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FACTORS AFFECTING BACTERIAL BIOMASS AND GROWTH IN THE DUPLIN RIVER ESTUARY AND COASTAL ATLANTIC OCEAN

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ABSTRACT - Bacterial numbers and growth rates were measured in coastal, planktonic systems of the western Atlantic ocean. Seasonal responses in growth rates of the bacterial assemblage were present at some sites, but these may be related to seasonal changes in substrate supply rather than to changes in temperature. Bacterial growth rates tended to decline with increasing distance from shore. There was evidence for predator-prey population cycles in the bacteria and $<17\mu\text{m}$ protozoa in the Duplin River estuary which may indicate that the bacterial assemblage serves as prey for protozoan populations. In summer, a significant negative correlation between μ (instantaneous growth rate) and cell numbers in the bacterial assemblage indicated that substrate limitation of the bacteria may sometimes occur.

Key words : bacteria, growth rate, thymidine, grazing, protozoan

RÉSUMÉ - Les nombres de bactéries et les taux de croissance ont été mesurés dans des systèmes planctoniques sur la côte ouest de l'Atlantique. Des réponses en fonction de la saison dans le taux de croissance des amas bactériens sont observées sur certains sites mais elles sont plus reliées aux variations saisonnières des apports nutritifs qu'aux variations de la température. Les taux de croissance bactériens ont tendance à décroître avec l'éloignement par rapport à la côte. Des cycles prédateurs-proies sont mis en évidence entre les bactéries et les protozoaires $<17\mu\text{m}$ dans l'estuaire de la rivière Duplin, ceci peut indiquer que les amas bactériens servent de proie pour les populations de protozoaires. En été une corrélation négative significative, entre μ (taux de croissance instantané) et le nombre de cellules dans les amas bactériens indique que le substrat peut parfois être un facteur limitant pour les bactéries.

Mots clés : bactéries, taux de croissance, thymidine, broutage, protozoaires.

INTRODUCTION

We have measured bacterial biomass and growth in a variety of marine environments around Sapelo Island, Georgia, USA. In addition, by evaluating predator populations and physico-chemical factors in relation to the bacterial data, we can try to better understand what major controlling factors are influencing the bacterial populations. Generally, of course, one might predict that physico-chemical factors will dominate in temperate habitats. But, as microbial ecologists, we are interested in the specifics. What is the relative importance of these controlling factors? How rapidly do the microbial populations respond to their influences? Some of our progress to date in addressing these questions is described below.

METHODS

Nearshore ocean sampling was done on four quarterly cruises in 1981 and 1982. Stations were located at 0.25, 3, 6, 9, and 15 km offshore. The Duplin River sampling was concentrated over two intensive three week sampling periods in late summer 1983 and

mid-winter 1984. One station near the mouth and one station 11 km up the estuary were sampled. Detailed physical descriptions of these coastal areas may be found in Imberger *et al.* (1983), and Oertel and Dunstan (1981). Bacterial biomass was estimated by acridine orange direct counts (AODC) and microscopic cell sizing (Hobbie *et al.* 1977; Newell and Fallon, 1982). Bacterial growth rates were estimated using the ³H-thymidine incorporation technique (Fuhrman and Azam, 1980, 1982; Moriarty and Pollard, 1981; Newell and Fallon, 1982). Changes in cell numbers were also used to estimate growth rates in calibration experiments used to establish the factor for cells produced per mole of thymidine incorporated. Tritiated thymidine (New England Nuclear, 50 Ci/mmole) was added to a final concentration of 20 nmolar. Protozoan biomass was estimated by the direct count epifluorescence method of Sherr and Sherr (1983).

RESULTS AND DISCUSSION

Instantaneous growth rates of the nearshore planktonic bacteria based on tritiated thymidine incorporation, μ (thy), and temperature showed a moderately strong correlation (Fig. 1). With all four cruises included, $r^2 = 0.329$, showing that temperature (or perhaps an unknown covarying factor) accounted for about 1/3 of the variation in μ (thy) for all nearshore data. One may also note the tendency for growth rates to decrease with increasing distance from shore. Also of interest is the difference in seasonal response for station 1 and station 5. Station 1 shows a strong seasonal response, whereas station 5 shows little or no seasonal response. Stations between 1 and 5 show an intermediate seasonal response.

