Application of Elemental Fingerprinting to Evaluate the Dynamics of Larval Exchange

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LONG-TERM GOALS

A major goal of this research is to develop a complete methodology for estimating inter- and intra-bay larval exchange rates that can be applied to several invertebrate taxa and to a range of bays and estuaries. This methodology will employ larval and recruit origin determinations using elemental 'fingerprints' imparted to the shell by the waters in which larvae develop. Mussels are selected as the model taxon. An ultimate goal is to incorporate dispersal information into population dynamic models to examine the consequences of different dispersal patterns and population connectivity.

OBJECTIVES

Objectives of this research are to: (1) extend the elemental fingerprinting approach developed for crab zoea to mussels in southern California, (2) assess the role of development and settlement location, as well as mussel species, shell size, shell zone, on elemental composition, (3) apply the technique to the detection of bay-ocean and inter-bay larval exchange, (4) evaluate physical connectivity of bay and ocean habitats (and thus dispersal potential) using thermistor, elemental, drifter and current meter data, (5) compare realized dispersal patterns to those predicted from physical transport models, and (6) model consequences of bay-ocean exchange for population dynamics using *P. crassipes* as a test organism. For southern California mussel populations, we hope to determine whether bay-released larvae develop inside or outside a specific bay, whether populations are self seeding, and whether there is larval exchange with other bay or coastal populations.

APPROACH

Elemental “fingerprinting” utilizes a naturally induced tag which characterizes specific environmental signals such as trace elements or temperature, to track movements of animals. This type of tag is expected to be imparted to all animals in a given environment and therefore overcomes many of the difficulties experienced with artificial tags. While organisms are forming, they incorporate various

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trace elements (such as metals introduced to the water through pollution) into their body tissues or hard parts in relation to levels found in ambient seawater (with additional signals reflecting local temperature and salinity). If the water composition is sufficiently different at different locations, it is possible to determine where larvae came from by analyzing the chemical composition of hard parts formed earlier in their lives.

For this study, we are collecting larvae and newly settled, post-larval recruits in San Diego Bay, neighboring embayments, and surrounding nearshore coastal habitats. We are characterizing the elemental composition of several species of mussel larvae and newly settled recruits using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and more recently, an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Discriminant function analyses are used to determine canonical variables (linear combinations of sampled trace elements) that serve as 'fingerprints' to identify larval origin. The purpose is to directly observe exchange of larvae within bays, between different embayments, and with the open coast.

Dispersal probability estimates for larvae originating from southern California bays are being established through a physical connectivity analysis of within-bay longitudinal dispersion, bay-ocean exchange and along- shore coastal dispersion. Larval and recruit sampling is being conducted in conjunction with long-term temperature measurements and real-time trace elemental analyses of seawater.

The population-level consequences of observed larval dynamics and developmental influences of bay vs. coastal water are being assessed through whole-life cycle and metapopopulation modeling approaches. Ultimately, information about growth, and survival as a function of water type (bay vs coastal) will be integrated with element-based quantification of larval origins and flux rates to evaluate the consequences of different larval migration strategies, release sites, residence times and population connectedness.

WORK COMPLETED

Initial research efforts have involved: (1) Development of methods to analyze the trace element composition of newly recruited mussels in bay and coastal habitats of southern California by ICP-OES and more recently, by Laser Ablation (LA)-ICP MS. Three species have been targeted: *Mytilus galloprovincialis* (Bay mussel), *M. californianus* (California mussel), and *Musculista senhousia* (Asian mussel). (2) Evaluation of species, site, size and shell-region dependence of shell composition in these mussels (based on ICP-OES), (3) Characterization of temperature regimes (with thermistor records) at bay and coastal sites to interpret temperature-sensitive elements (e.g., Sr, Mg) and to assess environmental variability at the scale of the dispersal process, (4) Evaluation of the bay-ocean-bay dispersal kernel (connectivity matrix) for simplified flow patterns, and (5) Population modeling of *Pachygrapsus crassipes* to determine consequences of larval development and dispersal in bay versus open coast habitats.

During the past year Scripps has been awarded equipment grants from NSF and ONR to purchase an ICP-MS and accompanying Laser Ablation unit, respectively. Development of protocols for mussel shell analyses with the LA-ICP MS are in progress. These instruments promise lower elemental detection limits and finer spatial resolution than were possible with the ICP-OES.

