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Spatial and Temporal Variation of Marine Bacterioplankton in Florida Bay, U.S.A.

John B. C. Bugden, Maria A. Guerrero and Ronald D. Jones

Southeast Environmental Research Program
Department of Biological Sciences
Florida International University
University Park, Miami, FL 33199, U.S.A.

ABSTRACT

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Physical, chemical and biological parameters of the water column of Florida Bay were examined at seven study sites over an 18-month period. At the time, Florida Bay was experiencing a seagrass die-off. The data collected indicated that the presence of seagrass significantly affected the overlying water column, especially with respect to biological parameters such as, heterotrophic activity and bacterial numbers. Microbial activity and numbers were also significantly higher over areas of dying seagrass than over healthy or dead areas. The exact nature of the relationship between seagrass and bacterioplankton is not known. Correlation analyses yielded significant associations ($P < 0.05$) between the biological parameters (heterotrophic activity and bacterial numbers) and dissolved organic matter, chlorophyll, primary productivity, and ammonium. The possibility that other variables might have compounded and added to this effect is discussed.

ADDITIONAL INDEX WORDS: *Seasonal changes, bacterioplankton properties, temperate seagrass systems, carbon flow, seagrass beds, detritus-based ecosystems, DOM.*

INTRODUCTION

In south Florida, Florida Bay is an important ecosystem with seagrass beds carpeting most of its bottom. Nearly 80% of the bay (1660 km^2), within Everglades National Park (ENP), is occupied by three species of seagrass: *Thalassia testidinum, Halodule wrightii, Syringonium filiform.* Not surprisingly, ODUM *et al.*, (1973) attributed the high productivity of the bay to seagrasses, which are regarded by the authors as "probably the most productive of the marine phototrophic communities of south Florida". Seagrass distribution and leaf biomass in Florida Bay is dominated by *Thalassia* (FOURQUREAN, 1992) and increases from the northeast to the southwest (ZIEMAN *et al.*, 1989). These seagrasses play an important role in the growth and survival of many commercially important marine species (ROBBLEE *et al.*, 1991) as well as many types of waterfowl (HECK and ORTH, 1980). Few organisms feed directly on the seagrass itself, KLUG (1980) has estimated that when devoid of direct grazing approximately 95% of seagrass biomass is turned into detritus. Similarly, MANN (1988) postulated that most of the primary production in aquatic ecosystems is conveyed to other trophic levels through detrital processes rather than through direct grazing.

It has been confirmed repeatedly (MORIARITY *et al.*, 1990; MORIARITY *et al.*, 1985; FENCHEL, 1970; BENNER *et al.*, 1986a and 1986b; MORAN and HODSON, 1989; ODUM and HEALD, 1972) that heterotrophic bacteria mediate the coupling of detritus and higher trophic levels by "conditioning" the detritus with their own biomass so that it acts as a food source (MARVALIN *et al.*, 1989; FENCHEL, 1970; CHIN-LEO and BENNER, 1991, KLUG, 1980). This is the established way in which dissolved organic matter (DOM) is conveyed to higher trophic levels in pelagic ecosystems (WRIGHT and COFFIN, 1984).

During the period of this study (January 1990–June 1991) Florida Bay was experiencing a major seagrass die-off event. The cause of the die-off was unclear but it may have involved a combination of: abnormally high salinity; reduction in the frequency of hurricanes; presence of a pathogenic slime mold (*Labyrinthula* sp.) related to the eelgrass wasting disease, and a chronic hypoxia of the *Thallasia testidinum* roots and rhizomes (ROBBLEE *et al.*, 1991).

There is limited data on the bacterial biomass and production for temperate seagrass systems, and specially for those in south Florida. A study of the factors that alone or in concert can affect bacterioplankton was needed, because as stated above bacteria are crucial in both decomposition processes as well as transfers of energy. The aim of this study was to evaluate the role of bacterioplankton in the water column above the seagrass system of Florida Bay and to investigate its relationship to specific physical, chemical and biological parameters. We hypothesized that bacterioplankton activity and numbers would be higher over seagrass dominated areas than over areas with little or no seagrass. Furthermore, we believe that microbial parameters will change according to the health of the seagrass.

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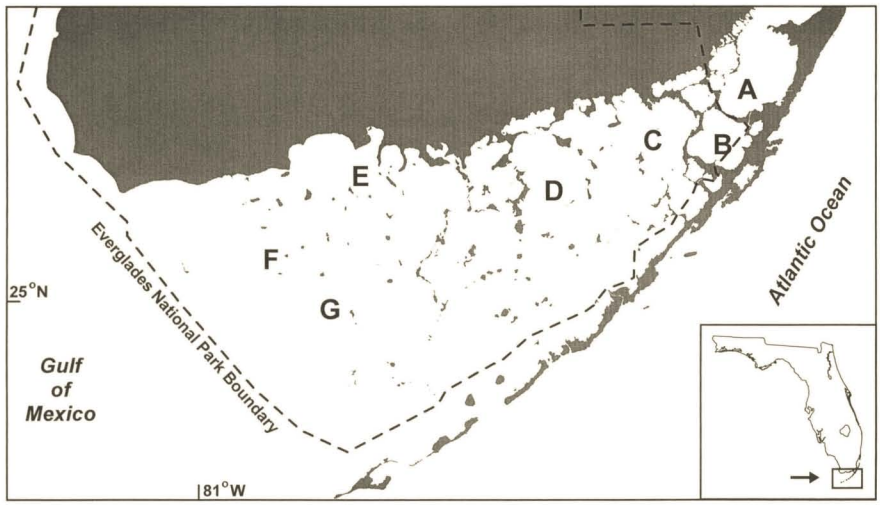

Figure 1. Map of Florida Bay showing study site locations. (A) Barnes Sound; (B) Blackwater Sound; (C) Duck Key; (D) Park Key; (E) Rankin Lake; (F) Johnson Key Basin; (G) Rabbit Key Basin.

