RAPID IDENTIFICATION AND SORTING OF MARINE LARVAE

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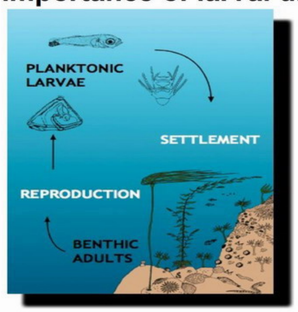
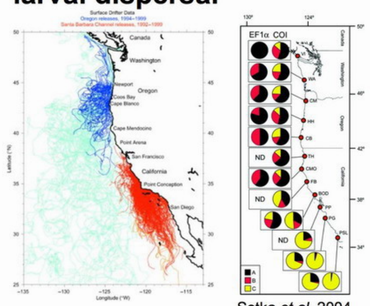
MOTIVATION: INSUFFICIENT METHODOLOGY LIMITS OUR UNDERSTANDING OF LARVAL DISPERSAL

The importance of larval dispersal



Drives population & community dynamics
(Gaines & Roughgarden1985)
Can establish:
-genetic population structure (Sotka *et al.* 2004)
-species' range edges (Gaylord & Gaines 2000)

Reliance on indirect methods to estimate larval dispersal



Sotka *et al.* 2004

Genetic estimates
-Pro: estimates realized dispersal
-Con: time averaged - misses stochastic events & temporal variability, and changes in population connectivity will take a long time to detect.

Manually sorting plankton samples is difficult & slow

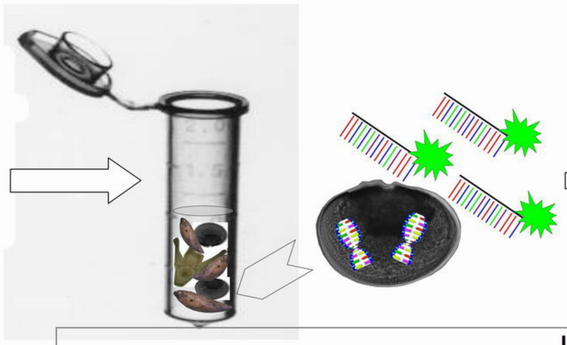
- ID to genus/species difficult or impossible (Levin 1990)
- Manual sorting can be prohibitively slow for high resolution or large scale sampling (Levin 1990, 2006; Palumbi 2004)

To directly sample larvae, need

Accurate identification
More efficient sample sorting

NOVEL METHOD: RAPIDLY ID & COUNT PLANKTON SAMPLES USING FLUORESCENCE *IN SITU* HYBRIDIZATION & A CELL SORTER

Sample larvae
Preserve in Modified Salt Ethanol Buffer
(Miller & Scholin 2000)



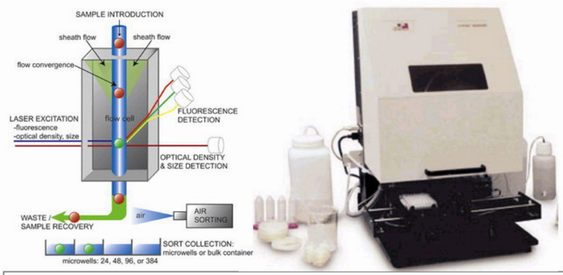
Label larvae using FISH

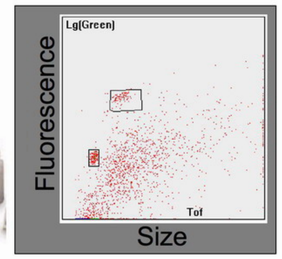
Concentrate plankton sample

Resuspend in salt buffer with fluorescently-labeled probe

Cook for 3 hrs

Glowing larvae





Count and Sort Larvae
Union Biometrica COPAS cell sorter
Flow cell size up to 1.2mm diameter
Sorts 200 objects/sec by fluorescence, size, and density
www.unionbio.com

