Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

Project Information

1. **Proposal Title:**

   Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

2. **Proposal applicants:**

   Edward Carpenter, San Francisco State University, Romberg Tiburon Center
   Douglas Capone, University of Southern California

3. **Corresponding Contact Person:**

   Bruce Macher
   San Francisco State University, Office of Research
   1600 Holloway Avenue HSS-204 San Francisco, CA 94132
   415 338 7094
   macher@sfsu.edu

4. **Project Keywords:**

   Habitat Restoration, Wetland
   Restoration Ecology
   Wetlands, Tidal

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: 38.0727

   Longitude: -122.3225

   Datum:

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

San Pablo Bay and Suisun Bay Marshes

10. **Location - Ecozone:**

    2.1 Suisun Bay & Marsh, 2.2 Napa River, 2.5 San Pablo Bay

11. **Location - County:**

    Marin, Napa, Solano, Sonoma

12. **Location - City:**

    Does your project fall within a city jurisdiction?

    No

13. **Location - Tribal Lands:**

    Does your project fall on or adjacent to tribal lands?

    No

14. **Location - Congressional District:**

    6th & 7th

15. **Location:**

    California State Senate District Number: 3,4,7

    California Assembly District Number: 3,4,7

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

    3

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: 1,253,188.00

    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)

Dr. Hans Paerl	**University of North Carolina Institute of Marine Sciences, Morehead City NC 28557**	**2527266841 x 133**	**hpaerl@email.unc.edu**
Dr. Walter Boynton	**Chesapeake Biological Lab, U. Maryland, Solomons MD 20688**	**4103267275**	**boynton@cbl.umd.edu**
Dr. Louis Codispoti	**Horn Point Lab, U. Maryland, Cambridge MD 21613**	**4102218479**	**codispoti@hpl.umces.edu**
Dr. Peggy Fong	**OBEE University of California, Los Angeles, Los Angeles CA 90024-1606**	**3108255444**	**pfong@biology.ucla.edu**

21. **Comments:**

Environmental Compliance Checklist

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

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.

      This research will not result in any significant environmental impact. The research only seeks to carry out limited sampling of biota in Bay marshes.

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

   CEQA Lead Agency:
   NEPA Lead Agency (or co-lead:)
   NEPA Co-Lead Agency (if applicable):

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

   CEQA
   -Categorical Exemption
   -Negative Declaration or Mitigated Negative Declaration
   -EIR
   Xnone

   NEPA
   -Categorical Exclusion
   -Environmental Assessment/FONSI
   -EIS
   Xnone

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

4. **CEQA/NEPA Process**
   a) Is the CEQA/NEPA process complete?

      Not Applicable

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

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

LOCAL PERMITS AND APPROVALS

Conditional use permit

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.
Agency Name:

Permission to access private land.
Landowner Name:

6. **Comments.**

Land Use Checklist

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

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

   No

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

   No

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

   No

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

   Research Only

4. **Comments.**

Conflict of Interest Checklist

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

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):

Edward Carpenter, San Francisco State University, Romberg Tiburon Center
Douglas Capone, University of Southern California

Subcontractor(s):

Are specific subcontractors identified in this proposal? Yes

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

Douglas G. Capone	University of Southern California

None	None
None	None
None	None
None	None

Helped with proposal development:

Are there persons who helped with proposal development?

Yes

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

Douglas G. Capone	**University of Southern California**

Comments:

None

Budget Summary

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

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	Stable isotopes	1600	15229.50	2533	1334	1417	25000	11000	334	56847.5	22924	79771.50
2	N2 fixation & denitrification	1200	15229.50	2533	1334	1417	32044	11000	334	63891.5	10423	74314.50
3	Nitrification & reduced N regeneration	1500	15229	2532	1333	1417	32044	11000	333	63888.0	10422	74310.00
4	N Fertilization & macrovegetation productivity	1800	15229	2532	1333	1417	32044	11000	333	63888.0	10422	74310.00
5	Sediment characteristics	1000	15229	2532	1333	1416	32043	11000	333	63886.0	10422	74308.00
6	Data workup & synthesis	2020	15229	2532	1333	1416	32043	11000	333	63886.0	10422	74308.00
		9120	91375.00	15194.00	8000.00	8500.00	185218.00	66000.00	2000.00	376287.00	75035.00	451322.00

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	Stable isotopes	1600	15991	2659	1384	1500	32413		334	54281.0	10934	65215.00
2	N2 fixation & denitrification	1200	15991	2659	1384	1500	32413		334	54281.0	10934	65215.00
3	Nitrification & N remineralization	1500	15991	2659	1383	1500	32413		333	54279.0	10933	65212.00
4	N fertilization & macrovegetation productivity	1800	15991	2659	1383	1500	32413		333	54279.0	10933	65212.00
5	Sediment characteristics	1000	15990	2659	1383	1500	32413		333	54278.0	10933	65211.00
6	Data workup & synthesis	2020	15990	2659	1383	1500	32413		333	54278.0	10932	65210.00
		9120	95944.00	15954.00	8300.00	9000.00	194478.00	0.00	2000.00	325676.00	65599.00	391275.00

Year 3												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** ***(per year)***	**Benefits** ***(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Stable isotopes	1600	16791	2792	1434	1584	34034		334	56969.0	11468	68437.00
2	N2 fixation & denitrification	1200	16790	2792	1434	1584	34034		334	56968.0	11467	68435.00
3	Nitrification & N remineralization	1500	16790	2792	1433	1583	34034		333	56965.0	11466	68431.00
4	N fertilization & macrovegetation productivity	1800	16790	2792	1433	1583	34034		333	56965.0	11465	68430.00
5	Sediment characteristics	1000	16790	2792	1433	1583	34033		333	56964.0	11465	68429.00
6	Data workup & synthesis	2021	16790	2792	1433	1583	34033		333	56964.0	11465	68429.00
		9121	100741.00	16752.00	8600.00	9500.00	204202.00	0.00	2000.00	341795.00	68796.00	410591.00

Grand Total=1253188.00

Comments.

Budget Justification

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

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

Edward J. Carpenter Yr 1 160 hr yr 2 160 hr Postdoctoral Investigator RTC Yr 1 2080 hr Yr 2 2080 hr Yr 3 2080 hr Graduate Student RTC Yr 1 2080 hr Yr 2 2080 hr Yr 3 2080 hr Undergraduate Aide RTC Yr 1 480 hr Yr 2 480 hr Yr 3 480 hr D.G. Capone USC Yr 1 160 hr Yr 2 160 hr yr 3 160 hr Postdoctoral Associate USC Yr 1 1040 hr Yr 2 1040 hr Yr 3 1040 hr Research Assistant USC Yr 1 1040 hr Yr 2 1040 hr Yr 3 1040 hr Graduate Student USC Yr 1 2080 hr Yr 2 2080 hr Yr 3 2080 hr

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

Edward J. carpenter Yr 1 $84.35/hr Yr 2 $87.50/hr Yr 3 $90.63/hr Postdoctoral Investigator RTC Yr 1 $19.23/hr Yr 2 $20.19/hr Yr 3 $21.15/hr Graduate Student RTC Yr 1 $11.06/hr Yr 2 $11.54/hr Yr 3 $12.01/hr Undergraduate Aide RTC Yr 1 $8.85/hr Yr 2 $9.17/hr Yr 3 $9.58/hr D.G. Capone USC Yr 1 $93.75/hr Yr 2 $98.44/hr Yr 3 $103.36/hr Postdoctoral Investigator USC Yr 1 $18.52/hr Yr 2 $19.44/hr Yr 3 $20.42/hr Research Assistant USC Yr 1 $16.67 Yr 2 $17.50/hr Yr 3 $18.38/hr Graduate Student USC Yr 1 $9.61/hr Yr 2 $10.10/hr Yr 3 $10.60/hr

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

Romberg Tiburon Center Benefit rate for the PI is 12%, for the Research Assistant 37%% and for the Graduate Student 1.5% University of Southern California Benefit rate for all is 32.5%

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

Romberg Tiburon Center Only Local travel is budgeted and this is to trailer the boat to sample site boat launching ramps. University of Southern California has travel to come up to San Francisco to sample in the study.

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

Romberg Tiburon Center Year 1: Office $250, Lab $5000, Computing $500, Field $2750 Year 2: Office $300, Lab $5500, Computing $500, Field $2700 Year 3: Office $300, Lab $5750, Computing $600, Field $2850 University of Southern California Year 1: Office $500, Lab $8000, Computing $500, Field $1000 Year 2: Office $500, Lab $8500, Computing $500, Field $1000 Year 3: Office $500, Lab $9025, Computing $500, Field $1000

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.

