



# Microbial Biogeography Along an Estuarine Salinity Gradient

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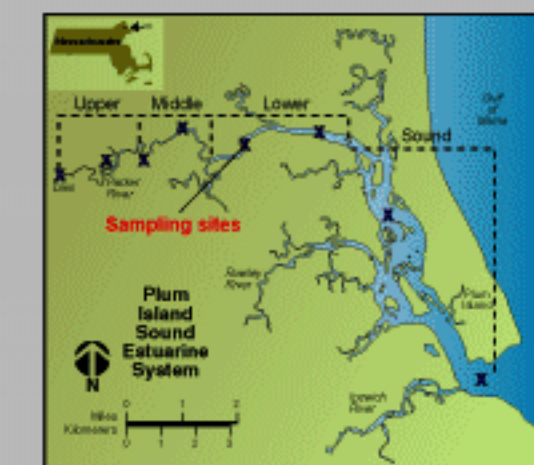
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## Abstract

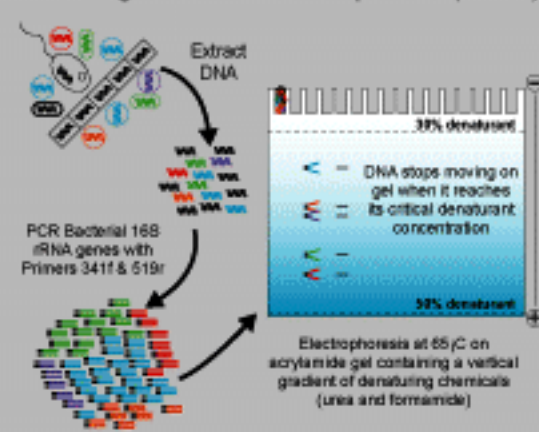
The growth and diversity of planktonic bacteria was investigated in the water column of the Parker River Estuary and Plum Island Sound, Massachusetts with samples collected along the salinity gradient from the freshwater Parker River to the coastal ocean. DGGE analyses of 16S rRNA genes showed persistent bacterial communities in the Parker River and in the coastal ocean during the summer and fall. In the estuary, these two communities mixed along the salinity gradient. An estuarine bacterial community developed at certain times of the year, but only in intermediate salinity waters. Sequencing of DGGE bands and environmental clone libraries indicated that these estuarine bacteria were related to the genera *Roseobacter*, *Thiothrix*, and *Cytophaga*, as well as to several uncultivated *Cytophaga*-like organisms. Associated biological measurements, which included bacterial production, and chlorophyll-*a* concentrations, coupled the development of the estuarine community to seasonal changes. The estuarine bacterial community only appeared in the summer and fall when bacterial community doubling time was short, estuarine residence time was long, and phytoplankton bloomed in the low salinity region of the estuary.

## Plum Island Long Term Ecological Research Site



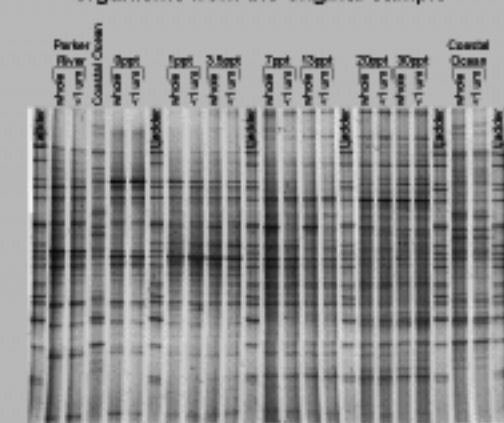
## Methods

### Denaturing Gradient Gel Electrophoresis (DGGE)

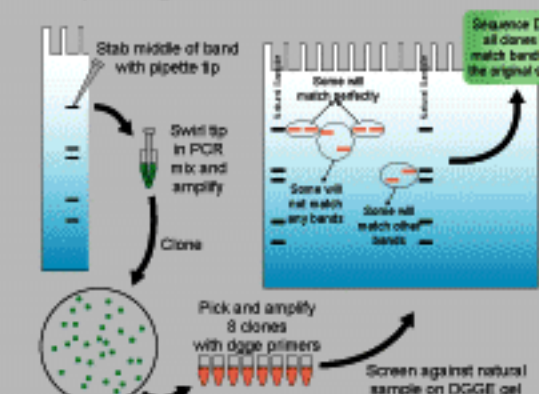


- Gel analysis procedure:
1. Identify and align bands
  2. Score bands as present or absent in each lane = binary dataset
  3. Create distance matrix (Dice)
  4. Multidimensional scaling cluster analysis

