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Carbon Production and Growth Physiology of Heterotrophic Bacteria in a Subtropical Coral Reef Ecosystem

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Carbon Production and Growth Physiology of Heterotrophic Bacteria in a Subtropical Coral Reef Ecosystem

A Thesis Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

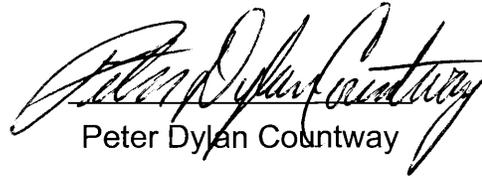
by
Peter Dylan Countway

1999

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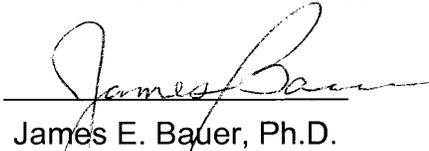
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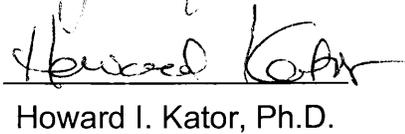
Master of Science


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I dedicate this project and my masters degree to my parents, David and Sylvia Countway; to my sister Melissa, her husband Thad and their son Zander; to my grandfathers, Russell Countway and Edward Hammond; to the memory of my grandmothers, Ruth Countway and Marian Hammond; to my second family, Tommy and Anne Hoopes and their son Josh; to my mother and father in-law, Jon and Janice Little; to my sister in-law Susie and her husband Scott and especially to my wife and best friend Becky. Some of the happiest times of my life have been spent on, in or under the water with all of you.

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ABSTRACT

Heterotrophic bacterial growth and removal was measured in the coral reef ecosystem of Bermuda, located in the western Sargasso Sea. The goal of this project was to examine the effect of the coral reef community on bacterial organic matter cycling. Samples were collected from the reef water-column, the coral surface-microlayer and the reef surface-microlayer. Bacterial abundance ranged from $3.6 - 12.8 \times 10^8$ cells \cdot l⁻¹. Bacteria were most abundant at locations with a high concentration of detrital particles in the water-column and from the reef surface micro-layer habitat. Compared to the water-column, bacterial abundance in the coral surface-microlayer appeared to be slightly depleted. Bacterial biomass ranged from $5.7 - 20.3$ μ g C \cdot l⁻¹ across habitats despite a uniform cell-specific biovolume (0.053 μ m³ \cdot cell⁻¹).

Site-specific conversion factors were empirically determined to calibrate rates of bacterial ³H-Thymidine (TdR) and ³H-Leucine (Leu) incorporation to cell production. Conversion factors averaged $5.03 (\pm 2.46, \text{SD}) \times 10^{17}$ cells \cdot Mol TdR⁻¹ and $0.86 (\pm 0.33, \text{SD}) \times 10^{17}$ cells \cdot Mol Leu⁻¹. Carbon production based on TdR ranged from $0.2 - 29.3$ μ g C \cdot l⁻¹ \cdot d⁻¹, while Leu based estimates ranged from $0.3 - 45.1$ μ g C \cdot l⁻¹ \cdot d⁻¹. Bacterial growth efficiency ranged from 10-30% based on bacterial consumption of ambient DOC. Bacterial growth rates and bacteriovore grazing rates were estimated to be $0.37 (\pm 0.05, \text{SD}) \cdot$ d⁻¹ and $0.30 (\pm 0.06, \text{SD}) \cdot$ d⁻¹ respectively, which suggests that bacterial production is nearly balanced by protozoan grazing. Cell biomass turnover times were 2-3 days for reef bacteria, which is four to five times faster than oceanic bacteria at the Bermuda Atlantic Time-series Station.

Cell-specific TdR incorporation rates increased with increasing TdR concentration, while cell-specific Leu incorporation rates demonstrated little response to increasing Leu concentration except when detrital particles were abundant. Trends in cell-specific saturation curves may indicate the small-scale heterogeneity of bacterial communities on coral reefs. Rates of TdR and Leu incorporation for a given range of substrate concentrations may also serve as an index of the relative importance of attached bacteria at a particular site.

When a natural reef-water bacterial assemblage was enclosed in a bottle with a coral specimen for 24 hours, bacterial production rates increased by 1800% over the initial rate compared to 400% in a control bottle. Bacteria from the reef surface-microlayer, over a 6 m depth profile, displayed a 300% increase in absolute rates of TdR and Leu incorporation as depth increased and a 300-1000% enhancement over rates from the coral surface-microlayer. Particle attachment and settlement of pre-colonized detrital particles onto the reef surface-microlayer may provide a mechanism by which heterotrophic bacteria are able to achieve high rates of carbon production (~ 20 μ g C \cdot l⁻¹ \cdot d⁻¹), while maintaining their position in close proximity to DOM releasing reef organisms.

**Bacterial Carbon Production and Growth Physiology
in a Subtropical Coral Reef Ecosystem**

INTRODUCTION

The spatial transition from oligotrophic seawater to highly productive coral reefs has intrigued scientists since the middle of the 19th century. Darwin (1842) was among the first to conclude that the growth rate of coral reefs was quite high, which countered the prevalent thinking of the time. As the body of coral reef literature developed, a mechanism was needed to explain how reef systems were able to achieve high rates of secondary production given the relative nutrient depletion of their surrounding waters compared to other productive coastal ecosystems. The theory invoked to explain this apparent paradox has generally been highly efficient nutrient and organic matter cycling within the reef community. Although gross primary productivity on the reef may be high, net productivity is typically much lower (Odum and Odum, 1955; D'Elia and Wiebe, 1990). The absence of large inputs of allochthonous nutrients to coral reef ecosystems prevents high net rates of benthic algae primary productivity, which would smother corals in comparatively more eutrophic reef waters (Hunter and Evans, 1995).

Marine microbial ecologists have been interested in the role of bacteria in nutrient and organic matter (OM) cycling within coral reef ecosystems since the 1950's when Odum and Odum (1955) first categorized the trophic status of various living compartments on reefs at Eniwetok. Heterotrophic bacterial processes have been shown to contribute significantly to the recovery of dissolved organic matter (DOM) and dissolved inorganic nutrients (DIN) lost from

coral reef communities (DiSalvo and Gundersen, 1971; Sorokin, 1973; Linley and Koop, 1986; Sorokin 1994). Bacterial communities were discovered to be thriving in the mucopolysaccharide (MP) layer on the surface of living corals (Ducklow and Mitchell, 1979; Rublee et al., 1980; Paul et al., 1986), where they use the MP as their organic matter growth substrate (Ducklow and Mitchell, 1979; Hoppe et al., 1988; Vacelet and Thomassin, 1991; Ritchie and Smith, 1995). In the water-column of coral reefs, free-living bacterioplankton and bacteria attached to suspended particles represent a significant source of organic carbon (Moriarty, 1979; Linley and Koop, 1986; Torr ton and Dufour, 1996a) and nitrogen, given the low C:N ratio of bacteria.

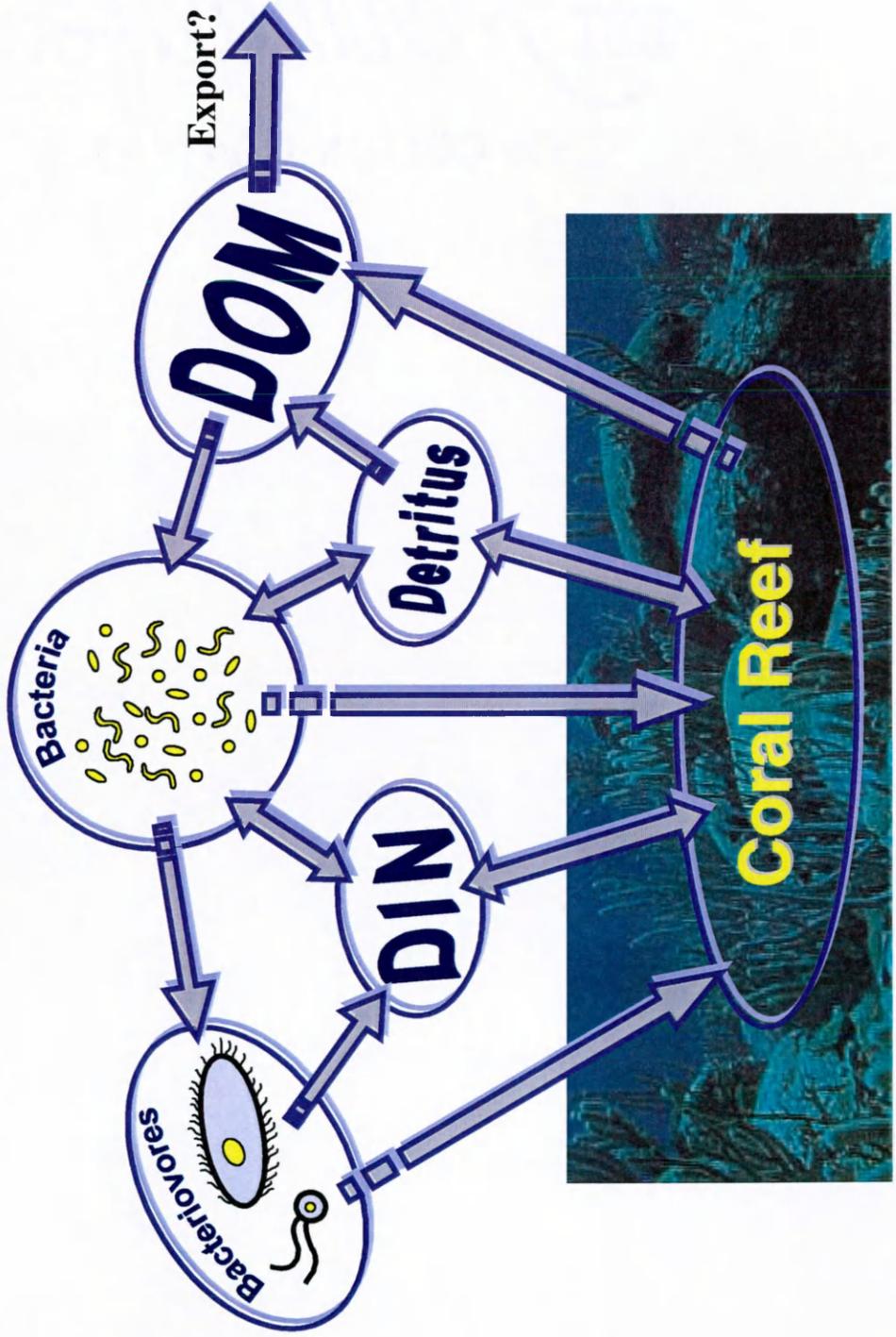
Marine bacteria may serve as a link between DOM and higher trophic levels via the microbial loop (Azam et al., 1983) or they may tend to be more of a net sink for this material (Pomeroy, 1974; Williams, 1981; Ducklow et al., 1986). A third possibility, short-circuiting the microbial loop, suggests that bacteria may be directly ingested by reef corals (Sorokin, 1978; Paul et al., 1986, Ducklow, 1990). Whether the link or sink route is dominant in a particular environment may be due in part to the relative C:N ratio of bacterial growth substrate with bacteria remineralizing nitrogen (sink) at low C:N and protozoan bacteriovores dominating nitrogen remineralization (link) at high C:N (Caron et al., 1988). Total microbial ammonium regeneration (sum of bacterial and heterotrophic nano-plankton) in the water-column above a coral reef is reportedly low ($1-11 \text{ nMol N} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) with highest rates occurring where bacteria were most closely associated with the reef surface (Hopkinson et al., 1987). Colloidal organic matter from reef-

waters has been demonstrated to be of similar amino acid composition to coral mucus exudate (Means and Sigleo, 1986). Compared to the Redfield ratio (6.6), coral mucus with relatively low C:N (5.6 - 5.9) has been detected in corals from Nouméa (Vacelet et al., 1991), though these estimates define the lower range of mucus C:N (5.6 -8.9) among literature values surveyed by the previous authors. Determining the trophic relationships among compartments of the microbial loop on a coral reef (**Fig. 1**) is likely to be considerably more complicated than what may be predicted by relatively simple C:N ratios of bacterial growth substrates.

Considering the central role of heterotrophic bacterial processes to the cycling of organic matter and nutrients within the reef environment, relatively few studies have measured bacterial growth and removal terms simultaneously. One objective of this project was to provide a detailed study of the magnitude and variability of bacterial carbon production and loss due to microbial grazers in the Bermudian reef ecosystem. The ultimate goal was to describe the relative contribution of the microbial population to organic matter cycling in the coral reef ecosystem of Bermuda. I hypothesized that bacterial production and biomass would be higher in habitats closer to the reef surface and at sites closer to the Island, where bacteria were closer to both natural and anthropogenic sources of DOM. A corollary to this hypothesis is that bottom up (nutrient supply) processes should regulate bacterial populations at sites with more oligotrophic water conditions, while populations at relatively more eutrophic sites should be controlled by top-down (grazing) processes (Billen et al.,1990). To help explain some of the inter-site and inter-habitat variability in bacterial production, I

Figure 1. A simplified model of the microbial loop for a coral reef ecosystem. Arrows represent direction of flux among various compartments. The dissolved inorganic nutrient (DIN) pool includes inorganic nitrogen and phosphorous compounds, while the dissolved organic matter (DOM) pool includes dissolved organic carbon, nitrogen and phosphorous.

Simplified Microbial Loop on a Coral Reef



hypothesized that different populations of bacteria, with variable growth physiologies were dominant at different locations.

WHY THIS SITE IS IMPORTANT

Despite the abundance of oceanographic data collected from the Sargasso Sea over the past 45 years, a comparatively small amount of water-column data exists for the reef platform comprising the Bermuda Islands. Darwin (1842) noted, from a description of Bermudian reef geology by Nelson (1837), that Bermuda had the distinction of being the farthest point from the equator capable of supporting coral reefs, made possible by the warm waters of the Gulf Stream. Bermuda's central location in the mid-Atlantic gyre has more recently provided a base for the development of a sophisticated research program (Michaels et al., 1994; Michaels and Knap, 1996) on the biogeochemical processes of the surrounding oligotrophic ocean. These references detail the history and nature of oceanographic work initiated offshore of Bermuda at Station "S" in 1954 by Henry Stommel, and at the Bermuda Atlantic Time-series Station (BATS) in 1988.

With the exception of work conducted as part of the Bermuda Inshore Waters Investigation (Barnes and Bodungen, 1978) very little information exists on whether and/or how the chemical and biological water-column properties of the Island platform differ from those in the Sargasso Sea. In general, there is a paucity of data relating how planktonic organisms in oligotrophic seawater react to transport into relatively nutrient replete, shallow coastal systems. This is

especially true for heterotrophic bacterial populations which may be comprised of different dominant groups, or have completely different growth physiology across scales of centimeters (Mitchell and Fuhrman, 1989) to kilometers (Lee and Fuhrman, 1991; Yoshinaga et al., 1991).

Natural assemblages of heterotrophic bacteria from oligotrophic systems are capable of responding to rather small experimental amendments of nutrients and organic matter (Carlson and Ducklow, 1996). This result supports the idea that oligotrophy is only a temporary condition for at least some fraction of the "oligotrophic" bacterial population (Schut et al., 1997). Therefore, measuring bacterial biomass and production in the Bermudian coastal environment contributes not only to the understanding of coral reef microbial ecology, but also suggests the magnitude by which the reef environment modifies the physiology (and perhaps composition) of oligotrophic Sargasso Sea bacteria. Fuhrman et al. (1989) calculated that heterotrophic bacteria comprise greater than 70% of the microbial carbon biomass in the Sargasso Sea off of Bermuda. Therefore, when a parcel of Sargasso Sea water is advected onto the Bermuda reef platform a large fraction of the microbial standing stock may respond to changes in the nutrient and organic matter supply.

ESTABLISHING CONVERSION FACTORS

Bacterioplankton production in the environment or in seawater culture is most simply estimated by monitoring cell abundance (Ducklow, 1983) over times ranging from a few hours (e.g., in cultures) to seasons (e.g., in the environment).

Using abundance as an indicator of bacterial production may be complicated by bacterial removal processes such as grazing and viral lysis (Fuhrman and Noble, 1995). Production estimates from culture experiments require considerably larger sample volumes (liters vs. milliliters) and longer incubation times (days vs. hours) than near instantaneous (< 1 hr) isotope-based incorporation assays (see Materials and Methods below). Although the modern techniques for estimating bacterial production are rapid and sensitive, the incorporation of ^3H -Thymidine (Fuhrman and Azam, 1980) and ^3H -Leucine (Kirchman et al., 1985) require calibration with an independent estimate of bacterial production from changes in bacterial abundance or biomass (Fuhrman and Azam, 1982; Kirchman et al., 1982). To observe net cell growth in seawater cultures it is generally desirable to reduce or remove nanoplanktonic bacteriovores from the microbial assemblage. The result of the calibration process is the determination of a specific TdR or Leu conversion factor which translates a rate of precursor incorporation ($\text{pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) into a rate of cell production ($\text{cells or biomass} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$). In the absence of performing the actual calibration procedure, researchers using the incorporation of tritiated molecules to estimate bacterial production in the environment have relied on conversion factors from the literature. Ever since the ^3H -Thymidine technique was first applied to marine samples it has been suggested that the application of a single conversion factor over a broad range of environmental conditions or oceanographic realms may be inappropriate (Fuhrman and Azam, 1982).

The premise of using the incorporation of ^3H -TdR into DNA and ^3H -Leu

into protein to estimate the rate of bacterial production implies that the amount of labeled substrate added must overwhelm environmental concentrations. It also assumes that the substrate is supplied to bacterial biochemical pathways at a rate fast enough to shut down *de novo* synthesis. Failing to saturate ^3H -TdR and ^3H -Leu incorporation can lead to isotope dilution by non-radioactive precursor pools which may underestimate bacterial production (Bell, 1986,1993; Kirchman, 1993a).

DILUTION-GRAZING STUDIES

Bacterial removal from seawater by heterotrophic micro-grazers is well documented (Caron et al. 1988; Sanders et al., 1992) and may serve as a significant sink for bacterial biomass in the environment (Leakey et al., 1996; del Giorgio et al., 1996; Coffin and Connolly, 1997). The removal of heterotrophic bacteria from water flowing over coral reefs (Ayukai, 1996) and by organisms (sponges) characteristic of reef environments (Pile, 1996) has recently been documented. As the coupling of the microbial loop to higher trophic levels becomes increasingly well defined, measuring bacterial removal from reef-water by micro-grazers will help constrain benthic-pelagic trophodynamics in these complex systems. Determining the magnitude of grazing on bacterioplankton by heterotrophic microbes is important to linking the cycling of dissolved organic matter (DOM) in the reef environment from bacteria, to micro-grazers, to corals and back to the DOM pool.

Three of the most common techniques for estimating bacterial removal from seawater include; 1) ^3H -TdR labeling of bacteria and subsequently following its transfer to larger size fractions (Servais et al., 1985); 2) Incubation of natural microbial assemblages with fluorescently labeled bacteria (Sherr et al., 1987) and (3) Serial dilution of grazing pressure (Landry and Hassett, 1982). The dilution technique is the least invasive to natural bacteria (no isotopic labeling), and is the most realistic for bacterivores (no ingestion of chemically treated heat-killed bacteria required). It also minimizes the number of additional samples from concurrent conversion factor incubations (one parallel undiluted seawater culture). Landry and Hassett (1982) used a modification of the exponential growth equation (Equation 1) to develop the dilution-grazing formula. The linear representation of this relationship is presented below (Equation 2). The apparent bacterial growth rate for each culture (Equation 3) is plotted against the undiluted fraction of cells (D) in culture to obtain estimates of bacterial growth (μ) and bacterivory (g).

$$N_t = N_0 \cdot e^{(\mu - gD)T} \quad (\text{Eqn. 1})$$

$$\frac{1}{T} \cdot \text{LN}\left(\frac{N_t}{N_0}\right) = \mu - g \cdot D \quad (\text{Eqn. 2})$$

$$\frac{1}{T} \cdot \text{LN}\left(\frac{N_t}{N_0}\right) = \text{apparent growth rate} \quad (\text{Eqn. 3})$$

The ratio of bacterial cell abundance at Time-t (N_t) and Time-zero (N_0) represents the fractional change in cell numbers over initial conditions, while g is

represented by the slope and μ by the Y-intercept of the linear relationship. An important assumption to consider when applying this technique is that dilution should affect the protozoan grazing rate in a linear manner but should not affect the bacterial growth rate at all. The dilution technique was originally developed for estimating the micro-zooplankton grazing rate on phytoplankton (Landry and Hassett, 1982) but is equally applicable to protozoan-bacteria predator-prey relationships (Kirchman et al., 1982).

MATERIALS AND METHODS

FIELD SITES

Research expeditions to Bermuda were conducted in July of 1997 and May-June of 1998. The majority of my sampling effort was focused on four primary reef sites (**Fig. 2**) over the two field seasons. These sites were among a number of coral reefs being monitored by the Benthic Ecology Research Program (Dr. S.R. Smith) at the Bermuda Biological Station. The locations for this study were chosen for their potentially contrasting water-column characteristics, differences in reef development as well as geographic distribution across the Bermuda platform. Sites included: **1**) Seabright East (32° 16' 50" N, 64° 45' 25" W) located approximately one kilometer off the south shore of Bermuda, **2**) John Smith's Bay East (32° 18' 56" N, 64° 42' 32" W), northeast of Seabright, approximately one kilometer off the south shore, **3**) Crescent Reef

Figure 2. Map of the Bermuda Islands showing the location of the primary study sites; Hog Breakers (HB) 32° 27' 32" N 64° 49' 54" W, Crescent Reef (CR) 32° 24' 04" N 64° 47' 57" W, John Smith's (JS) 32° 18' 56" N 64° 42' 32" W and Seabright (SB) 32° 16' 50" N 64° 45' 25" W. Additional samples were collected at Castle Harbor (CH) and NASA Reef (NR).

● Hog Breakers

● Crescent Reef

BERMUDA ISLANDS

● Castle Harbor

● NASA

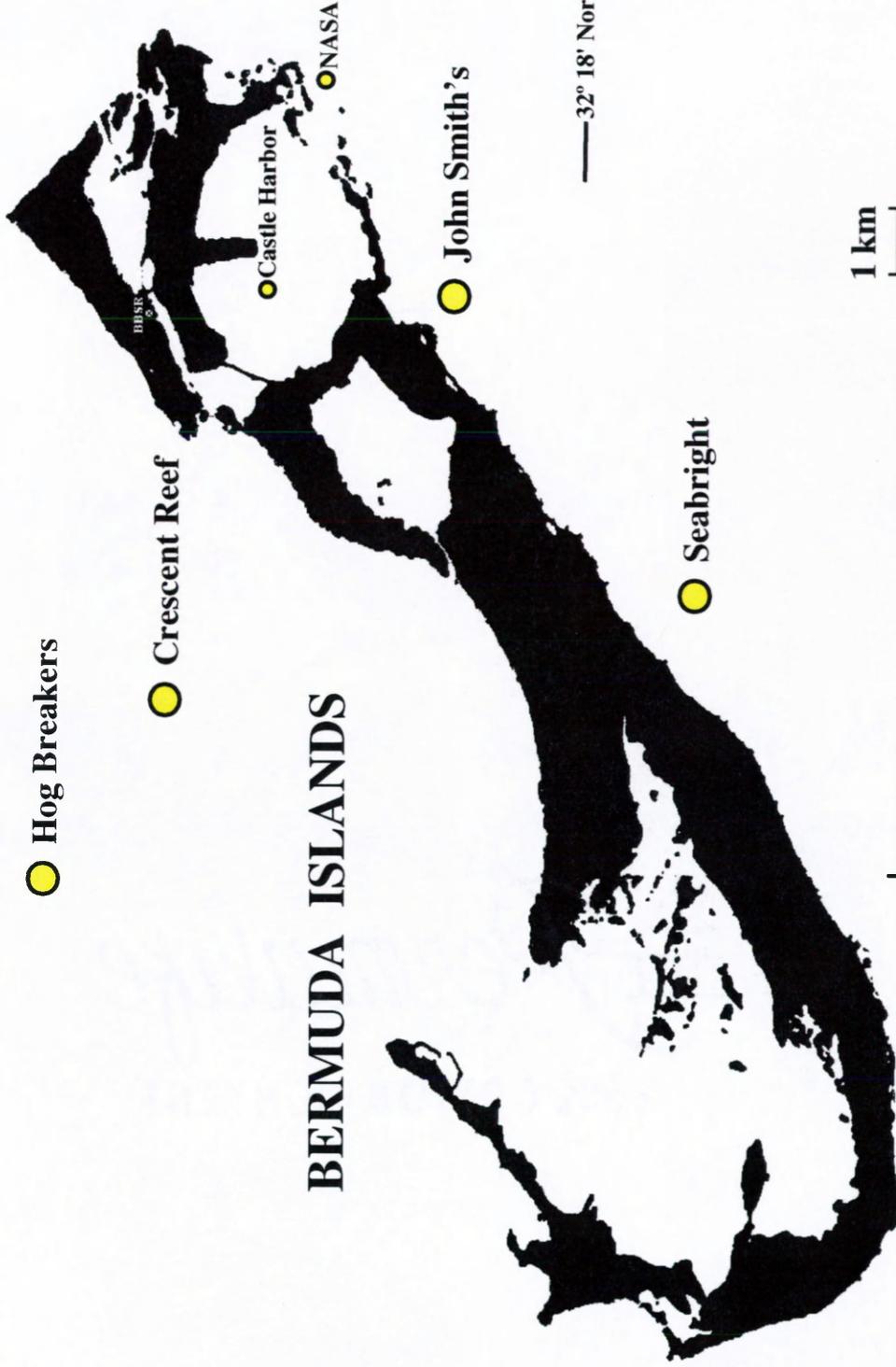
● John Smith's

● Seabright

— 32° 18' North

1 km

64° 48' West



(32° 24' 04" N, 64° 47' 57" W), in the middle of the north shore lagoon and 4) Hog Breakers (32° 27' 32" N, 64° 49' 54" W) at the northern perimeter of the lagoon. Two additional sites were visited during the 1998 field season, including a reef in the middle of Castle Harbor (CH) and NASA Reef (NR) at a site of water exchange between Castle Harbor and the open ocean.

Seabright (SB) was the site with the deepest water-column (approximately 20 m) with coral reefs rising 5 -10 m off the sandy bottom. This study site also had the distinction of being located in close proximity (100 - 200 m) to the primary submarine sewage-outfall system serving the Island of Bermuda. The John Smith's Bay (JS) site was used as a nearby control (no sewage outfall) for comparison to SB bacterial properties. The JS site was similar to SB in distance offshore but slightly shallower (15 m). Reef structure and benthic topography at JS were both similar to SB. Hog Breakers reef (HB) was characterized by having the greatest horizontal underwater visibility (25 - 30 m) of any of the sites. Its distance from the Island and proximity to the open Sargasso Sea contributed to the pristine water quality at HB. Water-column depth at HB was 10 m, with reef structures rising several meters off the bottom, surrounded by channels of coarse coralline sand. The shallow (5 m) water-column at Crescent Reef (CR) was high in particle load (low horizontal underwater visibility) compared to all sites except CH. Corals at the CH site were subjected to a heavy load of suspended particulate matter (visibility just 3 - 5 meters). This shallow reef rose from a depth of 7 m to within 1 m of the surface. NASA Reef (NR) on the

perimeter of Castle Harbor was 5-7 m in depth and characterized by heavy wave action and strong tidal currents.

SAMPLING STRATEGY

During July of 1997, water samples were collected from mid-depth (10 m) at SB on two occasions and at JS on a third date. Syringe samples (described below) were collected from the reef surface at JS as well. Sampling during the 1998 May-June field season was concentrated on the north shore sites (CR and HB), with a return to SB as well. Unless specifically noted, water samples were collected from mid-depth at the various sites and/or from the layer of water in immediate contact with the reef surface (**Fig. 3**). Fieldwork was conducted from the R/V Entropy, which allowed for the relatively rapid collection of samples and transit back to the laboratory facilities at the Bermuda Biological Station for Research (BBSR).

Individual water samples for bacterial incubation studies were collected into 20 liter LDPE collapsible I-CHEM cubitainers by SCUBA divers at mid-depth in the reef water-column (**Fig. 4**). Additional water samples were collected from the coral surface-microlayer (CSM), a term used by Paul et al. (1986) to describe the water in direct contact with the living coral surface, and the reef surface-microlayer (RSM). I define the RSM as the water in immediate contact with locations on the reef structure not colonized by living corals. This habitat frequently contained a fine layer of flocculent detrital material. The CSM and RSM were sampled with 60-ml plastic syringes fitted with silicone tips to protect

Figure 3. Reef habitats and sampling gear. A hand-operated pump and collapsible (20 liter) cubitainers were used to collect large volume samples from the reef water-column (Bulk) for culture experiments. Samples were collected from all three habitats (water-column, coral surface-microlayer and reef surface-microlayer) with 60 ml syringes.