None

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.

Romberg Tiburon Center Shimadzu Electron Capture Gas Chromatograph for denitrification Measurements $$26,000 Boston Whaler 19 ft Guardian with 75 hp engine and trailer $40,000 University of Southern California None

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

We estimate that Project Management accounts for 20% of the PI salaries listed under Direct Labor and Salaries

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

Romberg Tiburon Center Publication Costs $1000 per year, and Copying and Communication is also $1000 per year. University of Southern California Publication costs $1000 in Yr 1, $1050 in Yr 2 and $1103 in Yr 3. Xerox and Communication is $1000 in Yr 1, $1050 in Yr 2 and $1103 in Yr 3.

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.

Romberg Tiburon Center Indirect Cost Rate is 50% of MTDC (equipment is not included) University of Southern California Indirect Cost rate is 62.5%. The indirect costs reflect federally negotiated rates which include building upkeep and maintenance, heating and electric plus Research Foundation proposal processing and accounting costs, furniature, and lab maintenance.

Executive Summary

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

Nitrogen is typically the limiting nutrient in natural salt marshes, and nitrogen addition results in dramatic increases in growth of macrovegetation. This proposal seeks to investigate the role of nitrogen availability and cycling processes in the restoration of San Francisco Bay Marshes. Marshes undergoing restoration have high variability in success of restoration, and reasons for this variability are not understood. Furthermore, there has been little measurement of fundamental marsh ecosystem nutrient processes to date in CALFED-sponsored research. We propose to mesh our nutrient research with the ongoing CALFED-sponsored BREACH II program which involves research on marsh restoration as related to geomorphology and bathymetry, sediment accretion, tidal channel geomorphology, marsh vegetation, benthic, planktonic and neustonic invertebrates, fish, and birds in five marshes (and six control sites) in which levees were broached at different periods. Our research on nitrogen would be done in the same "restored" and natural marshes as studied in the BREACH II program in concert with scientists from this program, and both studies would benefit from each other's findings. Regarding CALFED management needs, this research would be an indicator of marsh restoration rate and effectiveness, and will provide information on how to accelerate restoration. Furthermore, the proposed research would aid management by documenting the contribution of tidal marsh restoration to the Bay. To study nutrient limitation, we propose controlled marsh fertilization experiments with N, P, and a fertilizer mix. Measurements in treated and control sites would involve above and below-ground macrophyte growth, porewater nutrients, organic matter and particulate nitrogen (PN) & particulate carbon (PC) content in sediments and macrovegetation. Rates of N2 fixation by surface cyanobacteria and N2 fixation, denitrification, and nitrification by bacteria in rhizosphere of submerged aquatic vegetation (SAV) and sediments will be quantified in transects through the marshes. These data would be related to information gathered in the BREACH II study as well as to additional measurements of sediment characteristics made by us. Since a major goal of marsh restoration is provision of habitat for invertebrates, fish and bird populations, we will use natural isotopic ratios (d15N) to trace the cycling of N in marsh food webs. Again, this research would mesh with observations made in the BREACH II study on invertebrate and vertebrate organisms, and it will extend beyond the objectives of the BREACH II study. The natural isotope studies would provide information on length of food chains and sources of these elements. Our overall goal would be to determine how nitrogen cycling and availability is related to restoration of the Bay's marshes.

Proposal

San Francisco State University, Romberg Tiburon Center

Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Bay Region

Edward Carpenter, San Francisco State University, Romberg Tiburon Center
Douglas Capone, University of Southern California

NITROGEN TRANSFORMATIONS IN RESTORATION OF SALT MARSHES IN THE SAN FRANCISCO BAY REGION

Primary Contact:

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A. Project Description: Project Goals and Scope of Work

1. PROBLEM

Salt marshes are important habitats at the interface between land and sea. They are highly productive zones, providing organic matter to support estuarine food webs, and they can serve as important nurseries for juvenile fish. Intimately tied to the substantial productivity of salt marshes is a highly dynamic nitrogen cycle. With respect to N, salt marshes serve as important buffers, often intercepting nitrogen loads from upland and thereby contributing to estuarine water quality. Improvement of estuarine water quality through marsh restoration is thus a major benefit of the CALFED program.

Since the mid-1800s, developers in the San Francisco Bay area have filled or diked about 2075 km^2 or 94% of the Bay's intertidal marshland (Nichols et al. 1986). Today, government agencies and public groups are striving to return almost half of this land to working wetlands through salt marsh restoration projects. A major goal of the restoration effort is reestablishing marsh macrovegetation and fish habitat.

Restoration involves removal or broaching of the dikes plus in some cases replanting of marsh macrophytes. There are currently 35 projects either planned or underway throughout the Bay area to restore thousands of acres by breaching dikes and rerouting waterways (Kay 2001). However, marsh restoration is variable in terms of returning habitat to "natural" conditions. Some marshes appear to be highly productive soon after restoration, while others require years to recover.

Marsh Nitrogen Cycling

Nitrogen is essential for protein and nucleotide synthesis, and primary production in most salt marshes is nitrogen limited (Anderson et al. 1997). Inputs of N to the marsh can be through groundwater, tidal input, rainfall, and microbial N_2 fixation (Valiela and Teal 1978). Losses are through denitrification, sedimentation and tidal flushing, and these latter processes generally result in a net loss of nitrogen from marshes. In many marshes, where groundwater input is low, N_2 fixation is a major source of combined N. For example, in Great Sippewissett Marsh, MA (Carpenter et al. 1978, Valiela & Teal 1979) and Sapelo Island, GA (Haines et al. 1977), N_2 fixation supplied 9 and 23% of the total N input, respectively. Nitrogen fixation in marshes is mediated by bacteria (including sulfate reducing bacteria) in the rhizosphere (sediment surrounding the rhizome) of *Spartina spp.*, and submerged aquatic vegetation (SAV). It is also associated with other aboveground macrovegetation (epiphytic cyanobacteria and bacteria), and on living and dead plant tissue, as well as by cyanobacteria on the marsh mud surface. Heterocystous cyanobacteria (the heterocyst is the site of N_2 fixation in many cyanobacteria and protects nitrogenase from O_2 deactivation) have been shown to be active in N_2 fixation in daytime in microbial mats of many marsh ecosystems (Paerl & Zehr 2000). At night, nonheterocystous species can also be active and can contribute 3-4 times more N_2 than the heterocystous species (e.g. Bebout et al. 1993). Marsh herbivores feed upon benthic microalgae such as cyanobacteria, diatoms and other algal classes on the marsh surface (Brenner et al. 1976), and through their feces, molts and being grazed upon, the fixed N is cycled to fish and other marsh organisms.

Denitrification in anaerobic sediments can be a significant N loss, and averaged 17% of the total N output from Great Sippewissett Marsh, MA (Valiela and Teal 1978), with the major N loss being through tidal flushing. Denitrification is an anaerobic process, and occurs in marsh sediments in which a source of NO_3 (i.e. groundwater) is present.

Salt marshes are often nitrogen limited, and availability of this element can greatly affect primary productivity of terrestrial plants and phytoplankton. Nitrogen addition as N fertilizer can result in dramatic increases in growth of macrovegetation. (Valiela et al. 1976). The results of N addition can be complicated, however. For example, increased primary production followed N fertilization of short *Spartina* in six different marsh studies, but similar fertilization had no effect on the tall form of Spartina (review by Whitney et al. 1981). In a coastal salt marsh in the Netherlands, young (15 yr) and older (100 yr) marshes were compared over a 3 yr period, and both N and P additions resulted in enhanced growth (van Wijnen & Baaker 1999). After 15 years of N fertilizer addition to a marsh on Cape Cod, production was higher in treated (N fertilized) compared with controls, and this higher production resulted in higher macrofaunal density and production (Sarda et al. 1996). Fertilizer nitrogen addition to a salt marsh restoration site in California resulted in improved height growth of *Spartina foliosa*, but it favored the growth of *S. bigelovii* (600% increase in biomass, branching and seed production) so that it out competed *S. foliosa* (Boyer & Zedler 1999). Soil N increased where *S. bigelovii* was present, suggesting that this species may aid accumulation of N at restoration sites with poor soils.

Marsh Restoration

Marshes can be restored, and the major restoration factor obviously concerns allowing tidal exchange. Comparisons of macroinvertebrate populations in a Connecticut marsh 13 years after restoration with a natural marsh indicated that populations were similar and the restoration was successful (Peck et al. 1994). Hydrologic regimes are important in restoration (Roman et al. 1995), and marsh elevation relative to sea level can be important. Some research suggests that restoration should be gradual (Portnoy and Giblin 1997). Ten years after restoration of Malibu Lagoon in Southern California, fish species richness, density and composition was somewhat lower than other natural marshes in the area, but comparable to other area marshes with similar hydrodynamics (Ambrose & Meffert 1999).