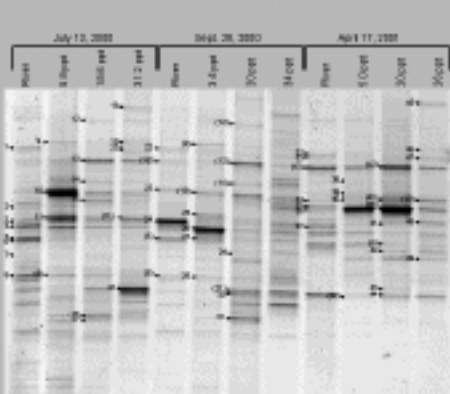
DGGE gel, September 28, 2000: Each band represents an organism or group of organisms from the original sample



### Sequencing DGGE Bands

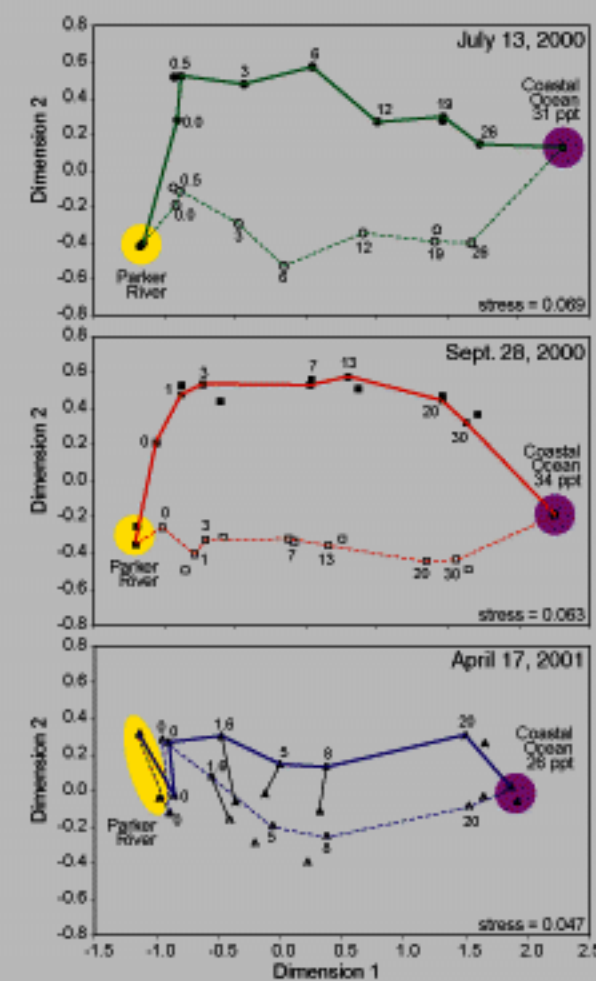


### DGGE bands sequenced



(Crump et al., 1999; Crump et al., 2003)

## Bacterioplankton Community Composition

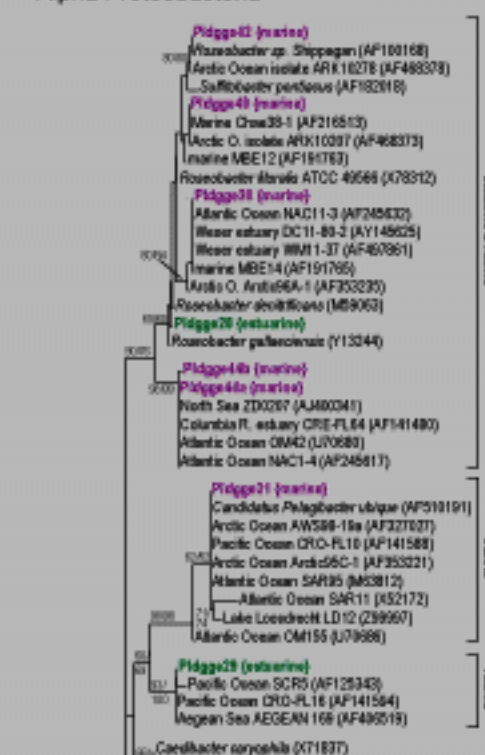


Multidimensional scaling diagrams (with stress values) of Dice distance matrices calculated from DGGE banding patterns. Salinity of samples is indicated.

