DYNAMICS OF MARINE PELAGIC BACTERIAL COMMUNITIES

ON THE TEXAS-LOUISIANA SHELF

A Thesis

by

ERIN COLLEEN ANITSAKIS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Oceanography

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Approved by:

Chair of Committee,	Richard A. Long
Committee Members,	Steven F. DiMarco
	Michael D. Manson
Interim Head of Department,	John W. Morse

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ABSTRACT

Dynamics of Marine Pelagic Bacterial Communities
on the Texas-Louisiana Shelf.
(December 2006)
Erin Colleen Anitsakis, B.S., Texas A&M University
Chair of Advisory Committee: Dr. Richard A. Long

Microbial community interactions within many ecosystems are still relatively unknown. Investigating links between environmental dynamics and shifting pelagic bacterial community structures on the Texas-Louisiana shelf, Eubacterial community profiles of Operational Taxonomic Units (OTUs) were generated using Automated Ribosomal Intergenic Spacer Analysis (ARISA) of the 16S rDNA and 23S rDNA intergenic spacer region. This ITS region is highly variable in both length and sequence. Community diversity was assessed by the comparison of ARISA-generated community fingerprints of samples collected from four distinct regions along the Texas-Louisiana shelf in a cross-shelf pattern on 10m, 20m, and 40m isobaths.

Incubations of samples with a thymidine analog, 5-Bromodeoxyuridine (BrdU), allowed for the isolation and analysis of the actively growing subset within the total bacterial population. Community composition was determined through the construction of clone libraries for sequencing and putative phyla affiliation of community 16 rRNA genes. Hydrographic data were also collected for analysis of shifts in microbial community diversity correlated with a variety of influential environmental factors. ARISA profiles of Eubacterial species richness suggest strong distinction between the two communities found within Zones A and C along the Texas-Louisiana Shelf.

Further analysis of salinity gradients originating from the two main fluvial sources, the Mississippi and the Atchafalaya Rivers, identified possible sources of variation between the individual communities. Whereas composition of these

communities remains discrete between regions, the active subset of the population becomes more similar across the shelf through the summer. Possibly due to under-sampling of hypoxic sites, no relationship could be determined between hypoxia formation and the Eubacterial community dynamics. Several OTUs within the communities were identifiable as α- and β- *Proteobacteria*, *Actinobacteria*, *Synechococcus*, *Prochlorococcus,* and Cytophaga/Flavobacterium/Bacteroides.

Through validation studies of 5-Bromodeoxyuridine field sampling, this study indicates the power of BrdU incorporation and ARISA analysis to study a dynamic environmental system and explore the factors that determine the structure of the pelagic community on the Texas-Louisiana Shelf.

DEDICATION

This work is dedicated to my better half,
who at times during this work endured my lesser half.

ACKNOWLEDGMENTS

Many thanks are due to my committee chair, Dr. Richard Long for his support and patience, and to my committee members, Dr. Steve DiMarco and Dr. Mike Manson, for insight and guidance throughout this program. Specific thanks go to Dr. Steve DiMarco for enabling the collection of these samples during his two year Mechanisms Controlling Hypoxia program. Thanks also to Dr. Dan Thornton for his assistance and recommendations during this work.

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Many thanks also to Chrissy Stover and Shelli Meyer for assistance in sampling aboard the MCH cruises, and again to Shelli for her willingness to co-inhabit our lovely abode, and to Dr. Matt Howard and Sudeshna Lahiry for access to hydrographic data and graphs collected during this project

Throughout this project I received support from the National Science Foundation awarded to Richard A. Long, Texas A&M University Graduate Merit Fellowship, the Louis and Elizabeth Scherck Endowed Scholarship in Oceanography, the Partnership for Environmental Education and Rural Health Program and National Science Foundation GK-12 Fellowship. Ship time was provided by NOAA funding to Steven F. DiMarco.

I would not have ever reached this point if it was not for the support and guidance from my loving parents, family and husband. Only through their patience and humor was I able to maintain both my sense of humor and sanity.

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INTRODUCTION

PROJECT OBJECTIVES

Marine microbial communities are dynamic systems. Changes in the physical and chemical system can affect the diversity of both the active metabolizing bacteria and the microbial community as a whole. Hypoxic regions, characterized by dissolved oxygen concentration levels that fall below 1.4 mL L^{-1} due to the depletion of dissolved oxygen by microorganisms at the bottom of the water column, are a reoccurring phenomenon on the Texas-Louisiana continental shelf. Although they are believed to be linked, little is known about the relationship between these fluctuating dissolved oxygen concentrations and bacterial community dynamics of the Texas-Louisiana Shelf. Analyses of microbial communities are beginning to focus on the role of active bacteria with community dynamics. Metabolically active bacteria have recently been analyzed using 5-Bromodeoxyuridine (BrdU). However, the application of BrdU to entire marine assemblages has not been validated. This project investigates the incorporation of BrdU into natural marine assemblages found on the Texas-Louisiana Shelf. The overall goal of this project is to explore the link between marine bacterial community structure and composition with chemical and physical parameters on the Texas-Louisiana Shelf.

Two main hypotheses driving this investigation are:

- *Bromodeoxyuridine incorporation effectively identifies the active Eubacteria in a marine microbial population.*
- *Spatial and temporal variation of the Eubacterial community structure and community composition of the Texas-Louisiana Shelf can be linked with the developing hydrological conditions of salinity gradients and hypoxia formation.*

This thesis follows the style of Limnology and Oceanography.

To address these hypotheses, this project explores the application of BrdU in natural marine microbial assemblages, the microbial community structure and composition of the Texas-Louisiana Shelf and the microbial community variation with changing environmental conditions.

PROJECT SUMMARY

5-Bromodeoxyuridine Application in Natural Marine Eubacterial Assemblages

This work tested the applicability of 5- Bromodeoxyuridine (BrdU), a thymidine analog, in environmental studies, using a seawater incubation and subsequent molecular analysis of the incorporation of BrdU into the bacterial community using Automated Ribosomal Intergenic Spacer Analysis (ARISA). This component is to validate the incorporation of BrdU into all dominant bacterial species detected by our analysis for use in identification of the actively growing fraction of the Eubacterial community.

Eubacterial Community Structure and Composition

Microbial communities were sampled from the Texas-Louisiana continental shelf west of the Mississippi River in a grid from 28 ^{o}N to 30 ^{o}N and 89 ^{o}W to 94 ^{o}W. During the summer of 2004, three distinct Zones (determined from Rowe and Chapman (2002) conceptual model) (A, B and C) were repeatedly sampled from surface, middle and bottom waters. In the summer of 2005 a forth Zone (D) was also sampled. Spatial and temporal variation of community structure was determined through analysis of intergenic transcribed spacer regions (ITS) found within the community, using ARISA. Composition of selected Eubacterial communities was determined through a clone library of PCR- amplified 16S rDNA coupled with the neighboring ITS region and 23S rDNA. The cloned PCR products were sequenced to identify Eubacterial species within the natural assemblages. To complement this work, we implemented BrdU incorporation into DNA to identify rapidly growing populations within the Eubacterial community.

Eubacterial Community Variation with Environmental Developments

This work studied the phylogenetic richness and composition of microbial communities in relationship to the development of hypoxia and salinity gradients on the Texas-Louisiana Shelf. For a comprehensive analysis of these communities, the vertical dynamics of the system were investigated by sampling throughout the water column. BrdU incubations were conducted to identify the actively growing bacteria.

BACKGROUND

Bacteria play an important role in the global carbon cycle (Azam et al. 1993; Kirchman and Williams 2000). On average, bacterial cycles are responsible for processing of one-half of the ocean's primary production (Azam et al. 1983; Ducklow 1983; Ducklow et al. 2000). Fluctuations within the microbial community can have profound implications for the global carbon cycle and ecosystem balances (Pomeroy and Deibel 1986; Williams 1998). Despite the importance of the microbial population in carbon cycling, specific Eubacterial activity in carbon cycling in marine environments is relatively unknown.

Bacterial Diversity and Community Structure

The variability in temporal and spatial components of freshwater and saltwater systems is large, both in terms of bacterial community biomass (Ducklow and Carlson 1992) and community composition (Riemann et al. 1999; Yannarell et al. 2003). Eubacterial community compositions are diverse at global geographic scales (Troussellier et al. 2002; Yannarell and Triplett 2005) and within the water column (Hewson et al. 2003). Eubacterial community diversity is not limited to macro-scales (meters to kilometers); Long and Azam (2001) have discovered diversity at micro-environmental scales. The diversity of a heterotrophic microbial community can be defined by the physical parameters of the system (Cottrell and Kirchman 2004, Hewson and Fuhrman 2004, Kirchman et al. 2005) and the availability of dissolved organic

carbon (DOC)(Amon and Benner 1994; Amon and Benner 1998). Microbial communities are exposed to a variety of environmental parameters and pressures, each which elicits different responses in community diversity and structure.

Determining Diversity and Community Structure

Assessing microbial diversity is difficult because the majority of bacteria (>99%)in the ocean have not been cultivated (Giovannoni and Rappe 2000). Therefore, studies of microbial diversity and community structure have increasingly relied on molecular analysis of bacterial DNA (DeLong 2004). Studied to infer bacterial phylogeny, the most widely used locus for such studies is the 16S rRNA gene of prokaryotes. This gene is viewed as a molecular clock with highly conserved regions that can be deeply rooted within a phylogenetic tree. However, hyper-variability of other regions within rRNA provides a source of information about more recent genetic developments (Giovannoni and Rappe 2000).

A variety of molecular fingerprinting methods use fragments of the 16S rRNA gene to estimate community composition. Denaturing Gradient Gel Electrophoresis (DGGE)(Muyzer et al. 1993) and Terminal Restriction Fragment Length Polymorphism (TRFLPS)(Avaniss-Aghajani et al. 1994) are two techniques commonly used for analysis of various loci, including 16S rDNA. While DGGE typically has high phylogenetic resolution for major taxa, this method is labor intensive and is difficult to standardize between gels. TRFLPs offer less phylogenetic resolution because they rely on relatively few heterogeneous sequences in the 16S rDNA, so that one peak could be shared by multiple species. Both of these complications can have implications in other molecular analyses of the 16S rDNA.

Another DNA fingerprinting technique, Automated Ribosomal Intergenic Spacer Analysis (ARISA)(Fisher and Triplett 1999), takes advantage of the variation in length of the intergenic transcribed spacer (ITS) region among species. ITS regions are primarily noncoding regions, which allow for faster accumulation of change and mutations, not only in sequence composition but also in sequence length. ARISA is a

robust tool to elucidate community structure by selectively amplifies the ITS region between the 16S and 23S rDNA of eubacteria (Fig. 1.1). Clone libraries of the 16S rDNA, the ITS region and 23S rDNA can then be sequenced, providing high phylogenetic resolution and identification (Brown et al. 2005).

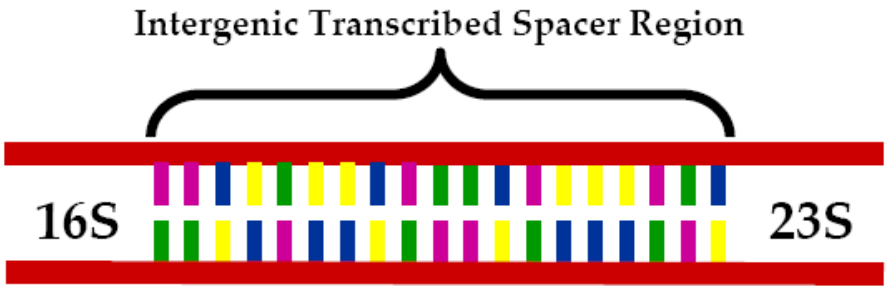


Fig. 1.1. The Intergenic Transcribed Spacer (ITS) region is located between the 16S rRNA and 23S rRNA genes. Nucleotide composition and length of the ITS region is inherently variable between bacterial species.

ARISA provides high phylogenetic resolution and sensitivity even for minor taxa (Hewson and Fuhrman 2004). Compared to other fingerprinting techniques (e.g. DGGE and TRFLPs), ARISA is much easier to standardize and reproduce both between runs, and between laboratories (Fisher and Triplett 1999; Ranjard et al. 2001). ARISA is a beneficial tool for comparative spatial and temporal analysis between communities.

Influences of Salinity Gradients on Eubacterial Community Structure

Phylogenetic differentiation of Eubacterial communities along estuarine salinity gradients have been observed worldwide (Troussellier et al. 2002; Cottrell and Kirchman 2004; Crump et al. 2004; Hewson and Fuhrman 2004; Kirchman et al. 2005). Comprising almost 74% of the total bacterial population, the dominate phylotypes in populations of the Delaware Estuary are α- and β-*Proteobacteria*, Cytophaga-like bacteria, *Actinobacteria,* and γ-*Proteobacteria* (Cottrell and Kirchman 2004; Kirchman

et al. 2005). Shifts of composition and metabolism activity, defined by the uptake of tritiated Thymidine and Leucine, among these prominent groups correlate strongly with salinities along the estuarine gradient. Thymidine incorporation reveals replicating DNA, whereas Leucine incorporation signifies protein synthesis or biomass production. Though more abundant at higher salinities, α-*Proteobacteria* actively incorporate thymidine and leucine throughout estuaries (Cottrell and Kirchman 2004; Kirchman et al. 2005). Both β-*Proteobacteria* and *Actinobacteria* abundances show a negative correlation with salinity in the estuary (Cottrell and Kirchman 2004) and decline of thymidine and leucine incorporation of β–*Proteobacteria* with increased salinity (Cottrell and Kirchman 2004; Kirchman et al. 2005). γ-*Proteobacteria* are evenly distributed throughout estuaries, though in lower abundances as a moderately active component of the community. However, *Cytophaga*-like bacteria, which are abundant throughout the estuary, exhibits no clear relationship with salinity (Cottrell and Kirchman 2004; Kirchman et al. 2005).

Bacterial phylotypes introduced to an estuary from riverine and oceanic sources become diluted across the estuarine gradient away form their source. Oceanic and riverine communities are phylogenetically distinct from each other, while communities within intermediate saline waters (between salinities of 25 and 33 on the practical salinity scale) are comprised of a mixture of phylotypes from both fresh and saline environments (Crump et al. 2004). Hewson and Fuhrman (2004) determined Eubacterial species richness was greater at intermediate salinities than at the river mouth or in the open ocean. These findings suggest that geographical location and salinity gradients influence the community composition and structure.

The fore mentioned studies that incorporated salinity gradients into community analysis have focused primarily on surface waters of estuarine systems (Crump et al. 2004; Hewson and Fuhrman 2004; Kirchman et al. 2005). As noted by Troussellier and colleagues (2002), estuarine systems and salinity gradients could be used to differentiate surface communities from those at depth. Previous studies of salinity gradient influences on microbial communities have limited their observations to surface

waters in estuaries, thereby overlooking microbial community development within salinity gradients sustained on continental shelves.

Analysis of Active Microbial Populations

As noted by Steward and Azam (1999), it is necessary to obtain information about bacterial activity to understand bacterial roles within the carbon cycle. The mere presence of a bacterium does not signify that it is actively influencing the surrounding environment. Investigations by Höppe (1978), later supported by Fuhrman and Azam (1982), found that during the summers 50% of all aquatic bacteria are metabolically active, while only 10% are metabolically active during winter months. Cultivation techniques to enumerate the active population of the community were inadequate and still are, since the colony forming bacteria comprised only 0.01%-12.5% of the active population (Höppe 1978), placing an emphasis on metabolic analyses to distinguish active bacterium.

The importance of utilizing bacterial metabolism to distinguish active bacteria was also noted by Zweifel and Hagström (1995), who discovered the absence of nucleoids in some marine bacteria. These "ghost" cells were believed inactive when cells containing visible nucleoids were correlated with colony forming units (CFU)(Zweifel and Hagström 1995). However, when electron transport system activity was determined by the reduction of cyanoditolyl tetrazolium chloride (CTC)(Del Giorgio et al. 1997; Ducklow 2000), some cells containing visible nucleoids were not actively respiring (Choi et al. 1996; Ducklow 2000).

Microautoradiography uses the uptake and metabolism of tritiated compounds to identify active bacteria. One of the more common techniques takes advantage of ^{3}H – Thymidine (^{3}H –TdR) incorporation into newly synthesized DNA of active bacteria (Fuhrman and Azam 1982). Recently, micro-autoradiography fluorescence in-*situ* hybridization (Micro-FISH/ STAR-FISH) combines the uptake of radioactively labeled compounds, dissolved organic matter (DOM), and fluorescently labeled rRNA probes to determine abundance and phylogeny (Lee et al. 1999; Ouverney and Fuhrman 1999;

Cottrell and Kirchman 2000; Cottrell and Kirchman 2004). However, working with radioactive tracers often comes with many complications; such as increased processing costs and safety restrictions for laboratory and environmental use.

5-Bromodeoxyuridine as an Identifier of Active Bacteria

5-Bromodeoxyuridine (BrdU) is a thymidine analog (Fig. 1.2) that is incorporated in place of thymidine into actively replicating DNA (Hakala 1959). Originally, studies have focused on BrdU incorporation into eukaryotic cells for analysis of cell proliferation combining immunochemical detection of BrdU with a multitude of techniques (autoradiography; Moran et al. 1985; flow cytometry; Rizzoli et al. 1988; Dolbeare 1995b; cell isolation and microscopy; Dolbeare 1995a). As BrdU is not found naturally in the environment, BrdU has recently been applied to prokaryotes as an alternative to radioactive tracers in the analysis of active Eubacterial assemblages. Studies in natural marine assemblages have shown that incorporation rates of BrdU and TdR are strongly correlated, but that BrdU uptake was lower than TdR (BrdU:TdR = 0.7; r = 0.98; Steward and Azam 1999; r = 0.96; Nelson and Carlson 2005). DNA synthesis has been monitored, not only for natural assemblages but also at the single cell level using BrdU incorporation into marine bacteria (Hamasaki et al. 2004). Other work has revealed the ability to identify metabolically active bacteria directly from sediments and environmental communities with BrdU incubations of bacterial assemblages *in situ* (Yin et al. 2000; Artursson and Jansson 2003) and seawater (Urbach et al. 1999) including those responding to stimuli (Borneman 1999) and immunochemically isolate the active BrdU-labeled assemblages for community analysis and identification.

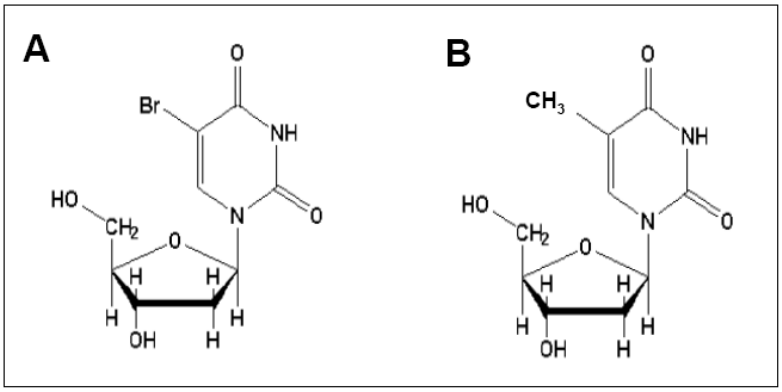


Fig. 1.2. Chemical structure of (A) BrdU an analog of the nucleoside, (B) TdR. *Modified from www.biotium.com.*

Incorporation of BrdU has been used with mixed success on bacterial environmental cultures. While two of these studies have found that all of isolates (n=37) examined incorporated BrdU (Pernthaler et al. 2002; Hamasaki et al. 2004), a previous one by Urbach and colleagues (1999) found one Gram- positive bacterium and a Flavobacterium that do not incorporate BrdU. By finding 2 isolates out of 4 that do not incorporate BrdU, the work done by Urbach and colleagues (1999) brings into question the ability of BrdU to identify all of the active bacteria within a marine community. One goal of this study was to determine if all dominant bacteria present within a marine community are capable of incorporating BrdU.

Gulf of Mexico Sampling Sites

The northwest continental shelf of the Gulf of Mexico has two main fluvial sources of fresh water and nutrients, which constitutes the Mississippi and the Atchafalaya River systems. The Mississippi River is the 6th largest river (by freshwater discharge) in the world and has a river basin encompassing almost 40 % of the continental United States, with a river flow rate ranging between 10 - 35 x 10^3 m^3s^{-1}. It delivers 70% of the total fresh water input into the Gulf of Mexico (Walker and Rouse 1994). Before reaching the Mississippi River delta, 30% of the Mississippi River's water is diverted away from the delta to the Atchafalaya River just south of Baton

Rouge. The Red River joins with the Atchafalaya River which empties into Atchafalaya Bay near Morgan City, LA and opens onto the Texas-Louisiana Shelf. Roughly half of the river water passing through the bird-foot Mississippi River Delta is advected offshore into the deep Gulf of Mexico or moves east onto the Mississippi-Alabama shelf (Etter et al. 2004). Therefore, the Mississippi and the Atchafalaya Rivers contribute nearly equal amounts of fresh water onto the eastern Texas-Louisiana shelf.

Hypoxia on the Texas-Louisiana Shelf in the Gulf of Mexico

Hypoxia is defined by dissolved oxygen concentrations below 2 mg L^{-1} (or 1.4 mL L^{-1}). At this concentration, benthic macrofauna are adversely stressed (Rabalais et al. 1994; Wiseman et al. 1997). The seasonal development and frequency of hypoxia on the Texas-Louisiana continental shelf (Fig. 1.3) is hypothesized to be driven by two mechanisms, which compete and combine across the continental shelf to produce the observed patterns of hypoxia (Hetland and DiMarco *In review*). Based on the relative strength of respiration and stratification, the system can show considerable spatial and temporal variability.

The first and mostly widely accepted process behind the development of hypoxia is the input of nutrients onto the continental shelf (Rabalais et al. 1994). High quantities of phosphate, nitrogen and other nutrients from the Mississippi River are introduced to the shelf with river freshwater discharge. Nutrient loading of coastal waters leads to increased primary productivity and phytoplankton blooms in surface waters. As these large phytoplankton blooms die off and release DOC, they fuel bacterial respiration, which consumes dissolved oxygen (Rabalais et al. 1994). Hypoxia driven by mid-water bacterial respiration, utilizing dissolved oxygen, is thought to occur primarily in the eastern area of the shelf, between 89^{o} W and 91^{o} W (Hetland and DiMarco *In review*).

The second mechanism of hypoxia formation focuses on the input of fresh water onto the continental shelf. Stratification, or density driven layering of the water column, is directly correlated with fresh water input into the Gulf of Mexico

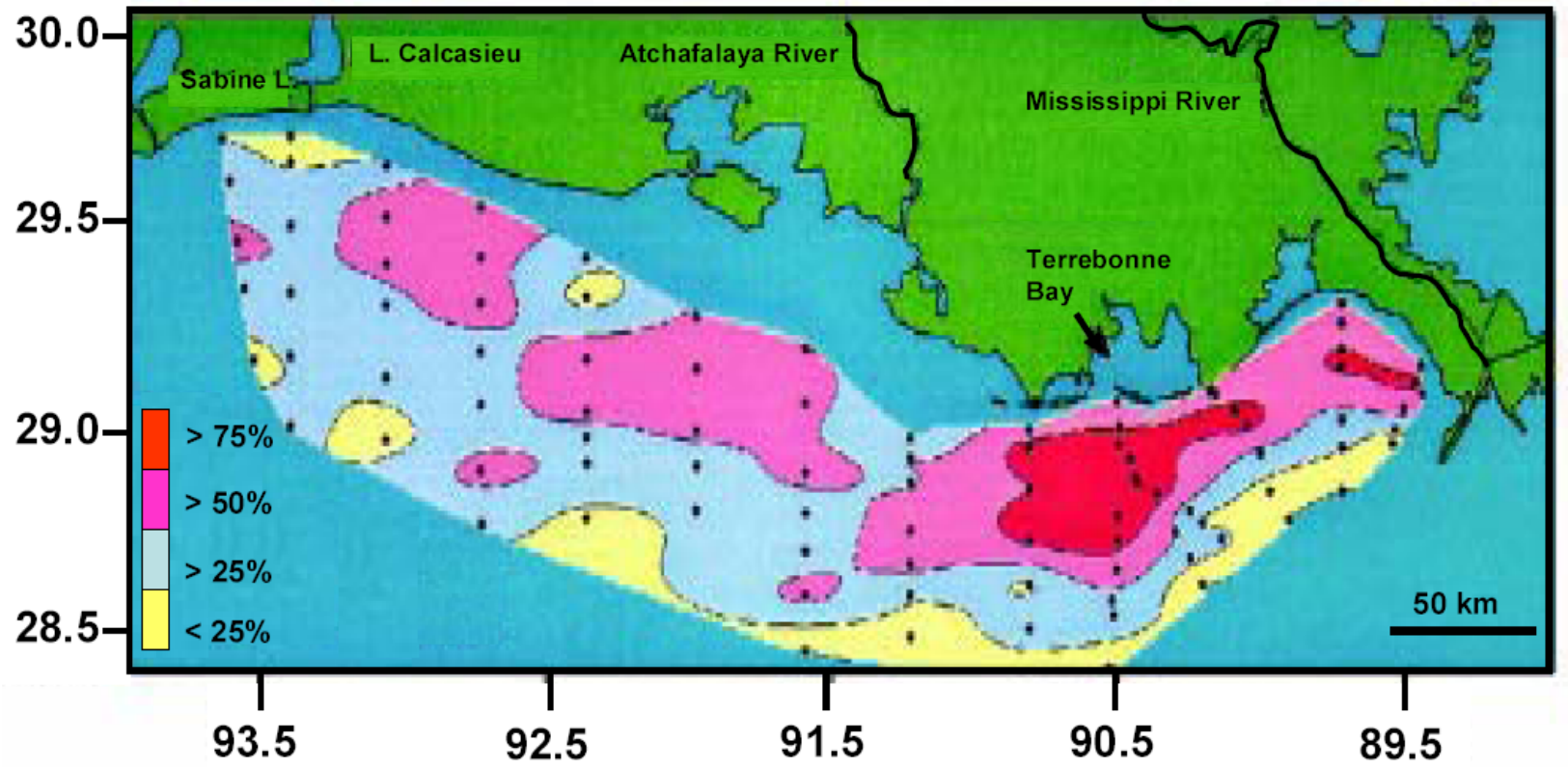


Fig. 1.3. Seasonal frequency of bottom-water hypoxia occurrence between 1985–2001 in the "Dead Zone". The term "Dead Zone" refers to an area of recurring hypoxia (Dissolved Oxygen Concentration < 2 mg L^{-1} in this diagram) on the Texas Louisiana Shelf from the Mississippi River to Sabine Pass. Bottom water (~1m above the seafloor) was collected from ~ 70 sites (black dots) on 4 day cruises every July during the years 1985-2001. The Texas-Louisiana Shelf ranges from 10-50m in depth over the highlighted sampling area. *Modified from Rabalais et al. 2002.*

(Rabalais et al. 2002). Stratification inhibits vertical mixing and halts the replenishment of dissolved oxygen to oxygen depleted bottom waters (Wiseman et al. 1997; Hetland and DiMarco *In review*). The presence of a strong halocline from freshwater input is necessary for the development of hypoxia on the Texas-Louisiana shelf and partially establishes the extent of the hypoxic area (Wiseman et al. 1997). West of 91^{o} W, the shelf is thought to be primarily influenced by the Atchafalaya River where hypoxia is driven by respiration of benthic bacteria below a strong pycnocline (Hetland and DiMarco *In review*).

Areas of the continental shelf affected by hypoxia have been found to be patchy in the spring and become more evenly distributed as stratification of the water column increases in summer. The area experiencing hypoxia in the bottom meter of the water-column can exceed 20, 000 km^2 stretching unbroken from Southwest Pass, LA to the Texas border (Rabalais et al. 2002). Typically, only bottom waters become hypoxic, but in intensely stratified waters, hypoxia can even occur in mid depth waters (Rabalais et al. 2002; Hetland and DiMarco *In review*). Late summer vertical mixing events, driven by winds and changing currents, break up the stratification leading to reoxygenating the water column (Hetland and DiMarco *In review*).

Riverine input onto the Texas-Louisiana continental shelf is believed to control the development of hypoxia through three distinct mechanisms (Rowe and Chapman 2002). The combination of these processes (physical, biological and chemical) controls the establishment of three areas of distinct areas of hypoxic development. The dynamics of these of hypoxic development working both independently and together across areas of the Texas-Louisiana Shelf (Rowe and Chapman 2002), is the basis for the distinction between the three Zones A, B and C (the forth Zone, D, was opportunistically added later) in this sampling area.

Eubacterial Communities on the Continental Shelf of the Gulf of Mexico

Microbial communities of the Texas-Louisiana shelf play an important role in the utilization of DOC and POC. Recent studies on the Texas-Louisiana continental shelf

have examined linkages of planktonic and benthic microbial communities to environmental conditions (i.e., hypoxia and salinity). Strong salinity gradients, established off the Mississippi River and Atchafalaya Rivers, influence microbial abundances through out the water column. Greater bacterial abundances are found at intermediate salinities (Amon and Benner 1998) and towards the eastern Texas-Louisiana shelf (closer to the Mississippi River delta) (Pakulski et al. 2000). Findings by both Amon and Benner (1998) and Pakulski (2000) determined higher Leucine incorporation rates at intermediate salinities. Bacterial production rates were also 3 fold higher off the Atchafalaya River than the Mississippi River (Pakulski et al. 2000). As dissolved oxygen concentrations decreased, microbial abundances of both benthic (Rowe et al. 2002) and water column (Pakulski et al. 2000) bacterial population declined. However, during the break up of hypoxic regions at the end of the summer, benthic microbial communities flourished (Rowe et al. 2002). In spite of these studies, analyses of microbial populations have been limited to bacterial abundances and production rates.

This thesis explores Eubacterial community structure and composition of the total and active communities throughout the water column along the Texas-Louisiana Shelf to address the two main hypotheses driving this investigation:

- *Bromodeoxyuridine incorporation effectively identifies the active Eubacteria in a marine microbial population.*
- *Spatial and temporal variation of the Eubacterial community structure and community composition of the Texas-Louisiana Shelf can be linked with the developing hydrological conditions of salinity gradients and hypoxia formation.*

CHAPTER II
5-BROMODEOXYURIDINE APPLICATION IN NATURAL MARINE BACTERIAL ASSEMBLAGES

OBJECTIVE

5-Bromodeoxyuridine (BrdU), a thymidine analog, has routinely been used in both eukaryotes (Moran et al. 1985; Rizzoli et al. 1988; Dolbeare 1995a; Dolbeare 1995b) and prokaryotes (Steward and Azam 1999; Urbach et al. 1999; Pernthaler et al. 2002; Hamasaki et al. 2004) to identify cells that are actively growing and replicating DNA. The application of BrdU to identify active bacterial populations provides a safe and less expensive alternative to the use of radioactive tracers in microbial ecology. The inability of some isolates to successfully incorporate BrdU (Urbach et al. 1999) calls into question the accuracy of determining the growing fraction of marine bacteria with BrdU. To validate the application of BrdU in environmental studies, the incorporation of BrdU into natural bacterial assemblages must first be established.

MATERIALS AND METHODS

Labeling Natural Bacterial Assemblages with BrdU

Seawater was collected from surface waters of the Gulf of Mexico at 28.9^{o} N and 92.0^{o} W on the *R/V Gyre*. Seawater was pre-filtered through 10 μm nitex and 47 mm GF/F filters (Whatman), to remove most protists and other grazers, into acid-washed and seawater rinsed 40 L carboys. A seawater culture was prepared by further filtering 18 L of seawater through 0.2 μm Sterivex filters (Millipore) to remove bacteria, and then inoculated with 2 L of GF/F filtered seawater to dilute the natural marine bacterial assemblage tenfold. The assemblage was inoculated with 20 nM (final concentration) BrdU and then aliquoted into acid-washed 1 L polycarbonate bottles. All samples were incubated at *in situ* temperatures in the dark. Duplicate samples of the seawater dilution

cultures were collected onto 0.2 μm Sterivex filters (Millipore) after 0, 6, 12, 24, 48, and 72 hours and stored onboard the ship at -20 ^{o}C. Samples were stored at -80 ^{o}C for further processing.

Bacterial Enumeration

Five mL samples were taken at each time site and preserved with filtered borate buffered formalin (2% final vol:vol) for bacterial counts. Preserved samples were stained with 4'6-diamidino-2-phenylindole solution (DAPI, 1 μg mL^{-1}, Sigma) and then 2 mL was concentrated onto a 0.22 μm prestained black polycarbonate filter (Isopore/Millipore) (Höppe 1978; Porter and Feig 1980). Slides were prepared onboard and stored at -20 ^{o}C. Following Turley and Hughes (1992), slides were counted for at least 200 cells or 20 fields by epifluorescent microscopy on a Zeiss Axioplan Imagining 2 universal microscope (Zeiss filter set 02) using Zeiss KS-300 3.0 image analysis software.

DNA Extraction and Purification

Sterivex filters were thawed and DNA was extracted following a modification of the method of Somerville et al. (1989). Filters were filled with 1.8 mL STE buffer (10 mM Tris-Hydrocloride [pH 8.0], 1 mM EDTA, 10 mM NaCl) containing fresh Lysozyme (5 mg mL^{-1}) and sealed with a syringe and cap for a one hour incubation at 37 ^{o}C. A 180 μL aliquot of Proteinase K (20 mg mL^{-1}) and 100 μL of 10% SDS solution were added to the filter and incubated for two hours at 60 ^{o}C. The filters were boiled in a microwave for 5-10 seconds. The sample was pulled into the syringe and transferred into 15 mL Corex tubes (Kimble Glass Inc.). The filters were rinsed with 1 mL TE buffer (10 mM Tris-hydrochloride [pH 8.0], 1mM EDTA) and the sample was pooled into 15 mL Corex tubes. A volume of 30 μL of RNase (10 mg mL^{-1}) was added to the sample and incubated for 10 minutes at room temperature. A half-volume of 7.5 M ammonium acetate was added and incubated for 15 minutes at room temperature. The sample was centrifuged at 10,000 *x g* for 5 minutes and the supernatant was transferred

to a clean tube. The DNA was precipitated by the addition of 9 mL 100% ethanol overnight at -80 ^{o}C. The precipitated DNA was collected by centrifugation at 14,500 *x g* for 30 min. After removal of the supernatant, the DNA was resuspended in 400 μL TE buffer and incubated at 37 ^{o}C for one hour. Extracted DNA was purified by phenol-chloroform extraction (Sambrook and Russell 2001).