Nitrogen dynamics in marshes can be manipulated to enrich N input. In North Carolina, nitrogenase activity by surface sediment cyanobacteria in a transplanted *S. alterniflora* marsh exceeded rates in an adjacent natural marsh by 5 to 10 fold. Overall, denitrification rates were three orders of magnitude less than N_2 fixation rates, thus the balance between these two processes resulted in a significant input of N to the marsh (Currin et al. 1996).

However, it is clear from a search of the salt marsh restoration literature, that there have been few scientific studies published in peer-reviewed journals on the role of N nutrient cycling and limitation on marsh restoration. We hope to monitor the scientific success, and advance the science of marsh restoration through a study on nitrogen transformations and budgets in selected San Pablo/Suisun Bay marsh area restoration projects.

Natural Abundance of Nitrogen Isotopes.

Analysis of the natural abundance of the nitrogen isotopes, ^{14}N and ^{15}N, in organic matter provides a useful and powerful *in situ* tracer for nitrogen sources and cycling. For instance, the contribution of different potential sources of N entering a system can be inferred if those sources are isotopically distinct. Most biologically mediated reactions discriminate slightly against molecules containing the heavy isotope of N, leading to measurable differences in the isotopic composition of different, biologically active pools. Within ecosystems, the small differences in reaction rate for the different isotopes often generate characteristic patterns of isotopic variation that can be used as an index to those processes.

The natural abundance of ^{15}N ($\delta^{15}N$) in a sample is generally expressed as the per mil (‰) deviation of that sample from the isotopic composition of a reference compound:

$$\delta N\ (‰) = 1000 \cdot [(R_{sample}/R_{reference}) - 1] \quad (1)$$

where R is the isotope ratio(^{15}N:^{14}N) and the reference compounds is atmospheric N_2 for $\delta^{15}N$ measurements.

Natural abundance ratios of nitrogen isotopes can be used to identify the fate of N entering marshes, as well as important processes occurring within the marsh. The isotopic composition of nitrogen entering a system often sets a baseline $\delta^{15}N$ for that ecosystem. For example, sewage derived NH_4^+ and NO_3^- (which may enter a marsh through creeks or

groundwater) is typically highly enriched in ^{15}N relative to marine nitrogen (Heaton 1986, Costanzo et al. 2001, Rau et al. 1981, Van Dover et al. 1992).

Against the backdrop of inputs of nitrogen, are internal processes that can be revealed through their effect on specific N pools. The distribution of isotopes within particular pools is affected by the fractionation effects of biological processes within the system. For instance, trophodynamic processes result in characteristic increases in the $\delta^{15}N$ signature with about a 3 ‰ enrichment per trophic step in biomass (Montoya et al. 1992, Fry & Quinones 1994). Nitrogen assimilation, nitrification and denitrification all often result in enrichment of the residual pools of their respective substrates.

All these processes are in sharp contrast to combined N in the marsh arising from N_2 fixation that, unlike most other biological nitrogen transformations, does not discriminate between the two isotopes of nitrogen (^{15}N & ^{14}N) present in the environment. Air contains 99.635% ^{14}N and 0.365 % ^{15}N. N_2 fixation results in low $\delta^{15}N$ values, very close to that present in the atmosphere. Any organism which consumes the fixed N will have a relatively low $\delta\ ^{15}N$. Thus, by measuring N isotopic ratios in marsh organisms, we can infer the major sources of their N (e.g. N_2 fixation, groundwater, river water etc).

Similarly, the natural abundance of the stable isotopes of carbon, ^{12}C and ^{13}C, can also provide important information on on sources of C, carbon cycling and trophic relationships within an ecosystem and complement N studies (Peterson and Howarth 1987). Many modern mass spectrometers can simultaneously analyze for both (including ours, see below).

In two California salt marshes (Tijuana Estuary and San Dieguito Lagoon) $\delta^{15}N$ ratios indicated that there are 4 trophic levels in the former and 3 in the latter marsh (Kwak & Zedler 1997). The natural isotopic values indicated that inputs from intertidal microalgae, marsh microalgae and *Spartina foliosa* (which all occupy tidal channels, low and mid salt marsh habitats), rather than high marsh productivity, supports invertebrates, fishes and the Light Footed Clapper Rail. The study also showed that the restoration of marshes for endangered birds and other biota is compatible with enhancement of coastal fish populations (previously assumed to be competing). In San Francisco Bay wetlands, the natural abundance of carbon (expressed as $\delta\ ^{13}C$) indicates a strong correlation between modern plant cover and the $\delta\ ^{13}C$ of underlying sediments (Malamud-Roam & Ingram 2001). In Delaware Bay, both N and C natural abundances were used to trace food sources in a restored wetland (Weinstein et al. 2000).

Productivity and limiting factors in Bay Area Marshes

According to the CALFED Ecosystem Restoration Program (Technical Apex, June 1999), in reference to the Bay-Delta Ecosystem, "For most aquatic species, the factors that limit abundance and production are unknown." The report also states "Productivity at the base of the food web has declined throughout the Delta and northern San Francisco Bay." In part, this decline is due to the introduced Asiatic Clam, but the report notes that this does not explain the whole of the decline. Since fixed nitrogen is a central element which controls productivity in marshes and many other marine ecosystems, a study of the

sources, losses and transformations of nitrogen would appear to be logical in light of the unknowns and the decrease in productivity rates. While there is a good understanding of the processes affecting planktonic primary production in San Francisco Bay primarily from research by J.E. Cloern (i.e. Cloern 1996) and colleagues, there is little information on marsh production.

2. JUSTIFICATION

We justify this research on the basis of the central role that fixed nitrogen availability plays in regulating productivity of salt marsh vegetation and the productivity of higher trophic levels. Knowledge of nutrient limitation, sources and sinks and cycling of nitrogen will provide information for better management of marsh recovery.

Hypothesis: We hypothesize that:

1) Nitrogen availability is a major factor in recovery of salt marshes.
2) Nitrogen cycling varies in different stages of restoration

Major questions (key uncertainties) we will address are:

1) What is the role of availability of nitrogen (and to a lesser extent, phosphorus) in marsh restoration, and how does this affect vegetation?
2) What are major source and loss terms of nitrogen? Quantify these terms.
3) How does N cycling differ between natural marshes and marshes which are being restored?
4) How is N and C cycled through food chains in natural and restored marshes?
5) How trophically open (to Bay waters) are natural and restoring marshes?
6) Can nitrogen addition be used to "jump start" marsh restoration processes?

This research program will reduce the uncertainty regarding the availability of nutrients in marsh restoration and will define the major routes of macronutrient cycling. Information gained in the research will aid managers in understanding basic nutrient processes that affect marsh restoration.

Objectives

1) To prepare a synthesis document, peer reviewed publications, and oral presentations for management on the results of our nutrient cycling study.

Relation to Adaptive Management Concept

This proposed research applies to the conceptual model and objectives of ecosystem restoration as defined in the Draft Stage 1 Implementation Plan. The research would be "targeted" and "necessary to resolve critical issues about ecosystem and function". The results of the research will generate information that can be used for future decision making on marsh restoration.

3. APPROACH

We propose a study that will provide a critical baseline to evaluate the trends in N cycle dynamics in restored marshes and thereby to allow comparison with natural marsh ecosystems. In order to understanding restoration of formerly diked marshland, we propose that N cycling may vary among Delta wetlands undergoing restoration. In a wide range of selected Bay Area restored (and, for comparison, natural) salt marshes, we will specifically investigate:

1) Rates of N_2 fixation and denitrification and nitrification, to delineate major inputs, losses and transformations of N within the marsh and compare these parameters with age of restoration, and geomorphology of the marshes.
2) Fertilization studies to aid in understanding factors limiting macrovegetation in restored and natural marshland.
3) Sediment characterization (organic content, grain size, and distribution of inorganic N species (DIN, DON) in marsh sediments pore waters.
4) The distribution of natural isotopic ratios of nitrogen in marsh sediments, plants, invertebrates, and selected fish as a means of understanding food chains in restored and natural marshes.

