Title

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Authors

Levin, Lisa A.
Rasmussen, Linda L.

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Understanding connectivity to sustain and manage coastal resources

Lisa A. Levin
UCSD/Scripps Institution of Oceanography
858-534-3579
llevin@ucsd.edu

Linda L. Rasmussen
UCSD/Scripps Institution of Oceanography
858-534-3579
raz@ucsd.edu

Project Hypothesis
We seek to test hypotheses about rates of self seeding, the role of passive circulation and behavior in larval transport, and to identify regional source and sink populations in mytilid mussels. We assessed temporal variability in connectivity patterns for Mytilus spp, using experiments conducted previously (in 03, 04, 05, 06, and 07) and during this grant (08-10), compared results to passive transport predictions corrected for larval supply, and synthesize the information to generate generalized conceptual models of open coast, inner and outer bay connectivity patterns in Southern California.

Project Goals and Objectives
The overall project objective was to determine patterns of mussel connectivity in Southern California to provide information relevant for invasive species, aquaculture, and marine reserve management. We targeted open coast, outer bay and inner bay mytilid species. Goals were to:
(1) develop of trace elemental fingerprinting approaches for the invasive mussel *Musculista senhousia*; (2) generate and compare Fall and Spring fingerprinting –based connectivity patterns for *Mytilus californianus*, *M. galloprovincialis* and *Musculista senhousia* in Southern California, and (3) analyze and compare connectivity matrices based on realized (elemental fingerprinting) and simulated (ROMS models) bay to-bay and bay-ocean larval exchange.

Briefly describe project methodology
Larval connectivity was assessed through trace elemental fingerprinting. This approach assumes that trace elements in carbonate shell deposited by developing mussel larvae reflect the chemistry of the water masses they are in. Different bay and coastal waters impart different chemical signatures to mussel shell. To determine time- and space-specific larval shell signatures, developing larvae were 'outplanted' (reared in situ) at 18 coastal and bay sites. We analyzed the larval shell retained on newly recruited individuals and match these against outplant signatures to determine site or region of larval development for new settlers. This methodology was developed in our laboratory recently for *M. californianus* and *M. galloprovincialis*; we focused on developing comparable techniques for the invasive Asian mussel *M. senhausia*.

Connectivity (outplant/recruit) studies were conducted over 2 years, focusing on the reproductive season of each species. This involved moored larval outplants at ~18 locations in San Diego County during Spring and Fall with recruit collections approximately 2-3 weeks later. Larval shells of outplanted larvae and new recruits were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to determine concentrations of metals (e.g., Pb, Cu, Ba, Mg, Mn, Sr) ratioed to a rare Ca isotope (48Ca). Larval elemental data were analyzed by discriminant function analysis to create site- or region-specific algorithms that serve as the reference map for assessing recruit origins. Trace elemental signatures of larval shells of new recruits were compared against the reference signatures to determine their site or region of origin and generate connectivity matrices. These results were compared to real time predictions of passive coastal dispersal and origin probabilities using a nested 3-D ROMS coastal circulation model for study of larval transport in Southern California. The terrain following model, with a 600-m grid of outplanted larvae and new recruits were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to determine concentrations of metals (e.g., Pb, Cu, Ba, Mg, Mn, Sr) ratioed to a rare Ca isotope (48Ca). Larval elemental data were analyzed by discriminant function analysis to create site- or region-specific algorithms that serve as the reference map for assessing recruit origins. Trace elemental signatures of larval shells of new recruits were compared against the reference signatures to determine their site or region of origin and generate connectivity matrices. These results were compared to real time predictions of passive coastal dispersal and origin probabilities using a nested 3-D ROMS coastal circulation model for study of larval transport in Southern California. The terrain following model, with a 600-m grid size, is forced by climate and winds. We attempted to simulate larval transport for the specific time period studied during outplanting and recruit collections, allowing a more detailed understanding of transport mechanisms.

