Research Summary
Proposal Reference Number: 01T CEQI 04 1083

Names of Participants: Risa Cohen, Peggy Fong

Project Title: **The effects of runoff on the physiology of *Enteromorpha intestinalis*: implications for use as a bioindicator of freshwater and nutrient influx to estuarine and coastal areas**

Project Goals:

1) To increase understanding of the environmental conditions that lead to blooms of macroalgae.
2) To start development of a tool that can be used by management agencies to measure water quality and allow early detection of eutrophic conditions.

1) The first major goal of this study has been to increase understanding of the environmental conditions that lead to blooms of macroalgae. Southern California is a highly populated region with developed watersheds. The climate consists of distinct wet and dry seasons, and therefore rainfall, runoff and stream flow are generally limited to pulsed events within the winter months (Young et al. 1978, Page et al. 1995). Urbanized watersheds in the region are highly channelized, thereby serving as conduits for increased volumes and speeds of runoff to southern California estuaries (Zedler 1996, Eyre and Balls 1999). Further, anthropogenic changes have resulted in year-round nutrient inputs from urban runoff and treated wastewater (Zedler et al. 1992, Dalkey and Shisko 1992, Johnson 1993). Therefore natural pulse rainfall events ameliorated by anthropogenic inputs transport freshwater and nutrients into estuaries and contribute to algal blooms and eutrophic conditions.

We hypothesized that the bloom-forming green macroalga, *Enteromorpha intestinalis*, has physiological mechanisms for short-term survival under decreased salinity and light availability associated with nutrient pulses. To understand the mechanisms that result in these algal blooms, short-term physiological responses of *E. intestinalis* after to exposure to one, then two, and finally three environmental factors associated with eutrophication were quantified. We measured tissue water and potassium content to examine short-term (48-hour) effects of reduced salinity, and nutrient content (nitrate ($NO_3^-$), ammonium ($NH_4^+$), and total nitrogen (N)) of tissue and water to see how decreased salinity and light availability affected nutrient uptake and storage.

We found that *E. intestinalis* osmoregulated effectively at all salinities tested (range = freshwater to oceanic salinity). The mechanisms that result in this salinity tolerance include altering tissue water content and potassium concentration to maintain osmotic balance with the surrounding medium; tissue water content decreased and potassium concentration increased with increasing external salinity. Salinity tolerance of *E. intestinalis* was further enhanced by high $NO_3^-$ uptake ability; although the role of potassium in osmoregulation was not affected by differing nutrient concentrations, the pattern was less pronounced in the presence of higher water column nutrient concentrations. These results suggest that inorganic nitrogen stored in algal tissue may function in short-term osmoregulation.

We also determined that *E. intestinalis* has the ability to take up and store nutrients for future growth, even during times of environmental stress due to short-term reduction in salinity and light availability. Tissue nutrients, an indicator of nutrient status, increased with increased nutrient supply regardless of variations in salinity or light availability. In the short-term, light availability did not affect tissue potassium or total nutrient concentration. However, light influenced whether the nutrients were present in the tissue as inorganic or organic forms. With reduced light, we hypothesized that the assimilation of inorganic to organic nitrogen was energy-limited. Thus the alga has physiological mechanisms for tolerating conditions that co-occur with eutrophication, including decreased salinity, increased nutrient concentration and decreased light availability, which aid in our understanding of why algal blooms proliferate in extremely variable estuarine environments.

The ability of *E. intestinalis* to take up pulses of different species of nitrogen ($NH_4^+$, $NO_3^-$) simultaneously would be an important mechanism to enhance bloom ability in estuaries with many different nutrient sources. To assess mechanisms that may enhance the bloom potential of *Enteromorpha intestinalis* during pulses of multiple nitrogen species typical of southern California estuaries, we quantified uptake and assimilation of $NH_4^+$ and $NO_3^-$ offered simultaneously. Stable nitrogen isotopes ($^{15}N$) were used to determine the preferred form of nitrogen ($NO_3^-$, $NH_4^+$) taken up and incorporated into the tissue. Uptake rates and preference for $NH_4^+$ or $NO_3^-$ following 1, 3, 6, 9, 12 or 24 hours of exposure to either $^{15}NH_4$-$NO_3$ or $NH_4$-$^{15}NO_3$ were determined by disappearance of N from the medium and accumulation in algal tissue.