Salt Marsh Restoration Research Sites

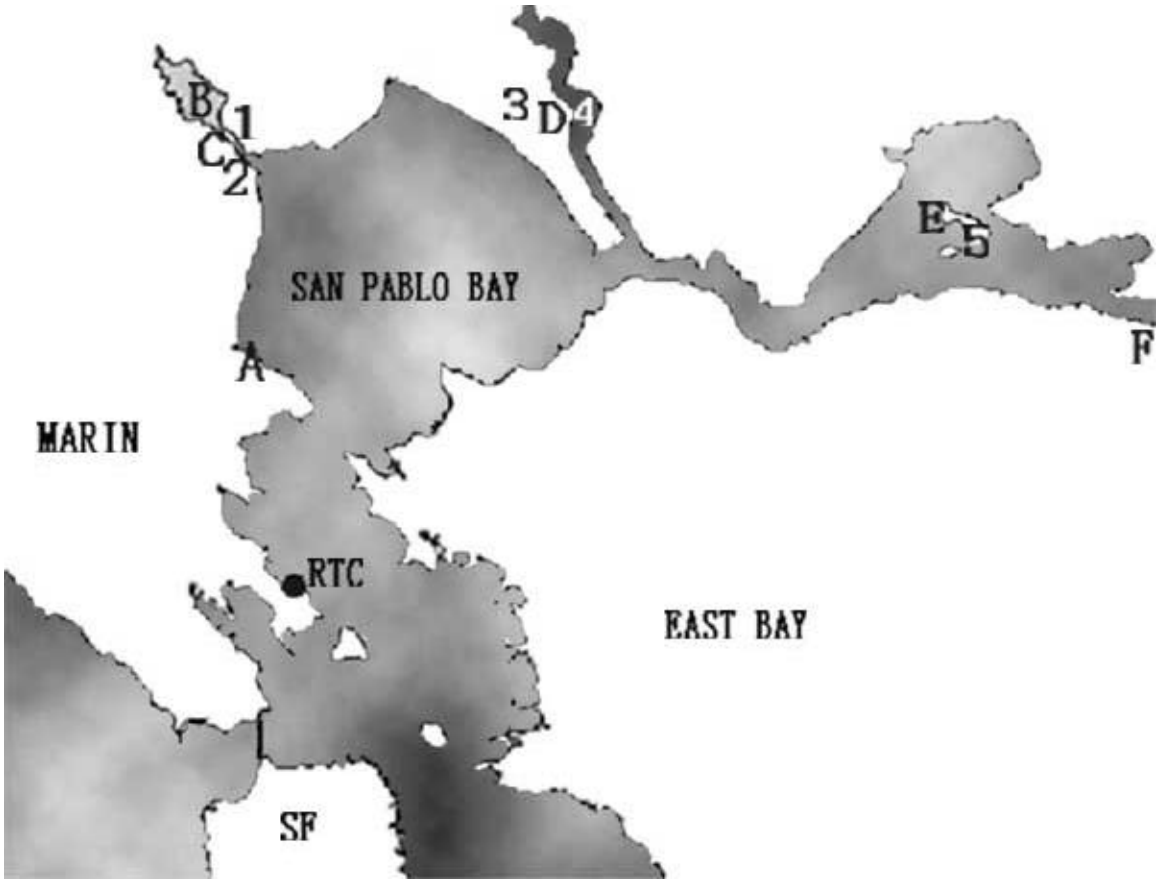
We propose to work at five marsh restoration sites that are currently being studied in the CALFED-funded San Pablo/Suisun Bay Breached Levee Wetland Study (BREACH II) program. The study is being carried out by scientists from the University of Washington (PI Charles (Si) Simenstad), Romberg Tiburon Center, Point Reyes Bird Observatory, University of New Orleans, and Philip Williams and Associates. See attached letter from Simenstad at end of proposal. The fundamental goal of the BREACH II study is to analyze historically-breached levee wetlands as a means to predict the feasibility, patterns, and rates of restoration to natural ecological function. This is an interdisciplinary study which involves measurements of hydrological, geomorphological, biogeochemical and ecological indicators. The sampling regime consists of geomorphology and bathymetry, sediment accretion rate and structural changes, tidal channel geomorphology, marsh vegetation complexity and structure, benthic, planktonic and neustonic invertebrate populations, fish assemblage and life history structure and behavior, food web linkages and bird populations.

Table 1. Restoration sites and control (natural) study areas in the BREACH II study.

Region	ID	Marsh Name	Date Breached	Area (acres)
Western	A	China Camp	Control	250
Petaluma	B	Upper Petaluma	Control	2800
	C	Centennial Greenpoint	Control	49

	1	Carl's Marsh	1994	39
	2	Greenpoint Toy	1986	54
Napa	D	Centennial Napa	Control	180
	3	Pond 2A	1995	550
	4	White Slough	1977-8	260
Suisun	E	Ryer Island West	Control	200
	5	Ryer Island	1983-4	730
Eastern	F	Browns Island	Control	848

Figure 1. Location of marshes under restoration and control sites. See Table 1 for Marsh areas. Numbers are marshes under restoration and letters are control marsh sites. RTC is location of Romberg Tiburon Center.



The BREACH II study and our proposed research on nutrient research within the same marshes would be complementary. Our research on N_2 fixation and denitrification would benefit from sediment accretion, geomorphology, marsh vegetation and hydrodynamics research. The existing BREACH II vertebrate/invertebrate research would aid our N and C natural abundance studies. Our research would relate well to the BREACH II data on composition and distribution of vegetation and in particular to the spring 2002 vegetation

index data. Furthermore, our data on N cycling and fertilization would enhance the BREACH II biological research.

We will also establish plots for manipulation by N, P, and fertilizer addition of marsh macrophyte to determine whether either or both limits production, to determine effects on N cycling and on standing crop and CHN content of marsh above and below ground vegetation, and to observe whether some form of nutrient addition might be valuable in "jump starting" marsh restoration. The same suite of measurements will be conducted on these transects.

3. APPROACH

Task 1: ***Stable isotope studies****:* To help determine the source of N (N_2 fixation, NO_3 or NH_4 input from tidal flushing) within marsh biota and to trace food webs, samples of above and belowground vegetation, sediment surface cyanobacteria, invertebrates (isopods, shrimp, crabs, shellfish and fish) will be collected at each wetland for assay of natural abundances of $\delta^{15}N$ and $\delta^{13}C$. Samples are dried, then analyzed on a Micromass IsoPrime mass spectrometer at USC. We have two systems configured for continuous flow with a CHN elemental analyzer interfaced to the mass spectrometer so that particulate C and N values are obtained for each sample along with the natural isotopic abundance values for each element. The N isotopic ratio of selected marsh-collected fish will be used as an integrative measure of the contribution of different inputs (e.g. N_2 fixation, groundwater nitrate) to their N nutrition.

Task 2*: N_2 fixation and Denitrification assays:* We routinely determine N_2 fixation by the C_2H_2 reduction method (Capone 1993, Capone & Montoya 2001) as applied to salt marsh habitats (Carpenter et al. 1978). In general, samples are contained in a sealed bottle of appropriate size, exposed to a 10-20% atmosphere of C_2H_2, and the gas phase monitored by flame ionization gas chromatography over brief periods for the production of C_2H_4, the result of reduction of C_2H_2 by nitrogenase. We also have deployed chambers as assay devices. In parallel with C_2H_2 reduction, limited direct assays of $^{15}N_2$ uptake will be performed for calibration (Montoya et al. 1996, Capone & Montoya 2001).

Denitrification is routinely assayed in tandem with C_2H_2 reduction during field studies by the C_2H_2 blockage procedure (Sorensen 1978, Joye & Paerl 1993, Capone & Montoya 2000) using an electron capture detector (ECD) gas chromatograph. Cores will be collected, and sediments will be incubated under anaerobic conditions in sealed containers. Tracer methods (^{15}N) will also be done on a limited basis to assess the relative importance of reduction of NO_3^- to N_2 and to NH_4^+ (Koike & Hattori 1978a,b) and to assess the relative importance of nitrification-denitrification coupling to denitrification along the gradients (Nielson 1992, Rysgaard et al. 1993).

N_2 fixation can occur by cyanobacteria in surface sediments of the marsh and in the rhizosphere of marsh macrophytes. Cyanobacterial mats, plant material (roots and leaves), sediment (depth profiles) will be examined for nitrogenase activity. Subsystems with high rates of N_2 fixation generally exhibit low $\delta^{15}N$ signatures in biological material and material will be collected where high nitrogenase activity is detected for $\delta^{15}N$ (see

above). Samples of cyanobacteria will be preserved in Lugols solution for determination of species composition to see whether there are major species differences from one restored marsh to another.