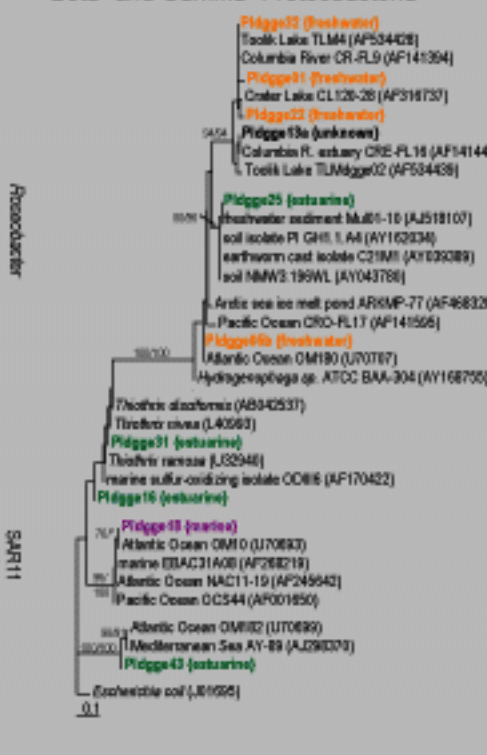
1. Solid symbols represent real DGGE banding patterns.
2. Open symbols represent artificial banding patterns from which estuarine bands were removed.
3. Symbols representing unfiltered water samples are connected with colored lines.
4. Symbols representing 1 micron screened samples are either the closest symbols to the unfiltered samples or are connected to the unfiltered samples with a line.

1. Marine and Freshwater bacterioplankton communities are very different.
2. Bacterioplankton communities mix along the salinity gradient.
3. Unique estuarine bacterioplankton community is evident in summer and fall, but not in spring.
4. Almost no difference between filtered and unfiltered banding patterns.