Describe progress and accomplishments toward meeting goals and objectives.
Several *Musculista senhousia* rearing experiments were performed to evaluate life history, growth behavior, and survival relevant to connectivity. These studies revealed that (a) eggs are approximately 65 microns in diameter at release, (b) adults release gametes within 20 min of exposure to natural and artificial light (c) larval shell formation begins ~15-20 h after fertilization, (d) larvae remain suspended in the water due to active swimming for ~12 to 14 days, (e) duration of active swimming seems to depend on food availability, (f) larvae drop to the beaker bottom (arguably looking for suitable settlement habitat) after 12-14 days and remain alive for up to one additional week, and (g) within 2-3 weeks of fertilization they double in length from a D stage to a more rounded protoconch of approximately 120 microns.

Surveys and literature reviews were conducted with the purpose of identifying Musculista populations (i.e., larval source sites). Nine embayments from Oceanside Harbor to the Tijuana Estuary were surveyed from 2007-2009. We were unable to find adults during our collections in Oceanside Harbor, Agua Lagoon, Batiquitos Lagoon, San Elijo Lagoon, Peñasquitos Lagoon, and the Tijuana Slough National

Wildlife Refuge. However, Musculista populations had been reported in these embayments previous to our surveys. Populations were identified in San Dieguito Lagoon, Mission Bay, and San Diego Bay.

A series of embryo outplants were conducted at 8 locations in San Diego County during May and November 2008 and the trace elemental signatures imparted to larval shell during the first week of development were examined. A parallel laboratory study was conducted in May 2008, in which embryos were reared in seawater from 5 of the outplant locations to assess the feasibility of mapping elemental fingerprints for different bays in the laboratory.

Newly settled Musculista recruits (0.5mm– 2mm; N=13) were collected 2-3 weeks after outplanting to conduct shell chemistry analysis and assign natal origins. Trace elemental analysis on both larvae and recruits was completed using laser ablation inductively coupled plasma–mass spectrophotometry (LA–ICP–MS). Using Discriminant Function Analysis (DFA), the greatest classification success was obtained when outplant sites were grouped into four regions: North County (Oceanside Harbor, Agua Hedionda Lagoon, and San Dieguito Lagoon), Open Coast (Scripps Pier), Mission Bay (Dana Landing and CPMS), and San Diego Bay (Shelter Island and Coronado). Region-specific classification success (jack-knifed) averaged 61% and 64% for May and November, respectively. Key elements that help distinguish larvae are Mg/Ca, Mn/Ca, Cu/Ca, Sr/Ca, Ba/Ca, Pb/Ca, and U/Ca. Analysis of laboratory–reared larvae in different water treatments also indicated differences in their shell chemistry. However, a lower region-specific classification success (43% jack-knifed) was obtained when laboratory treatments were grouped into four regions. The most important discriminant element ratios in this case were Sr/Ca, Ba/Ca, Pb/Ca, and U/Ca.

Musculista senhousia exhibited a high degree of self-recruitment. Musculista recruits (N=13) collected in San Diego Bay after the May 2008 originated in San Diego Bay (N=10) or the Open Coast (N=3). In November, all recruits exhibited self recruitment from San Diego Bay (N=9) and San Dieguito Lagoon (N=1). Because three recruits were assigned to the Open Coast in May, there is an indication that at least a portion of the population can recruit from external sources.

Analysis of laboratory–reared larvae indicated that differences in their shell chemistry were not sufficient to discriminate among study sites (six water treatments). When laboratory treatments were grouped into the same four regions as the outplants, classification success (jack-knifed) was 43%, with Ba, Mn, Sr, Pb, Cu, and U being the most important discriminant elements. While Ba and Mn were useful to distinguish the North County treatment, Sr and Pb were important for the Coast, and Cu for San Diego Bay.

PROJECT MODIFICATIONS:
Comparisons of ROMS and elemental fingerprinting–based connectivities led to major discrepancies. Acquisition of ADCP data overlapping study periods revealed that ROMS failed to accurately reproduce water velocities or directions beyond the immediate surface due to inadequate resolution of offshore wind forcing data. Thus a decision was made to abandon ROMS–based null hypotheses and draw directional comparisons between fingerprinting connectivity and ADCP data. ADCP instruments were deployed at La Jolla at two depths

(14 and 30 m) for 2-3 month periods coincident with the larval outplantings and recruit collection. Additional ADCP data were acquired from the City of San Diego, which has a continuously operated instrument at Pt Loma at 100 m depth.

