

## APPROACH AND SCOPE OF WORK

We propose a collaborative program of research that will integrate field and laboratory studies. The primary research focus is to gain basic biological information on invasive hydrozoans in order to understand both what leads the invasions to be successful and how the invasions may be negatively affecting the SFE ecosystem.

### TASK 1—PROJECT MANAGEMENT

This research program will be managed as an inter-disciplinary collaborative effort between two Primary Investigators, Dr. Bernie May and Dr. Peter Moyle at the University of California, Davis. Dr. Bernie May will be the overall project director and the lead PI for Tasks 2 and 4 and Dr. Peter Moyle will be the lead PI for Task 3. Two doctoral students, Mariah Meek and Alpa Wintzer, will be responsible for executing the research. Weekly meetings will be held among Dr. May, Dr. Moyle, Ms. Meek, and Ms. Wintzer to review progress and make management decisions. All laboratory work will be completed at the University of California, Davis genetic and aquatic facilities. Deliverables will be produced as outlined in Table 1.

### TASK 2—GENETIC STUDIES

#### *2.1: Marker Development*

We will extract DNA from several positively identified representatives of *M. marginata*, *Moerisia* sp., *B. virginica*, and *C. caspia*. The extracts will be pooled and four enriched microsatellite libraries produced for tetranucleotide and dinucleotide repeats (CTAT, CTGT, AG, CA) using established methods (Jones et al. 2002). Recombinant clones will be sequenced and nucleotides will be aligned in the program Sequencher (GeneCodes) to screen for microsatellites. Clones containing microsatellites will be selected and primers will be designed from flanking regions using the online software program Primer 3. Initial PCR amplification using primers from candidate microsatellite loci will be conducted for six positively identified individuals from each species. Products will be

evolving microsatellite markers developed in Task 2.1 to determine the role of asexual versus sexual reproduction in the polyp and medusa populations.

We will determine clonal diversity and the relative contribution of asexual reproduction within each sample by employing the program MGLSim (Stenberg et al. 2003) to estimate clonal diversity and predominant reproductive mode. This program calculates significance values for the likelihood that a multilocus genotype observed more than once in a population is the result of sexual reproduction (Stenberg et al. 2003). We will compare the clonal diversity of the polyp and hydromedusae phase to determine the role of sexual reproduction to recruitment in the polyp phase. We will calculate both the multilocus genotype (clonal) diversity (D) and the clonal evenness (E). We will use a modification of the Simpson Index as used by Ellstrand and Roose (1987) and Novak and Mack (2005) to calculate D:

$$D_{\text{obs}} = 1 - \sum_{i=1}^G (n_i(n_i-1)) / (N(N-1))$$

where  $n_i$  is the number of individuals of genotype I, N is the number of individuals sampled, and G is the number of multilocus genotypes detected in the population. Values for D can range from zero (only one multilocus genotype in the population) to 1.0 (every individual sampled contains a unique multilocus genotype). We will calculate evenness to evaluate the distribution of clonal lines throughout the population using the following equation (Ellstrand and Roose 1987 and Fager 1972):

$$E = (D_{\text{obs}} - D_{\text{min}}) / (D_{\text{max}} - D_{\text{min}})$$

where  
and

$$D_{\text{min}} = ((G-1)(2N-G)) / (N(N-1))$$
$$D_{\text{max}} = (N(G-1)) / (G(N-1))$$

Values of E range from zero in populations with only one genotype present to 1.0 when each genotype present is equally represented in the population.

We will analyze our microsatellite data for potential population substructure with the program STRUCTURE (Pritchard et al. 2000). We will remove all repeat copies of multilocus genotypes to avoid biasing the data. STRUCTURE uses a model-based clustering method to assign individuals to groups in which Hardy-Weinberg equilibrium is realized. If our initial analyses at the sample level determine there are multiple clonal lines present in the population, we will then evaluate clonal diversity and the relative contribution of asexual reproduction in each subpopulation if sub-structuring exists or on the population as a whole if no substructure exists. Additionally, we will determine the amount of genetic diversity both in the polyp and medusae phase to evaluate whether there is differential recruitment of clonal lines to the medusae population. We will use ARLEQUIN to execute Analysis of Molecular Variance to calculate nucleotide diversity. Microsatellites are ideal for this type of investigation as they evolve rapidly, are distributed throughout the genome, generally don't code for structural genes, and are not thought to be subject to strong selection pressure. This leads to increased polymorphisms.

Polyp sampling: Due to the paucity of information regarding all aspects of polyp ecology, this portion of the study will be a preliminary attempt at documenting their distributions and abundances. We will suspend fouling plates made of 0.15m<sup>2</sup> sanded sheet PVC in quadruplicate from various docks in Suisun Marsh beginning in July of 2007 and 2008. At the time of set-up and collection, water quality parameters will be measured as described in the previous section. One plate will be removed from each site at the end of each month, July-October. We will preserve the plates in 95% ethanol and transport them to the lab where hydrozoan polyps will be identified using molecular markers, as described in Task 2.2, and counted. *C. caspia* forms complex branching colonies, making it a difficult subject to assign an abundance per unit area. Hence, its abundance will be estimated as a function of weight per area. It is unclear whether polyps prefer fresh or saline conditions (Mills and Sommer 1995), so fouling plates will also be placed around boat launches and docks at John F. Kennedy Park in Napa, CA, the Turning Basin and Shollenberger Park in Petaluma, CA, and Suisun City Marina in Suisun City, CA. Additional plates will be hung at the Carquinez Straits and near Chipps Island. In addition, we will collect three benthic grab samples from each slough surveyed during monthly medusae collections. The densities of any polyps within the samples will be estimated and the types of substrate upon which polyps are attached will be noted.