Task 3: Nitrification and Reduced N Regeneration: Several approaches for the determination and quantification of reduced N (NH_4^+ and DON) regeneration and nitrification will be examined. Chamber assays will be conducted, some amended with the nitrification inhibitor, C_2H_2, and changes in the flux of NO_3^- and NH_4^+ from the sediment to the water column will be determined (Sloth et al. 1992). Small vial assays and other inhibitors (e.g. N-Serve, CH_3F) will be examined with respect to their effect on NH_4^+ oxidation and dark $^{14}CO_2$. Direct tracer ($^{15}NH_4^+$ oxidation) and $^{15}NO_3^-$ isotope dilution assays (Koike & Hattori 1978a) will also be conducted on a limited basis (Glibert & Capone 1993).

Task 4: N Fertilization and macrovegetation productivity experiments: These fertilization experiments will be carried out to determine what nutrients limit marsh productivity. Plots (10 m diameter circles) will be established in the mid-elevation marsh onto which fertilization studies will be done following the protocol used by Valiela et al. (1976) for Sippewisset Marsh on Cape Cod. Valiela et al. fertilized at low tide with either N alone, as urea (46% N as weight), P alone as granules (20%) or with a commercially available fertilizer (10% N, 6% P_2O_5, 4% K_2O). Plots will be in duplicate, and control plots are established as well. Valiela's experiments are still ongoing over a 30-year period with bi weekly nutrient additions. Fertilization, effects are still dramatically evident, and nutrients added have been retained within plots. In these studies, for the commercial fertilizer, additions are either at 25.2 (HF) or 8.4 (LF) g m^{-2} wk^{-1}. Combined N will be added as urea (5.6 g m^{-2} wk^{-1}) that is comparable to the N dosage in the HF commercial fertilizer addition. Phosphorus addition will be added at a rate of 6.5 g m^{-2} wk^{-1}.

In control and treated plots, measurements of underground and aboveground vegetation will be made at bi-weekly intervals through the growing season (ca. March-October). Underground plant tissue will be sampled by coring with a 6.5 cm ID plastic corer to 25 cm in depth (Valiela et al. 1976). Cores will be cut in half vertically, with one half used for chemical analysis (CHN and $\delta^{15}N$ and $\delta^{13}C$) and the other half sectioned horizontally at intervals of 0-2, 2-5, 5-10, 10-15, 15-20 and 20-25 cm. Sections are washed with a 0.5 mm mesh sieve and the roots, rhizomes and dead vegetation are separated after blotting.(live roots and rhizomes are pearl white and translucent and dead ones are dull gray and flaccid). Wet (blotted) weights of live material are then recorded. Aboveground vegetation is measured within random 0.1 m^{-2} quadrats, and the heights of the ten tallest plants are recorded. These values will then be converted to biomass using a regression of height on dry wt m^{-2} that will be established empirically. In August, a harvest of 0.1 m^{-2} plots will be done to assess the accuracy of the nonharvest method.

Regarding the placement of nutrient addition plots, we plan to first review restoration research on the marshes being studied in the BREACH II study, and then select two divergent marshes for these experiments.

Task 5: Sediment characteristics and pore water determination: Within each marsh, transects will be established for defining the gradients of sediment organic content, particulate C and N, and pore water NO_3, NH_4 in cores by standard methods. Research on sediment accretion by the BREACH II study will compliment these data. Nutrient concentrations (NO_3, NH_4,) will be measured with a Lachat Auto Analyzer system on GF/F filtered pore water samples which will be collected at same vertical depth intervals as used in N_2 fixation (acetylene reduction) and denitrification assays. Particulate CHN content will be measured with the elemental analyzer that is in-stream with the mass spectrometer. Percent organic matter in sediments will be determined by measuring difference between dry weight (90°C) and ash free dry weight (490°C) on sections of sediment cores that are separated from plant tissue.

Task 6: ***Data workup and synthesis***: This final task will involve the synthesis of data from the five previous tasks, preparation of manuscripts and reports for presentation to scientific journals and the public.

N input from tidal exchange and groundwater: We will not measure N input from tidal exchange. Measurement of N flux through tidal flushing of dissolved and particulate N is complex and requires studies to be done through the tidal cycle. It also requires precise measurement of tidal flow in and out. Tides in San Francisco Bay are complex, and it is our opinion that measurement of tidal input is beyond the scope (and budget) of this study. Tidal flushing typically results in net loss of N (Woodwell et al. 1979, Valiela 1984). Since the delta region is so flat and far from uplands, and since rainfall is low in this region, we assume that groundwater input to restored marshes in the region is minimal.

Possible relation to control of an invasive species… a potentially unexpected outcome of the research. The Atlantic salt marsh cordgrass *Spartina alterniflora* has become abundant in some areas, particularly the southern region, of San Francisco Bay, and it competes with the native cordgrass *S. foliosa*. *S. alterniflora* has more rigid stems, greater stem and rhizome density, and it is thought that it may change habitat for native wetland animals and infauna (Cohen and Carlton 1995). This species is noted for having high rates of N_2 fixation associated with the rhizosphere (Carpenter et al. 1978). N_2 fixation also occurs in the native west coast cordgrass *S. foliosa* (Gibson et al. 1994) but the rates appear to be much lower. Among other competitive attributes of *S. alterniflora* (Callaway & Josselyn 1992), N_2 fixation by *S. alterniflora* may give it a competitive advantage. It is known that addition of fixed N to a marsh (as a fertilizer) will depress N_2 fixation in the *S. alterniflora* rhizosphere (Bagwell & Lovell 2000). Furthermore, N fertilization has been shown to increase growth of *S. foliosa* (Gibson et al. 1994). Aboveground biomass and stem densities of *S. foliosa* were proportional to the amount of N added. However, the sandy soil of the constructed marsh prevented the retention of added N. While we do not want to draw this line of reasoning regarding control of S. alterniflora out too far, it is possible that N fertilizations could play a role in aiding the establishment of *S. foliosa* vs. *S. alterniflora* in restored marshes. Our proposed research will provide initial data that could be useful in future species competition studies.

4. FEASIBILITY
The research outlined in this proposal is straightforward and the PIs have extensive experience. The work will be done on marshes that are already being studied through the BREACH II program, so access is not an issue. The home laboratory, Romberg Tiburon Center is on San Francisco Bay and there is easy access to all marshes by boat. Virtually all of the equipment (mass spectrometer, nutrient auto-analyzer, acetylene reduction gas chromatograph & standard lab instrumentation) is at the two labs involved (see below). The investigators are highly experienced with N and C cycling research in a variety of sites ranging from open ocean to seagrass beds, coral reefs and salt marshes. There are no contingencies (dependence on outcome of other projects) that would affect the execution of the research. The research timetable is noted in following pages.
Lastly, there is no requirement for the physical construction of any structures on the research sites.

Facilities, RTC: Almost all equipment necessary for the completion of this research is present in the labs of the investigators at the Romberg Tiburon Center. In Carpenter's lab, a new Shimadzu GC-8A FID gas chromatograph is present for the acetylene reduction assays. The lab has standard equipment necessary for the research such as drying ovens, muffle furnace, MilliQ DDI system, hood, filtration units, microscopes, balances, three desktop computers for data storage and analysis and lighted, constant temperature enclosures (Hotpack) for incubation of cores and surface samples.

Facilities: **USC:** Capone's laboratory has two Micromass IsoPrime mass spectrometers for $\delta^{15}N$ and $\delta^{13}C$ measurement. Each mass spectrometer has an elemental analyzer interfaced for continuous flow while one can also be configured for dual inlet for very high precision work. Capone's laboratory also has a Lachat Nutrient Auto Analyzer, and a Dionex Ion Chromatograph for measurement of nutrient concentrations in pore water, as well as a Waters HPLC, several gas chromatographs, scintillation counters, microscopes and spectrophotometers.

5. PERFORMANCE MEASURES The proposed work primarily consists of research, and as such, research products will be used as the primary performance measure. The six tasks listed each have as the output, the collection and analysis of samples. The successful collection of these data and subsequent analysis and understanding will be the criteria for measuring performance. We have laid out timelines for each of the six tasks, and successful performance will be assessed on an annual basis by writing an annual report that includes the scientific results. The final metric will be publication of results in peer reviewed scientific journals.

6. DATA HANDLING AND STORAGE Data will be entered into Excel Spreadsheets and stored on both hard drives and diskettes. The raw and graphed data will be made accessible to the scientific, management and public communities by posting it on a website

7. EXPECTED PRODUCTS/OUTCOMES. We expect to publish our research results in peer reviewed scientific journals. Carpenter and Capone have a record of achieving

scientific goals and disseminating their results to the scientific community and public through scientific journal publications and by oral presentation of results at national and international scientific meetings. Furthermore, we plan to disseminate the results of this research program to management officials through seminars at management offices.