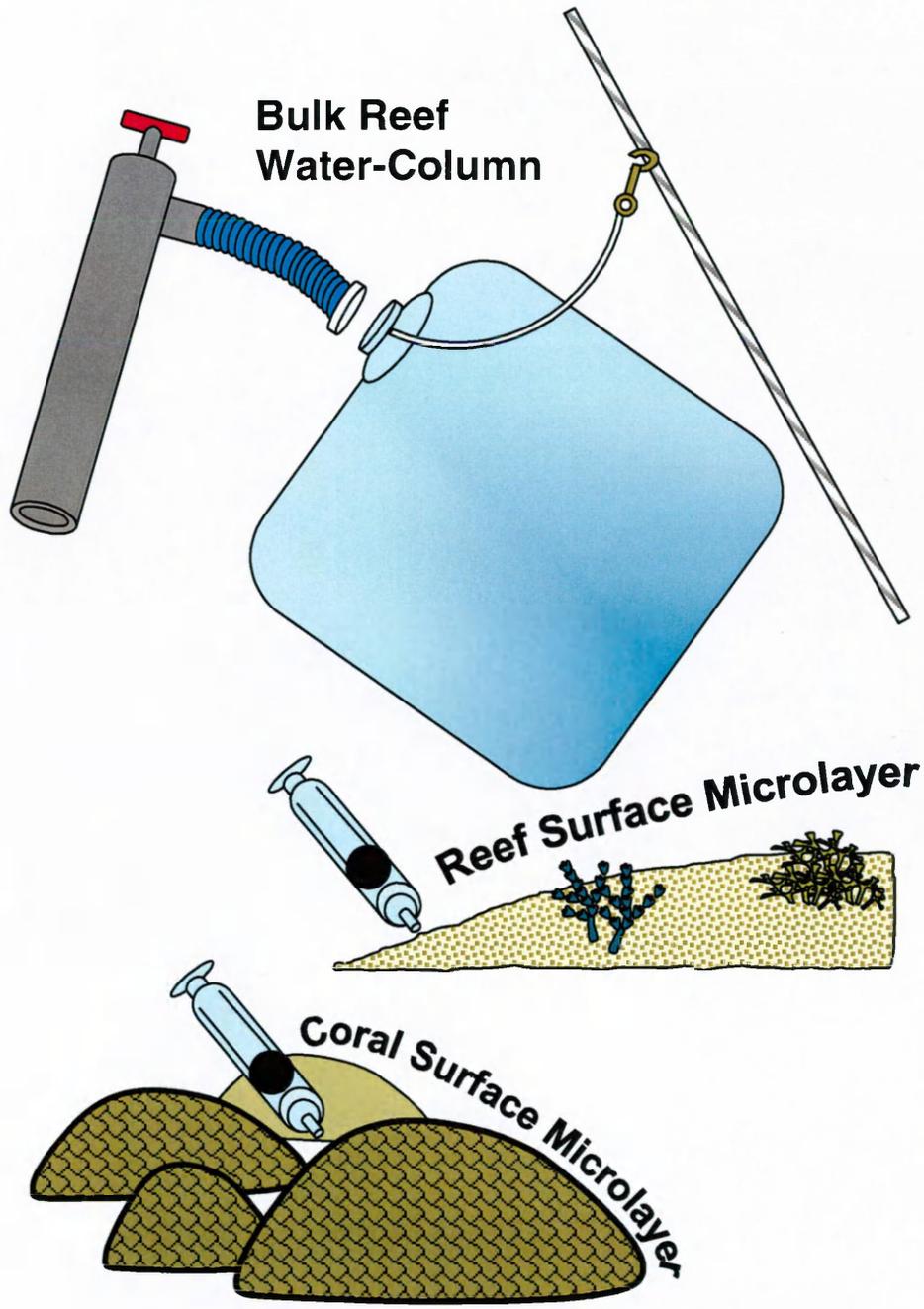
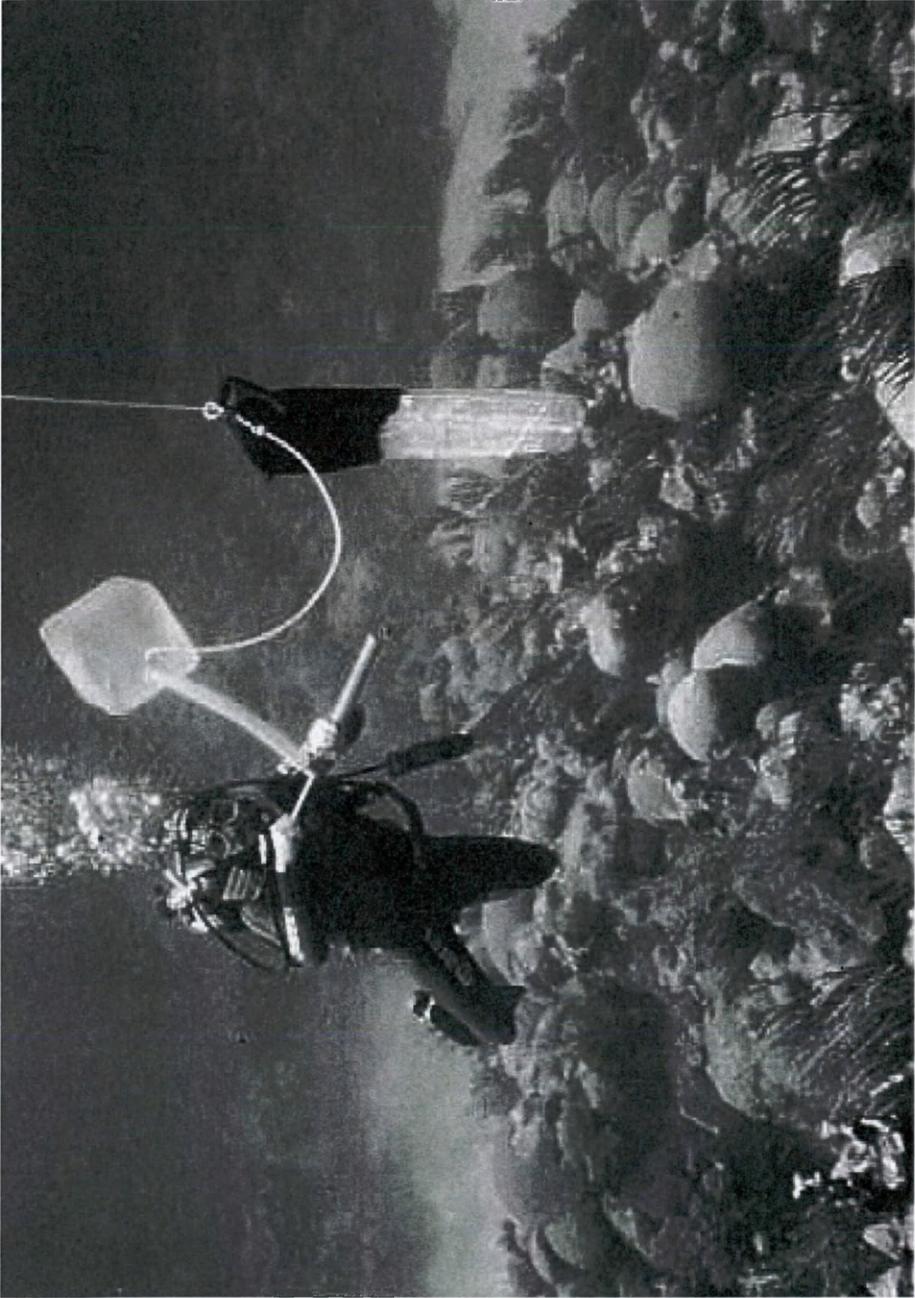


Figure 4. SCUBA diver collecting a large volume water sample at the Hog Breakers site using the pump and cubitainer apparatus.



the coral tissue when collecting water within several millimeters of its surface. Samples were collected as the syringe was drawn across the surface of interest, usually covering a distance of < 10 cm. Prior to use in the field, all water sampling gear was soaked in 1.2 Molar HCl (10% of stock concentration) and rinsed with copious amounts of 18 MΩ resistivity, de-ionized Milli-Q (Millipore) water. To collect the large volume water samples, the outflow hose of a hand-operated bilge pump was fitted with a cubitainer cap which allowed for rapid filling of cubitainers underwater. Three collapsed cubitainers, the pump and syringes were lowered from the boat to the desired depth in a mesh dive bag clipped to a weighted line. Once submerged, a diver attached the individual cubitainers to the weighted line with bungee-cord lanyards and screwed the pump onto a cubitainer to begin the filling process. Before filling, care was taken to ensure that residual air bubbles trapped in the pump and cubitainers at the surface were shaken out of these sampling devices. The large bore (3 cm) of the hand pump provided gentle transfer of the water sample from the environment to the cubitainer. Preliminary experiments with this sampling procedure demonstrated that the pump and cubitainer apparatus did not adversely affect the heterotrophic bacterial component of the water sample compared to parallel incubations in polycarbonate bottles. No significant difference ($p > 0.05$) was detected in the bacterial TdR incorporation rate among samples collected by this method compared to samples collected into two liter Nalgene polycarbonate carboys by using either the same pump apparatus or by

uncapping (no pumping) a duplicate polycarbonate carboy below the surface (Table 1).

When filled, the cubitainers were neutrally buoyant and remained attached to the weighted line while syringes were filled at the reef surface. Prior to diving, silicone tips (2 cm) were placed on the end of each 60 ml syringe and the end of each plunger was numbered with indelible ink. Water samples drawn from the surface of living coral colonies (*Diploria*, Fig. 5) were collected at the area of underlying reef-structure nearest the weighted line and cubitainers. Each syringe sample was rinsed with at least one full volume of water before the final aliquot was drawn. Water-tight luer locking caps were placed on the syringe tips immediately after collection to prevent sample loss upon transport to the surface. Cubitainers and syringes were stored in a large plastic crate filled with surface seawater for transport back to BBSR, thus keeping the samples in the dark at ambient temperature (22-27°C). Transit times from reef to lab were usually less than one hour, with experiments generally beginning within three hours of water collection. Shielding reef-water samples from exposure to sunlight during the transit was necessary to prevent potential photoinhibition of bacterial production in T-0 bioassays (Pakulski et al., 1998).

DILUTION EXPERIMENTS

Bacterial growth on ambient dissolved organic matter (DOM) and removal by nanoplankton grazing, were estimated for each study site using the seawater culture technique (Ammerman et al., 1984; Ducklow and Hill, 1985; Ducklow et

Table 1. Comparison of sampling techniques to test the suitability of water collection by pumping into a collapsed cubitainer. Samples were obtained from nearshore waters close to BBSR and left to sit in shaded collection vessels for 90 minutes to simulate transit from the reef, prior to sampling for TdR incorporation. Values are averages (\pm SD) of single time-point determinations performed in triplicate.

Sampling Technique	TdR (pMol \cdot l ⁻¹ \cdot h ⁻¹)	t-Test ¹
1. Pump and Cubitainer	35.8 (\pm 3.7)	*
2. Pump and Polycarbonate	46.1 (\pm 12.0)	*
3. Uncap Polycarbonate	45.4 (\pm 5.0)	*

1. Rates of TdR incorporation using the three sampling techniques were not statistically different from one another indicated by the same number of asterisks ($p > 0.05$) in the t-Test column.

Figure 5. Colonies of the brain coral *Diploria* on a Bermuda reef. Discrete water samples were collected from the coral surface-microlayer of *Diploria strigosa*, and *Diploria labyrinthiformis*.



al., 1992). Using this experimental design it was possible to estimate specific bacterial growth rate (μ), bacterial specific removal rate (g), bacterial growth efficiency (BGE) and both Thymidine and Leucine based conversion factors (TdR-CF and Leu-CF). To minimize the effects of container surface-area to volume ratio on natural microbial populations, four dilution experiments (SB2, JS1, HB1, and CR1) were carried out in 20 liter Nalgene polycarbonate carboys. A preliminary dilution-grazing experiment (SB1) was also conducted at the SB site using 10 liter Nalgene polycarbonate carboys. Biological oceanographers generally recommend that surface-area to volume ratios of incubation vessels be as low as possible to avoid experimental artifacts due to containment effects (e.g., Sanford and Ducklow, submitted). Incubations of seawater in 20 liter polyethylene containers (Lee and Fuhrman, 1991) demonstrated a high degree of similarity (84%-95%) between T-0 and T-F (32-48 hours) samples based on bacterioplankton DNA composition. The previous study was the first direct test showing that the natural bacterial composition did not change in 20 liter vessels over 2 days. Inadvertent or deliberate enhancement of DOC can lead to a significant shift in the bacterial community structure (Dr. Craig Carlson, personal communication).

The primary reason for monitoring trends in substrate incorporation and bacterial abundance over time in dilution cultures was to derive factors to convert TdR and Leu incorporation rates into bacterial production estimates. Additionally, the comparison of trends in incorporation rates across sites is potentially

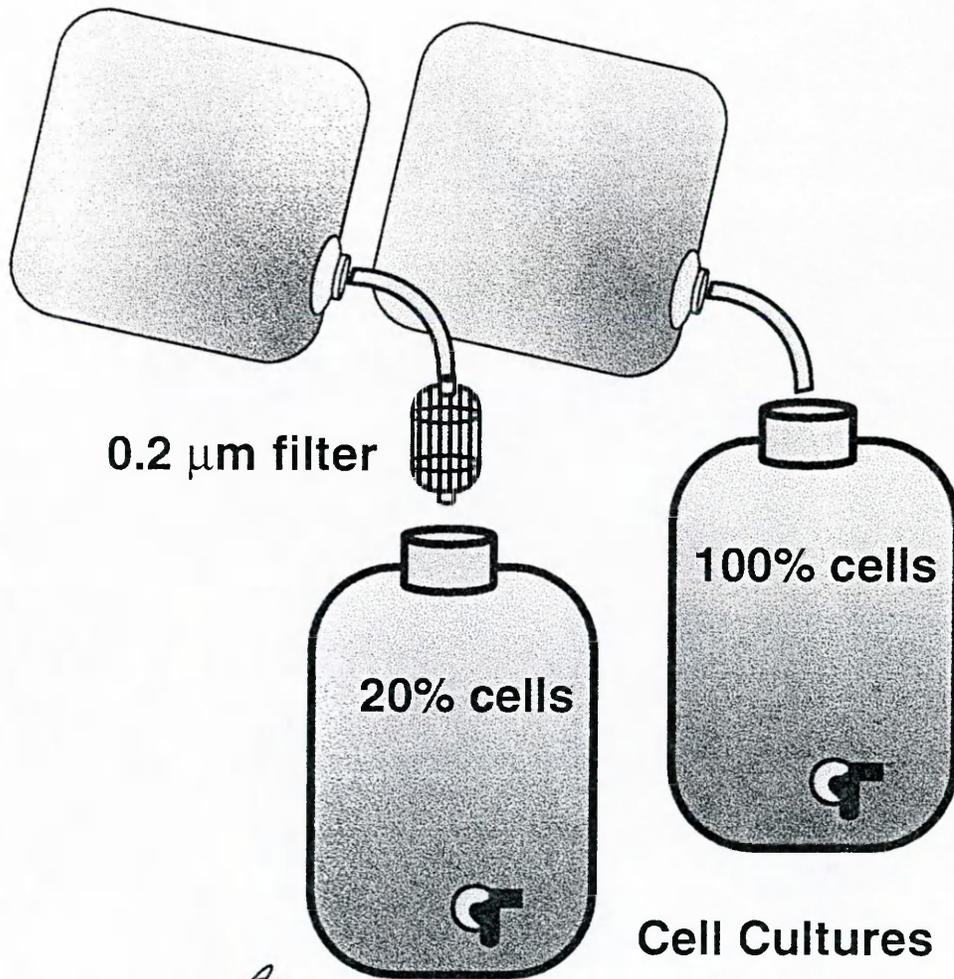
informative regarding the degree of top-down (removal) or bottom-up (substrate limitation) control of bacterial populations. Calibration of the TdR and Leu methods requires that positive changes in bacterial cell numbers (cell division) or biomass (cell growth) be related to the rate of substrate incorporation. Therefore, only diluted cultures where cell abundance increased for at least the initial 48 hours were used for conversion factor estimates.

Gelman Mini-Capsule filters (0.2 μm) were used to prepare particle-free reef-water. Prior to their use on fresh reef-water, the Mini-Capsules were leached overnight with 1.2 M HCl to remove organic contaminants bound to the filtration matrix. Capsules were rinsed with large volumes (~ 15 liters) of Milli-Q water, until the dissolved organic carbon (DOC) concentration (High Temperature Combustion method, Carlson et al., submitted) of the filtrate was identical to that of unfiltered Milli-Q water. While the 0.20 μm filtration theoretically removed 100% of the nanoplanktonic bacteriovores, the virioplankton component most likely passed through the cartridge unaffected. Therefore, estimates of bacterial growth rate might be slightly underestimated due to the inclusion of a viral lysis component in the bacterial growth terms.

Experimental treatments for dilution-grazing experiments included a whole water treatment (undiluted) and whole water diluted by 80% with the 0.20 μm filtrate (**Fig. 6**). Most dilution-grazing experiments (SB, JS and HB) were conducted with 100% (undiluted) and 20% (80% diluted) reef water-column microbial assemblages, while the CR experiment was performed with a single

Figure 6. Sample processing upon returning reef water to the lab. Cubitainer samples were partitioned into 20 liter polycarbonate carboys for dilution experiments to monitor cell growth and removal over several days. A 20% cell culture was composed of 16 liters of 0.20 μm filtered reef water plus 4 liters of unfiltered reef water. Syringe samples from the field were processed for bacterial activity, abundance and biovolume as well as DOC concentration immediately following the setup of dilution cultures.

Cubitainer Samples



30% (70% diluted) reef-water assemblage. It was not possible to calculate removal rates (g) for the CR site since a second cell culture (at a different dilution factor) was not prepared for this experiment. To initiate the 100% culture, 20 liters of reef-water was transferred from a cubitainer to one of the 20 liter polycarbonate carboys using acid washed silicone tubing. Gentle sample transfer was accomplished by extending the tubing to the bottom of the carboy and controlling the flow with a spigot at the cubitainer. Diluted reef-water cultures (20% and 30%) were prepared by inoculating either 16 or 13 liters of particle-free (0.20 μm filtrate) reef-water with 4 or 6 liters of 100% unfiltered reef-water respectively.

After transfer and mixing by gentle inversion, carboys were moved to darkened incubators that were set to the ambient temperature of reef-water. Experiments conducted in July of 1997 were incubated at 28 (± 1) $^{\circ}\text{C}$, while May 1998 experiments were cultured at 22 (± 1) $^{\circ}\text{C}$. Incubations were sub-sampled at intervals of 6-24 hours, depending on the stage of bacterial growth, as determined by microscopic examination. Carboys were mixed before sampling to ensure homogenous sample composition. Samples for bacterial abundance, biovolume and production were collected at each time-point, along with T-0 and T-F DOC samples. Additional bacterial production samples were incubated at T-0 and T-F time-points to determine the concentration of substrate (thymidine or leucine) required to saturate the incorporation physiology. Between T-0 and T-F the total volume of water removed from the 20 liter incubations was less than 5

liters, thus maintaining a low surface-area to volume relationship within the incubation vessel.

FIELD SAMPLES

Multiple syringe samples (usually ≤ 3) from the same point of collection on the reef-surface were combined into one pre-cleaned polycarbonate bottle upon return to the lab. Bacterial parameters, including abundance, production, biovolume and substrate saturation, were measured in reef-surface samples, for comparison with the bulk reef-water T-0 samples from dilution-grazing experiments. Additionally, DOC samples were collected from several of the benthic CSM habitats for comparison to water column values to determine if this property displayed any vertical concentration gradient away from the reef.

MEASUREMENTS

BACTERIAL ABUNDANCE AND BIOVOLUME

Measurements of bacterial abundance and biovolume were estimated via epifluorescence microscopy using acridine orange to obtain visual counts (Hobbie et al., 1977) and video image analysis (Bjørnsen, 1986; Viles and Sieracki, 1992) techniques for biovolume determinations. Seawater samples for bacterial enumeration and sizing were preserved with 0.2 μm Acrodisc (Gelman) filtered 25% glutaraldehyde solution (Sigma-Aldrich), added to seawater samples to a final concentration of 1.25%. Bacterial samples for abundance and biovolume were refrigerated at 4°C and slides were prepared within one week of

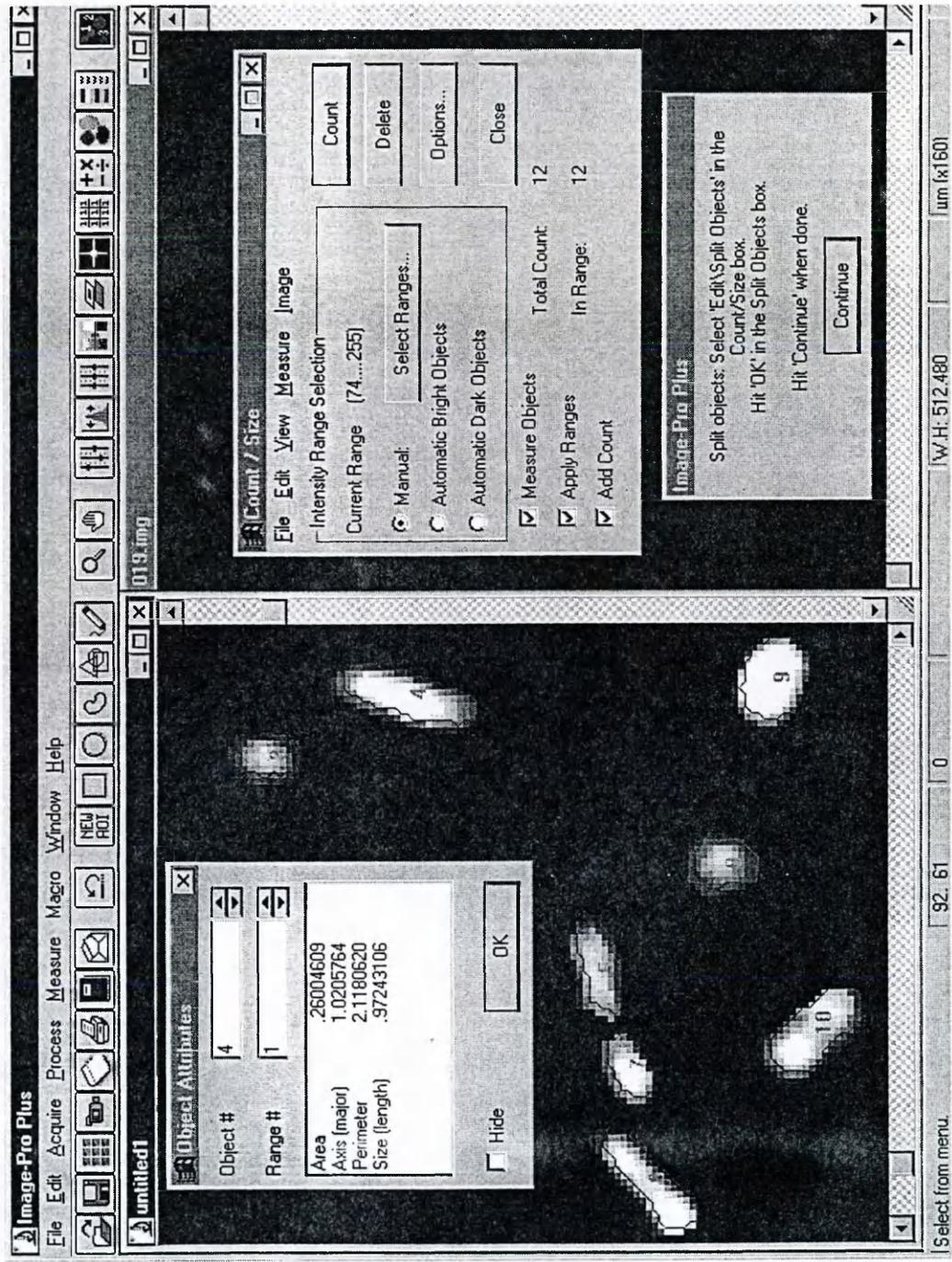
collection. Preserved water samples were filtered onto 25 mm, 0.2 μm , black polycarbonate Nuclepore filters which were backed by 25 mm, 0.45 μm , mixed ester Nuclepore filters. Ground glass microanalysis vacuum filter holders (25 mm) were employed to support the filters and provide gentle filtration across their entire surface area. The filtration of 3 ml of reef water was sufficient for visual abundance estimates, while 10 ml was an ideal sample volume for image analysis. When the sample volume remaining above the filter was reduced to 2 ml, 200 μl of 0.2 μm Acrodisc filtered, 90% dye content, acridine orange (Sigma-Aldrich) solution (0.05% w/v) was pipetted directly into the filtration funnel. At vacuum pressures of <125 mm Hg this technique allowed sufficient time for brilliant cell staining. Resolve (Stephens Scientific) low viscosity immersion oil was used for both slide preparation and subsequent microscopy. Slides were stored at -20°C until they were analyzed and then archived at -80°C .

Bacterial enumeration was accomplished with an Olympus BX-40 epifluorescence microscope using the acridine orange direct count (AODC) technique cited above. Total magnification of 1500x was achieved with a 100x oil immersion objective and 15x eyepiece. A count of autofluorescent picoplankton (Olympus Filter-Cube Set U-MNG; green excitation filter BP530-550 nm, dichroic mirror DM570 nm and emission filter BA590 nm) was subtracted from the total count of fluorescent cells (Olympus Filter-Cube Set U-MNB; blue excitation filter BP470-490 nm, dichroic mirror DM500 nm and emission filter BA515 nm) to obtain the heterotrophic bacterial component. The

trophic characteristics of microbial populations stained with only acridine orange (AO) are difficult to identify by simply using the difference in cell counts between blue vs. green excitation due to the masking effects of the emission spectra of AO on photo-pigments (Sherr et al., 1993). Therefore, only the brightest red-fluorescing cells under green excitation were scored “autotrophic” and subtracted from the total count. Sieracki et al. (1995) demonstrated that the inclusion of the phototroph *Prochlorococcus* (~0.7 μm long) in heterotrophic bacterial counts may lead to overestimation of heterotrophic bacterial biomass by 2 - 40% in samples from the euphotic zone of two Sargasso Sea stations. Autotrophic cells were generally 5 - 15% of total cell counts, which suggests the maximum percentage by which bacterial abundance determinations may be overestimated in this data set if I failed to account for all of the autotrophs.

Image analysis was performed on bacterial slide preparations using a Zeiss Axiophot epifluorescence microscope with a Photometrics CH250 charge coupled device (CCD) camera system. Images were captured at 1600x magnification with a 100x oil immersion objective, 1.6x optivar and 10x eyepiece. The components of blue-light excitation for the Zeiss microscope system consisted of an excitation (BP 450-490 nm), dichroic mirror (FT 510 nm), and emission (LP 520 nm) filter set. Green-light excitation components included excitation (BP 546/12 nm), dichroic mirror (FT 580) and emission (LP 590) filters. Images were collected and processed with Image-Pro Plus (version 3.0) image analysis software (**Fig. 7**). The pixel to micron ratio was 0.04264 for all image

Figure 7. Screen snapshot of Image-Pro biovolume determination. Cells were not processed at this image magnification (400% of normal size). Cell number four is approximately $0.06 \mu\text{m}^3$, for comparison to literature values and average volumes detected in this study.



analysis samples. Objects with a diameter less than the filter pore size, 0.20 μm , were automatically removed during image processing. Image-Pro Plus bacterial abundance estimates were generally found to be in good agreement with direct visual counts from the Olympus microscope. However, cell density of the slide preparation greatly affected the degree of agreement between the two techniques. Abundance estimates of low-density bacterial slides were more variable and generally greater when obtained by image analysis compared with direct counts of the same slide. The image area produced by the CCD attached to the Zeiss microscope was 20.3 μm x 21.4 μm compared with the counting reticule area of 100 μm x 100 μm on the Olympus system. It was therefore easier to obtain statistically meaningful abundance estimates (Kirchman, 1993b) using the Olympus microscope when cell density on slide preparations was low. For consistency, the only abundance data presented in this study are those obtained by direct counts with the Olympus BX-40. Biovolume estimates were calculated from Image-Pro Plus measurements of the CCD images using the biovolume algorithm of Sieracki et al. (1989). Biomass estimates merged these two data sets (e.g., abundance \times mean cell volume \times carbon conversion factor).

BACTERIAL PRODUCTION

Rates of heterotrophic bacterial production were estimated by using the ^3H -Thymidine (Fuhrman and Azam, 1980;1982) and ^3H -Leucine (Kirchman et al., 1985; Simon and Azam, 1989) incorporation methods as modified by Smith and Azam (1992). Microliter quantities of aqueous [Methyl- ^3H]-Thymidine, (90 Curies

• mMol^{-1} (July 1997) and 88.5 Curies • mMol^{-1} (May 1998); NEN Life Science Products, Inc.) and L-[3,4,5- $^3\text{H}(\text{N})$]-Leucine (179 Curies • mMol^{-1} (same specific activity both field seasons); NEN Life Science Products, Inc.) were pipetted into 2 ml capacity, non-sterile, screw-cap microcentrifuge tubes. Sterile microcentrifuge tubes were not recommended due to their apparent antimicrobial properties (Dr. Craig Carlson, personal communication).

At the beginning and end of every dilution-grazing experiment a series of incorporation rate bioassays was performed to determine the physiological state of the bacterial community. For TdR, this included a range of concentrations from 5 -100 nM, while Leu spanned the range of 5 - 75 nm. During the July 1997 field season, both TdR and Leu were added to a final concentration of 20 nM for the bacterial production assays. In May-June of 1998 the TdR concentration was increased to 30 nM. Since both metabolic precursors were used at their NEN-supplied concentration, a working volume was transferred to a microcentrifuge tube to avoid contaminating stock solutions. A micro-pipettor (0.5 – 10.0 μl) was used to load isotope into the 2 ml micro-centrifuge tubes for incubation with reef-water. Care was taken to ensure that all isotope solution was ejected from the micro-pipette tip by dispensing the desired volume (e.g., 4.66 μl TdR and 1.25 μl Leu for routine bioassays during the 1998 field season) against the inside wall of the microcentrifuge tube. Using undiluted isotopes avoided the possibility of contamination by organic-matter during the preparation of diluted working solutions. Control samples (blanks) were incubated in parallel

with TdR and Leu bioassay samples and subtracted from the experimental replicates to obtain final rate estimates. Blanks contained the same volume of isotope as bioassay samples plus 100 μ l of 100% Trichloroacetic Acid (TCA) solution (to eliminate microbial activity) which mixed with the isotope solution as it was loaded into the tube. It was possible to obtain blanks of lower activity by adding the 100% TCA to the sample followed by the isotope, but this technique added time to the procedure and introduced the possibility of contaminating the working volume of isotope with the acid. Reef-water samples of 1.75 ml were gently loaded into the microcentrifuge tubes with an Eppendorf 2100 pipettor (0.5-5.0 ml) without splashing the preloaded isotope or isotope/TCA (blanks) out of the tubes. Microcentrifuge tubes were loaded into floating racks and incubated in a darkened cooler filled with ambient temperature nearshore water. Incubations lasted approximately 60-90 minutes and were stopped with the addition of 100 μ l of 100% TCA.

The cold TCA-insoluble ^3H -labeled macromolecule fraction of the reef-water samples was extracted by the centrifugation technique described by Smith and Azam (1992). This procedure involved centrifugation of the micro-tubes at 14,000 rpm ($20,800 \times g$) for 7 minutes at 2 - 4°C in an Eppendorf 5417R Microcentrifuge, aspiration of supernatant, extraction in 5% (v/v) ice-cold TCA, centrifugation (same settings), aspiration, extraction in 80% (v/v) ice-cold ethanol, centrifugation and a final aspiration. Precipitated macromolecules in the microcentrifuge tubes were resuspended in 1.6 ml of Ultima Gold (Packard

Instrument Co.) liquid scintillation cocktail. Samples were allowed to sit in the dark for >24 hours at room temperature before being counted on a Packard Tri-Carb LSC (Model A2300-1600 TR) at BBSR. Counting the ^3H samples at least an entire day after processing generally resulted in higher sample DPM and closer agreement among replicates.

Heterotrophic bacterial cell production ($\text{cells} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) was calculated from measured rates of TdR and Leu incorporation using empirically determined conversion factors. To obtain estimates of bacterial carbon production (BCP), bacterial cell production was multiplied by a carbon conversion factor (CCF). A power function [$\text{fg Carbon per cell} = 92.01 \cdot (\mu\text{m}^3)^{0.598}$] was determined using the measured and derived data of Simon and Azam (1989) to calculate a cell-specific CCF, as suggested by Torréton and Dufour (1996a). Simon and Azam's technique of estimating CCF for a broad range of biovolumes ($0.03\text{-}0.40 \mu\text{m}^3$) relied on determinations of $\text{protein} \cdot \text{cell}^{-1}$ and constrained estimates of carbon in the macromolecular constituents of a bacterial cell. Based on the previous relationship and an average cell volume of $0.053 (\pm 0.008, \text{SD}) \mu\text{m}^3$ in the reef environment (see Results), a CCF of $15.9 \text{ fg C} \cdot \text{cell}^{-1}$ was used to calculate BCP for production except for cells from experimental cultures where cell-specific biovolume was variable. Gundersen et al. (1994) measured a range of $80 - 220 \text{ fg C} \cdot \mu\text{m}^3$ for Sargasso Sea bacteria, however the median cell volume they detected was $0.10 \mu^3$ which produced an average CCF of $14 \text{ fg C} \cdot \text{cell}^{-1}$.

DISSOLVED ORGANIC CARBON

DOC samples were collected into acid washed 40 ml borosilicate glass vials (I-CHEM Certified) which were baked for 4-5 hours at 550°C. Both plastic vial-caps and their silicone septa were soaked in acid (1.2 Molar HCl) for ≥ 12 hours and subsequently rinsed with Milli-Q water. Caps and septa were allowed to air dry before use. At all stages of vial clean-up, care was taken to avoid any form of organic contamination. Samples were collected from dilution cultures at time-points throughout the incubations to relate bacterial cell growth to DOC draw-down and allow for the calculation of bacterial growth efficiency (BGE). Upon collection, samples were chilled in a bucket of ice before transfer to the lab freezer. Samples remained frozen at -20°C until they were analyzed at BBSR via the High Temperature Combustion method (Carlson et al., 1998) which has a precision of $\pm 0.6 \mu\text{M C}$. All DOC samples were actually total organic carbon (TOC) samples since they were not filtered prior to collection. Since the POC fraction is approximately 5 percent of the TOC measurement in samples from the oligotrophic Sargasso (Dr. Craig Carlson, personal communication) I decided to forego filtration, thus avoiding the risk of lysing cells and adding an unknown amount of DOC to the sample.

SMALL SCALE EXPERIMENTS

SURFACE- MICROLAYER DEPTH PROFILE

On a single date, syringe samples were collected by SCUBA from the

CSM and RSM of Mills Reef, located in the center of Castle Harbor (CH).

Sampling was conducted at four depths from the base of the reef at a depth of 7 m, up to its crest at 1 m depth below the surface of the water. This survey was conducted to examine bacterial production at the reef-surface over a depth gradient of a highly particle-impacted coral reef. The water-column of this reef had a heavy particulate matter load (characterized by M. Mills, Ph.D. Thesis in preparation) with large amounts of detritus raining down on the reef surface.

Unlike other sites, CH coral were observed to be actively sloughing off aggregates of mucus and detrital particles. This process was less evident moving towards the surface where wave action apparently kept the reef surface clean.

CORAL INCUBATION

In addition to the samples and experiments already described, an experiment involving the incubation of a coral (*Madracis mirabilis*) in a polycarbonate bottle was performed at the end of the May-June 1998 field season to determine the effect of coral derived DOC on bacterioplankton properties. *Madracis mirabilis* is a vigorous branching coral found over a large area of the Bermuda platform. A small (3 cm long, 1 cm diameter) healthy branch of this coral, acclimated to the BBSR wave-tank for several weeks, was placed in 500 ml of freshly collected reef-water from the HB site. Prior to immersion in the incubation bottle, the specimen was gently rinsed with a large volume (≥ 500 ml) of fresh reef-water to remove any loosely-associated coral-

surface microbial population. The reef-water and coral were maintained at *in situ* water temperature (28 °C) in a darkened laboratory incubator for the time course of the experiment. A control bottle, without coral, was incubated in parallel under identical conditions. Over 24 hours, bacterial production, bacterial abundance, bacterial biovolume and DOC were monitored in both the coral and control treatments. The coral's individual polyps remained extended for the entire time of its bottle-containment indicating, in a qualitative sense, a lack of stress upon the colony. Upon completion of the experiment, the coral branch was returned to the wave-tank.