RESULTS

Trace element analyses. Water samples have been collected from three sites in San Diego Bay, CA, two sites inside Mission Bay, CA, and eight sites along the open coast of southern California during July 2001. Samples have been analyzed using solution based ICP-MS with only filtration, acidification and dilution necessary to prepare samples. Among the 17 elements examined, concentrations of Ba, Cd, Cr, Ca, Sr, Mn, Mg and Li , along with temperature and salinity, were useful in discriminating locations (Fig. 1A). These water data, to be obtained quarterly, will aid interpretation of mussel shell data.

Newly settled mussels have been sampled and analyzed from 14 sites in different southern California embayments and coastal regions. Trace element analyses by ICP-OES and subsequent discriminant function analyses have indicated our ability to distinguish mussels based on site, species, and size.

Overall, the ability to distinguish small (<250,000 ppb Ca) *Mytilus* individuals from among Mission Bay, San Diego Bay and the open coast is very good (Fig. 1B). Among *Mytilus* recruits, concentrations of Ag, Cd, Cu, Fe, Ni, Pb, Sr, and Zn were elevated in samples from Dana Landing in Mission Bay and in most cases, from Harbor Island in San Diego Bay relative to open coast sites. Elements such as Mn and Mg do not show a difference among sites for *Mytilus* spp. Within the outer portion of San Diego Bay, Harbor Island mussels are distinct from those on Shelter Island and Coronado Tidelands, mainly due to elevated Al, Ba, and Cu concentrations. Among open coast sites, Crystal Pier (Pacific Beach) mussels are distinct, driven by Mn, Al, Cd and Sr, but this could be due to the fact that these animals were considerably smaller than the others.

Mytilus species are distinct from *Musculista senhousia* (Fig. 1C), largely due to differences in Cd, Ba, Sr, Mg and Mn, but at present, *M. galloprovincialis* and *M. californianus* have not been considered separately. To confirm identifications, *Mytilus* soft parts are being identified to species by molecular (PCR) methods while shells are analyzed for trace element composition.

Mussel shell size, inferred by Ca content, is related to elemental concentration. High concentrations of trace elements were found only in the smallest *Mytilus spp.* for all elements examined except those known to be sensitive to temperature and salinity (Mg, Sr), and except for Zn and Mn, for which the pattern is more ambiguous. Similar size-trace element relationships were observed in *Mytilus* spp. and *Musculista senhousia*. Size alone is able to discriminate among mussel shell compositions from a single site. For example, differences in Al, Fe, Sr, Cd, and Mn drive size-related variation in *Mytilus* from the Scripps Pier (Fig. 1D).

Solution based elemental techniques employed to date have limited our ability to target only the larval portion of bivalve shells (about 200 μm diameter) or to distinguish larval versus newly settled juvenile shell. A newly acquired 213 nm Laser Ablater combined with a double focusing, single collector, magnetic sector ICP MS now permits elemental analysis of specific regions of the shell (10-20 μm diameter) to be analyzed on individuals of varied ages. This methodology should allow us not only to determine the site of origin, but also to construct possible trajectories for individual larvae (e.g., Swearer et al. 1999).

We acquired this configuration this summer, and have been developing a protocol for its use. Experiments to determine analytical details, such as laser settings, standards, machine configuration,

mounting techniques for small mussel samples, and calibration with solution-based ICP-MS data, have been conducted. We will calibrate solution-based and LA ICP-MS results with previous data collected using the ICP-OES.

Connectivity Model. A model of connectivity between different larval source habitats has been constructed in the San Diego region (Fig. 2A). Exchange rates between modules are estimated based on results of field studies in San Diego Bay, Mission Bay and coastal waters. Box sizes are scaled according to typical excursion lengths of the exchange flows (e.g., tidal excursion lengths in the bays with internal mixing of box every tidal cycle). Preliminary results are presented for an initial case of mussel larvae with 30-day passive dispersal, with no mortality or mean flow (e.g., no river inflow to bays and no alongshore mean coastal current). Connectivity is shown in Fig. 2B, with connectivity defined as the probability that larvae released in box X are found in box Y after 30 days (for a given origin, the sum of values is one).