The presence of $^{15}N$ or $^{14}N$ had no effect on uptake of either $NH_4^+$ or $NO_3^-$; $^{15}N$ was removed from the water column at an almost identical rate and magnitude as for $^{14}N$. There were differences in accumulation of $^{15}NH_4^+$ and $^{15}NO_3^-$ in the tissue reflecting disappearance from the water; $^{15}N$ from $NH_4^+$ increased faster and reached an atom % twice that for accumulation of $^{15}N$ from $NO_3^-$. These results suggest that when $NH_4^+$ and $NO_3^-$ were available in equal amounts, $NH_4^+$ was taken up and assimilated preferentially, but $NO_3^-$ still pooled in algal tissue. These are important findings because often $NO_3^-$ is the most readily available form of N in eutrophic conditions (Page et al.1995, McGlathery et al.1996, Fong and Zedler 2000). Further, the ability to take up high concentrations of $NH_4^+$ and $NO_3^-$ is a very important trait for bloom-forming species of estuarine macroalgae, as they are usually subject to multiple nutrient species from various sources (Litaker et al. 1987, Valiela et al. 1992, McGlathery et al. 1996).

Natural abundance of stable nitrogen isotopes ($\delta^{15}N$) has become increasingly useful for determining nitrogen (N) sources and sinks (Peterson and Fry 1987). Several studies have shown that the $\delta^{15}N$ reveals whether the N source is from a terrestrial-based or a marine-derived source (Van Dover et al. 1992, Page 1995, Fourqurean et al. 1997, Rogers 1999). A general assumption is that the $\delta^{15}N$ of primary producers reflect the $\delta^{15}N$ of the sources. However, differential partitioning of isotopes, or fractionation, can create differences between $\delta^{15}N$ of sources and sinks especially since $^{14}N$ is thought to be preferred over $^{15}N$ in biological processes (Waser et al. 1998).

We examined the relationships between N supply and macroalgal tissue responses to assess whether fractionation of nitrogen isotopes ($^{14}N$ vs. $^{15}N$) occurs under different isotopic ratios and concentrations. Using both traditional nutrient analysis techniques and stable isotopes for water and algal tissue and labeled $^{15}NO_3^-$ and $^{15}NH_4^+$, we measured uptake rates, tissue $NO_3^-$, $NH_4^+$, total N and $\delta^{15}N$ and which form of inorganic N was preferred. When *E. intestinalis* was exposed to a range of water $\delta\ ^{15}N$ values, but the same N concentration, more fractionation occurred for $NO_3^-$ when it was the primary N source than for $NH_4^+$. Under high nutrient concentrations, the alga removed more N from the water column when the $\delta^{15}N$ was low (less heavy N). Across all treatments, more of the lighter isotope ($^{14}N$) was taken up by algal tissue.

*E. intestinalis* exposed to a range of water N concentrations with the same $\delta^{15}N$ value did not reveal differences in fractionation with increased water N availability. Tissue $\delta^{15}N$ did not change with increased $NO_3^-$ or $NH_4^+$ availability over the range of concentrations tested (50-500 μM), but may be important at lower concentrations. Although fractionation occurred, it was still possible to detect differences in $\delta^{15}N$ available by examining the $\delta^{15}N$ of the algal tissue. Results from these experiments with $^{15}N$ provide an understanding of how the $\delta^{15}N$ of water column dissolved inorganic N is reflected in macroalgal primary producers and suggests that *E. intestinalis* would be a good indicator of N sources to estuarine and coastal communities.

2) The second aim of this project has been to start development of a tool that can be used by management agencies to measure water quality and allow early detection of eutrophic conditions. A major limitation of current methods of freshwater and nutrient measurement is the samples are discrete. Most water samples provide salinity and nutrient information for a given instant, and changes may occur over a variety of spatial and temporal scales. There is also the problem of relating the physical data to biological data. Therefore an integrative way to quantify terrestrial inputs to estuarine and marine communities is necessary. One possibility is to develop biological measures of conditions associated with eutrophication. Studies designed to determine whether algae, including *Enteromorpha intestinalis*, can be used to assess ambient nutrient (N) concentrations have recently begun to appear in the literature (e.g. Jones et al. 1996, Fong et al. 1998, Lyngby et al. 2000).

Using information from experiments where the responses of *E. intestinalis* to factors associated with influx of freshwater and nutrients from terrestrial sources were quantified, we began to assess this alga's usefulness as a bioindicator. Though analyses have not yet been completed, data from field experiments where cultured algae was outplanted into three different southern California estuaries known to receive runoff input year-round appear to support the laboratory findings. It was possible to detect salinity and nutrient changes in algal tissue due to changes in freshwater and nutrient inputs across seasons by analyzing the tissue water, potassium, $NH_4^+$, $NO_3^-$, and total N content of *E intestinalis* tissue after 24 hours in the field. In addition, the natural abundance of stable isotopes ($\delta\ ^{15}N$) sequestered by algae placed in the field was used to establish whether differences in the sources and amount of processing of the nutrients available to the algae in these estuaries during both wet and dry seasons can be detected.

This research has increased our understanding of the environmental conditions that lead to blooms of macroalgae. In addition, this alga's physiological responses may be used as an inexpensive, straightforward method for measuring the magnitude of the freshwater and nutrient inputs associated with runoff and the sources of the bioavailable nutrients.