MATERIALS AND METHODS

Study Sites

Florida Bay is a triangular, shallow water embayment located south of the Florida peninsula (Figure 1). It comprises an area of approximately 2200 km^2 and is defined by the Florida peninsula to the north, the Florida Keys to the south and east, and the Gulf of Mexico to the west. It is characterized by small mangrove islands and carbonate mudbanks which surround shallow basins with the average depth being slightly less than one meter (TILMANT, 1989). These mudbanks serve to restrict circulation and attenuate the lunar tidal influence of the Gulf (ROBBLEE *et al.*, 1991). Florida Bay has historically been described as varying between a positively functioning estuary and a tropical hypersaline lagoon (TILMANT, 1989), but due to the effects of water management (surface water flow controlled by channeling through canals) the bay now functions more often as hypersaline lagoon.

Seven study sites were picked in Florida Bay (Figure 1). The sites were designated Barnes Sound (BRN—25° 12.8N, 80° 23.9W); Blackwater Sound (BLK—25° 10.5N, 80° 25.8W); Duck Key (DUK—25° 10.8N, 80° 29.5W); Park Key (PRK—25° 07.0N, 80° 35.6W); Rankin Lake (RKN—25° 06.7N, 80° 47.2W); Johnson Key Basin (JKB—25° 59.6N 80° 57.4W); and Rabbit Key Basin (RKB—25° 59.6N, 80° 57.4W). Collections were made once a month starting in January 1989 and ending in June 1990 at approximately 4 week intervals. Water samples were collected from the side of an ENP research boat at a depth of 15 cm. All the bottles were rinsed three times with sample prior to actual collection.

Analytical Methods

Salinity and temperature were measured in the field using an Orion model 140 conductivity salinity meter (Orion Research Corp., Boston, MA) by immersing the electrode in surface waters to a depth of 0.5 m. The readings were allowed to stabilize before readings were recorded. Salinity was registered in parts per thousand (ppt), and temperature in °C.

Heterotrophic activity was determined from samples collected in 500 ml Nalgene bottles stored at ambient temperature in a cooler until returned to the laboratory, within 8 h of collection. Laboratory incubations were performed with ^{14}C-glucose (specific activity: 4.074 μCi ml^{-1}; 275 mCi mmole^{-1}) following the method of STRICKLAND and PARSONS (1972). Samples (10 ml) were placed in 60 ml serum bottles followed by the addition of 25 μl of label. The bottles were then sealed with rubber caps and incubated at 25°C on a shaker table at 200 rpm for 3 h. Incorporation of label was terminated by addition of 1 ml of 5N NaOH injected through the cap with a syringe; thorough blending was ensured by mixing the bottles on a shaker table for 30 min.

Respiration (HA_{resp}) was determined by evolution of $^{14}CO_2$. This was performed by removing the caps and replacing them with caps and buckets containing a filter paper wick (Whatman CHR 1, Maidstone, England) soaked with β-phenylethylamine. The bottle was sealed and 1 ml of 10 N H_2SO_4 was injected through the cap with a syringe. The samples were placed on a shaker table (200 rpm) for 1 h after which the wicks were removed from the cap and bucket assembly and placed into scintillation vials to which 5 ml of Omnifluor (New England Nuclear, Boston, MA) scintillation cocktail was added. The scintillation vials were capped and then placed into a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Fullerton, CA) for determination of radioactivity. Incorporation of label into cellular material (HA_{inc}) was determined by filtering the remaining sample in the serum bottle through a 0.45 μm Gelman GA-6 Metricel membrane filter (Gelman Sciences Inc., Ann Harbor, MI), placing the filter in a scintillation vial, and allowing it to dry overnight. For these filters only three ml of Omnifluor scin-

tillation cocktail were added to the vials. Total heterotrophic activity (HA_{tot}) was determined by adding both HA_{resp} and HA_{inc}.

Samples for acridine orange direct counts (AODC) were collected in the field by adding 10 ml of sample to a scintillation vial containing 100 μl of 37% formaldehyde. The sample was stored at 5°C until analyzed. Counting was done following the procedure of Strickland and Parsons (1972). Acridine orange was obtained from Sigma Chemical Co. (St. Louis, MO) and made to a concentration of 0.1%. The filters used were prestained black 0.2 μm polycarbonate membrane filters (Poretics Corporation, Livermore, CA). At least 10 fields or 300 bacteria were counted per filter.

Chlorophyll samples were collected in the field by filtering 120 ml of water through a GF/F glass fiber filter (Whatman International Ltd., Maidstone, England). The filter was placed into a 1.8 ml microcentrifuge tube and 1.5 ml of 90% acetone was added. Samples were kept in the dark and on ice for transportation to the laboratory. Upon arrival, samples were stored at −15°C until analyzed. Analysis was performed using the method of Strickland and Parsons (1972) by first pushing the filter down to the bottom of the microcentrifuge tube and centrifuging for approximately 3 min to settle any particulate matter in a Hermle Z 230 M microcentrifuge (National Labnet, Woodbridge, NJ). A 750 μl subsample of the acetone extract was taken into a cuvette and diluted with 2.25 ml of 90% acetone. Relative fluorescence was measured in a Fluoro IV fluorometer (excitation 435 nm, emission 667 nm; Gilford Instruments, Oberlin, OH). A standard curve for chlorophyll-a was generated by measuring different concentrations of a chlorophyll-a stock solution prepared in 90% acetone (final concentration: 1 mg L^{-1}).