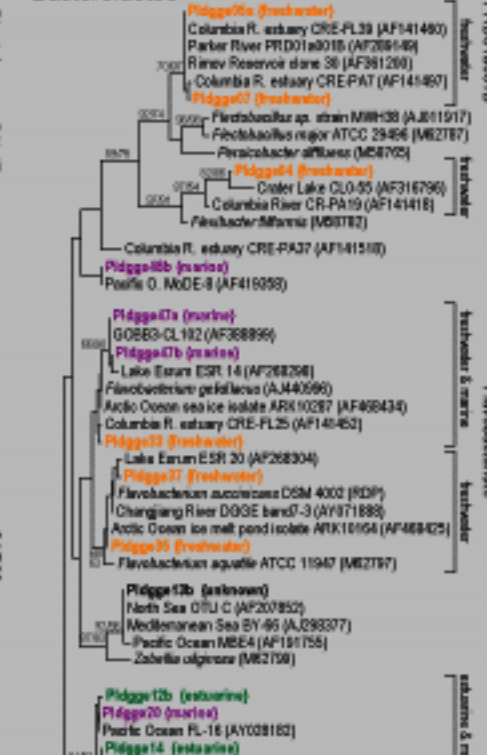
### Alpha Proteobacteria



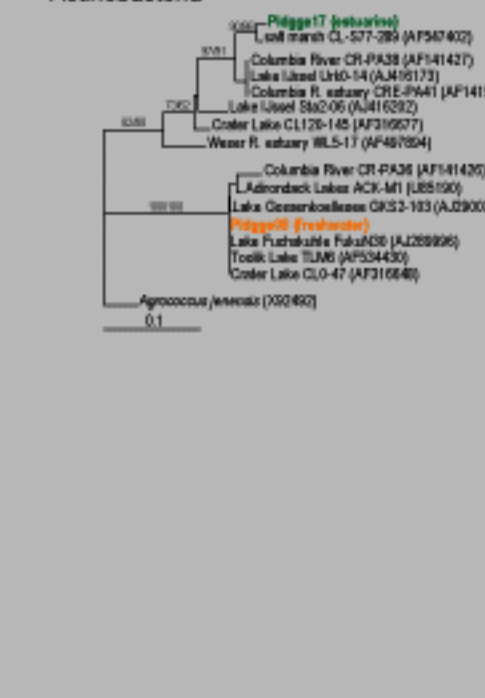
### Beta- and Gamma- Proteobacteria



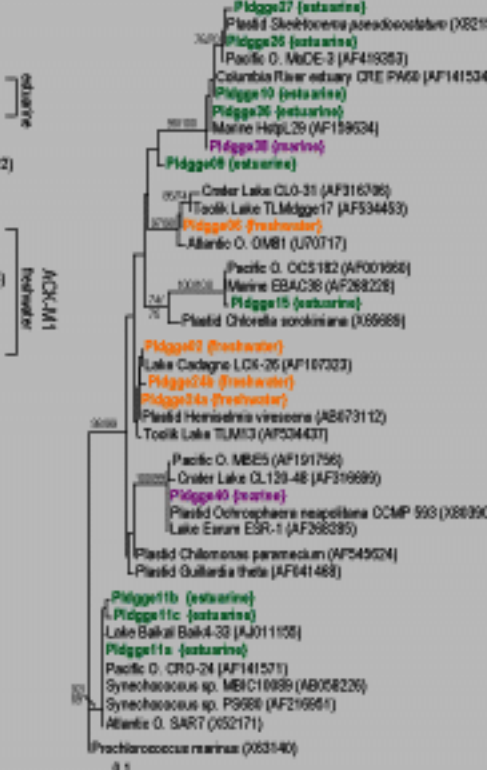
### Bacteroidetes



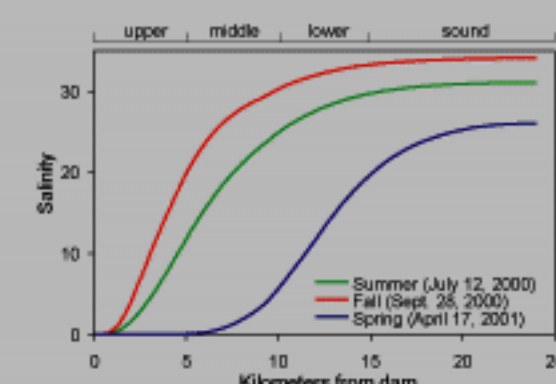
### Actinobacteria



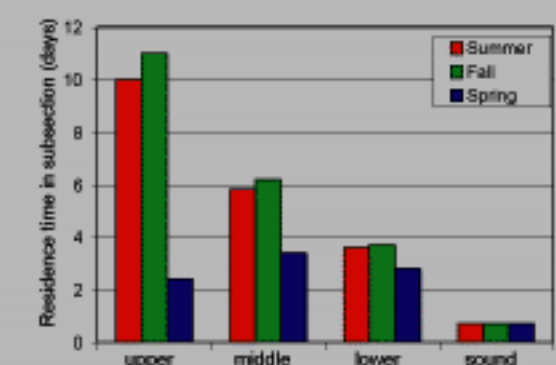
### Plastids



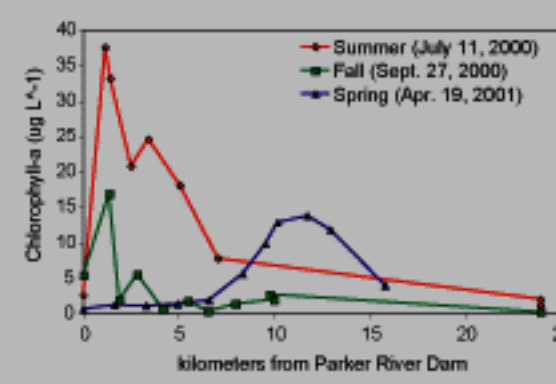
## Salinity and Residence time\*



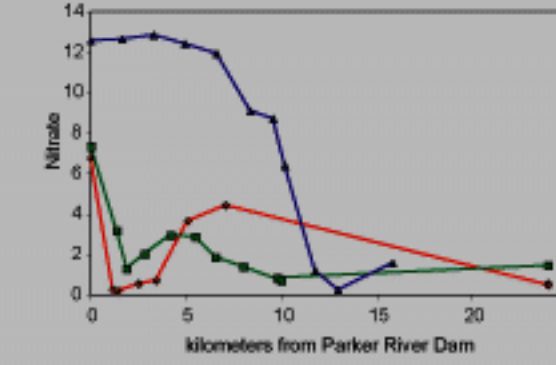
1. The majority of the salinity gradient is located in the upper and middle estuary in spring and summer
2. High river flow rate pushed the majority of the salinity gradient down to the lower estuary in spring
3. Residence time of water was longer than 10 days in the upper estuary in summer and fall, but was never greater than 4 days in the spring.