RESULTS

OVERVIEW

Heterotrophic bacterial populations from mid-depth in the water-column of the four primary reef sites (HB, CR, JS and SB) were remarkably similar to one another with respect to abundance, biovolume, and the incorporation of both TdR and Leu. Bacterial production estimates from reef water-column sites (separated by kilometers) were generally more similar to one another than they were to bacterial production estimates in the layer of water immediately overlying coral colonies and adjacent non-living reef just meters below. Dissolved organic carbon concentration, measured in T-0 samples from SB and JS conversion factor studies during July of 1997, was within the annual range (1996) of euphotic zone (0-140 m) values at the BATS oceanographic research site 75 km

to the south-east. Although bacterial abundance estimates between the reef sites and BATS were similar, rates of TdR incorporation were significantly higher on the reef.

COMPARISON OF FIELD SITES

BACTERIAL ABUNDANCE

The mean of average heterotrophic bacterial cell abundance (duplicate determinations) in freshly collected reef water samples was $7.4 (\pm 2.2) \times 10^8$ cells $\cdot l^{-1}$ ($n = 25$). This average includes samples collected by both techniques described above from a variety of depths (1 - 20 m) and habitats (Bulk reef-water, CSM and RSM). Bacterial abundance in the upper water column (0 - 140 m) at the oceanic BATS station (Jan. 1996 - Jan. 1997) ranged from $1.4 - 10.6 \times 10^8$ cells $\cdot l^{-1}$, with an average of $4.5 (\pm 1.6) \times 10^8$ cells $\cdot l^{-1}$ (BATS data obtained from; <http://www.bbsr.edu>, Carlson et al., 1996). Partitioning the 25 reef-water determinations into their individual categories (Bulk, CSM and RSM) showed some differences among individual mean cell abundance. Bacterial abundance from mid-depths of the various reef water-columns had a mean of $7.2 (\pm 1.4, SD) \times 10^8$ cells $\cdot l^{-1}$ ($n = 9$), while the mean of CSM was $6.9 (\pm 2.3, SD) \times 10^8$ cells $\cdot l^{-1}$ ($n = 12$) and for RSM was $9.6 (\pm 2.9, SD) \times 10^8$ cells $\cdot l^{-1}$ ($n = 4$). Four particularly high abundance determinations from the CH reef site were initially included in the CSM data set. Horizontal underwater visibility at CH was greatly reduced (5 - 10 m) compared to other sites (20 - 30 m) due to the greater particulate load

at this inshore reef. When CH samples were removed from the CSM data set, average cell abundance of CSM samples dropped to $5.7 (\pm 1.6, \text{SD}) \times 10^8 \text{ cells} \cdot \text{l}^{-1}$ ($n = 8$). Performing pair-wise two-tailed T-tests on average bacterial abundance showed significant differences ($p < 0.05$) between individual comparisons (**Table 2**). Abundance was different between CSM and RSM, Bulk and RSM, but was not significantly different between CSM and Bulk until the four CSM samples from the CH site were removed from the data set. When separated from the other samples in the CSM group, average bacterial abundance of CH-CSM samples was not significantly different from the RSM group. Thus, RSM and CH-CSM samples had statistically greater bacterial abundance than samples from the other habitats.

BACTERIAL BIOVOLUME

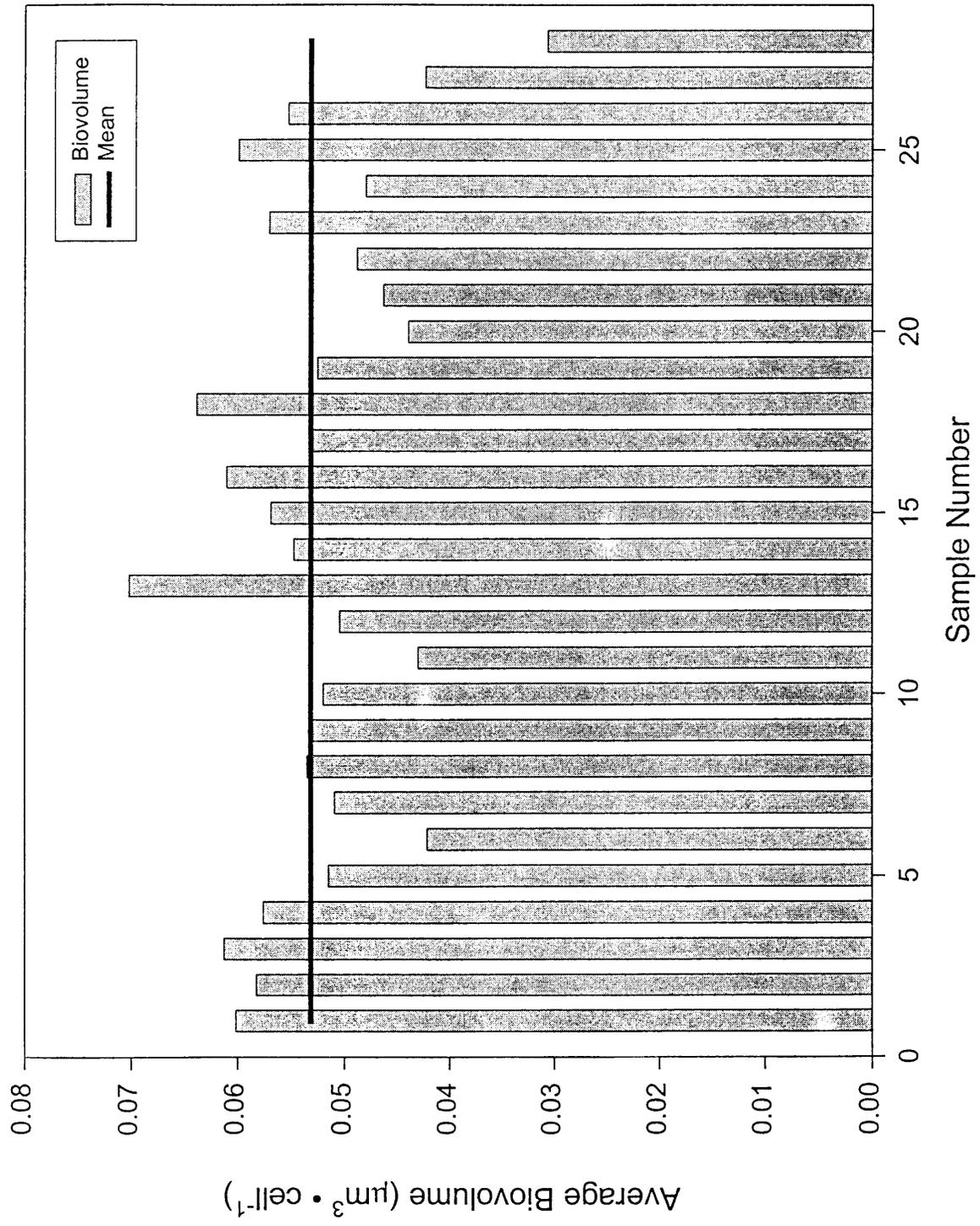
The average volume of freshly collected bacterial cells from the reef environment was remarkably uniform. Cell-specific biovolume of freshly collected reef-water bacteria (T-0 dilution-culture samples and syringe-collected samples) was $0.053 (\pm 0.008, \text{SD}) \mu\text{m}^3$ (mean of average replicate determinations, $n = 28$ paired samples) over both field seasons (**Fig. 8**). On average, approximately 300 cells (minimum = 50, maximum = 1150) were imaged for each biovolume replicate. Of the 28 average *in situ* biovolume determinations, no difference ($p = 0.77$, single factor ANOVA) in this parameter was detected among the three intra-site sampling locations. Reef water-column bacteria demonstrated the greatest range in cell-specific biovolume ($0.031 - 0.070 \mu\text{m}^3$) with an average of

Table 2. Bacterial abundance (\pm SD) of freshly collected reef-water from various habitats, including the Coral Surface-Microlayer (CSM), Bulk and Reef Surface-Microlayer (RSM). Two-tailed t-test performed on the average bacterial abundance within a habitat.

Samples	Abundance ($\times 10^8$) cells \cdot l⁻¹	n	t-Tests¹
CSM	6.9 (\pm 2.3)	12	*
Bulk	7.2 (\pm 1.4)	9	*
RSM	9.6 (\pm 2.9)	4	**
CSM²	5.7 (\pm 1.6)	8	***
CSM³	9.3 (\pm 1.0)	4	**

1. Same number of asterisks (*) indicates NS difference ($p > 0.05$) while different numbers indicate significant difference ($p < 0.05$) in bacterial abundance between habitats.
2. CH samples removed from CSM data set.
3. CSM from CH only.

Figure 8. Average cell-specific biovolume of bacteria in freshly collected reef-water samples. Each bar represents the average of 200-500 individual cell biovolume determinations for a particular sample. Samples represent all three habitats: Coral Surface Microlayer (CSM), Reef Surface Microlayer (RSM) and Bulk Reef Water (Bulk). Mean cell-specific biovolume equals $0.053 (\pm 0.007) \mu\text{m}^3$, $n=28$.



0.053 (± 0.011 , SD) μm^3 ($n = 11$), while CSM bacteria had a narrower range (0.042 - 0.061 μm^3) averaging 0.052 (± 0.007 , SD) μm^3 ($n = 12$) and RSM bacteria narrower still (0.050 - 0.064 μm^3) averaging 0.055 (± 0.005 , SD) μm^3 ($n = 5$).

BACTERIAL THYMIDINE AND LEUCINE INCORPORATION

Incorporation rates of thymidine (**Fig. 9A**) and leucine (**Fig. 9B**) across different habitats (Bulk, CSM, RSM) within a particular reef-site (SB, JS, HB and CR) were highly variable during any particular sampling excursion. One interesting trend among these intra-site samples is the suggestion of lower absolute rates of TdR and Leu incorporation at the CSM compared to overlying Bulk water. However, when all CSM ($n = 12$), Bulk ($n = 14$) and RSM ($n = 4$) rate samples were pooled and compared on a cell-specific level, no significant differences ($p > 0.05$) in rate of substrate incorporation were detected between CSM and Bulk bacterial populations. Both CSM and Bulk cell-specific rates were significantly different ($p < 0.05$) from pooled RSM samples.

Although the data set of sampling trips, to habitats within a site on different dates, is small (3 \times CSM at HB and CR, 3 \times Bulk at HB and SB, 2 \times RSM at CR and HB), some trends in the small scale variability of absolute rates of substrate incorporation are suggested (**Fig. 10**). In general, the samples from sites and habitats with a high particle load in the water-column (visual estimate from underwater horizontal visibility) and on the reef surface (relative amount of detrital material in syringe sample) displayed higher absolute rates of TdR and

Figure 9. Thymidine (A) and Leucine (B) incorporation rates (\pm SD) across the Bermuda Reef Platform on various dates and habitats within a site. SB – May 15, 1988; JS – July 23, 1997; HB – May 7, 1998; HB2 – May 29, 1998; CR – May 29, 1998.

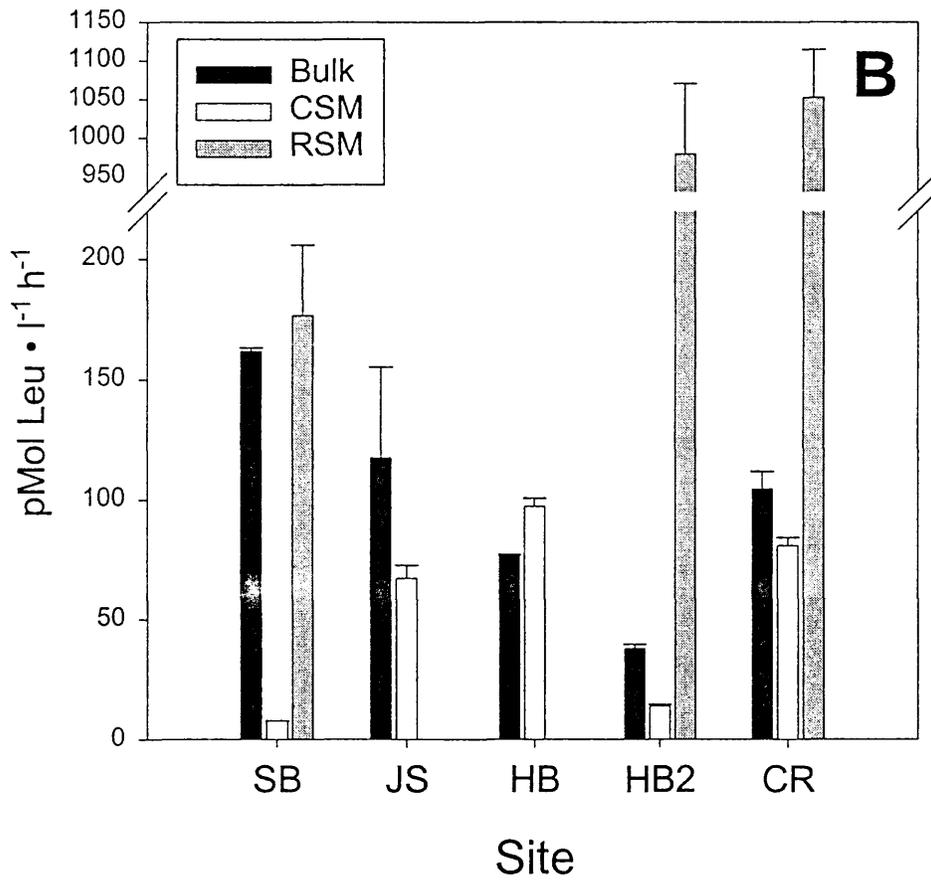
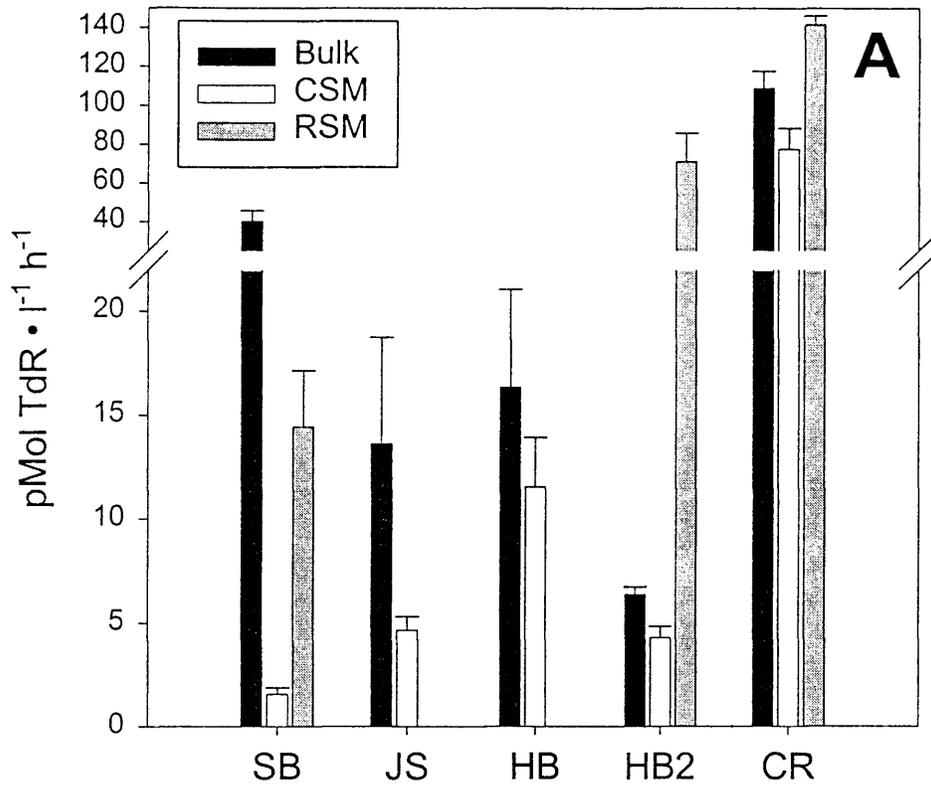
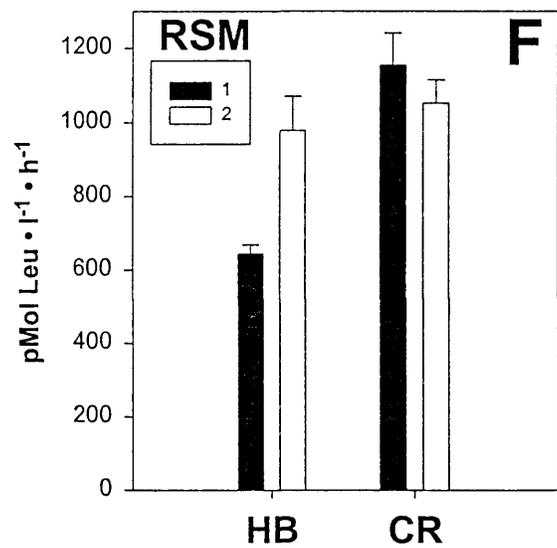
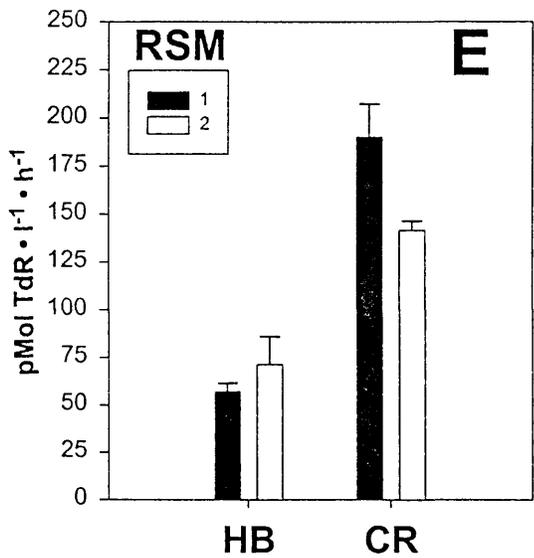
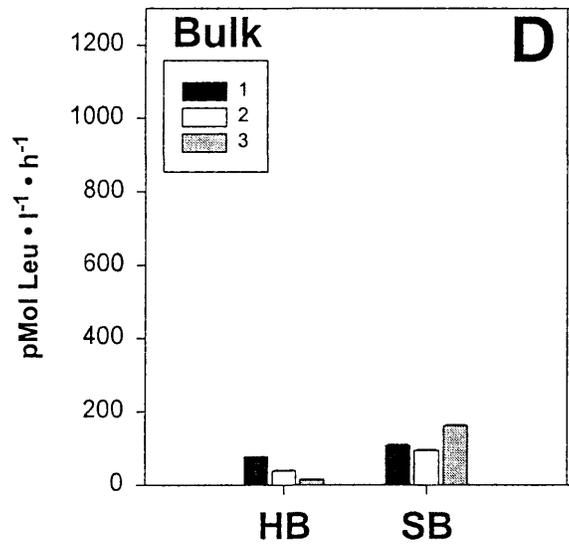
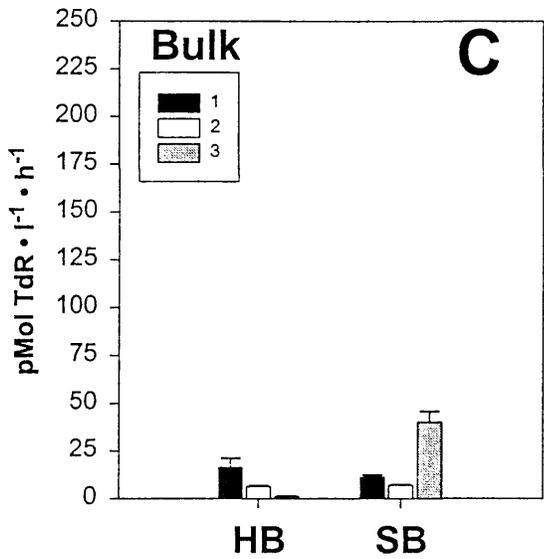
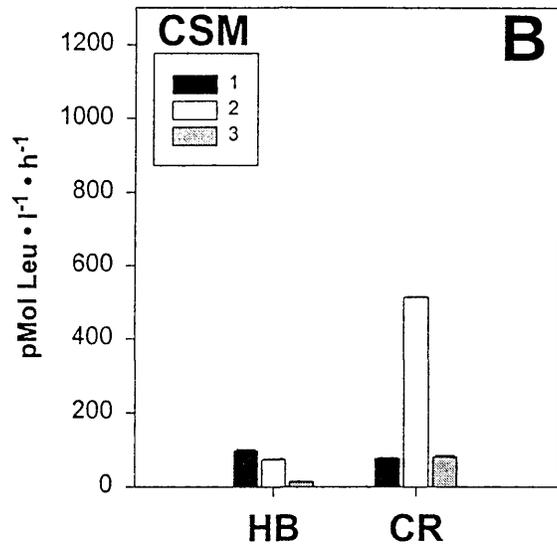
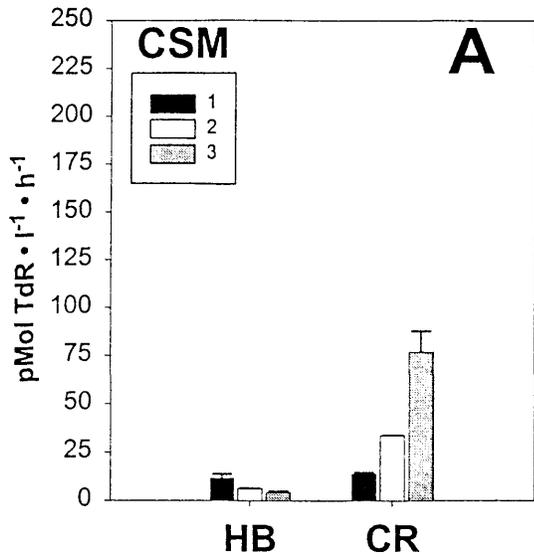


Figure 10. Variability of Thymidine (A, C, E) and Leucine (B, D, F) incorporation rates within a particular habitat (CSM, Bulk, RSM) across reef sites (HB, CR, SB) on different sampling dates (1,2,3).



Leu incorporation. This result suggests the importance of particle-attached bacteria to bacterial carbon production in the reef environment.

Absolute rates of Log_{10} Leu and Log_{10} TdR incorporation by heterotrophic reef bacteria were significantly correlated ($p < 0.001$, $n = 39$) to one another (Model II Regression). Samples were compared on a Log-Log scale to homogenize the variances in the Leu and TdR incorporation data. Each rate estimate represents the average of triplicate determinations on freshly collected samples from the reef environment (**Fig. 11**). The slope of this log-log relationship was 0.991, which yields a Leu to TdR incorporation ratio of 9.79.

DISSOLVED ORGANIC CARBON

Time-zero DOC concentrations (**Fig. 12**) in undiluted reef-water cultures were assumed to be indicative of *in situ* DOC concentration. The average DOC concentration of T-0 reef-water samples (undiluted cultures) from the 1997 conversion-factor experiments (SB1, SB2 and JS) was $64.0 (\pm 1.1) \mu\text{M C}$. Dissolved organic carbon samples collected from various reef-sites during the 1998 field season displayed a decreasing concentration gradient away from the coral surface-microlayer (CSM) to the Bulk reef-water (**Fig. 13**). The average DOC concentration of Bulk reef-water was $69.8 (\pm 4.7) \mu\text{M}$ ($n = 5$), while DOC in the CSM was $109.1 (\pm 27.8, \text{SD}) \mu\text{M}$ ($n = 5$). The greatest difference in DOC concentration between Bulk and CSM water occurred at the HB and CR sites with DOC concentration at the CSM, 67.1 and 70.5 μM greater than the Bulk water, respectively. The smallest difference in DOC concentration between

Figure 11. Model II linear regression of log Leucine versus log Thymidine indicating an incorporation ratio (Leu/TdR) of 9.79, $n=39$. Data points represent TdR and Leu bioassays from field samples only.

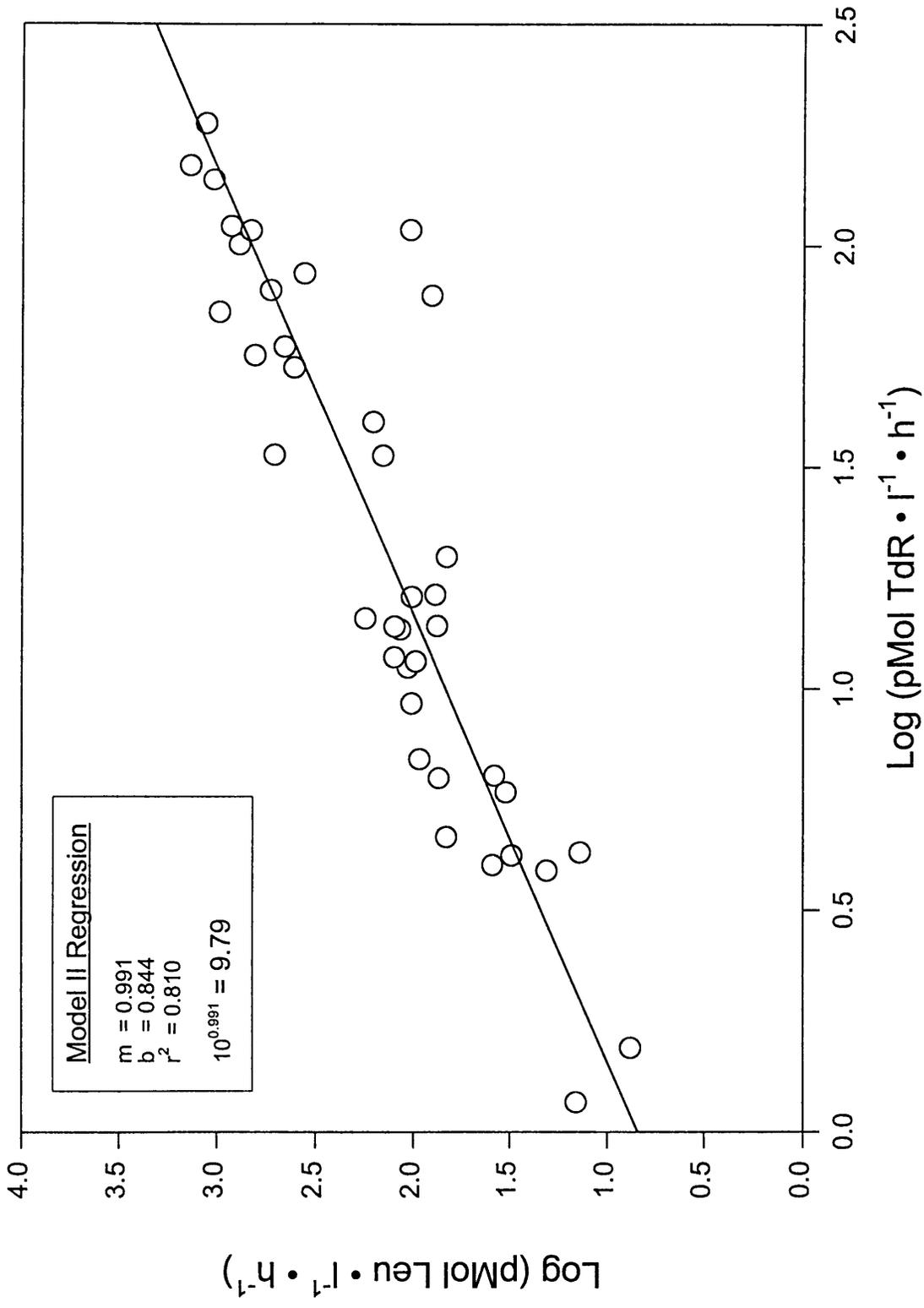


Figure 12. Dissolved Organic Carbon (DOC) change in dilution cultures. Error bars (JS, HB, and CR) represent \pm SD on triplicate determinations.

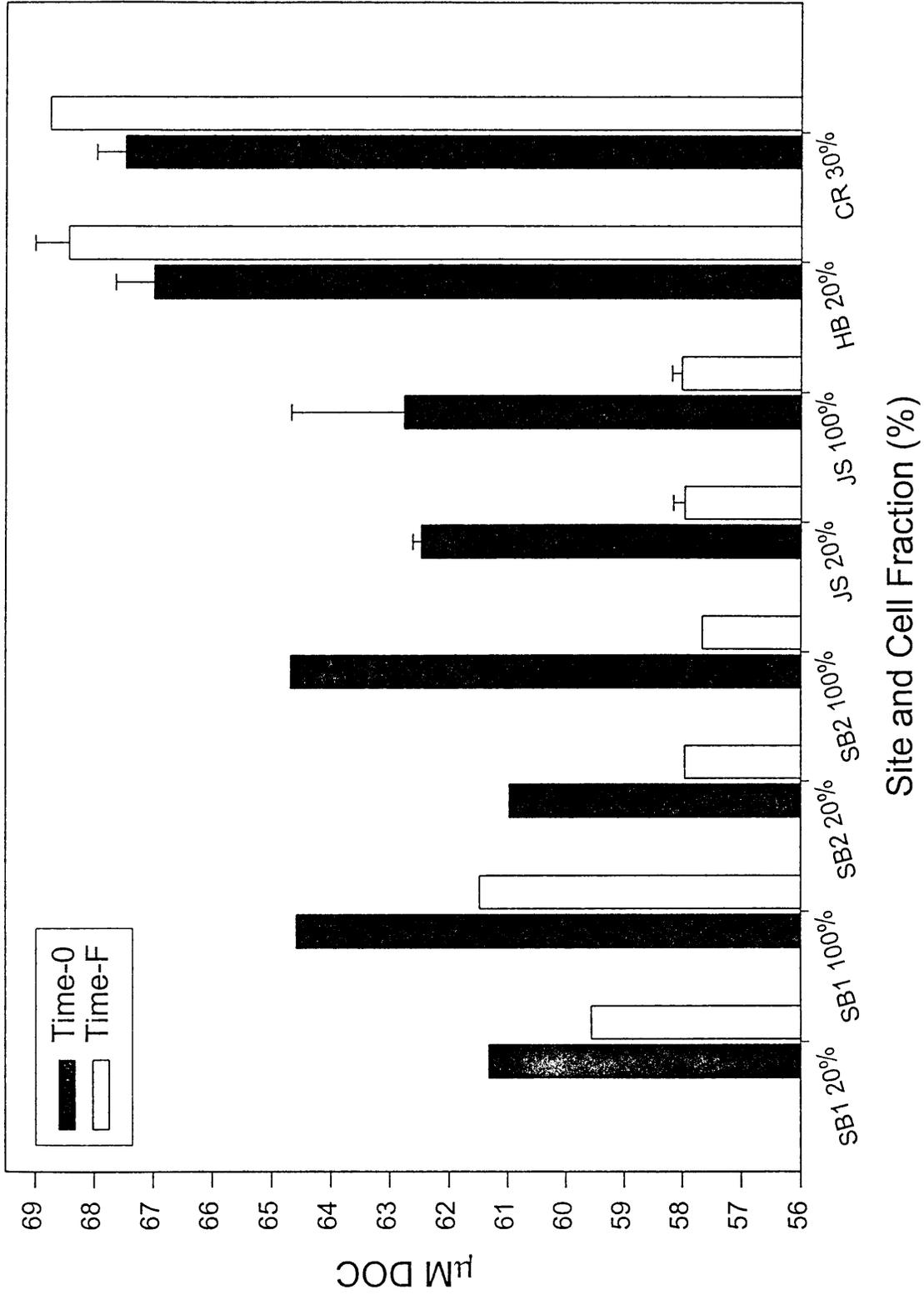
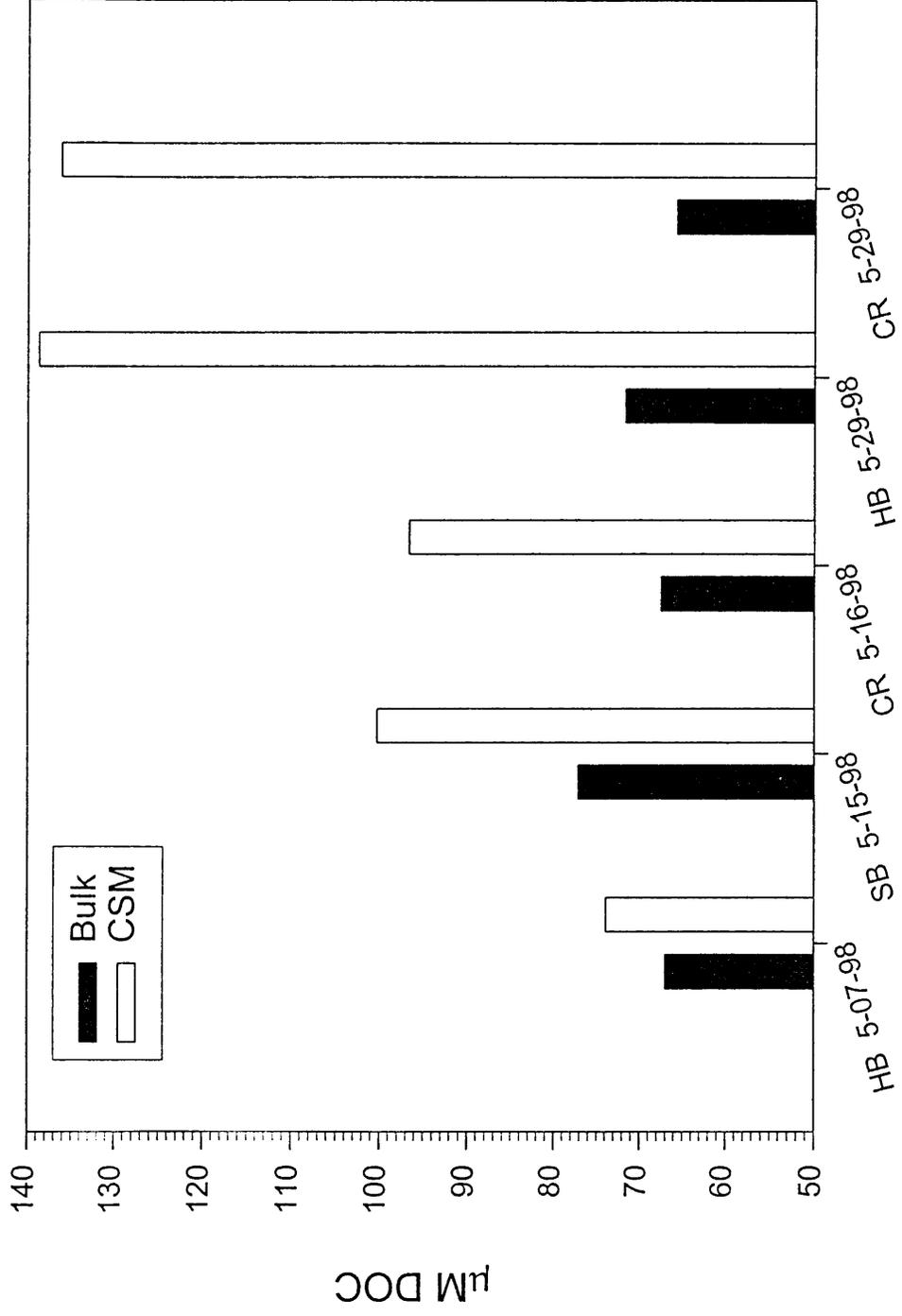


Figure 13. Dissolved Organic Carbon (DOC) in Bulk reef water (filled bars) and in Coral Surface Microlayer (CSM) water (open bars) showing enhanced DOC concentration at the coral surface.