DOM samples were obtained from the filtrates from chlorophyll collection and placed in 60 ml Nalgene (HDPE) bottles which had first been rinsed three times with the filtrate. The samples were stored on ice until they reached the laboratory. Relative DOM concentrations were estimated fluorometrically using a Hoefer Scientific Instruments TKO 100 (emission 360 nm, excitation 490 nm; Hoefer Scientific Instruments, San Francisco, CA). Duplicates of each sample were recorded and the values averaged. The concentrations were recorded as relative fluorescence units (RFU). The instrument was calibrated against a standard of o-methylfluorescein (final concentration: 0.01 mM). The standard was measured before and after the samples, and the values compared from trip to trip to ensure stability of standard stock solutions.

Primary Productivity was determined in samples held in 300 ml Biological Oxygen Demand (BOD) bottles (dark and light) and stored in the dark in a cooler at ambient temperature until returned to the laboratory for analysis. In the lab, the bottles were processed following the procedure of Strickland and Parsons (1972). Both bottles were injected with 0.5 ml of ^{14}C-sodium bicarbonate (2.5 μCi ml^{-1}), and incubated submerged in a distilled water bath at 25°C for 2 h, while irradiated under daylight fluorescent light (Phillips F30T12/DS/R). Irradiance was measured with a Photosynthetically Active Radiation (PAR) detector. The irradiance was determined to be approximately 25 W m^{-2}. Thus, there is a considerable difference between the intensity of surface sunlight (appr. 650 W m^{-2}) and that of artificial sunlight (25 W m^{-2}). After incubation, samples were removed from the water bath and filtered through a GF/F filter. The filter was placed in a scintillation vial to which 10 ml of Triton-X Omnifluor scintillation cocktail (New England Nuclear, Boston, MS) was added. Samples were allowed to quench overnight to eliminate any water interference before the counting of radioactivity.

Nutrient samples for nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) analysis were obtained from the filtrate of chlorophyll collection. The samples were stored on ice until returned to the laboratory, and were frozen at −4°C until analyzed. Analysis for nutrients was performed by wet chemical analysis using a four channel Alpkem RFA-300 (Rapid Flow Analyzer) Nutrient Analyzer (Alpkem Corp., Clackamas, OR). All nutrients were determined colorimetrically; NO_3^- by the cadmium reduction to NO_2^- method; NO_2^- by the sulfanilamide method; NH_4^+ by the sodium hypochlorite-phenolate-nitroferrocyanide method and PO_4^{3-} by the ascorbic acid-ammonium molybdate method (see Alpkem Methodologies Manual for analytical techniques and references). Internal standards were prepared from stock standard solutions and were used at various concentrations to generate calibration curves.

Total phosphorus was determined according to the procedure of Solorzano and Sharp (1980). Ten ml of the sample placed in a scintillation vial was mixed with 200 μl of 0.17 M $MgSO_4$ and then evaporated to dryness in a 80°C oven. Once dried, the samples were ashed at 550°C for 3.5 h, hydrolyzed by incubation with 10 ml of 0.25 N HCl at 80°C for 3 h with shaking at approximately 0.5 h intervals. After hydrolysis the pH of the samples were adjusted to approximately 3–4 with 1 N HCl or 1 N NaOH as required (pH higher or lower than this range interfered with the chemicals). The processed samples were analyzed in the Alpkem RFA-300 like the rest of the nutrients.

Statistical analyses were done with Statgraphics version 4.0 (Statistical Graphics Corporation, Princeton, NJ). Seasonal statistical analyses was not practical due to the short length of data collection. For all other statistical analyses a one time-averaged mean value was calculated for each measured parameter at each study site and sub-site. These means were used to generate one-way analysis of variance (ANOVA) and also correlation matrices. ANOVA analysis was performed as the best possible test to determine the relationship between the presence of seagrass and health of the seagrass and the overlying water column. Those means that were not significantly different ($P > 0.05$) were placed together as a homogeneous group. A significantly different mean was displayed by having another number: the higher the mean, the higher the number. Factor analysis was also performed as a supplement to the ANOVA and to help assess any underlying patterns or causal relationships.

RESULTS

Temperature patterns were typical; high (31°C) in the summers and low (21.3°C) in the winters, with only occasional

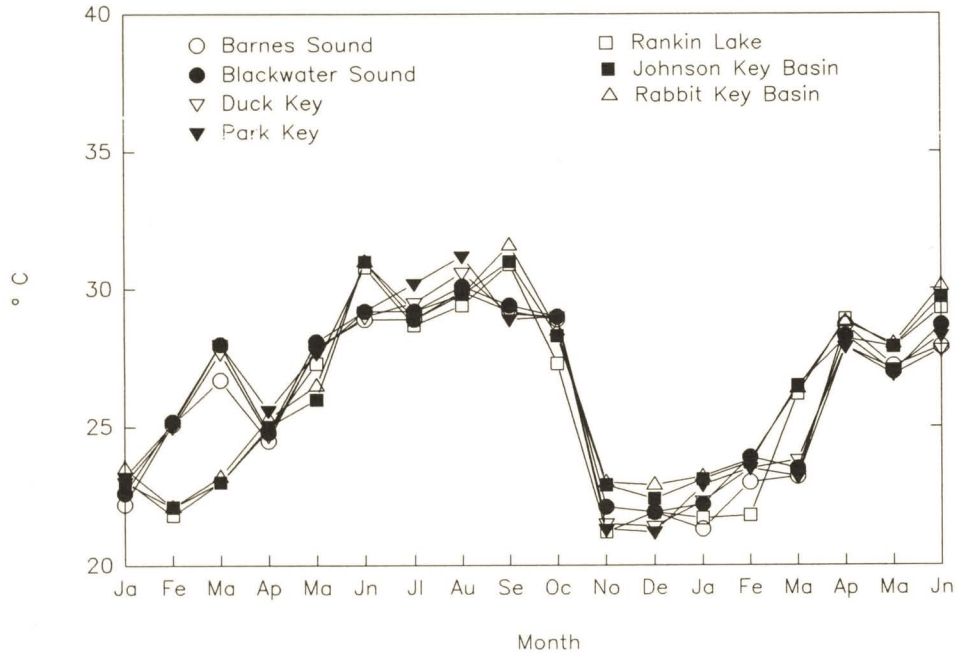

Figure 2. Plot of temperature *vs.* time for all study sites.

fluctuations from the norm (Figure 2). Salinity (range: 38 to 60 ppt) exhibited seasonality to a lesser extent, yet it was the only parameter that displayed spatial variation (Figure 3). Typically higher salinities were found in the central part of the bay (i.e. Duck Key, Park Key and Rankin Lake).