Immunochemical Isolation of BrdU-labeled DNA

Following DNA extraction and purification, immunoglobulin paramagnetic beads (MagnaBind, Pierce) were used to immunocapture BrdU-labeled DNA from assemblages of labeled and unlabeled communities. Following a modification of the procedure of Urbach and colleagues (1999), all incubations were performed at room temperature. Herring Sperm DNA (1.25 mg mL^{-1} in phosphate buffered saline [PBS]) was denatured in boiling water for one minute and then quickly cooled in ice water. Monoclonal anti-BrdU antibody solution (4.5 mg IgG mL^{-1} Clone BU33 mouse ascites fluid (Sigma) diluted 1:10 in PBS) was diluted 9:1 with Herring Sperm DNA and incubated for 30 minutes. Sample DNA (1.0 μg DNA in PBS) was denatured for one minute in boiling water and then quickly cooled in ice water. The sample was mixed with 50 μL of the diluted anti-BrdU antibody mixture and incubated for 30 minutes. IgG-coated paramagnetic beads were washed once in a PBS-BSA solution (1 mg mL^{-1} immunohistochemical grade bovine serum albumin (Vector Inc.) in PBS) using a magnetic particle concentrator (MagnaBind), and resuspended in PBS-BSA to their initial concentration. A 25 μL volume of paramagnetic beads were added to each sample and incubated for 90 minutes with constant agitation. Using the magnetic concentrator, the beads-sample mixture was washed with 300 μL PBS-BSA seven times to remove unlabeled DNA. The BrdU-labeled DNA was eluted from the paramagnetic beads by adding 100 μL 1.7 mM BrdU (in PBS-BSA) and incubated for 30 minutes with constant agitation. After removing the beads with the magnetic concentrator, the BrdU-labeled DNA was precipitated out of the supernatant by the addition of 2 μL glycogen (20 mg mL^{-1}), 20 μL10 M ammonium acetate, and 200 μL cold ethanol, and then

incubated at -80 ^{o}C for 15 minutes. After a second ethanol precipitation, the DNA was resuspended in TE buffer.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA (Fisher and Triplett 1999) was performed following modifications of the method detailed by Brown et al. (2005). Primers used in ARISA targeted the universal 3' 16S rDNA and the Eubacterial-specific 5' 23S rDNA (16S-1392F (5'-G[C/T] ACACACCGCCGCCCGT-3'); 23S-125R (5'-6-Carboxyfluorescein (6-FAM) – GGGTT[C/G/T] CCCCATTC [A/G] G-3')) after Hewson and colleagues (2003). The 5' 6-FAM labeling was for the detection of the PCR products. Duplicate 20 μL reactions contained final concentrations of 1x PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 200 μM of each dNTP (Qiagen), 200nM of each primer, 40 ng μL^{-1} BSA, 2.5 U *Taq* DNA polymerase (Qiagen), and 1-10 ng μL^{-1} template DNA. Reaction mixtures were held at 94 ^{o}C for 2 min followed by 35 cycles of amplification at 94 ^{o}C for 40 s, 56 ^{o}C for 40 s, and 72 ^{o}C for 90 s, with a final extension step at 72 ^{o}C for 5 min. PCR products were purified using DNAce Strip Kit with sephadex columns (Bioline) and then quantified with SybrGold fluorescence on a SpectraMax Gemini EM spectrofluorometer (Molecular Devices). Concentrations of the PCR products were then diluted to 5 ng μL^{-1}.

Diluted PCR products were prepared for ARISA analysis by adding 1-2 μl diluted PCR product to a Marker-dye master mix consisting of 2.5 μL deionized formamide, 0.5 μL Blue Dextran Tracking Dye in EDTA (Bioventures), and 0.18 μL custom Rhodamine X-labeled MapMarker 50-1500 Size Standard (Bioventures). After denaturing at 95 ^{o}C for 5 min, PCR-Marker-dye mixes were immediately cooled on ice. Using ficoll loading solution (Gel Co), a 0.8 μL aliquot of PCR-Marker-dye mix was loaded onto a 36 cm long 5.0% polyacrylamide gel (Long Ranger) and run on an ABI 377XL automated sequencer for 5.5 hrs. Electropherogram output was aligned with scanned size fragment bands and analyzed using ABI Genescan 3.1 software and exported to Microsoft Office excel for further analysis.

ARISA Gel Analysis and Statistical Analysis

Electropherogram peaks were interpreted operational taxonomic units (OTUs) as analyzed by GeneScan 3.1 Software. Outputs from duplicate runs of samples were combined together to determine total community structure. Peaks that were <0.09% of the total amplified DNA, seen by the total relative fluorescent area for each sample, were determined to be background noise and discarded, following of Hewson and Fuhrman (2004). Sample peaks were then assigned to bins to compensate for decreased resolution of the base number of larger fragments. Fragments smaller that 700 bp were grouped into bins of 3 bp, bins of 5 bp for fragments between 700-999, and bins of 10 bp for fragments greater than 1000 bp in length (Brown et al. 2005).

Un-weighted pair-group mean average (UPGMA) cluster analysis was conducted from the matrix generated from the comparison of bacterial communities analyzed by ARISA using NTSYSpc 2.2 software program (Rohlf 2000). The matrix was generated by comparing communities based upon the presence or absence of OTUs using the Jaccard coefficient of similarity (S_j);

$$S_j = \frac{W}{(a_1 + a_2) - W},$$

where W is the number of shared OTUs between two communities and a_1 and a_2 are the total number of OTUs within the respective communities. The Jaccard coefficient ranges from 0 to 1, where communities that have a value of 1 are 100% similar. This comparison allows for a relative determination of the degree of similarity between groups of bacterial communities.

Clone Library and Sequencing

The total community at 48 hrs was chosen for cloning and sequencing for identification of bacterial species within the community. Fresh 20 μL reactions using bacteria specific 16S primer 27F (5'-GTTGATCCTGGCTCAG-3')(Hugenholtz and

Goebel 2001) and unlabeled 23S primer (23S-125R (5'–GGGTT[C/G/T]CCCCATTC [A/G] G-3') after (Hewson et al. 2003)) were prepared containing final concentrations of 1x PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 200 μM of each dNTP (Qiagen), 200nM of each primer, 40 ng μl^{-1} BSA, 2.5 U *Taq* DNA polymerase (Qiagen) and 1-10 ng μL^{-1} template DNA. Reaction mixtures were held at 94 ^{o}C for 2 minutes, followed by 35 cycles of amplification at 94 ^{o}C for 40 s, 56 ^{o}C for 40 s and 72 ^{o}C for 90 s, with a final extension step of 72 ^{o}C for five minutes. PCR products were pooled together from communities to construct two libraries consisting of dominant and rarer OTUs. PCR products were then inserted into the pCR8/GW/TOPO vector using the TA Cloning kit (Invitrogen), and transformants containing the plasmid were selected by plating on LB plates containing 100 μg mL^{-1} spectinomycin. Positive clones successfully grew on the LB plates. Transformed clones were picked and lysed in ddH_2O at 92 ^{o}C for 5 minutes, following Kilger et al. (1997), and dereplicated by ARISA. Multiple OTUs found within each clone are from the *E. coli* clone, while the unique OTU is attributed to the targeted sequence. This procedure allowed putative phylum affiliations to be aligned with specific OTUs to identify a communities' composition. Picked clones were also cultured at 37 ^{o}C for 12 hours in LB broth containing 100 μg mL^{-1} spectinomycin. Plasmids were purified and sequenced using the 27F primer (Hugenholtz and Goebel 2001) by Agencourt Bioscience Corporation (Beverly, MA). Sample sequences were analyzed using Chromas and were phylogenetically affiliated using the online BLAST tool on the GenBank website (Altschul et al. 1990).

RESULTS AND DISCUSSION

Seawater Culture Growth

Natural marine assemblages were diluted tenfold with 0.2 μm filtered seawater to stimulate bacterial growth. Starting at $2.0x10^5$ cell mL^{-1}, bacterial abundance increased

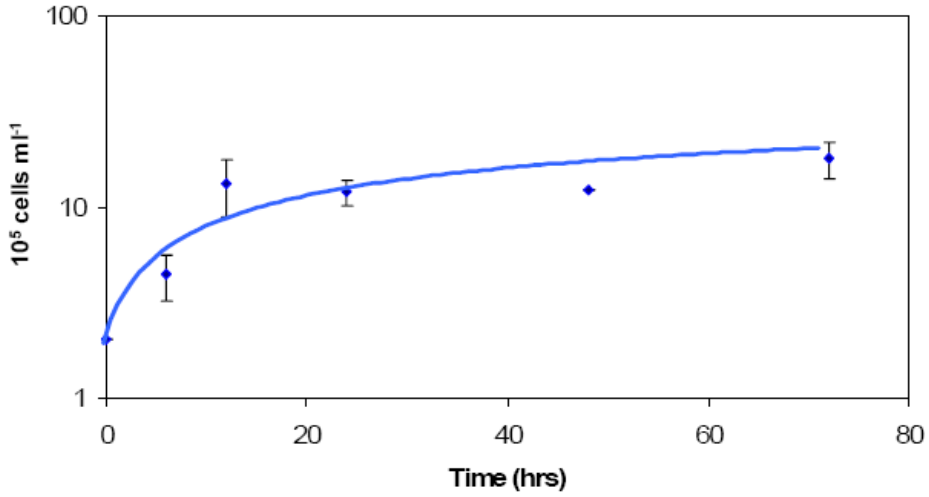


Fig. 2.1 Seawater-culture bacterial abundances over incubation. The seawater-culture was prepared by creating a ten-fold dilution of the natural microbial marine assemblage by inoculating 18 L of 0.2 μm seawater with 2 L of GF/F filtered seawater. The seawater-culture was incubated in the dark at *in situ* temperatures for 72 hours. Duplicate sub-samples were taken at each interval and fixed with 2% borate buffered formalin, stained with 4'6-diamidino-2-phenylindole (DAPI) and concentrated onto a 0.22 μm polycarbonate filter. Bacteria were enumerated by epifluoroscopy. Logarithmic regression trend line ($r^2 = 0.86$) is shown by the blue line. Error bars represent standard deviation.

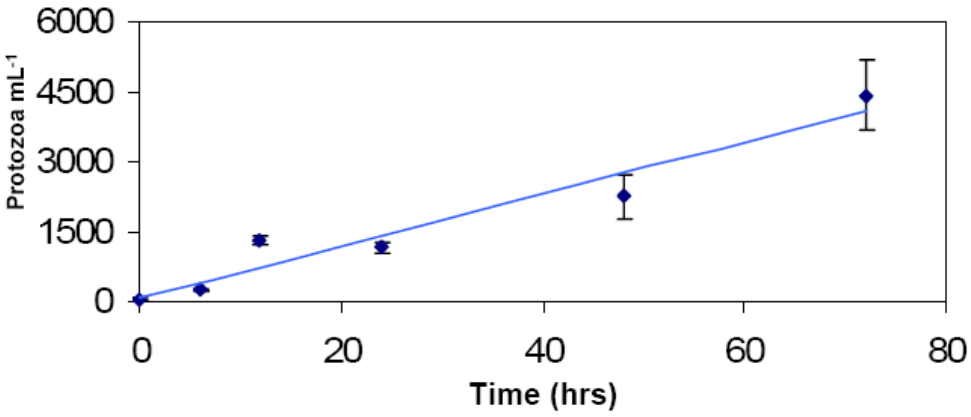


Fig. 2.2 Seawater-culture protozoa abundances over incubation. The seawater-culture was prepared by creating a ten-fold dilution of the natural microbial marine assemblage by inoculating 18 L of 0.2 μm seawater with 2 L of GF/F filtered seawater. The seawater-culture was incubated in the dark at *in situ* temperatures for 72 hours. Duplicate sub-samples were taken at each interval and fixed with 2% borate buffered formalin, stained with 4'6-diamidino-2-phenylindole (DAPI) and concentrated onto a 0.22 μm polycarbonate filter. Protozoa were enumerated by epifluoroscopy. Linear regression trend line ($r^2 = 0.94$) is shown by the blue line. Error bars represent standard deviation.

during the seawater culture incubation to $1.8x10^6$ cell mL^{-1} (Fig. 2.1). Grazing influences of protozoa, which could not be completely removed, were also monitored during the course of the incubation. Protozoa abundance increased from $6.3x10^1$ mL^{-1} to $4.4x10^3$ mL^{-1} (Fig 2.2).

Temporal Changes of Community Fingerprints

BrdU-labeled DNA was isolated by immunochemical purification from all sub-samples of total bacterial community from the seawater culture, with the exception of 0 hours. Seawater culture samples were analyzed by ARISA to investigate the incorporation of BrdU into natural mixed marine assemblages. ARISA allows the assessment of OTUs present and provides a 'fingerprint' of the community. Both total and BrdU-positive communities were analyzed using ARISA to allow the comparison of the two community fingerprints. ARISA-detected OTUs ranged in length from 418 bp to 1020 bp (Fig. 2.3). Species richness was determined from the number of unique OTUs. During the seawater culture, species richness ranged from 3 to 17 (Fig 2.4). Total community species richness increased from 6 to 16, while BrdU community richness increased from 3 to 17. At 12 and 48 hrs, total and BrdU-labeled communities shared the same value of species richness. BrdU community richness was greater than total community richness at 72 hours.

The assessment of species richness of the seawater culture could be limited by the dilution of the natural assemblage prior to incubation with BrdU. This dilution could have reduced, or even removed, more rare species populations too small to be detected by ARISA. This dilution allowed more dominant species to thrive and potentially overpower signals of less dominant species. The larger increase in species richness of the BrdU-labeled community than that of total community richness at 72hrs (Fig. 2.4) indicates that some of the less dominant species, masked in the total community, can be distinguished in the BrdU positive community. In conjunction with the increase of bacterial abundance at that time there could also be species that grew to numbers that

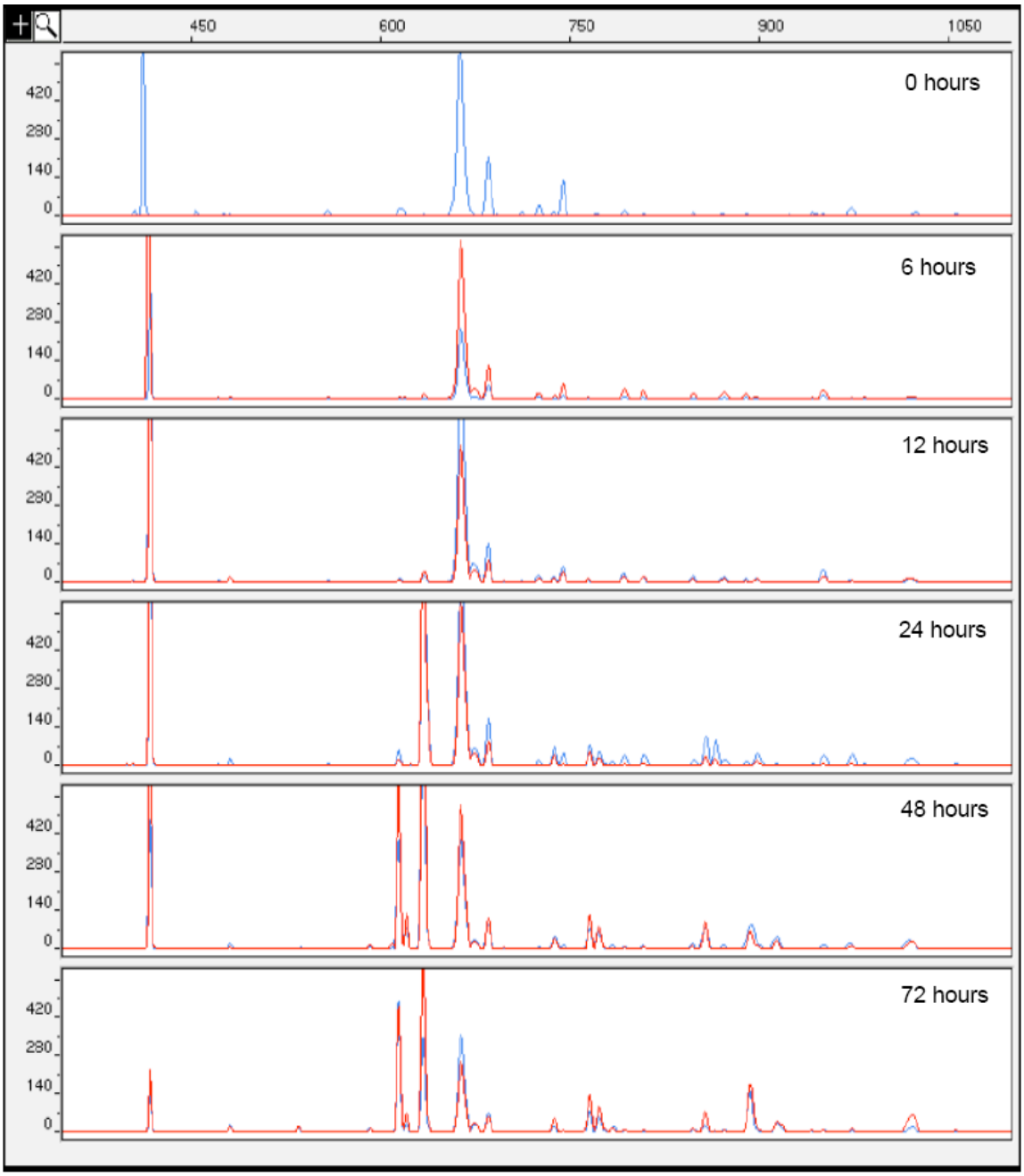


Fig. 2.3 Fingerprints of total (blue) and BrdU-labeled (red) seawater-culture eubacterial communities. ARISA (Fisher and Triplett 1999) was performed targeting the universal 3' 16S rDNA and the eubacterial-specific 5' 23S rDNA (16S-1392F (5'-G[C/T] ACACACCGCCGCCCGT-3'); 23S-125R (5'-6-FAM – GGGTT[C/G/T] CCCCATTC [A/G] G-3')). Duplicate PCR products were pooled together and normalized to 5ng μL^{-1}. ITS-PCR samples were run on an ABI 377xl automated sequencer and analyzed using GeneScan 3.1. No BrdU-label community was detected at 0 hours.

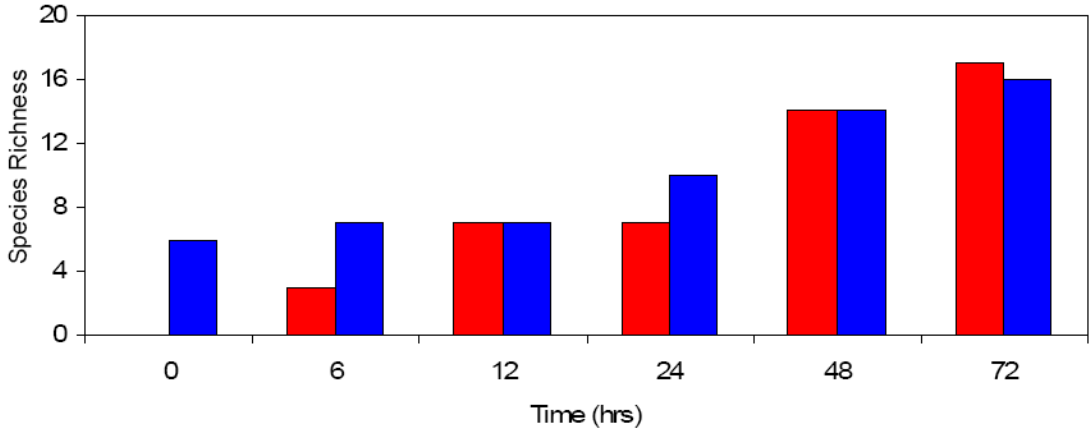


Fig. 2.4. Species richness of total (blue) and BrdU-labeled (red) seawater culture Eubacterial communities. Duplicate 16S rDNA and 23S rDNA intergenic spacer region (ITS) PCR products (16S-1392F (5'-G[C/T] ACACACCGCCGCCCGT-3'); 23S-125R (5'-6-FAM – GGGTT[C/G/T] CCCCATTC [A/G] G-3')) were pooled together, normalized to 5 ng μL^{-1}, and run on an ABI 377xl automated sequencer and analyzed using GeneScan 3.1. Species richness was determined from the number of unique OTUs. No BrdU-label community was detected at 0 hours.

Table 2.1. Similarity of total and BrdU-labeled seawater culture communities. The pair-wise Jaccard similarity coefficient was calculated from the presence of OTUs of Total and BrdU-labeled communities during the seawater-culture using NTSYSpc.

Time (hrs)	Jaccard Similarity Coefficient
0	0.000
6	0.429
12	0.750
24	0.720
48	1.000
72	0.950

could not yet be distinguished from the total community but were large enough to be seen in the BrdU community sub fraction.

Total and BrdU-labeled community structures were compared using the Jaccard's coefficient of similarity (Table 2.1). This index determines the similarity between two communities based upon the presence or absence of OTUs as seen in Fig 2.3. Following Hewson and Fuhrman (2006) , pair-wise comparisons of Monte Carlo generated random communities based upon random number of fragments (from 10 to 76) and random fragment sizes (from 350-1250 bp) determined that a Jaccard's Similarity Coefficient of 0.21 can be expected by chance alone. Therefore, Jaccard's coefficients greater than 0.21 are significant for this sample set. Total and BrdU communities at the beginning of the culture were the least similar, with a Jaccard's coefficient of 0. Similarity between total and BrdU-labeled communities increased until the total and BrdU-labeled communities were identical with a Jaccard's coefficient of 1.0 at 48 hrs. The greatest increase in similarity between the total and BrdU-labeled communities corresponds with the highest net rate of growth from 6 to 12 hrs (Fig 2.1). At 72 hrs, the similarity between the total and the BrdU- labeled communities slightly decreased to 0.95. The slight decline in similarity between the total and BrdU-labeled bacterial communities at 24 hrs and 72 hrs could be accounted for by the increase of bacterial growth (Fig. 2.1), increased species richness (Fig. 2.4), or increased grazing by more abundant protozoa (Fig. 2.2). Any change in bacterial abundance due to growth or grazing can change the relative abundances of Eubacterial species in the community. A slight shift in relative abundances can affect the detection of a particular species and influence the Jaccard's coefficient of similarity at that point.

The Jaccard coefficients were used to analyze the characteristics of the seawater culture incubation. The pair-wise comparisons for UPMGA cluster analysis revealed that there was a shift in community structure between 12 and 24 hrs of the culture incubation (Fig 2.5). Communities at 0 to 12 hrs are clustered together, while samples from 24 to 72 hrs are clustered together. In the second phase of the community (24 to 72 hrs), total

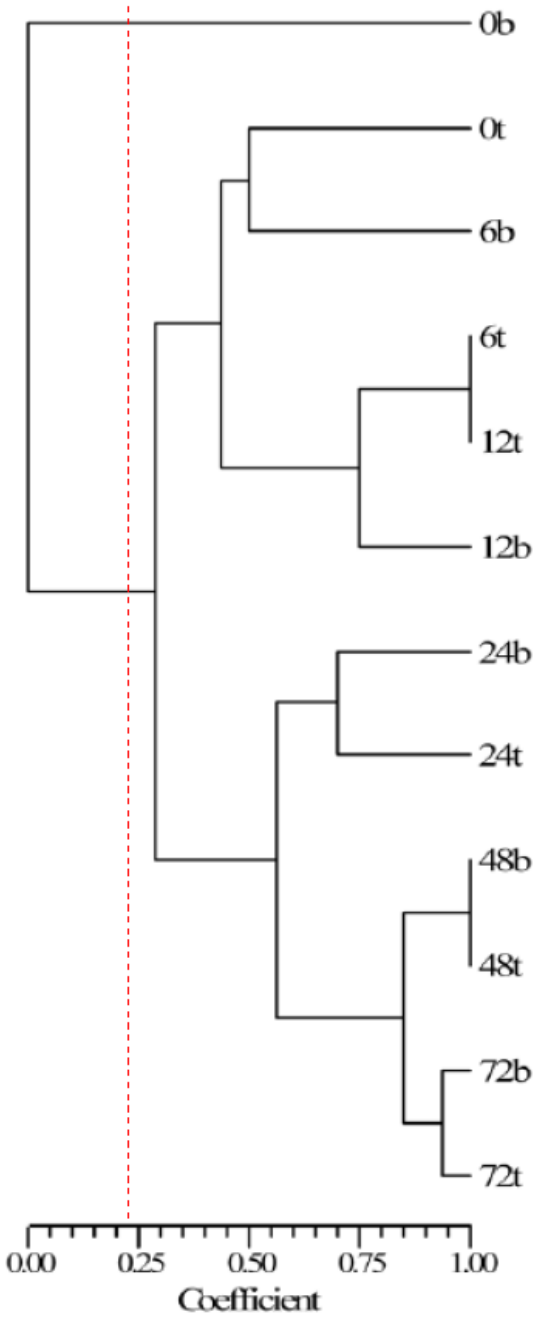


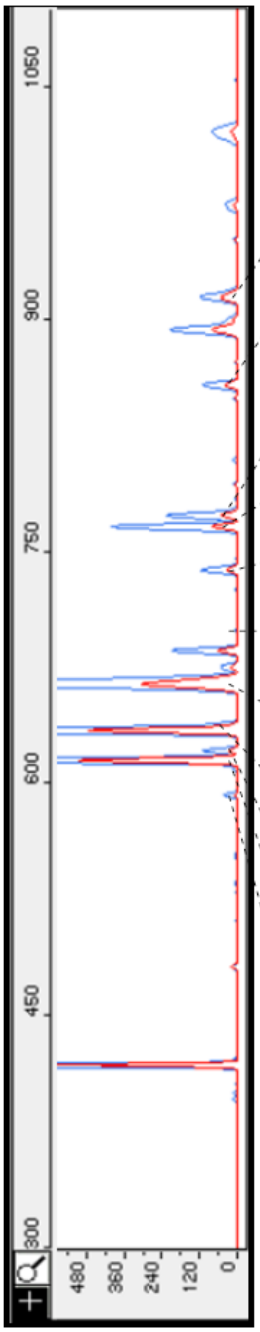
Fig. 2.5. Comparison of seawater culture Eubacterial community fingerprints. BrdU-labeled (b) and total (t) eubacterial assemblages over 72 hour culture were clustered using Jaccard Index un-weighted pair-group-mean average (UPGMA) of 16S rDNA and 23S rDNA ITS region with NTSYSpc 2.2 software package. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

and BrdU-labeled samples at the same time are more similar to each other than samples from other times. BrdU-labeled community at 0 hrs clusters outside of all other samples, as no BrdU-labeled DNA was purified from the start of the culture. The closest clustering was of the total communities from 6 and 12 hrs and the total and BrdU-labeled communities from 48 hrs. The Jaccard's coefficient for total communities at 6 and 12 hrs indicated that the community composition did not change while bacterial abundance (Fig 2.1) and BrdU species richness increased (Fig 2.3). However, at 24 hrs the total community species richness increased, and BrdU-labeled community species richness remained the same. A possible explanation is that slowly replicating species slower could be detected in the purified BrdU-labeled community but were not abundant enough to be detected in the total community until 24 hours. Shifts in community growth rates can be seen in this incubation.

BrdU allows the detection of those bacteria that were actively replicating within the seawater culture. This accumulation of BrdU allows the analysis of relative growth rates of bacteria within the community. The power of BrdU is that the correlation of BrdU incorporation with growth can reveal short timescales of change. This snapshot of the active community is dependant on the time for incubation as shorter incubation times have the potential to omit bacteria that are more slowly growing.

Phylogenetic Analysis of Eubacterial Community

The total bacterial community at 48 hrs of the seawater culture was selected for construction of the clone library because the BrdU-labeled and the total bacterial communities were identical in composition at that time. The 16S rRNA gene was sequenced for 35 of 47 clones. Fig. 2.6 shows the putative phyla, with the most closely related organism and accession numbers, aligned with the corresponding peak. Phylogenetic assignments could be made to many lineages of Eubacteria, including the α –, β –, and γ –*Proteobacteria*, low G + C Gram-positives, and Cytophaga/Flavobacterium/Bacteroides (CFB). CFB, α–, and γ –*Proteobacteria* have



Putative Phylum	Closest database match	ARISA Length	Accession	% Similarity
γ -Proteobacteria	*Idimarinia sp.*	930	DQ234155.2	99
CFB	*Polaribacter sp.**	858	AF493676.1	94
CFB	*Bacteroidetes bacterium*	793	AY580597.1	100
CFB	*Bacteroidetes bacterium*	766	AY580597.1	100
γ -Proteobacteria**	*Shewanella gaetbuli*	745	AY190533.1	98
γ -Proteobacteria	*Alteromonas sp.*	738	AY664213.1	98
α-Proteobacteria**	α – Proteobacterium*	686	AF151249.1	100
β-Proteobacteria	β - Proteobacterium	621	DQ189650.1	99
Gram-positive	*Exiguobacterium antarcticum*	615	DQ019164.1	85
CFB	*Bacteroidetes bacterium*	609	AY794159.1	99
CFB	*Bacteroidetes bacterium*	592	AY830012.1	100

* Sequence also matches *Bacteroidetes bacterium*
** Sequence also matches CFB

Fig. 2.6. Putative Phyla affiliations of seawater culture Eubacterial community. Cloned 16s rRNA genes (using universal primer 27F - 5'-GTTGATCCTGGCTCAG-3') from the total eubacterial community at 48 hours of the seawater culture experiment. Clones were phylogenetically affiliated using the online BLAST tool on the GenBank website. Clones were reanalyzed with ARISA (Fisher and Triplett 1999) to align putative phyla with OTUs.

been shown to incorporate BrdU by Pernthaler and colleagues (2002) and Hamasaki and colleagues (2004). While a strain of gram-positive bacteria was previously found to not incorporate BrdU (Urbach et al. 1999), both this study and Hamasaki and colleagues (2004) identified BrdU incorporation into at least one gram-positive species.

One peak (ARISA length 850) corresponded with two phylogenetically related organisms within CFB. Another peak (ARISA length 634) corresponded to two phylogenetically distinct organisms within the α – *Proteobacteria* and CFB. Although this study does not allow for the phylogenetic differentiation of these two ARISA lengths, others have suggested to further dereplicate these OTUs using phylogenetic specific primers with ARISA (Brown et al 2005).

SUMMARY

The dynamics of the bacterial community were captured both by BrdU incorporation and the total community structure in the seawater-culture experiment. BrdU incorporated into all of the major OTUs present in the natural community over the course of the incubation, suggesting that all of the major types of bacteria present were capable of replicating under these conditions. Shorter BrdU incubations may exclude some bacteria because of slower growth rates. These results suggest that the pairing of BrdU with the sensitive fingerprinting tool ARISA validates the use of BrdU in environmental studies.

CHAPTER III

MARINE BACTERIAL COMMUNITY STRUCTURE AND COMPOSITION ON THE TEXAS-LOUISIANA SHELF

OBJECTIVE

Bacterial communities are dynamic systems that play a major role within the global carbon cycle and degradation of primary producers. However, few studies have examined the community dynamics and impacts of the bacterioplankton community on the Texas-Louisiana Shelf. To determine the spatial and temporal variation of Eubacterial community structure, the total and active fraction of the Texas-Louisiana Shelf bacterial community was determined with BrdU incubations and analyzed with ARISA.

MATERIALS AND METHODS

Sample Collection

Samples were collected aboard the *R/V Gyre* during 7 cruises in spring and summer of 2004 (April, June and August) and 2005 (March, May, July and August). Sampling sites were located on the Texas-Louisiana continental shelf, west of the Mississippi River delta in a grid from 28.0^{o} N to 30.0^{o} N and 89.0^{o} W to 94.0^{o} W (Fig. 3.1). The sampling region was divided into four Zones (A, B, C, and D). During the summer of 2004, five sites were sampled from each of the Zones A, B and C, with samples taken along the 20m isobath, and also cross-shelf, on the 10m, 20m and 40m isobaths. During the summer of 2005, three sites were sampled from each of Zones A, B, C, and D with sampling cross-shelf, on 10m, 20m and 40m isobaths. At each site, seawater was sampled from three depths, surface (1.5m deep), middle (below the pycnocline) and bottom (~ 0.5 m above sea floor). Surface and mid- water-

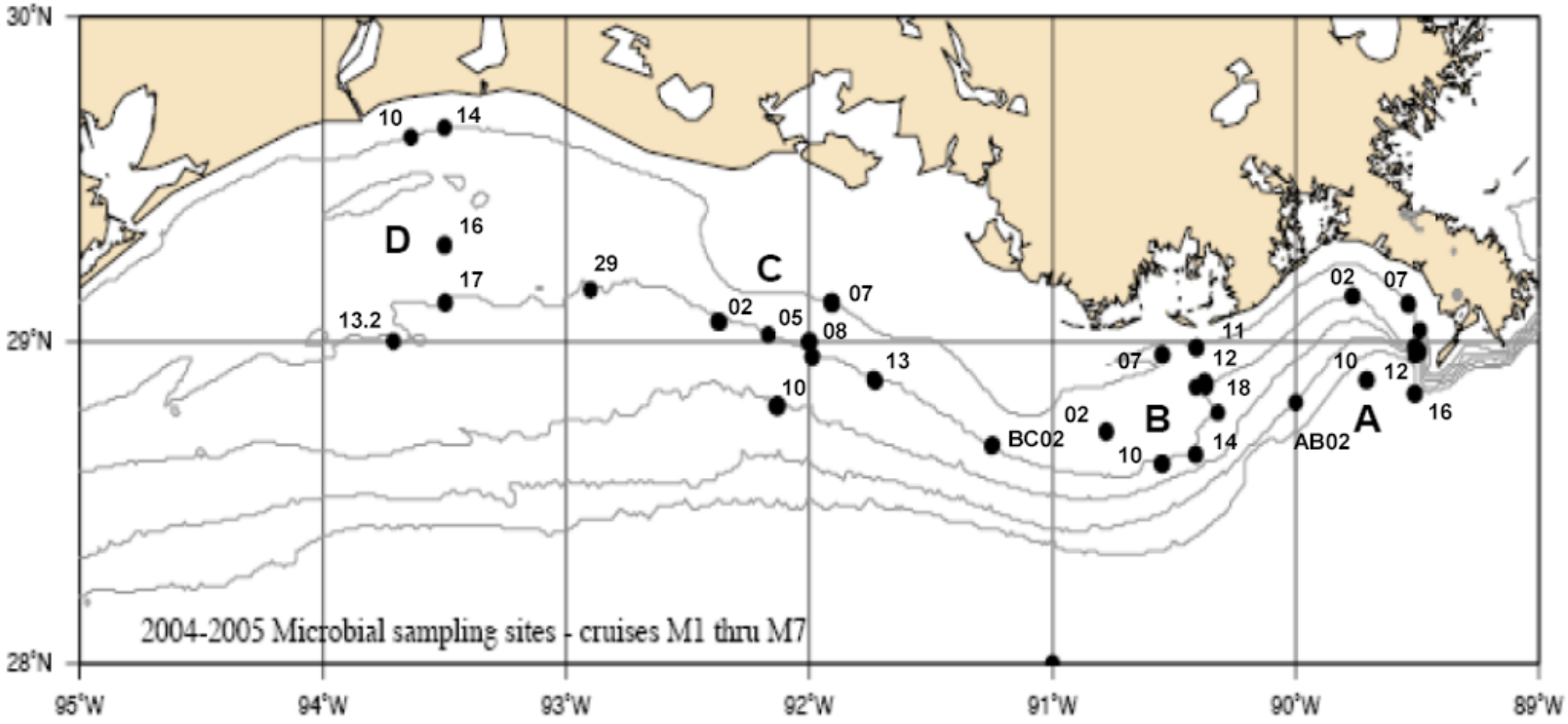


Fig. 3.1. Sample locations on the Texas-Louisiana Shelf during 2004 and 2005. Samples were collected in April, June and August of 2004 and in March, May, July and August of 2005. Sites are grouped into four zones (A,B,C,D). At each site, seawater samples were collected by Niskin bottles from surface (1.5 m), middle (below the pycnocline) and bottom waters (~0.5 m above the seafloor). Hydrographic measurements were also collected with a CTD package (SBE-9), flourometer, transmissometer (Seatech) and oxygen probe (SBE-43). Bathymetry lines denote 10m, 20m, 30m, 40m and 50m water column depth.

column samples were collected in 10 L Niskin bottles attached to a rosette with an attached CTD package (Seabird, SBE-9) for hydrographic measurements (i.e. conductivity, temperature, and depth). Bottom water samples were collected with specially fashioned 5 L Niskin bottles triggered by contact with the seafloor (Mason and Rowland 1990).

To sample the natural bacterial population, 2 L of seawater were collected from surface and middle waters and 1 L from the bottom waters at each site. Bacterial samples were collected onto 0.22 μm Sterivex filters (Millipore), sealed and stored onboard the ship at -20 ^{o}C.

During 2005, the actively growing bacterial populations were assessed through BrdU incubations. At each site an additional 2 L was collected from surface and middle waters, and an additional 1 L from bottom waters. The samples were transferred into acid-wash, seawater rinsed polycarbonate bottles for the incubation. Samples were inoculated with a final 20 nM BrdU and incubated at *in situ* temperatures for two hours in the dark. At termination of the incubation, samples were collected onto 0.22 μm Sterivex filters as above were processed as above. In the lab, all samples were stores at -80 ^{o}C until further processing.

