energy on restoration projects within the watershed. The results of this study will help focus their efforts on key wetland landscapes, critical species required to maintain desired functional attributes as well as will help prioritize invader eradication projects.

Because the project is coupled with the first stages of an invasive plant control program at the Solano Land Trust and within the SF NERR, it is designed to provide critical input into future management efforts. Our experimental approach of testing the efficacy of various eradication techniques and evaluating subsequent ecosystem recovery will provide essential input into adaptive management for the long-term control of invasive species in the reserve and in the general SF Bay area. Specifically, the results of this research can be used for invasive species management within the delta as follows:

YEAR 1

- Through our detailed examination of aerial surveys and on the ground vegetation distribution, we will provide fine-scale information on the location and extent of invasive weeds. This will complement the NERR and DWR maps and will aid in future control and monitoring efforts.
- Describing the impact of several species of invaders in different habitats and growing in different patch sizes will create metrics by which to help managers determine eradiation priorities. Using the Solano Land Trust as an test scenario, we can help them decide which patches of which species in which habitat (upland transition zone versus marsh) should be eradicated first.
- Identifying giant reed, common reed, and pepperweed as important salt marsh invaders and spreading knowledge of their impacts throughout the system to the public and managers is a crucial element of controlling future spread.
- Description of roles of three invasive plants in the food web and detrital cycles of a salt marsh using natural abundance stable isotopes analysis is an important and often overlooked aspect of ecosystem functioning. Understanding the influence of these plants on higher trophic levels, such as birds and fish can eventually help predict how invaders will do under future scenarios of change (climate, water management, development).

YEAR 2

- Quantitative tracking of invasive plants through the food web using a novel stable isotope enrichment technique will potentially provide information to managers and scientists about role of detritus in diets of invertebrates, fish, and other organisms of concern to managers.
- From a larger perspective, although the immediate goal is localized control of the invader itself, the ultimate goal is the recovery of native communities. We will directly address how chosen management practices achieve this goal.
- The information generated from this research could become an important component of a comprehensive invasive species management plan incorporating multiple species and habitats in both SF NERR and local non-profit organizations.
- By being based at a NERR site, with staff dedicated to education and outreach, we will also use the results of this research to stress importance of invasions and their control to the general public. In addition, we will highlight the many important functions of reserve areas such as the SF NERR and the Rush Ranch Preserve, including maintaining ecosystem integrity and translating sound science into action.

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