Site and Date of Collection

habitats at a particular site also occurred at HB, with a 6.9 μM DOC enhancement in the CSM.

DEPTH GRADIENT

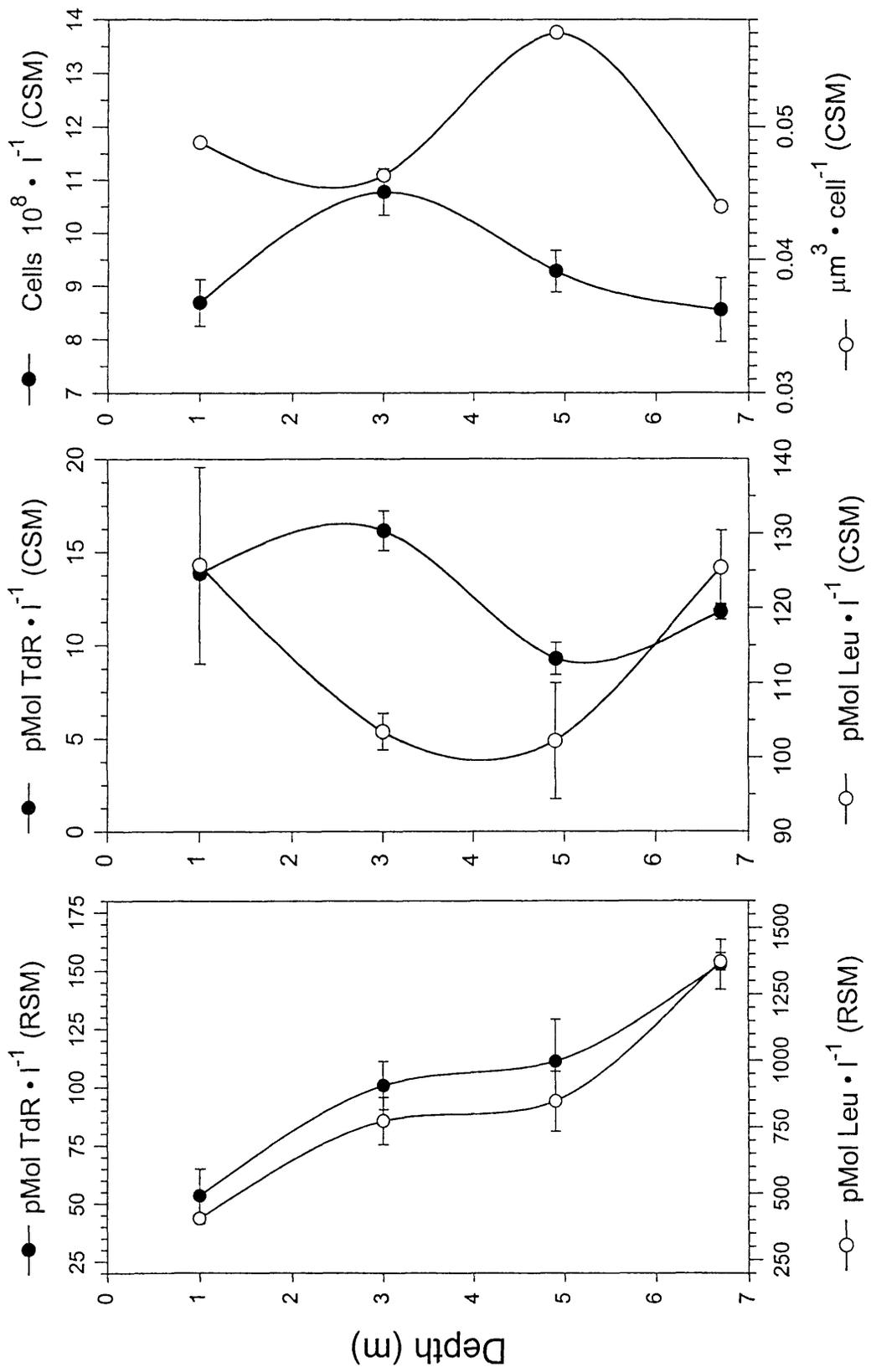
Bacteria sampled from the CSM of a reef in the middle of Castle Harbor (CH) showed no discernible differences with depth (1 - 7 m) for abundance, TdR incorporation, Leu incorporation or biovolume (**Fig. 14**). Comparison of the relative rates of TdR and Leu incorporation from RSM with those in adjacent CSM suggests high bacterial abundance and activity in the RSM. Thymidine and Leu incorporation rates of RSM samples were approximately 4 -10 \times greater than corresponding CSM samples collected at the same depth (**Fig. 14**). The one trend visible in the RSM data set is the suggestion of increasing TdR and Leu rates with depth. Detritus on the RSM of relatively shallower sites was not as densely distributed, contributing to syringe samples with lower percentages of attached bacteria (visual estimate). Increasing rates of TdR and Leu incorporation with depth could therefore be the result of a greater amount of detrital accumulation at the base of the reef, providing a flocculent matrix for the development of a concentrated microbial community.

EXPERIMENTAL RESULTS

CELL CULTURE EXPERIMENTS

Cell abundance increased for at least the initial 48 hours in all diluted cultures, indicating that bacterial growth and cell division were not initially limited

Figure 14. Bacterial properties at the Coral Surface Microlayer (CSM) and the Reef Surface Microlayer (RSM) in Castle Harbor across a 7m depth gradient. Error bars represent \pm SD. No data for RSM cell abundance or biovolume due to interference by detrital material.



by the supply of dissolved organic matter or nutrients. Rates of TdR incorporation (**Fig. 15**) generally followed trends in bacterial abundance (**Fig. 16**) in diluted cultures, where cells grew at a lower bacteriovore grazing pressure than that experienced by cells in undiluted culture. The trend towards balanced growth was not perfect, e.g. incorporation rates sometimes leveled off or declined (SB1, HB and CR) while bacterial abundance continued to increase. Two experiments (SB2-20% and JS-20%) demonstrated a lag-phase in TdR incorporation, while bacterial abundance doubled, before incorporation increased exponentially over the later time-intervals. One experiment (CR-30%) displayed completely unbalanced growth, with bacterial abundance increasing by 37% between T-0 and T-3, while TdR and Leu rates declined by 68% and 42% respectively. After 18 h, rates of incorporation declined exponentially in this experiment, followed by a similar trend in bacterial abundance two days later. The incorporation of Leu in diluted cultures (**Fig. 17**) followed trends in bacterial abundance over the first three to four time-points but declined (SB1 and HB) or oscillated (SB2 and JS) thereafter. The oscillation between peak Leu incorporation rates in SB2 and JS was 2.2 and 2.4 days respectively, which is nearly identical to the experimentally determined turnover times (2.4 and 2.6 days) for bacterial cells in these cultures.

Undiluted (100%) treatments of SB1 and SB2 increased in TdR incorporation rate (**Fig. 15**) over the initial time-interval, then steadily declined for the rest of the incubation. Bacterial abundance (**Fig. 15**) continued to increase, for an additional 1.5 days (SB1) and 1 day (SB2) before reaching stationary

Figure 15. Thymidine incorporation rates (\pm SD) in cell culture experiments. Experiments began on the following dates: Seabright one (SB1), July 9, 1997; Seabright two (SB2), July 17, 1997; John Smith's (JS), July 23, 1997; Hog Breakers (HB), May 7, 1998; Crescent Reef (CR), May 17, 1998.

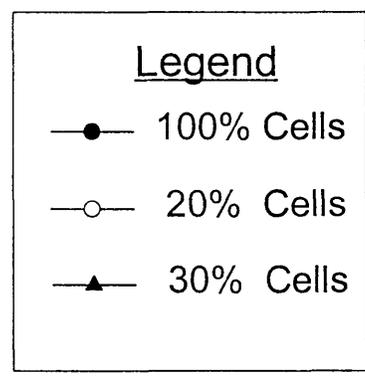
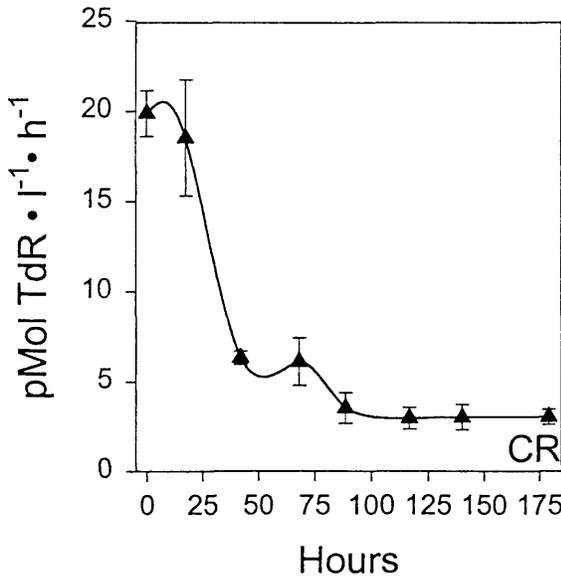
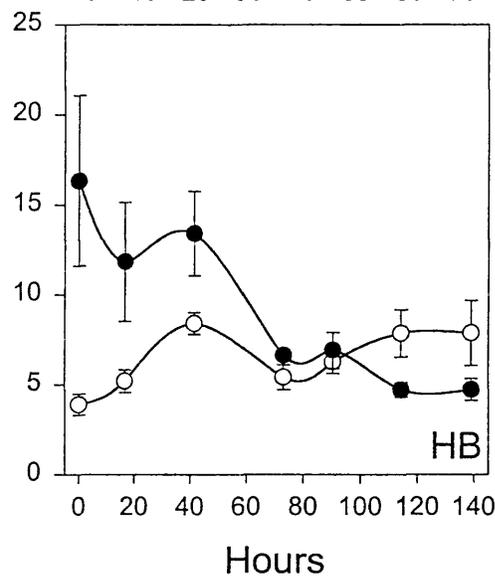
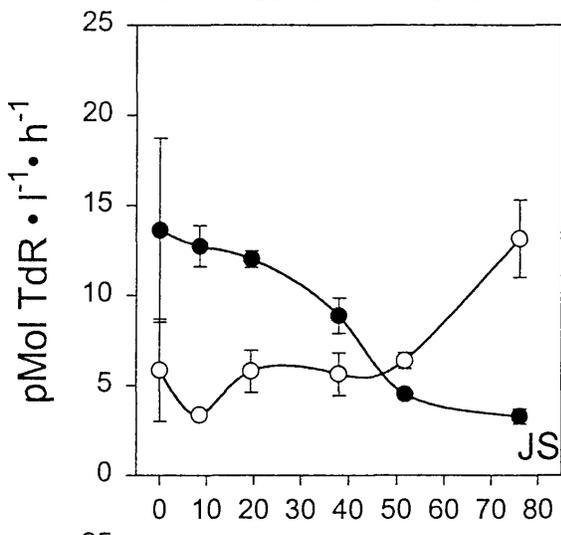
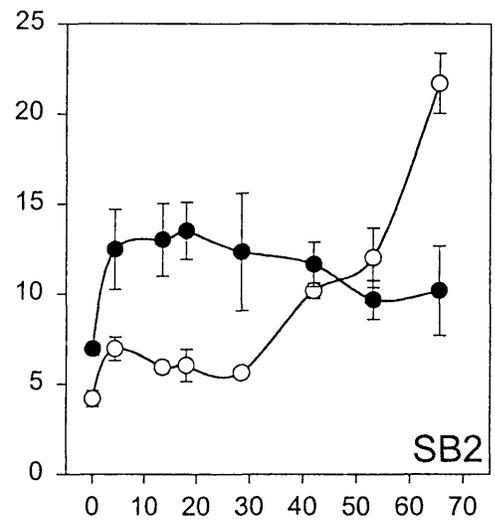
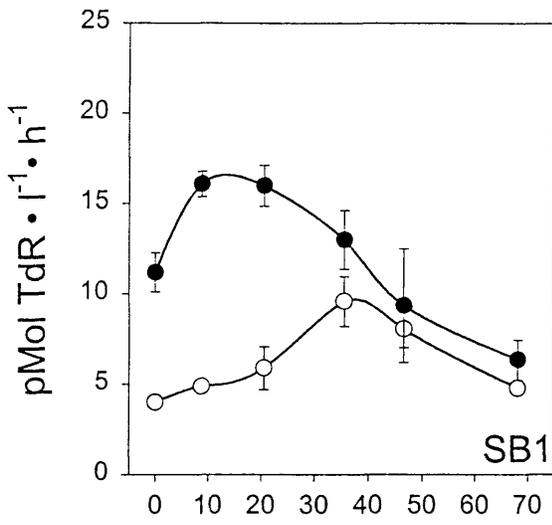


Figure 16. Bacterial abundance (\pm SD) in cell culture experiments. Experiments began on the following dates: Seabright one (SB1), July 9, 1997; Seabright two (SB2), July 17, 1997; John Smith's (JS), July 23, 1997; Hog Breakers (HB), May 7, 1998; Crescent Reef (CR), May 17, 1998.

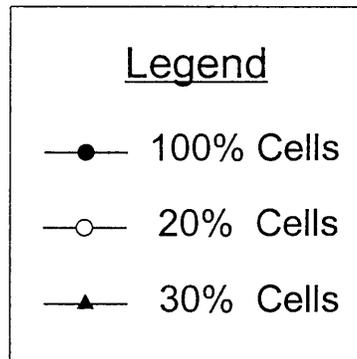
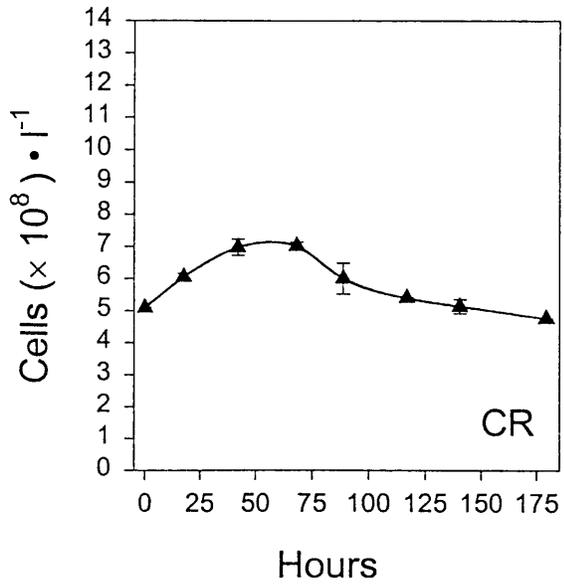
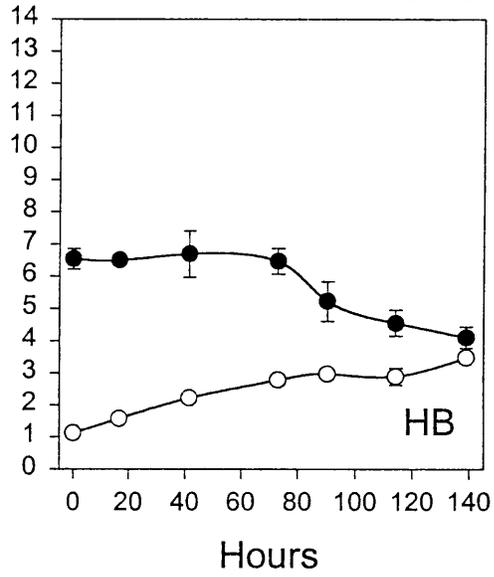
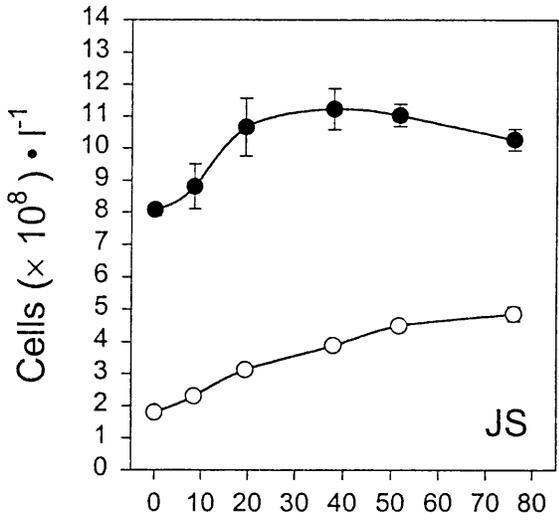
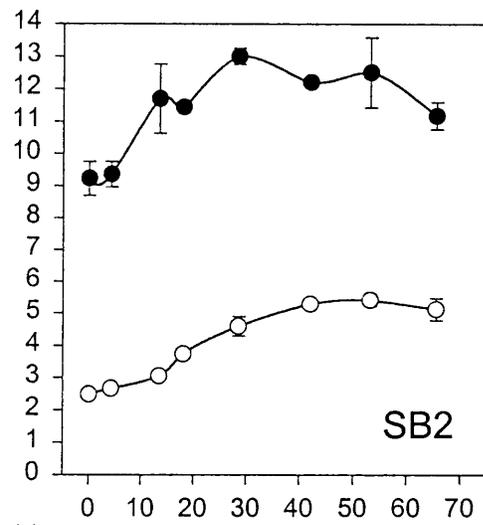
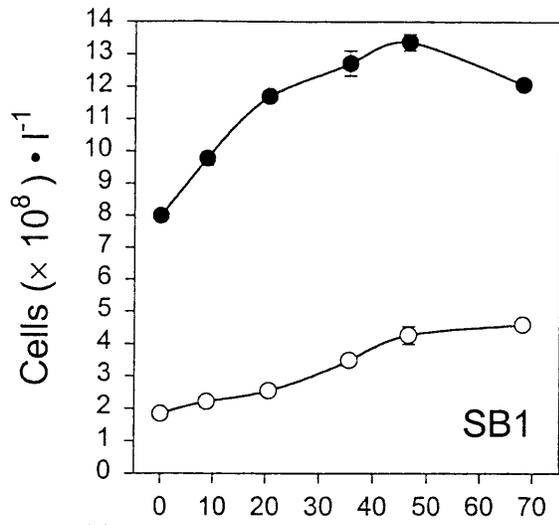
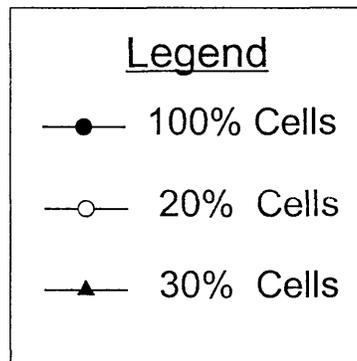
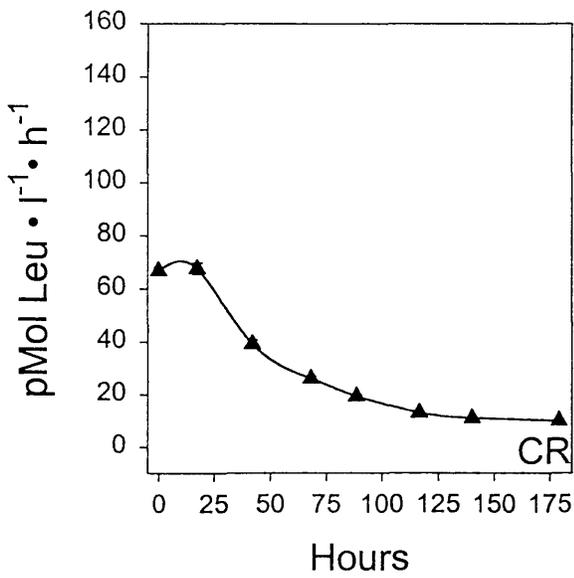
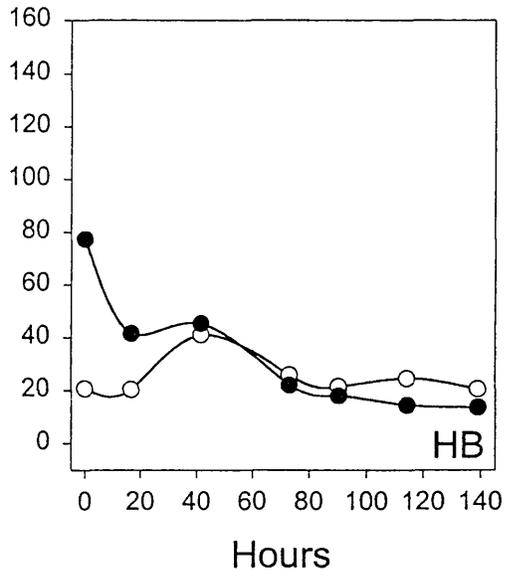
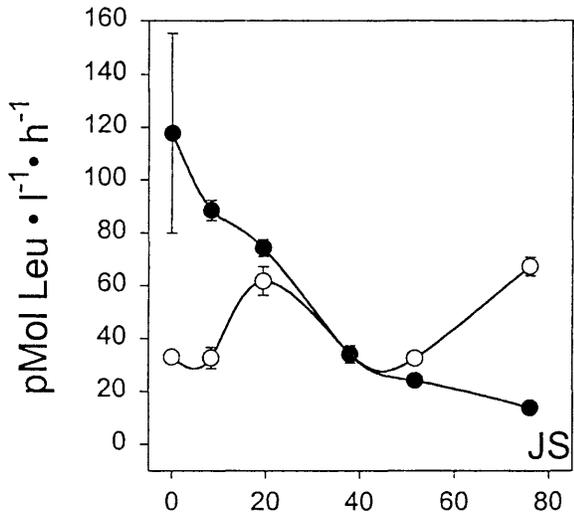
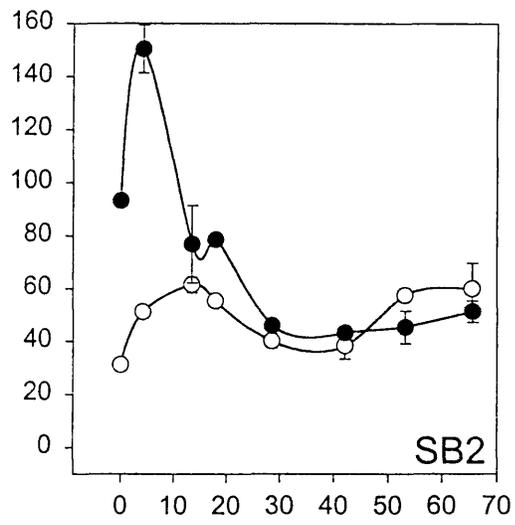
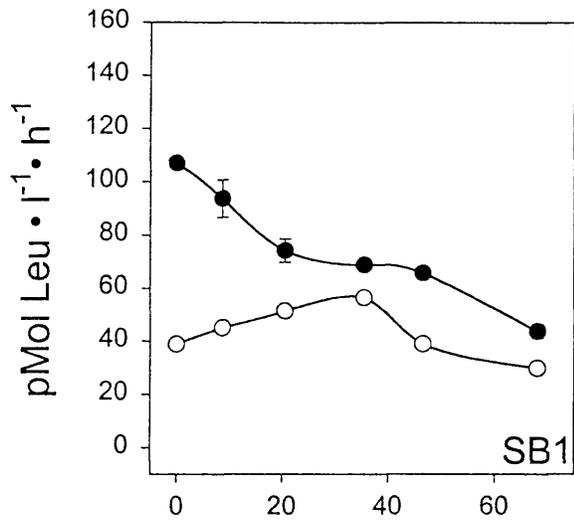


Figure 17. Leucine incorporation rates (\pm SD) in cell culture experiments. Experiments began on the following dates: Seabright one (SB1), July 9, 1997; Seabright two (SB2), July 17, 1997; John Smith's (JS), July 23, 1997; Hog Breakers (HB), May 7, 1998; Crescent Reef (CR), May 17, 1998.



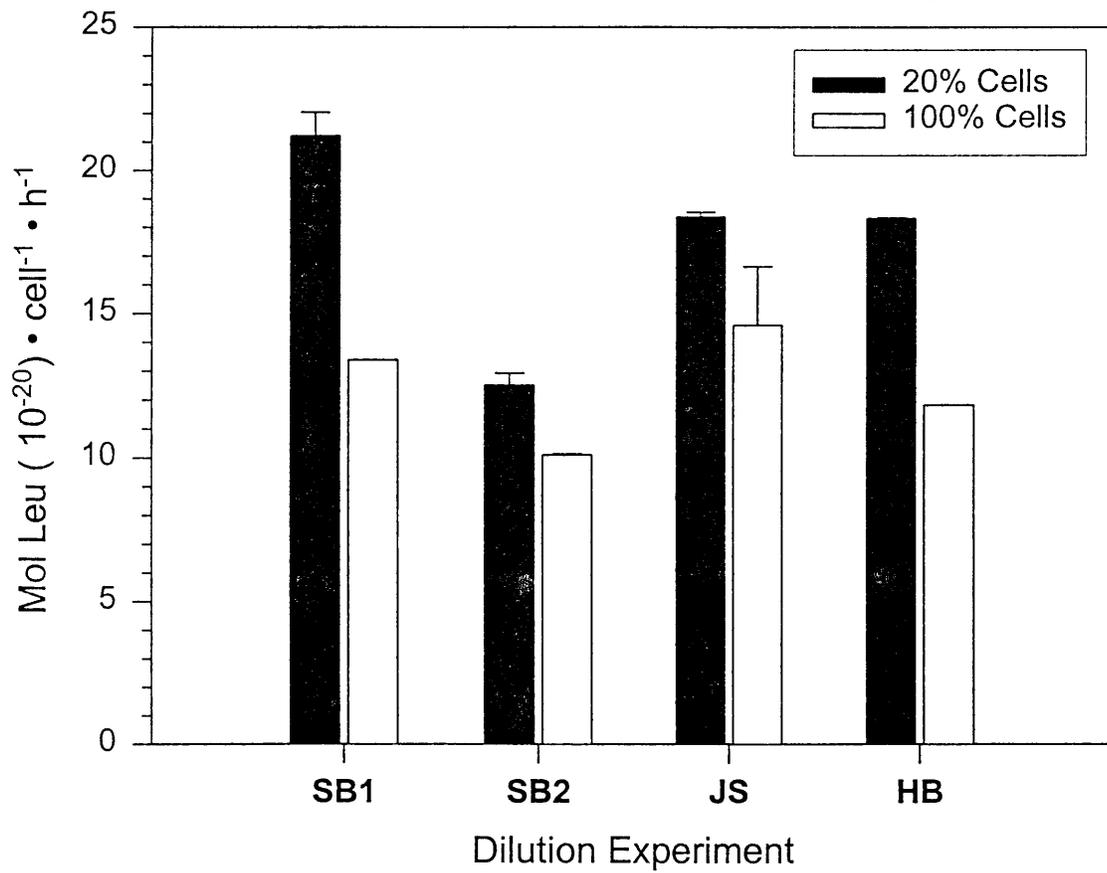
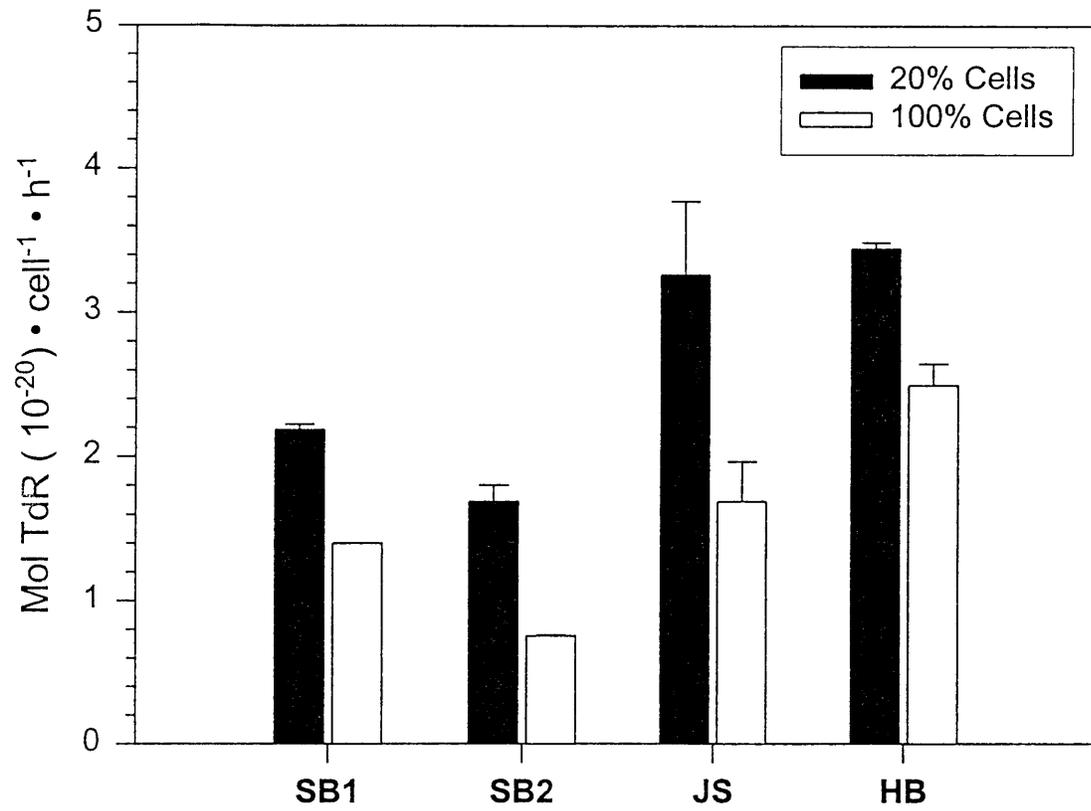
phase. The other 100% cultures (JS and HB) exhibited their highest TdR incorporation rates at T-0, followed by precipitous decline. Although the undiluted SB2 cells showed a dramatic increase in Leu rate over the first time interval (similar to TdR) the general trend for Leu in all other undiluted cultures was declining rate of incorporation with time (**Fig. 17**).

Both TdR and Leu showed higher cell-specific rates of incorporation in 20% T-0 cultures (SB1, SB2, JS, and HB) than those calculated for the respective 100% T-0 cultures (**Fig. 18**). The cell-specific rates of TdR incorporation in T-0 100%-culture samples were 44 - 72% of T-0 20%-culture samples, while T-0 100%-culture Leu incorporation rates were 65 - 81% of the 20%-culture samples. This result was somewhat perplexing since initial DOC concentration tended to be lower in 20% cultures than corresponding 100% cultures (**Fig. 12**), possibly indicating some other control besides DOC supply on precursor incorporation rate between the two treatments. It suggests that dilution may affect bacterial growth rates due to a decrease in cell to cell competition for ambient DOC (same amount of DOC, lower cell density in culture) or that cell-specific DOC removal was uncoupled from growth.