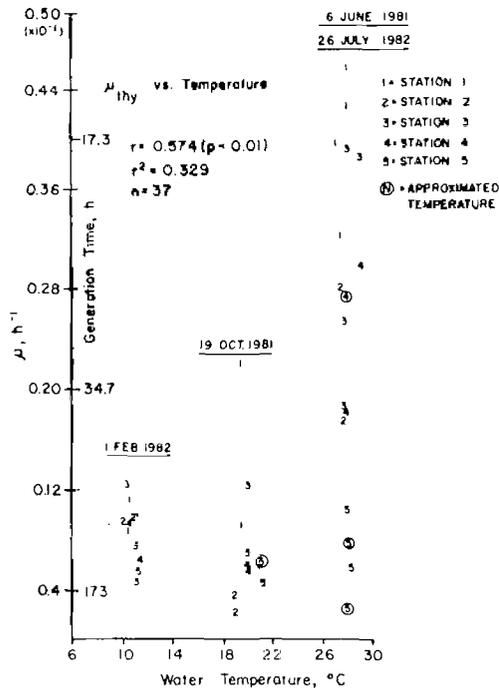


Figure 1 : Temperature vs. μ (thy) for nearshore ocean studies. Stations 1, 2, 3, 4, & 5 are 0.25, 3, 6, 9, & 15 km is offshore, respectively. A logarithmic growth model is assumed for μ values throughout this work.

The cause for this difference is unclear, but our current hypothesis is that perhaps it is not temperature but some underlying factor correlated with temperature that is responsible for this seasonal response. For example, reducing sugar-release from *Spartina* peaks in the warmer months (Pakulski, University of Georgia, Institute of Ecology, pers. comm.) Also, phytoplankton production peaks in mid to late August (Thomas, 1966). Input of bacterial substrates could thus yield a growth response which is positively correlated with temperature, but not necessarily directly dependent on temperature. One would expect this response to be dampened as we move farther offshore, since both the marsh-estuary influence and phytoplankton production decline with distance from shore (Thomas, 1966, 1970). Growth rates in Duplin River populations appeared to show no significant correlation with temperature (Fig. 2). Growth rates were generally higher than in the nearshore ocean and rather similar during both the summer and winter sampling periods. Although winter growth rates tended to be lower than summer growth rates at the lower station, they were not significantly lower.

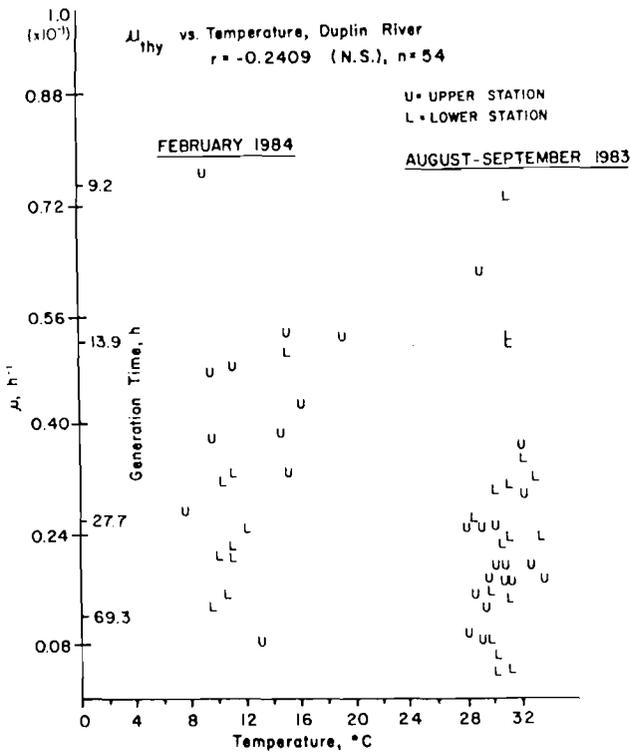


Figure 2 - Temperature vs. μ (thy) for Duplin River stations.

Thus, seasonal factors appeared to be of only moderate importance, but what of short term biological factors? Is there any evidence for classical population dynamics in these bacterial assemblages? Fenchel (1982) has described classical, out-of-phase predator-prey cycles with periods of 16 days in flagellate and bacterial populations in Limfjord, Denmark. Fuhrman *et al.* (1983) have reported that bacterial grazers annually consume a major fraction of bacterial production in nearshore waters of Long Island Sound. Our data also indicate that the bacterial assemblages in the Georgia estuaries may often be under intense grazing pressure. Indications for periodic changes in biomass in the

bacterial and protozoan populations are especially strong in upper station data (Fig. 3). Summer data show out-of-phase cycles with a period of about 8-10 days. Although a similar period is evident in the winter data, the cycles appear to be almost in phase. Examining the lower station, we see once again that summer data show more evidence for periodicity than do the winter data, but in both cases the amplitude is smaller than in upper station data for the corresponding season.