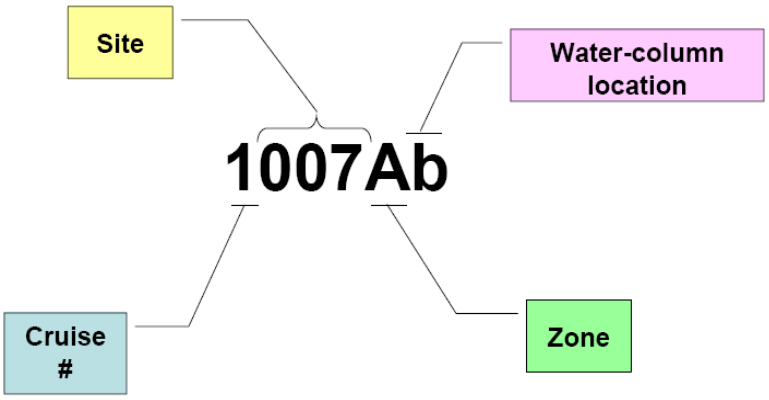


Fig 3.2. Key to community sample identifiers. Cruises numbers were categorized by April (1), June (2) and August (3) of 2004 and March (4), May (5), July (6) and August (7) of 2005. Sampling sites were numbered 1 thru 16 within each of the zones (A, B, C and D). Sampling occurred as described above at the surface (S), middle (M) and bottom (B) depths of the water-column. This example sample would have been collected in April 2004, at site 007 in zone A bottom water.

Samples were labeled by the cruise number, sample site, Zone, and the location in the water column at which they were collected (Fig 3.2). Cruises were numbered 1 thru 7 in order of date (April (1), June (2) and August (3) of 2004 and March (4), May (5), July (6) and August (7) of 2005). Sampling sites were numbered 1 thru 16 within each of the Zones (A, B, C and D). Sampling occurred as described above at the surface (S), middle (M) and bottom (B) depths of the water-column.

Bacterial Enumeration

Five mL samples were taken at each time site and preserved with filtered borate buffered formalin (2% final vol:vol) for bacterial counts. Preserved samples were stained with 4'6-diamidino-2-phenylindole solution (DAPI, 1 μg mL^{-1}, Sigma) and then 2 mL was concentrated onto a 0.22 μm prestained black polycarbonate filter (Isopore/Millipore) (Höppe 1978; Porter and Feig 1980). Slides were prepared onboard and stored at -20 ^{o}C. Following Turley and Hughes (1992), slides were counted for at least 200 cells or 20 fields by epifluorescent microscopy on a Zeiss Axioplan Imagining 2 universal microscope (Zeiss filter set 02) using Zeiss KS-300 3.0 image analysis software.

DNA Extraction and Purification

Sterivex filters were thawed and DNA was extracted following a modification of the method of Somerville et al. (1989). Filters were filled with 1.8 mL STE buffer (10 mM Tris-Hydrocloride [pH 8.0], 1 mM EDTA, 10 mM NaCl) containing fresh Lysozyme (5 mg mL^{-1}) and sealed with a syringe and cap for a one hour incubation at 37 ^{o}C. A 180 μL aliquot of Proteinase K (20 mg mL^{-1}) and 100 μL of 10% SDS solution were added to the filter and incubated for two hours at 60 ^{o}C. The filters were boiled in a microwave for 5-10 seconds. The sample was pulled into the syringe and transferred into 15 mL Corex tubes (Kimble Glass Inc.). The filters were rinsed with 1 mL TE buffer (10 mM Tris-hydrochloride [pH 8.0], 1mM EDTA) and the sample was pooled into 15 mL Corex tubes. A volume of 30 μL of RNase (10 mg mL^{-1}) was added to the

sample and incubated for 10 minutes at room temperature. A half-volume of 7.5 M ammonium acetate was added and incubated for 15 minutes at room temperature. The sample was centrifuged at 10,000 *x g* for 5 minutes and the supernatant was transferred to a clean tube. The DNA was precipitated by the addition of 9 mL 100% ethanol overnight at -80 ^{o}C. The precipitated DNA was collected by centrifugation at 14,500 *x g* for 30 min. After removal of the supernatant, the DNA was resuspended in 400 μL TE buffer and incubated at 37 ^{o}C for one hour. Extracted DNA was purified by phenol-chloroform extraction (Sambrook and Russell 2001).

Immunochemical Isolation of BrdU-labeled DNA

Following DNA extraction and purification, immunoglobulin paramagnetic beads (MagnaBind, Pierce) were used to immunocapture BrdU-labeled DNA from assemblages of labeled and unlabeled communities. Following a modification of the procedure of Urbach and colleagues (1999), all incubations were performed at room temperature. Herring Sperm DNA (1.25 mg mL^{-1} in phosphate buffered saline [PBS]) was denatured in boiling water for one minute and then quickly cooled in ice water. Monoclonal anti-BrdU antibody solution (4.5 mg IgG mL^{-1} Clone BU33 mouse ascites fluid (Sigma) diluted 1:10 in PBS) was diluted 9:1 with Herring Sperm DNA and incubated for 30 minutes. Sample DNA (1.0 μg DNA in PBS) was denatured for one minute in boiling water and then quickly cooled in ice water. The sample was mixed with 50 μL of the diluted anti-BrdU antibody mixture and incubated for 30 minutes. IgG-coated paramagnetic beads were washed once in a PBS-BSA solution (1 mg mL^{-1} bovine serum albumin (Vector Inc.) in PBS) using a magnetic particle concentrator (MagnaBind), and resuspended in PBS-BSA to their initial concentration. A 25 μL volume of paramagnetic beads were added to each sample and incubated for 90 minutes with constant agitation. Using the magnetic concentrator, the beads-sample mixture was washed with 300 μL PBS-BSA seven times to remove unlabeled DNA. The BrdU-labeled DNA was eluted from the paramagnetic beads by adding 100 μL 1.7 mM BrdU (in PBS-BSA) and incubated for 30 minutes with constant agitation. After removing the

beads with the magnetic concentrator, the BrdU-labeled DNA was precipitated out of the supernatant by the addition of 2 μL glycogen (20 mg mL^{-1}), 20 μL10 M ammonium acetate, and 200 μL cold ethanol, and then incubated at -80 ^{o}C for 15 minutes. After a second ethanol precipitation, the DNA was resuspended in TE buffer.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA (Fisher and Triplett 1999) was performed following modifications of the method detailed by Brown et al. (2005). Primers used in ARISA targeted a universal 3' region of the 16S rDNA and a Eubacterial-specific 5' 23S rDNA (16S-1392F (5'-G[C/T] ACACACCGCCGCCCGT-3'); 23S-125R (5'-6-Carboxyfluorescein (6-FAM) – GGGTT[C/G/T] CCCCATTC [A/G] G-3')) after Hewson and colleagues (2003). The 5' 6-FAM labeling was for the detection of the PCR products. Duplicate 20 μL reactions contained final concentrations of 1x PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 200 μM of each dNTP (Qiagen), 200nM of each primer, 40 ng μL^{-1} BSA, 2.5 U *Taq* DNA polymerase (Qiagen), and 1-10 ng μL^{-1} template DNA. Reaction mixtures were held at 94 ^{o}C for 2 min followed by 35 cycles of amplification at 94 ^{o}C for 40 s, 56 ^{o}C for 40 s, and 72 ^{o}C for 90 s, with a final extension step at 72 ^{o}C for 5 min. PCR products were purified using DNAce Strip Kit with sephadex columns (Bioline) and then quantified with SybrGold fluorescence on a SpectraMax Gemini EM spectrofluorometer (Molecular Devices). Concentrations of the PCR products were then diluted to 5 ng μL^{-1}.

Diluted PCR products were prepared for ARISA analysis by adding 1-2 μl diluted PCR product to a Marker-dye master mix consisting of 2.5 μL deionized formamide, 0.5 μL Blue Dextran Tracking Dye in EDTA (Bioventures), and 0.18 μL custom Rhodamine X-labeled MapMarker 50-1500 Size Standard (Bioventures). After denaturing at 95 ^{o}C for 5 min, PCR-Marker-dye mixes were immediately cooled on ice. Using ficoll loading solution (Gel Co), a 0.8 μL aliquot of PCR-Marker-dye mix was loaded onto a 36 cm long 5.0% polyacrylamide gel (Long Ranger) and run on an ABI 377XL automated sequencer for 5.5 hrs. Electropherogram output was aligned with

scanned size fragment bands and analyzed using ABI Genescan 3.1 software and exported to Microsoft Office excel for further analysis.

ARISA Gel Analysis

Electropherogram peaks were interpreted as present operational taxonomic units (OTUs) as analyzed GeneScan 3.1 Software. Outputs from duplicate runs of samples were pooled together for total community structure. Peaks that were <0.09% of the total amplified DNA, seen by the total relative fluorescent area for each sample, were determined to be background noise and discarded following Hewson and Fuhrman (2004). Sample peaks were then assigned to bins to compensate for decreased resolution of the base calling of larger fragments. Fragments smaller that 700 bp were grouped in to bins of 3 bp, bins of 5 bp for fragments between 700-999, and bins of 10 bp for fragments greater than 1000 bp in length (Brown et al. 2005).

Statistical Analysis

Un-weighted pair-group mean average (UPGMA) cluster analysis was conducted from the matrix generated from the comparison of bacterial communities analyzed by ARISA using NTSYSpc 2.2 software program (Rohlf 2000). This matrix was generated by comparing communities based upon the presence or absence of OTUs using the Jaccard coefficient of similarity (S_j);

$$S_j = \frac{W}{(a_1 + a_2) - W},$$

where W is the number of shared OTUs between two communities and a_1 and a_2 are the total number of OTUs within the respective communities. This comparison allows for a relative determination of the degree of similarity between bacterial communities. To determine if a relationship exists between bacterial abundance, community richness and the sample location (Zones A, B, C and D and sampling depth, surface, middle, and

bottom), correlation analysis and model I analysis of variance (ANOVA) was conducted using SPSS (SPSS Inc, Release 13.0).

Clone Library and Sequencing

Communities chosen because of their complex and unique structure were cloned and sequenced for identification of bacterial species. Six communities were selected for two clone libraries. The first library combined the communities 2002bs, 5012as and 5012am to identify dominant OTUs seen more frequently within all samples (Fig 3.3). The second library combined the communities of 5007cb, 1016ab, 7017db to determine the affiliations of rarer OTUs (Fig 3.4). Fresh 20 μL reactions using Eubacterial specific 16S primer 27F (5'-GTTGATCCTGGCTCAG-3')(Hugenholtz and Goebel 2001) and unlabeled 23S primer (23S-125R (5'–GGGTT[C/G/T]CCCCATTC [A/G] G-3') after (Hewson et al. 2003)) were prepared containing final concentrations of PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 200 μM of each dNTP (Qiagen), 200nM of each primer, 40 ng μL^{-1} BSA, 2.5 U *Taq* DNA polymerase (Qiagen) and 1-10 ng μL^{-1} template DNA. Reaction mixtures were held at 94 ^{o}C for two minutes followed by 35 cycles of amplification at 94 ^{o}C for 40 s, 56 ^{o}C for 40 s and 72 ^{o}C for 90 s, with a final extension step of 72 ^{o}C for 5 min. PCR products were pooled together from the communities for two libraries consisting of dominant and rarer OTUs. PCR products were pooled together from the communities for two libraries consisting of dominant and rarer OTUs. Pooled PCR products were then inserted into the pCR8/GW/TOPO vector using the TA Cloning kit (Invitrogen) and clones containing the plasmid were selected for by plating on LB plates containing 100 μg mL^{-1} spectinomycin. Positive clones were picked and lysed in ddH_2O at 92 ^{o}C for 5 min, following Kilger et al. (1997), and dereplicated by ARISA. Multiple OTUs found within each clone are from the *E. coli* clone, while the unique OTU is attributed to the targeted sequence. This allowed sequence putative phylogenetic affiliations to be aligned with specific OTUs and to identify communities' composition. Picked clones were also cultured at 37 ^{o}C for 12 hours in LB broth containing 100 μg mL^{-1} spectinomycin. Plasmids were

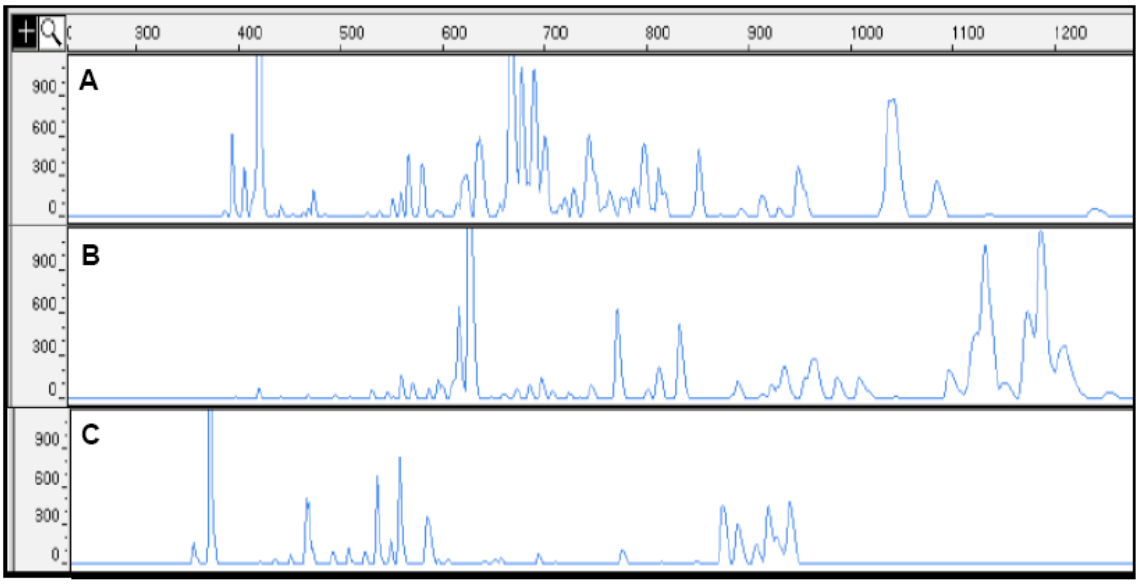


Fig 3.3. ARISA derived community fingerprints of dominant OTUs. 5 ng μL^{-1} normalized duplicate PCR products (using 16S rDNA 1392F and 23S rDNA TET-labeled 125R primers) were pooled and run on an ABI 377xl automated sequencer from each of the samples (A) 2002bs (B) 5012as and (C) 5012 am . Electropherograms were generated using GeneScan 3.1 software. Samples were chosen for 16S-ITS clone library to determine phylogenic affiliations of dominant OTUs.

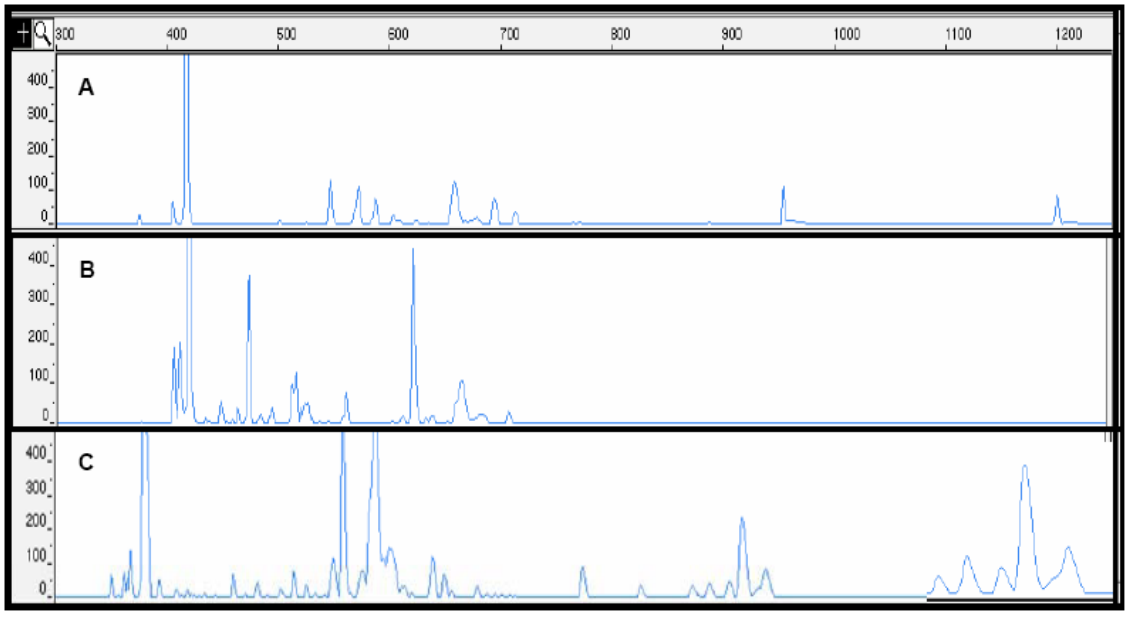


Fig 3.4. ARISA derived community fingerprints of rare OTUs. 5 ng μL^{-1} normalized duplicate PCR products (using 16S rDNA 1392F and 23S rDNA 6-FAM-labeled 125R primers) were pooled and run on an ABI 377xl automated sequencer from each of the samples (A) 7017dm (B) 1016ab and (C) 5007cb . Electropherograms were generated using GeneScan 3.1 software. Samples were chosen for 16S-ITS clone library to determine phylogenic affiliations of rare OTUs.

purified and sequenced by Agencourt Bioscience Corporation using universal 16S primer 27F (Hugenholtz and Goebel 2001) for phylogenetic affiliation using the online BLAST tool on the GenBank website (Altschul et al. 1990).

RESULTS AND DISCUSSION

Sample Collection

During 2004 and 2005, 28 sites on the Texas-Louisiana shelf were studies: 212 samples of total communities and 64 samples of BrdU-labeled communities from the surface, middle and bottom of the water column. Zone C was sampled during each cruise, but the frequency of sampling of the other Zones varied during the project. Zone A, and to a lesser extent Zone B, were not routinely sampled throughout 2005 due to time and safety restrictions brought about by approaching hurricanes. The loss of sampling at these sites was to some extent compensated by the addition of Zone D in the latter half of 2005.

Bacterial Abundances on the Texas-Louisiana Shelf

Bacterial abundance of the 195 communities enumerated from 2004 (Fig 3.5) and 2005 (Fig 3.6) ranged from 2.06×10^5 to 6.98×10^6 cells mL^{-1}, with a mean abundance of 1.72×10^6 cells mL^{-1}. These abundances are similar to those previously determined for Atlantic coastal waters (Azam et al. 1983) and those determined in the coastal waters off of the Mississippi River (Amon and Benner 1998; Pakulski et al. 2000). Abundances and the richness of the communities were affected by sampling date, Zone and depth within the water column. Non-parametric Spearman's Rank Order correlation analysis revealed that bacterial abundance and the geographical and temporal variables listed in Table 3.1 were significantly correlated ($p > 0.01$). Significance was conservatively determined at the 0.01 level since the data were not normally distributed (and not significantly affected by transformation). R ranges from +1 to -1 where zero signifies

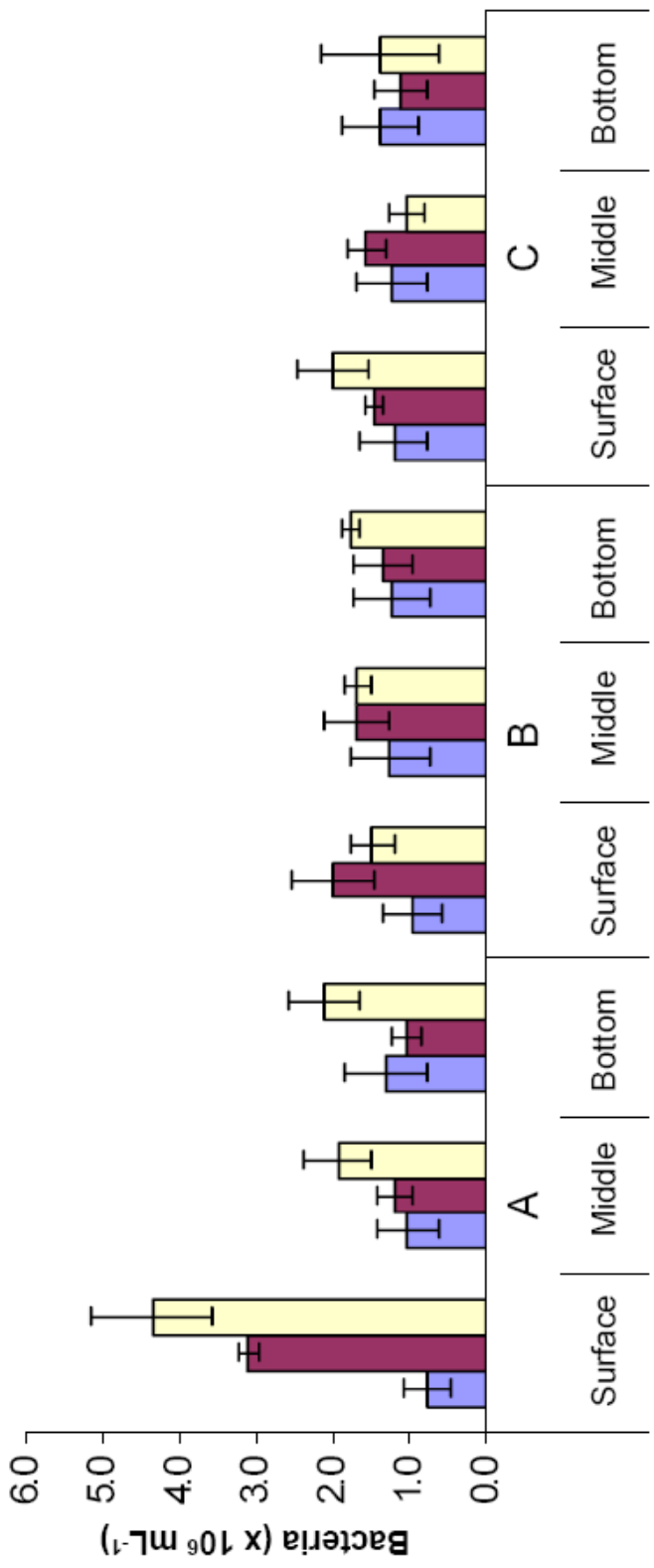


Fig. 3.5. Average bacterial abundance of each zone (A, B, C) at each depth (surface, middle, bottom) for April (blue), June (red) and August (yellow) of 2004. Formalin preserved and DAPI stained (1 mg mL-1) samples were concentrated onto a 0.22 µm prestained black polycarbonate filters and enumerated by epifluorescent microscopy on a Zeiss Axioplan Imagining 2 universal microscope (Zeiss filter set 02). Bacterial abundances were averaged for sampling zone and depth at each sampling date. Error bars represent standard deviation.

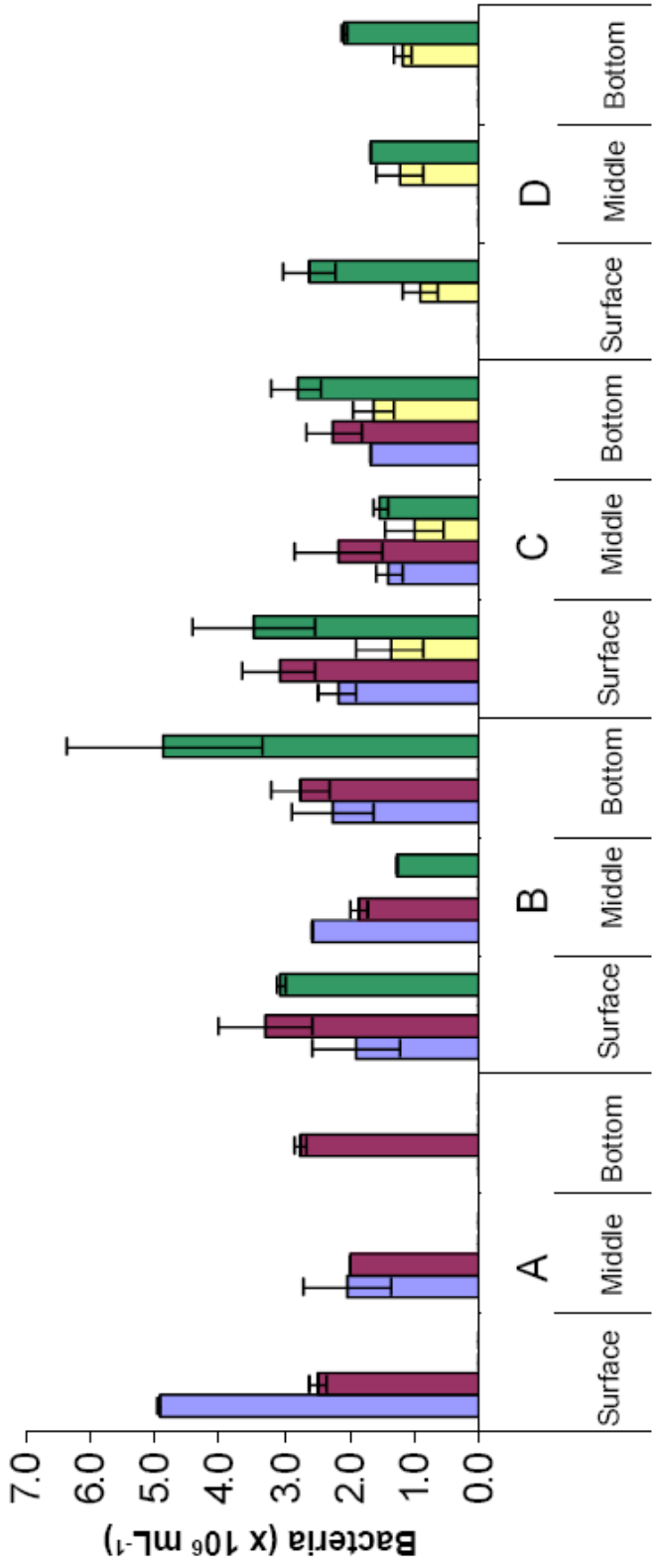


Fig. 3.6. Average bacterial abundance of each zone (A, B, C, D) at each depth (surface, middle, bottom) for March (blue), May (red), July (yellow) and August (green) of 2005. Formalin preserved and DAPI stained (1 mg mL-1) samples were concentrated onto a 0.22 μm prestained black polycarbonate filters and enumerated by epifluorescent microscopy on a Zeiss Axioplan Imagining 2 universal microscope (Zeiss filter set 02). Bacterial abundances were averaged for sampling zone and depth at each sampling date. Error bars represent standard deviation. Sampling of zone D was added is July and August of 2005, due to approaching hurricanes that forced the sampling west, omitting sampling of zones A and B.

Table 3.1 Significant correlations of bacterial abundance vs. geographical and temporal variables. Non-parametric Spearman's Rank Order correlation analysis was performed with bacterial abundance and the geographical variables listed with a significance level of 0.01 as the data were not normally distributed. N is the number in the sample set. A positive r signifies a direct correlation, while a negative r is an inverse correlation.

Variable	r	N	p-value
Sampling Month	0.44	195	0.000
Latitude	0.19	195	0.008
Site Depth (m)	-0.30	195	0.000
Depth (m)	-0.20	186	0.002

* Longitude was also analyzed and was not found to be significantly correlated with bacterial abundance.

no correlation, an R value of +1 signifies a perfect direction correlation, and an R value of -1 value is a perfect inverse correlation. The strongest positive correlation with bacterial abundance was found with the sampling month. There is also a positive relationship between latitude and bacterial abundance. Latitude could also be interpreted as a proxy for distance from shore, where the distance of the site from shore increases as the latitude decreases. The depth of the site also increases with distance from shore, perhaps accounting for the negative correlation between bacterial abundance and site depth. Bacterial abundances are greater closer to shore where the site depth is shallower. However, the correlations with geographic parameters are relatively weak, suggesting that additional parameters influence the bacterial population size.

A three factor model I analysis of variance, with location (Zones A, B, C and D), sampling depth (surface, middle, and bottom), and sampling date (April, June and August of 2004, March, May, June and August of 2005) as main factors was used to

determine the effects of location, sampling date and depth on bacterial abundance by comparing the means of the data sets. The ANOVA indicated that the interaction terms sampling date*depth and sampling date*location were significant ($p < 0.01$), while the interaction terms location*sampling depth and sampling date*depth*location were both not significant ($p > 0.01$). The ANOVA also suggested that both main factors, sampling date and depth, were significant (date: $F_{3, 188} = 14.691$, $p < 0.05$; depth: $F_{2, 192} = 8.459$, $p < 0.05$), while the main factor location did not have a significant effect ($F_{3, 191} = 3.722$, $p > 0.05$) on bacterial abundance. Post Hoc analysis revealed the relationship of these factors with bacterial abundance, as seen in Fig 3.7.

Bacterial abundances were significantly different between two clusters of sampling dates (August 2004, March, May and August 2005; April, June 2004 and July 2005). Higher bacterial abundances were found later in the summer, after peaks of primary production are known to occur (Lohrenz et al. 1990; Lohrenz et al. 1997). When analyzing sampling depth, surface and bottom water column samples were significantly higher than samples from the middle of the water column. Amon and Benner (1998) also reported higher bacterial abundances in surface and bottom waters connected with higher bacterial productivity than in middle waters. The highest bacterial abundances are associated with primary production in the surface waters. Although the different locations of the Zones did not have a significant affect on the mean bacterial abundance, latitude did have a significant correlation with bacterial abundance (Table 3.1) and as seen in Fig 3.5 and Fig 3.6, there is a trend of decreasing bacterial abundance from Zone A to Zone D. This trend agrees with previous studies where bacterial abundance was found to be higher in the eastern Texas – Louisiana shelf (Amon and Benner 1998; Pakulski et al. 2000).

Overall bacterial abundance was higher in 2005 than 2004, with the exception of July 2005. This discrepancy could be due to fewer sites close to the delta were sampled during July 2005. In July 2005 only Zones C and D, which were usually characterized by lower bacterial abundances, were sampled due to weather restrictions. Kent and

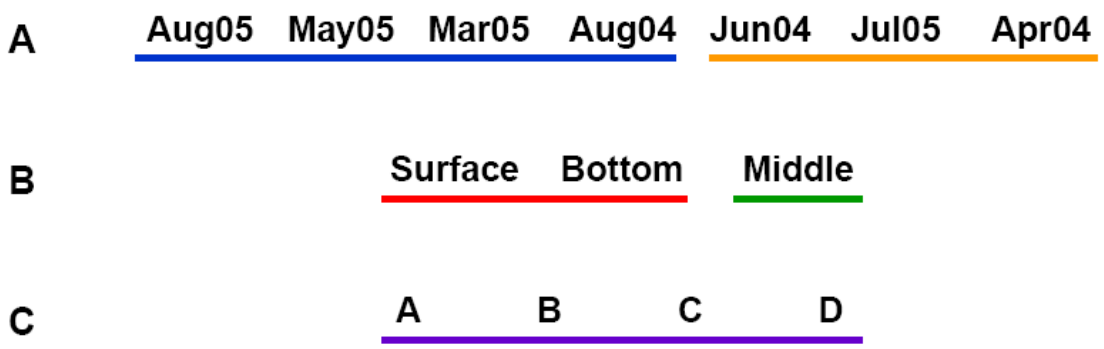


Fig 3.7. Homogeneousness of group averages of bacterial abundance by sampling date (a), depth (b) and Zone (c). A three factor model I analysis of variance, with location (Zone A, B, C and D), sampling depth (surface, middle and bottom), and date (April, June and August of 2004, March, May, June and August of 2005) as main factors was used to determine the effects of location, sampling date and depth on bacterial abundance by comparing the means of the data sets. Underlined groups depicts homogenous bacterial abundances. Groups are ranked in order of highest to lowest average of bacterial abundance.

colleagues (2004) found much variation in freshwater bacterioplankton community from year to year. This annual variability could be due to many environmental factors influencing the sampling area such as fluctuating river discharge (Appendix C), continental shelf currents or weather. It should also be noted that just previously to sampling, during July 3-7th 2005, Hurricane Cindy passed directly through Zone B, possibly diluting bacterial communities with excess rain and also disrupting the bacterial communities with winds and storm surges.

Eubacterial Community Structure Richness

Two-hundred and seventy-six unique community fingerprints (Total, n=212; BrdU-labeled, n=64) were discriminated by ARISA analysis of collected samples across the Texas-Louisiana Shelf. Sixty-four active communities were distinguished by pairing BrdU incorporation with ARISA analysis. Intergenic spacer regions detected from these bacterial communities ranged from 350-1250 base pairs (bp) in length (Fig 3.3 and 3.4). The breadth of ITS regions in this study supports and slightly expands previous findings

by Brown and colleagues (2005) of marine communities ranging from 400-1200 bp, showing that marine communities have a wider range of ITS length than those found previous. These findings also expand upon studies finding bacterial ITS in freshwater lakes (Fisher and Triplett 1999; Yannarell and Triplett 2005) and sediments (Ranjard et al. 2001; Schloss et al. 2003). Our study detected a more diverse community than those previous studies. While bacterial communities in sediments are typically more diverse that bacterioplankton communities, earlier sediment studies might have excluded more rare bacteria by using a higher detection threshold of 5% of the community in their ARISA analysis (Ranjard et al. 2001; Schloss et al. 2003).

Species richness of total community fingerprints ranged from 10 to 76, with an average value of 32. Although much greater values (150-240) of species richness have been found in and near marine sediments (Ranjard et al. 2001; Hewson et al. 2003), the OTU richness found in this study is similar to values found in freshwater bacterial communities (Fisher and Triplett 1999; Yannarell and Triplett 2005) and marine systems (Suzuki et al. 1998; Brown et al. 2005; Schwalbach et al. 2005). As seen in Fig 3.8, 12.3% of total communities had equal or greater than 50 OTUs, 82.1 % of total communities had equal or greater than 20 OTUs. BrdU-labeled community fingerprints ranged in species richness from 10 to 57 OTUs, with an average richness value of 28. 68.8% of BrdU-labeled communities have greater than 20 OTUs. Eubacterial community richness of BrdU-labeled communities was less than for the total community, supporting previous findings that BrdU-labeled communities are different from the total community (Borneman 1999) and less rich than the total bacterial community (Yin et al. 2000). Presumably because not all bacterial species within the total community were actively growing, supporting previous findings of Hoppe (1978) and Fuhrman and Azam (1982) , allowing us to distinguish between the active and the non-active bacterial communities.