CONVERSION FACTORS

Methods for calculating conversion factors (CF) using cell abundance or cell biovolume estimates and rates of substrate incorporation vary greatly in the literature. I have chosen to use the model free (exponential cell growth not assumed) cumulative approach of Bjørnsen and Kuparinen (1991). This method

Figure 18. Cell-specific Thymidine and Leucine incorporation rates (\pm % error) at T-0 of various dilution experiments.



uses a Model II linear regression to relate cumulative positive changes in bacterial abundance to cumulative integrated thymidine (**Fig. 19**) and leucine (**Fig. 20**) incorporation. An assumption of the cumulative approach is that changes in cell abundance must be positive between all time points over which the factor is calculated and that removal of cells is not a significant fraction of the growth term. For this reason, it was only possible to calculate factors for the diluted (20 - 30% of *in situ* cell abundance) cultures. The slopes of Model II Regressions (**Fig. 19, Fig. 20**) for cumulative cell production vs. cumulative integrated TdR and Leu incorporation are the conversion factors (**Table 3**) for the isotope-based bacterial production measurements.

Empirical determinations of TdR and Leu conversion factors were uniformly low across the reef platform (**Table 3**). Values for thymidine ranged from 2.3×10^{17} cells \cdot Mol TdR⁻¹ at the CR site in May of 1998 (22 °C) to 8.3×10^{17} cells \cdot Mol TdR⁻¹ at the SB site in July of 1997 (28 °C). The lowest leucine conversion factor, 5.0×10^{16} cells \cdot Mol Leu⁻¹, was also detected during May of 1998 (22 °C) at the CR site with the highest estimate, 1.30×10^{17} cells \cdot Mol Leu⁻¹, also occurring at the SB site in July of 1997. The average TdR-CF measured at the Bermudian reef sites was $5.0 (\pm 2.5, \text{SD}) \times 10^{17}$ cells \cdot Mol TdR⁻¹, and the Leu-CF averaged $8.6 (\pm 3.3, \text{SD}) \times 10^{16}$ cells \cdot Mol Leu⁻¹.

Conversion factor data were analyzed by performing an ANCOVA followed by pair-wise Tukey Tests to compare the regression coefficients (the conversion factors) of the individual experiments (**Table 3**). Results of this

Figure 19. Model II linear regressions of cumulative cell production versus cumulative integrated Thymidine incorporation for diluted cell cultures (20-30%) from the various study sites. Thymidine conversion factors equal the slopes of the individual regression lines.

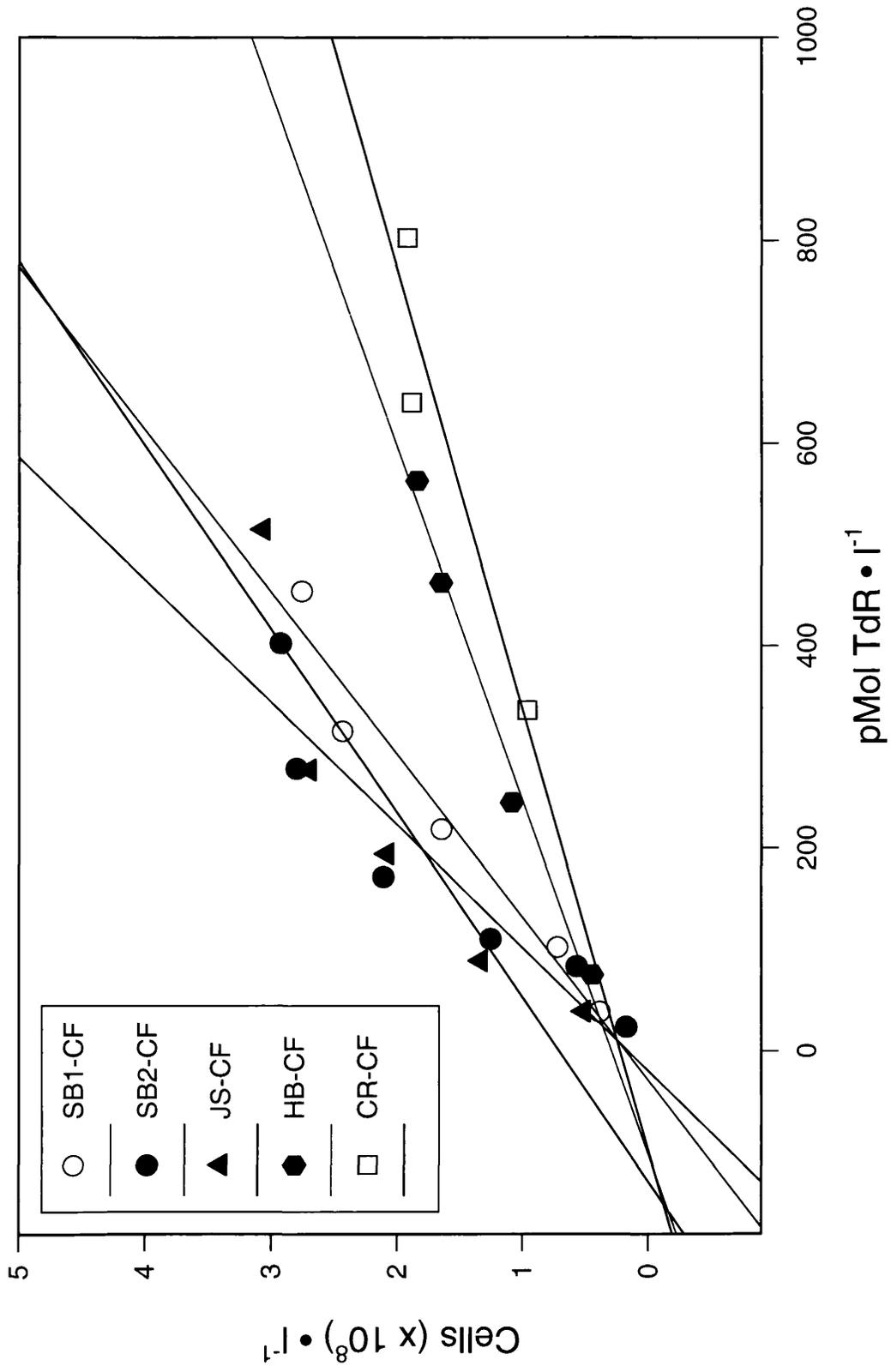
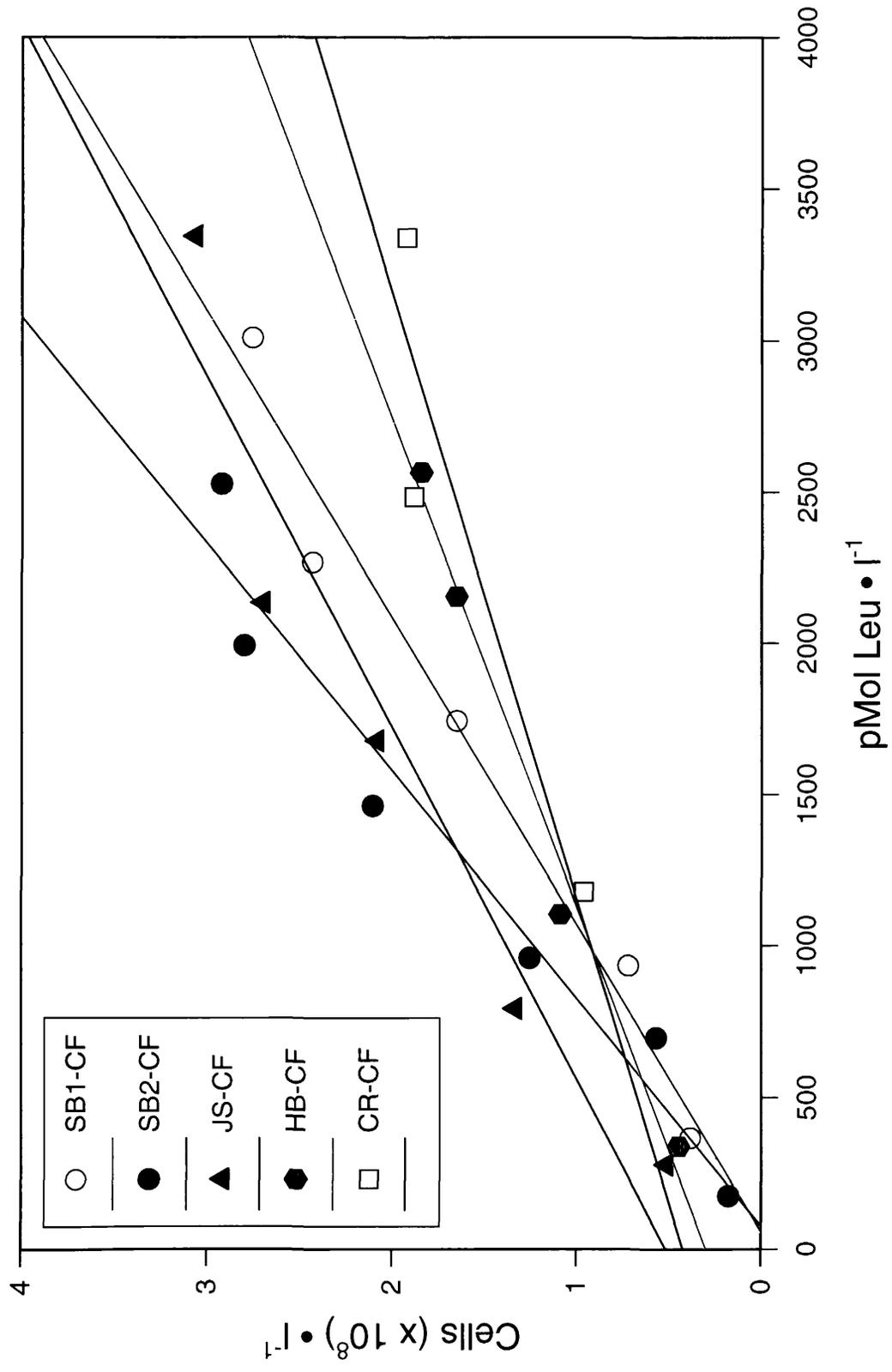


Figure 20. Model II linear regressions of cumulative cell production versus cumulative integrated Leucine incorporation for diluted cell cultures (20-30%) from the various study sites. Leucine conversion factors equal the slopes of the individual regression lines.

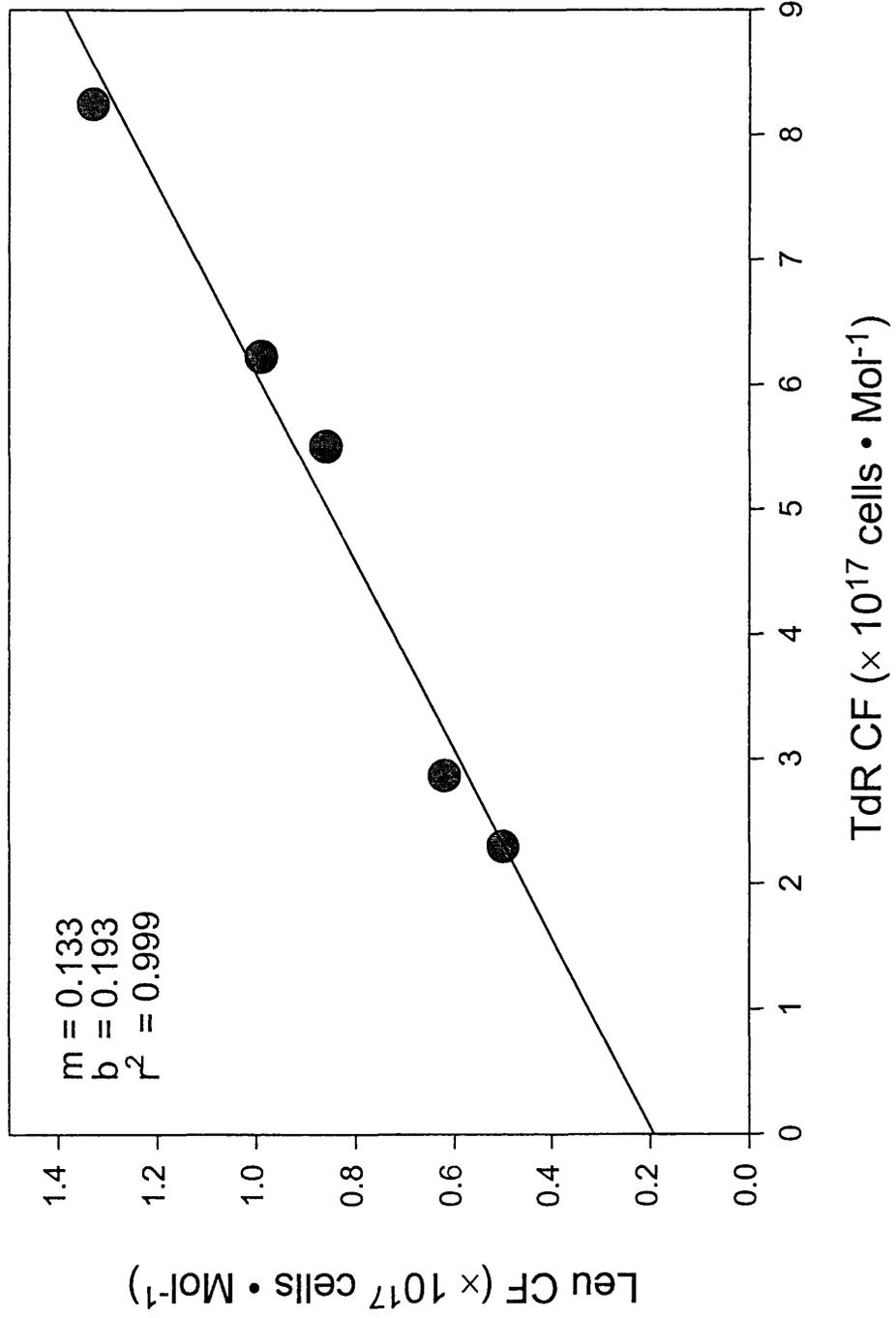


statistical comparison were identical for both thymidine and leucine within a site. Both TdR and Leu conversion factors were statistically indistinguishable ($p > 0.05$) for the three south shore experiments (SB1, SB2 and JS), the two north shore stations (HB and CR) and the three non-outfall stations (JS, HB, CR). The conversion factors from the three south shore experiments were statistically different ($p < 0.05$) from the two north shore sites, and the three non-outfall stations were different ($p < 0.05$) from the outfall samples. Model II regression of the Leu conversion factors vs. the corresponding TdR conversion factors (**Fig. 21**) demonstrated that the ratio of Leu-CF to TdR-CF is consistent across the Island platform, suggesting that cell growth (Leu incorporation) is balanced to cell division (TdR incorporation) over the time scale of several days.

DILUTION-GRAZING EXPERIMENTS

Grazing by bacteriovores appears to be a significant component of the bacterial removal term in the reef environment. Bacterial abundance in undiluted (100%) cell cultures increased (SB1, SB2, and JS) for a shorter time-interval than diluted cultures or steadily declined (HB) over time (**Fig. 16**) showing that removal exceeded growth rates. Regardless of dilution factor, cell abundance in all culture experiments (except 100% HB) increased over several sampling intervals. Undiluted cultures in all of the July 1997 experiments (SB1, SB2 and JS) demonstrated initial increases in bacterial abundance (40 - 70% change) over the first 24 - 48 hours, followed by a period of leveling off and gradual decline (**Fig. 16**). Changes in the bacterial abundance of 20% cultures were

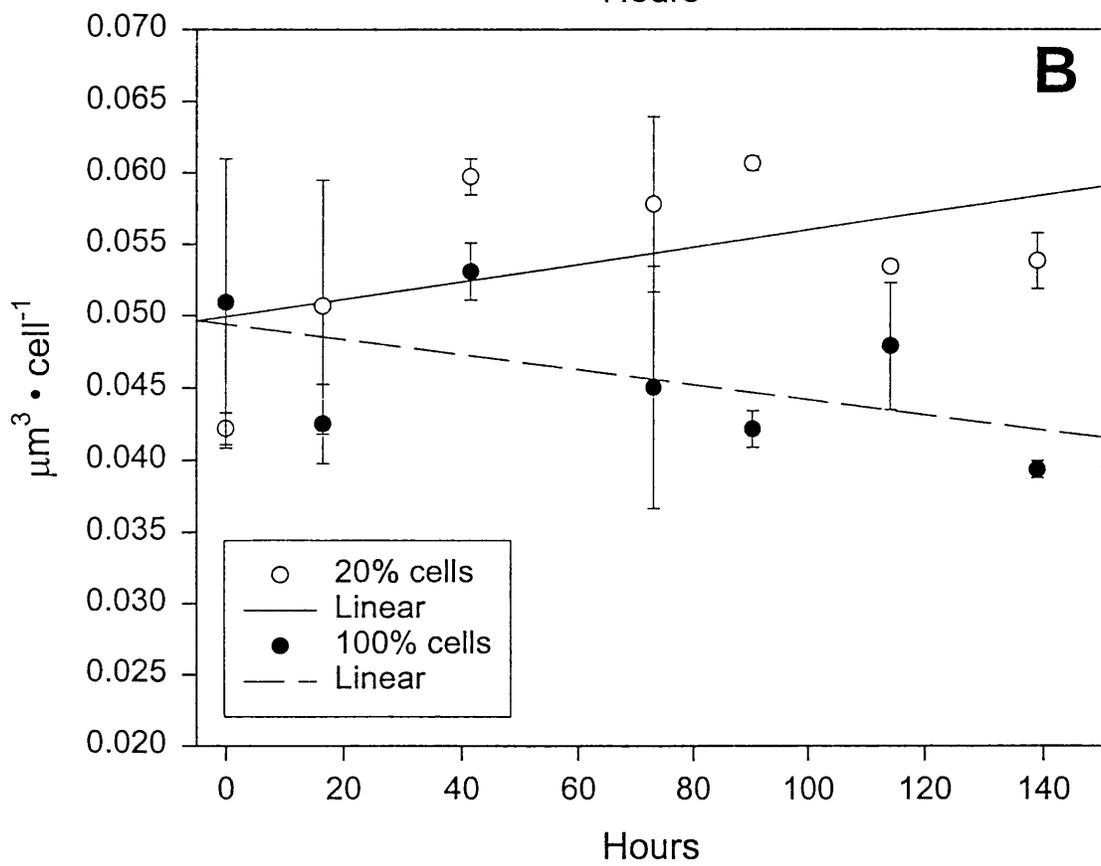
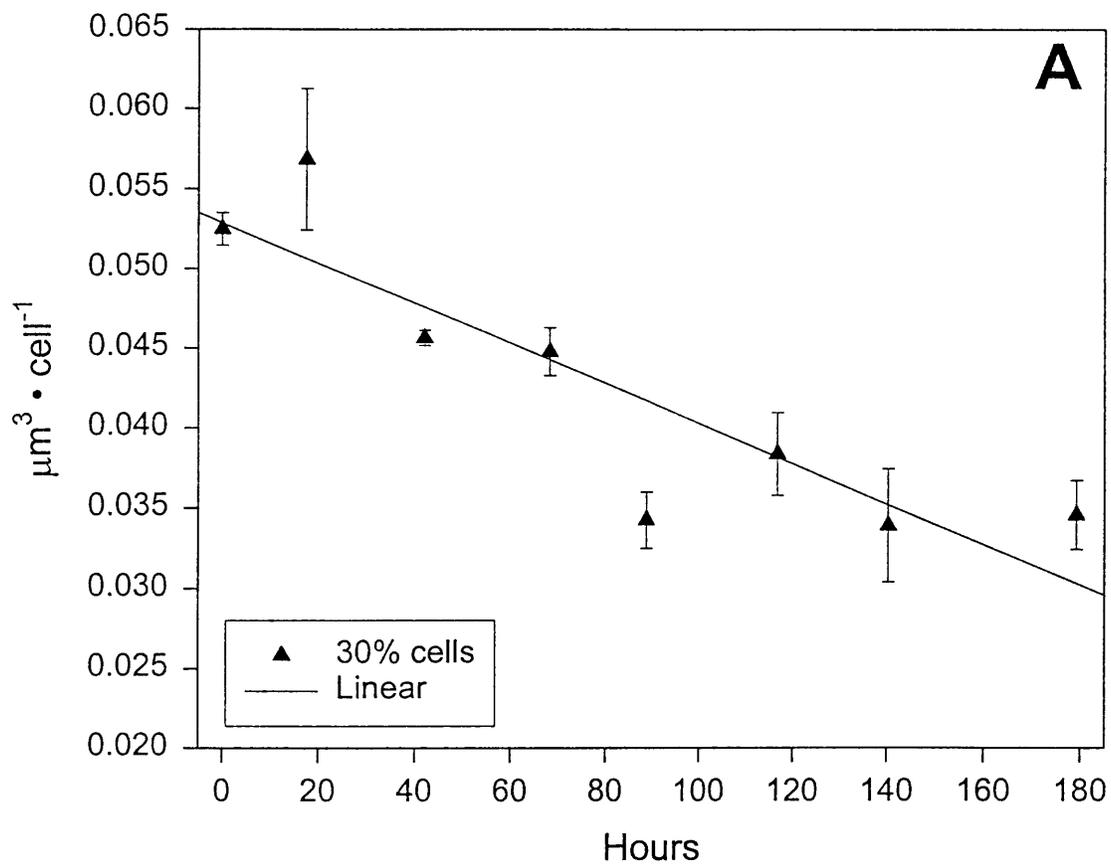
Figure 21. Model II linear regression of Leucine versus Thymidine conversion factors from dilution cultures (20-30% cells).



always positive and ranged from 100% to 150% of initial abundance over the time-course of the experiments. Experiments performed during May of 1998 (HB and CR) showed both positive and negative trends in bacterial abundance over time. The 100% cell culture of HB declined in bacterial abundance by 37% over initial values, while the bacterial abundance of the 20% culture increased by over 200%. The single 30% culture comprising the CR experiment increased in bacterial abundance by 38% for the initial 48 hours, before declining to less than its starting cell concentration 132 hours later.

In the two dilution-culture experiments (HB and CR) for which cell volume was extensively monitored, average cell biovolume tended to either decline through the time-course of the experiment (CR) or remain stable (HB) compared to initial time-point cell volume determinations. CR cell volume in culture was monitored for a total time interval of 179 hours, extending well beyond the initial 68 hour interval used to determine TdR-CF and Leu-CF. Over one week, CR cells declined from 0.053 to 0.035 μm^3 , with a significant ($p < 0.05$) negative regression coefficient (**Fig. 22A**). When biovolume was examined over the initial three day interval, the regression coefficient was non significant ($p > 0.05$), indicating no change in cell size. Regression coefficients of biovolume vs. time (0 - 139 hours) for both 20% and 100% HB cultures (**Fig. 22B**) were not significantly different than zero ($p > 0.05$). This result indicates that factors regulating bacterial cell biovolume in both culture vessels, such as bacteriovore grazing (size selectivity), DOC and nutrient supply, were proportionately similar

Figure 22. Trends in bacterial cell-specific biovolume incubations. Hog Breakers 20% and 100% cultures (A) and Crescent Reef 30% culture (B). Both slopes for Hog Breakers cultures NS ($p > 0.05$), while slope for Crescent Reef is significant ($p < 0.05$).



between the two treatments. Furthermore, it suggests that the filtration and dilution process did not disproportionately affect cell size in the 20% culture compared to the relatively unperturbed 100% culture.

Bacterial growth rates (μ) and the bacteriovore grazing rates (g) were simultaneously estimated for each dilution experiment for which parallel dilution (20% and 100% *in situ* cell abundance) cultures were monitored (**Fig. 23**). The specific bacterial growth rate μ (d^{-1}) was very consistent across the four experiments ranging from $0.34 \cdot d^{-1}$ to $0.40 \cdot d^{-1}$ while g varied by a factor of two, from $0.22 \cdot d^{-1}$ to $0.40 \cdot d^{-1}$ (**Table 4**). The relatively consistent growth rates yielded turnover times (μ^{-1}) of intermediate length, ranging from 2.5 to 3 days. Bacterial cell growth was most closely balanced by grazing rate at JS ($\mu / g = 1.2$) and HB ($\mu / g = 0.9$). The two dilution-grazing experiments conducted with Bulk reef-water collected near the Seabright sewage outfall (SB1 and SB2) were the most imbalanced with respect to the ratio of growth to grazing (1.7 and 1.5 respectively). Growth and grazing rates derived from the four independent regressions could not be compared statistically since only two cell fractions with single apparent growth rate estimates were available for each experiment. When the dilution experiment data were pooled into one data-set the common regression was significant ($p < 0.05$) and indicated a growth rate of $0.37 (\pm 0.05, SD) \cdot d^{-1}$, with a grazing rate of $0.30 (\pm 0.06, SD) \cdot d^{-1}$ (**Fig. 24**). In general, this data suggests a balanced microbial loop in the water-column of the reef environment. Increases in cell abundance were observed in several of the 100%

Figure 23. Apparent change in bacterial abundance (Y-axis) versus the fraction of cells in culture (20% cells and 100% cells). The slopes of the lines equal the bacteriovore grazing rate (h^{-1}) and the Y-intercepts equal the specific bacterial growth rates (h^{-1}).

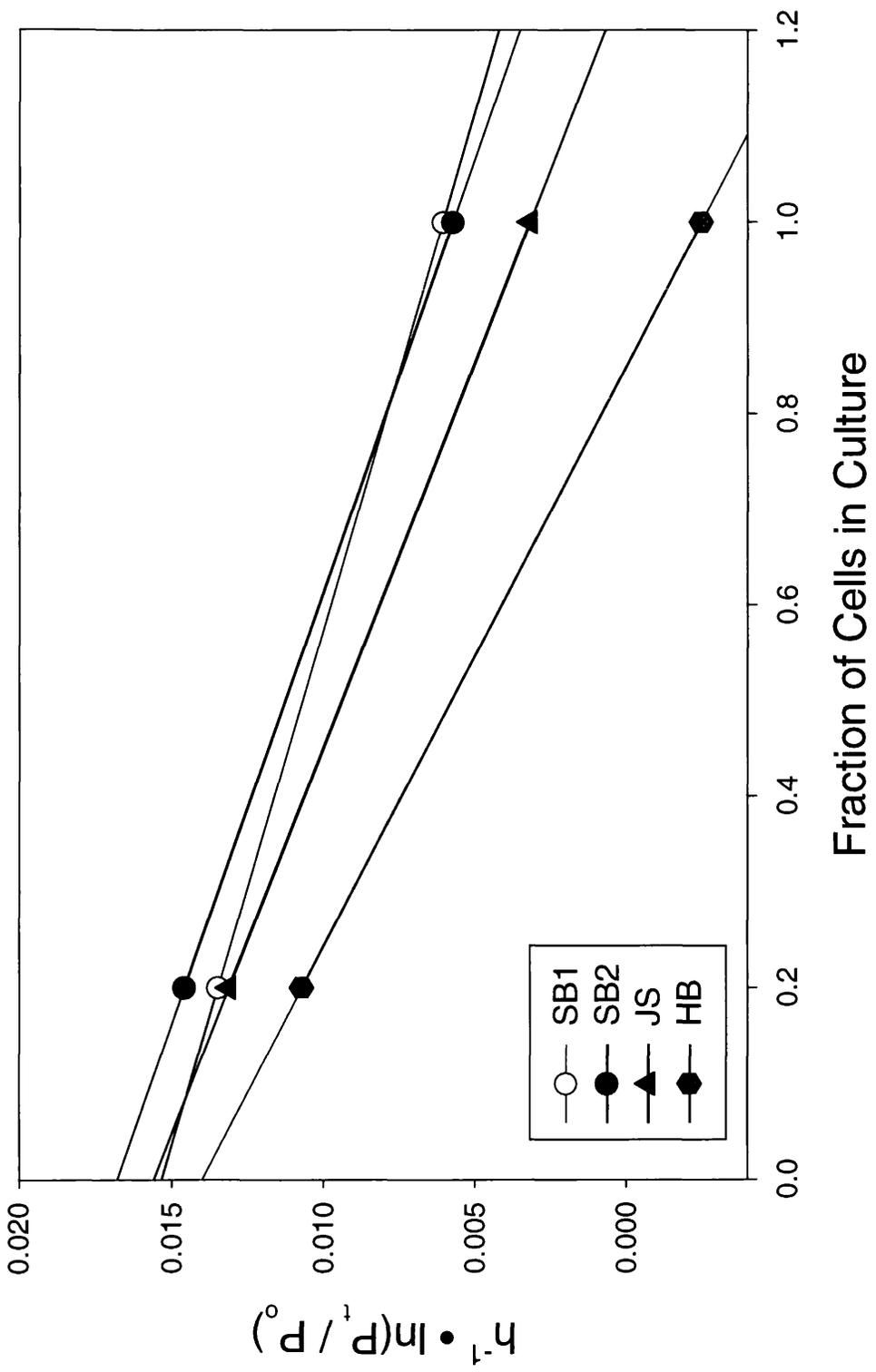
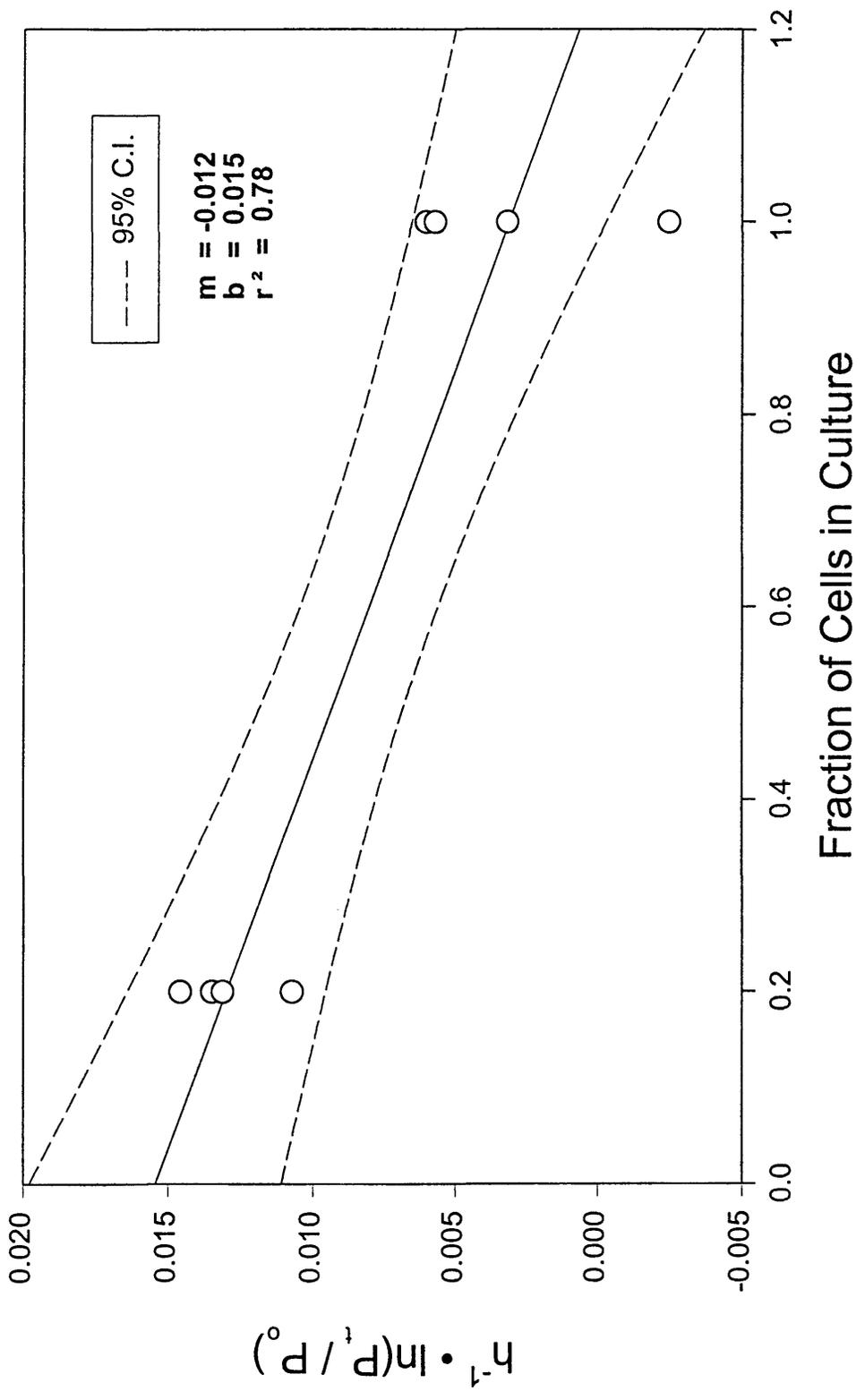


Table 4. Specific rates of bacterial growth (μ) and bacterivory (g), ratio of growth to bacterivory (μ/g) turnover time (tt) and generation time (gt) by the dilution technique. Turnover time equals μ^{-1} and generation time equals $\ln 2 \cdot \mu^{-1}$.