References:

Dalkey, A. and J.F. Shisko. 1993. Water Quality. In: Marine monitoring in Santa Monica Bay: annual assessment report for the period July, 1991 through June 1992. Environmental Monitoring Division, Bureau of Sanitation, DPW, Los Angeles.

Eyre, B. and P. Balls. 1999. A comparative study of nutrient behavior along the salinity gradient of tropical and temperate estuaries. Estuaries. 22(22A): 313-326.

Fong, P., Boyer, K.E. and J.B. Zedler. 1998. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). Journal of Experimental Marine Biology and Ecology 231: 63-79.

Fong, P. and J.B. Zedler. 2000. Sources, sinks, and fluxes of nutrients (N+P) in a small highly modified urban estuary in southern California. Urban Ecosystems. 4: 125-144.

Fourqurean, J.W., Moore, T.O., Fry, B. and J.T. Hollibaugh. 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}N$, and $\delta^{13}C$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Mar Ecol Prog Ser. 157: 147-157.

Johnson, S.C. 1993. Introduction. In: Marine monitoring in Santa Monica Bay: annual assessment report for the period July, 1991 through June 1992. Environmental Monitoring division, Bureau of Sanitation, DPW, Los Angeles.

Jones, A.B., Dennison, W.C. and G.R. Stewart. 1996. Macroalgal responses to nitrogen source and availability: Amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). Journal of Phycology 32(5): 757-766.

Litaker, W., Duke, C.S., Kenney, B.E. and J. Ramus. 1987. Short-term variability and phytoplankton abundance in a shallow tidal estuary. I. Winter and summer. Marine Biology. 96: 115-121.

Lyngby, J.E., Mortensen, S. and N. Ahrensberg. 2000. Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecosystems. Marine Pollution Bulletin 39: 212-223.

McGlathery, K.J., Pedersen, M.F. and J. Borum. 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). J. Phycol. 32: 393-401.

Page, H.M. 1995. Variation in the natural abundance of 15N in the halophyte, *Salicornia virginica*, associated with groundwater subsidies of nitrogen in a southern California salt marsh. Oecologia. 104(2): 181-188.

Page, H.M., Petty, R.L. and D.E. Meade. 1995. Influence of watershed runoff on nutrient dynamics in a southern California salt marsh. Estuarine, Coastal and Shelf Science. 41: 163-180.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293-320.

Rogers, K. 1999. Effects of sewage contamination on macro-algae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. New Zealand Journal of Marine and Freshwater Research. 33: 181-188.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-Andreson, B., D'Avanzo, C., Babione, M., Sham, C.H., Brawley, J. and K. Lajtha. 1992. Couplings of watersheds and coastal waters sources and consequences of nutrient enrichment in Waquoit Bay Massachusetts. Estuaries. 15: 443-457.

Van Dover, C.L., Grassle, J.F., Fry, B., Garritt, R.H. and V.R. Starczak. 1992. Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. Nature. 360: 153-156.

Waser, N.A.D., Harrison, P.J., Nielsen, B., Calvert, S.E. and D.H. Turpin. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnol. Oceanogr. 43(2): 215-224.

Young, D. R., T-K. Jan and T. C. Heesen. 1978. Cycling of trace metal and chlorinated hydrocarbon wastes in the Southern California Bight, p. 481-496. In M. L. Wiley (ed.), Estuarine Interactions. Academic Press, Inc., New York.

Zedler, J.B., Nordby, C.S. and B.E. Kus. 1992. The ecology of Tijuana Estuary, California: A National Estuarine Research Reserve. NOAA Office of Coastal Resource Management, Sanctuaries and Reserves Division, Washington, D.C.

Zedler, J.B., Principal Author. 1996. Tidal wetland restoration: a scientific perspective and southern California focus. La Jolla, CA: California Sea Grant College System, University of California.

Titles of Publications:

Below are the titles of manuscripts to be submitted for publication. Copies of the publications will be sent when they become available.

Cohen, R. A. and P. Fong. In review. Factorial experiments of increasing complexity to quantify physiological responses of a bloom-forming green macroalga to short-term change in salinity, nutrients, and light. *Estuaries*

Cohen, R. A. and P. Fong. In prep. Nitrogen uptake and assimilation in *Enteromorpha intestinalis*: Using $^{15}N$ to determine preference for simultaneous pulses of nitrate and ammonium.

Cohen, R. A. and P. Fong. In prep. The effects of different nitrogen isotopic ratios and concentrations on fractionation by the opportunistic green macroalga *Enteromorpha intestinalis*.

Cohen, R. A. and P. Fong. In prep. Measurement of salinity and nutrient changes in the field using the macroalga *Enteromorpha spp*: Implications for use as a bioindicator of freshwater and nutrient influx to estuarine and coastal areas.