8. WORK SCHEDULE. Regarding milestones of success, the results of marsh fertilization should be evident by midsummer in the first year of the grant. By the end of the first year, we should have a basic conceptual framework of the importance of nitrogen inputs and losses in control and restored marshes plus the relationship between these processes and sediment characteristics. During the first winter, we will work with BREACH II scientists regarding our results and the relationship to marsh geomorphology and sedimentation characteristics. The stable isotope samples will be collected in the first year and analyses will proceed to the end of the second year. We will continue fertilization and N cycling research through the second and third years, and will adjust added concentrations to optimize results on growth of marsh vegetation. N_2 fixation, denitrification and nitrification studies will be carried out both in fertilization and control plots through the entirety of the study to establish seasonality of rates and environmental effects on rates. During the third year, results should be evident, and manuscripts will be written for submission to peer reviewed journal and we will present papers at scientific meetings.

The six tasks listed above form a comprehensive study of the role of nitrogen availability in restoration of Bay Area salt marshes. While it would be possible to separate or eliminate any one of these tasks and still carry out a viable research program, the value of the other remaining tasks would be diminished since the results from one task provides comparative information for the other tasks.

Timelines of tasks through the three year study.

	Year 1	Year 2	Year 3
Task 1	----------		
Task 2	----------	----------	----------
Task 3	----------	----------	----------
Task 4	----------	----------	----------
Task 5	----------		
Task 6			----------

B. Applicability to CALFED ERP and goals.

1. ERP Science Program and CVPIA priorities The research will be done in marshes adjacent to San Pablo Bay and in Suisun Bay which are identified as a priority areas for CALFED funding. Our proposal specifically addresses items 1, 2, and 4 of the Bay Region Restoration Priorities, namely: 1. “Restore wetlands in critical areas throughout the Bay either via new projects or improvements that add to or help sustain existing projects.” 2. “Restore uplands in key areas of Suisun Marsh and San Pablo Bay.” 4: “Understand performance of wetlands restoration efforts on a local and regional scale.”

In item 4, the priorities specifically state "…advance understanding of optimal restoration approaches…", and (p. 47) call for "…advance understanding of optimal restoration approaches…" The fertilization and N dynamics research in restored and control marshes will fit into this category. Furthermore, the document calls for research to "…understand poorly known aspects of the food webs of Grizzly Bay, San Pablo Bay, and South Bay." Our proposed research on food chains via the stable isotope research will address this last point.

C. QUALIFICATIONS

EDWARD J. CARPENTER

Education:

B.S. State University of New York, College at Fredonia, 1964
M.S. North Carolina State University, 1966
Ph.D. North Carolina State University, 1969

Experience:

Woods Hole Oceanographic Institution, 1969-1975
Marine Sciences Research Center, SUNY at Stony Brook, 1984-2000
Associate Program Manager, Office of Polar Biology and Medicine, National Science Foundation, 1995-1997.
Romberg Tiburon Center, San Francisco State University, 2000-present

Research Interests:

Phytoplankton ecology, nutrient cycling in marine waters, photosynthesis, nitrogen fixation by marine cyanobacteria, phytoplankton nuisance blooms, cyanobacterial symbioses, bacterial ecology. Carpenter has extensive experience on research ships and has logged over 50 research cruises. He has been Principal Investigator on over 50 Federal government (NSF, NASA, SeaGrant) grants. He is a reviewer for major scientific journals and has published over 100 papers in scientific journals and edited five scientific books. Courses have been taught in Biological Oceanography, Microbial Ecology, Phytoplankton Ecology, Phycology, and General Oceanography.

DOUGLAS G. CAPONE

Education:

Ph.D., Marine Sciences, December 1978. Rosenstiel School of Marine and Atmospheric Sciences, Univ. of Miami, FL.
B.S., Biology, December, 1973. Univ. of Miami, Coral Gables, FL.
----, Biology, 1967-1970. Seton Hall University, So. Orange, New Jersey.

Experience:

Wrigley Professor of Environmental Biology, 1998-present. Department of Biological Sciences & Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, CA

Professor, 1989-1999; Associate Professor, 1987-1989. University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, MD
Associate Professor, 1986-1987; Assistant Professor, 1984-1986; Assistant Research Professor, 1979-1984. Marine Sciences Research Center, SUNY at Stony Brook.
Research Collaborator, 1984-1991. Department of Chemistry, Brookhaven National Laboratory, Upton, NY.

Research Interests:
Marine biochemistry, microbiology, biogeochemistry and microbial ecology. The microbial ecology of nitrogen transformations. Pollutant impact on the microbiota of marine sediments and the role of microorganisms in environmental detoxification.

Staff organization and workload are as follows. The RTC laboratory will be headed by E.J. Carpenter, and the study will be conducted by himself, a full time Research Assistant (RA), Graduate Student (GS) and a summer Field Aide. The RTC lab will carry out the bulk of the field program in the marshes. This will involve the nutrient fertilization experiments, sediment collection, sampling for stable isotopes within marsh biota, measurement of above and below ground vegetation, vegetation growth, as well as the N_2 fixation and denitrification assays. Carpenter will initially go on field samplings, but once the staff are trained, all field work will be done by the two other personnel (plus field aide in summer and personnel from USC through the year) and USC investigators. The RA will do data entry onto spreadsheets, and Carpenter will be involved in data interpretation and statistical testing. The GA will be involved heavily in the fertilization study, and this would appear to be a suitable thesis topic. During summer, when the biological effects are intensified and sampling will be more frequent, the field aide (an SFSU undergraduate student) will help with sampling.

The USC laboratory will be headed by D.G. Capone and will consist of a half-time Postdoctoral Investigator, half-time Research Assistant and full time Graduate Student. This lab's involvement in the study will be analytical and experimental, and will carry out all isotopic measurements, nutrient analyses, and particulate CHN analyses. USC personnel will also help with the field program. The direct tracer studies for measurement of nitrification will be done by USC as well as the NO_3 and NH_4 measurements associated with the nitrification research. These measurements are critical, and the utmost care will be taken to assure that the highest level of quality control is attained. To achieve this, the USC lab will make daily measurements of standards and blanks for the nutrient and isotope studies. It is expected that the GS will (under Capone's supervision) do his/her thesis research on trophic relationships as determined from the measurement of natural isotopic ratios. The ½RA will be involved with nutrient analyses with the Lachat system (automated nutrient analysis), and the ½postdoctoral investigator will do the mass spectrometer analyses.

D Cost.
Budget summary RTC: This research program is labor-intensive, and requested are funds for one Research Specialist, one Graduate Student, one Summer Field Aide (3 months/yr) and one 20% of Carpenter's salary per year. The benefits rates are 37% for the RA, 1.5%

for the student, and 2.5% for the PI. Regarding Equipment, a Shimadzu Electron Capture gas chromatograph GC-8A series and a data integrator are requested for the denitrification studies. Sampling of marshes is done by boat, and for some marshes, the only possible access is by boat. For the field program, we request a commercial grade boat (Boston Whaler 19 ft Guardian with 4 cycle 75 HP engine and trailer). Supplies are for serum bottles and caps for acetylene reduction and denitrification assays syringes, pipetters, tips, filters, chemicals, gas chromatograph gasses and demurrage, and computer supplies. A modest amount is for communication and for publication costs. Travel is for car and boat trailer mileage to and from study sites plus to attend one scientific meeting per year. Overhead rate at SFSU is 50% of MTDC.

Budget Summary USC: Capone requests one month of funding per year, plus a half-time postdoctoral investigator, a half-time Research Assistant and a Graduate Student. Benefit rate is 32.5%. Supplies are for sample bottles, stable isotopes, filters, mass spectrometer supplies (gasses, maintenance), computer supplies, and reagents and replacement parts for the Lachat auto analyzer. Travel is from Los Angeles to San Francisco for sampling and meetings plus to attend one scientific meeting per year. A modest amount is budgeted for communication and copying and for publication costs. The overhead rate at USC is 62.5%.

E. Local Involvement

We will work with local landowners and who are associated with the study sites and will explain the goals and purposes of the research program. This will be done both on an individual basis and through public forums (i.e. Rotary Clubs etc.).

F. Compliance with Standard Terms and Conditions

The applicants will comply with standard State and Federal contract terms

G. Literature Cited

Ambrose, R.F. and D. J. Meffert 1999. Fish-assemblage dynamics in Malibu Lagoon, a small hydrologically altered estuary in southern California. Wetlands 19:327-340.