Table 1 shows the results of correlation analyses on the time averaged grouped data. All of the raw data from this work could be found in BUGDEN (1993). In general, biological parameters like total heterotrophic activity (HA_{tot}), and its individual components bacterial respiration (HA_{resp}) and

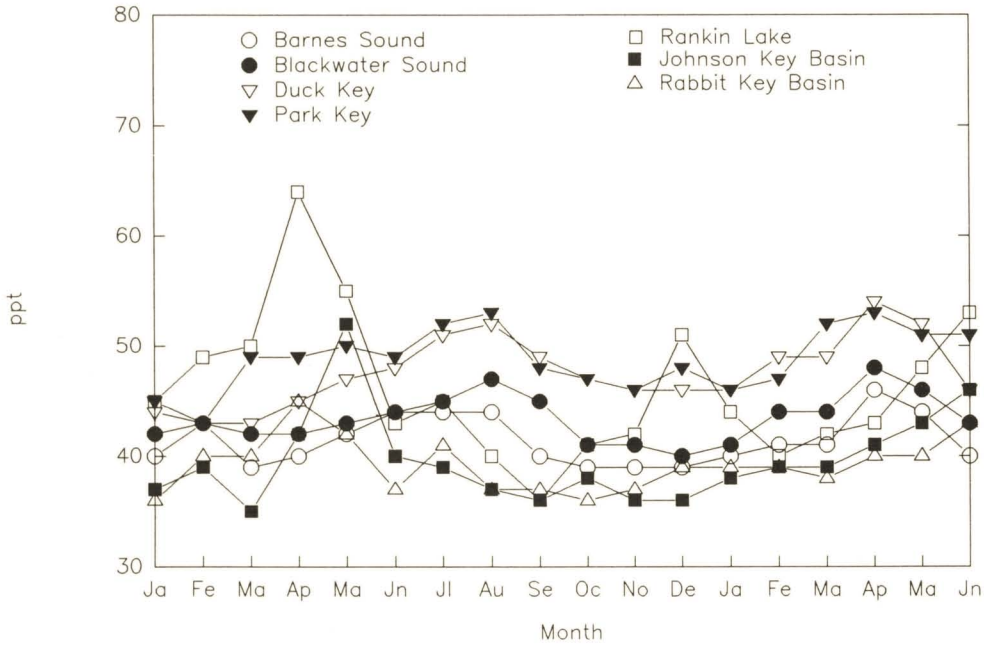

Figure 3. Plot of salinity *vs.* time for all study sites.

Table 1. *Matrix of correlation coefficients of grouped data for Florida Bay. Those coefficients in bold are significant to the 0.05 confidence level.*

	SAL.	TEMP.	HA_{tot}	HA_{inc}	HA_{resp}	AODC	DOM	CHL	PP	NO_2	NO_3	NH_4	PO_4
TEMP.	0.061												
HA_{tot}	−0.052	0.118											
HA_{inc}	−0.147	**0.229**	**0.756**										
HA_{resp}	0.024	0.025	**0.918**	**0.435**									
AODC	0.110	−0.107	**0.741**	**0.407**	**0.774**								
DOM	−0.158	−0.041	**0.415**	0.174	**0.474**	**0.420**							
CHL	**0.326**	0.137	**0.291**	**0.262**	**0.241**	0.199	**0.155**						
PP	0.063	0.001	**0.421**	**0.433**	**0.315**	**0.298**	**0.300**	**0.467**					
NO_2	**0.337**	−0.021	**−0.205**	−0.086	**−0.230**	−0.180	**−0.317**	0.035	−0.055				
NO_3	**0.304**	−0.008	−0.0194	−0.069	**−0.227**	−0.178	**−0.303**	0.015	−0.039	**0.935**			
NH_4	0.127	**−0.230**	**0.353**	0.063	**0.445**	**0.534**	0.195	−0.127	0.182	0.064	0.066		
PO_4	0.002	0.019	0.053	0.063	0.037	0.001	0.069	0.126	0.067	−0.026	−0.003	0.012	
TP	0.067	−0.139	**0.221**	0.061	**0.282**	0.199	**0.455**	0.168	**0.275**	−0.088	−0.103	0.064	−0.026

incorporation into biomass (HA_{inc}) exhibited a significant correlation with chlorophyll (CHL) and primary productivity (PP). Dissolved organic matter (DOM) only correlated well with total heterotrophic activity and respiration, bacterial numbers, and primary productivity, but not with chlorophyll.

With respect to the dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3+} and TP) only NH_4^+ correlated in a positive and significant way with heterotrophic activity (r = 0.353), respiration (r = 0.445) and bacterial numbers (r = 0.534). Both NO_2^- and NO_3^- exhibited significant negative correlations. For instance, total heterotrophic activity and respiration were inversely correlated with NO_2 (r = −0.205 and r = −0.230, respectively) and respiration was inversely correlated with NO_3 (r = −0.227). No significant correlation was found between temperature and salinity with either heterotrophic activity or bacterial numbers. Only in one instance temperature correlated well with incorporation into biomass (r = 0.229).