In our study, OTU richness was independent of bacterial abundance. Non-parametric Spearman's Rank Order correlation analysis revealed that bacterial abundance and species richness was not significantly correlated ($r = 0.34$, $n = 195$, $p =$

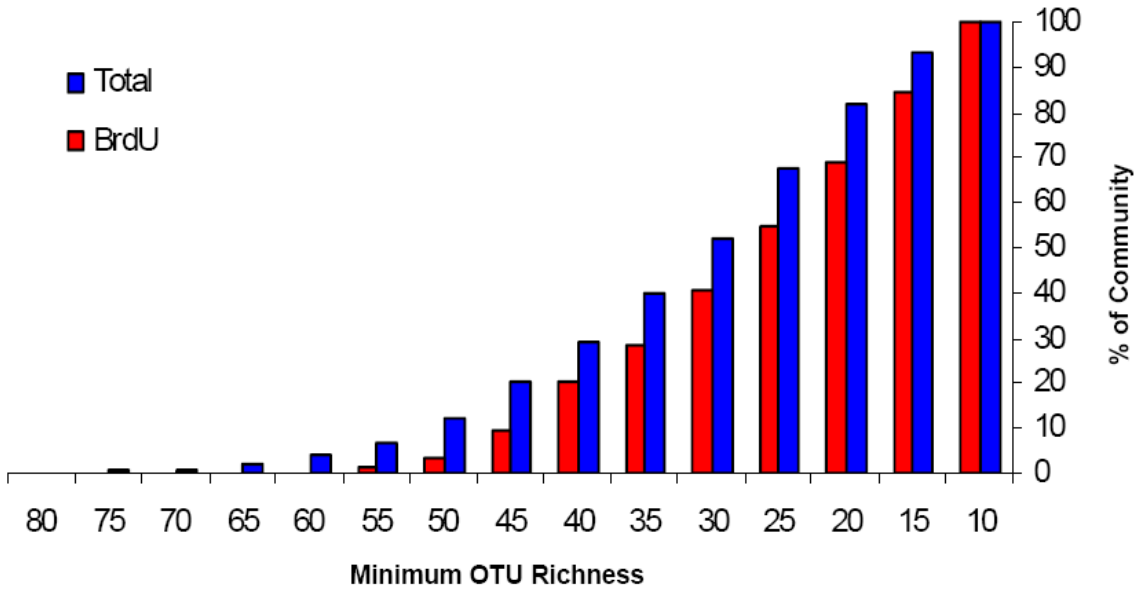


Fig 3.8. Distribution of total (blue) and BrdU-labeled (red) communities with a minimum OTU richness . ARISA determined OTU richness of total (n=276; $\bar{x}$ = 32) Eubacterial communities ranged from 10-76. ARISA determined OTU richness of BrdU-labeled (n=64; $\bar{x}$ = 28) eubacterial communities ranged from 10-57. 100% of both BrdU-labeled and total communities had a species richness of at least 10 unique OTUs. Total Eubacterial community richness was greater than BrdU-labeled communities.

0.933). This analysis also revealed that species richness was not significantly correlated with any of the geographical parameters (site depth, sampling date and depth, longitude and latitude: n = 210, p > 0.10). Kent and colleagues (2004) had found a pattern of OTU richness that varies with seasons. Kent's study, however, was conducted within the closed system of a fresh water lake as opposed to this study which was on a continental shelf. On the continental shelf much more movement of bacterial communities can occur due to oceanic currents, possibly making seasonal variation more difficult to detect among other influencing parameters.

A limitation, not unique to ARISA, arises because of possible biases during PCR amplification. When amplifying DNA from mixed communities, species ratios can be altered due to less specific primers, inefficient annealing of template strands and the

formation of chimeric sequences (recombinant sequences from two or more template strands) (Suzuki and Giovannoni 1996; Suzuki et al. 1998; Hugenholtz and Goebel 2001). By limiting primers to universal and Eubacterial specific for ITS analysis, Archaea are overlooked and not included in the community analysis.

ARISA imposes other limitations due to the focus of analysis on the ITS region. Multiple species have the potential to have corresponding ITS region lengths, making them indiscernible through ARISA and underestimating the diversity of the community (Fisher and Triplett 1999; Boyer et al. 2001). Conversely, a single species can have multiple ITS regions of varying lengths and ARISA may also overestimate the diversity of the system (Jensen et al. 1993; Fisher and Triplett 1999; Boyer et al. 2001; Schloss et al. 2003). Assuming that these biases are constant and balance out throughout the course of a study, despite these problems, ARISA remains a beneficial tool for comparative spatial and temporal analysis between communities.

Eubacterial Community Structure Clustering

ARISA generated community fingerprints were compared to one another using the Jaccard coefficient of similarity and UPMGA cluster analysis to look at relationships between the Eubacterial community structures. During April 2004 (Fig. 3.9), Zone C clearly clustered more closely with itself, while Zones A & B are intermingled together. This trend continues through June 2004 (Fig 3.10) and August 2004 (Fig 3.11). In April (Fig 3.10) and August (Fig 3.11) of 2004, there is also a subset of communities from Zone A that are independently clustered from both Zone B and Zone C. Only once in August of 2004 (Fig 3.11) is there a subset of communities from Zone B that are grouped more closely with each other. Community fingerprints from Zone B regularly clustered with both Zones A and C throughout 2004. However, communities from Zones A and C never clustered together, with the exception of a few sites. This

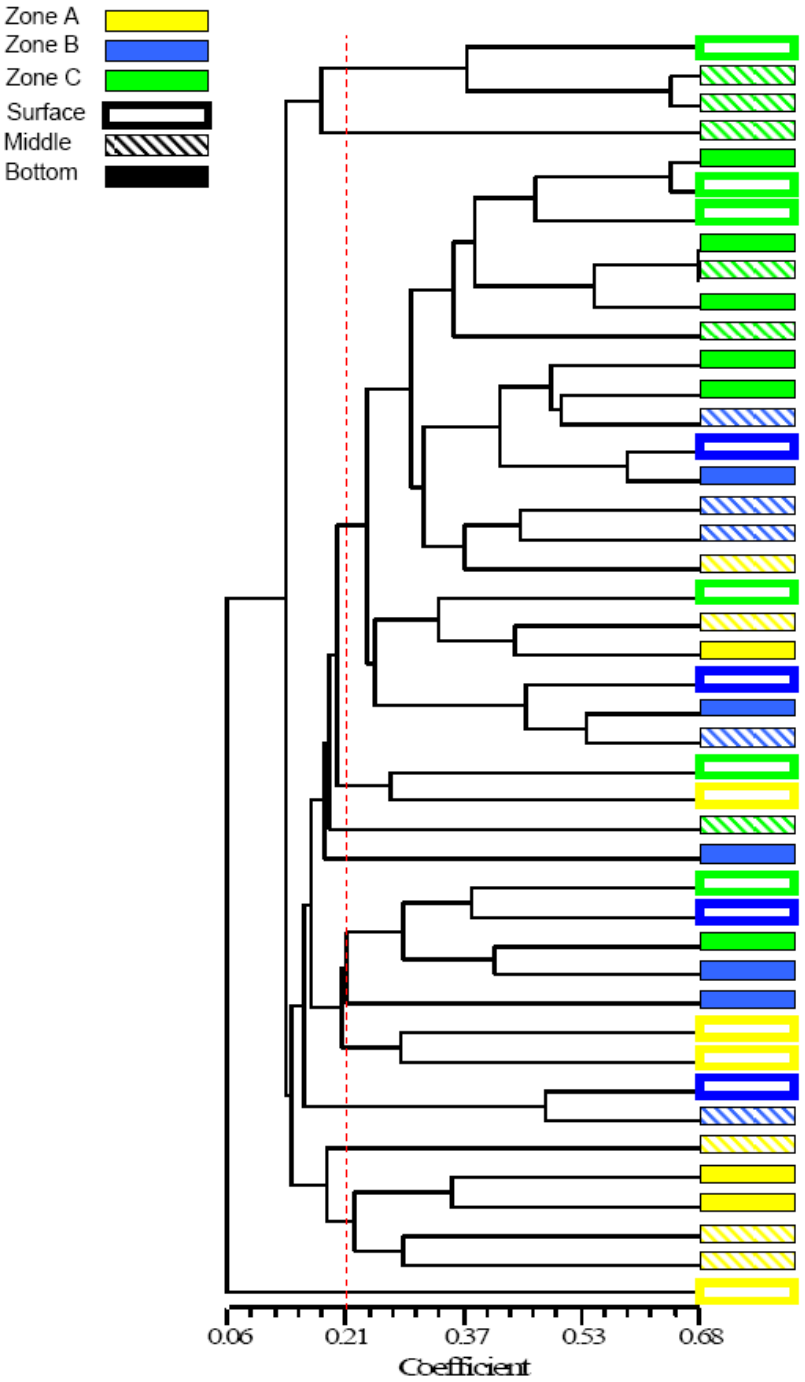


Fig. 3.9. Comparison of total Eubacterial community structures from April 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity Coefficient and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

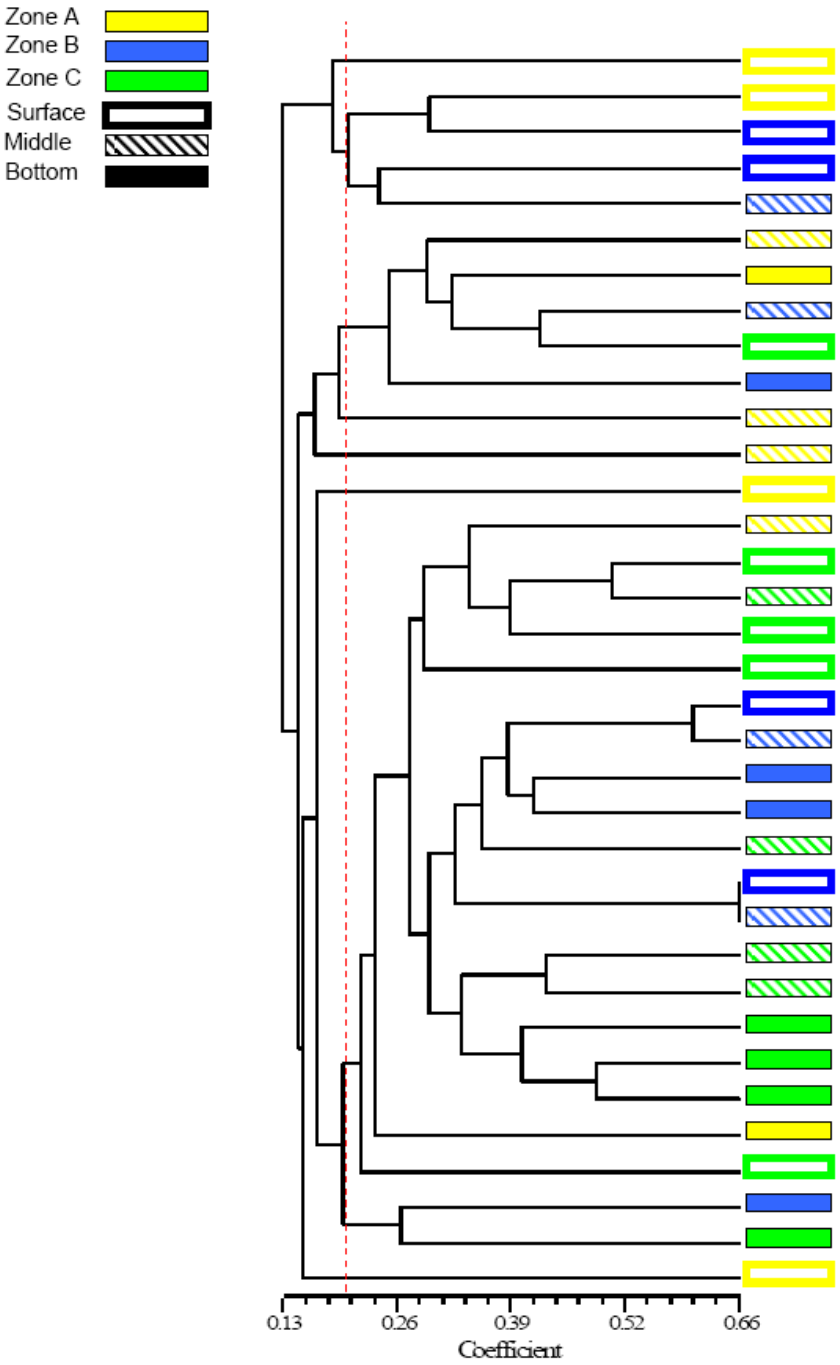


Fig. 3.10. Comparison of total Eubacterial community structures from June 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity coefficient and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

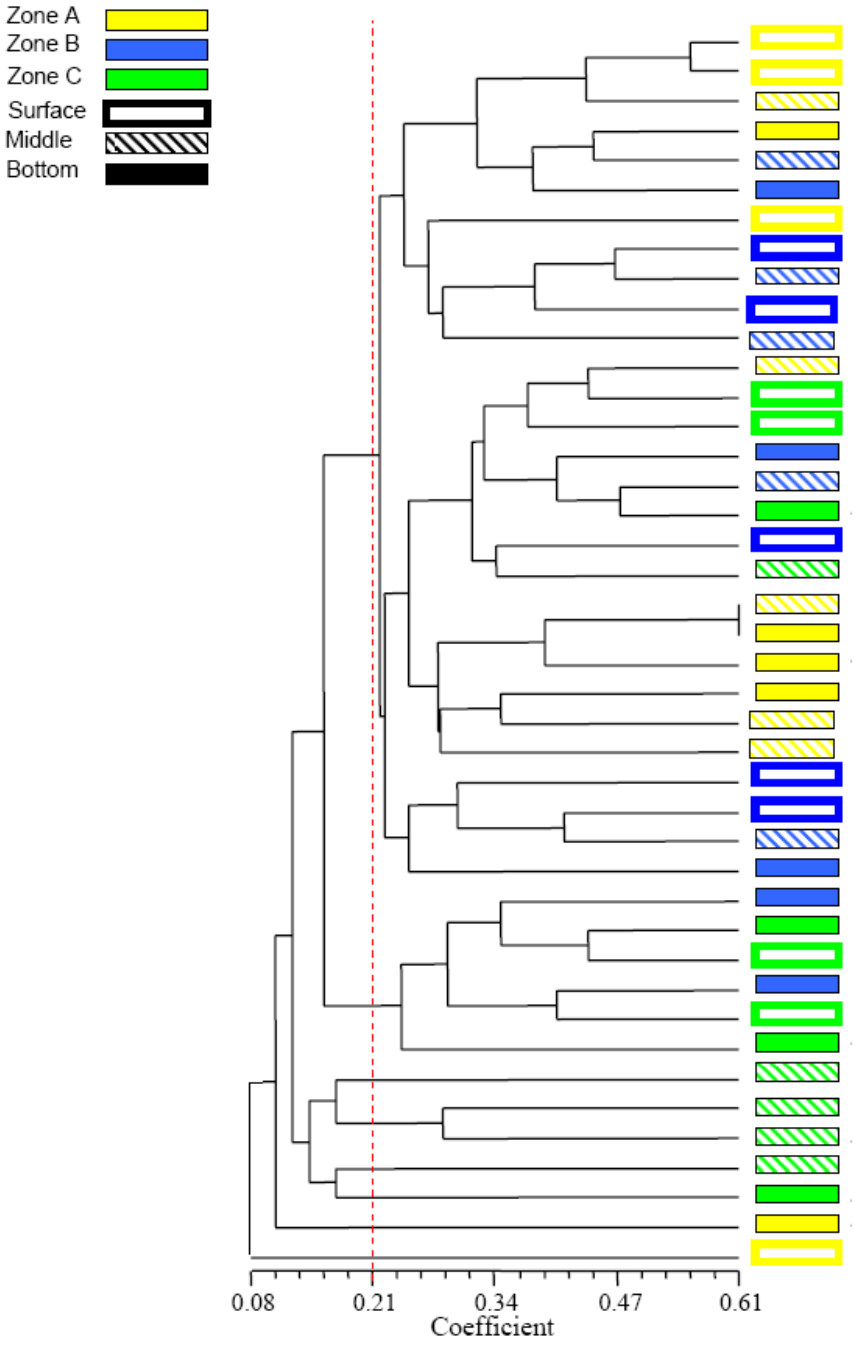


Fig. 3.11. Comparison of total Eubacterial community structures from August 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity Coefficient and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

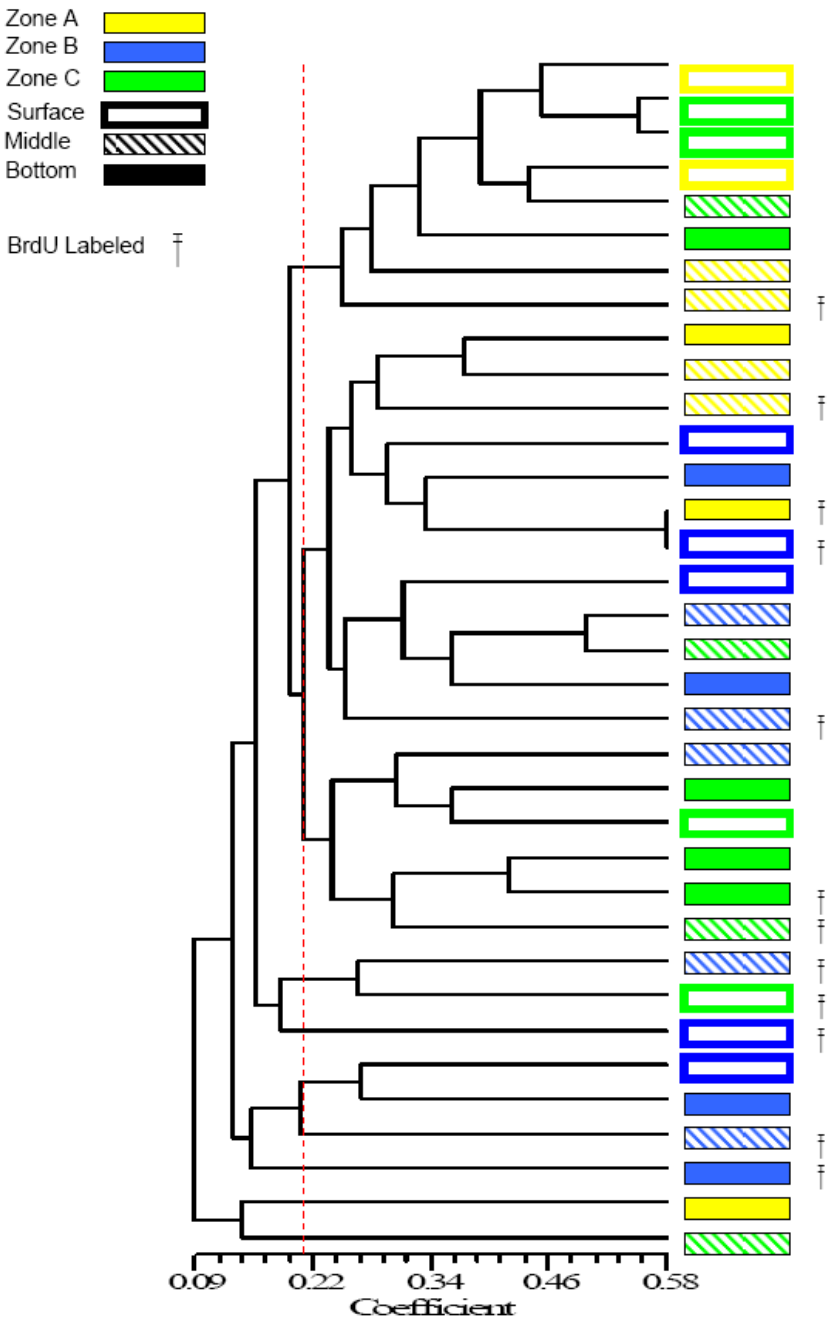


Fig. 3.12. Comparison of total Eubacterial community structure and active subset of community from March 2005. Total and BrdU-labeled (†) eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

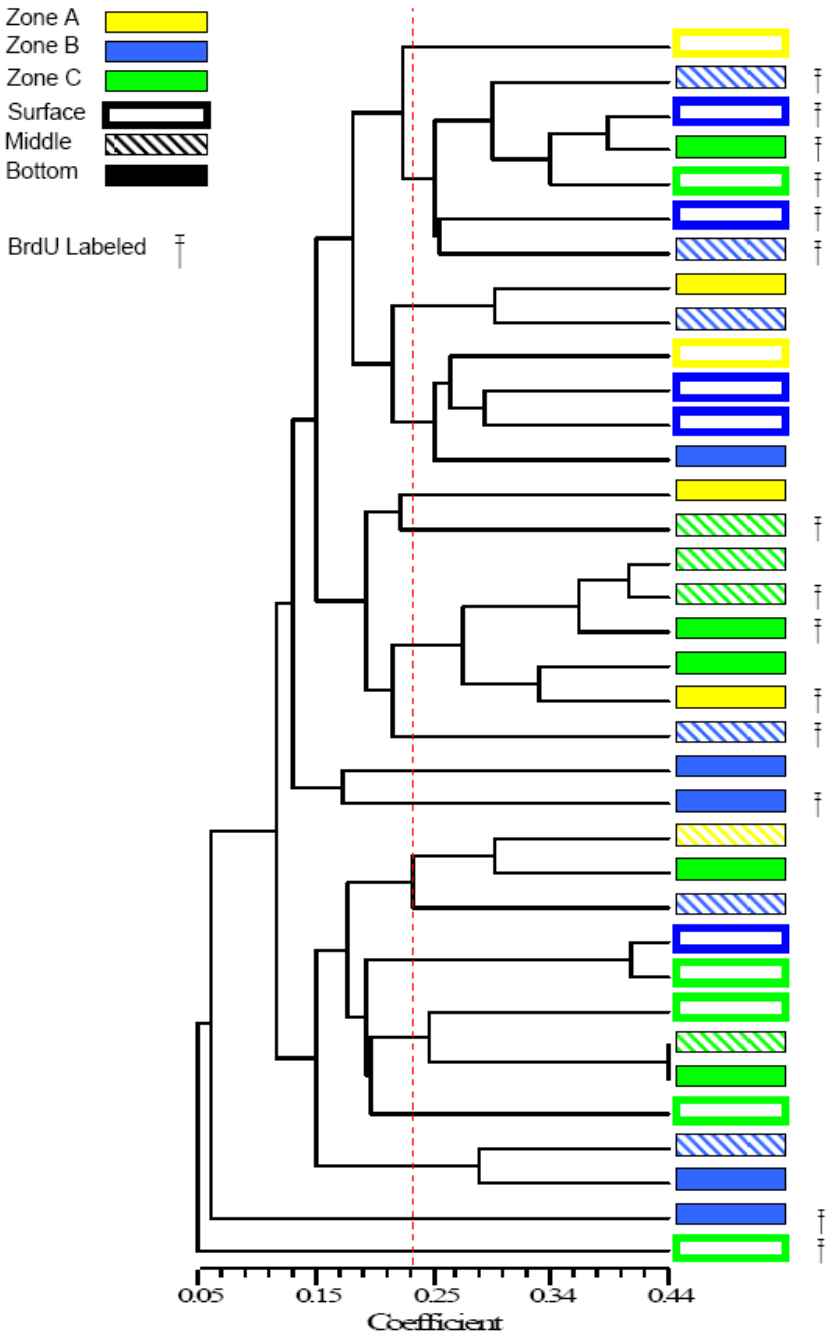


Fig. 3.13. Comparison of total Eubacterial community structure and active subset of community from May 2005. Total and BrdU-labeled (†) eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

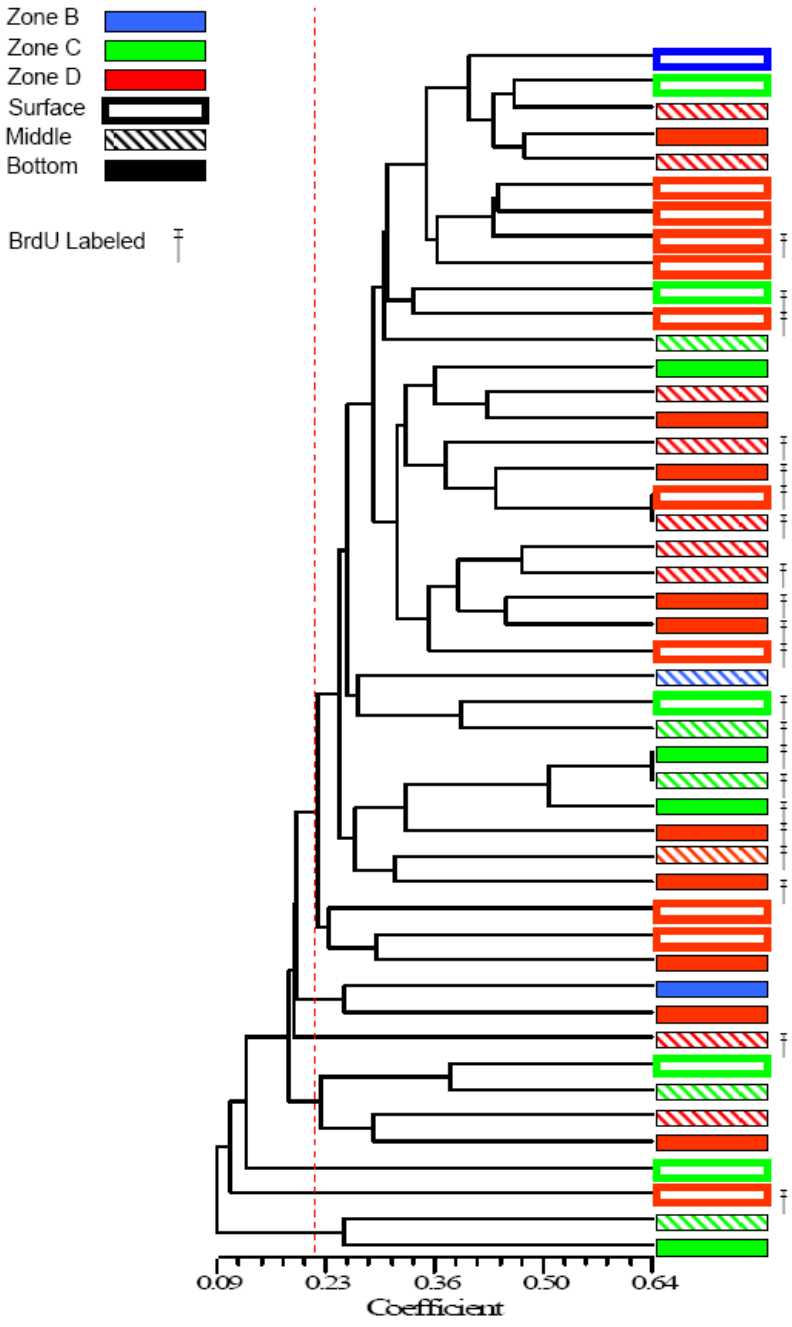


Fig. 3.14. Comparison of total Eubacterial community structure and active subset of community from July 2005. Total and BrdU-labeled (†) eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

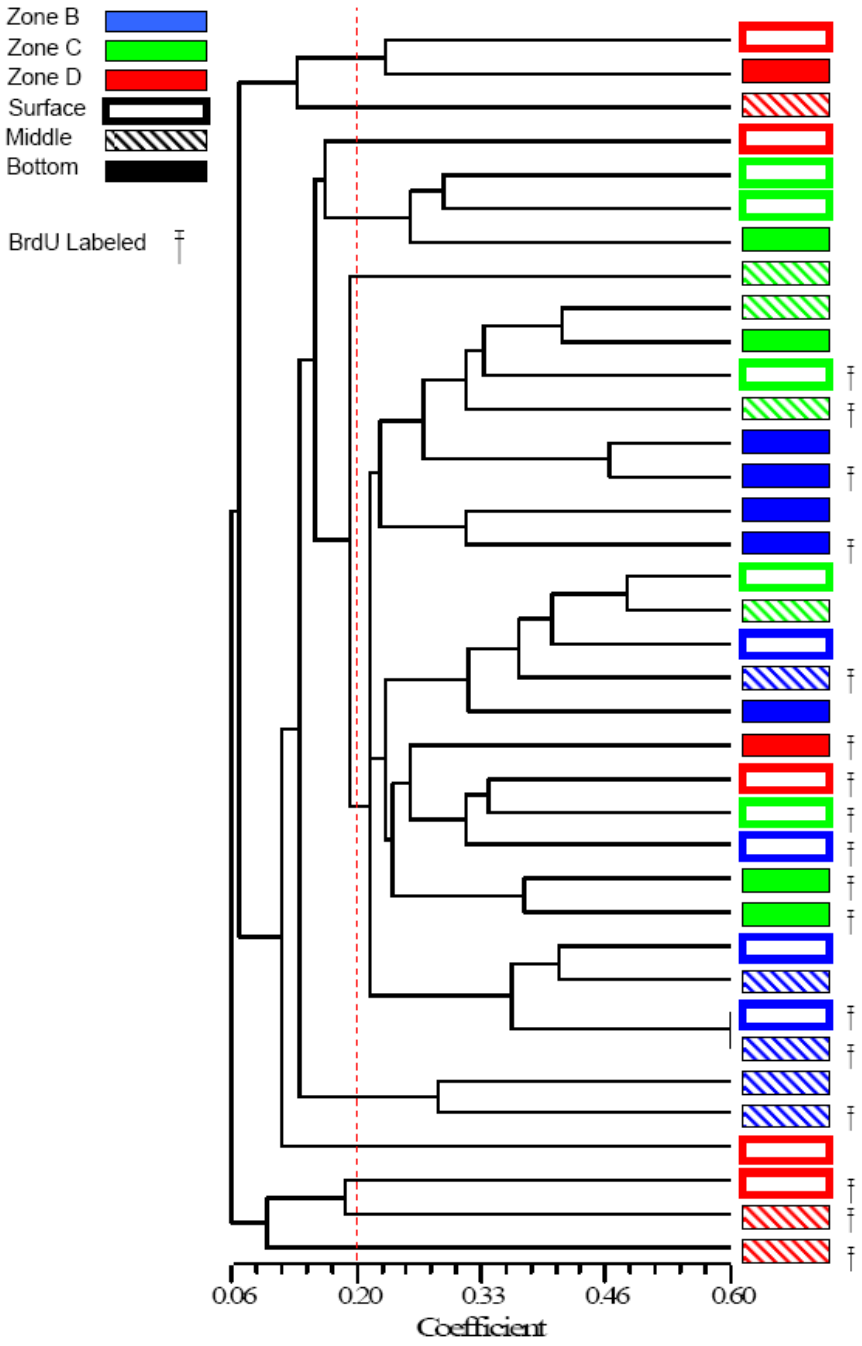


Fig. 3.15. Comparison of total Eubacterial community structure and active subset of community from August 2005. Total and BrdU-labeled (†) eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. A Jaccard's Coefficient of 1.0 is 100% similar, a coefficient of 0 is the most dissimilar. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone.

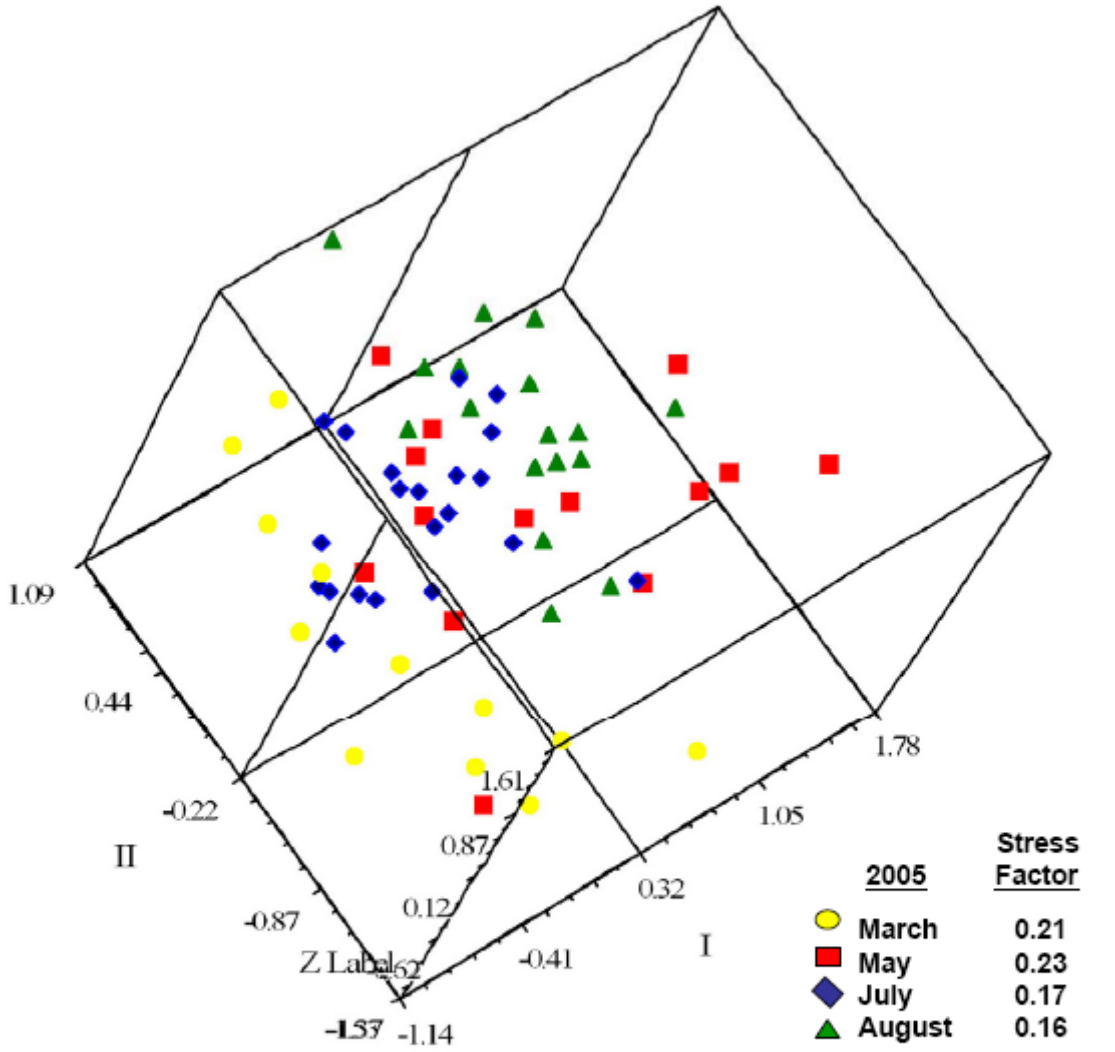


Fig 3.16. 3-dimensional multidimensional scaling (MDS) plot depicting the similarity of all BrdU-labeled communities. Communities are grouped by samples collected in March (yellow), May (red), July (blue) and August (green) 2005. MDS plot was generated from Jaccard coefficient of similarity of ARISA derived community fingerprints by NTSYSpc 2.2. Stress Factor ranges from 0 to 1, with the closeness of proximities of samples shown by smaller values.

might suggest that Zone B could be considered a transitional Zone between the two distinct communities within Zones A and C, with no direct influence on the community structure other than interaction with the connecting Zones. Also during 2004 the surface community at site 02a was never clustered with other communities in Zone A and was the least similar community to all other Zones. Site 02a is however the closest site to Barataria Bay (half-way between the Mississippi River Delta and Terrebonne Bay) and could be affected by its proximity to another freshwater source.

In March 2005 (Fig. 3.12), fingerprints from Zones A and B again cluster together. While some of the samples from Zone C are also clustered with samples from A, there is also a subset of samples from Zone C that are clustered apart from A and B. The intensity of clustering of Zone C increased in May 2005 (Fig. 3.13) with two distinct clusters of Zone C with Zones A and B spread throughout. In July 2005 (Fig. 3.14), the majority of samples from Zone D and Zone C clustered independently of each other. During August 2005 (Fig. 3.15) Zone C again clusters together, but also groups with a subset of samples from Zone B while Zone D clusters apart from the other samples.

Within these clustered groups, communities from the same depth were the majority of the time more closely related. However, this trend is only seen within clusters of communities. This suggests that, overall; the clustering of communities is more dependent on the location across the shelf than the location within the water column. The prominent separation of communities between Zones A and C could be due to the unique riverine signals from the Mississippi and Atchafalaya Rivers. Distinct chemical signals were determined around each river, as seen in separation of nutrients within bottom waters of Zones A and C, supporting previous work by Pakulski and colleagues (2000). The Mississippi River plume primarily impacts Zones A and B, while the Atchafalaya River plume effects Zone C. These riverine waters remain separate and the plumes off shore do not typically mix due to the western current during the summer (Hetland and DiMarco *In review*).

During the summer of 2005, samples were also inoculated with BrdU for the analysis of the active Eubacterial community fraction of the total community. In March

2005 (Fig. 3.12) the BrdU-labeled active community clusters throughout the samples. However, during May through August 2005 (Fig. 3.13, 3.14 and 3.15) the active communities become more closely clustered together and independent of the total community. The clustering of active communities is more prominent than that of the Zones, suggesting that the active communities are much more distinct from the total communities.