Anderson, I.C., To bias, C.R., Neikirk, B.B., and Wetzel, R.L. 1997. Development of a processed-based nitrogen mass balance for a Virginia (USA) *Spartina alterniflora* salt marsh. Marine Ecol. Prog. Ser. 159: 13-27.

Bagwell, C.E., & C.R. Lovell. 2000. Persistence of selected *Spartina alterniflora* rhizoplane diazotrophs exposed to natural and manipulated environmental variability. Appl. Environ. Microbiol. 66:4625-4633.

Bebout, B.M., M.W. Fitzpatrick, and H.W. Paerl. 1993. Identification of the sources of energy for nitrogen fixation and physiological characterization of nitrogen fixing members of a marine microbial mat community. Appl. Environ. Microbiol. 59:1495-1503.

Boyer, K.E., & J.B. Zedler. 1996. Damage to cordgrass by scale insects in a constructed salt marsh: effects of nitrogen additions. Estuaries 19:1-12.

Boyer, K.E., and J.B. Zedler. 1999. Nitrogen addition could shift plant community composition in a restored California salt marsh. Restoration Ecology 7:74-85.

Boynton, W.R., J.H. Garber, R. Summers and W.M. Kemp. 1995. Inputs, transformations and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries 18:285-314.

Brenner, D., Valiella, I., VanRaalte, C.D. & Carpenter, E.J. 1976. Grazing by *Talorchestia longicornis* on an algal mat in a New England salt marsh. J. exp. Mar. Biol. Ecol. 22:161-169.

Callaway, J.C., & M.N. Josselyn. 1992. The introduction and spread of smooth cordgrass (*Spartina alterniflora*) in South San Francisco Bay. Estuaries 15:218-226.

Capone, D., and J. Montoya. 2000. Nitrogen fixation and denitrification. In: J. Paul (ed.) Methods in Microbiology: Marine Microbiology. Academic Press.

Capone, D.G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. Handbook of Methods in Aquatic Microbial Ecology. P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole. Boca Raton, Lewis Press**:** 621-631.

Capone, D.G. 1996. Microbial nitrogen cycling. Manual of Environmental Microbiology, Section IV: Aquatic Environments. S. Newell and R. Christian, ASM Press pp. 334-342.

Carpenter, E.J., VanRaalte, C.D., & Valiella, I. 1978. Nitrogen fixation by algae in a Massachusetts salt marsh. Limnol. Oceanogr. 23:318-327.

Cloern, J.E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Review of Geophysics 34:127-168.

Cohen, A.N. & J.T. Carleton. 1995. Biological Study. Nonindigenous aquatic species in a United States estuary: A case study of the biological invasions of the San Francisco Bay and Delta. A report for the US Fish and Wildlife Service.

Costanzo, S., M. O'Donohue, W. Dennison, N. Loneragan and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42: 149-156.

Currin, C.A., S.B. Joye, & H.W. Paerl. 1996. Diel rates of N_2 fixation and denitrification in a transplanted *Spartina alterniflora* marsh: implications for N-flux dynamics. Est. Coast. And Shelf Sci. 42:597-616.

Fry, B. and R. B. Quinones. 1994. Biomass spectra and stable isotope indicators of tropic level in zooplankton of the northwest Atlantic. Mar. Ecol. Prog. Ser. **112**: 201-204.

Fry, B and E. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. 27: 13-47.

Fry, B., Brand, W., Mersch, F.J., Tholke,K., Garritt, R. 1992. Automated analysis system for coupled $\delta^{13}C$ and $\delta^{15}N$ measurements. Anal. Chem.64: 288-291.

Garber, J. 1984. Laboratory study of nitrogen and phosphorus remineralization during the decomposition of coastal plankton and seston. Estuar. Coast. Shelf Sci. 18: 685-702.

Gibson, K.D., J.B. Zedler, & R. Langis. 1994. Limited response of cordgrass (Spartina foliosa) to soil amendments in a constructed marsh. Ecological Applications 4:757-767.

Glibert, P.M. & D.G. Capone. 1993. Mineralization and assimilation in aquatic, sediment, and wetland systems. Nitrogen Isotope Techniques. R. Knowles and H. T. Blackburn. San Diego, Academic Press, Inc.**:** 243-272.

Haines, E. B., A. Chalmers, R. Hanson & B. Sherr. 1977. Nitrogen pools and fluxes in a Georgia Salt Marsh. Pp. 241-254. In: M. Wiley (ed) Estuarine Processes. Vol II. Academic Press.

Heaton, T.H.E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem.Geol. 59: 87-102.

Hopkinson, C., B. Fry and A. Nolin. 1997. Stoichiometry of dissolved organic matter dynamics on the continental shelf of the northeastern USA. Continental Shelf Research **17**: 473-489.

Jackson, G and P.M. Williams. 1985. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. Deep Sea Res. 32:223-235.

Joye, S.B. & H.W. Paerl. 1993. Contemporaneous nitrogen fixation and denitrification in intertidal microbial mats: rapid response to runoff events. Mar. Ecol. Prog. Ser. **94**: 267-274.

Kana, T. et al. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Anal. Chem. **66**: 4166-4170.

Kay, J. 2001. Will wetlands return? San Francisco Chronicle March 12, 2001 pp 1 & 8-9.

Koike, I. & A. Hattori. 1978a. Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a ^{15}N-dilution technique. Appl. Environ. Microbiol. **35**: 853-857.

Koike, I. & A. Hattori. 1978b. Denitrification and ammonia formation in anaerobic coastal sediments. Appl. Environ. Microbiol. **35**: 278-282.

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

Malamud-Roam, F. and B.L. Ingram. 2001. Carbon isotopic compositions of plants and sediments of tide marshes in the San Francisco Estuary. J. Coastal Res. 17:17-29.

Miller, L.G., M.D. Coutlakis, R.S. Oremland & B.B. Ward. 1993. Selective inhibition of ammonium oxidation and nitrification-linked N_2O formation by methyl fluoride and dimethyl ether. Appl. Environ. Microbiol. 59: 2457-2464.

Montoya, J. P., M. Voss, P. Kaehler and D.G. Capone. 1996. A simple, high precision tracer assay for dinitrogen fixation. Appl. Environ. Microbiol. **62**: 986-993.

Montoya, J.P., Wiebe, P.H., McCarthy, J.J. 1992. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Gulf Stream region and Warm Core Ring86A. Deep-Sea Res. 39, Suppl. 1:S363-S392.

Nichols, F.H., J.E. Cloern, S.N. Luoma & D.H. Peterson. 1986. The modification of an estuary. Science 231:567-573.

Nielsen, L.P. 1992. Denitrification in sediments determined from nitrogen isotope pairing. FEMS Microb. Ecol. **86**: 357-362.

Oremland, R.S. & D.G. Capone. 1988. Use of specific inhibitors in microbial ecological and biogeochemical studies. Advanced Microbial Ecology **10**: 285-383.

Owens, N.J.P., Rees, A.P. 1989. Determination of nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. Analyst 114: 1655-1657.

Paerl, H. and J. Zehr. 2000. Marine nitrogen fixation. Microbial Ecology of the Oceans. D. Kirchman, Ed. NY, Wiley-Liss**,** pp. 387-426.

Peck, M.A., Fell, P.E., Allen, E.A., Gieg, J.A., Guthke, C.R., & Newkirk, M.D. 1994. Evaluation of tidal marsh restoration – comparison of selected macroinvertebrate populations on a restored impounded valley marsh and an unimpounded valley marsh within the same salt-marsh system in Connecticut, USA. Environmental Management 18:283-293.

Piceno, Y.M., & Lovell, C.R. 2000. Stability in natural bacterial communities: II. Plant resource allocation effects on rhizosphere diazotroph assemblage composition. Microb. Ecol. 39:41-48.

Portnoy, J.W., & Giblin, A.E. 1997. Biogeochemical effects of seawater restoration to diked salt marshes. Ecol. Appl. 7:1054-1063.

Rau, G.H., Sweeney, R.E., Kaplan, I.R.,Mearns, A.J., Young, D.R. 1981. Differences in animal ^{13}C,^{15}N, and D abundance between a polluted and an unpolluted coastal site: Likely indicators of sewage uptake by a marine food web. Est. Coast. Shelf Sci. 13: 701-707.

Roman, C.T., Garvine, R.W., & Portnoy, J.W. 1995. Hydrologic modeling as a predictive basis for ecological restoration of salt marshes. Environ. Management. 19:559-566.