For this scenario (no behavior), model results suggest that a significant portion of larvae spawned in Mission Bay (about 10% released from the back of the bay) and in San Diego Bay (about 50% released from the back of the bay) would be retained within their bays. Of the remainder, a relatively large fraction are wasted (leave the area of interest), and for San Diego Bay, some will accumulate outside the Bay mouth. Most larvae released from populations along the outer coast are lost. The remainder of coastal spawned larvae are distributed along the shore; few enter the bays. Sensitivity analyses and estimates of uncertainty are underway. Other model runs include mean flows (advection), non-uniform spawning, non-uniform mortality, and exchange rates that are adjusted to account for larger tides and or estuarine circulation effects.

Population modeling. Stage-structured matrix models were constructed for the crab *Pachygrapsus crassipes* to explore population-level consequences of transport to and development in bay versus coastal waters of southern California. A 4-stage model (zoea 1, zoea 2-6, megalopae, post settlement stage) yielded highest population growth rates for larvae that were spawned and developed along the open coast, lowest rates for larvae spawned and developed in SDB, and intermediate/similar values for larvae spawned in one place and developed in the other. Elasticities suggest that population growth rate is most sensitive to changes in fecundity and larval growth rates. C. DiBacco's participation in an NCEAS workshop led to a pilot dispersal-population model linking an invasion kernel (bay source entering coastal waters) to population matrix modes.

IMPACT/APPLICATIONS

This research will advance understanding of marine invertebrate dynamics by (a) further development of techniques to evaluate larval origins and exchange, (b) relating physical exchange probabilities to actual estimates of bay-ocean and bay-bay larval exchange and (c) linking elements of larval behavior and transport to larval success and population dynamics. Expansion of element-based tagging approaches to identification of invertebrate recruit origins, and to questions of bay-bay exchange, should open up a wide range of applications including assessment of the interdependence of different habitats, evaluation of controls on population dynamics, and assessment of pollution consequences.

TRANSITIONS

During the award period we are making a technological transition from ICP-OES solution-based analysis to LA - ICP-MS solid phase analyses, opening an enormous opportunity for the study of invertebrate shells as recorders of their environmental history.

RELATED PROJECTS

We are working closely with J Gieskes (Scripps), C. Mahn (Scripps) and B. Chadwaick (SPAWAR) who are examining trace metal concentrations in waters and sediments of San Diego Bay. We are also collaborating with Scripps graduate student Bonnie Becker, who is studying the influence of the Point Loma kelp forest on scales of dispersal and recruitment in mussels at the Cabrillo National Monument, San Diego, and with graduate student Joel Fodrie, who is using trace element fingerprinting to study utilization of estuaries by juvenile halibut. Our thermistor data and transport patterns described along the open coast will compliment similar efforts by J. Largier to synthesize historical hydrographic data for the region.

PUBLICATIONS AND PRESENTATIONS

DiBacco, C. and L.A. Levin. 2000. Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae. Limnology and Oceanography 45: 871-880

DiBacco,C., D. B. Chadwick. 2001. Use of elemental fingerprinting to assess net flux and exchange of brachyuran larvae between regions of San Diego Bay, California and nearshore coastal habitats. Journal of Marine Research 59: 53-78.

DiBacco, C., Sutton, D. McConnico L. 2001. Vertical migration behavior and horizontal distribution of brachyuran larvae in a low-inflow estuary: implications for bay-ocean exchange. Mar. Ecol. Progr. Ser. 217: 191-206.

Levin, Lisa A., Becker, Bonnie J., McMillan, Pat, Fodrie, F. Joel, Walther, Shelly. Use of elemental fingerprinting to evaluate the dynamics of larval exchange in southern California mussels. Poster presentation. Western Society of Naturalists (2001).

Becker, Bonnie J. 2001. Extent of self seeding in a rocky intertidal ecosystem: The effects of a large kelp forest on hydrodynamics and retention of invertebrate larvae off of Point Loma, San Diego, California. Poster presented at the opening conference for the Center for Marine Biology and Conservation.

Becker, Bonnie J. 2001. "Mussels, lasers, and marine reserves: Why we care about the chemistry of baby mussel shells" (North County Sierra Club, September 2001). Invited presentation.