For more detailed comparisons of active Eubacterial communities, Jaccard coefficient similarity matrices were used to generate a 3-dimensional multidimensional scaling (MDS) plot of active community profiles from 2005 (Fig 3.16). MDS plots provide visual representations of patterns and trends of dissimilarities and similarities of the Eubacterial communities. While the axis and orientation of the graph are arbitrary, the distance between points reveals the relatedness of two communities; therefore the closer points are more similar. The MDS plot, with a overall stress of 0.19, depicts a good representation of relatedness between the communities, as defined by Kruskal (1964). When all of the active communities from 2005 are compared to each other a clear trend can be seen from March to August. In March and May 2005, the active communities are distinct from each other, but relatively broader in relation to themselves. However, the active communities in July and August 2005 are much more closely related and significantly narrow the degree of dissimilarity within each other. Active Eubacterial communities, as labeled by BrdU, became more closely clustered as summer 2005 progressed.

An ANOVA also suggested that the main factor, sampling date, was a significant (date: $F_{3, 62} = 5.595$, $p < 0.05$) factor on the similarity of the active and total bacterial community at the same site (Table E.1). However, Post Hoc analysis revealed that the only significant difference of the mean similarity between the total and BrdU-labeled community at each site was during May 2005 (March 2005 = 0.211, May 2005 = 0.129, July 2005 = 0.267, and August 2005 = 0.268).

Eubacterial Community Composition

The total bacterial communities from 2002bs, 5012as and 5012 were selected for the clone library to determine the species affiliation of dominant OTUs present in at least 25% of all Eubacterial communities. The 16S rDNA was successfully sequenced from 23 of 49 colonies. Due to few sequences from this clone library, it is suggested that screening using ARISA be done prior to sequencing and possibly that more antibiotics be added to the LB plates to minimize false-positive clones from growing. Fig. 3.17 and Table 3.2 show the putative phyla, with the closest related organism and accession numbers, aligned with the identified peak. Phylogenetic alignment placed sequences within many lineages of Eubacteria including α– and γ–*Proteobacteria*, Actinobacteria, *Synechococcus*, *Prochlorococcus* and Cytophaga/Flexibacter/Bacteroides (CFB). One peak (ARISA length 880) corresponded with both α – and γ - *Proteobacteria,* both of which are dominant throughout marine systems (Urbach et al. 1999). Another peak (ARISA length 634) corresponded with two phylogenetically distinct organisms within *Synechococcus* and Actinobacteria. When including clone libraries from previous studies, more OTUs were discovered that were shared between two phylogenetically distinct organisms. γ - *Proteobacteria* (Schwalbach et al. 2005) and CFB both were affiliated with and 785bp OTU.

However, other affiliations of an OTU 1050 bp with *Synechococcus* determined in this study supported previous work (Brown et al. 2005; Schwalbach et al. 2005). The total bacterial communities from 5007cb, 1016ab and 7017db were selected for the clone library to determine the species affiliation of rarer OTUs present in fewer than 25% of all Eubacterial communities. The 16S rDNA was successfully sequenced from 16 of 48 colonies. Fig. 3.18 and Table 3.3 show the putative phyla, with the closest related organism and accession numbers, aligned with the identified peak. Phylogenetic alignment placed sequences within many lineages of Eubacteria including α– and γ–*Proteobacteria*, *Actinobacteria*, *Synechococcus* and *Prochlorococcus*. One peak (ARISA length 1200 bp) corresponded with two phylogenetically distinct organisms within *Prochlorococcus* and α – *Proteobacteria*. Schwalbach and colleagues (2005)

Table 3.2. Closest match of ARISA derived dominant OTUs. 16s rDNA sequences from clone library of sites with dominant OTUs (2002bs, 5012as and 5012am; Fig. 3.3) were affiliated with a putative phylum from the closest match using BLAST search tool (Genbank).

ARISA length	Closest match by BLAST	Accession	% similarity	Putative Phylum
601	*Bacteroidetes bacterium*	AY509315.1	85	CFB
607	Actinobacterium	AY922105.1	93	Actinobacteria
613	*Actinomycetales bacterium*	AY370630.1	97	Actinobacteria
625	Actinobacterium	AY712522.1	99	Actinobacteria
634	*Synechococcus sp.*	AY172825.1	99	Cyanobacteria
634	*Actinomycetales bacterium*	AY370630.1	99	Actinobacteria
695	*Prochlorococcus sp.*	AY712385.1	99	Cyanobacteria
720	*Synechococcus sp.*	AF448060.1	99	Cyanobacteria
785	*γ - Proteobacterium*	AY830033.1	97	γ - Proteobacteria
880	*Paracoccus sp.*	DQ421707.1	99	α - Proteobacteria
880	*Legionella sp.*	AY924070.1	99	α - Proteobacteria
885	*Brevundimonas sp.*	AY526714.1	99	α - Proteobacteria
930	*Acinetobacter johnsonii*	DQ257426.1	99	γ - Proteobacteria
960	*α - Proteobacterium*	AY921960.1	97	α - Proteobacteria
995	*Synechococcus sp.*	AY172826.1	99	Cyanobacteria
1000	*Synechococcus sp.*	AY663929.1	99	Cyanobacteria
1030	*Prochlorococcus sp.*	AY712385.1	99	Cyanobacteria
1050	*Synechococcus sp.*	CP000110.1	99	Cyanobacteria
1070	*Synechococcus sp.*	AY663929.1	100	Cyanobacteria

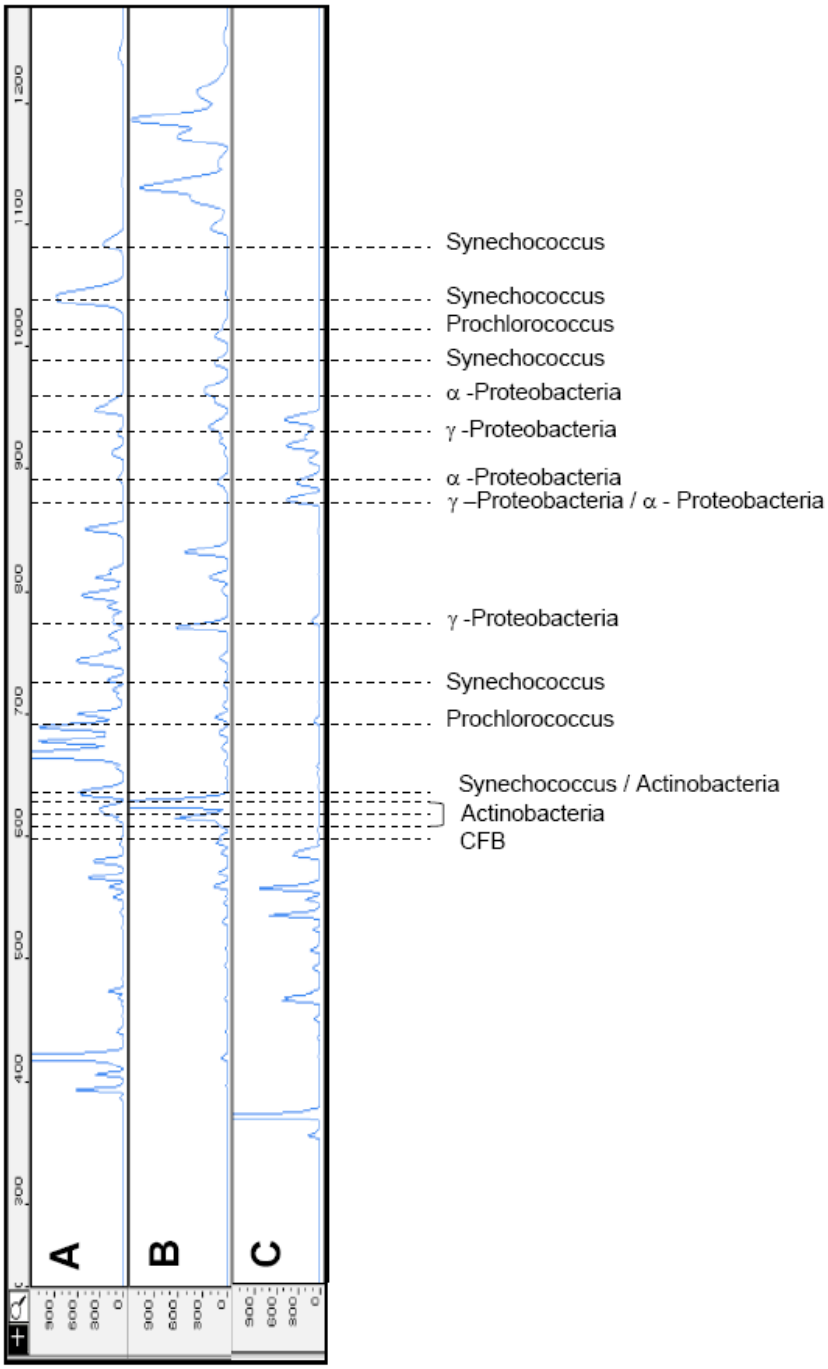


Fig 3.17. Putative phylum affiliations of dominant OTUs. 16S-ITS clone library was generated with pooled PCR products of (A) 2002bs (B) 5012as and (C) 5012am (16S rDNA 27F and unlabeled 23S rDNA 125R primers) inserted into pCR8/GW/TOPO vector (Invitrogen). Purified and sequenced (Agencourt Bioscience, primer 16s rDNA 27F) vector inserts were phylogenetically affiliated using BLAST (GenBank). Putative phyla were affiliated with ARISA peaks by dereplication of clone inserts. Clone inserts were reamplified (using 16S rDNA1392F and TET-Labeled 23S rDNA 125R primers),5 ng mL-1 normalized and run on an ABI 377xl automated sequencer to determine the ARISA base pair length and then associated with the putative phyla.

Table 3.3. Closest match of ARISA derived rare OTUs. 16s rDNA sequences from clone library of sites with rare OTUs (7017dm, 1016ab and 5007cb; Fig. 3.4) were affiliated with a putative phylum from the closest match using BLAST search tool (Genbank).

ARISA length	Closest match by BLAST	Accession	% similarity	Putative Phylum
361	*Actinomycetales bacterium*	AY370630.1	97	Actinobacteria
421	*γ - Proteobacterium*	AY663906.1	97	γ - Proteobacteria
460	Actinobacterium	DQ070787.1	99	Actinobacteria
568	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
628	*Actinomycetales bacterium*	AY370630.1	97	Actinobacteria
685	*α - Proteobacterium*	AB235387.1	98	α - Proteobacteria
740	*α - Proteobacterium*	AB235387.1	99	α - Proteobacteria
825	*Synechococcus sp.*	CP000110.1	99	Cyanobacteria
890	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
1070	*α - Proteobacterium*	AB235387.1	98	α - Proteobacteria
1100	*Synechococcus sp.*	AY664041.1	100	Cyanobacteria
1200	*Prochlorococcus sp.*	AY712368.1	99	Cyanobacteria
1200	*α - Proteobacterium*	AY664073.1	99	α - Proteobacteria

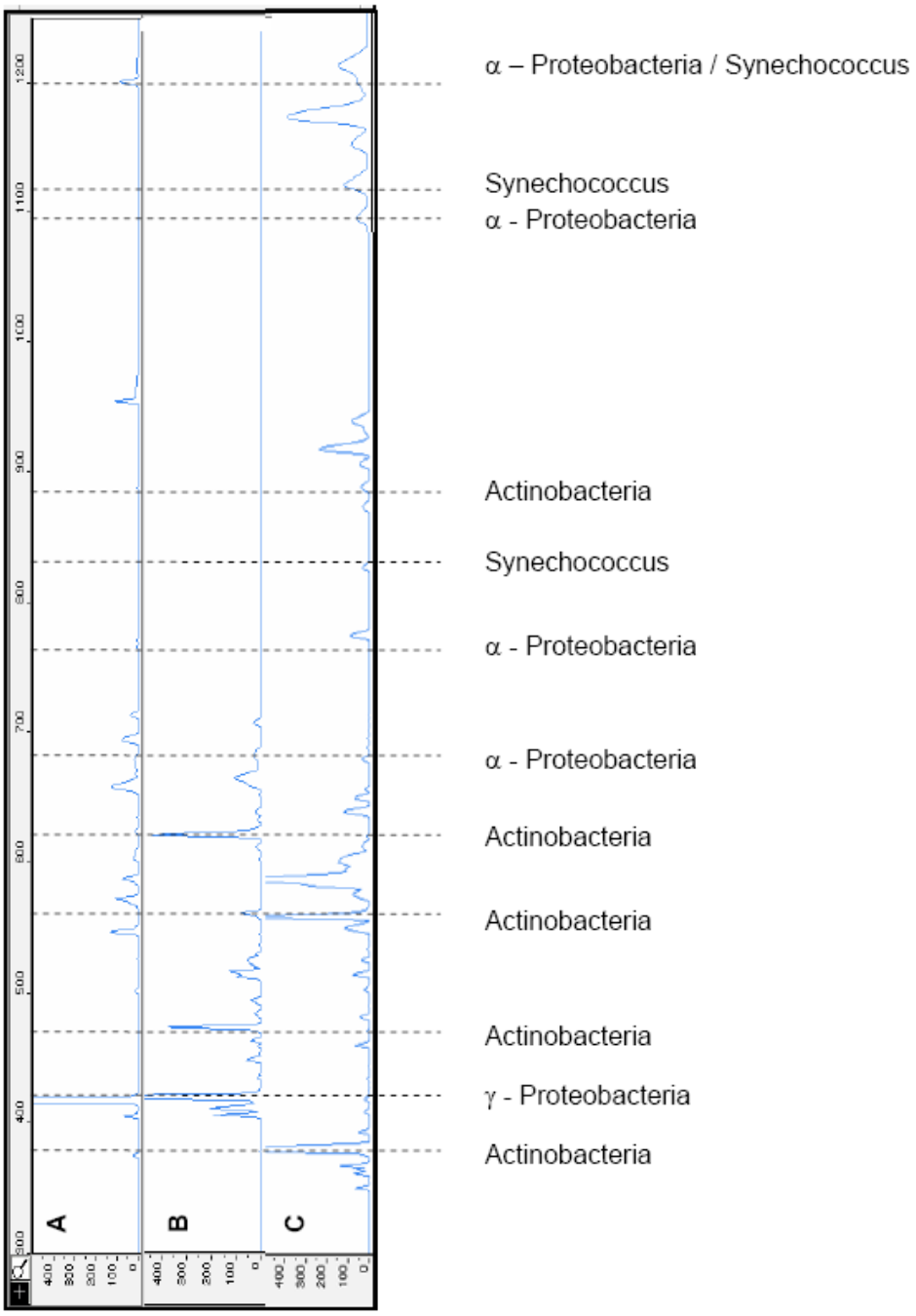


Fig 3.18. Putative phylum affiliations of rare OTUs. 16S-ITS clone library was generated with pooled PCR products of (A) 7017dm (B) 1016ab and (C) 5007cb (16S rDNA 27F and unlabeled 23S rDNA 125R primers) inserted into pCR8/GW/TOPO vector (Invitrogen). Purified and sequenced (Agencourt Bioscience, primer 16s rDNA 27F) vector inserts were phylogenetically affiliated using BLAST (GenBank). Putative phyla were affiliated with ARISA peaks by dereplication of clone inserts. Clone inserts were reamplified (using 16S rDNA1392F and TET-Labeled 23S rDNA 125R primers),5 ng mL-1 normalized and run on an ABI 377xl automated sequencer to determine the ARISA base pair length and then associated with the putative phyla.

also affiliated *Synechococcus* with the same ARISA determined OTU (length 1000 bp). Actinobacteria, though seen with in both clone libraries, appeared more often within the clone library of rarer OTUs. Actinobacteria are both diverse and dominant within marine sediments (Maldonado et al. 2005) and have been found in the pelagic system in lesser abundances (Urbach et al. 1999; Rappe et al. 2000). This suggests that the pelagic communities composed of Actinobacteria, could be influenced by sediment runoff and resuspension from the seafloor.

Between both clone libraries, only one OTU (601 bp in the dominant clone library) was phylogenetically affiliated with CFB. While Urbach and colleagues (1999) have determined that CFBs are not dominant within marine systems, others have raised the possibility that clone libraries and PCR amplification analysis might underestimate the presence of CFBs due to weak binding with universal primers (Cottrell and Kirchman 2000). Their finding must therefore be taken into consideration when determining the composition, and possible underestimation of CFBs, of the microbial communities in this study. Another consideration that must be accounted for is the possible overestimation of *Synechococcus* and *Prochlorococcus*, as both possess multiple ITS regions (Palenik et al. 2003)

ARISA has also been used as a quantitative tool to determine the relative abundance of species within a community based upon the amount of fluorescence measured for each labeled operational taxonomic unit (Hewson et al. 2003; OTU; Hewson and Fuhrman 2004; Schwalbach et al. 2004). While Brown (2005) even correlated *Prochlorococcus* abundances with fluorescence, only 1 OTU was used in the analysis, questioning the applicability to other OTUs and taxa. Yannarell and colleagues (2003) have questioned the quantitative ability of ARISA due to reports by others on the potential for PCR bias and the alteration of PCR product ratios (Suzuki et al. 1998). For these reasons, we did not implement ARISA as a quantitative tool for species, but rather for the relative presence of OTUs and taxa within the community.

SUMMARY

The Texas-Louisiana Shelf had a mean bacterial abundance of 1.72×10^6 cells mL^{-1} (Zone A: 1.89×10^6 cells mL^{-1}, Zone B: 1.85×10^6 cells mL^{-1}, Zone C: 1.72×10^6 cells mL^{-1}, Zone D: 1.38×10^6 cells mL^{-1}). While the bacterial abundance did not significantly differ between Zones along the shelf, the largest values were nearest the Mississippi River delta in Zone A. However, bacterial abundance did differ significantly with depth and also by the sampling month during the summer. Higher bacterial abundances were found in surface and bottom waters during later months of the summer (July and August). Eubacterial communities varied across the eastern Texas-Louisiana Shelf in both composition and also community richness. Eubacterial communities of Zones A and C were rarely similar to each other and clustered independently of the other. However, communities of Zone B often clustered with communities within Zones A and C, suggesting that Zone B is a transitional Zone between Zones A and C. Eubacterial communities within Zone D clustered independently. Hydrographical data suggests that the Mississippi River plume influences Zones A&B, while the Atchafalaya River impacts Zone C. The river plumes from these rivers remain separate on the shelf and appears to establish two distinct bacterial communities. Taking into consideration the location of the shelf to the Mississippi and Atchafalaya Rivers, this suggests fluvial source strongly influences the bacterial community structure. Eubacterial communities grouped more closely by location on the shelf rather than by location within the water column, suggesting influences on Eubacterial community structure affects the whole water-column. Community composition was dominated by *Synechococcus* and *Prochlorococcus*, and *Proteobacteria,* with rarer occurrences of Actinobacteria and CFB. With the addition of BrdU in 2005, active communities could be isolated from the total Eubacterial community. Active communities became more closely related to each other than to the total Eubacterial community during summer 2005.

CHAPTER IV

MARINE BACTERIAL COMMUNITY VARIATION WITH ENVIRONMENTAL DEVELOPMENTS

OBJECTIVE

Hypoxia, defined at dissolved oxygen concentrations less than 1.4 mL L^{-1}, is a seasonal reoccurrence on the Texas-Louisiana Shelf. The development of hypoxia is due in part to eutrophication of the continental shelf by freshwater inputs of the Mississippi and Atchafalaya Rivers. This work further studies the phylogenic relationships of the bacterial community with environmental dynamics surrounding dissolved oxygen and salinity concentrations on the Texas-Louisiana Shelf.

MATERIALS AND METHODS

Measurement of Environmental Parameters

Environmental parameters of the sampling site were obtained through a combination of electronic, chemical, and physical measurements of the water column and water samples. Discrete water column samples collected using a 12 Niskin bottle rosette. Salinity was calculated using the Practical Salinity Scale (PSS) from the conductivity of the water using a salinometer (Guildline Conductive). Dissolved Oxygen concentrations were obtained through automated potentiometric titration using a titroprocessor (Brinkman model 682) and then calibrated with Winkler Titration. A 6-channel Autoanalyzer-II system (Technicon) was configured for automated analyses of nitrate, nitrite, phosphate, silicate, ammonium, and urea. Profiles of temperature, photo-synthetically available radiation (PAR), dissolved oxygen concentration, salinity, light transmission, optical backscatter and fluorescence measurements were made using a Seabird SBE-9 CTD package and SBE-43 oxygen probe, a transmissometer (SeaTech) LSS and flourometer.

Statistical Analysis

Community fingerprints and bacterial abundances used in the statistical analyses were collected and processed as previously described in the Methodology and Materials section of Chapter III.

Un-weighted pair-group mean average (UPMGA) cluster analysis was conducted from the matrix generated from the comparison of bacterial communities analyzed by ARISA using NTSYSpc 2.2 software program (Rohlf 2000). This matrix was generated by comparing communities based upon the presence or absence of OTUs using the Jaccard coefficient of similarity (S_j);

$$S_j = \frac{W}{(a_1 + a_2) - W},$$

where W is the number of shared OTUs between two communities and a_1 and a_2 are the total number of OTUs within the respective communities. This comparison allows for a relative determination of the degree of similarity between groups of bacterial communities. To determine if a relationship exists between bacterial abundance, community richness and the environmental parameters (salinity, nutrients and dissolved oxygen), correlation analysis and regression analysis was conducted using SPSS (SPSS Inc, Release 13.0).

Clone Library and Sequencing

Communities (7008cb, 4011bb and 5011bb) (Fig 4.1) chosen for their complex and unique structure in conjunction with the sample site also being hypoxic (dissolved oxygen concentration < 1.4 mL L^{-1}) were cloned and sequenced for the identification of bacterial species within the community. Fresh 20 μL reactions using bacteria specific 16S primer 27F (5'-GTTGATCCTGGCTCAG-3')(Hugenholtz and Goebel 2001) and unlabeled 23S primer (23S-125R (5'–GGGTT[C/G/T]CCCCATTC [A/G] G-3') after (Hewson et al. 2003)) were prepared containing final concentrations of 1x PCR buffer

(Qiagen), 2.5 mM $MgCl_2$, 200 μM of each dNTP (Qiagen), 200nM of each primer, 40 ng μl^{-1} BSA, 2.5 U *Taq* DNA polymerase (Qiagen) and 1-10 ng μL^{-1} template DNA. Reaction mixtures were held at 94 ^oC for 2 min followed by 35 cycles of amplification at 94 ^oC for 40 s, 56 ^oC for 40 s and 72 ^oC for 90 s, with a final extension step of 72 ^oC for 5 minutes. PCR products were pooled together from communities for two libraries consisting of dominant and rarer OTUs. Pooled PCR products were then inserted into the pCR8/GW/TOPO vector using the TA Cloning kit (Invitrogen) and clones containing the plasmid were selected for by plating on LB plates containing 100 μg mL^{-1} spectinomycin. Positive clones were picked and lysed in ddH_2O at 92 ^oC for 5 min, following Kilger et al. (1997), and dereplicated under the above PCR conditions, purified using DNAce Strip Kit with sephadex columns (Bioline), quantified with SybrGold fluorescence on a SpectraMax Gemini EM spectrofluorometer (Molecular Devices), and diluted to 5 ng μL^{-1} for ARISA analysis as detailed in Chapter III. Multiple OTUs found within each clone are from the *E. coli* genome, while the unique OTU is attributed to the inserted sequence. This allowed sequence putative phylum affiliations to be aligned with specific OTUs and to identify communities' composition. Picked clones were also cultured at 37 ^oC for 12 hours in LB broth containing 100 μg mL^{-1}spectinomycin. Plasmids were purified and sequenced by Agencourt Bioscience Corporation using universal 16S primer 27F (5'-GTTGATCCTGGCTCAG-3') (Hugenholtz and Goebel 2001). Clones were phylogenetically affiliated using the online BLAST tool on the GenBank website (Altschul et al. 1990).

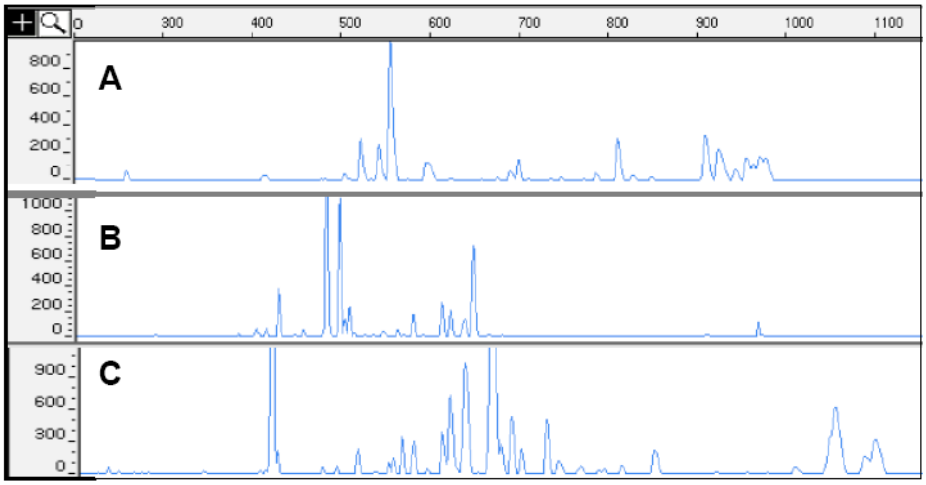


Fig 4.1. ARISA derived community fingerprints from hypoxic sites. 5 ng μL^{-1} normalized duplicate PCR products (using 16S rDNA1392F and 23S rDNA 6-FAM-labeled 125R primers) were pooled and run on an ABI 377xl automated sequencer from each of the samples (A) 7008cb (B) 4011bb and (C) 5011bb. Electropherograms were generated using GeneScan 3.1 software. Samples were chosen for 16S-ITS clone library to determine phylogenic affiliations of hypoxic communities.

RESULTS AND DISCUSSION

Hydrographic Developments on the Texas-Louisiana Shelf

Development of Hypoxia

To visualize the extent of bottom water hypoxia, contour plots were generated using Generic Mapping Tools (GMT; Wessel and Smith 1991) of calculated dissolved oxygen concentrations from 2004 (Fig B.2) and 2005 (Fig. B.3) (DiMarco *Unpublished*). Bottom water Dissolved Oxygen concentrations were not hypoxic during April 2004 (Fig. B.2). However, in both June and August 2004, bottom waters were hypoxic and sometimes anoxic (devoid of oxygen) in areas of water depths between 10 and 30m. In June 2004, hypoxic bottom waters were primarily found at stations more inshore. By August 2004, hypoxic bottom waters were further offshore and their distribution had become patchier across the shelf. Hypoxia was present during all samplings in 2005

(Fig. B.3). The spread of hypoxic bottom waters in March and May 2005 closely resembled that seen in June 2004 (Fig. B.2), with hypoxic waters located across the shelf from Zones A through C and primarily close in shore while oxygenated waters were further offshore. In July 2005, Zone C becomes completely oxic, and the only hypoxic waters detected were located throughout Zone D. This shift is mostly likely due to mixing of the water column and reintroduction of oxygen to bottom waters by the passing of Hurricane Cindy directly through Zone B just prior to the July sampling and to wave action caused by Hurricane Dennis in July 2005 (S.F. DiMarco personal communication). By August 2005, hypoxic and anoxic waters were distributed from Zone B through Zone D. During both summers, the largest amount of hypoxic bottom waters was found at the end of the summer in August.

Development of Salinity Gradients

Zone C was chosen for the analysis of bacterial communities with salinity gradients primarily due to its location directly off of the Atchafalaya Bay (Fig. 4.2). On Line C3 Eubacterial community samples were taken from sites 07C, 08C and 10C every sampling time, with the exception of site 10C in June 2004. This consistency and location allows for the analysis along a salinity gradient over the course of summers 2004 and 2005, establishing a temporal study of the Eubacterial community along a salinity gradient on a continental shelf. Salinity profiles were generated on the transect Line C3 from measured salinities and plotted using GMT (Wessel and Smith 1991) and PSS (Practical Salinity Scale; DiMarco *Unpublished*). In April 2004 surface salinities are low, developing a surface freshwater wedge and a highly stratified water column (Fig 4.3). During June and August 2004 the freshwater wedge was less distinct and intermediate salinity waters (between 29 and 33) became mixed throughout the water-column at depths less than 30m.

A larger freshwater wedge developed again in March 2005 (Fig. 4.4) stratifying the water column on transect C3. During May and July 2005 the freshwater wedge was more mixed. Intermediate salinity waters (between 29 and 33) were mixed throughout

the water-column at depths less than 20m. In August 2005, the water-column was weakly stratified with intermediate and strong salinity waters.

These variations in surface water salinities can be explained in the context of the flow rate and output of the Atchafalaya River. Discharge rates of the Atchafalaya River fluctuate throughout the year (USACE 2005). In 2004, the Atchafalaya River discharge rate remained near average (blue dashed line) with the exception of high flow in June/July and in December (Fig. C.1). The Atchafalaya River discharge was above average with peak flow during January through March of 2005 and then fluctuated below average rates (with the exception of September when Hurricane Katrina made landfall in Louisiana)(Fig. C.2). Pokryfki and Randall (1987) previously determined that there is a 1 month lag between peak discharge rate and minimum surface water salinity off of the Atchafalaya River. This delay is then translated into a two month

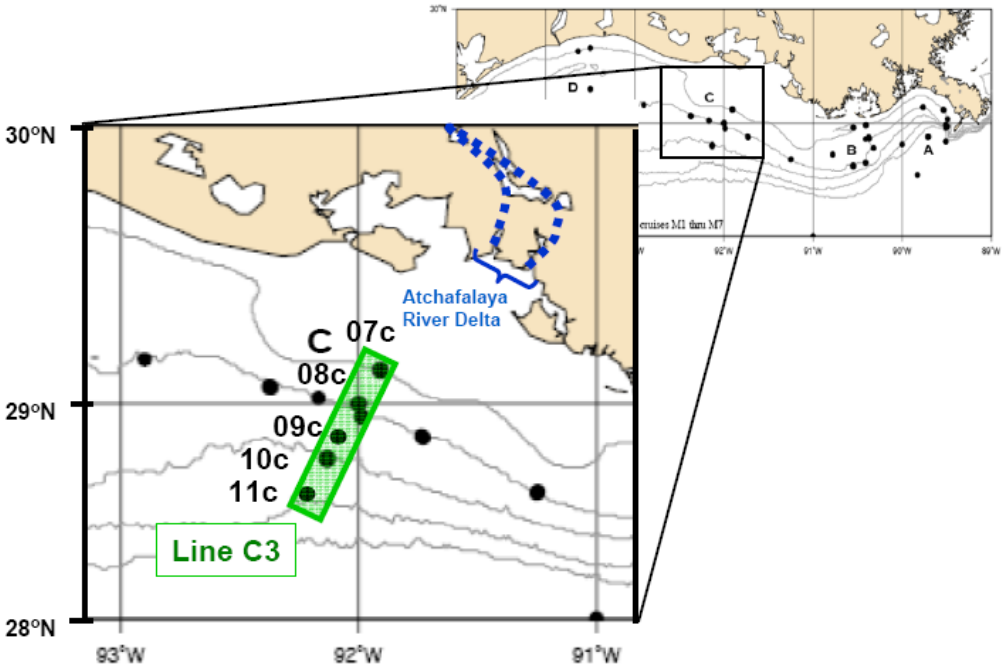


Fig. 4.2 Location of sampling line C3 in Zone C on the Texas-Louisiana Shelf. Line C3 is located directly off of the Atchafalaya River Delta. Contour lines represent the 10m, 20m, 30m, 40m and 50m isobaths. Salinity gradient profile plots (Fig 4.3 and Fig 4.4) were generated from sites 11c, 10c, 9c, 8c and 7c located along line C3.