Rysgaard, S., N. Risgaard-Petersen, L.P. Nielsen & N.P. Revsbech. 1993. Nitrification and denitrification in lake and estuarine sediments measured by the ^{15}N dilution technique and isotope pairing. Appl. Environ. Microbiol. **139**: 2093-2098.

Sarda, R., I. Valiela, & K. Foreman. 1996. Decadal shifts in a salt marsh macrofaunal community in response to sustained long-term experimental nutrient enrichment. J. Exp. Mar. Biol. & Ecol. 205:63-81.

Short, F.T., W.C. Dennison & D.G. Capone. 1990. Phosphorus-limited growth of the tropical seagrass *Syringodium filiforme* in carbonate sediments. Mar. Ecol. Prog. Ser. **62**: 169-174.

Sloth, N.P., L.P. Nielsen & T.H. Blackburn. 1992. Nitrification in sediment cores measured with acetylene inhibition. Limnology & Oceanography **37**: 1108-1112.

Sorensen, J. 1978. Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl. Environ. Microbiol. **35**: 301.

Valiela, I, and J. Teal. 1978. Inputs, outputs, and interconversions of nitrogen in a salt marsh ecosystem. Pp 399-414 In: R.K. Jefferies and A.J. Davy (eds), Ecological Processes in Coastal Environments. Brit Ecol. Soc. Symp., Blackwell.

Valiela, I. 1984. Marine Ecological Processes. Springer-Verlag. 546 pp.

Valiela, I., & J. Teal. 1979. The nitrogen budget of a salt marsh ecosystem. Nature. 280:652-656.

Valiela, I., J.M. Teal, and N.Y. Persson. 1976. Production and dynamics of experimentally enriched salt marsh vegetation: below ground biomass. Limnol. Oceanogr. 21:245-252.

Van Dover, C.L., Grassle, J.F., Fry, B.,Garritt, R.H., Starczak, V.R. (1992). Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. Nature 360:153-155.

VanWijhen, H.J. and J.P. Baaker 1999. Nitrogen and phosphorus limitation in a coastal barrier salt marsh: the implication for vegetation succession. J. Ecology 87:265-272.

Weinstein, M.P., S.Y. Litvin, K.L. Bosley, C.M. Fuller, S.C. Wainright. 2000. The role of tidal salt marsh as an energy source for marine transient and resident finfishes: a stable isotope approach. Trans. Amer. Fisheries Soc. 129:797-810.

Whitney, D.M., A.G. Chalmers, E.B. Haines, R.B. Hanson, L. R. Pomeroy and B. Sherr. 1981. The cycles of nitrogen and phosphorus. Pp 163-181 In: L.R. Pomeroy & R.G. Weigert, eds. The Ecology of a Salt Marsh. Springer-Verlag.

Woodwell, G. M., R.A. Houghton, C.A. Hall, A.S. Whitney, R.A. Moll, D.W. Juers 1979. The Flax Pond Ecosystem study: The annual metabolism and nutritient budgets of a salt marsh. Pp 491-511 IN R.L. Jeffries & A.J. Davy, eds. Ecological Processes in Coastal Environments. Blackwell.

Attachments:

School of Aquatic and Fishery Sciences — Box 355020 — University of Washington — Seattle, Washington 98195-5020 USA

10 September 2001

Dr. Edward J. Carpenter
San Francisco State University, Romberg Tiburon Center
3152 Paradise Drive
Tiburon, CA 94920

Subject: Support of CALFED proposal

Dear Ed,

This letter is to express full support and cooperation of the BREACH II research project and team for your and Doug Capone's proposed CALFED proposal, "Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Delta." Your proposal is both timely and extremely relevant to the overall goals of CALFED, addresses a serious gap in our understanding of shallow-water habitat restoration in the Bay-Delta, and effectively takes advantage of and expands our BREACH II studies.

The fundamental premise of our BREACH research is that by studying historically-restored and remnant natural wetland sites in the Sacramento-San Joaquin Delta and northern San Francisco Bay we can determine directions, rates and patterns of current and future CALFED estuarine restoration. Our research goals are to: (1) systematically address the present status, rates, and patterns of tidal ecosystem restoration in recognizably different Bay-Delta ecosystems; (2) evaluate factors that promote rapid restoration of shallow-water habitat *versus* factors that have potentially inhibited natural rates and patterns of functional development; (3) evaluate the contribution of shallow water habitats to food webs supporting Bay-Delta ecosystems; and (4) assess the overall outcome of breached-levee restoration in the different Bay-Delta regions and recommend optimum strategies and spatial distribution of future restoration initiatives.

As much as we are trying to be comprehensive in our approaches to investigating the processes governing restoration trajectories in the Bay-Delta, we realize that we are unable to evaluate all likely important factors. This is particularly the case with nutrient cycling, which involves critical processes supporting marsh and mudflat primary production and the estuary's food webs. Your proposed investigations of nutrient limitation, nitrification and denitrification, and stable isotope tracing of nitrogen and carbon in marsh food webs would address many of these nutrient cycling "gaps," and build considerably toward our understanding of the contribution of emergent marsh restoration to the ecosystem functions of the Bay-Delta. Because we will be characterizing only the pattern and rate of emergent vegetation change through restoration, developing a better understanding of the role of nutrient limitation in restoring marshes would be extremely valuable to BREACH and CALFED. Your studies would be important to our interpretation of controls on revegetation and vegetation succession rates and patterns, thereby helping us to:

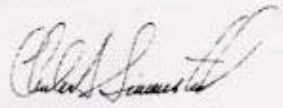
- refine and extend applicability of BREACH conceptual model by (a) elucidating rates of transition between vegetated and non-vegetated habitats, and rates of transition from one floral community to another, and (b) adapting it for processes, conditions and floral/faunal communities in more saline regions of Bay-Delta.

- prepare synthesis of patterns, rates and short-term and long-term endpoints of tidal marsh restoration predicted from refined conceptual model for breached-levee restoration along the Bay-Delta continuum.
- assess food web contributions of restoring marshes to consumer organisms, in conjunction with collaborating existing/proposed CALFED studies.

Positioning the proposed studies at our BREACH II study sites should particularly enhance and extend the interpretability and power of both studies' results. We offer all of our background information, assistance in interfacing with our study and sampling sites, and direct collaboration whenever possible. In addition to integration of our actual research, we should also strive to collaborate on data handling and management, dissemination of integrated work products and outreach.

All of us associated with the BREACH II research in the Bay-Delta look forward to the opportunity to collaborate.

Sincerely,



Charles A. Simenstad
Research Associate Professor

Charles Simenstad
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USC
UNIVERSITY OF SOUTHERN CALIFORNIA

September 10, 2001

Department of Contracts and Grants

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

Subject: Proposal Entitled:	**"NITROGEN TRANSFORMATIONS IN RESTORATION OF SALT MARSHES IN THE SAN FRANCISCO DELTA"**
Principal Investigator:	**Dr. Douglas Capone**
Amount Requested:	**$608,206**
Period:	**9/1/02 thru 8/31/05**

We are pleased to forward the enclosed proposal for your consideration and approval. This proposal has been approved by the administration of the University and signed by Lloyd Armstrong, Jr., Provost and Senior Vice President for Academic Affairs.

Should you have any questions of a technical nature regarding this proposal, please contact the Principal Investigator. Information of a business or administrative nature should be directed to the attention of the undersigned at the address below or at (213) 740-6064. My E-Mail address is nbennett@bcf.usc.edu.

Sincerely yours,

Nann L. Bennett

Nann L. Bennett
Contract and Grant Administrator

Enclosures

University of Southern California
Los Angeles, California 90089-1147
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www.usc.edu/dept/contracts/

Proposal Cover Sheet
San Francisco State University – Romberg Tiburon Center

Title: **Nitrogen Transformations in Restoration of Salt Marshes in the San Francisco Delta**

Principal Investigator(s):

Name: **D.G. Capone**
Department: **Department of Contracts and Grants**
Institution: **University of Southern California**
Street: **University Park**
City: **Los Angeles** **State: CA** **Zip: 90089-1147**
Country: **USA**
E-mail: **capone@usc.edu**
Telephone: **(213) 740-2772**
Fax: **(213) 740-6720**

Period of Performance: **September 1, 2002 to August 31, 2005**

Budget: **$608,206**

Authorizing Official: Lloyd Armstrong (Signature) 9/10/2001 (Date)
Lloyd Armstrong, Jr.
Provost and Senior Vice President
For Academic Affairs

Principal Investigator(s): D G Capone 11 SEP 01 (Date)
Douglas Capone
Professor