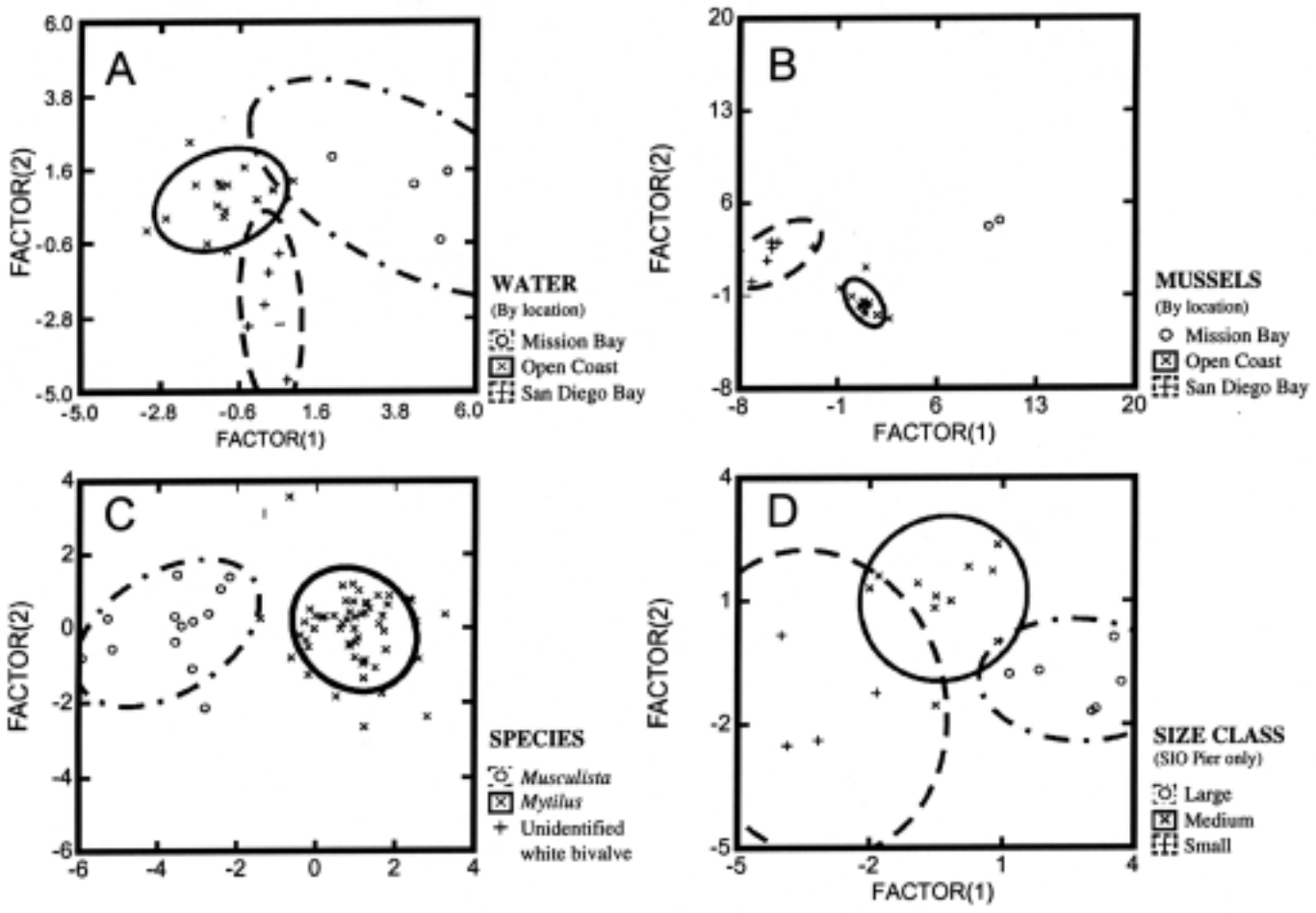

Figure 1. Canonical scores plot of discriminant function analyses (all solution-based). A. Seawater analysis grouped by location (includes temperature and salinity), summer 2001; B. Analysis of Mytilus, grouped by location, Summer 2000, Winter 2001; C. Analysis of mussels grouped by species; D. Analysis of Scripps Pier Mytilus, grouped by size class (inferred from Calcium values)