PROJECT OUTCOMES
Our results suggest that trace elemental fingerprinting is feasible for *Musculista senhousia*, an invasive, back-bay species. Initial testing of recruits suggests that 77-89% of San Diego Bay recruits originate in and develop in San Diego Bay, but a small fraction either originate from another bay or exit San Diego Bay, obtain an open coast signature, and re-enter the San Diego Bay prior to settlement. Sample sizes from other lagoons are too small at this point to assess connectivity, in part due to the unexpected population crash in Mission Bay during the study period. Larval planktonic durations of 12-14 days appear to restrict persistent populations to larger bays such as San Diego Bay or Mission Bay, with episodic recruitment resulting from influxes of larvae likely to be responsible for populations in smaller lagoons experiencing large exchanges with ocean water.

Assessment of Mytilus connectivity patterns between 2003 and 2008 revealed consistent autumn poleward movement and spring equatorward movement for both *M. californianus* and *M. galloprovincialis*, consistent with currents measured at La Jolla and Pt Loma. However, because the major reproductive seasons differ, the dominant source-sink dynamics of these two congeneric species were nearly opposite.

A final analysis in Nov. 2009 incorporated Baja populations as possible sources for the first time, and examined transport during an El Niño year. We hypothesized more extensive south to north transport during this year. *M. Californianus* exhibited 59% of larvae moving northward (vs. 12% southward), while *M. galloprovincialis* larvae exhibited 73% northward movement (vs. 17% southward), with 29% and 10% self recruitment respectively. Approximately 20% of *M. californianus* recruits originated in Mexico whereas no *M. galloprovincialis* originated in Mexico.

IMPACTS OF PROJECT:
This project has promoted interdisciplinary investigations blending physical, ecological and geochemical approaches to the study of population connectivity. We have provided an unprecedented examination of connectivity patterns over time in three confamilial species, revealing significant seasonal and interannual variation in connectivity patterns but unexpectedly coherent connectivity patterns of outer bay and open coast mussel species in Southern California. This work highlights the importance of reproductive timing and contributes information of value to spatial planning and MPA design in the region. It also confirms the tendency for successful estuarine invaders like *Musculista senhousia* to exhibit high self recruitment."

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:
The methodology developed has been adopted by other laboratories involved in connectivity research.

ECONOMIC BENEFITS
NA

Issue-based forecast capabilities
Results for Mytilus spp. suggest that the connectivities of species in Southern California will be sensitive to climate change via alteration of reproductive timing (phenology). Demonstration of feasibility of elemental fingerprinting in *M. senhousia* expands the potential applications of this method to invasive species. High self recruitment in *M. senhousia* and the boom/bust population cycles observed indicate that irradication of this invasive species may be feasible. However fingerprinting results also suggest that the species is capable of surviving along the open coast and thus may re-invade estuaries following erradication.

Tools, technologies and information services developed
We have developed geochemical 'maps' of trace elements imparted to larvae developing in coastal and embayment waters. While these must be generated each season there appear to be coherent spatial patterns in elemental signals that transcend taxa (two fish species, three mussel species).

Publications
Technical reports
Integrating approaches for studying larval connectivity within mussel metapopulations: for comparing species, times and orthogonal techniques. Lisa A. Levin, Linda Rasmussen, Bonnie .J. Becker, F. Joel Fodrie, Pat McMillan. Unpublished manuscript

Conference papers, proceedings, symposia
June 2008. Am. Soc. Limnol. Oceanogr. Connectivity Conundrums: The benefits and challenges of integrating connectivity approaches, L. Levin and L. Rasmussen, St. Johns, Newfoundland. Invited tutorial

July 2008. International Larval Ecology Conference. Connectivity metrics for mussels: Integrating multiple approaches to the study of larval connectivity. Lisa Levin, Linda Rasmussen, Pat McMillan, Bonnie Becker, Kristen Gruenthal, F. Joel Fodrie, Lisbon, Portugal. (Invited)

November 2009. Coastal and Estuarine Research Federation. Linking population connectivity and demography to assess metapopulation fitness of coastal and estuarine mytilid mussels. Henry S. Carson and Lisa A. Levin, Portland, OR.
November 2009. Coastal and Estuarine Research Federation. Larval Connectivity of the Invasive Asian Mussel, *Musculista senhousia*, in Southern California. Paola C. Lopez-Duarte, Christina A. Tanner, Henry S. Carson, Linda L. Rasmussen, Lisa A. Levin, Portland, OR.