Canonical Correspondence Analysis will be performed with the CANOCO software program (ter Braak and Smilauer 1998) to examine the relationship between species distributions and abiotic habitat variables. Additionally, Principle Components Analysis will be used to compare differences in jellyfish abundance among sample sites (or water depth) and over time.

### 3.2: Temporal Feeding Behavior on Larval Fish and Zooplankton

During monthly daytime medusae sampling in Suisun Marsh (described above in Task 3.1), up to 30 individuals per species per tow will be preserved in 5% formalin. Non-medusae zooplankton from the 500µm mesh plankton tow will also be preserved in 5% formalin. Finally, an additional 150µm mesh plankton tow will be conducted to collect microzooplankton, and its contents will be preserved. This sampling effort will be repeated during four nights over the seasonal bloom. In the lab, the bell lengths of jellyfish will be measured and gut contents will be identified to the lowest taxon possible and counted. Zooplankton and ichthyoplankton from subsamples of plankton tow collections will be identified and counted. Additionally, medusae will be collected from the Carquinez Straits in March and May. Salinities may allow the earliest medusae appearances here and gut contents will be examined for predation on native fish larvae.

Selection patterns for prey (including larval fishes) by the three jellyfish species will be examined, using Pearre's selectivity index (Pearre 1982), C:

$$C = \pm [((|a_d b_e - a_e b_d| - (n/2)^2) / abde)]^{0.5}$$

where,  $a_d$  is the number of a specific prey type ingested,  $a_e$  is the number of that prey in the environment,  $a$  is the total number of that prey type (ingested + environment),  $b_d$  is

The level of dietary overlap between Suisun Marsh fishes and hydromedusae will be evaluated with Pianka's symmetric niche coefficient (Pianka 1974).

$$\phi_{ij} = (\sum P_{ij}P_{ik}) / (\sqrt{\sum P_{ij}^2 \sum P_{ik}^2})$$

where,  $P_{ij}$  is the proportion of prey type  $i$  found in the diet of species  $j$  and  $P_{ik}$  is the proportion of prey type  $i$  in the diet of species  $k$  (Freyer et al. 2003). We will also apply this analysis to individual fish species over time to examine the possibility of a diet change during bloom periods.

#### TASK 4—ECOLOGICAL LABORATORY STUDIES

##### 4.1: Feeding Rates

We will conduct laboratory experiments to determine predation rates on zooplankton and fish larvae for medusae and/or polyps of *M. marginata* and *Moerisia sp.* We will use individuals of average size from either our laboratory cultures outlined in Task 4.2 or collected from the field under Task 3. We will allow the medusae and polyps to acclimate to the container and conditions for 24 prior to beginning the experiment. A single medusae or polyp of average size will then be carefully placed in a 250-2000 ml glass container, containing the average density of zooplankton as in Suisun Marsh using *Artemia* or copepods, as available. Size of the experimental container will depend on the size of the specimens used. Conditions will be as is typical for Suisun Marsh in September during the height of the blooms. After medusae and polyps have fed for 1 hour, medusae/polyps will be removed from 1/3 of the replicates for each medusae/polyp x species combination and fixed in 5% formalin. All remaining live zooplankton will then be removed from the container and fixed in 5% formalin for quantification. Dead zooplankton remaining in the container will also be quantified. We will examine medusae and polyps for zooplankton attached to outer portions of their bodies and add this count to the count of zooplankton found dead but not consumed in the container. We will repeat this procedure for the remaining 2/3 of the replicates after 2 and 4 hours of feeding in order to determine if feeding rate changes over time. This entire process will also be conducted using cultured fish larvae to determine the rate at which each species can consume larval fishes. We will conduct 60 replicates each for the medusae and polyps of each species, as specimens are available. There will be 60 replicate controls for the experiment using the same techniques and density of prey with the exclusion of the hydrozoan predator. These organisms are amenable to rearing under laboratory conditions (Dr. C. Mills and Dr. J. Rees, per. comm.) and will, therefore, make experimental research feasible. Future extensions of this work may include the determination of feeding rates under different temperature and salinity conditions, zooplankton densities, and medusae sizes, as well as repeating this experiment using *C. caspia*.

newly formed polyp and medusa buds on each polyp and remove newly liberated medusae and polyps from the jar. We will additionally record survival of polyps and medusae each day and remove any dead individuals from the experiment. Future work may include testing responses at a finer scale of temperature and salinity at the experimental condition extremes, as well as repeating the above described experiments using *C. caspia*.

All data will be analyzed using ANOVA to test for differences in reproduction and survival among temperature and salinity treatments and clonal lines within species. We will also test for differences in survival and reproduction in temperature and salinity treatments among species as well as any possible interactions among factors.

#### DELIVERABLES

Research findings and progress from these tasks will be distributed in quarterly reports, our final report, at presentations during national and local meetings, and in articles submitted to both the IEP Newsletter and peer-reviewed publications (Table 1). In addition, data collected from all midwater trawl surveys will be added to the existing long-term sampling database of otter trawl and seine information from Dr. Peter Moyle's Suisun Marsh Sampling Program, which is posted on the IEP website. This pairing will lead to further understanding of this system.