Site	Time (h)	μ (day^{-1})	g (day^{-1})	μ / g	tt (days)	gt (days)
SB1	68.1	0.37	0.22	1.7	2.7	1.9
SB2	53.2	0.40	0.27	1.5	2.5	1.7
JS	76.0	0.37	0.30	1.2	2.7	1.9
HB	90.3	0.34	0.40	0.9	2.9	2.0

Figure 24. Linear regression of grouped dilution data yielding the average bacterial growth (h^{-1}) and bacteriovore grazing (h^{-1}) rate for the four experiments. Dashed lines represent 95% confidence intervals.



cultures (SB1, SB2 and JS) indicating that the bacteriovore grazing rate was lower than the bacterial growth rate. However, while bacterial abundance increased by a factor of 2-3 in the 20% cultures, it only increased by a factor of 1.5 in 100% cultures over the time course of incubation.

SATURATION CURVES

Bacteria from the reef environment incorporated TdR (5-50 nM) and Leu (5-50 nM) at variable rates across the five sites. Saturation profiles for TdR and Leu were generated at T-0 of each reef-water culture experiment (**Fig. 25**) and with freshly collected syringe samples (**Fig. 26.**, **Fig. 27**) from various locations on the reef (Bulk, CSM, and RSM) at three of the sites (SB, HB, and CR). Incorporation rates were normalized to cell abundance for comparison across sites (**Fig. 25**, **Fig. 27**). The rate of bacterial TdR incorporation into macromolecules increased steadily through the tested concentration range, demonstrating an incorporation plateau only for SB1-CF (**Fig. 25**). For the HB-CF and CR-CF experiments, the concentration range was extended out to 75 nM TdR (not shown), with bacteria still increasing their rates of incorporation. Maximal Leu incorporation rates were generally achieved at the lower end (5-10 nM) of the saturation curves. Two curves (HB and NS) did demonstrate a Michaelis-Menten type (rectangular hyperbola) relationship from 5-30 nM but jumped from the plateau rate between 10 and 30 nM to a greater cell-specific rate at 50 nM.

When saturation curves were compared at different points in the SB

Figure 25. Cell-specific Thymidine (A) and Leucine (B) saturation curves measured at T-0 from 100% cell cultures (SB1, SB2, JS, NS and HB) and the single 30% cell culture (CR).

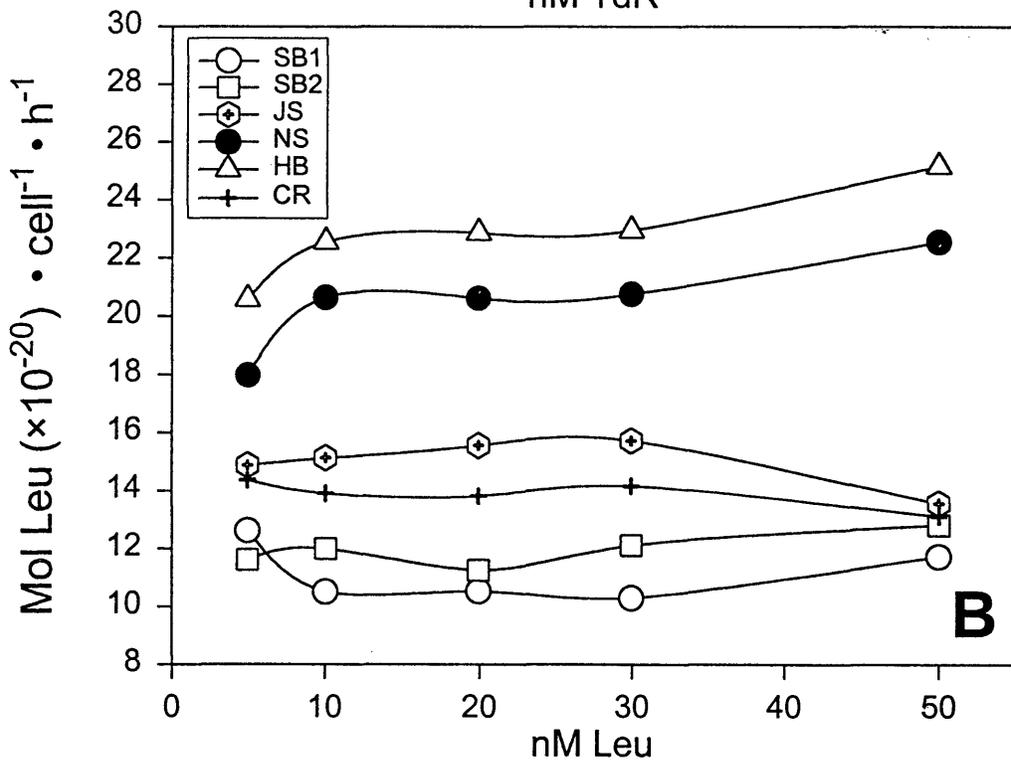
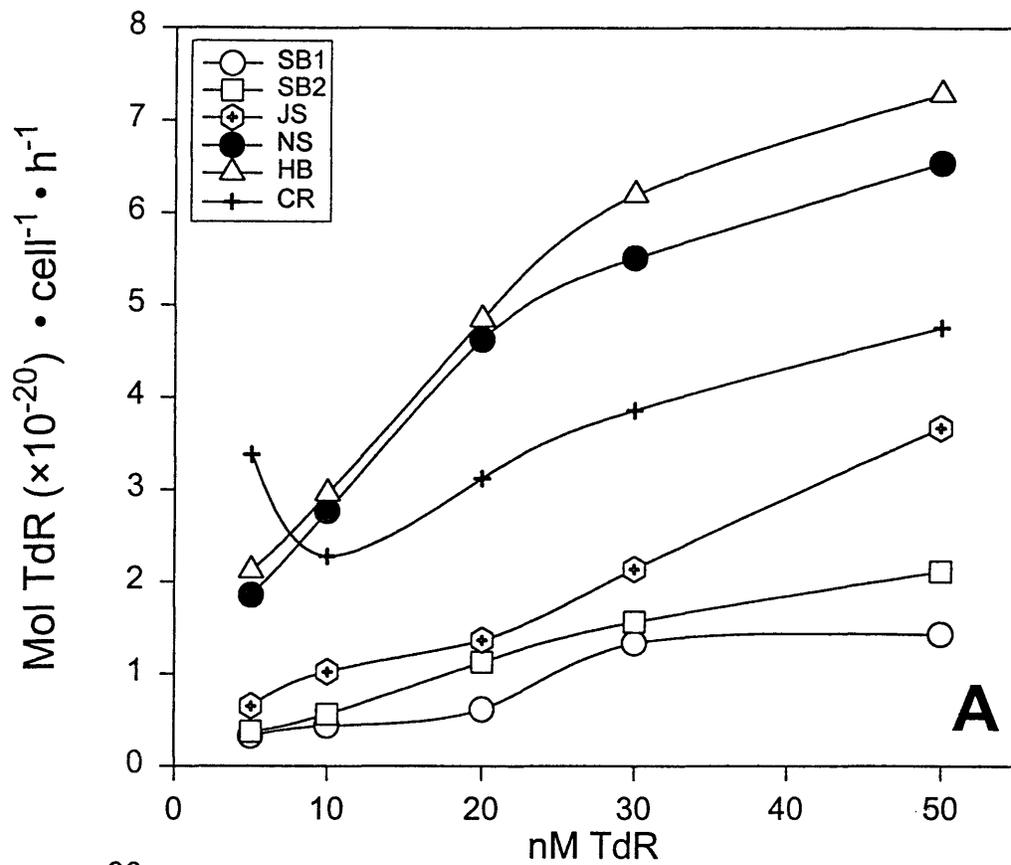


Figure 26. Absolute rates of bacterial Thymidine (A, C, E) and Leucine (B, D, F) incorporation across a range of substrate concentrations for three study sites (SB, HB, CR) grouped by habitat (CSM, Bulk, RSM).

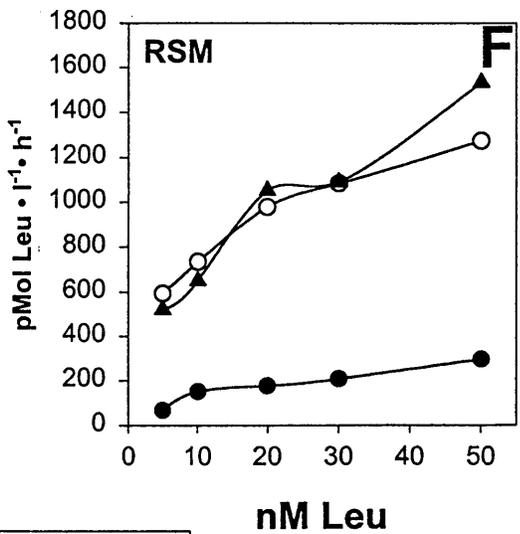
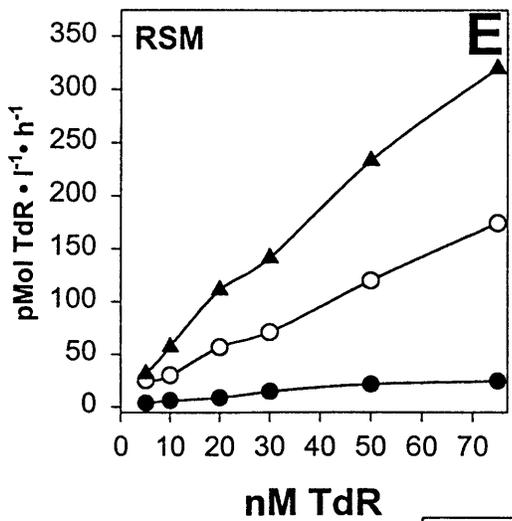
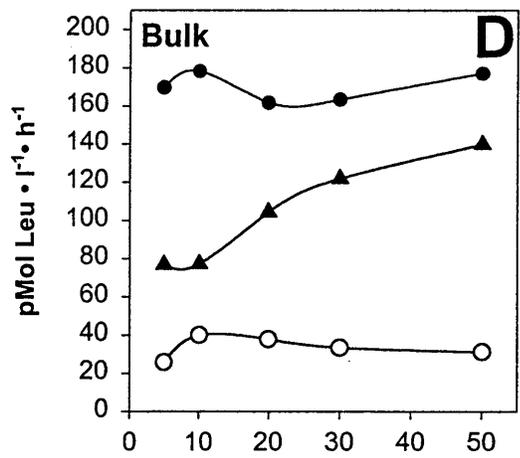
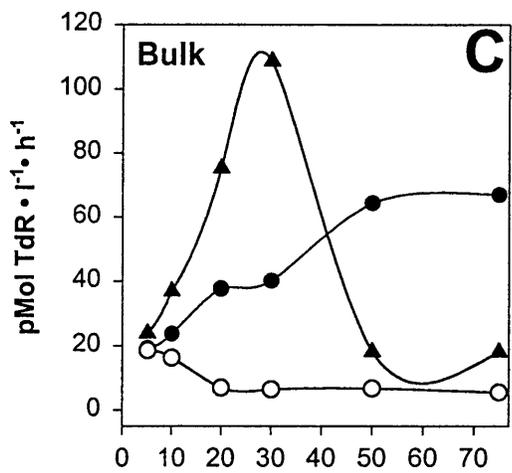
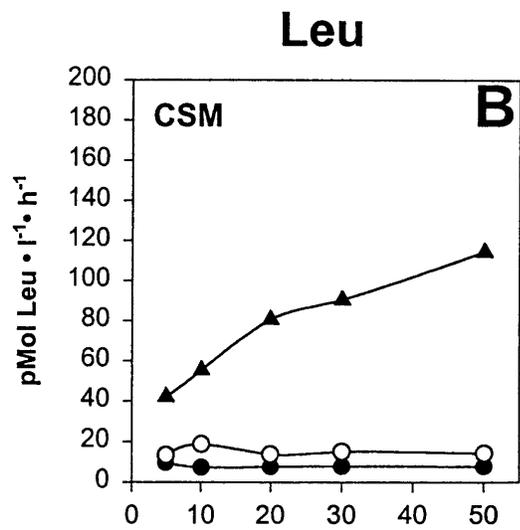
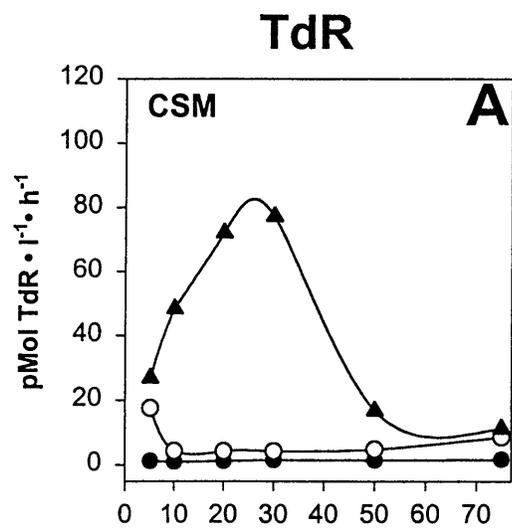
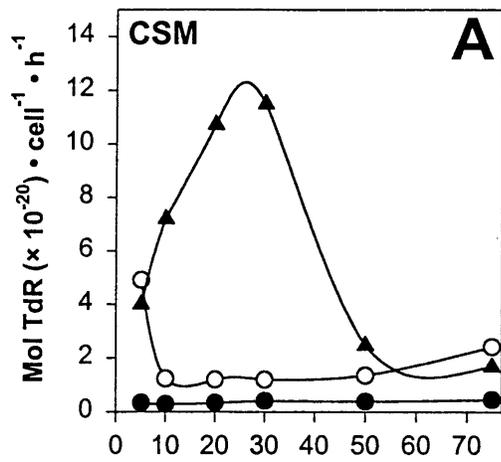
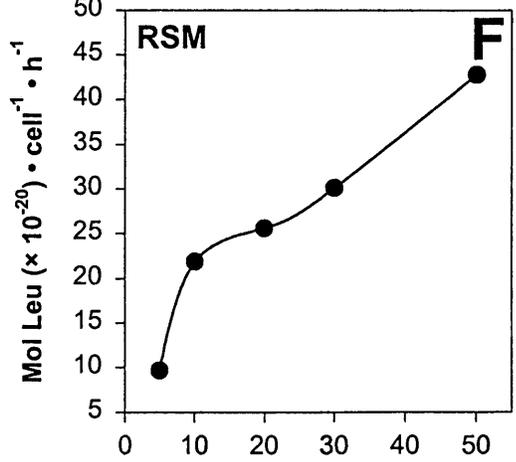
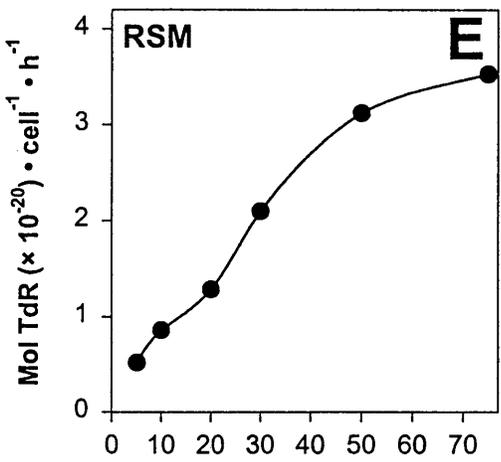
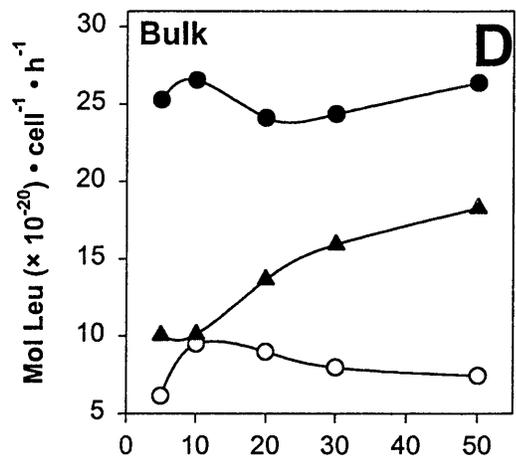
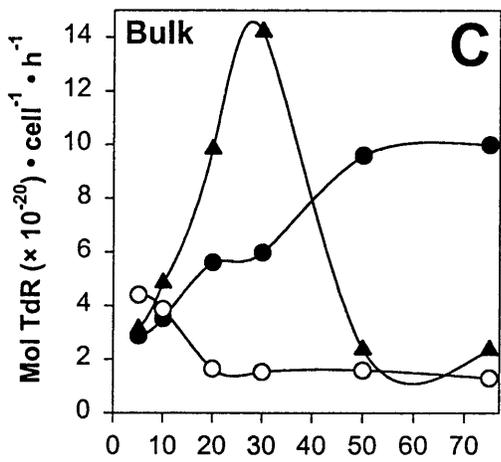
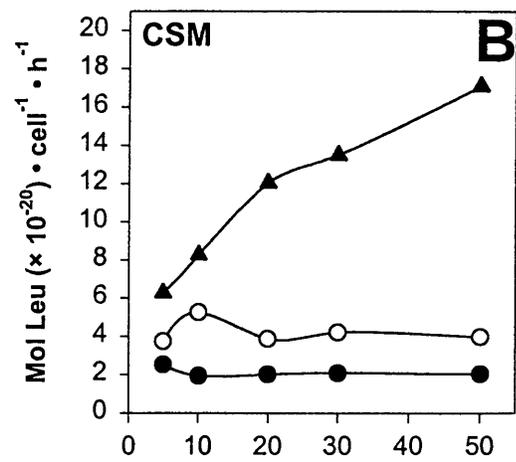


Figure 27. Cell-specific rates of bacterial Thymidine (A, C, E) and Leucine (B, D, F) incorporation across a range of substrate concentrations for three study sites (SB, HB, CR) grouped by habitat (CSM, Bulk, RSM).

TdR



Leu



nM TdR

nM Leu



water-column there was some indication of different bacterial TdR and Leu incorporation physiology (**Fig. 26**). The samples from the CSM and RSM of SB were from the deepest reef (15 m) and deepest water-column (20 m) surveyed during the entire study. Of all saturation curves obtained, the SB-CSM displayed the lowest cell-specific incorporation rates for both thymidine (**Fig. 26A**) and leucine (**Fig. 26B**). Rates of precursor incorporation in this sample were only weakly dependent on substrate concentration and were uniformly low across the concentration range tested (5-75 nM TdR, 5-50 nM Leu). A water sample collected from the SB-RSM, less than a meter from the CSM site, demonstrated enhanced rates of incorporation with increasing thymidine concentration (**Fig. 26E**) and enhanced, but uniform rate of incorporation across the range of leucine concentrations (**Fig. 26F**). A bulk water sample from a depth of 1m, with bacterial abundance similar to that of the RSM, demonstrated a third unique profile (**Fig. 26C, D**).

Similar measurements performed at HB and CR on water from Bulk, CSM and RSM demonstrated kinetic similarities in the CR-CSM and CR-Bulk profiles and HB-CSM and HB-Bulk profiles (**Fig. 26A-D**). It appears that on this date, the bacterial physiological characteristics in the bulk water-column of HB and CR were similar to the CSM (**Fig. 26**). It should be noted that the depth differences between CSM and Bulk samples at HB (~3 m) and CR (~2 m) were much shallower than at SB (~15 m). While cell-specific Leu incorporation rates of CR-Bulk and CR-CSM samples displayed a lack of saturation kinetics (increasing rate, with increasing thymidine concentration), profiles from HB were more typical

of Leu curves (maximum rate at lower leucine concentration).

Significant inhibition of substrate incorporation into TCA-insoluble macromolecules was observed beyond certain thymidine (**Fig. 26A, C**) and leucine (**Fig. 26B, D**) concentrations. The similarity in TdR profiles of CR-CSM and CR-Bulk included a rapid rise in incorporation rate between 5 and 30 nM followed by a precipitous decline to 50 nM with little to no change out to 75 nM. HB-CSM and HB-Bulk demonstrated peak rates at the lowest (5 nM) tested thymidine concentration, followed by declining rates at higher concentrations.

Comparison of the RSM saturation curves on a cell-specific basis was not possible due to a high percentage of particle-attached bacteria, making accurate bacterial abundance estimates impossible. Direct visual examination of the HB-RSM and CR-RSM slides showed relatively few free bacteria, far outnumbered (visual estimate) by cells comprising dense aggregates around detrital material. The high cell density in the RSM samples was reflected in the non cell-specific saturation curves, with absolute rates of enhanced incorporation from 1 - 30× (TdR curves) and 10 - 90× (Leu Curves) that of CSM and Bulk profiles (**Fig. 27**).

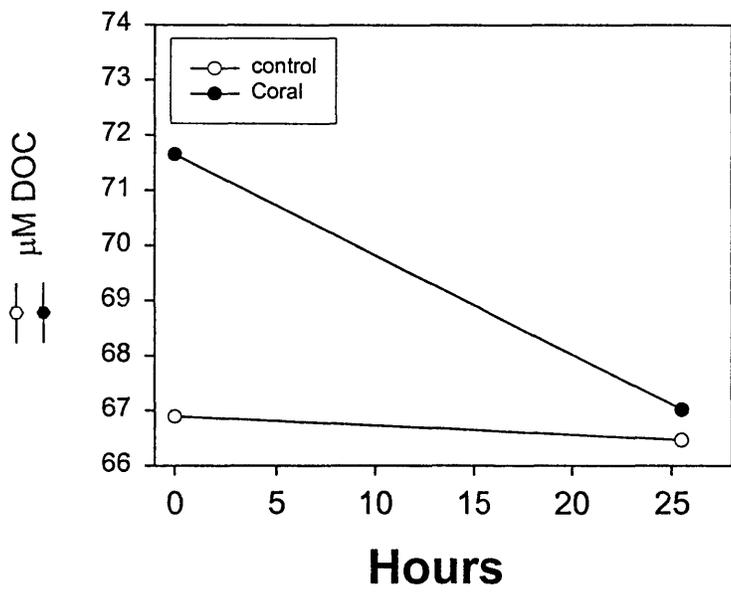
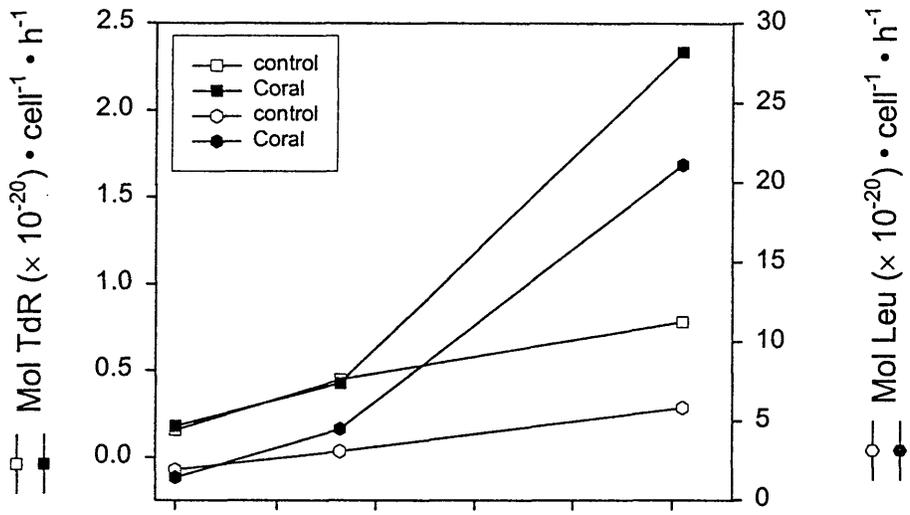
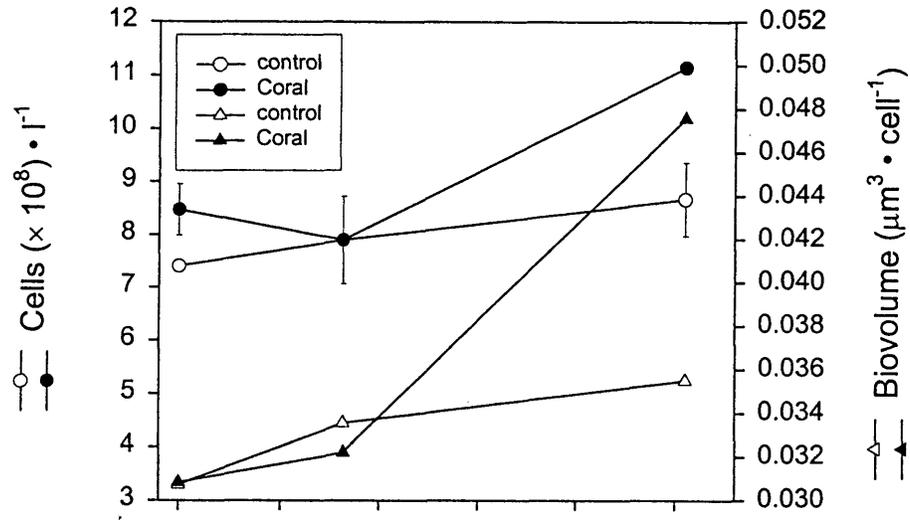
CORAL EFFECT

The presence of a living coral specimen in a reef-water incubation study had profound effects on bacterioplankton growth, biovolume and incorporation rates of TdR and Leu. The bacteria in unfiltered water from HB exhibited relatively high abundance compared to previously collected samples from this site. Bacterial abundance in the control bottle at T-0 was $7.41 (\pm 0.07) \times 10^8$

cells \cdot l⁻¹ compared to $8.47 (\pm 0.5) \times 10^8$ cells \cdot l⁻¹ in the bottle with the coral, indicating a small potential source of cells from the coral surface. For both experimental and control treatments, bacteria present at T-0 of the incubation were among the smallest cells ($0.031 \mu\text{m}^3 \cdot \text{cell}^{-1}$) detected during the entire research project. Despite an average bacterial abundance (7.4×10^8 cells \cdot l⁻¹), the initial rate of TdR incorporation was among the lowest detected ($1.2 \text{ pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) at any site.

Rates of cell-specific substrate incorporation increased gradually between the first two time intervals (8 hours) in both experimental and control bottles. During this same interval, average cell-specific biovolume increased by only $0.001 - 0.002 \mu\text{m}^3$ in both treatments. Compared to the population in the control bottle, the bacteria incubated with the coral colony underwent a substantial physiological change between 8 and 24 hour time-points (**Fig. 28**). Using the average TdR (5.03×10^{17} cells $\cdot \text{Mol}^{-1}$) and Leu (0.86×10^{17} cells $\cdot \text{Mol}^{-1}$) conversion factors and a carbon conversion factor based on the average biovolume over the three time-points, bacterial carbon production (BCP) in the experimental treatment increased from $0.28 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ (average of TdR and Leu based estimates) to $5.11 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ in just 24 hours. This corresponds to an order of magnitude change in specific growth rate from $0.02 \cdot \text{d}^{-1}$ to $0.36 \cdot \text{d}^{-1}$. Over the same time interval BCP in the control bottle increased from $0.28 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ to just $1.19 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, with specific growth rates rising to $0.11 \cdot \text{d}^{-1}$ from $0.03 \cdot \text{d}^{-1}$. Bacterial carbon production in the presence of coral was significantly

Figure 28. Changes in bacterial properties and incorporation rates in HB water due to incubation with a branch of the coral *Madracis mirabilis* (open symbols) versus a control treatment without coral (filled symbols).



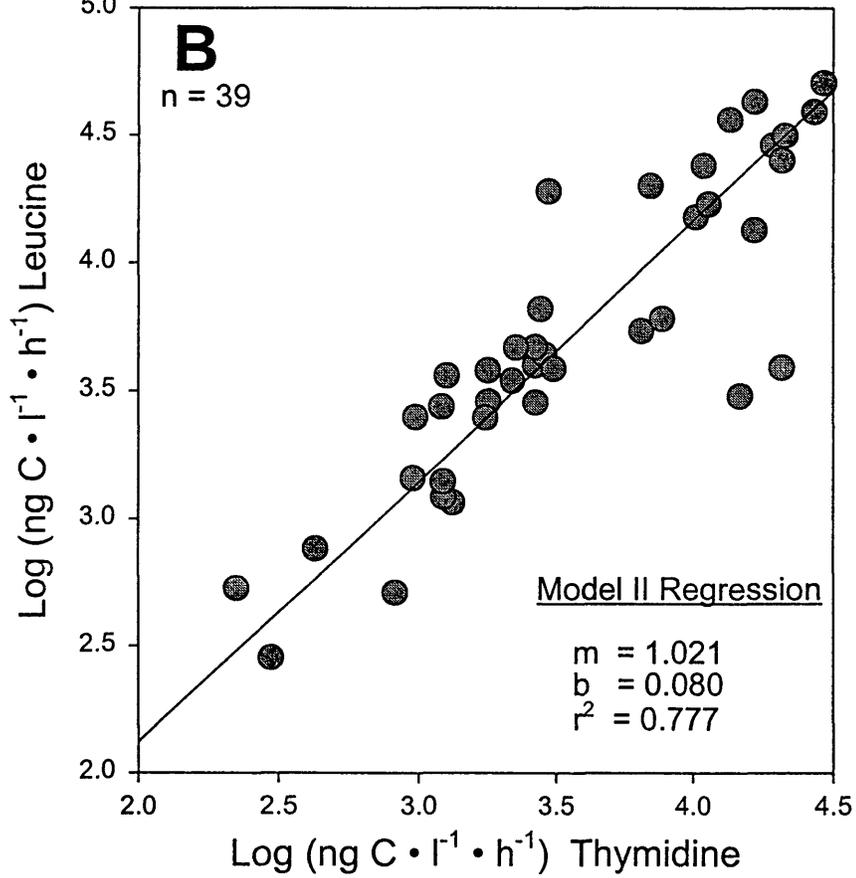
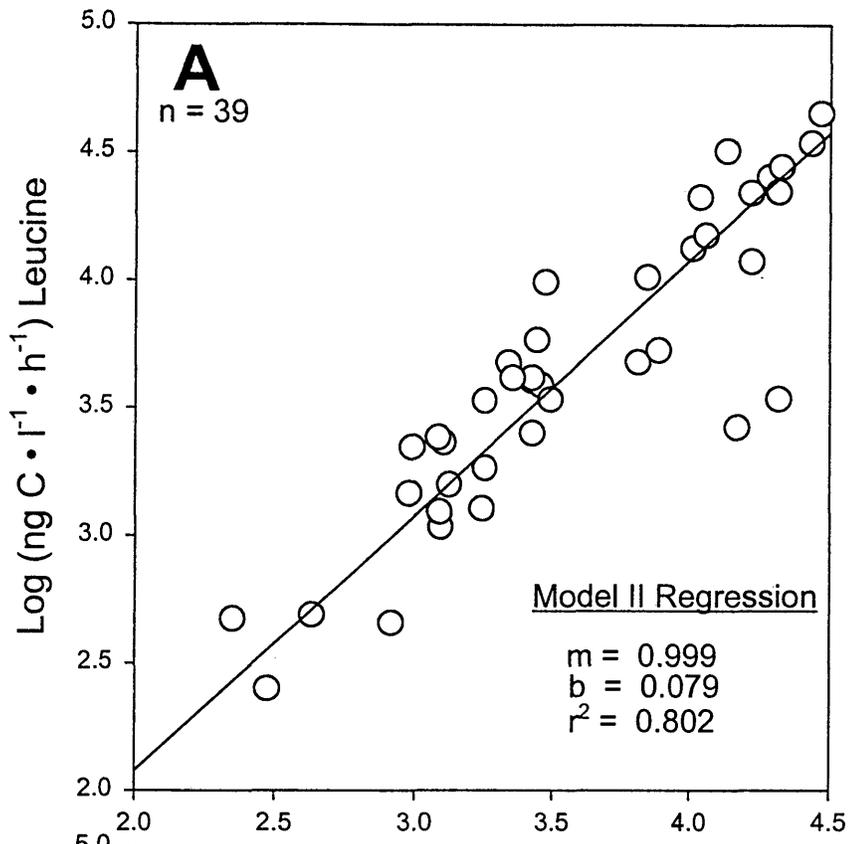
enhanced over that of the control indicating that organic molecules and nutrients released into the water by the coral stimulated bacterial growth.

EXTRAPOLATION TO BACTERIAL CARBON PRODUCTION

THYMIDINE VS LEUCINE ESTIMATES

Bacterioplankton carbon production estimates were calculated based on site-specific, empirically determined thymidine and leucine conversion factors. Average thymidine and leucine conversion factors (5.03×10^{17} cells \cdot Mol⁻¹TdR and 0.86×10^{17} cells \cdot Mol⁻¹Leu) were used to calculate bacterial production for samples collected from sites without a specific conversion factor determination (NS and CH). A carbon conversion factor (15.9 fg C \cdot cell⁻¹) based on the average cell volume ($0.053 \mu\text{m}^3$) detected in the reef environment and the relationship between cell volume and cell carbon determined by Simon and Azam (1989) was used to compute bacterial carbon production. Production estimates based on both the TdR and Leu incorporation techniques were in good agreement with one another. To visualize the entire range of production estimates, data were plotted on a log-log scale that indicated a relationship of unity between the two production measurements (**Fig. 29A, Fig. 29B**). Bacterial production estimates based on Leu incorporation were checked with a direct conversion factor (1546 g C \cdot Mol leucine⁻¹) presented by Simon and Azam (1989) based on the Mole percent of leucine in bacterial protein. Using this conversion factor, the scaling between leucine and thymidine based carbon

Figure 29. Model II regressions of log (Leucine based bacterial carbon production rate) versus log (Thymidine based bacterial carbon production rate) for field samples. Carbon production was computed using 16 fg C per cell and empirically derived Thymidine and Leucine conversion factors (A) and using 16 fg C per cell with empirically determined Thymidine conversion factors and the Leucine conversion factor of 1546 g C per Mole of Leucine incorporation presented by Simon & Azam (1989), assuming no isotope dilution (B). Slopes and Y-intercepts of regressions are equivalent ($p < 0.05$).



production remained identical with the same slope and Y-intercept (**Fig. 29B**) as the plot using both empirically determined factors (**Fig. 29A**). The use of a Carbon to Leucine conversion factor of 1546, instead of 3092 g C • Mol leucine⁻¹ (suggested by Simon and Azam), is justified since the rate of leucine incorporation was saturated at the standard bioassay concentration (20 nM).