February 2010. AGU/ASLO Ocean Sciences. Temporal variability in mytilid mussel larval exchange: insights from a population connectivity time series. Henry S. Carson, Paola C. López-Duarte, Lisa A. Levin, Portland, OR.

February 2010. AGU/ASLO Ocean Sciences. Assessing Population Connectivity to Inform Invasive Species Eradication and Control Programs. Paola C. López-Duarte, Christina A. Tanner, Henry S. Carson, Linda L. Rasmussen, Lisa A. Levin, Portland, OR.

February 2010. AGU/ASLO Ocean Sciences. Observational and computational perspectives on regional population connectivity: Synthesizing and quantifying interdisciplinary results. Rasmussen, L., Levin, L.A., B. Cornuelle, B. Becker, J. Fodrie. et al., Portland, OR.

July 2010. American malacological Society. Population connectivity of *Mytilus galloprovincialis* in Southern California. Henry S. Carson, M. Paola López-Duarte, and Lisa A. Levin, San Diego, CA.

Peer-reviewed journal articles or book chapters
Rasmussen, L., Cornuelle, B.D., Levin, L.A. , Largier, J.L. DiLorenzo, E. 2009. Effects of small-scale features and local wind forcing on tracer dispersion and estimates of population connectivity in a regional scale circulation model. J. Geophys. Res. 114, C01012, doi:10.1029/2008JC004777.

Carson, H.S., P.C. Lopez-Duarte, L. Rasmussen, D. Wang and L. Levin. 2010. Reproductive timing aletrs population connectivity in marine metapopulations. Current Biology 20: 1-6.

Henry S. Carson, Geoff Cook, M. Paola López-Duarte and Lisa A. Levin Demographic modeling of marine metapopulations using realized larval exchange. In revision. Ecology

López-Duarte P. C., H.S., Carson and L.A. Levin. Assessment of Larval Connectivity of the Invasive Asian Mussel, *Musculista senhousia*, through Elemental Fingerprinting. In preparation.

Theses, dissertations
"Rocky Reef Fish Connectivity: Patterns, Processes, and Population Dynamics" by Geoff Cook

Dissemination of results
Presentation Title – Larvae on the Loose: Connectivity of Southern California Mytilid Mussel Populations. Invited seminar speaker: Lisa A. Levin, California State University, Los Angeles, California. February 2009. Target audience: faculty and students.

Presentation Title – Mussel Madness: Connectivity of Mytilid Mussel Populations in Southern California. Presented by: Paola López-Duarte, Cabrillo National Monument, National Park Service, San Diego, California. March 2009. Target audience: general public (all ages) who attended Cabrillo Science Day

Students
Geoffrey S. Cook
UCSD/Scripps Institution of Oceanography
Integrative Oceanography Department
Degree program enrolled in: Biological Oceanography
Theses/dissertation title: Rocky Reef Fish Connectivity: Patterns, Processes, and Population Dynamics
Supported by Sea Grant funds? [x] yes [] no
Start date: 01/01/2009
End date: 03/31/2009

Christina Tanner
UCSD/SCripps `institution of Oceanography

Integrative Oceanography Division
Degree program enrolled in: Biological Oceanography
Theses/dissertation title: TBA
Supported by Sea Grant funds? [] yes [x] no

Volunteer Count: 3

Cooperating organizations
Academic Institutions
The University of California Marine Council (UCMC) contributed
additional funds for this project through the Coastal Environmental
Quality Initiative (CEQI).

International implications
Dexiang Wang, a visiting scientist from China assisted with the
research in 2008-9, There was an exchange of ideas and methodologies.
Dexian led the PCR identification of recruit species, essential to
the successful analysis of connectivity. Contact: Dexiang Wang –
College of Oceanography and Environmental Science, Xiamen University,
Xiamen, Fujian, China 361005.

Awards
Carson et al. 2010 (Reproductive timing alters population
connectivity in marine metapopulations) was selected for special
mention by the Faculty of 1,000 as a 'must read' factor 8.

Keywords
mussel, dispersal, larvae, connectivity, invertebrate, transport,
invasive species, Mytilus, Musculista, aquaculture, marine reserve,
self seeding