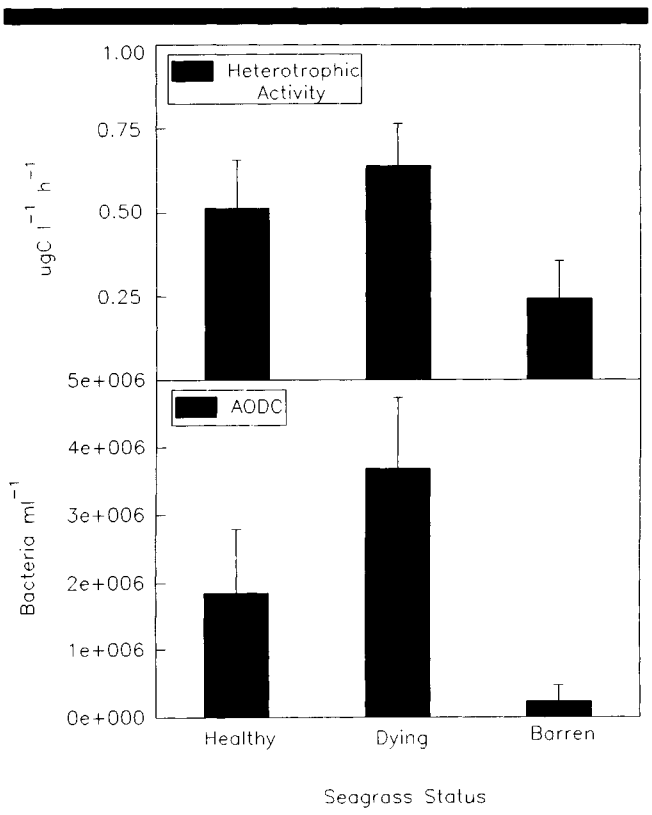

Figure 4. Time averaged data for heterotrophic activity and acridine orange direct counts (AODC) for sites describing seagrass health in Florida Bay.

The condition of the underlying seagrass had an effect on the bacterioplankton in the water column above. Typically a dying site (*i.e.* Rankin Lake), showed higher values for both heterotrophic activity and bacterial numbers than either the healthy (*i.e.* Johnson Key Basin) or barren site (*i.e.* Park Key; Figure 4). This pattern of: dying > healthy > barren, was repeated with respect to DOM, PP, and CHL (Figure 5), but only DOM values were significantly different. If these same variables were plotted by study site, the trend of higher values for the seagrass covered western areas (RKN, JKB, RKB) and lower values for the barren eastern areas (BRN, BLK, DUK, PRK) was reproduced (Figures 6 and 7). This is in agreement with the correlations that were obtained previously between bacterial activity/numbers and the abovementioned factors (Table 1). ANOVA analyses clearly indicated that biological parameters displayed the same pattern: highest at dying RKN (HA_{tot}: 0.640 μg l^{-1} h^{-1}; HA_{resp}: 0.485 μg l^{-1} h^{-1}; DC: 3.68e6 bacteria ml^{-1} and DOM: 421 relative fluorescence units), intermediate at healthy JKB (HA_{tot}: 0.515 μg l^{-1} h^{-1}; HA_{resp}: 0.306 μg l^{-1} h^{-1}; DC: 2.04e6 bacteria ml^{-1} and DOM: 303 RFU) and lowest at dead PRK (HA_{tot}: 0.243 μg l^{-1} h^{-1}; HA_{resp}: 0.182 μg l^{-1} h^{-1}; DC: 9.09e5 bacteria ml^{-1} and DOM: 136 RFU). All differences were significant ($P < 0.05$, Tables 2 and 3).

With the aid of factor analysis, it was possible to gather the variables that were highly correlated with one another. This correlation was indicated by some underlying variable (referred to as the "factor"). The extent to which any variable correlated with the factor was determined by a number which approached 1.0, this was referred to as the "factor loading". The parameters that showed an association with factor 1 were total heterotrophic activity, bacterial numbers (AODC), primary productivity and DOM. Salinity was negatively associated with factor 2, while chlorophyll and ammonium were

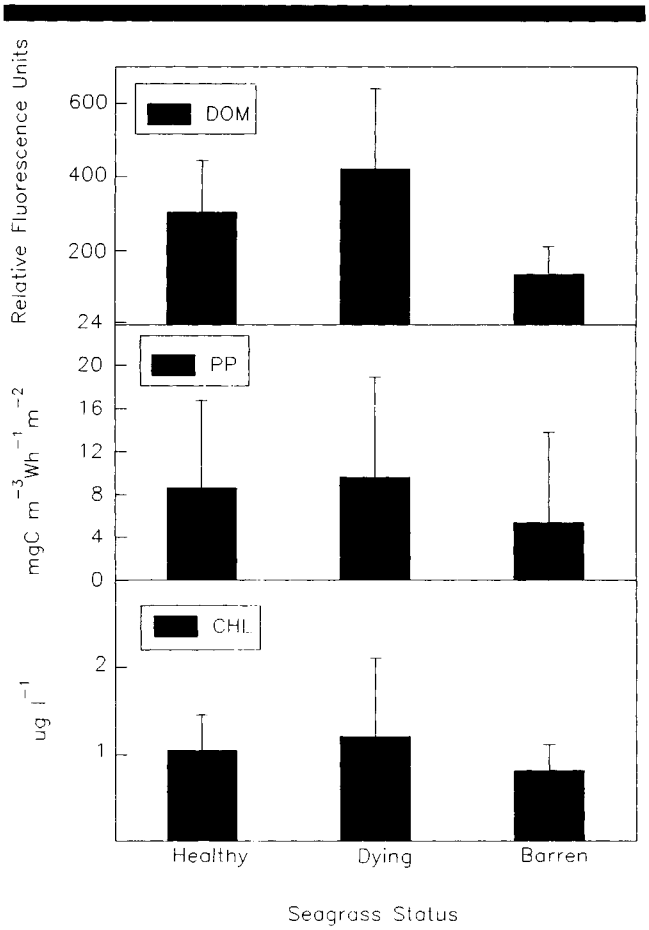

Figure 5. Time averaged data for dissolved organic matter (DOM), primary productivity (PP) and chlorophyll (CHL) for sites describing seagrass health in Florida Bay.