For comparison across reef habitats, carbon production was taken to be the average of thymidine and leucine based estimates. Comparing bacterial carbon production among all freshly collected, undiluted reef water samples (**Fig. 30**) indicated relatively high (compared to Sargasso Sea, **Fig. 31**) and variable rates of carbon synthesis across the reef. Carbon production by bacteria from the coral surface-microlayer (CSM) was similar to that of Bulk reef-water bacteria, ranging from 0.3 – 8.7 $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ and 0.4 – 12.2 $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ respectively. Estimates of bacterial carbon production from the reef surface-microlayer (RSM), with its high concentration of detrital particles, had the widest range and greatest magnitude (4.3 – 37.2 $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) of all reef habitats. Performing t-Tests on the mean production values from the three sites indicated that CSM (n = 12) and Bulk reef-water (n = 9) had identical bacterial carbon production ($p > 0.05$), while both of these habitats were different ($p < 0.05$) from the RSM (n = 13) samples (**Table 5**).

GROWTH RATE BASED ON BACTERIAL CARBON PRODUCTION AND BIOMASS

The ratio of bacterial production (P) to bacterial biomass (B) defines the growth rate (μ) of a population at a given time-point. The highest specific

Figure 30. Average bacterial carbon production (\pm SD) grouped by reef habitat (CSM, Bulk, and RSM) across the six study sites. Each n represents the average of an individual sample for which Leucine and Thymidine based carbon production were assayed in triplicate.

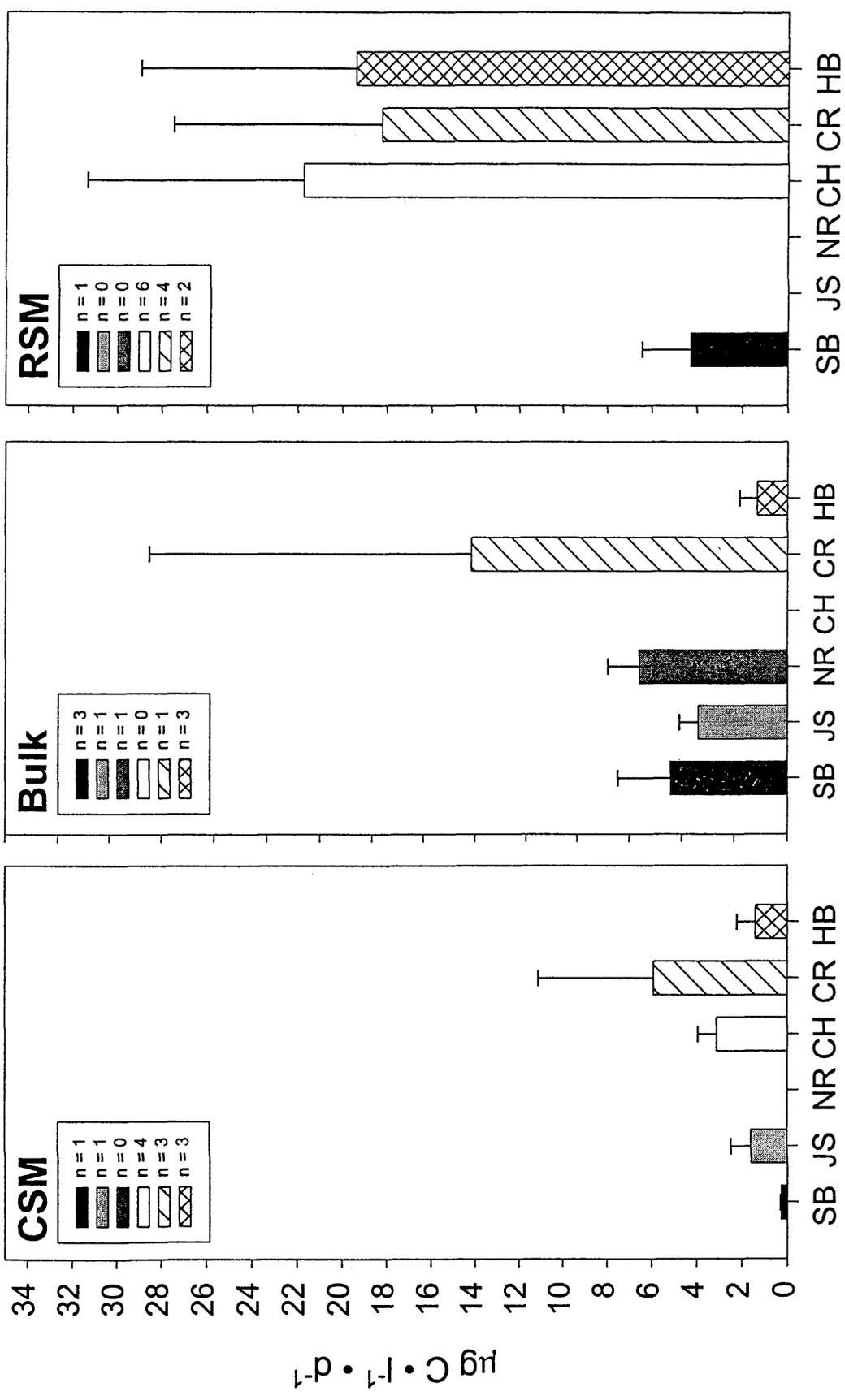


Figure 31. Bacterial growth rate at the Bermuda Atlantic Time-series Station (BATS) estimated from the slope of Model II regression of Production (P) versus Biomass (B) measurements in the top 40 meters of the water column for the year 1996. Production estimates calculated from Thymidine incorporation rates assuming 1×10^{17} cells per mole Thymidine and 15 fg C per cell. Data obtained from <http://www.bbsr.edu>.

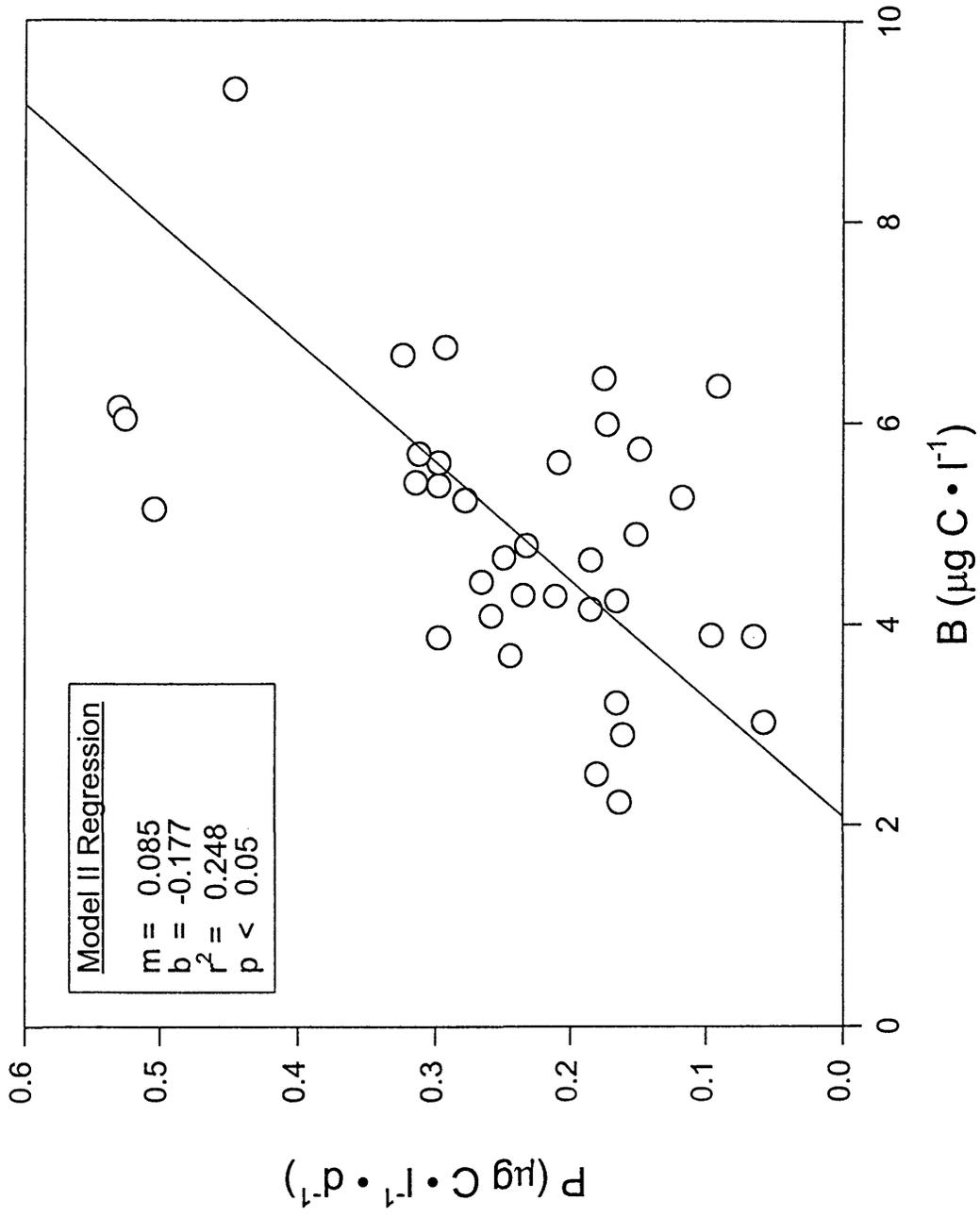


Table 5. Average bacterial carbon production rates across reef habitats using 5.03×10^{17} cells \cdot Mol TdR, 0.86×10^{17} cells \cdot Mol Leu and 15.9×10^{-15} g C \cdot cell. Two-tailed t-Test on average TdR and Leu bacterial carbon production.

Habitat	n	TdR ($\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$)	Leu ($\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$)	Average ($\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$)	t-Tests ¹
CSM	12	2.9 (\pm 3.9)	3.1 (\pm 2.4)	3.0 (\pm 2.4)	*
Bulk	9	5.1 (\pm 6.4)	3.3 (\pm 1.7)	4.2 (\pm 3.6)	*
RSM	13	15.9 (\pm 7.7)	22.0 (\pm 11.1)	19.0 (\pm 9.0)	**

1. Same number of asterisks (*) indicates NS difference ($p > 0.05$) while different numbers indicate significant difference ($p < 0.05$) in bacterial carbon production rates between habitats.

bacterial growth rates were generally found to occur in diluted reef-water cultures at T-0 time-points and in cultures from sites along the south shore of Bermuda (**Table 6**). The ratio of P/B ($n = 10$) in reef-water cultures (T-0) varied within a narrow range from $0.20 - 0.50 \cdot d^{-1}$ compared to the range of values from the literature (**Table 7**). Time-zero growth rates of bacteria from 20% cultures were close to the growth rate values calculated by the dilution technique. This is a major finding for reducing the uncertainty of bacterial growth rates based on the linear plots of the dilution equations (**Fig. 23**). The three dilution experiments conducted during July of 1997 were similar with respect to the ratio of P/B among cultures of similar dilution factor.

BACTERIAL GROWTH EFFICIENCY

Bacterial growth efficiency (BGE) can be estimated by dividing the amount of bacterial carbon production by the sum of bacterial carbon production plus the respiration term. The denominator in this equation is also known as the assimilated ration and can be estimated from the amount of DOC removed from a bacterial incubation (del Giorgio and Cole, 1998). Bacterial growth efficiency ranged from 9% at John Smith's Bay (JS) to 24% at the Seabright (SB) site (**Table 8**). Results from the SB1 and SB2 incubations (separated by one week) were in good agreement with one another. Calculating BGE using TdR incorporation, Leu incorporation or the increase in cell abundance as the bacterial production estimate produced similar growth efficiencies. The exception to this result was the SB2 experiment (factor of 2 lower based on the

Table 6. Bacterial growth rates calculated as the ratio of P/B using average P, from initial time point (T-0) of reef-water cultures. Growth rate from dilution cultures (Y-intercept of the apparent change in growth rate vs. the fraction of cells in culture) for comparison. ND indicates no dilution series conducted at site.

Incubation	Date	Cells (%)	$P_{(TdR)}$ $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$	$P_{(Leu)}$ $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$	$P_{(Avg.)}$ $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$	$B_{(cells)}$ $\mu\text{g C} \cdot \text{l}^{-1}$	μ (P/B)	μ (Dilution)
SB1	7-9-97	20	0.95	1.46	1.21	2.91	0.41	0.37
		100	2.66	4.03	3.34	12.71	0.26	
SB2	7-15-97	20	1.33	1.58	1.45	3.97	0.37	0.40
		100	2.19	4.73	3.46	14.67	0.24	
JS	7-23-97	20	1.23	1.08	1.16	2.85	0.41	0.37
		100	2.87	3.87	3.37	12.83	0.26	
NR	5-1-98	100	6.47	4.77	5.62	11.11	0.51	ND
		20	0.43	0.49	0.46	1.79	0.26	
HB	5-7-98	100	1.79	1.83	1.81	10.40	0.17	0.34
		30	1.74	1.28	1.51	8.09	0.19	
CR	5-17-98	30	1.74	1.28	1.51	8.09	0.19	ND

Table 7. Summary of measured and derived bacterial parameters and growth from coral reef habitats. Numbers in the column headers correspond to the following: 1. Water-Column (WC), Particle Attached (PA), Coral Surface Microlayer (CSM), Interstitial Reef Space (IRS), Oceanic (OCE), Reef Flat (RF), Reef Surface Microlayer (RSM); 2. Bacterial cell abundance ($\times 10^6$ cells $\cdot l^{-1}$); 3. Biovolume ($\mu m^3 \cdot cell^{-1}$); 4. TdR Conversion Factor ($\times 10^{18}$ cells $\cdot Mol^{-1}$); 5. Leu Conversion Factor ($\times 10^{18}$ cells $\cdot Mol^{-1}$); 6. Carbon Conversion Factor ($fg C \cdot cell^{-1}$) and Muramic Acid (MA); 7. Bacterial Carbon Production ($\mu g C \cdot l^{-1} \cdot d^{-1}$); 8. Bacterial Biomass ($\mu g C \cdot l^{-1}$); 9. Specific Growth Rate (d^{-1})

Habitat ¹	Abundance ²	Biovolume ³	TdR-CF ⁴	Leu-CF ⁵	CCF ⁶	P ⁷	B ⁸	μ ⁹	Citation	
WC	-	-	-	-	-	8-28	19-43	0.4-0.7	Sorokin (1973)	
WC	1-6	-	-	-	80-100x MA	-	17-48	-	Moriarty (1979)	
WC	3-5	-	-	-	80-100x MA	-	36-62	-		
PA	3-5	-	-	-	80-100x MA	-	28-50	-		
WC	1.2-13.1	0.10	-	-	10-25	0.8-147	1.3-16.2	0.6-9.1	Linley & Koop (1986)	
WC	1.7-6.3	0.08	4	-	45	2.8-19.8	7.7-28.6	0.1-1.9	Paul et al. (1986)	
CSM	7.5-19.4	0.13	4	-	73	58.8-1608	54.8-141.6	1.1-2.5	Hopkinson et al. (1987)	
WC	1.8	0.05	4	-	11	0.6-4.2	2	0.3-2.1		
WC	0.1-3	0.45	-	-	170	-	3.5	-	Schiller and Herndl (1989)	
IRS	3-18	0.58	-	-	220	-	219	-	Herndl (1991)	
WC	5-23	-	-	-	20	3.7-17.8	10.1-46.4	0.4		
WC	8-14	-	1.7	-	15	2.7-18	12-21	0.2-0.9	Yoshinaga et al. (1991)	
WC	7.8-16	-	1.7	-	20	4.8-125	15.6-32	0.2-5.7		
WC	3.6-9.3	-	-	-	20	9.2-15.5	7.2-18.6	0.83-15.7	Rath et al. (1993)	
WC	2.6-9.1	-	-	-	50-119	9.3-68	15-83.3	0.3-1.5	Sorokin (1994)	
OCE	2.2-3.6	-	-	-	59-68	17.6-20.2	12.9-20.7	0.9-1.6		
WC	2.3-5.8	-	-	-	25-67	5.4-31.9	11.4-27.5	0.3-1.5		
OCE	3.1	-	-	-	34	6.3	10.4	0.6		
WC	3.1-1.04	-	-	-	23-43	5.6-26.6	9-39.3	0.4-0.9		
WC	3.4-12.8	-	-	-	20-52	4.8-15.6	11.4-45.9	0.3-0.8		
WC	2-11	-	-	-	20	-	4-22	-		Ayukai (1995)
WC	10-60	-	-	-	20	-	2-12	-		
WC	18.4	0.05	0.798	0.091	15.3	3.6-5.24	27.6	0.1-0.2	Torrón and Dufour (1996a)	
WC	6.4	0.05	0.798	0.091	15.3	-	9.6	-	Torrón and Dufour (1996b)	
WC	9.7-23.7	0.05	0.99	0.076	15.3	4.3-4.4	14.8-36.3	0.1-0.4		
RF	3	0.05	0.99	0.076	15.3	1-2.2	4.6	0.2		
OCE	4.5	0.05	0.99	0.076	15.3	0.4-0.8	6.9	0.1	Ferrrier-Pages & Gattuso (1998)	
WC	4-8.5	-	-	-	15	2.7-28.2	6-13	1.3-2.7		
WC	7.2	0.053	0.503	0.086	15.9	4.2	11.4	0.4	This Study (1999)	
CSM	6.9	0.052	0.503	0.086	15.9	3.0	11	0.3		
RSM	9.6	0.055	0.503	0.086	15.9	19	15.3	1.24		

Table 8. Bacterial growth efficiency (BGE) calculated for 20% cultures by relating Bacterial carbon production (BCP) to the removal of dissolved organic carbon (DOC). Site specific conversion factors were used to calculate TdR and Leu based bacterial production, assuming $17.1 \text{ fg C} \cdot \text{cell}^{-1}$ (based on the average T-0 cell volume of $0.060 \mu\text{m}^3$ from the three experiments). TdR and Leu based BCP represent total integrated carbon production over the time interval indicated. Cell based BCP represents the absolute difference in cell carbon over the same time.

Site (CF Expt.)	Time (hours)	ΔDOC ($\mu\text{g C} \cdot \text{l}^{-1}$)	Production	BCP ($\mu\text{g C} \cdot \text{l}^{-1}$)	BGE (%)
SB1	68.1	21.1	TdR	4.8	23
			Leu	5.0	24
			Cell	4.7	22
SB2	65.5	36.2	TdR	8.5	24
			Leu	7.3	20
			Cell	4.5	12
JS	76.0	56.6	TdR	4.8	8
			Leu	4.9	9
			Cell	5.2	9

cell production estimate). Efficiency estimates were based on bacterial production from 20% cultures only, since cell abundance did not increase continually in 100% cultures over the time-course of DOC removal. Bacterial growth efficiency calculations were not possible for CR and HB culture experiments since T-F DOC concentrations were not significantly different from the concentrations at T-0.

In the case of the coral incubation experiment, BGE was calculated based on production estimates from the 100% (undiluted) culture water and loss of DOC between T-0 and T-F. Average reef platform TdR and Leu conversion factors and a carbon conversion factor based on the average cell volume in each incubation bottle were used to calculate bacterial production. The bottle with reef water and the coral specimen exhibited a loss of 4.6 μM DOC ($55.7 \mu\text{g C} \cdot \text{l}^{-1}$) compared to only 0.4 μM DOC ($5.2 \mu\text{g C} \cdot \text{l}^{-1}$) in the control bottle (**Fig. 28**). Although the magnitude of bacterial production calculated by three different methods (TdR, Leu and Cells) over the 24 hours of the experiment was greater in the experimental bottle ($1.8 - 2.8 \mu\text{g C} \cdot \text{l}^{-1}$) than the control ($0.7 - 1.5 \mu\text{g C} \cdot \text{l}^{-1}$), BGE was higher in the control bottle. Bacterial growth efficiency was 2-3% in the experimental bottle and 8-17% in the control bottle (**Table 9**), based on the three types of production estimates (TdR, Leu and Cell).

Table 9. Bacterial Growth Efficiency (BGE) for the coral incubation experiment. BGE was calculated for undiluted cultures by relating bacterial carbon production (BCP) to the removal of Dissolved Organic Carbon (DOC). Average reef conversion factors were used to calculate TdR and Leu based bacterial production, assuming $12.8 \text{ fg C} \cdot \text{cell}^{-1}$ for the experimental treatment and $12 \text{ fg C} \cdot \text{cell}^{-1}$ for the control, based on the average cell volume of 0.037 and $0.033 \text{ } \mu\text{m}^3$ respectively). TdR and Leu based BCP represent total integrated carbon production over the time interval indicated. Cell based BCP represents the absolute difference in cell carbon over the same time.

Treatment	Time (hours)	Δ DOC ($\mu\text{g C} \cdot \text{l}^{-1}$)	Production	BCP ($\mu\text{g C} \cdot \text{l}^{-1}$)	BGE (%)
Experimental (coral)	25.5	55.7	TdR	1.8	3
			Leu	2.8	5
			Cell	2.7	5
Control (no-coral)	25.5	5.16	TdR	0.7	13
			Leu	0.8	16
			Cell	1.5	29

DISCUSSION

BACTERIAL PROPERTIES AND GROWTH PARAMETERS ON THE REEF

BACTERIAL ABUNDANCE, BIOVOLUME AND BIOMASS

Bacterioplankton abundance from reef sites around the world is typically less than 10×10^8 cells \cdot l⁻¹, with a range of 1 - 45×10^8 cells \cdot l⁻¹ reported in the literature, though most values are below 10×10^8 cells \cdot l⁻¹ (**Table 7**). Cell abundance in the Bermuda reef environment rarely exceeded 10×10^8 cells \cdot l⁻¹, with an average of $7.4 (\pm 2.2, \text{SD})$ cells \cdot l⁻¹. Cell abundance was greatest in benthic water-layer or sediment samples. A high degree of small-scale variability in the distribution of bacterial abundance can be expected across different habitats in coral reef waters (**Table 2**). Bacterial abundance can vary by as much as an order of magnitude between bulk reef water and from samples collected within the interstitial spaces of branching corals (Schiller and Herndl, 1989).

In Bermuda, concentrations of bacterial cells were the least variable in Bulk reef water samples. Cell abundance at mid-depth (~10 m) in the reef water-column approximately 100 m from the Seabright sewage outfall (SB) was nearly identical to that of the water-column at the John Smith's Bay control site (**Fig. 16**). This pattern suggests that the outfall contributes little to the heterotrophic bacterial biomass on the reef, or that the relatively buoyant plume

(lower salinity) does not immediately mix with bulk reef-water. Mean bacterial cell abundance in the reef water-column (Bulk) was found to be slightly elevated, compared to samples collected from the CSM, when samples from a highly sediment influenced inshore reef (CR) were removed from the CSM data set (**Table 2**).

The observation of cell depletion at the reef surface suggests that reef organisms may selectively remove a fraction of the heterotrophic bacteria from the benthic boundary layer. On coral reefs with a prevailing direction of water flow (usually wind-driven) the abundance of heterotrophic bacteria in the water-column has been shown to decline from windward to leeward, presumably after contact with the reef surface (Moriarty, 1979; Ayukai, 1995). Although, their small size (usually $< 1\mu\text{m}$) would seem to preclude heterotrophic bacteria from the diet of macrofaunal reef organisms, recent studies have demonstrated highly efficient ($> 70\%$) removal from the water column by sponges (Pile, 1996). Additionally, several species of coral, have been shown to remove ^{14}C -labeled bacterioplankton from suspension (Sorokin, 1978). A more thorough study of *in situ* bacterial removal processes (e.g., one that included both protozoan and coral reef macrofauna bacterivory) on the coral reefs of Bermuda would help elucidate the observed pattern of bacterial depletion from the CSM.

The observation of uniform cell volume across sites, habitats and field-seasons (**Fig. 8**) may indicate that a system-wide process controls this parameter. Bacteria of similar biovolume to those detected in Bermuda coastal

water have been reported from the Great Barrier Reef (Hopkinson et al., 1987) and from Tuamotu Atolls (Torréton and Dufour, 1996a; 1996b). Carlson and Ducklow (1996) report heterotrophic bacterial cells of $0.04 \mu\text{m}^3$ from a depth of 10 m in the Sargasso Sea near Bermuda. Surveying the literature, Schut et al. (1997) report a range of cell-specific biovolumes from $0.02 - 0.12 \mu\text{m}^3$ for oligotrophic waters, and $0.34 - 6.0 \mu\text{m}^3$ for marine bacterial isolates. The dominance of cells of a particular size may indicate a high level of grazing on cells above a certain size threshold (for this system $0.05 \mu\text{m}^3$) below which cells are afforded some degree of grazing refuge (Juergens and Guede, 1994). Limitation of removal below a certain minimum threshold could apply equally to protozoan bacteriovores and macrobiotic reef filter feeders.

Assuming that the mean of average bacterial abundance determinations ($7.4 \times 10^8 \text{ cells} \cdot \text{l}^{-1}$) is typical of reef waters the biomass of bacteria in this ecosystem is $11.8 (\pm 3.6) \mu\text{g C} \cdot \text{l}^{-1}$. This estimate is slightly lower than those from other reef systems (**Table 7**), but other studies have generally used higher carbon conversion factors, justified by larger cell-specific biovolume or simply by assumption. There are very few studies of bacterial cell volume determined by video image-analysis from other reef environments. Video image-analysis eases the sizing of many cells using a uniformly applied measurement algorithm, contributing to accurate estimates of *in situ* cell biovolume. Torréton and Dufour (1996a) detected cells of $0.05 \mu\text{m}^3$ in Tuamotu atoll lagoons using projected photographic images combined with manual digitization of images to determine

cell length and width. Tuamotu cell-specific biovolume was equivalent to estimates from Bermuda, but bacterial biomass was generally higher in the water column of the atolls due to greater cell abundance than the average for Bermudian coastal waters.

Reef surface-microlayer bacteria are least represented in the biovolume data set due to interference by reef detrital material with cell detection during image analysis. Moriarty and Hansen (1990) used 20% (v/v) acetic acid to dissolve particles of CaCO_3 in their reef substrate samples, followed by buffer addition, to obtain an aliquot suitable for epifluorescence microscopy. I used 5% (v/v) acetic acid to dissolve CaCO_3 in RSM samples followed by 10× dilution (and buffering) with 0.2 μm filtered reef water. Although this technique worked well for removing the carbonate particles, most bacterial cells remained attached to particulate organic material (POM) in dense aggregates, which made accurate visual counts impossible. Treatment of the pre-filtered RSM water by sonication could have possibly detached cells from POM and produced a sample more amenable to AODC and image analysis.

The topic of appropriate carbon conversion factors (CCF) to use for calculating bacterial biomass continues to be of considerable debate. Average literature values range from 106 $\text{fg C} \cdot \mu\text{m}^{-3}$ (Carlson et al., in press) to 560 $\text{fg C} \cdot \mu\text{m}^{-3}$ (Bratbak, 1985). The CCF of Carlson et al. is unique in that it was derived from the construction of carefully measured budgets of carbon mass balance within single incubation vessels. It is likely that future estimates of bacterial

biomass will need to include site and seasonal specific carbon conversion factor determinations.

BACTERIAL THYMIDINE AND LEUCINE INCORPORATION

The incorporation rate of both thymidine and leucine into bacterial macromolecules has been used to estimate bacterial production in the coral reef environment for almost as long as the techniques have been available. The factor of greatest interest resulting from these bioassays is the production estimate, however the ratio of Leu:TdR incorporation may be suggestive of the dominant bacterial growth physiology in the environment (i.e., the balance of cell division vs. biomass increase. The ratio of Leu:TdR for freshly collected reef-water samples was 9.79 (**Fig. 11**). This ratio is slightly lower than the average Leu:TdR detected in Tikehau lagoon (14) but considerably lower than that of the oceanic water (24) surrounding Tikehau (Torréton and Dufour, 1996b).

Shifting Leu:TdR ratios over seasons or throughout the time course of bacterioplankton culture studies is generally believed to be indicative of a physiological response by bacteria to the available DOM, whereby higher ratios are indicative of a reduction in the DOM quality or supply (Chin-Leo and Kirchman, 1990; Servais, 1992; Shiah and Ducklow, 1997). A high Leu:TdR may also indicate a relatively short term response to increased DOM quality (shift-up), where protein production should respond before cell division (Chin-Leo and Kirchman, 1990). At temperatures > 25 °C, Leu:TdR ratios (6 -14) from a tidal creek are reportedly lower and less variable than Leu:TdR (6 - 24) at

temperatures < 25 °C (Shiah and Ducklow, 1997). Observations of Leu:TdR from Bermuda where incubation temperatures were 22 - 28 °C agree well with the previous range of values, but are slightly higher than the cross system average (7.5 - 9) determined by Servais (1992). If bacterial growth is nearly balanced then the mean Leu:TdR ratio of incorporation rates should approximate the mean TdR-CF:Leu-CF ratio, 5.84 (**Fig. 21**). Since Leu:TdR is approximately 2x the ratio of conversion factors, the comparison suggests that bacteria in the freshly collected reef-water samples initially use the ambient growth substrate for biomass synthesis. The ratio of Leu:TdR from Bermuda reefs decreases slightly when incubated samples (those past T-0) are removed from the data set. Based on this result, and the observation that DOC was removed from bacterial cultures, it is probable that DOM depletion may be reflected by the slight Leu:TdR shift.

DISSOLVED ORGANIC CARBON

The role of bacterial organic matter cycling in coral reef ecosystems is a relatively under-investigated field. Specifically, very little is known about the sources and sinks of DOC on reefs. Background levels of DOC from the water-column of the Bermuda coastal environment (61 - 68 μM) (T-0 samples, **Fig. 12** and Bulk reef-water, **Fig. 13**) were not dissimilar to the range of values (58 - 83 μM DOC) detected in Tuamotu Atoll lagoons (Pages et al., 1997).

Very few estimates of DOC flux from corals are reported in the literature however the release rate of DOC-lipid by one coral species has been shown to

be faster at a depth of 5 m than at 23 m (Crossland, 1987). DOC concentrations in the water column have been shown to be enriched in the presence of coral reefs (Schiller and Herndl, 1989) compared to surrounding oceanic waters (Yoshinaga et al., 1991; Sorokin, 1994). This was not the case for two sites along Bermuda's southern coast where the reef water-column DOC concentration (61 - 65 μM) at 10 m was identical to the yearly average (1996) euphotic zone DOC concentration (62.6 ± 3.7 , SD) at the BATS site. It is not surprising that the reef DOC values are similar to those from the BATS site, considering the rapidity at which water depth increases providing little to no barrier to water exchange between coastal and open ocean water masses.

DOC samples from the reef water-column collected during 1998 field season were notably higher than values from the previous year. A majority of the 1998 samples (**Fig. 13**) were collected from the lagoon side of the Island where the residence time of reef water permits longer contact time with the reef surface compared to the south shore of Bermuda. The strong gradient in DOC concentration away from the coral reef surface was the most striking result from the 1998 DOC samples (**Figure 13**). It is probable that when labile DOC is released by reef organisms (plant or animal) that it may be rapidly metabolized by the heterotrophic bacteria in closest association with the reef surface, thereby preventing its detection in the reef water-column. An alternative explanation is that the flux of DOC from the reef is low enough and physical mixing processes strong enough to prevent DOC accumulation. Sampling of DOC and bacterial

production at sites across the reef and along transects from the reef platform into the surrounding Sargasso Sea is necessary to explain some of the larger scale processes which may be important to the microbial ecology of the reef.