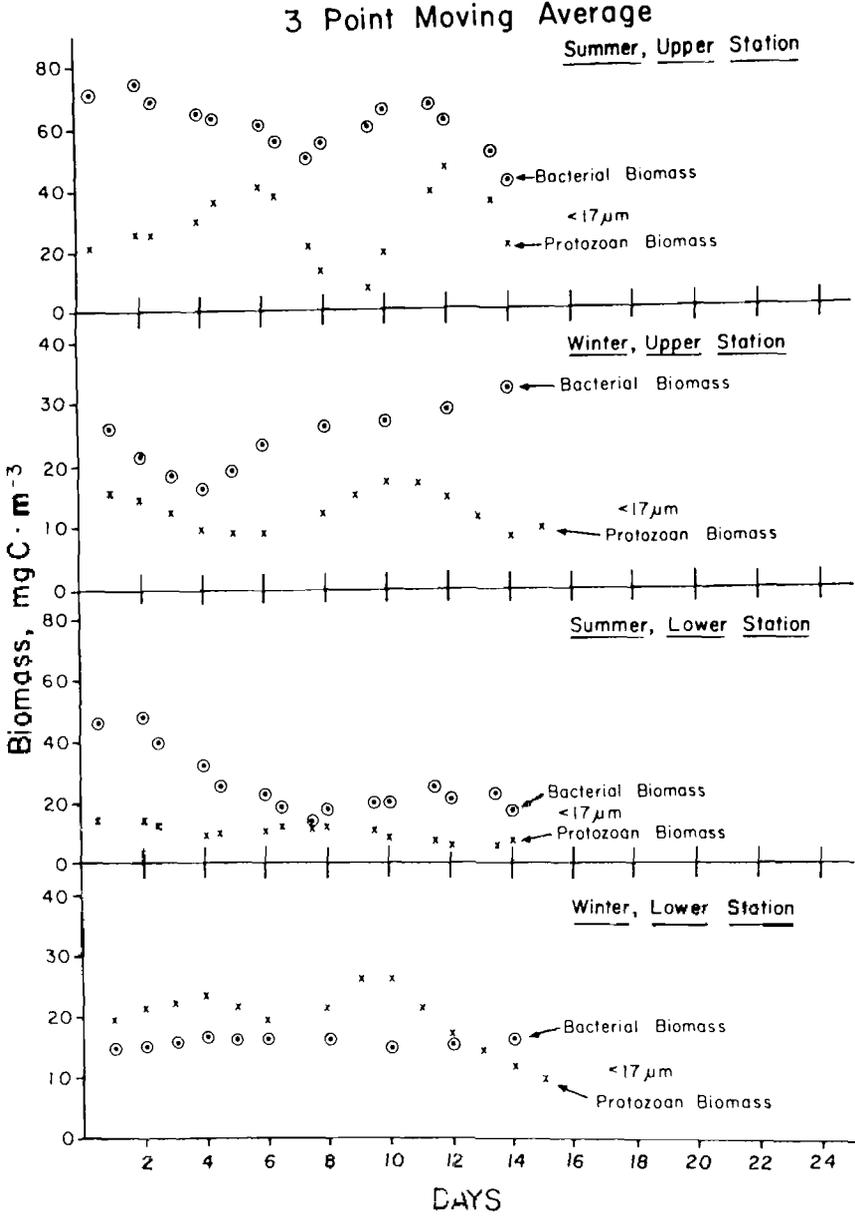


Figure 3 : Three-point moving average plot of bacterial and protozoan biomass for the Duplin River stations. Data have been smoothed using a three-point moving average technique (Arkin and Colton, 1970).

With this evidence in mind we have also tested the relationship between μ (thy) and numbers of bacteria. Except for the one summer thunderstorm data point, there was generally a negative correlation between total numbers and μ (thy) (Fig. 4). Summer data show this very strongly and are responsible for the negative relationship seen when all data are compared. Without the thunderstorm point, $r = -0.455$, indicating a highly significant negative correlation. Although weaker with the thunderstorm point included, the relationship is still significant. If we then examine the individual data sets, winter sets show no significant relationships. In contrast, without the thunderstorm data point, both summer sets show significant negative correlations.