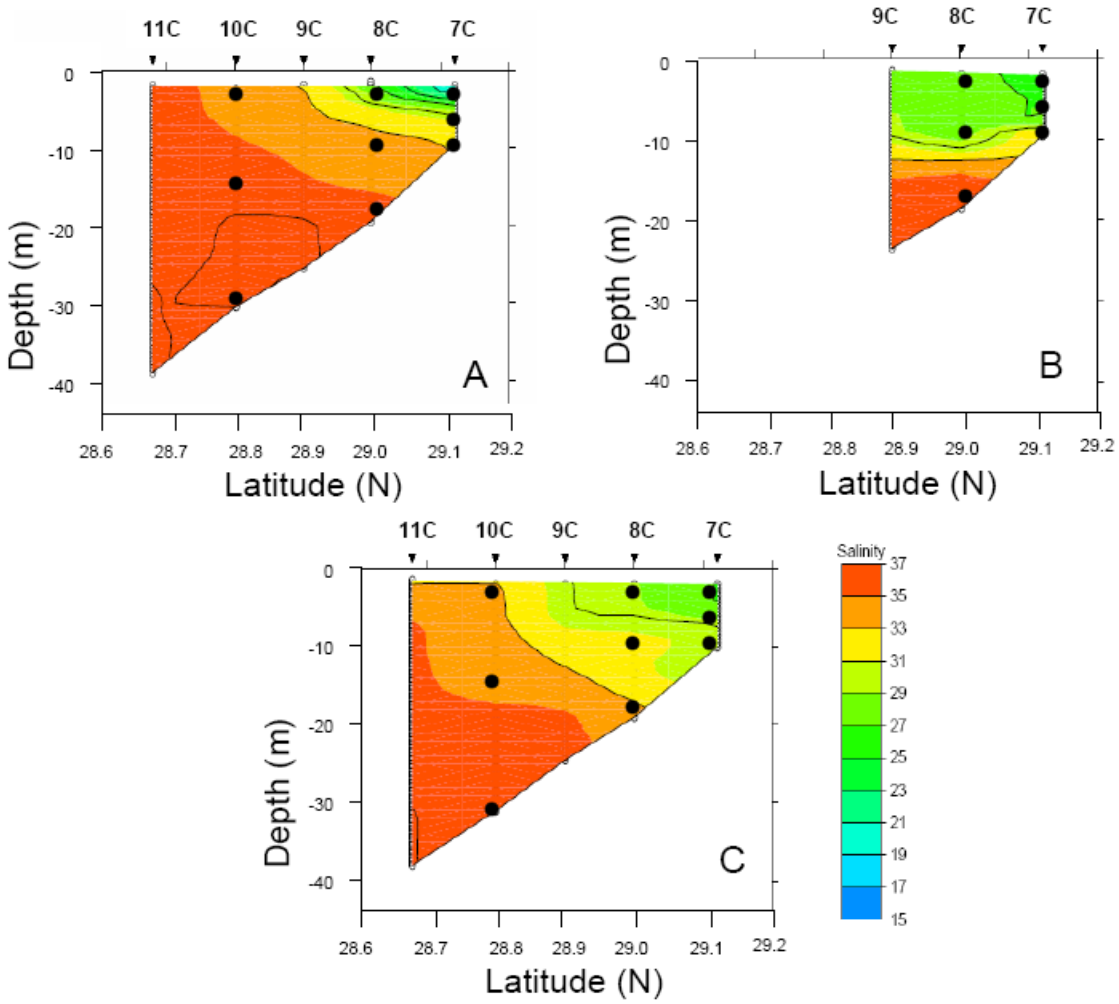


Fig 4.3. Salinity gradient profiles along line C3 during 2004. Profiles were generated from water column casts of sites 11c, 10c, 9c, 8c and 7c along line C3 (Fig. 4.4) during (A) April, (B) June and (C) August of 2004. Salinity samples were drawn at 5m intervals in the vertical profile. Salinity was calculated from the conductivity of the water using a salinometer (Guildline Conductive) and the Practical Salinity Scale. Closer isopycnals are representative of a highly stratified water column due to an increased fresh water wedge. Black points represent locations of microbial community sampling. *Modified from DiMarco Unpublished.*

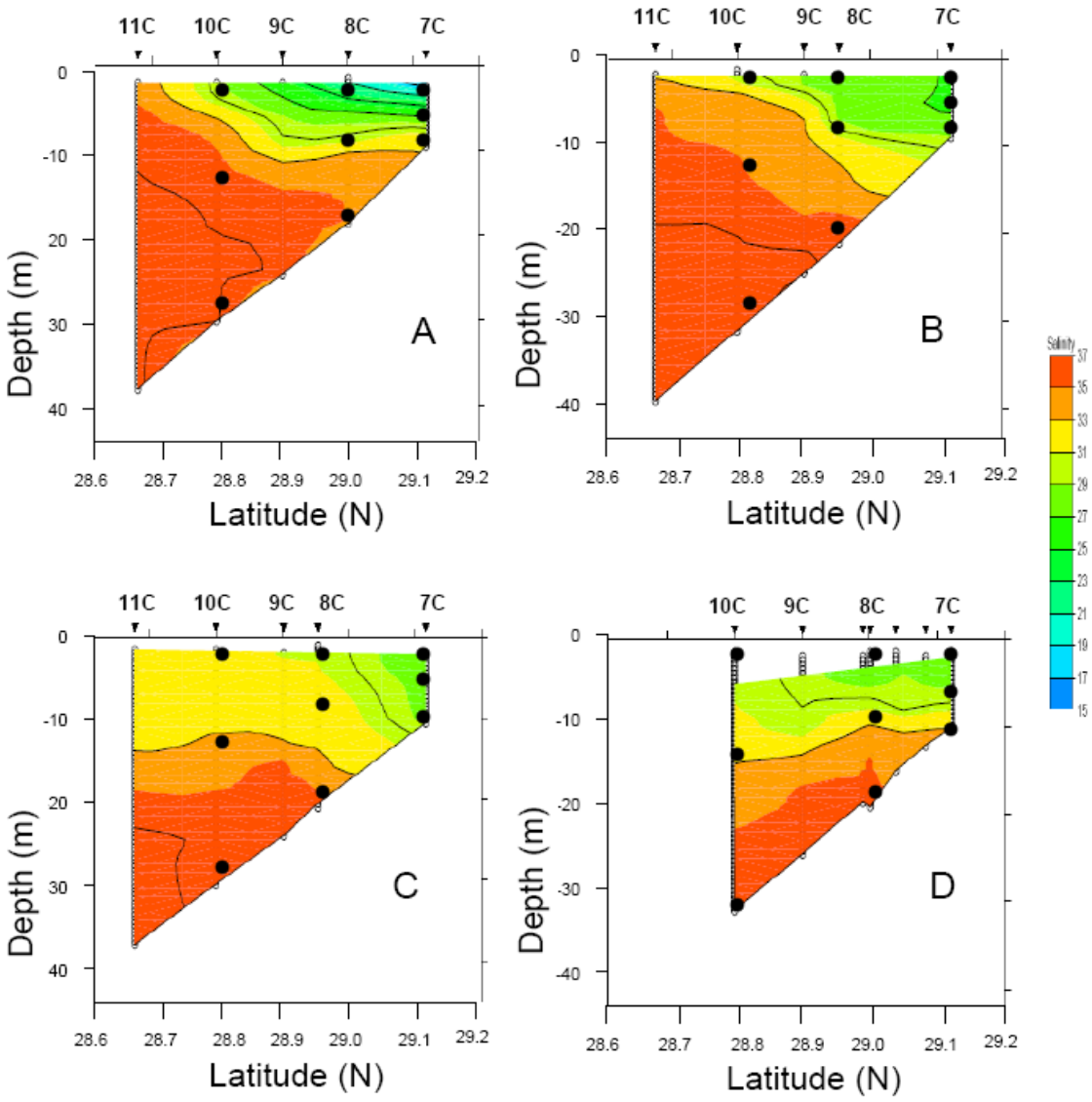


Fig 4.4. Salinity gradient profiles along line C3 during 2005. Profiles were generated from water column casts of sites 11c, 10c, 9c, 8c and 7c along line C3 (Fig. 4.4) during (A) March, (B) May, (C) July and (D) August of 2005. Salinity samples were drawn at 5m intervals in the vertical profile. Salinity was calculated from the conductivity of the water using a salinometer (Guildline Conductive) and the Practical Salinity Scale. Closer isopycnals are representative of a highly stratified water column due to an increased fresh water wedge. Black points represent locations of microbial community sampling. *Modified from DiMarco Unpublished.*

delay of peak discharge with the development of hypoxic bottom water off of both the Atchafalaya and Mississippi Rivers (Pokryfki and Randall 1987; Justic et al. 1996). This delay is seen in March 2005 with the early development of hypoxia (Fig. C.2).

Community Abundances with Environment

As previously discussed in Chapter III, bacterial abundance ranged from 2.06 x 10^5 to 6.98 x 10^6 cells mL^{-1}, with a mean abundance of 1.72 x 10^6 cells mL^{-1}(Fig. 3.5 and 3.6). Non-parametric Spearman's Rank Order correlation analysis revealed that bacterial abundance and the hydrological variables listed in Table 4.1 were significantly correlated ($p > 0.01$). Significance was conservatively determined at the 0.01 level since the data were not normally distributed. R ranges from +1 to -1 while zero signifies no correlation, an R value of +1 signifies a perfect direction correlation, while a -1 value is a perfect inverse correlation.

A negative correlation was seen with bacterial abundance and dissolved oxygen concentration, salinity, and % transmission. A one way model I analysis of variance, with dissolved oxygen concentration (hypoxic and not hypoxic) as the main factor was used to determine the effects dissolved oxygen concentration on bacterial abundance by comparing the means of the data sets. The ANOVA also suggested that the main factor, dissolved oxygen concentration, was significant ($F_{0, 186} = 13.572$, $p < 0.01$) on bacterial abundance. Bottom water-column non hypoxic samples had an average bacterial abundance of 2.6 x 10^6, while bottom-water column samples that were hypoxic had an average bacterial abundance of 1.6 x 10^6. Amon and Benner (1998) also found that bacterial abundance decreased with decreasing concentration of Dissolved Oxygen. A negative correlation of bacterial abundance and salinity (Table 4.1) suggests that as salinity decreases, bacterial abundances increase. These findings agree with other studies that determined higher bacterial abundances were found within intermediate salinities (Amon and Benner 1998; Pakulski et al. 2000; McManus et al. 2004). In those studies intermediate salinities were classified as 10-30, and no sites samples in this study

Table 4.1 Significant correlations of bacterial abundance vs. hydrological variables. Non-parametric Spearman's Rank Order correlation analysis was performed with bacterial abundance and the hydrographical variables listed with a significance level of 0.01 as the data were not normally distributed. N is the number in the sample set. A positive r signifies a direct correlation, while a negative r is an inverse correlation.

Variable	r	N	p-value
Temperature (°C)	0.24	186	0.001
Salinity (PSS)	-0.30	186	0.000
Dissolved oxygen concentration (mL L^{-1})	-0.30	186	0.001
% Transmission	-0.30	186	0.000
Fluorescence (μg *Chl a* L^{-1})	0.22	186	0.003
Optical Backscatter (mg particle L^{-1})	0.34	186	0.000
NO_2 + NO_3 (μmol L^{-1})	0.31	173	0.000
NO_3 (μmol L^{-1})	0.30	173	0.000
$HSiO_3$ (μmol L^{-1})	0.30	171	0.000

* Photosynthetically Available Radiation (PAR), HPO_4 (μmol L^{-1}), NH_4 (μmol L^{-1}), NO_2 (μmol L^{-1}) and Urea (μmol L^{-1}) were also analyzed and were not found to be significantly correlated with bacterial abundance.

fell below 10. A positive correlation was seen between bacterial abundance and fluorescence, optical backscatter, total nitrogen and $HSiO_3$. Optical backscatter measures particulate matter within the water column. As particulate matter suspension increases within the water column, the transmission of light decreases. Previous studies by Pakulski et al. (2000) and McManus et al. (2004) did not find any correlation between nutrient measurements and bacterial abundances. However, both projects have much smaller sample sets. During Pakulski's single sampling event, concentrations of nutrients did not vary across the shelf.

Specific interactions of these hydrological parameters were also correlated with the bacterial abundances at depth, as determined in Chapter III, bacterial abundances significantly vary with depth (Table. 4.2). Surface bacterial populations densities are influenced primarily by the particle load of the riverine output, which varies through the summer. However, the size of bottom water communities is hindered by the decrease of dissolved oxygen concentrations at depth.

Even though bacterial populations are not significantly variable in size between Zones, different hydrological parameters may influence the population size within each Zone (Table. 4.3). The size of bacterial communities within Zones A and B are highly connected with hydrological parameters, many stemming from the introduction of the Mississippi River onto the shelf. However, the only variable influencing the population size of Zone C is the sampling month, suggesting that this area is less impacted by the riverine effects. An explanation for this could be that community size within Zone C has relatively stronger influences, in comparison to Zones A and B, from offshore variables rather than from the Atchafalaya River. Abundances of communities within Zone D do not correlate with any of the hydrological variables; nevertheless, this is most likely due to the limited sampling of Zone D at the end of summer 2005.

Regression analysis was performed using variables that showed a significant correlation with bacterial abundance (listed in Tables 3.1 and 4.1) to determine their combined relationship with bacterial abundance. A significant linear relationship was found between the dependant variable bacteria abundance (A) and the independent variables salinity (S), % transmission (T), sampling depth (D) and dissolved oxygen concentration (O) using the stepwise regression analysis (n=195, adj. $r^2 = 0.231$, $p < 0.01$). The linear equation of this relationship is:

$$A = 5.3 \times 10^{6} - 9.6 \times 10^{3} [T] - 2.5 \times 10^{4} [D] - 5.1 \times 10^{4} [S] - 2.7 \times 10^{5} [O]$$

A significant linear relationship was also found between the dependant variable bacteria abundance (A) and the independent variable $HSiO_3$ using the stepwise

Table 4.2. Significant correlations of bacterial abundance with depth vs. hydrological variables. Bacterial abundances were separated into three groups for surface (~1m), middle (below pycnocline), and bottom waters (less than 1m above seafloor). Non-parametric Spearman's Rank Order correlation analysis was performed with bacterial abundance and the variables listed with a significance level of 0.01 as the data were not normally distributed. N is the number in the sample set. A positive r signifies a direct correlation, while a negative r is an inverse correlation.

SURFACE WATER	**r**	**N**	**p-value**
% Transmission	-0.38	64	0.002
Optical Backscatter (mg particle L^{-1})	0.46	64	0.000
Sampling Month	0.47	60	0.000
MIDDLE WATER			
% Transmission	-0.46	60	0.000
Salinity (PSS)	-0.42	60	0.001
Site Depth (m)	-0.40	62	0.001
Optical Backscatter (mg particle L^{-1})	-0.39	60	0.002
Depth (m)	-0.38	60	0.003
Fluorescence (μg *Chl a* L^{-1})	-0.04	60	0.000
BOTTOM WATER			
Site Depth (m)	-0.48	66	0.000
Dissolved oxygen concentration (mL L^{-1})	-0.43	61	0.001
Depth (m)	-0.41	61	0.001
Sampling Month	0.46	66	0.000

Table 4.3. Significant correlations of bacterial abundance by Zones vs. hydrological variables. Non-parametric Spearman's Rank Order correlation analysis was performed with bacterial abundance and the variables listed with a significance level of 0.01 as the data were not normally distributed. N is the number in the sample set. A positive r signifies a direct correlation, while a negative r is an inverse correlation. No correlations were found with Zone D.

ZONE A	**r**	**N**	**p-value**
% Transmission	-0.44	44	0.003
Site Depth (m)	-0.44	49	0.002
Salinity (PSS)	-0.42	44	0.004
Longitude	0.40	49	0.001
Temperature (^{o}C)	0.41	44	0.005
Latitude	0.48	49	0.004
Optical Backscatter (mg particle L^{-1})	0.53	44	0.000
Sampling Month	0.69	49	0.001
ZONE B			
Site Depth (m)	-0.40	62	0.001
% Transmission	-0.40	59	0.002
Salinity (PSS)	-0.35	59	0.006
Optical Backscatter (mg particle L^{-1})	0.37	59	0.003
Flourescence (ug *Chl a* L^{-1})	0.38	59	0.004
Latitude	0.51	62	0.000
Sampling Month	0.59	62	0.000
ZONE C			
Sampling Month	0.35	67	0.004

regression analysis (n=165, adj. $r^2 = 0.082$, $p < 0.01$). The linear equation of this relationship is:

$$A = 1.5 \times 10^{6} + 2.3 \times 10^{4} [HSiO_3]$$

Many of these relationships between physical and chemical parameters and bacterial abundances can be linked together through interactions with the phytoplankton populations. Phytoplankton abundances on the Texas-Louisiana Shelf are also the greatest at intermediate salinities (Lohrenz et al. 1990; Gardner et al. 1994; Pakulski et al. 2000; McManus et al. 2004). At lower salinities (less than 10), phytoplankton growth can be light limited due to high concentrations of suspended sediments within riverine waters. However, nutrients become more dilute as both salinity and distance from the riverine sources increase. Therefore, phytoplankton biomass reaches a maximum at intermediate salinities where both light and nutrients are available (Lohrenz et al. 1990; McManus et al. 2004). Even though enough nutrients and organic matter enter the shelf from the rivers to sustain bacterial growth (McManus et al. 2004), the bacterial communities on the Texas-Louisiana Shelf depend on phytoplankton production for organic matter rather than terrigenous sources (Gardner et al. 1994; Pakulski et al. 2000; McManus et al. 2004). This dependence links the microbial population to physical and chemical parameters that regulate the phytoplankton community.

Linking Community Structure with the Environment

Eubacterial Community Richness

Two-hundred and seventy-six unique community fingerprints (Total n = 212; BrdU n = 64) were discriminated by ARISA analysis of collected samples across the Texas-Louisiana Shelf. Nineteen of these communities were collected under hypoxic conditions. OTU species richness of total community fingerprints ranged from 10 to 76,

with an average richness value of 32 (Fig. 3.8). Hypoxic communities generally had lower species richness than total communities and ranged from 13 to 53 OTUs, with an average richness value of 33. A one-way model I analysis of variance, with dissolved oxygen concentration (hypoxic vs. not hypoxic) as the main factor was used to determine the effects dissolved oxygen concentration on species richness by comparing the means of the data sets. The ANOVA suggested that the main factor, dissolved oxygen concentration, was not significant ($p > 0.05$) on species richness and that community richness for all sites and hypoxic sites was not statistically different. Species richness is independent of environmental parameters. Non-parametric Spearman's Rank Order correlation analysis indicated that species richness was not significantly correlated with any of the hydrographical parameters (salinity, temperature, dissolved oxygen concentration, PAR, % transmission, fluorescence, and optical backscatter: $n = 202$, $p > 0.01$).

Hypoxic Eubacterial Community Structures

As described in Chapter III, ARISA generated community fingerprints were compared to one another using the Jaccard coefficient of similarity and UPMGA cluster analysis to examine relationships between the Eubacterial community structures and their relationships with hypoxia and salinity gradients. During April 2004 (Fig. 4.5), no sites sampled were hypoxic. Six sites were hypoxic in June 2004 (Fig. 4.6) and seven sites were hypoxic in August 2004 (Fig. 4.7).

In March 2005 (Fig. 4.8) only three sites were hypoxic and none of the communities were labeled with BrdU. Three communities in May 2005 (Fig. 4.9) were hypoxic, one of which was also BrdU-labeled. Only one community in July 2005 (Fig. 4.10) was hypoxic and the total and BrdU-labeled community was not closely related, even though it was a hypoxic site. . During August 2005 (Fig. 4.11) four communities were hypoxic and each was also BrdU-labeled. Three of the hypoxic communities were from Zone B and Total and BrdU-labeled hypoxic communities clustered closely with

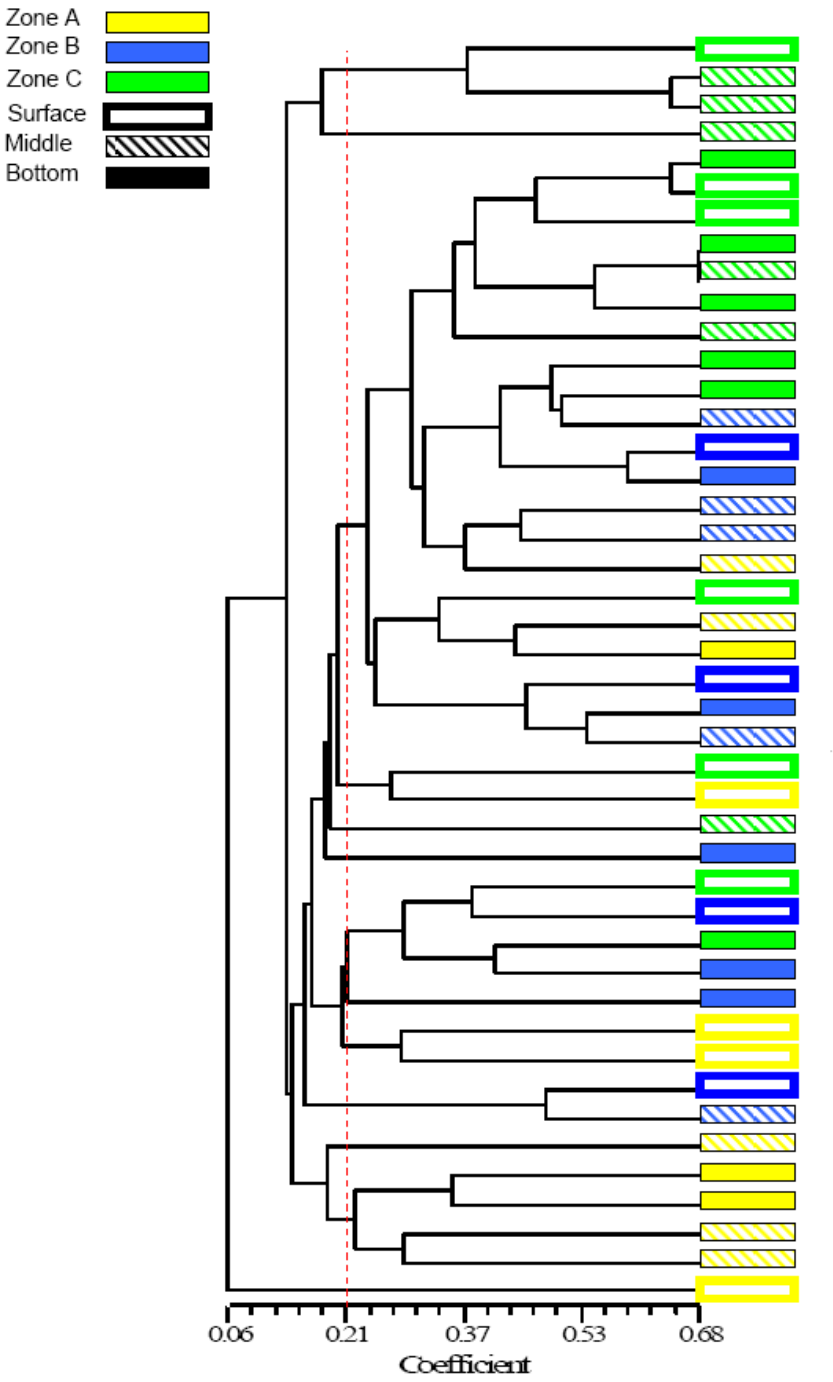


Fig. 4.5. Comparison of total Eubacterial community structures from April 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Index of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. No sites sampled in April 2004 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

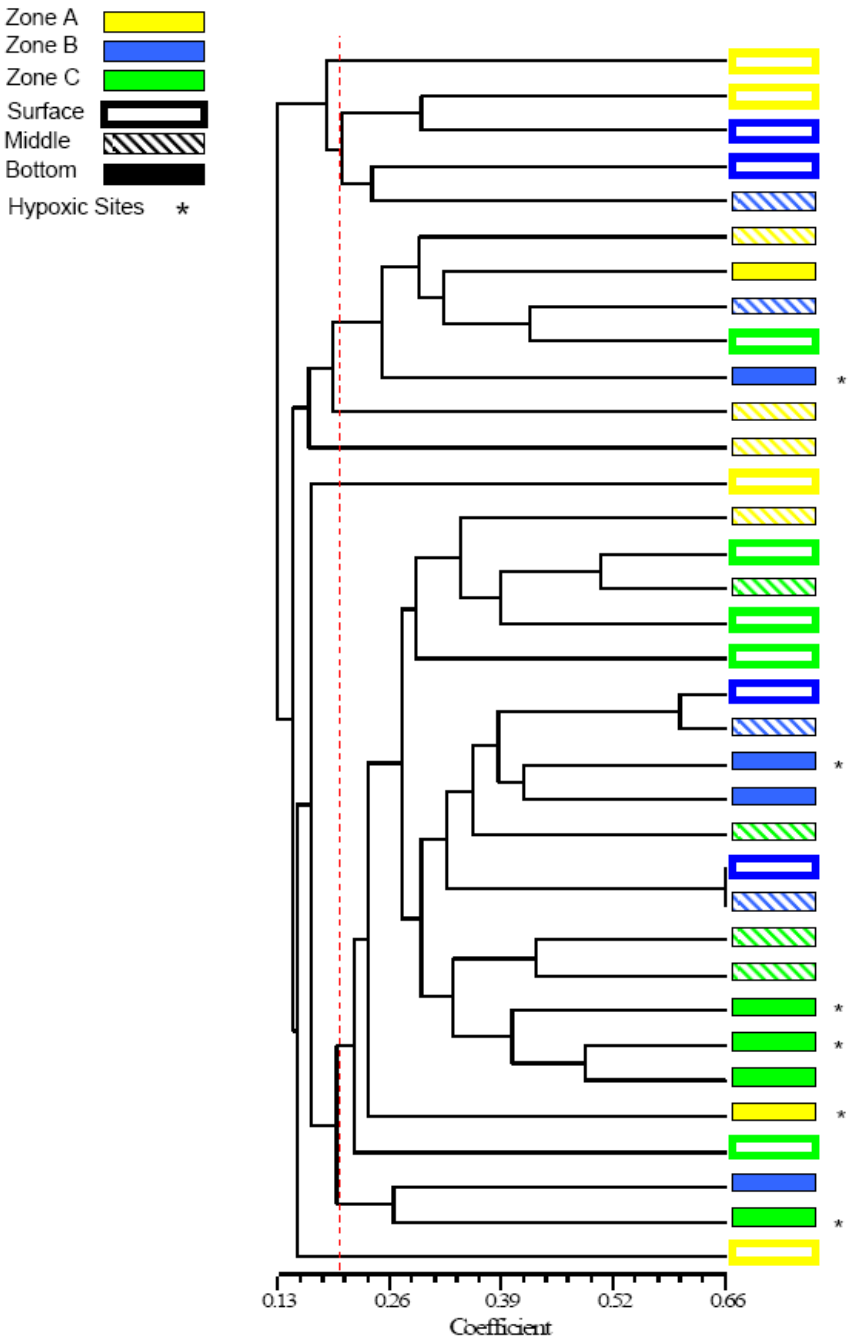


Fig. 4.6. Comparison of total Eubacterial community structures from June 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 6 sites sampled in June 2004 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

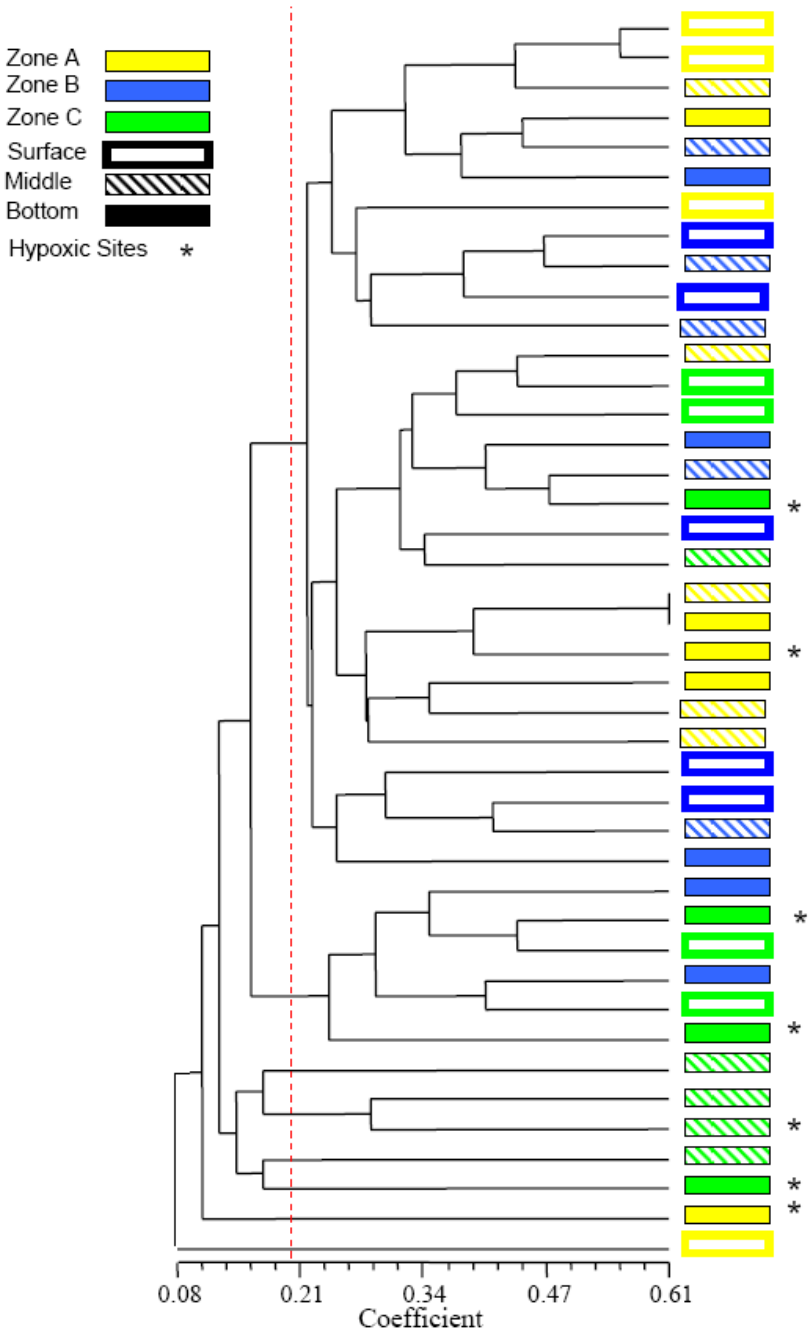


Fig. 4.7. Comparison of total Eubacterial community structures from August 2004. Total eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 7 sites sampled in August 2004 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

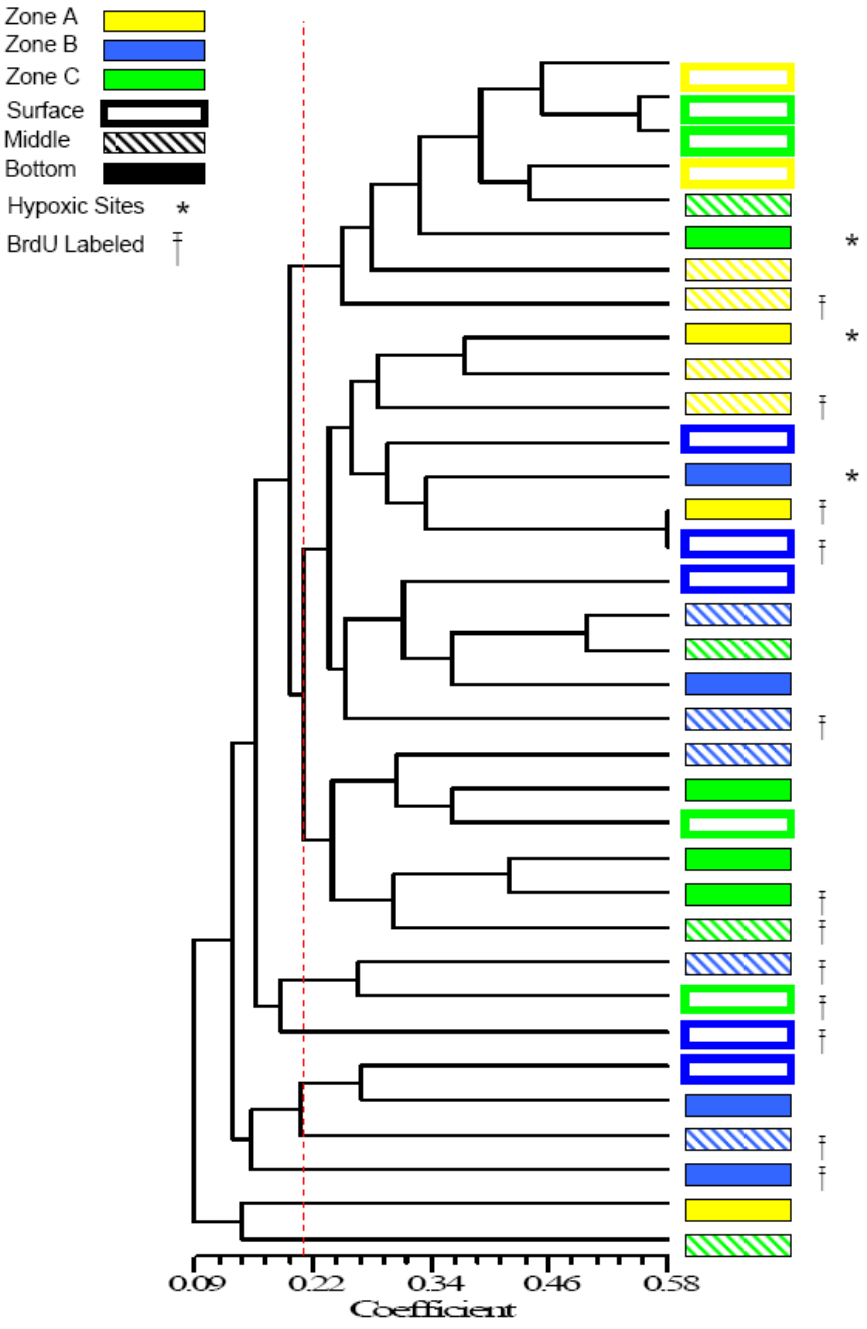


Fig. 4.8. Comparison of total and active Eubacterial community structures from March 2005. Total and BrdU-labeled eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 3 sites sampled in March 2005 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

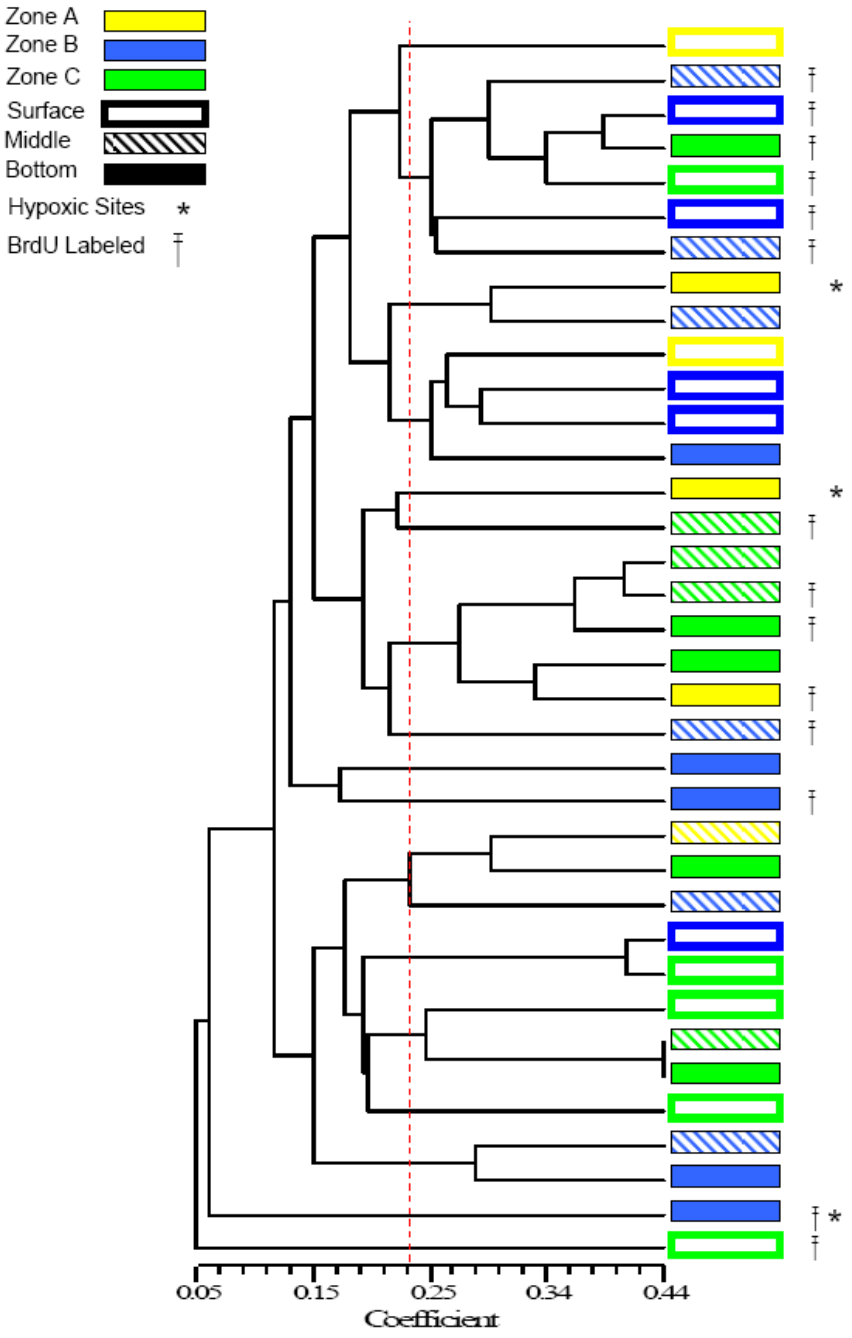


Fig. 4.9. Comparison of total and active Eubacterial community structures from May 2005. Total and BrdU-labeled eubacterial communities from zones A, B and C were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 3 sites sampled in May 2005 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

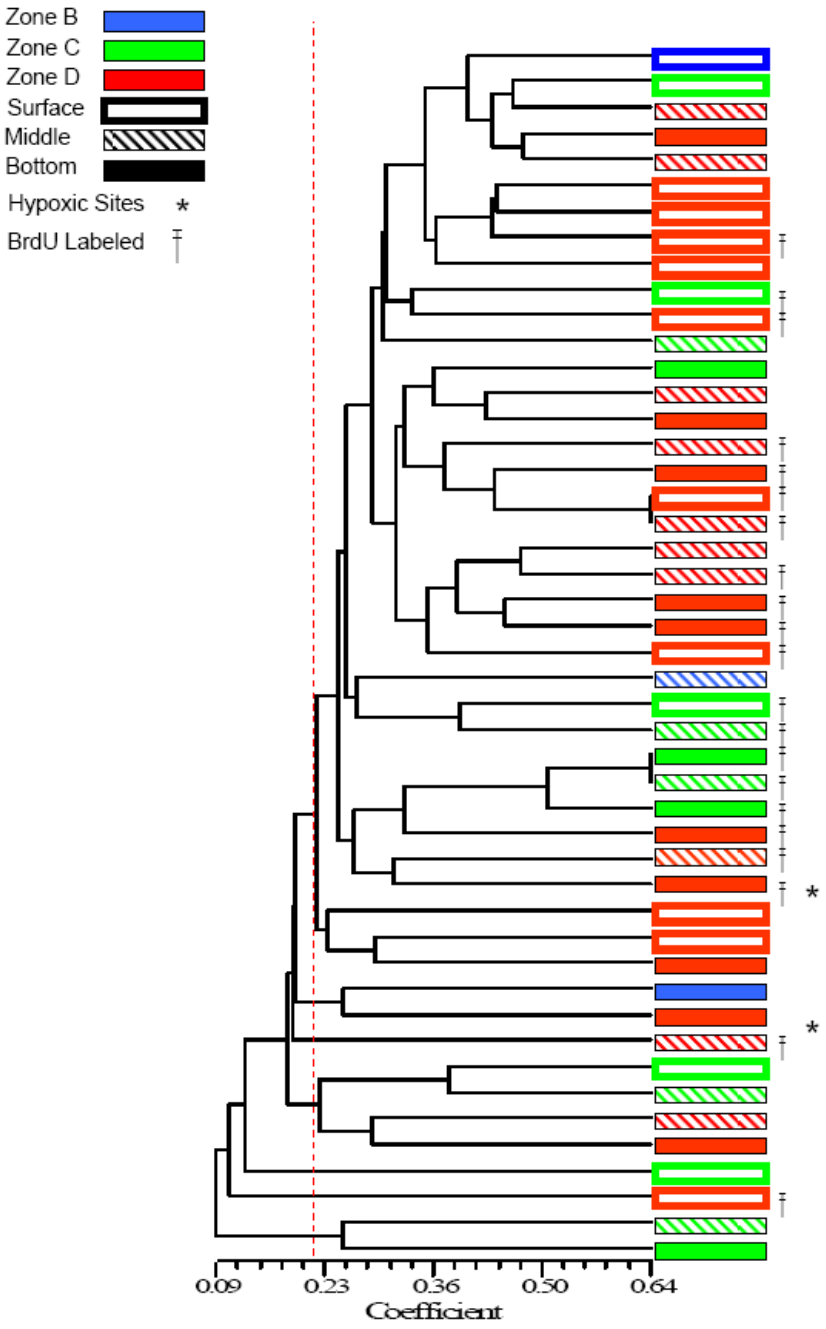


Fig. 4.10. Comparison of total and active Eubacterial community structures from July 2005. Total and BrdU-labeled eubacterial communities from zones B, C and D were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 2 sites sampled in July 2005 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