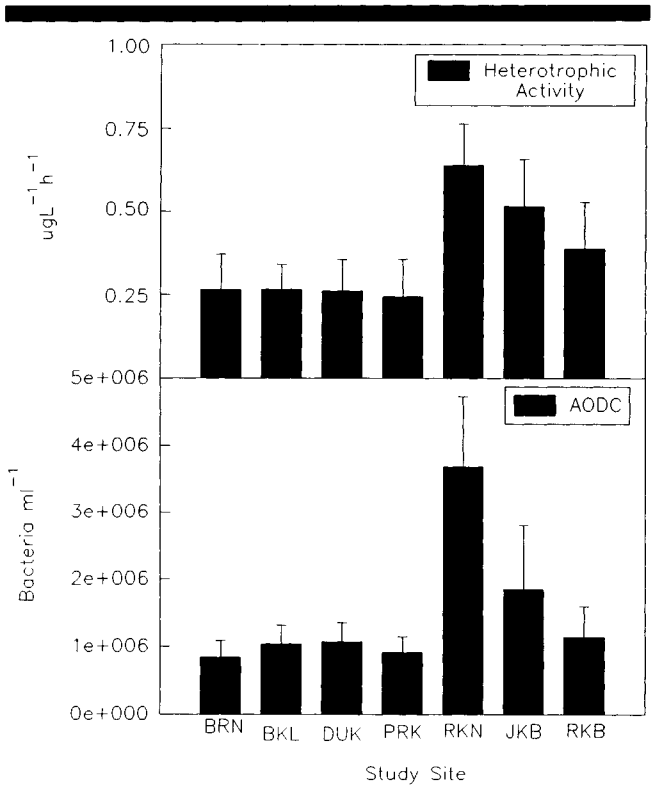

Figure 6. Time averaged data for heterotrophic activity and acridine orange direct counts (AODC) for each study site in Florida Bay.

associated with factor 3, although chlorophyll's association was negative (Table 4).

DISCUSSION

Seasonality

Seasonality in Florida Bay was only evident for water temperature. Salinity also displayed seasonal variation, yet the pattern of higher salinities in the summer and lower in the winter was much less apparent (Figures 3 and 4).

Temperature was only associated with bacterial incorporation of labelled substrate (HA_{inc}; Table 1). This correlation ($r = 0.229$; $P < 0.05$) indicates that temperature may be controlling the anabolic processes of bacterial growth rather than the catabolic ones (*i.e.* bacterial respiration). Temperature effects on bacterial productivity have been well documented. There have been reports on its positive influence (MARVALIN *et al.*, 1989; MORIARITY *et al.*, 1990; CHIN-LEO and BENNER, 1991; SHIAH and DUCKLOW, 1994; DANOVARO *et al.*, 1994) as well as, reports that show increased bacterial production due to factors other than temperature (*i.e.* phytoplankton release of DOC; CHIN-LEO and BENNER, 1991; VELIMIROV and WALENTA-SIMON, 1992). Considering that Florida Bay is exposed to a variety of factors, it is likely that anyone of these may have concealed an actual temperature effect. The possibility that temperature may have had a more pronounced effect on bacterioplankton activity and numbers but on a shorter time scale than the monthly sampling interval can not be discarded.

Elevated salinities in Florida Bay, in the range of 35 ppt- (JKB, March and September 1990) to 64 ppt (RKL, April '90), had no significant correlation with microbial activities as measured by bacterial numbers and heterotrophic potentials. Salinity has been shown to effect enzymes by either inhibiting their activity at low substrate concentration, or by activating them at saturating concentrations (UNEMOTO *et al.*, 1974). Apparently higher salinities are in the normal range for the bay (ZIEMAN *et al.*, 1994) and thus resident bacterioplankton communities are adjusted to them.

Spatial Variation

One of the parameters measured that displayed a true geographic pattern was salinity. The highest measured salinities were clustered in the central portion of the bay in RNK, DUK, and PRK. This agrees with earlier research from FOURQUREAN (1992) who found that the highest salinities were in the center part of the bay; lower values in the northeast and near to normal in the western edge. The decline in salinity in the last two instances was attributed to freshwater runoff from