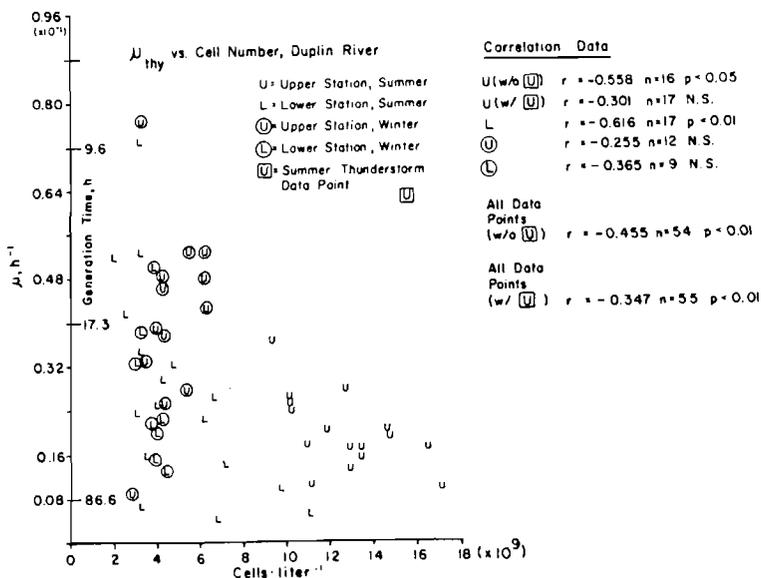


Figure 4 : Bacterial numbers vs. μ (thy) for the Duplin River stations. Various correlation data are shown at right.

Our interpretation of these data is as follows. In the summer, when top-level consumers such as fish and invertebrate larvae are present (Daiber, 1982), bacteria may sometimes escape from protozoan predation, because protozoa, the bacterial predators, are being eaten by higher level consumers. It may sometimes then be possible for bacterial populations to build up to a level where substrate becomes limiting. Thus, high numbers can mean slow generation time. In the winter, when these higher consumers are absent, protozoan numbers are never reduced to levels low enough to allow bacteria to escape predation. Minimum biomass of protozoa was lowest in the summer at both stations (Fig. 3), supporting the hypothesis of possibly less consistent protozoan grazing pressure in the summer. This also leads to an explanation of the summer thunderstorm point. Storms have been shown by Chalmers *et al.* (in press) to be very important in the overall DOC (dissolved organic carbon) balance in the marsh-estuarine system. Thunderstorms at low tide, as this one was, wash large amounts of DOC into the Duplin River. Thus, high numbers and high growth rates were possible in the bacterial assemblage because of substrate inputs from the thunderstorm. Comparisons of Figs 3 & 4 on Day 13 (date of the thunderstorm). Summer, Upper Station also support our argument for bacteria being under strong grazing pressure, although the bacteria were growing at a rapid rate (Fig. 4),

the overall population was in a decline phase (Fig. 3). One possible cause for such observations would be harvesting by the expanding protozoan assemblage (Fig. 3) which kept the bacterial assemblage in check. In general, considering the relatively rapid growth of the bacterial populations, some persistent loss mechanism (grazing?) must be present in the Duplin, since bacterial biomass showed no long term increase over the two sampling periods.

A negative correlation between numbers and growth rate in the bacterial population may not always be the case. Nearshore data show a significant positive correlation between these two variables (Fig. 5). Our data on protozoan numbers are, unfortunately, not sufficient to analyse protozoan-bacterial relationships in the nearshore ocean. Perhaps here bacteria never escape protozoan predation (we note that bacterial numbers were never as high as in the upper Duplin), and the whole system is more in phase, with predators and prey populations increasing and decreasing as nutrient pulses move through the system.

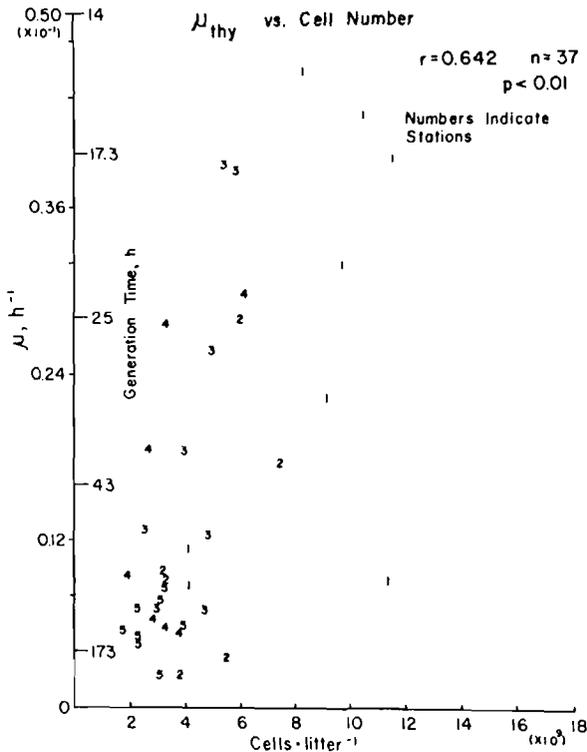


Figure 5 : Bacterial numbers vs. μ (thy) for the nearshore stations.