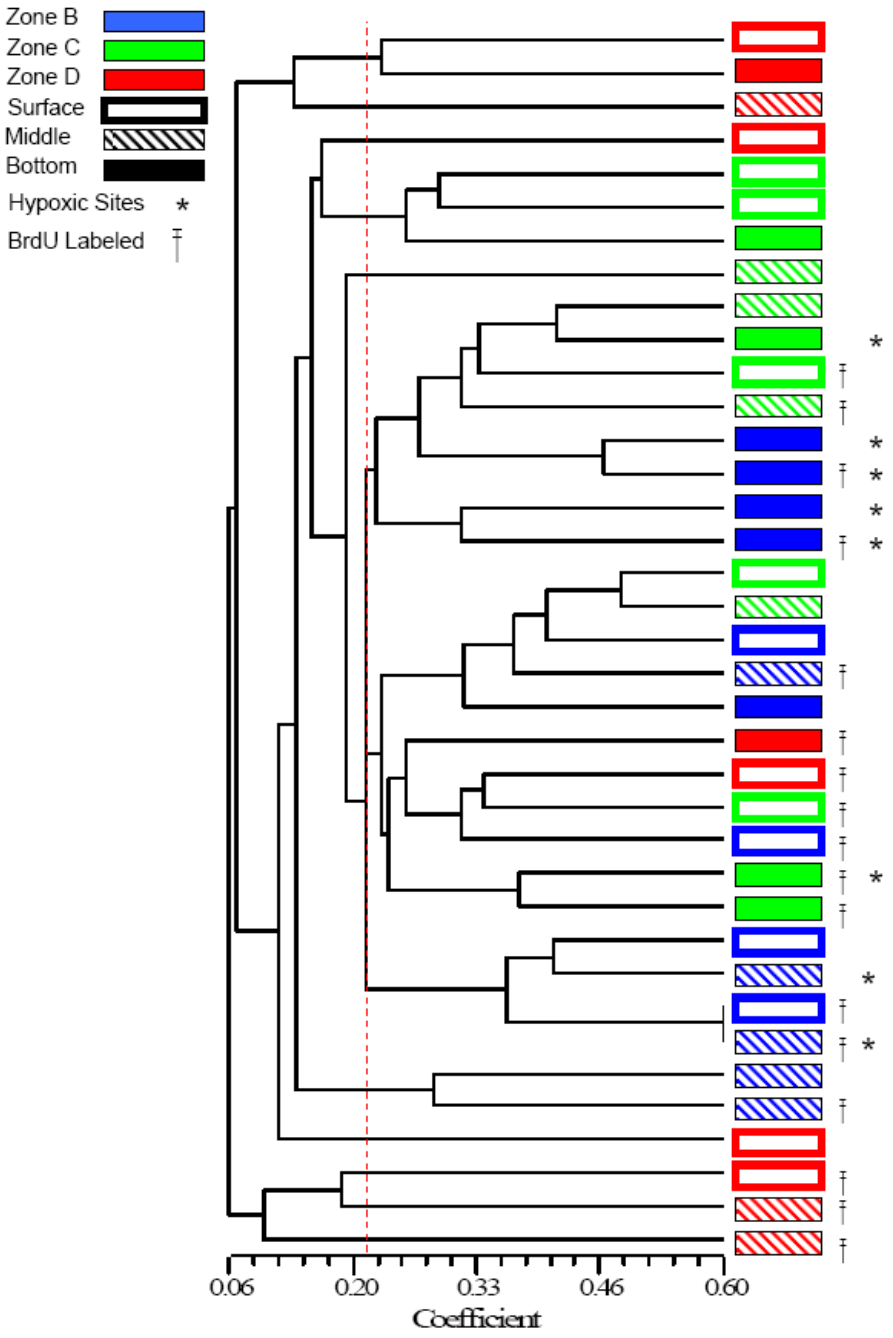


Fig. 4.11. Comparison of total and active Eubacterial community structures from August 2005. Total and BrdU-labeled eubacterial communities from zones B, C and D were sampled from Surface, Middle and Bottoms waters. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Coefficient of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. 8 sites sampled in August 2005 were hypoxic (Dissolved Oxygen Concentration < 1.4 mL L^{-1}).

each other. However, one hypoxic community from Zone C did not cluster close together. These hypoxic communities showed no clear pattern or clustering together. While this could be an effect of so few hypoxic communities, this might also suggest that hypoxic formation does not significantly alter the Eubacterial community from other affiliations, especially when considering the patchiness and development of hypoxia across the continental shelf.

Hypoxic BrdU-labeled communities must be evaluated carefully as some anaerobic bacteria cannot successfully take up BrdU. Not all anaerobic bacteria are capable of the uptake of thymidine (McDonough et al. 1986; Gilmour et al. 1990) including some methanogens and sulfate-reducing bacteria (Winding 1992). This brings into question the ability to incorporate BrdU by those bacterium incapable of thymidine uptake since BrdU is taken up into the cell using thymidine transporters (Hakala 1959). Therefore, it has been suggested by these findings that BrdU and thymidine incorporation could potentially underestimate bacteria activities in anoxic conditions. However this project has no strong findings concerning hypoxic community structure. There was no strong clustering of hypoxic sites. This could be due to the small sample set of hypoxic samples taken relative to the total sample set.

Eubacterial Community Structure with Salinity Gradients

ARISA generated community fingerprints for surface, middle and bottom waters collected from 07C, 08C, and 10C were compared to one another using the Jaccard coefficient of similarity and UPMGA cluster analysis to look at relationships between the Eubacterial community structures and their relationships with salinity gradients. Salinities were grouped by strength; Fresh (less than 25), Intermediate (between 25 and 33), Saline (greater than 33) (from Amon and Benner 1998). April 2004 (Fig. 4.12a) Eubacterial communities are grouped weakly by salinity. Communities in June 2004 (Fig. 4.12b) are closely clustered by salinity with clustering within the salinities by location. In August 2004 (Fig. 4.12c) communities are more closely clustered by site and location on the shelf. March 2005 was highly stratified (Fig. 4.4a) possibly due to

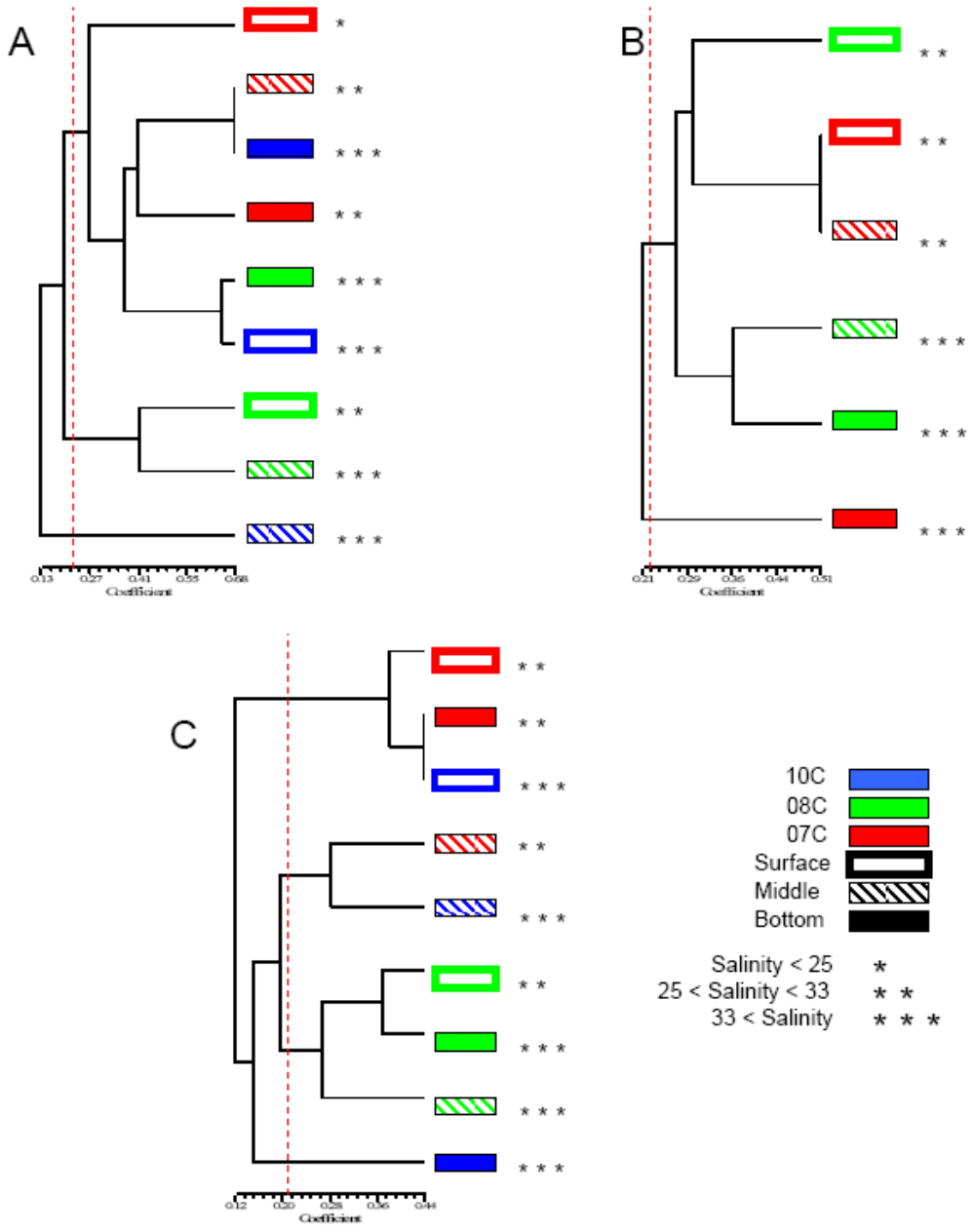


Fig. 4.12. 2004 Eubacterial communities on a salinity gradient. Total Eubacterial communities from (A) April, (B) June and (C) August of 2004 were sampled from Surface, Middle and Bottoms waters of sites 07C, 08C and 10C on line C3. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Index of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. ranges of the sites were grouped into three categories; Fresh (Salinity < 25), Intermediate (25 < Salinity < 33) and Saline (33 < Salinity) waters.

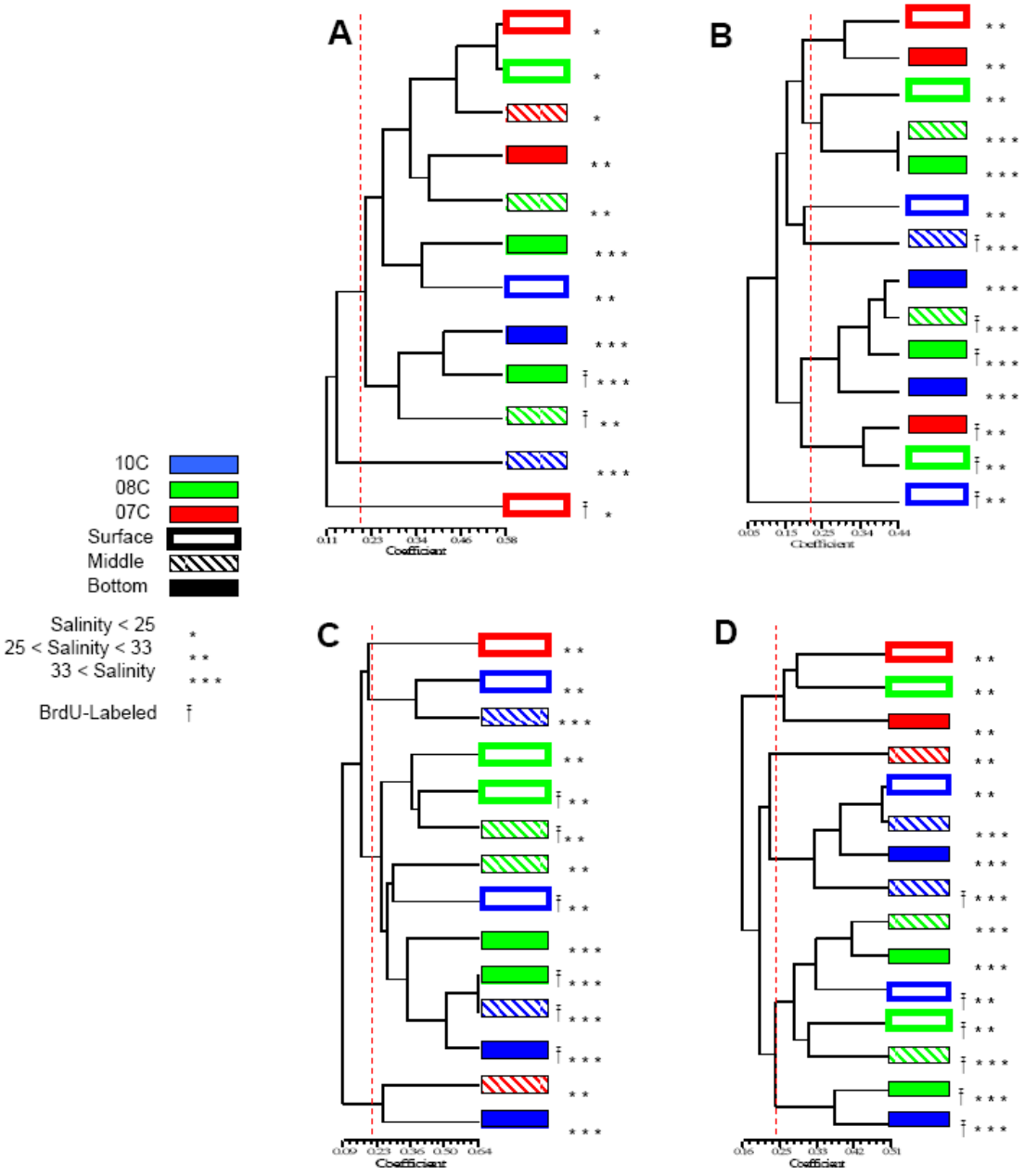


Fig. 4.13. 2005 Eubacterial communities on a salinity gradient. Total and BrdU-labeled Eubacterial communities from (A) March, (B) May, (C) July and (D) August were sampled from Surface, Middle and Bottoms waters of sites 07C, 08C and 10C on line C3. Cluster analysis was performed on ARISA derived community fingerprints of samples the using Jaccard Index of Similarity and un-weighted pair-group-mean average (UPGMA) with NTSYSpc 2.2. Dashed line indicates similarity of fingerprints determined by Monte Carlo simulations that may be expected by chance alone. Salinity ranges of the sites were grouped into three categories; Fresh (Salinity < 25), Intermediate (25 < Salinity < 33) and Saline (33 < Salinity) waters.

above average river discharges (Fig. C.2); and Eubacterial communities were closely clustered by salinity (Fig. 4.13a). Communities in May and July 2005 (Fig. 4.13b and c) are closely clustered by salinity with increased clustering within the salinities by sampling location. In August 2005 (Fig. 4.13d), Eubacterial communities were closely clustered by salinity and sampling location on the continental shelf. In 2005, samples were also incubated with BrdU to label active communities. Active BrdU-labeled communities became more closely clustered over summer 2005. In the presence of a strong salinity gradient, community structure is determined more by salinity, rather than the location on the shelf. As salinities became more uniform and stratification decreased, communities cluster more closely with communities form the same site and location on the shelf.

Linking Community Composition to the Environment

The total bacterial communities from 7008cb, 4011bb and 5011bb were selected for the clone library to determine the species affiliation of dominant OTUs present in hypoxic communities. 16S rDNA was successfully sequenced from 45 of 48 colonies. Fig. 4.14 and Table 4.4 show the putative phyla, with the closest related organism and accession numbers, aligned with the identified peak. Sequences were aligned with the corresponding ARISA peaks to determine the relative diversity and frequency of the composition of the Eubacterial communities. Phylogenetic alignment placed sequences within many lineages of Eubacteria including α– and γ– *Proteobacteria*, Actinobacteria, *Synechococcus* and Cytophaga/Flexibacter/ Bacteroides (CFB). One peak (ARISA length 631) corresponded with two phylogenically distinct organisms within *Synechococcus* and CFB. Another peak (ARISA length 634) corresponded with three phylogenetically distinct organisms within *Synechococcus*, α - *Proteobacteria* and Actinobacteria. Actinobacteria and Cyanobacteria were the most frequent phylum affiliations with ARISA peaks from the hypoxic sites.

The community composition of hypoxic sites does not notably vary from that of non-hypoxic sites (Fig. 3.17 and Fig. 3.18). All libraries are comprised of α– and

Table 4.4. Closest match of ARISA derived OTUs from hypoxic sites. 16s rDNA sequences from clone library of hypoxic sites (7008cb, 4011bb and 5011bb; Fig. 4.1) were affiliated with a putative phylum from the closest match using BLAST search tool (Genbank).

ARISA length	Closest match by BLAST	Accession	% similarity	Putative Phylum
418	Actinobacterium	AY033296.1	99	Actinobacteria
598	Actinobacterium	DQ200518.1	99	Actinobacteria
610	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
616	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
616	*Synechococcus sp.*	AY855313.1	99	Cyanobacteria
619	Actinobacterium	DQ200518.1	99	Actinobacteria
622	Actinobacterium	DQ200518.1	99	Actinobacteria
631	*Synechococcus sp.*	CP000110.1	99	Cyanobacteria
631	*Bacteroidetes bacterium*	AY580649.1	94	CFB
634	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
634	*Synechococcus sp.*	CP000110.1	99	Cyanobacteria
634	*Ruegeria sp.*	AY712025.1	99	α - Proteobacteria
649	*Legionella gresilensis*	AF122883.1	94	γ - Proteobacteria
667	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
676	*Synechococcus sp.*	CP000110.1	99	Cyanobacteria
745	*Bacteroidetes bacterium*	DQ189920.1	98	CFB
785	*Actinomycetales bacterium*	AY370630.1	98	Actinobacteria
810	*Photobacterium mandapamensis*	AY455873.1	99	γ - Proteobacteria
885	*Synechococcus sp.*	AY172806.1	99	Cyanobacteria
1010	*Synechococcus sp.*	AY172826.1	100	Cyanobacteria
1050	*Synechococcus sp.*	AY172826.1	99	Cyanobacteria
1090	*Synechococcus sp.*	AY172826.1	99	Cyanobacteria
1170	*Synechococcus sp.*	AY172806.1	97	Cyanobacteria

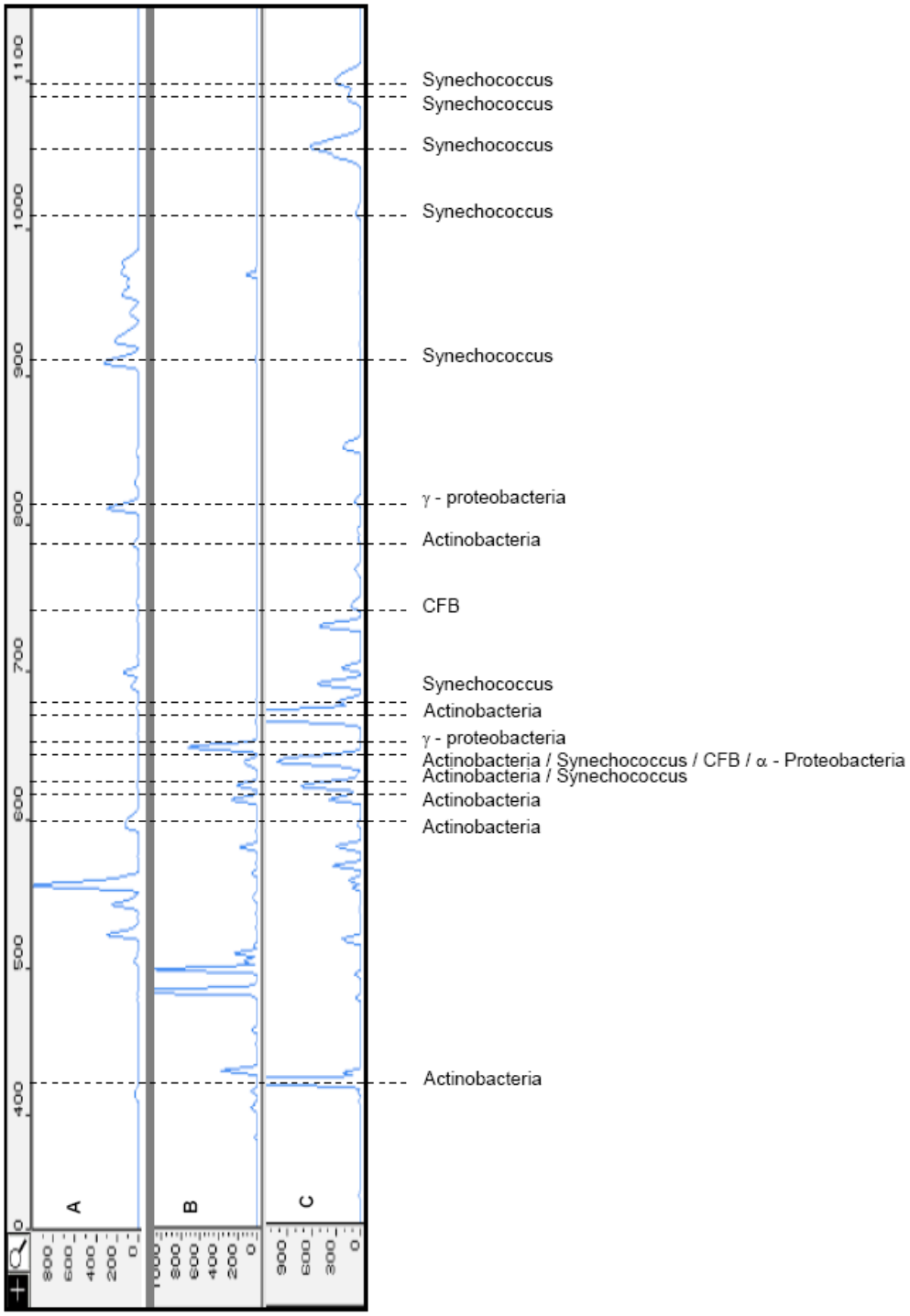


Fig 4.14. Putative phylum affiliations from hypoxic sites. 16S-ITS clone library was generated with pooled PCR products of (A) 7008cb, (B) 4011bb and (C) 5011bb (16S rDNA 27F and unlabeled 23S rDNA 125R primers) inserted into pCR8/GW/TOPO vector (Invitrogen). Purified and sequenced (Agencourt Bioscience, primer 16s rDNA 27F) vector inserts were phylogenetically affiliated using BLAST (GenBank). Putative phyla were affiliated with ARISA peaks by dereplication of clone inserts. Clone inserts were reamplified (using 16S rDNA1392F and TET-Labeled 23S rDNA 125R primers),5 ng mL-1 normalized and run on an ABI 377xl automated sequencer to determine the ARISA base pair length and then associated with the putative phyla.

γ– *Proteobacteria*, Actinobacteria, *Synechococcus* and CFBs. This is not surprising since the development of hypoxia is patchy and varies from month to month on the Texas-Louisiana shelf (Fig. B.1 and Fig. B.2). Also similarities of community structure are not limited to hypoxic waters (Fig. 4.5 – 4.11), and hypoxic communities can be more closely related to an oxic community.

Community composition is also very similar to that found within estuarine gradients by Cottrell and Kirchman (2004) and Kirchman and colleagues (2005) with some noted differences. While we did not identify β -*Proteobacteria* within the community, this could be accounted for by a negative correlation of abundance and Thy- and Leu- activity with salinity that was noted by Cottrell and Kirchman (2004). While found within coastal systems (Rappe et al. 1997; Cottrell and Kirchman 2000), β -*Proteobacteria* are predominantly located in freshwater environments (Hugenholtz et al. 1998; Glockner et al. 1999). Sequences identified from our systems were also dominated by marine Cyanobacteria, which were absent in the previous estuarine studies. The presence of Cyanobacteria can also be due to the increased overall salinity and location on the continental shelf, as *Synechococcus* abundances increase with salinity (Uysal 2001). Abundances of the Cyanobacteria sub-population increased during the summers of 2004 and 2005, with the highest abundances in mid-water column waters of Zone D (Table D.1). In August 2005, the chlorophyll maximum along the 20m isobath was located near the bottom of the water-column into hypoxic waters. Determined active by BrdU incorporation, *Synechococcus* populations could have peaked due to lessen grazing pressure in hypoxic waters.

However, Actinobacteria seen in our study does not appear to have the negative correlation with salinity that was seen in Cottrell and Kirchman (2004) , but it should also be noted that our study does not have the ability to determine absolute abundances of affiliations, as previously discussed in Chapter III. These methods do provide a good depiction of the relative abundances within a community based upon ARISA signal and clone frequency.

SUMMARY

Hypoxic waters were found during every sampling cruise, with the exception of April 2004, though the intensity and coverage area varied throughout the summers of 2004 and 2005. Both vertical and horizontal salinity gradients along Line C3 were present that the beginning of the summer, but began to lessen towards the end of the summers as the average flow of the Atchafalaya River also diminished. 2005 had higher than average water flow at the beginning of the year. This could account for earlier hypoxia formation in 2005. No pattern could be determined of community structure within hypoxic communities in this study. Hypoxic communities were dominated by Cyanobacteria and Actinobacteria and were not significantly different in composition from non-hypoxic sites. Salinity gradients were analyzed along line C3 due to the close proximity to the Atchafalaya River. In early summer Eubacterial communities were most similar to those communities located with similar salinities. As the summer progress and fresh water input onto the shelf decreased, communities were most similar to those communities closest regardless of salinity. Weak relationships of community structure with hypoxic waters and strong similarities among the sampling Zones suggest freshwater input influence the Eubacterial community structure on the shelf. The river sources support the foundation of the microbial community at on the Texas-Louisiana Shelf.

CHAPTER V

OVERVIEW AND CONCLUSIONS

5-Bromodeoxyuridine (BrdU), a thymidine analog, can be used to isolate actively DNA-replicating dominant phyla of a natural marine assemblage. BrdU was incorporated into all major phyla within the seawater-culture dilution experiment under aerobic conditions. The progression dynamics of the community were able to be captured since BrdU incorporation varied during the seawater-culture incubation. This enabled the differentiation of the growing fraction within the total Eubacterial community. However, slower growing species within microbial communities can limit the effectiveness of shorter BrdU incubations. This study supports the application of BrdU in natural marine assemblages for the detection and identification within the active fraction of the Eubacteria community. For analysis of the whole bacterial population, further work is still needed to determine the effectiveness of BrdU incorporation with Archaea and those phyla less commonly detected within the bacterial communities.

The pelagic Eubacterial community of the Texas-Louisiana Shelf is a variable system. While the bacterial population is slightly more abundant towards the eastern side of the Texas-Louisiana Shelf, it did not significantly vary cross-shelf in either 2004 or 2005. Bacterial abundances did significantly vary with depth, with the highest abundances located in surface waters. Eubacterial community structure and community richness varied throughout the Texas-Louisiana Shelf. Eubacterial communities were differentiated based upon their location to the Atchafalaya and Mississippi Rivers. Communities within Zones A & C were distinct from one another while communities within Zone B appeared to be a transitional Zone between Zones A & C. Those communities within Zone D were not closely related to other communities within other Zones. Within each Zone, Eubacterial communities resembled those found at similar depths within the water column. However this trend did do extend outside of each Zone, suggesting, when also considering the relationships between all the Zones, that the Eubacterial communities are more likely influenced by their location across the shelf

rather than by location within the water column. Community composition was dominated by Cyanobacteria, *Synechococcus and Prochlorococcus,* Actinobacteria and α - and β - *Proteobacteria*.

Hypoxic waters developed above the seafloor during the summers of 2004 and 2005. Hypoxic sites were found during at every sampling date, with the exception of April 2004. Hypoxia development early in 2005 could be accounted for by peak river flows early in the year.

In this study, no clear connection could be found with community structure and the development of hypoxia on the Texas-Louisiana Shelf. Bacterial abundances decreased with decreasing dissolved oxygen concentrations. Species richness of bacterial communities at hypoxic sites slightly decreased from overall total community species richness. However Eubacterial community structures from hypoxic waters were no more similar to each other than to communities in oxic waters. In addition, the community composition of hypoxic sites did not significantly differ from those that were not hypoxic. The findings of this study do not support a relationship between hypoxia and Eubacterial community structure; however, we are unable to rule out a connection due to two factors. First, sampling of hypoxic sites for microbial structures was relatively small (n=24) when compared to the total sampling set (n= 276). This large difference in the sampling sizes makes establishing trends and characteristics difficult. Second, hypoxia on the Texas-Louisiana Shelf is patchy and can quickly become oxic, as seen with Zone B due to Hurricane Cindy on 2005. These shifts in environment do not significantly alter the total Eubacterial Community composition. However, the metabolically active fraction of the community could possible be affected by these transitions between oxic and hypoxic water-column conditions. Unfortunately this study does not have sufficient samples from hypoxic active communities to explore this feature in more detail and BrdU has not been shown to be an effective tracer with anaerobic bacteria.

Findings in this study of weak relationships of community structure with hypoxic waters and strong similarities among the sampling Zones suggest that fluvial sources to

the Texas-Louisiana Shelf, the Mississippi and Atchafalaya Rivers, strongly influence the structures of pelagic Eubacterial communities. The Eubacterial community structure within the salinity gradient of the Atchafalaya River plume was explored. This region could possibly act as a buffering Zone between terrestrial and marine communities on the continental shelf. Salinity gradients directly off of the Atchafalaya River were strong at the start of spring and summer, and weakened as the average river flow diminished later in the summer. Early during each summer and spring Eubacterial communities were most similar to communities located within similar salinities. However as the summer progressed, communities became more distinct by their location off the shelf as the salinity profiles became more uniform.

BrdU incubations isolated the rapidly replicating and active fraction of the total Eubacterial communities. During summer 2005, active communities across the Texas-Louisiana Shelf, as determined by BrdU incorporation, became more closely related to each other regardless from their location in the shelf. Even though the total communities are distinct by their riverine source, the active fractions of the populations are most similar to other active Eubacterial communities on the Texas-Louisiana Shelf. One possibility is that as the fresh water input onto the shelf decreases towards the end of the summer (USACE 2005), the amount of freshwater bacteria introduced onto the shelf also decreases. The metabolism of bacteria present on the shelf could also select for similar communities on the shelf. Bacteria select phytoplankton derived organic matter over terrigenous material from the rivers (Gardner et al. 1996; Pakulski et al. 2000; McManus et al. 2004). Therefore it is possible that even though the rivers are depositing different bacterial populations, nutrients, and organic matter onto the shelf, the fraction of the bacterial population able to successfully respire phytoplankton derived organic matter remains similar across the shelf. This similarity could possibly be due to selection within the bacterial population due to chemical, physical, and biological availability and pressures. In addition to carbon availability, physical parameters also determine the activity of bacterial populations. Further on the shelf, away from riverine inputs, salinities increase and freshwater derived bacterial population are limited in successful

growth. Support for these findings could be found by directly sampling the bacterial community from each river and river delta before the 10m isobath and further detailing the transitional Zone from the river delta to off the shelf, which was limited in this study due to the inability of the R/V *Gyre* to enter waters < 10m depth.

Further analysis of the bacterial community of the Texas-Louisiana Shelf could elucidate the dynamics of these unique microbial communities off of the Mississippi and Atchafalaya Rivers and determine more specifically the role of these communities within regeneration of organic matter and the development of bottom-water hypoxia.