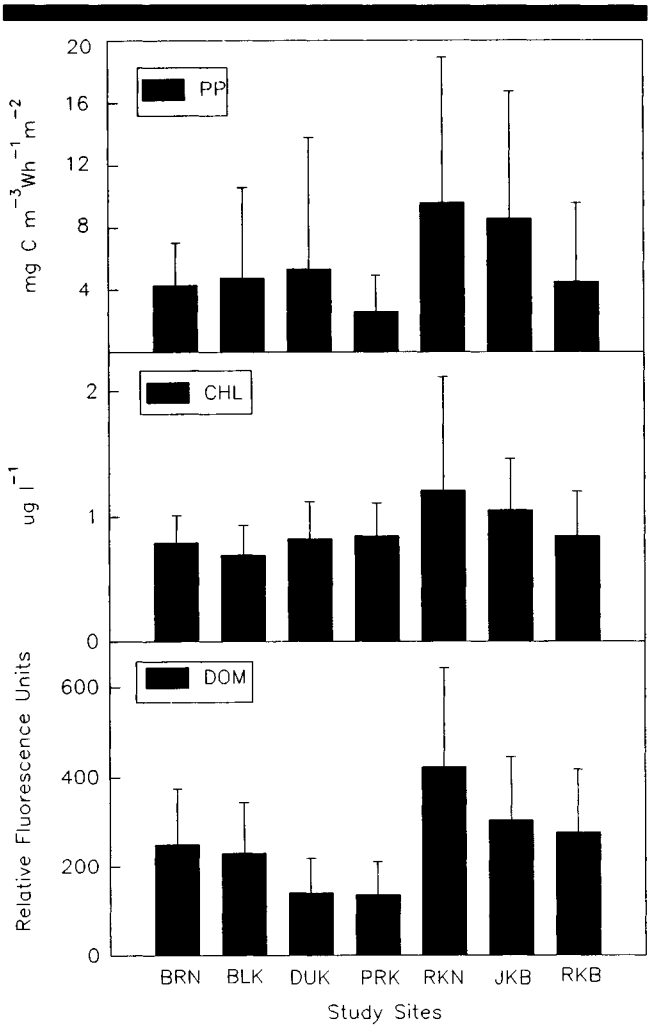

Figure 7. Time averaged data for dissolved organic matter (DOM), primary productivity (PP) and chlorophyll (CHL) for each study site in Florida Bay.

the Everglades and the periodic flushing from the Gulf of Mexico, respectively. Among the apparent reasons for the elevated salinities in central Florida Bay are: (1) the relative shallowness of the area; (2) evaporation effects increasing as depth decreases and (3) restricted exchange with water from lower salinity basins. In addition, the enclosive nature of the mud banks surrounding the basins can also favor the high salinity conditions.

It should be pointed out that during this study, there were no significant differences in bacterioplankton activity and numbers between seagrass covered areas and areas devoid of seagrass within the same basin (data not shown). A possible explanation for this is that the small size of the barren areas within these basins along with the continual mixing due to the action of winds, resulted in a homogenization of the water column.

Factors Affecting Microbial Activity

The two parameters that showed the highest correlations with respect to bacterioplankton activity and numbers were dissolved organic matter ($r = 0.415$ for HA_{tot} and $r = 0.420$ for AODC) and primary productivity ($r = 0.421$ for HA_{tot} and $r = 0.298$ for AODC). Bacterial utilization of phytoplanktonic exudates has been widely investigated and known to be crucial to the cycling of DOM in pelagic ecosystems (AZAM *et al.*, 1983; AZAM and AMMERMAN, 1984; KIRCHMAN, 1990). In fact, the theory behind the "microbial loop" is based on the commensalism between phytoplankton (DOM providers) and bacteria (DOM utilizers; AZAM *et al.*, 1983). There has been some controversy inasmuch as which DOM source affects bacterial activity. Some studies identified the DOM released by phytoplankton as the most influential on bacterial activity (*i.e.* growth) in different ecosystems (FUHRMAN *et al.*, 1980; KRAMBECK, 1984; SHIAH and DUCKLOW, 1994; MARVALIN *et al.*, 1989; VELIMIROV and WALENTA-SIMON, 1993; WRIGHT and COFFIN, 1984). In contrast, other investigations indicate that in some systems it is the DOM released by macrophyte photosynthetic activity, senescence and decay that is of primary importance for bacterial growth (KLUG, 1980; MORIAR-

Table 2. *Results of ANOVA analysis displaying homogeneous groups for each study site. Parameters measured were: salinity, temperature, primary productivity, chlorophyll, heterotrophic activity, acridine orange direct counts, dissolved organic matter, nitrite, nitrate, ammonium, orthophosphate, and total phosphorus showing resulting homogeneous groups. The 2 study sites (Park Key and Johnson Key Basin) have been bolded as representatives of a site almost totally devoid of seagrass, and one with a lush and healthy seagrass community respectively. (Please note: the numbers indicate an homogeneous group, or a group whose means are not significantly different ($P > 0.005$) from each other.)*

	Parameter					
	Salinity	Temperature	Prim. Prod.	Chlorophyll	Het. Act.	AODC
F-Ratio	18.367	0.160	3.444	3.798	41.072	56.216
Signif. Level	0.0000	0.9865	0.0036	0.0017	0.0000	0.0000
Homogeneous Groups						
Barnes Sound	1 2	1	1	1	1	1
Blackwater Sound	2	1	1	1	1	1
Duck Key	3	1	1 2	1	1	1
Park Key	**3**	**1**	**1**	**1**	**1**	**1**
Rankin Lake	3	1	3	3	4	3
Johnson Key Basin	**1**	**1**	**2 3**	**2**	**3**	**2**
Rabbit Key Basin	1	1	1	1	2	1

Table 3. *Results of ANOVA analysis relating to seagrass status (health). Parameters measured were: salinity, temperature, primary productivity, chlorophyll, heterotrophic activity, acridine orange direct counts, dissolved organic matter, nitrite and nitrate (combined as NO_x), ammonium, ortho-phosphate, and total phosphorus showing resulting homogeneous groups. (Please note: the numbers refer to an homogeneous group, or a group whose means are not significantly different ($P > 0.005$) from each other.)*

	Parameter					
	Salinity	Temperature	Prim. Prod.	Chlorophyll	Het. Act.	AODC
F-Ratio	16.350	0.076	7.266	6.976	57.233	53.416
Signif. Level	0.0000	0.9272	0.0017	0.0022	0.0000	0.0000
Homogeneous Groups						
Healthy (JKB)	1	1	2	2	2	2
Dying (RKN)	2	1	2	3	3	3
Barren (PRK)	2	1	1	1	1	1

	Parameter				
	DOM	NO_x	Ammonium	Phosphate	Total P
F-Ratio	13.994	8.681	7.761	0.673	1.981
Signif. Level	0.0000	0.0006	0.0011	0.5145	0.1493
Homogeneous Groups					
Healthy	2	1	1	1	1
Dying	3	1	2	1	1
Barren	1	2	1	1	1

ITY *et al.*, 1985; MANN, 1988; MORIARITY *et al.*, 1990; PEDUZZI and HERNDL, 1991; CHIN-LEO and BENNER, 1991).