REEF-WATER INCUBATION EXPERIMENTS

CONVERSION FACTORS

Aside from the results I have presented from Bermuda's reefs, there is only one other known empirical determination of both TdR and Leu conversion factors for reef ecosystems. The method of conversion factor calculation has varied in the literature with more recent studies (including the present) settling on the use of the "cumulative" approach proposed by Bjørnsen and Kuparinen (1991). This technique typically yields conversion factors lower than other methods because it may be insensitive to rapid changes in the bacterial TdR or Leu incorporation rate relative to changes in cell abundance. However, its calculation includes a greater percentage of the measured cell abundance and incorporation data than the other techniques (Ducklow et al., 1992; Torréton and Dufour, 1996a). Torréton and Dufour (1996b) conducted a study of conversion factors in Tuamotu atoll lagoons and report average values of 8×10^{17} cells \cdot Mol TdR⁻¹ and 0.9×10^{17} cells \cdot Mol Leu⁻¹, using the cumulative approach to factor calculation. These values agree exceptionally well with estimates from the Bermuda coral reefs (**Table 3**). The TdR-CF from these two reef-water studies is approximately 2-4 times lower than the median value from the literature of

2×10^{18} cells \cdot Mol TdR⁻¹ (Ducklow and Carlson, 1992). Carlson et al. (1996) have determined conversion factors for an annual cycle in the Sargasso Sea near the BATS station using the cumulative approach. The previous authors report a mean TdR-CF of $16.3 (\pm 4.6) \times 10^{17}$ cells \cdot Mol TdR⁻¹ (n = 13) and a mean Leu-CF of $0.78 (\pm 0.22) \times 10^{17}$ cells \cdot Mol Leu⁻¹ (n = 12), however these values include data from nutrient amended cell cultures. When only the unenriched cultures from the previous study are considered the TdR-CF drops to $7.0 (\pm 3.9) \times 10^{17}$ cells \cdot Mol TdR⁻¹ (n = 5) and the Leu-CF drops to $0.46 (\pm 0.36) \times 10^{17}$ cells \cdot Mol Leu⁻¹ (n = 4).

The similar conversion factors for TdR-CF and Leu-CF suggests similar bacterial incorporation physiology between the reef and surrounding oligotrophic waters. Noting the similarities of both TdR and Leu conversion factors to those derived in surrounding waters (Carlson et al., 1996) and in similar environments (Torréton and Dufour, 1996a, 1996b) I believe that the Bermuda reef-water conversion factors are both realistic and accurate for calculating heterotrophic bacterial production. When the reef TdR and Leu conversion factors were plotted against one another, a significant linear trend was detected ($p < 0.05$) suggesting that bacterial TdR and Leu incorporation physiology was uniform among sites and between the two field seasons (**Fig. 21**). It appears that some of the previous studies from other reef locations (**Table 7**) may have overstated the magnitude of bacterial incorporation-based production and growth rates by assuming unreasonably high TdR-CF. Using the site-specific conversion factors

to calculate bacterial production may lead to lower estimates compared to values reported from other nearshore environments (Fuhrman and Azam, 1982; Riemann et al., 1987; Iriberry et al., 1990; Leakey et al., 1996; Tuomi, 1997). This provides further evidence for the need to calculate site-specific conversion factors for estimating bacterial production and suggests that conversion factors in the reef environment are lower than conversion factors routinely used for the open ocean and continental margins.

A fixed concentration of substrate was used for individual time-point rate determinations (**Fig. 15, Fig. 17**). During the 1997 field season, maximal rates of TdR incorporation were probably not obtained by using a [TdR] of 20 nM, while using [Leu] of 20 nM readily saturated the bacterial incorporation physiology. One consequence of not saturating TdR incorporation is the potential to overestimate the TdR-CF (e.g., ratio of cells produced to an underestimate of cumulative TdR incorporation) as suggested by Bell (1990). Bell found that literature values of TdR-CF derived from production estimates obtained with > 10nM TdR addition were closer to the theoretical conversion factors of $3 - 5 \times 10^{17}$ cells • Mol TdR⁻¹.

Since the concentration of TdR used in the present study was at the high end of all reported values for water column studies, it must be concluded that the order of magnitude of the conversion factors is probably still correct. If the TdR saturation kinetics had been more Michaelis-Menten like (increasing to some plateau rate) it would have been possible to estimate the percentage by which

TdR incorporation was underestimated, however most saturation assays displayed curves that were more linear than hyperbolic (**Fig. 25A, B**).

To obtain accurate TdR and Leu conversion factors, it is necessary to avoid contamination of the culture-media by organic matter. Comparing DOC concentration of diluted (20% of *in situ* abundance) to undiluted cell cultures indicated either slight DOC removal in diluted cultures (SB1, SB2) or no difference (JS) between treatments (**Fig. 12**). The most likely reason for the consistent difference in DOC concentration between 20% and 100% cultures is due to the fact that the measurement was a TOC analysis. The lower starting DOC concentration in the 20% culture may reflect the particulate organic carbon (POC) removed by the 0.20 μm cartridge filtration.

DILUTION CULTURES: BACTERIAL GROWTH AND REMOVAL

Bacterial growth rates (μ) calculated by the dilution technique (**Fig. 23**) were remarkably similar to one another ($0.37 \pm 0.02 \cdot \text{d}^{-1}$, $n = 4$) across the three sites where these experiments were conducted (**Table 4**). This result suggests that the conditions for bacterial growth on the reef platform were relatively uniform during the two field seasons, perhaps indicating a constant supply of DOM of a particular quality. Bacterial removal by grazing (g) in these same cultures was slightly more variable ($0.30 \pm 0.08 \cdot \text{d}^{-1}$, $n = 4$) than the growth rate. Relatively low removal rates (compared to μ) for the two experiments conducted at the Seabright sewage outfall reefs suggest an imbalance between growth and grazing. The ratio of μ/g decreases from the outfall site (SB) to the outfall control

site (JS) to the pristine reef-site on the northern perimeter of the lagoon (HB), where a net loss of bacterial cells to grazing mortality was detected. The trends suggest that microbial bacteriovores play an increasingly important role in controlling bacterioplankton populations along the transect. Since only two dilution factors (80% and 0%) were used for each of these four experiments no statistical comparison between the growth and grazing rates among sites was possible. Therefore, the data were pooled to estimate common growth ($0.37 \cdot d^{-1}$) and grazing ($0.30 \cdot d^{-1}$) rates for the reef environment (**Fig. 24**).

Although the grazing rate of bacteriovores can be manipulated in dilution culture, viral lysis of bacterial cells should be constant in both undiluted and diluted treatments since their minute size precludes them from removal by 0.20 μm filtration. Therefore, the dilution technique should only estimate bacterial losses caused by bacteriovores, which is a critical assumption of the dilution technique (Landry and Hassett, 1982). Proctor and Fuhrman (1992) showed that enrichment of filtered seawater cultures with a concentrated viral component caused significant bacterial removal. Additionally, it has been demonstrated that rates of bacterial mortality by protozoan bacteriovores and viral particles are nearly identical (Fuhrman and Noble, 1995). If viruses are important to bacterial removal on the reef it would be difficult to maintain any bacterioplankton biomass given the already high protozoan grazing rates (relative to growth). One possibility is that the dilution technique somehow overestimates the grazing rate, or conversely that the actual bacterial growth rate is underestimated due to

significant viral lysis. A recent study of microbial dynamics in the water-column over a shallow coral reef in Japan found that a large fraction (60 - 70%) of daily bacterial production was removed by heterotrophic flagellates and ciliates (Ferrier-Pagès, 1998). These estimates are very similar to ratios of bacteriovore grazing to bacterial growth for Bermuda reefs (59 - 118%) determined in the present study.

Dilution of natural microbial assemblages has the advantage over other techniques for promoting cell growth in that all the components of the microbial loop remain intact, simply less concentrated than natural assemblages. A commonly used alternative to dilution is filtration of the culture water through some operationally defined pore size to remove certain size-classes of bacteriovores. Filtration removes a large percentage of microbial grazers and potentially cuts off a source of regenerated inorganic nutrients available to cells under natural conditions. A risk associated with both the dilution and filtration methods is that filtration (to prepare diluent or screen out bacteriovores) may cause cells to rupture, elevating the concentration of dissolved organic matter. However, this was not observed in the present study at the μM scale of resolution.

SATURATION CURVES

Incorporation of TdR and Leu (thus bacterial production) can be severely underestimated if a non-saturating concentration of precursor is used. Fuhrman and Azam (1980) developed the ^3H -TdR method for oceanographic research

purposes using a range of 2 - 28 nM thymidine additions to seawater. Substrate concentrations used for incubating samples from coral reef water-columns have ranged from 5 nM TdR and 5 nM Leu (Yoshinaga et al, 1991) to 20 nM TdR and 24 nM Leu (Torréton and Dufour, 1996a). Torréton and Dufour (1996b) found that 10 nM TdR was an acceptable concentration for routine bacterial production measurements.

Interestingly, the uncorrected rates of TdR incorporation (**Fig. 27**) for the three types of sample (CSM, RSM and Bulk) from both CR and HB show a high degree of similarity at the lower concentration range (5 - 10 nM at CR, 5 nM at HB). This result may be indicative of a bacterial group, common to all three zones, with a low K_m for TdR incorporation (e.g., able to out-compete other members of the bacterial assemblage for available substrate at the lower concentration range). At higher thymidine concentrations the incorporation physiology of another bacterial group could become dominant. Hollibaugh (1994) notes the likelihood that the paths of TdR metabolism (relative fraction incorporated into DNA vs. other macromolecules) may be an indicator of both taxonomic and physiological diversity within the heterotrophic bacterial community. Plots of TdR and Leu incorporation rates vs. concentration (**Fig. 25**) display a variety of possible trends for saturation curves including, non-saturating (Logan and Fleury, 1993), multiphasic (Azam and Hodson, 1981) and inhibitory (Hollibaugh, 1988). Given the variability in curve shape from these diverse habitats, uncorrected saturation curves (not cell-specific) compared among sampling sites could potentially be used as a crude indicator of the diversity of

the bacterial population structure. This proposed technique would theoretically require calibration with a molecular technique like denaturing gradient gel electrophoresis (Muyzer et al., 1993) or DNA-DNA hybridization (Lee and Fuhrman, 1990). Although I have used the term K_m , which suggests a Michaelis-Menten type of relationship, it is not meant to imply that TdR (or Leu) incorporation is governed by Michaelis-Menten uptake kinetics. When the added TdR concentration is high enough to shut down *de novo* thymidine synthesis in the cell, bacterial incorporation of TdR into DNA proceeds via the "Salvage" pathway. Incorporation involves the participation of at least five enzymes (Robarts and Zohary, 1993), therefore an equal number of classically defined K_m values for each enzyme would need to be defined to truly discuss incorporation kinetics.

Although not comparable at the cell-specific level, HB-RSM and CR-RSM were similar in terms of absolute rates of leucine incorporation (**Fig. 27F**). These non-saturating Leu curves responded with rates of $> 500 \text{ pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ for a 5 nM Leu addition up to $> 1200 \text{ pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ at 50 nM Leu addition. When multiplied by their Leu conversion factors (**Table 3**) and the carbon conversion factor ($15.9 \text{ fg C} \cdot \text{cell}$), the rates at 50 nM represent substantial bacterial carbon production ($27\text{-}28 \text{ } \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$). Bacterial production rates for the water-columns of the two sites (at a Leu concentration of 20 nM, typical for routine bioassays) was 1400% (CR) and 2800% (HB) lower than the RSM. Beyond 5 nM TdR, kinetic curves of HB-RSM produced rates which were almost exactly

50% of the CR-RSM values perhaps indicating a common population detected at the lower end of the concentration range, with divergence in population structure at higher concentrations. A plausible alternative explanation for non-saturating rates of incorporation is that diffusive transport drives the relatively high concentration of TdR and Leu (compared to *in situ*) into bacterial cells (Logan and Fleury, 1993). When measuring bacterial production with TdR and Leu, in ecosystems like coral reefs with diverse habitat structure, saturation curves should become an integral part of the measurement to potentially correct for the underestimation of production rates.

BACTERIAL CARBON PRODUCTION

THYMIDINE AND LEUCINE ESTIMATES

Bacterial production estimates calculated from TdR and Leu incorporation rates and site-specific conversion factors yielded identical rates of carbon production demonstrated by Model II regression (**Fig. 11**). Assuming a direct leucine to carbon conversion factor of $1546 \text{ g C} \cdot \text{Mol}^{-1} \text{ Leu}$ (Simon and Azam, 1989) yields carbon production rates identical to those based on my empirical Leu conversion factors and a carbon conversion factor of $15.9 \text{ fg C} \cdot \text{cell}^{-1}$. The equivalent slopes and Y-intercepts of **Fig. 29A** and **Fig. 29B** demonstrate this result. The previous result is significant because the Simon and Azam approach avoids reliance on a $\text{carbon} \cdot \text{cell}^{-1}$ conversion factor so the agreement between the two Leu based production estimates validates the $\text{carbon} \cdot \text{cell}^{-1}$ conversion

factor that I used for Bermuda reef bacteria. Both regressions yield slopes of 1, showing a direct correspondence between the two production estimates. This result suggests a balanced growth situation, where cell division is proportional to biomass production. Since it is valid to assume balanced growth, the average of the two production estimates (each the average of a triplicate determination) was used to compare bacterial production across sites (**Fig. 30**).

Bacterial carbon production (BCP) reached its highest average rate $19 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ in the RSM (**Fig. 30**). Unlike the CSM and Bulk samples that were relatively detritus-free, samples from the RSM invariably included organic detrital particles and extremely fine carbonate particles, which contributed to a complex matrix for bacterial colonization. Although detritus may be an important source of organic matter on coral reefs (Alongi, 1989), Hansen et al. (1992) detected no significant correlation between the amount of detrital input to a coral reef lagoon, and both bacterial production and the standing stock of bacterial biomass in lagoon sediments.

On Bermudian reefs, carbon production in the RSM was fairly consistent across sites, except for the single sample from SB which was collected at a depth of 20 m compared to a range of 3 - 10 m at the other sites. Bulk and CSM samples were essentially indistinguishable with respect to the magnitude of BCP (**Fig. 30, Table 5**), which is in contrast to the findings of Paul et al. (1986). The previous authors detected an average TdR incorporation rate of $2.3 \text{ pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ (± 1.4 , $n = 7$) in the water-column above Florida reefs and $58.3 \text{ pMol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$

(± 86.6 , $n = 5$) in the CSM, but failed to extend their estimate to rates of carbon production. Assuming a TdR-CF of 5×10^{17} cells \cdot Mol $^{-1}$ and a CCF of 15 fg C \cdot cell $^{-1}$, their carbon production rates are 10.5 μ g C \cdot l $^{-1}$ \cdot d $^{-1}$ in the CSM and 0.4 μ g C \cdot l $^{-1}$ \cdot d $^{-1}$. This reanalysis yields a similar carbon production estimate to those obtained for Bermuda reef bacteria.

The high end of bacterial carbon production (assuming 15 fg C \cdot cell $^{-1}$) estimates from reef-waters are those which were directly incubated with coral mucus, yielding 330 μ g C \cdot l $^{-1}$ \cdot d $^{-1}$ (Vacelet and Thomassin, 1991). Since unamended BCP estimates from coral reef waters do not approach this level, except in samples containing sediment and detrital particles (Moriarty and Hansen, 1990; Torr eton et al., 1997), it must be concluded that enhancement of bacterial carbon production by coral derived DOM is generally diffuse in the reef water-column. The results from the coral incubation experiment (**Fig. 28**) suggest that the contact time between a parcel of seawater and the reef surface will affect the relative magnitude by which bacterial production may be stimulated by DOM lost by the reef. This result may be applicable to the idea that microbial regenerative spaces within the reef structure, where water flow is reduced, are important locations for recovering reef-community DOM back to particulate form (DiSalvo and Gundersen, 1971).

GROWTH RATE BASED ON BACTERIAL CARBON PRODUCTION AND BIOMASS

Specific bacterial growth rates (ratio of P/B) from the reef water-column (0.20 - 0.50 d $^{-1}$) were within the range of growth rate estimates from other reef

systems (**Table 7**). Although there is no *a priori* reason to expect similar growth rates from one geographically distinct reef system to the next the fact that different studies, employing a variety of techniques, have converged on similar growth rates suggests a common growth physiology for reef bacteria. Ferrier-Pagès and Gattuso (1998) used a novel approach for conducting microbial incubations *in situ*, yielding moderate growth rates (1.32 - 2.76 d⁻¹). The experimental design used by the previous authors allowed natural DOM and DIN to diffuse through dialysis membrane into *in situ* incubation chambers containing size-fractionated reef-water microbial assemblages suspended above a shallow coral reef. Since the substrate supply never became limiting and grazing pressure was reduced or removed, bacterial growth and bacterial carbon production was substantial. This result suggests that traditional microbial culture techniques may be inadequate at estimating the true magnitude of bacterial growth and production on naturally available substrates.

Herndl (1991) estimated bacterial growth rates from 0.6µm seawater cultures collected along a trophic gradient crossing the Atlantic Barrier Reef off Belize. Progressing along the 2 km trophic gradient from seaward to inshore stations, the absolute magnitude of bacterial production and biomass each increased by a factor of 4.6 and 4.8 respectively, however the specific growth rate (μ) remained constant. A constant μ suggests a bacterial physiology able to rapidly adapt to changing nutrient or organic matter concentrations over a relatively short distance.

The ratio of P/B ratio in 20% cultures at T-0 was similar to the growth rates derived from all four dilution grazing experiments (**Table 6**), suggesting that the use of dilution series accurately estimates μ . The range of growth rates across sites (water-column only) and dilution factors was relatively constant ($0.30 \pm 0.10 \text{ d}^{-1}$, $n = 10$) but slightly lower than the rate computed by the average regression of the pooled dilution curves ($0.36 \pm 0.06 \text{ d}^{-1}$). The average growth rate translates into a generation time of 2.3 days, strikingly similar to that determined by Ducklow and Hill (1985) in the surface waters of Gulf Stream warm core rings. Sorokin (1973) measured some of the first bacterial growth rates for bacteria from a wide range of reef habitats. Sorokin's pioneering growth rate estimates ($0.47 \pm 0.37 \text{ d}^{-1}$, $n = 14$) for the reef environment span the range of more recent estimates.

The ratio of bacterial production rate (P) to bacterial biomass (B) is the growth rate (μ) of a population at a particular point in time. Plotting numerous P:B pairs and regressing the scatter plot of data is a technique for estimating a system wide bacterial growth rate. Bacterial production covaries with bacterial biomass depending on parameters such as DOM, nutrients and temperature (Azam et al., 1983; Daneri et al., 1994; Shiah and Ducklow, 1994; Carlson and Ducklow, 1996). The P/B ratio is highly variable and the strength of developing a system-wide growth rate lies in having a large number of samples to compare. When this analysis was performed for the reef field samples, the variables exhibited a high degree of scatter with respect to one another. A system wide

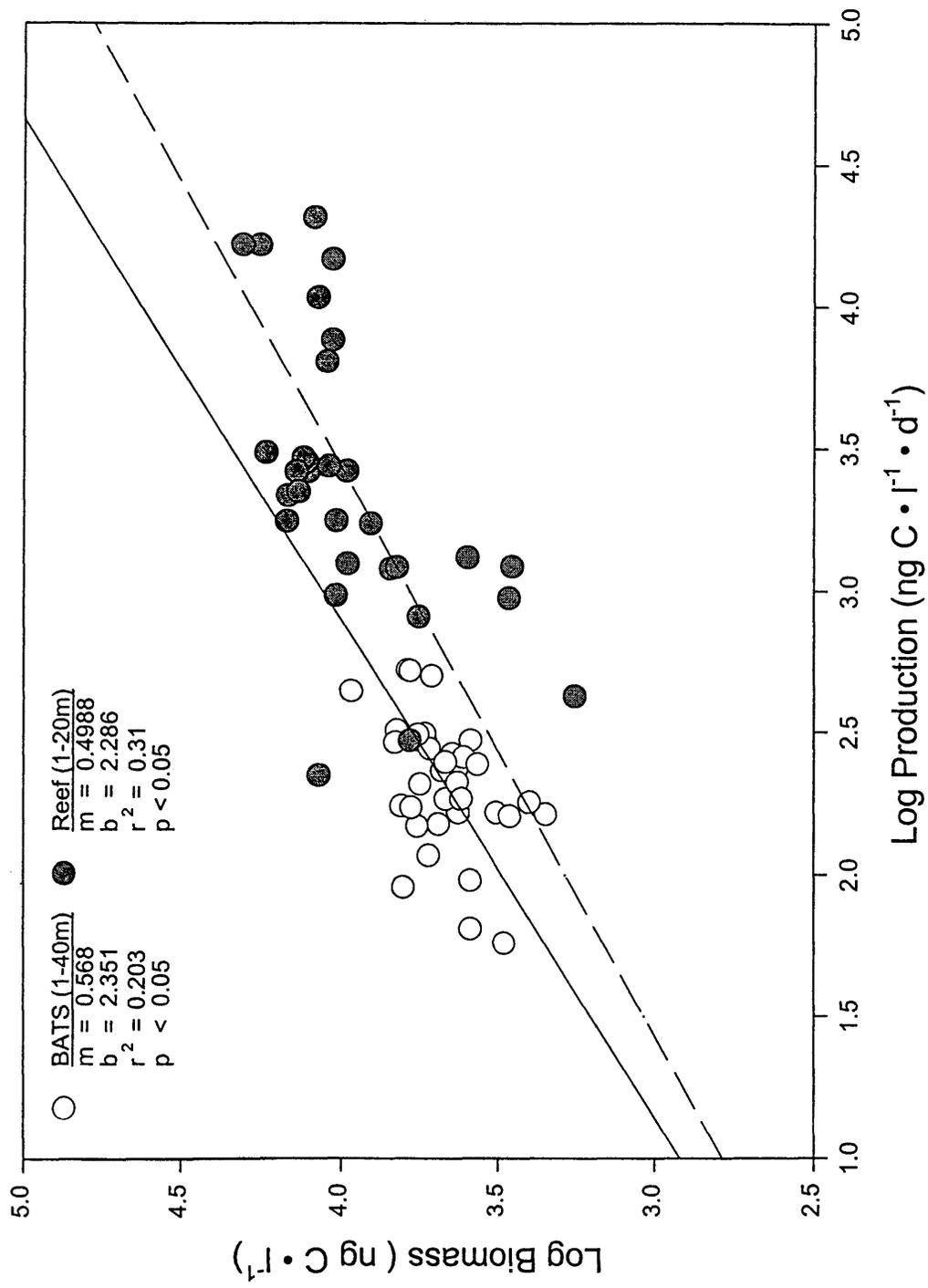
growth rate for bacteria in reef ecosystems may only be appropriate for each particular habitat studied.

The inverse of growth rate, B/P , has been used as an indicator of resource limitation, where P is used as an estimate of the flux of growth substrate into cells assuming uniform growth efficiency (Billen et al., 1990). Expanding on this theme, Ducklow (1992) examined ratios of B/P at the scale of oceanic basins and reached the conclusion that above a ratio of 0.6 (slope of Log_{10} - Log_{10} plots) bacterial populations in these systems were controlled by organic matter flux. Using the same reef samples described above B/P was computed via Model II linear regression to be 0.50 ($n = 30$) (**Fig. 32**). Based on this finding it is suggested that resource limitation is a controlling influence of intermediate effect for bacterial populations on the reef.

BACTERIAL GROWTH EFFICIENCY ON THE REEF

Growth efficiency of marine bacteria has been defined in many terms, with increasing refinement as techniques for measuring the total bacterial carbon budget have been developed (Carlson et al., submitted). Early estimates of bacterial growth efficiency were thought to be greater than 50% (Wiebe and Smith, 1977) a value which has commonly been used to scale the flow of organic matter through the bacterial component of the microbial loop (Ducklow, 1983). In a comprehensive review of the BGE literature, del Giorgio and Cole (1998) detected a trend of increasing BGE with increasing trophic richness. They found that BGE estimates were lowest in oligotrophic systems, where it was

Figure 32. Index of bacterial resource limitation ($\log B/\log P$) for the Bermuda Atlantic Time-series Station (BATS) and the Reef estimated from the slopes of Model II regressions of \log Biomass versus \log Production. Both the regressions are significant ($p < 0.05$). BATS data (<http://www.bbsr.edu>) represent samples to a depth of 40 m during 1996 (open circles) and data from the reef was collected between the surface and 20 m (filled circles). Regression coefficients (m and b) from BATS (solid line) and reef (dashed line) are not NS different from one another ($p > 0.05$).



hypothesized that high basal metabolic energy requirements and a shortage of both inorganic nutrients and DOC of high energy yield, contributed to inefficient bacterial biomass production (del Giorgio and Cole, 1998).

For this study I have utilized the relatively simple approach of dividing net bacterial cell production by the change in DOC concentration of the 20% cell cultures over the time course of the experiment (**Table 8**). If newly produced DOC enters the bulk pool within the culture vessel (from cell lysis or excretion) BGE may be underestimated since this technique only accounts for the net change in DOC. Bacterial growth efficiency (BGE) for the reef environment was 10-25% for all three experiments, which is a range of values typical for nutrient depleted waters (del Giorgio and Cole, 1998). These efficiencies suggest that cells were limited by some other growth factor aside from DOC since DOC was taken up over the relatively short time-scales of these culture experiments. Although relatively low, the magnitude of BGE detected in this study is similar to values reported from the Sargasso Sea (Hansell et al., 1995; Carlson and Ducklow, 1996). Relatively inefficient BGE suggests that bacteria would have a difficult time maintaining their observed biomass unless cell populations exhibit rapid turnover times. Turnover times of 2.5 to 3 days (calculated by dilution) and 2 to 4 days (calculated by P/B ratio) are not particularly rapid compared to the fastest rates from the literature (measured in hours). They may however be sufficient to prevent depletion of bacterial cells by viral lysis, bacteriovore grazing and potential removal by the reef filter feeders.

The hypothesis that bacteria may sacrifice growth efficiency for enhanced rates of growth (del Giorgio and Cole, 1998) is exhibited in the BGE calculations from the coral/reef-water incubation experiment (**Table 9**). By the final time-point (24 hours), the rate of bacterial production was 4-5 times greater in the treatment incubated with the coral specimen compared to the control treatment, but BGE was 3-7 times lower in the experimental bottle. At the scale of this experiment, bacterial growth clearly increases due to the process of coral derived DOC being released into a confined volume. However, the change of DOC concentration of only 0.4 μM in the control bottle is within the error of the measurement. This result suggests that a longer incubation is needed to detect bacterial utilization of DOC in unamended cultures. Further studies, with replication of experimental and control incubations are needed to provide a more complete description of the magnitude by which freshly produced DOC may stimulate bacterial growth in the coral reef environment.

REEF VS. BERMUDA ATLANTIC TIME-SERIES STATION (BATS) BACTERIAL PROCESSES

Since heterotrophic bacterial biomass on the reef is roughly equivalent to that in the top 40 m at BATS ($2 - 10 \mu\text{g C} \cdot \text{l}^{-1}$), but bacterial carbon production on the reef ($2 - 20 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$), is in excess of that at BATS ($0.1 - 0.5 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$), removal of bacteria must be relatively greater on the reef than in the oligotrophic Sargasso. This result also suggests that bacteria in the Sargasso Sea may undergo physiological changes when they impinge upon the Bermudian coastal zone. A second possibility is that the Island platform may be

a source of heterotrophic bacteria with growth characteristics unique to the reef environment. The growth rate of bacteria at the BATS station over an annual cycle (calculated from P/B) was $0.09 \cdot \text{d}^{-1}$ (**Fig. 31**) indicating a turnover time of 11 days compared to an average of 2.5 - 3.0 days on the reef. Some aspect of the bacterial growth condition changes along the 75 km between Bermuda reefs and the BATS station. It may be that the DOC quality on the reef is superior to the DOC at BATS, even though concentrations across the two systems are similar. The Model II regressions of $\text{Log}_{10} B$ vs $\text{Log}_{10} P$ for BATS and Reef samples (**Fig. 32**) are significant ($p < 0.05$) and show identical slopes of 0.57 and 0.50, suggesting that the bacterial populations at both sites are controlled to a relatively large degree by resource limitation.

CONCLUSIONS

Conversion factors (CF) to calculate bacterial production based on leucine and thymidine incorporation rates were fairly homogeneous (same order of magnitude) across the Bermuda reef platform. Statistical comparison of the five CF determinations showed the greatest difference between north-shore versus south-shore sites. The choice of CF for a particular system will greatly affect calculated rates of bacterial carbon production. This point is illustrated by the magnitude of the average reef-bacteria TdR CF ($5 \times 10^{17} \text{ cells} \cdot \text{Mol}^{-1}$) which is approximately 2 – 3x lower than the value commonly used for Sargasso Sea bacteria. Rates of bacterial carbon production in the coral reef water-column were similar to those in the coral surface-microlayer, despite higher

concentrations of dissolved organic carbon (DOC) in the at the coral surface. The reef surface-microlayer habitat, with its abundant detrital particles, supported bacteria capable of producing approximately $20 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, while coral surface-microlayer and water-column production rates were typically closer to $5 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$. This difference among habitats suggests the importance of particle-attached bacteria to carbon cycling in the reef environment. Bacterial growth efficiencies were approximately 10 – 30% based on the utilization of dissolved organic carbon during incubation. The higher bacterial growth efficiencies (20 – 30%) are more typical of systems that are not particularly depleted of nutrients and organic matter. These efficiencies suggest that a substantial amount of dissolved organic matter in the reef environment is returned to particulate form, before being washed off the reef platform and diluted in the oligotrophic Sargasso Sea.

Heterotrophic bacterial populations appear to maintain a surprisingly uniform cell-specific biovolume ($0.05 \mu\text{m}^3$) in the subtropical coral reef ecosystem of Bermuda. Since cell-specific biovolume was extremely consistent across habitats and field seasons, factors that controlled cell abundance dictated the magnitude of bacterial biomass. Uniform cell size suggests that the larger size classes of heterotrophic bacteria may be subject to higher rates of protozoan grazing pressure, although this theory remains untested in the coastal waters of Bermuda. Rates of bacteriovore grazing ($0.2 - 0.4 \text{ d}^{-1}$) were found to nearly balance bacterial growth rates ($0.3 - 0.4 \text{ d}^{-1}$), with grazing in excess of growth

detected during one of the four dilution experiments. Independent estimates of the specific bacterial growth rate, based on the ratio of production to biomass (P/B), support the growth rates determined by the dilution technique. Evidence is mounting that removal of bacteria by reef filter feeders is an important bacterial loss term and an important source of carbon for reef organisms. Future studies of coral-reef/bacteria interactions will need to consider a two-layered model of bacterial removal, e.g. loss to protozoan grazers and viruses in the water-column and loss to reef macrofauna in the benthic layer.

Rates of bacterial thymidine incorporation are generally 1- 2 orders of magnitude greater on Bermuda's reefs than at the BATS site (oligotrophic ocean). The turnover time of bacterial biomass (11-12 days) at BATS is 4 – 6x times slower than that of coral reef bacteria (2 – 3 days) despite similar bacterial abundance and dissolved organic carbon (DOC) concentrations between the two sites. This suggests a fundamental difference in the quality and source of DOC available to the reef bacteria. During a reef-water incubation experiment, the presence of a small coral specimen greatly enhanced bacterial abundance, cell-specific biovolume and production rates compared to a control treatment with no coral. The likely source of this stimulation in the coral bottle was labile dissolved organic matter, enriched by 5 μ M DOC compared to the control bottle, released by the coral. The slope of the relationship between bacterial biomass and production (the resource limitation index) was similar for historical BATS data and reef data from this study, suggesting that the supply or quality of growth

substrates limit bacterial populations at both sites. Although the two groups of data yield identical slopes, the reef samples displayed slightly higher bacterial biomass with much higher production rates. It is likely that different factors (grazing, viral lysis and resources) and different resources (organic matter and inorganic nutrients) control bacterial populations in the open Sargasso Sea versus the Bermuda coastal environment. A further likely scenario is that the two environments support genetically distinct bacterial populations.

This is one of the first studies to examine bacterial growth and removal, as well as DOC concentrations, in the Bermuda coral reef ecosystem. Results presented in this study (growth and grazing rates, growth efficiencies and carbon production estimates) could be used to parameterize models of bacteria/reef-community dynamics. Modeling the microbial loop for a coral reef ecosystem may provide some insight into the extent to which the reef community benefits from bacterial carbon cycling. Future studies of bacterial populations at the level of community structure may help to explain the processes that contribute to vastly different bacterial carbon production rates between the coral reef ecosystem and the surrounding Sargasso Sea.

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VITA

Peter Dylan Countway was born in Wolfeboro, New Hampshire on November 4th, 1968. As a boy, he was frequently found with diving mask and flippers, exploring Lake Winnepesaukee with his family on their sailboat, *Moonshadow*. Peter graduated high school as class salutatorian and went on to the University of New Hampshire, in Durham, NH. While enrolled at UNH he majored in Zoology with a minor in Marine Biology. Upon graduating *cum laude* from UNH with a BA degree, he departed immediately for Bermuda to participate in a volunteer program at the Bermuda Biological Station for Research (BBSR). Continuing on at BBSR as a research technician, Peter spent the next five years working on various oceanographic and marine biology projects, logging nearly 50 cruises on the R/V *Weatherbird II*. Leaving Bermuda with his future wife, Rebecca, the couple moved to Virginia to begin masters programs at the Virginia Institute of Marine Science, College of William and Mary. Peter earned his M.S. degree in Marine Science in August of 1999 and will begin a Ph.D. program in Biological Oceanography at the University of Southern California in Los Angeles in the fall of 1999.