Finally, Table 1 gives some idea of the importance of bacterial production in relation to gross primary production in this coastal region. One may note that, although volumetric production showed a strong decrease with distance from shore, areal production was rather similar across the whole region. In all cases the bacterial production is equal to a few percent of the estimated phytoplankton production, with the fraction tending to increase as one moves from offshore into the marsh. The bacterial production values are,

	DEPTH, m	PLANKTONIC BACTERIAL PRODUCTION, $\text{mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$	PHYTOPLANKTON PRODUCTION, $\text{mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (% DOC)	BACTERIAL AS % OF PHYTOPLANKTON
STATION 5	15.8	43 ^a	1500 (2) ^b	2.9
STATION 1	2.4	116 ^a	3000 (4) ^b	3.9
LOWER STATION	5	60 ^a	990 (N.D.) ^c	6.1
UPPER STATION	1.5	45 ^a	650 (N.D.) ^c	6.9

N.D. = no data

a - based on $100 \text{ mg C} \cdot \text{cm}^{-3}$ cell volume
(Fallon et al 1983)

b - from Thomas (1966,1970)

c - from Pomeroy & Wiegert
(1981); E. Sherr, pers. comm.

Table 1 : Comparison of bacterial and phytoplankton carbon production in the nearshore ocean and Duplin River estuary.

however, of the same order of magnitude as the estimated DOC output from phytoplankton (Thomas, 1970). Overall, in comparison with other studies of bacterial production based on thymidine incorporation, bacterial production in Georgia coastal planktonic systems appears to be quite small in relation to primary production. Our data fall at the bottom end of the wide range of estimates reported from the literature (bacterial production as cell C = 0.6 — 42 % of primary production) (Bell *et al.*, 1983; Fuhrman and Azam, 1982; Ducklow and Kirchman, 1983; Moriarty, in press; Moriarty and Pollard, 1982; Riemann *et al.*, 1982). Also, one must keep in mind that from the estuary out to about 10 km offshore, the planktonic community is receiving some DOC input from the marshes. Some of this too may be available for bacterial production. Thus, relative to other carbon fluxes in the planktonic system, planktonic bacterial production appears to be rather low. No clear explanation for this observation is available. However, one possible hypothesis is that in this shallow, well mixed system, much of the heterotrophic processing occurs in the benthos. Data on sedimentary bacterial production and nutrient regeneration support this hypothesis (Newell and Fallon, 1982; Fallon *et al.*, 1983); but more data are needed in order to fully explain these relationships.

		GROWTH RATE, h^{-1}	GENERATION TIME, h
<u>NEARSHORE OCEAN</u>	MEAN	0.016	43
	RANGE	0.0021 — 0.0458	330 — 15.1
<u>DUPLIN RIVER</u>	MEAN	0.028	24
	RANGE	0.004 — 0.076	173 — 9.1

Table 2 : Mean and ranges for planktonic bacterial growth rates and generation times across the coastal region.

In summary, bacterial growth rates sometimes showed little seasonal response in Georgia coastal planktonic systems. Although there was a strong seasonal response in the nearshore stations within 6 km of Sapelo Island, such a response was not evident at other sampling sites. The absence of a strong, consistent seasonal effect on growth implies that the populations can adapt to the seasonal temperature changes. With 1/2 the population being replaced every 1-2 days on average (Table 2), we would expect rapid physiological adjustments in the bacterial populations. Also, the limited annual temperature range, 7-30°C, minimizes the cost of adaptation. Bacterial growth rates are inversely related to distance from shore, probably due to decreasing substrate availability with increasing distance from shore. Data from the Duplin River studies indicate that some bacterial populations may undergo classical population fluctuations because of predator and/or substrate limitations. Planktonic bacterial production in these systems is equivalent to only a small percentage of ¹⁴CO₂ based estimates of phytoplankton production. Further data on the substrate inputs that actually drive the system are needed in order to better understand the behavior of the bacterial populations in these habitats.

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