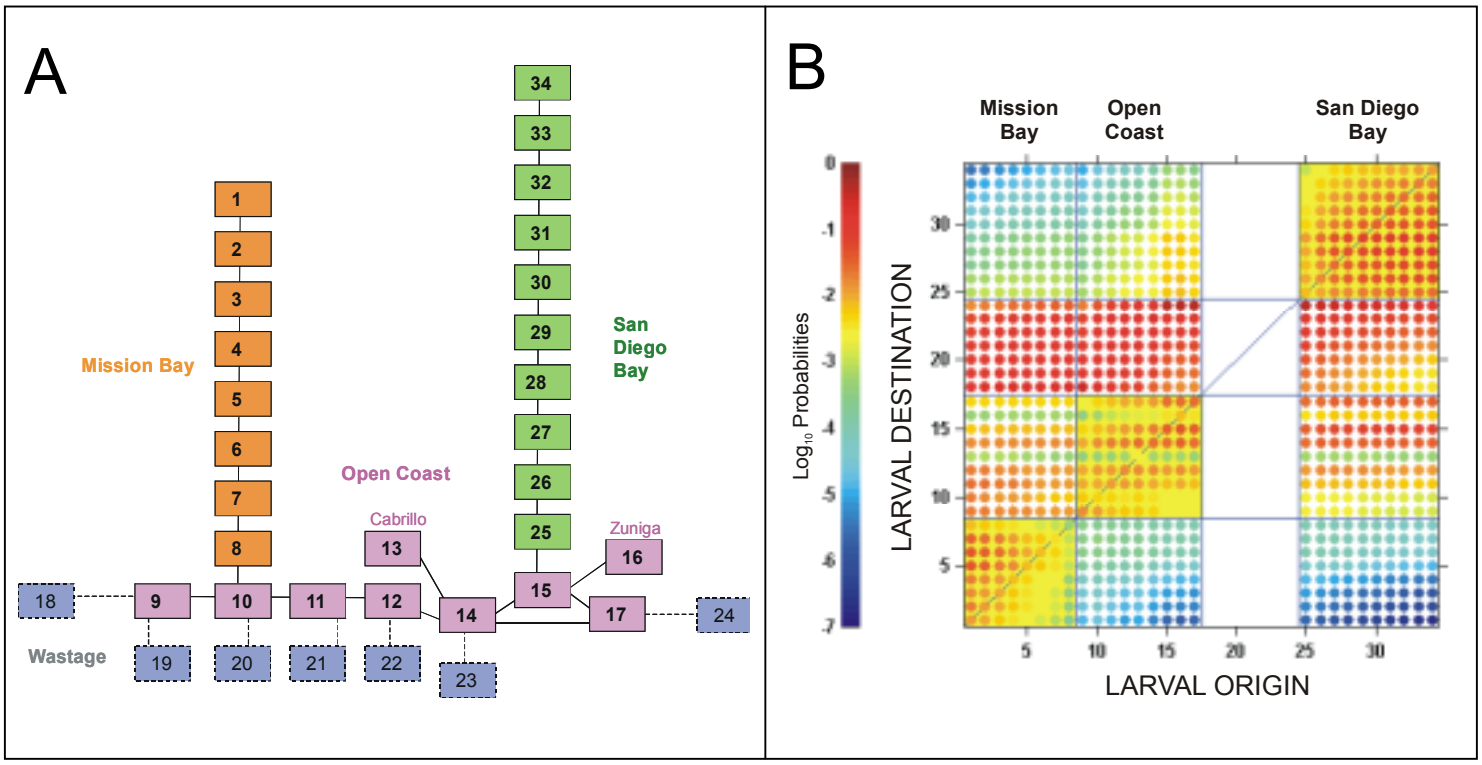

Figure 2. A. Schematic layout of boxes representing Mission Bay, San Diego Bay and coastal waters in the connectivity box model. Boxes have real volumes. Advective and diffusive exchange rates between boxes is estimated from field data. Connectivity between boxes is calculated by releasing larvae in one box and determining what percentage is found in each box at the end of the larval period; B. Color coded plot of connectivity matrix (probability of larva released at origin box X being in destination box Y at end of larval period). This base case is for passive larvae with a 30-day planktonic period, no behavior, zero mortality, and zero mean flow. No larvae are released in "wastage" boxes 18 to 24 (no spawning habitat). For small-volume boxes (e.g., 1 and 34), the final larval concentration may be high, but the probability of that box as a destination is low as the box volume is small. Strength of settlement is more closely related to larval concentration (connectivity/volume).