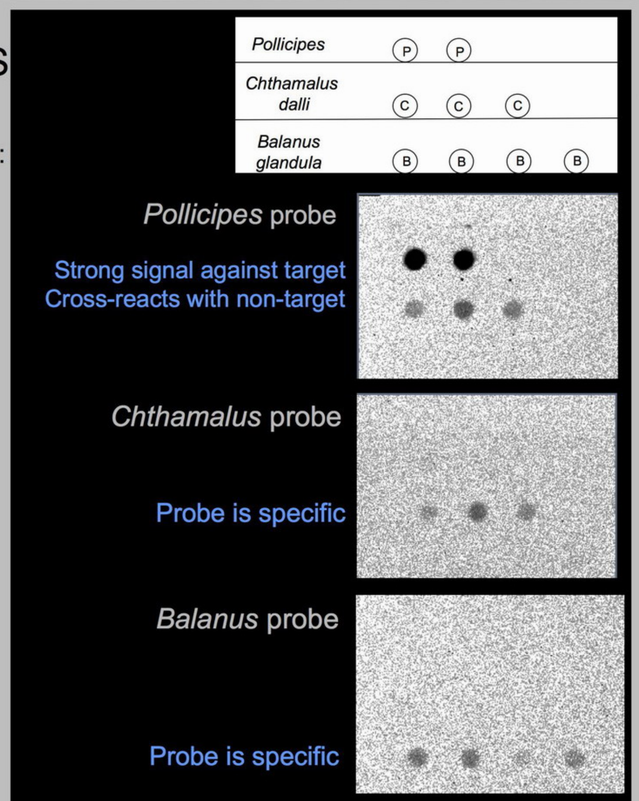
PROGRESS: PROBES DEVELOPED FOR MUSSELS & BARNACLES, SAMPLES SUCCESSFULLY ID'd & COUNTED ON CELL SORTER

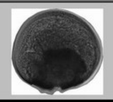
BARNACLES

Designed three barnacle probes:
1. *Pollicipes pollicipes*
2. *Chthamalus* sp.
3. *Balanus glandula/crenatus*

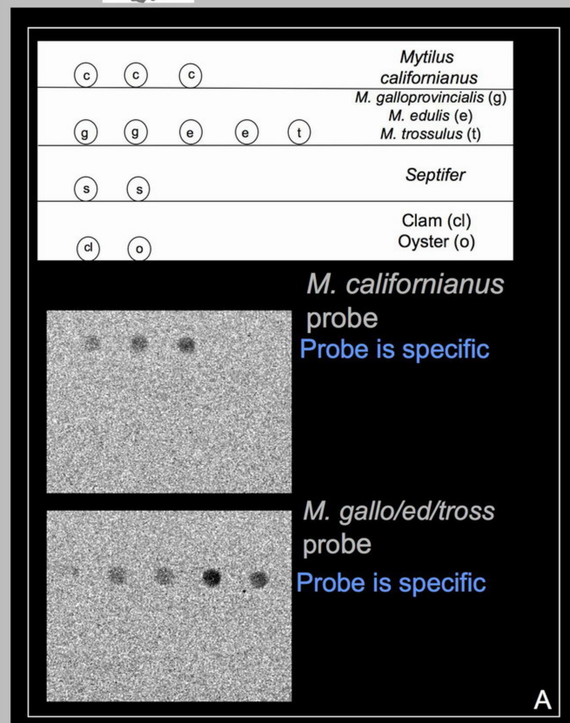
Probes designed against 18S RNA region with greatest differentiation between species & complete conservation between individuals within species.

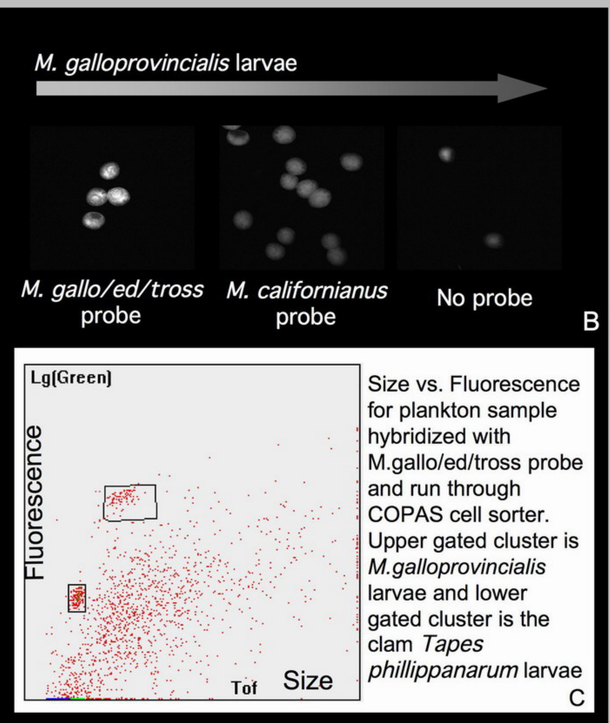
Tested probes with dot blots: DNA from adult tissue of all target species was spotted on nylon membranes and separate membranes with a full set of DNA each were hybridized with each probe.