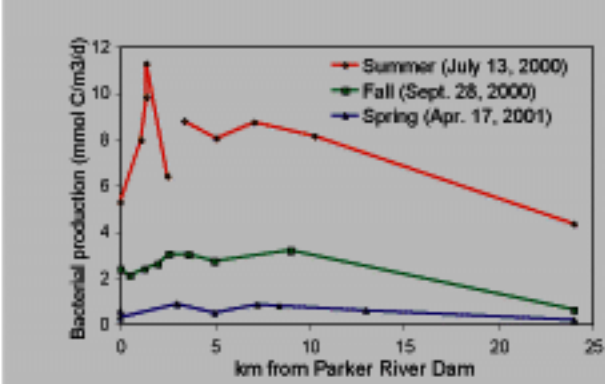
## Phytoplankton and Nitrate



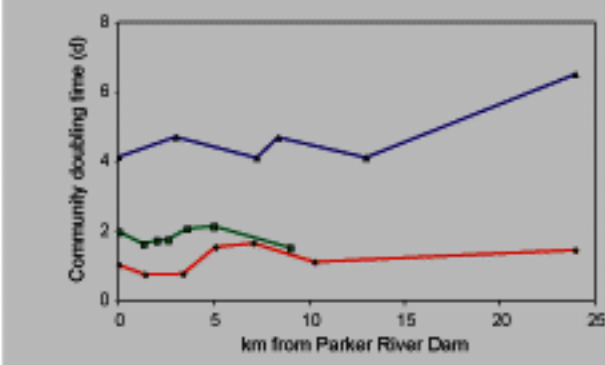
1. Seasonal phytoplankton blooms occurred in regions of longest residence time.
2. Nitrate concentrations were low where phytoplankton concentration was high
3. Nitrate concentration rises downstream of phytoplankton bloom in summer and fall probably because of inputs from marshes and nitrification



## Bacterial Production and Doubling Time



1. Bacterial Production was higher in the estuary than the river or coastal ocean
2. Production was even across the salinity gradient, except associated with the summer phytoplankton bloom
3. Bacterial community doubling time was much lower than residence time in summer and fall, but about the same as residence time in spring.



Bacterial carbon production (BP), calculated from leucine incorporation rate, used a ratio of cellular carbon to protein of 0.86, a fraction of leucine in protein of 0.073, and an intracellular leucine isotope dilution of 2 (Kirchman, 1993). Bacterial biomass (BB), estimated from cell concentration, assumed 25 fg carbon per cell. The equations  $\ln((BB+BP)/BB)$  estimated cell-specific exponential growth, and  $DT=\ln 2/u$  calculated doubling time (Barbosa et al., 2001)

## References

Barbosa, A. B., H. M. Galvao, P. A. Mendes, X. A. Alvarez-Salgado, F. G. Figueiras, and I. Joint. 2001. Short-term variability of heterotrophic bacterioplankton during upwelling off the NW Iberian margin. *Progress in Oceanography* 51:339-359.

Crump, B. C., E. V. Arntson, and J. A. Barros. 1999. Phylogenetic analysis of particle-associated and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. *Applied and Environmental Microbiology* 65:3192-3204.

Crump, B. C., O. W. Kling, M. Behr, and J. E. Hobbie. 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69:2253-2268.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria, p. 513-517. In P. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton.

Vallino, J. J., and C. S. Hopkins. 1998. Estimation of dispersion and characteristic mixing times in Plum Island Sound estuary. *Estuarine Coastal and Shelf Science* 46:333-350.

## Conclusions

- Estuarine bacterioplankton is composed of marine, estuarine, and freshwater organisms
- Residence time and bacterial doubling time influence the development of a unique estuarine community
- Estuarine community develops in association with phytoplankton blooms and/or with inputs of marsh-derived organic matter
- Estuarine bacterial community appears to be persistent throughout the summer

\* Calculated from a one-dimensional, intertidal, advection-dispersion hydrodynamic model developed for the Plum Island Sound (Vallino and Hopkins, 1998)