The primary source of DOM for Florida Bay has not yet been established. Because DOM showed a significant association with primary productivity and chlorophyll (r = 0.3 and 0.155) it could be assumed that in Florida Bay the primary source of DOM for bacterial utilization is derived from phytoplankton. Although a more probable situation would be a combination of phytoplankton and macrophyte sources (CHIN-LEO and BENNER, 1991). In this study, DOM was recorded as a relative fluorescence measurement, that neither differentiates nor quantifies the source of DOM.

Dissolved inorganic nutrients, for the most part, did not show a significant association with bacterial parameters. Past research have indicated that inorganic nutrients (HORRIGAN *et al.*, 1988) along with an adequate supply of C (AZAM *et al.*, 1983) are of most importance in determining the bacterial standing stock. The significant correlation of total heterotrophic activity and numbers to total phosphorus, but not to soluble reactive phosphorus (Table 1), suggests that other forms of this nutrient were preferred as the source of phosphorus for the bacterioplankton. It also indicates that phosphorus may be a limiting nutrient for bacterioplankton production. This is in general agreement with FOURQUREAN *et al.* (1992, 1993) who found that it was phosphorus and not nitrogen that limited phytoplankton biomass in Florida Bay. That phosphorus limits primary production in the bay is also supported by the findings of this study where there was a significant correlation between total phosphorus and primary productivity (r = 0.275). The bay-wide ratio of dissolved inorganic nitrogen to phosphate (data not shown) of 52:1 (maximum 160:1) is less than that reported by others (152:1, FOURQUREAN *et al.*, 1993), but it is still far in excess of the Redfield ratio of 16:1, thus indicating phosphorus limitation (BOYTON *et al.*, 1982).

Table 4. *Results of factor analysis displaying the underlying factors and factor loadings for each variable. Important factor correlations have been highlighted.*

	Factor Loading		
Variable	1	2	3
Salinity	−0.036	**−0.572**	−0.158
Temperature	0.038	−0.067	−0.480
Primary Productivity	**0.525**	−0.103	−0.197
DOM	**0.723**	0.273	0.054
Chlorophyll	0.345	−0.283	**−0.541**
Heterotrophic Activity	**0.792**	−0.159	−0.063
Total Phosphorus	0.405	0.048	−0.028
Nitrate + Nitrite	−0.350	−0.361	0.052
Ammonium	0.366	−0.428	**0.571**
Ortho-phosphate	0.070	−0.056	−0.071
AODC	**0.732**	−0.328	0.201

NH_4^+ showed a reasonable correlation with the biological parameters for Florida Bay as a whole, much more so than nitrate or nitrite. The significant correlation of ammonium to heterotrophic activity and bacterial numbers could be an indication of deamination of amino acids exuded from seagrasses or seagrass decay. BOON *et al.* (1986) found that substantial amounts of ammonium could be produced by deamination of aminoacids exuded from seagrass roots or by the action of proteolytic bacteria on proteinaceous matter. Yet, the actual relationship between the concentration of ammonium in the surface waters of Florida Bay, its source, and the bacterioplankton population awaits further investigation.

Relationship of Bacterioplankton to Seagrass

There is a remarkable difference in the bacterial parameters (total heterotrophic activity and direct counts) obtained from the lush seagrass areas and the areas free of seagrass. This is in agreement with other researchers who found bacterial growth rates, production and numbers to be signifi-

cantly higher over seagrass beds than over areas without seagrass (MORIARITY *et al.*, 1990; MORIARITY and POLLARD, 1982; KLUG, 1980). Seagrass communities are considered detrital based since direct consumer utilization of seagrasses is usually less than 5% annual production (KLUG, 1980). As mentioned earlier, microbes can utilize DOM from seagrasses as carbon and energy sources. It follows that the microbial community plays a pivotal role in bringing an otherwise low production to higher trophic levels (MANN, 1988). Most likely the spatial differences within Florida Bay are primarily due to the presence and density of seagrasses.

Relationship of Bacterioplankton to Health of Underlying Seagrass

Florida Bay was experiencing a seagrass die-off during the study period (ZIEMAN *et al.*, 1994; ROBBLEE *et al.*, 1991). The bacterioplankton, on the other hand, was taking advantage of the increase in organics (*i.e.* DOM; Figures 4 and 5) due to the processing of seagrass detritus (FENCHEL, 1970; KLUG, 1980; MORIARITY *et al.*, 1990; CHIN-LEO and BENNER, 1991; TRESHER *et al.*, 1992). Bacterial utilization of detritus is well documented (MANN, 1988; BENNER *et al.*, 1986a and 1986b; PEDUZZI and HERNDL, 1991) and it is believed to be only way in which higher trophic levels get access to the carbon found in highly refractory polymeric complexes, such as lignocellulose (BENNER *et al.*, 1986a; MORAN and HODSON, 1989). In this work, the results from total heterotrophic activity, bacterial numbers and DOM were significantly higher ($P > 0.05$) over the "dying" than over the "healthy" or "barren" sites (Table 3). This outcome supports our hypothesis; where bacterioplankton activity and numbers would be higher over seagrass dominated areas and would change accordingly depending on the health of the seagrass. What is difficult to discern from the data so far, is which of the parameters examined exerted the most control upon bacterioplankton in Florida Bay. That only one factor is responsible for the differences is unlikely. A more likely scenario would be a compound effect of several of the variables studied, as they all relate to the presence and health of seagrass in this ecosystem.

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