MUSSELS





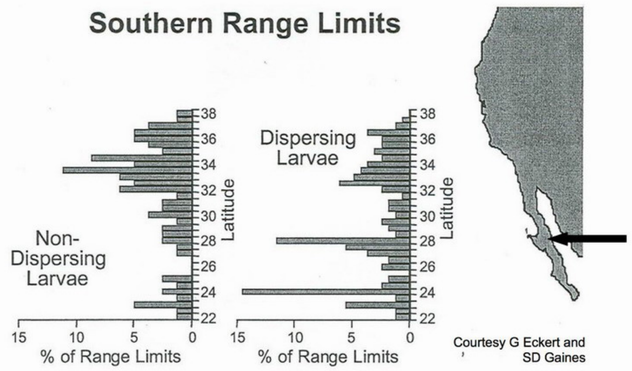
Size vs. Fluorescence for plankton sample hybridized with M.gallo/ed/tross probe and run through COPAS cell sorter. Upper gated cluster is *M.galloprovincialis* larvae and lower gated cluster is the clam *Tapes phillippanarum* larvae

Designed two mussel probes:
1. *Mytilus californianus*
2. *M. galloprovincialis/edulis/trossulus*

Tested probe specificity by dot blot, as with barnacles (A), and by hybridizing mussel larvae from pure cultures with multiple probes (B).

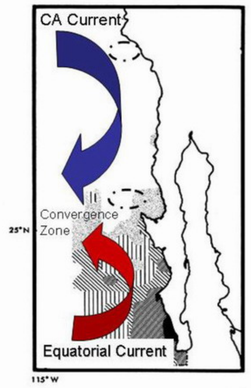
Tested sorting ability by supplementing an environmental plankton sample with *M. galloprovincialis* and clam larvae, hybridizing sample with M. gallo/ed/tross probe, and sorting with COPAS cell sorter (C).

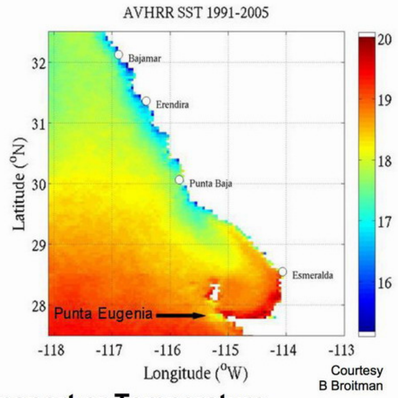
FUTURE DIRECTIONS: How does dispersal contribute to biogeographic patterns?



There is a concentration of species range edges at Punta Eugenia (PE), Baja California, Mexico (28°N). I plan to investigate the role that larval dispersal plays in establishing limits to species' distributions in this region.

Evaluate dispersal of Target Species around PE	
Mussels	S Range edge: *M. californianus* and *Septifer* N Range edge: *Modiolus*
Urchins	S Range edge: *Strongylocentrotus purpuratus* & *S. franciscanus* N Range edge: Black urchin
Barnacle	Spans Pta Eugenia: *Balanus glandula*





Transport or Temperature

Are species distributions limited by barriers to dispersal (Gaylord & Gaines 2000)?

Or by decreased survival in environmental conditions beyond adult distribution (Sanford *et al*. 2006)?

Approach

High spatial and temporal resolution plankton sampling around PE - evaluate potential of larvae to disperse beyond their adult distribution

References

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