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APPENDIX A

HYDROGRAPHIC DATA

Table A.1. Raw data from April 2004

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
1008c	4/3/2004	0:36	29.00	-92.0	C	2.7E+05	19.3	Surface	1.51
1008c	4/3/2004	0:36	29.00	-92.0	C	1.2E+06	19.3	middle	9.57
1008c	4/3/2004	0:36	29.00	-92.0	C	1.9E+06	19.3	bottom	ND
1010c	4/3/2004	19:47	28.80	-92.1	C	1.1E+06	31.1	Surface	3.02
1010c	4/3/2004	19:47	28.80	-92.1	C	7.2E+05	31.1	middle	15.10
1010c	4/3/2004	19:47	28.80	-92.1	C	1.3E+06	31.1	bottom	29.20
1002c	4/4/2004	5:39	29.06	-92.4	C	1.2E+06	21.3	Surface	1.51
1002c	4/4/2004	5:39	29.06	-92.4	C	1.7E+06	21.3	middle	10.57
1002c	4/4/2004	5:39	29.06	-92.4	C	1.5E+06	21.3	bottom	21.14
1005c	4/4/2004	0:59	29.02	-92.2	C	1.6E+06	20.0	Surface	1.51
1005c	4/4/2004	0:59	29.02	-92.2	C	1.5E+06	20.0	middle	9.57
1005c	4/4/2004	0:59	29.02	-92.2	C	1.2E+06	20.0	bottom	18.63
1016c	4/3/2004	7:52	28.88	-91.7	C	1.2E+06	21.6	Surface	2.01
1016c	4/3/2004	7:52	28.88	-91.7	C	1.4E+06	21.6	middle	10.57
1016c	4/3/2004	7:52	28.88	-91.7	C	1.5E+06	21.6	bottom	20.14
1007c	4/3/2004	15:05	29.12	-91.9	C	1.3E+06	9.9	Surface	2.01
1007c	4/3/2004	15:05	29.12	-91.9	C	1.5E+06	9.9	middle	5.03
1007c	4/3/2004	15:05	29.12	-91.9	C	1.5E+06	9.9	bottom	9.56
m1bc2	4/4/2004	16:34	28.68	-91.3	B	1.1E+06	20.3	Surface	2.01
m1bc2	4/4/2004	16:34	28.68	-91.3	B	6.1E+05	20.3	middle	10.07
m1bc2	4/4/2004	16:34	28.68	-91.3	B	1.3E+06	20.3	bottom	19.63
1018b	4/6/2004	8:41	28.78	-90.3	B	3.6E+05	22.1	Surface	2.01
1018b	4/6/2004	8:41	28.78	-90.3	B	9.0E+05	22.1	middle	10.57
1018b	4/6/2004	8:41	28.78	-90.319	B	8.2E+05	22.1	bottom	20.71
1012b	4/6/2004	18:28	28.86	-90.41	B	1.2E+06	20.2	Surface	2.01
1012b	4/6/2004	18:28	28.86	-90.41	B	1.0E+06	20.2	middle	10.07
1012b	4/6/2004	18:28	28.86	-90.41	B	9.7E+05	20.2	bottom	19.13
1007b	4/6/2004	13:26	28.96	-90.552	B	2.1E+06	12.5	Surface	2.01
1007b	4/6/2004	13:26	28.96	-90.552	B	1.5E+06	12.5	middle	6.04
1007b	4/6/2004	13:26	28.96	-90.552	B	1.2E+06	12.5	bottom	11.15
1010b	4/7/2004	1:06	28.62	-90.552	B	5.9E+05	21.9	Surface	2.55
1010b	4/7/2004	1:06	28.62	-90.552	B	1.2E+06	21.9	middle	10.57
1010b	4/7/2004	1:06	28.62	-90.552	B	1.3E+06	21.9	bottom	21.14
1002b	4/7/2004	9:01	28.72	-90.779	B	3.7E+05	17.8	Surface	ND
1002b	4/7/2004	9:01	28.72	-90.779	B	1.9E+06	17.8	middle	ND
1002b	4/7/2004	9:01	28.72	-90.779	B	1.9E+06	17.8	bottom	ND
1007a	4/5/2004	23:20	29.11	-89.536	A	1.5E+06	11.1	Surface	1.01
1007a	4/5/2004	23:20	29.11	-89.536	A	1.0E+06	11.1	middle	5.54
1007a	4/5/2004	23:20	29.11	-89.536	A	2.0E+06	11.1	bottom	ND
1012a	4/5/2004	20:13	28.97	-89.49	A	9.1E+05	28.5	Surface	2.01
1012a	4/5/2004	20:13	28.97	-89.49	A	9.7E+05	28.5	middle	13.59
1012a	4/5/2004	20:13	28.97	-89.49	A	1.3E+06	28.5	bottom	23.78
1016a	4/5/2004	10:54	28.84	-89.508	A	5.2E+05	59.1	Surface	2.52
1016a	4/5/2004	10:54	28.84	-89.508	A	4.2E+05	59.1	middle	29.20
1016a	4/5/2004	10:54	28.84	-89.508	A	4.9E+05	59.1	bottom	57.90
1002a	4/6/2004	3:03	29.14	-89.764	A	9.8E+05	19.8	Surface	2.01
1002a	4/6/2004	3:03	29.14	-89.764	A	1.0E+06	19.8	middle	9.57
1002a	4/6/2004	3:03	29.14	-89.764	A	1.3E+06	19.8	bottom	ND
1010a	4/5/2004	6:13	28.88	-89.706	A	5.3E+05	60.5	Surface	2.01
1010a	4/5/2004	6:13	28.88	-89.706	A	8.3E+05	60.5	middle	30.21
1010a	4/5/2004	6:13	28.88	-89.706	A	8.4E+05	60.5	bottom	57.82
m1ab2	4/5/2004	3:08	28.81	-90	A	7.4E+05	40.2	Surface	1.51
m1ab2	4/5/2004	3:08	28.81	-90	A	3.7E+05	40.2	middle	20.14
m1ab2	4/5/2004	3:08	28.81	-90	A	5.2E+05	40.2	bottom	39.77

Table A.1. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
1008c	1.50	21.93	30.07	20.50	5.56	74.80	2.46E+00	1.01	0.64
1008c	9.50	21.23	33.37	23.19	5.00	82.82	2.46E+00	0.58	0.44
1008c	ND	ND	ND	ND	ND	ND	ND	ND	ND
1010c	3.00	21.41	34.41	23.93	4.93	86.22	1.75E+03	0.15	0.00
1010c	15.00	20.73	35.93	25.28	4.85	90.70	2.84E+02	0.17	0.15
1010c	29.00	19.83	36.10	25.65	4.43	80.07	4.93E+01	0.64	0.87
1002c	1.50	21.97	27.24	18.34	5.16	86.18	2.46E+00	0.48	0.35
1002c	10.50	20.66	34.99	24.58	4.23	89.16	2.46E+00	0.30	0.24
1002c	21.00	20.43	35.51	25.04	4.31	61.21	2.46E+00	0.52	3.03
1005c	1.50	21.97	31.56	21.62	5.21	79.07	2.46E+00	0.66	0.45
1005c	9.50	20.98	34.30	23.96	4.69	84.60	2.46E+00	0.44	0.37
1005c	18.50	20.41	35.68	25.17	4.13	62.27	2.46E+00	0.61	3.58
1016c	2.00	21.68	31.41	21.58	6.04	72.07	2.46E+00	2.06	0.53
1016c	10.50	21.19	33.18	23.06	4.80	82.12	2.46E+00	0.76	0.46
1016c	20.00	20.73	35.71	25.11	4.09	59.78	2.46E+00	0.58	2.78
1007c	2.00	21.43	19.47	12.61	6.23	73.99	8.87E+02	1.32	0.99
1007c	5.00	21.36	29.31	20.07	4.02	83.80	2.16E+02	0.57	0.53
1007c	9.50	21.06	32.78	22.79	3.25	65.02	6.54E+01	0.57	2.38
m1bc2	2.00	21.96	28.89	19.59	7.12	59.67	1.85E+03	2.50	0.93
m1bc2	10.00	20.75	35.87	25.22	4.22	81.38	1.21E+02	0.33	0.62
m1bc2	19.50	20.58	35.98	25.35	3.89	48.27	7.86E+00	0.30	3.98
1018b	2.00	21.52	35.19	24.50	4.78	90.83	2.46E+00	0.09	0.16
1018b	10.50	21.63	35.96	25.05	4.78	90.73	2.46E+00	0.08	0.17
1018b	20.50	21.55	36.32	25.34	4.54	79.03	2.46	0.19	0.93
1012b	2.00	21.51	34.24	23.78	4.78	91.02	1780	0.05	0.04
1012b	10.00	21.59	35.33	24.58	4.75	90.54	565.2	0.10	0.17
1012b	19.00	21.32	36.09	25.24	3.75	21.42	246.2	0.83	6.65
1007b	2.00	21.58	31.36	21.57	5.16	79.68	583.5	0.69	0.64
1007b	6.00	21.60	31.47	21.65	5.14	80.28	145.4	1.00	0.63
1007b	11.00	21.68	32.59	22.48	5.03	78.56	38.52	1.02	0.68
1010b	2.50	21.65	35.77	24.90	4.71	89.35	2.46	0.12	0.27
1010b	10.50	21.65	35.76	24.89	4.70	89.39	2.46	0.13	0.35
1010b	21.00	21.29	36.01	25.18	4.12	56.81	2.46	0.43	1.36
1002b	ND	ND	ND	ND	ND	ND	ND	ND	ND
1002b	ND	ND	ND	ND	ND	ND	ND	ND	ND
1002b	ND	ND	ND	ND	ND	ND	ND	ND	ND
1007a	1.00	21.19	19.40	12.61	6.68	45.30	391	2.71	5.58
1007a	5.50	20.86	31.44	21.82	3.93	88.07	8.024	0.24	0.79
1007a	ND	ND	ND	ND	ND	ND	ND	ND	ND
1012a	2.00	21.11	25.45	17.21	6.65	81.24	1299	0.79	1.07
1012a	13.50	21.45	35.86	25.03	3.83	81.58	28.25	0.20	0.71
1012a	23.50	21.02	36.36	25.53	3.41	80.13	4.357	0.11	0.94
1016a	2.50	20.85	23.92	16.12	6.75	75.98	2.46	2.36	2.18
1016a	29.00	22.02	36.34	25.23	4.52	90.40	2.46	0.18	0.34
1016a	57.50	19.88	36.46	25.91	3.32	55.14	2.46	0.31	2.78
1002a	2.00	21.22	23.77	15.90	7.26	70.78	2.46	2.39	0.79
1002a	9.50	21.59	27.72	18.80	5.89	81.35	2.46	1.15	0.44
1002a	ND	ND	ND	ND	ND	ND	ND	ND	ND
1010a	2.00	21.58	22.97	15.21	7.69	57.85	2.46	4.80	1.58
1010a	30.00	21.96	36.53	25.39	4.56	91.12	2.46	0.15	0.23
1010a	57.50	20.22	36.45	25.81	3.51	53.78	2.46	0.12	1.86
m1ab2	1.50	22.53	30.81	20.89	6.64	80.67	2.46	0.74	0.37
m1ab2	20.00	21.99	36.31	25.22	4.72	91.28	2.46	0.18	0.16
m1ab2	39.50	21.65	36.46	25.42	4.49	54.32	2.46	0.28	3.43

Table A.2. Raw data from June 2004

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
2008c	6/26/2004	4:47	29.00	-92.0	C	2.1E+06	19.7	Bottom	19.63
2008c	6/26/2004	4:47	29.00	-92.0	C	1.8E+06	19.7	Middle	8.56
2008c	6/26/2004	4:47	29.00	-92.0	C	1.3E+06	19.7	Surface	1.51
2002b	6/26/2004	22:10	28.72	-90.8	B	0.0E+00	18.0	Bottom	17.12
2002b	6/26/2004	22:10	28.72	-90.8	B	0.0E+00	18.0	Middle	11.08
2002b	6/26/2004	22:10	28.72	-90.8	B	0.0E+00	18.0	Surface	1.51
2010B	6/27/2004	5:53	28.62	-90.5	B	7.0E+05	22.9	Bottom	21.14
2010B	6/27/2004	5:53	28.62	-90.5	B	7.4E+05	22.9	Middle	10.57
2010B	6/27/2004	5:53	28.62	-90.5	B	0.0E+00	22.9	Surface	2.52
2007b	6/27/2004	10:43	28.96	-90.5	B	1.9E+06	12.7	Bottom	10.57
2007b	6/27/2004	10:43	28.96	-90.5	B	2.5E+06	12.7	Middle	5.54
2007b	6/27/2004	10:43	28.96	-90.5	B	2.8E+06	12.7	Surface	2.01
2010A	6/28/2004	9:38	28.88	-89.7	A	7.7E+05	60.5	Bottom	59.91
2010A	6/28/2004	9:38	28.88	-89.7	A	6.5E+05	60.5	Middle	35.24
2010A	6/28/2004	9:38	28.88	-89.7	A	3.1E+06	60.5	Surface	3.02
2012a	6/28/2004	14:53	28.97	-89.5	A	1.0E+06	32.9	Bottom	31.21
2012a	6/28/2004	14:53	28.97	-89.5	A	1.6E+06	32.9	Middle	15.61
2012a	6/28/2004	14:53	28.97	-89.5	A	1.6E+06	32.9	Surface	2.52
2016A	6/28/2004	23:39	28.84	-89.5	A	0.0E+00	59.2	Bottom	56.89
2016A	6/28/2004	23:39	28.84	-89.5	A	9.0E+05	59.2	Middle	28.19
2016A	6/28/2004	23:39	28.84	-89.5	A	2.8E+06	59.2	Surface	2.52
2007a	6/29/2004	5:09	29.12	-89.5	A	0.0E+00	11.5	Bottom	8.62
2007a	6/29/2004	5:09	29.12	-89.5	A	1.9E+06	11.5	Middle	5.03
2007a	6/29/2004	5:09	29.12	-89.54	A	0.0E+00	11.5	Surface	2.52
2012b	6/29/2004	23:11	28.88	-90.374	B	1.3E+06	20.0	Bottom	17.62
2012b	6/29/2004	23:11	28.88	-90.374	B	1.3E+06	20.0	Middle	10.07
2012b	6/29/2004	23:11	28.88	-90.374	B	3.3E+06	20.0	Surface	1.09
2016C	6/30/2004	16:18	28.88	-91.727	C	7.2E+05	22.0	Bottom	21.65
2016C	6/30/2004	16:18	28.88	-91.727	C	1.1E+06	22.0	Middle	10.07
2016C	6/30/2004	16:18	28.88	-91.727	C	1.2E+06	22.0	Surface	2.01
2007c	7/1/2004	7:19	29.12	-91.909	C	1.3E+06	10.7	Bottom	9.57
2007c	7/1/2004	7:19	29.12	-91.909	C	0.0E+00	10.7	Middle	5.03
2007C	7/1/2004	7:19	29.12	-91.909	C	1.8E+06	10.7	Surface	2.01
2002c	7/1/2004	12:23	29.06	-92.367	C	1.4E+06	21.7	Bottom	20.64
2002c	7/1/2004	12:23	29.06	-92.367	C	0.0E+00	21.7	Middle	10.07
2002c	7/1/2004	12:23	29.06	-92.367	C	1.3E+06	21.7	Surface	2.52

Table A.2. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
2008c	19.50	26.02	35.38	23.32	1.33	-4.92	2.46E+00	0.53	6.82
2008c	8.50	28.56	33.41	21.02	2.50	88.76	2.46E+00	0.27	2.53
2008c	1.50	28.16	26.29	15.81	2.61	67.27	2.46E+00	0.64	3.81
2002b	17.00	27.46	35.78	23.16	2.01	10.33	2.46E+00	0.78	7.50
2002b	11.00	29.06	30.48	18.66	2.98	77.57	4.91E+00	1.73	2.68
2002b	1.50	28.93	26.50	15.72	3.37	63.87	6.37E+02	1.60	3.07
2010B	21.00	27.41	35.98	23.32	2.41	36.30	2.46E+00	0.38	6.47
2010B	10.50	28.97	34.43	21.64	3.00	83.90	2.46E+00	0.81	2.51
2010B	2.50	28.98	33.74	21.13	3.09	81.77	2.46E+00	0.82	2.46
2007b	10.50	26.83	36.12	23.61	0.62	25.01	2.46E+00	0.51	5.98
2007b	5.50	28.44	23.53	13.65	2.75	57.94	2.46E+00	2.06	4.30
2007b	2.00	28.36	22.86	13.18	2.80	59.97	2.46E+00	1.83	4.07
2010A	59.50	20.10	36.41	25.81	2.33	47.90	2.46E+00	0.08	6.44
2010A	35.00	22.26	36.40	25.21	3.38	92.25	2.46E+00	0.08	2.14
2010A	3.00	29.62	29.45	17.70	3.31	71.61	2.46E+00	0.66	3.60
2012a	31.00	21.49	36.38	25.41	1.90	38.32	2.46E+00	0.11	6.06
2012a	15.50	22.63	36.28	25.01	2.12	73.49	2.46E+00	0.09	3.71
2012a	2.50	29.88	19.39	10.11	3.31	37.62	9.63E+01	1.99	6.78
2016A	56.50	20.35	36.43	25.76	2.66	65.86	2.46E+00	0.08	4.53
2016A	28.00	22.20	36.37	25.20	2.99	79.09	2.46E+00	0.07	3.22
2016A	2.50	28.79	15.60	7.63	2.61	24.26	7.01E+01	1.60	7.50
2007a	8.50	27.90	34.68	22.19	1.35	74.09	2.46E+00	0.20	3.37
2007a	5.00	29.50	20.79	11.28	3.20	50.29	2.46E+00	1.70	4.91
2007a	2.50	29.75	18.71	9.64	4.07	37.81	2.46	2.80	5.41
2012b	17.50	27.10	35.99	23.43	0.81	58.60	2.46	0.27	4.64
2012b	10.00	28.67	32.90	20.60	1.83	77.18	4.44	0.56	2.88
2012b	1.00	30.29	24.61	13.86	4.46	65.47	1482	2.37	0.01
2016C	21.50	26.02	35.33	23.28	1.62	35.16	19.29	0.45	7.99
2016C	10.00	28.77	31.08	19.20	3.24	90.16	123.8	0.18	2.17
2016C	2.00	29.15	30.57	18.69	3.23	90.69	782.4	0.14	2.11
2007c	9.50	27.25	34.23	22.06	0.13	28.98	2.46	3.45	7.35
2007c	5.00	29.45	26.91	15.86	3.47	82.89	2.46	0.96	2.21
2007C	2.00	29.08	25.95	15.26	3.88	78.58	2.46	1.64	2.51
2002c	20.50	25.13	35.53	23.70	0.73	39.48	2.46	0.26	7.08
2002c	10.00	28.73	30.54	18.81	3.31	90.51	5.039	0.21	2.10
2002c	2.50	28.63	25.62	15.16	3.69	71.63	55.54	3.13	2.57

Table A.3. Raw data from August 2004

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
3002a	8/22/2004	8:19	29.14	-89.8	A	1.7E+06	20.2	Surface	1.51
3002a	8/22/2004	8:19	29.14	-89.8	A	1.2E+06	20.2	middle	10.07
3002a	8/22/2004	8:19	29.14	-89.8	A	1.3E+06	20.2	bottom	19.13
3007a	8/22/2004	19:20	29.12	-89.5	A	5.7E+06	11.1	Surface	nd
3007a	8/22/2004	19:20	29.12	-89.5	A	3.4E+06	11.1	middle	nd
3007a	8/22/2004	19:20	29.12	-89.5	A	3.7E+06	11.1	bottom	nd
3010a	8/22/2004	14:43	28.88	-89.7	A	4.6E+06	60.8	Surface	2.01
3010a	8/22/2004	14:43	28.88	-89.7	A	1.8E+06	60.8	middle	35.24
3010a	8/22/2004	14:43	28.88	-89.7	A	1.9E+06	60.8	bottom	58.90
3012a	8/23/2004	1:28	28.96	-89.5	A	5.2E+06	39.7	Surface	1.51
3012a	8/23/2004	1:28	28.96	-89.5	A	1.4E+06	39.7	middle	19.63
3012a	8/23/2004	1:28	28.96	-89.5	A	1.6E+06	39.7	bottom	38.26
3016a	8/23/2004	3:56	28.84	-89.5	A	0.0E+00	60.0	Surface	2.01
3016a	8/23/2004	3:56	28.84	-89.5	A	0.0E+00	60.0	middle	30.21
3016a	8/23/2004	3:56	28.84	-89.5	A	0.0E+00	60.0	bottom	56.89
3002b	8/24/2004	11:23	28.72	-90.8	B	8.0E+05	18.3	Surface	2.52
3002b	8/24/2004	11:23	28.72	-90.8	B	1.4E+06	18.3	middle	9.06
3002b	8/24/2004	11:23	28.72	-90.8	B	2.0E+06	18.3	bottom	17.12
3007b	8/21/2004	12:55	28.96	-90.5	B	1.5E+06	12.4	Surface	2.01
3007b	8/21/2004	12:55	28.96	-90.5	B	0.0E+00	12.4	middle	6.04
3007b	8/21/2004	12:55	28.96	-90.5	B	2.1E+06	12.4	bottom	9.50
3010b	8/24/2004	5:00	28.62	-90.6	B	2.3E+06	22.5	middle	10.07
3010b	8/24/2004	5:00	28.62	-90.552	B	1.6E+06	22.5	bottom	20.14
3008b	8/21/2004	16:50	28.87	-90.372	B	1.4E+06	20.5	Surface	2.24
3008b	8/21/2004	16:50	28.87	-90.372	B	1.5E+06	20.5	middle	10.57
3008b	8/21/2004	16:50	28.87	-90.372	B	1.5E+06	20.5	bottom	18.12
3018b	8/24/2004	0:22	28.78	-90.32	B	1.4E+06	22.2	Surface	2.01
3018b	8/24/2004	0:22	28.78	-90.32	B	1.5E+06	22.2	middle	10.57
3018b	8/24/2004	0:22	28.78	-90.32	B	1.7E+06	22.2	bottom	20.64
3002c	8/26/2004	3:17	29.06	-92.374	C	0.0E+00	21.3	Surface	1.51
3002c	8/26/2004	3:17	29.06	-92.374	C	1.6E+06	21.3	middle	10.07
3002c	8/26/2004	3:17	29.06	-92.374	C	0.0E+00	21.3	bottom	18.62
3007c	8/25/2004	7:58	29.12	-91.909	C	7.9E+05	10.7	Surface	2.01
3007c	8/25/2004	7:58	29.12	-91.909	C	7.5E+05	10.7	middle	5.03
3007c	8/25/2004	7:58	29.12	-91.909	C	0.0E+00	10.7	bottom	9.57
3008c	8/25/2004	13:21	29.00	-91.999	C	2.4E+06	19.7	Surface	2.01
3008c	8/25/2004	13:21	29.00	-91.999	C	0.0E+00	19.7	middle	9.57
3008c	8/25/2004	13:21	29.00	-91.999	C	2.0E+06	19.7	bottom	19.13
3010c	8/25/2004	16:50	28.80	-92.131	C	1.5E+06	31.4	Surface	2.01
3010c	8/25/2004	16:50	28.80	-92.131	C	1.5E+06	31.4	middle	16.11
3010c	8/25/2004	16:50	28.80	-92.131	C	0.0E+00	31.4	bottom	31.21
3016c	8/25/2004	0:05	28.88	-91.733	C	3.2E+06	21.1	Surface	1.51
3016c	8/25/2004	0:05	28.88	-91.733	C	5.5E+05	21.1	middle	10.07
3016c	8/25/2004	0:05	28.88	-91.733	C	7.1E+05	21.1	bottom	19.60

Table A.3. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
3002a	1.50	29.10	26.70	15.81	3.81	65.84	2.46E+00	1.97	2.87
3002a	10.00	28.96	31.50	19.45	2.98	86.74	2.46E+00	0.42	2.11
3002a	19.00	25.85	35.77	23.66	1.13	8.45	2.46E+00	0.29	7.50
3007a	nd	nd	nd	nd	nd	nd	nd	nd	nd
3007a	nd	nd	nd	nd	nd	nd	nd	nd	nd
3007a	nd	nd	nd	nd	nd	nd	nd	nd	nd
3010a	2.00	28.95	26.31	15.57	3.87	71.49	1.35E+02	1.36	2.68
3010a	35.00	23.88	36.27	24.64	2.13	87.54	2.46E+00	0.09	2.30
3010a	58.50	20.70	36.41	25.65	1.52	51.31	2.46E+00	0.16	5.91
3012a	1.50	29.96	26.83	15.63	4.24	70.02	2.46E+00	0.86	2.73
3012a	19.50	27.04	35.45	23.04	2.86	87.46	2.46E+00	0.15	2.42
3012a	38.00	24.11	36.27	24.57	1.80	31.74	2.46E+00	0.14	7.45
3016a	2.00	29.85	25.97	15.03	4.22	71.17	2.46E+00	0.79	2.68
3016a	30.00	24.36	36.22	24.46	2.87	88.12	2.46E+00	0.13	2.12
3016a	56.50	21.16	36.41	25.52	2.07	57.25	2.46E+00	0.14	4.89
3002b	2.50	29.76	29.90	17.99	3.37	86.69	2.46E+00	0.32	2.18
3002b	9.00	29.77	30.03	18.08	3.42	85.81	2.46E+00	0.40	2.19
3002b	17.00	28.75	34.53	21.79	1.58	38.22	2.46E+00	0.46	4.59
3007b	2.00	29.12	27.73	16.58	3.35	65.55	2.21E+02	1.02	3.46
3007b	6.00	29.13	27.84	16.66	3.34	67.43	2.96E+01	0.98	3.34
3007b	9.50	28.75	29.66	18.14	2.57	81.99	7.74E+00	0.37	2.84
3010b	10.00	29.17	31.69	19.52	3.02	88.63	2.46E+00	0.21	2.14
3010b	20.00	28.81	34.46	21.72	1.50	78.26	2.46	0.68	2.64
3008b	2.00	29.62	27.75	16.43	3.41	81.94	1087	0.31	2.44
3008b	10.50	28.88	30.86	19.00	2.04	62.51	39.59	0.78	4.55
3008b	18.00	28.38	34.47	21.87	1.82	17.55	2.468	0.33	5.85
3018b	2.00	29.68	28.80	17.19	3.09	81.43	23.51	0.47	2.31
3018b	10.50	29.50	33.60	20.84	2.97	88.62	2.504	0.25	2.22
3018b	20.50	26.77	35.67	23.30	2.37	71.20	2.46	0.54	3.42
3002c	1.50	29.61	27.73	16.41	3.14	86.74	2.46	0.23	2.28
3002c	10.00	28.83	30.45	18.71	2.75	86.46	2.46	0.36	2.25
3002c	18.50	27.63	33.94	21.72	0.81	75.88	2.46	0.49	2.96
3007c	2.00	29.39	26.88	15.85	3.11	80.17	2.46	0.82	2.56
3007c	5.00	29.39	26.88	15.85	3.14	80.46	2.46	0.80	2.58
3007c	9.50	28.20	30.61	19.04	0.98	20.47	2.46	0.52	7.50
3008c	2.00	28.76	29.19	17.79	2.92	87.74	80.1	0.33	2.24
3008c	9.50	29.01	31.22	19.22	2.90	89.45	12.86	0.24	2.19
3008c	19.00	28.18	33.43	21.16	1.51	76.31	2.444	0.27	3.03
3010c	2.00	29.72	33.47	20.68	2.97	90.65	1374	0.11	2.08
3010c	16.00	27.69	34.69	22.26	2.28	88.21	286.5	0.54	2.12
3010c	31.00	24.21	35.84	24.22	0.51	61.37	23.16	0.66	4.22
3016c	1.50	29.55	31.67	19.38	3.01	85.72	71.96	0.27	2.30
3016c	10.00	29.76	33.46	20.65	3.08	89.77	12.44	0.11	2.08
3016c	19.50	26.80	35.31	23.02	1.77	84.67	5.595	0.53	2.43

Table A.4. Raw data from March 2005

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
4007a	3/27/2005	0:58	29.12	-89.5	A	5.0E+06	11.0	bottom	9.57
4007a	3/27/2005	0:58	29.12	-89.5	A	3.0E+06	11.0	surface	1.53
4007a	3/27/2005	0:58	29.12	-89.5	A	0.0E+00	11.0	middle	5.04
4012a	3/27/2005	3:57	28.99	-89.5	A	4.9E+06	20.0	bottom	19.62
4012a	3/27/2005	3:57	28.99	-89.5	A	1.1E+06	20.0	middle	10.08
4012a	3/27/2005	3:57	28.99	-89.5	A	0.0E+00	20.0	surface	1.01
4011b	3/26/2005	13:50	28.98	-90.4	B	0.0E+00	13.0	bottom	1.54
4011b	3/26/2005	13:50	28.98	-90.4	B	2.6E+06	13.0	middle	6.55
4011b	3/26/2005	13:50	28.98	-90.4	B	3.2E+06	13.0	surface	12.08
4012b	3/26/2005	11:14	28.86	-90.4	B	2.8E+06	20.9	bottom	20.13
4012b	3/26/2005	11:14	28.86	-90.4	B	0.0E+00	20.9	middle	10.07
4012b	3/26/2005	11:14	28.86	-90.4	B	0.0E+00	20.9	surface	1.02
4014b	3/26/2005	7:59	28.65	-90.4	B	9.2E+05	23.3	bottom	22.14
4014b	3/26/2005	7:59	28.65	-90.4	B	0.0E+00	23.3	middle	10.07
4014b	3/26/2005	7:59	28.65	-90.4	B	1.3E+06	23.3	surface	1.52
4007c	3/24/2005	13:26	29.12	-91.9	C	1.6E+06	10.2	bottom	9.56
4007c	3/24/2005	13:26	29.12	-91.9	C	1.7E+06	10.2	middle	5.03
4007c	3/24/2005	13:26	29.12	-91.9	C	1.7E+06	10.2	surface	2.01
4008c	3/24/2005	11:22	28.00	-91.0	C	2.9E+06	19.2	bottom	18.62
4008c	3/24/2005	11:22	28.00	-91.0	C	0.0E+00	19.2	middle	9.57
4008c	3/24/2005	11:22	28.00	-91.0	C	0.0E+00	19.2	surface	1.51
4010c	3/24/2005	8:45	28.80	-92.1	C	2.2E+06	31.2	bottom	30.20
4010c	3/24/2005	8:45	28.80	-92.1	C	1.1E+06	31.2	middle	15.10
4010c	3/24/2005	8:45	28.80	-92.125	C	0.0E+00	31.2	surface	2.01

Table A.4. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
4007a	9.50	20.45	34.58	24.32	0.90	12.77	2.46E+00	0.44	7.13
4007a	1.50	20.90	22.68	15.17	6.41	71.21	2.46E+00	1.79	3.23
4007a	5.00	20.87	22.77	15.25	6.50	71.70	2.46E+00	1.91	3.25
4012a	19.50	20.94	35.99	25.27	2.46	53.46	2.46E+00	0.13	4.63
4012a	10.00	19.50	27.46	19.15	3.32	72.81	2.46E+00	0.85	3.32
4012a	1.00	20.58	20.47	13.57	6.81	56.56	2.46E+00	3.75	4.28
4011b	1.50	20.78	25.68	17.47	5.95	70.69	2.46E+00	1.68	3.05
4011b	6.50	20.21	26.69	18.38	6.15	71.47	2.46E+00	1.52	3.18
4011b	12.00	20.41	34.08	23.95	0.67	30.02	2.46E+00	0.61	7.46
4012b	20.00	20.82	35.83	25.17	1.96	27.78	2.46E+00	0.30	7.38
4012b	10.00	20.19	33.43	23.52	3.52	84.11	2.46E+00	1.01	2.58
4012b	1.00	20.28	25.09	17.15	6.61	69.42	2.46E+00	1.81	3.18
4014b	22.00	20.42	35.19	24.80	3.42	69.50	2.46E+00	0.38	3.34
4014b	10.00	20.31	33.34	23.41	4.90	84.99	2.46E+00	0.88	2.29
4014b	1.50	20.57	30.02	20.82	6.31	68.82	2.46E+00	3.09	2.77
4007c	9.50	19.20	32.39	22.97	1.16	35.72	2.46E+00	0.44	7.52
4007c	5.00	19.06	23.79	16.46	5.65	53.73	8.41E+00	4.47	3.95
4007c	2.00	18.45	17.69	11.96	6.60	44.52	7.20E+01	6.11	4.91
4008c	18.50	20.07	34.97	24.72	2.67	50.67	2.46E+00	0.24	5.89
4008c	9.50	19.12	31.45	22.28	4.00	89.00	2.46E+00	0.33	2.31
4008c	1.50	18.87	21.22	14.56	6.96	42.92	2.46E+00	6.36	5.10
4010c	30.00	20.35	36.15	25.55	3.85	65.52	2.46E+00	0.19	4.16
4010c	15.00	20.30	35.86	25.34	4.08	90.26	2.46E+00	0.25	2.22
4010c	2.00	19.23	29.64	20.87	5.44	78.21	2.46	1.36	2.56

Table A.5. Raw data from May 2005

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
5011a	5/23/2005	12:51	29.04	-89.5	A	2.3E+06	11.9	surface	2.01
5011a	5/23/2005	12:51	29.04	-89.5	A	2.0E+06	11.9	middle	6.04
5011a	5/23/2005	12:51	29.04	-89.5	A	2.9E+06	11.9	bottom	11.07
5012a	5/23/2005	14:40	28.99	-89.5	A	2.7E+06	20.9	surface	2.01
5012a	5/23/2005	14:40	28.99	-89.5	A	2.0E+06	20.9	middle	10.57
5012a	5/23/2005	14:40	28.99	-89.5	A	2.6E+06	20.9	bottom	20.64
5011b	5/23/2005	2:17	28.98	-90.4	B	5.1E+06	12.3	surface	1.51
5011b	5/23/2005	2:17	28.98	-90.4	B	2.1E+06	12.3	middle	6.04
5011b	5/23/2005	2:17	28.98	-90.4	B	3.6E+06	12.3	bottom	11.52
5012b	5/23/2005	0:21	28.87	-90.4	B	2.5E+06	20.2	surface	1.51
5012b	5/23/2005	0:21	28.87	-90.4	B	1.9E+06	20.2	middle	10.57
5012b	5/23/2005	0:21	28.87	-90.4	B	2.9E+06	20.2	bottom	19.13
5014b	5/22/2005	22:05	28.65	-90.4	B	2.3E+06	21.3	surface	1.51
5014b	5/22/2005	22:05	28.65	-90.4	B	1.5E+06	21.3	middle	10.07
5014b	5/22/2005	22:05	28.65	-90.4	B	1.7E+06	21.3	bottom	20.14
5007c	5/21/2005	17:06	29.12	-91.9	C	4.5E+06	10.3	surface	2.01
5007c	5/21/2005	17:06	29.12	-91.9	C	3.8E+06	10.3	middle	5.04
5007c	5/21/2005	17:06	29.12	-91.9	C	3.2E+06	10.3	bottom	9.06
5008c	5/21/2005	11:44	28.95	-92.0	C	2.6E+06	21.8	surface	2.01
5008c	5/21/2005	11:44	28.95	-92.0	C	1.5E+06	21.8	middle	11.07
5008c	5/21/2005	11:44	28.95	-92.0	C	2.2E+06	21.8	bottom	21.14
5010c	5/21/2005	7:13	28.80	-92.1	C	2.2E+06	31.6	surface	1.54
5010c	5/21/2005	7:13	28.80	-92.1	C	1.2E+06	31.6	middle	16.11
5010c	5/21/2005	7:13	28.80	-92.13	C	1.3E+06	31.6	bottom	31.21

Table A.5. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
5011a	2.00	27.89	20.07	11.25	5.04	73.72	1.43E+02	2.32	1.70
5011a	6.00	25.65	27.14	17.22	2.47	74.46	2.07E+01	2.61	1.94
5011a	11.00	24.37	35.84	24.17	0.69	23.12	3.57E+00	0.32	7.07
5012a	2.00	28.00	19.77	10.99	6.05	58.09	2.60E+02	5.20	2.99
5012a	10.50	24.08	35.31	23.85	2.13	86.76	8.99E+00	0.17	1.45
5012a	20.50	23.66	36.29	24.72	2.81	33.99	9.44E-01	0.13	6.47
5011b	1.50	28.02	27.00	16.39	4.08	47.45	6.87E-01	2.39	3.87
5011b	6.00	25.47	33.68	22.20	3.94	86.90	3.35E-01	0.33	1.57
5011b	11.50	22.74	35.28	24.22	0.17	56.66	3.37E-01	0.45	3.64
5012b	1.50	28.44	26.79	16.10	4.83	60.22	5.08E+01	2.66	2.23
5012b	10.50	23.98	34.93	23.59	3.79	88.71	1.69E+00	0.28	1.37
5012b	19.00	23.27	36.06	24.66	3.31	61.67	6.67E-01	0.34	3.22
5014b	1.50	27.90	24.83	14.80	5.03	59.16	2.52E+02	2.54	1.94
5014b	10.00	23.09	35.11	23.99	3.41	88.57	1.16E+01	0.43	1.33
5014b	20.00	22.35	36.13	24.98	2.85	74.04	5.35E+00	0.48	2.39
5007c	2.00	26.90	26.85	16.62	4.50	45.53	1.39E+03	2.76	3.34
5007c	5.00	26.85	26.88	16.66	4.50	45.96	1.93E+02	5.84	3.28
5007c	9.00	24.50	29.11	19.04	2.08	72.18	1.66E+01	0.38	2.86
5008c	2.00	25.99	28.50	18.15	4.15	88.47	1.21E+02	0.17	1.33
5008c	11.00	25.63	32.69	21.41	4.04	89.01	1.12E+01	0.17	1.33
5008c	21.00	21.63	35.71	24.87	2.59	51.28	1.86E+00	0.49	4.46
5010c	1.50	26.13	31.40	20.28	4.05	88.64	8.24E-01	0.14	1.30
5010c	16.00	22.11	35.41	24.50	4.00	90.66	3.35E-01	0.11	1.18
5010c	31.00	20.83	36.23	25.48	3.20	73.03	0.3319	0.57	2.53

Table A.6. Raw data from July 2005

Site	Date	Time (GMT)	LAT	LONG	Zone	Bacterial Abundance (cells mL^{-1})	Site Depth (m)	Water Column Location	Press.
6bc2	7/13/2005	1:12	28.68	-91.2	B	2.3E+06	20.8	surface	2.01
6bc2	7/13/2005	1:12	28.68	-91.2	B	1.7E+06	20.8	middle	10.07
6bc2	7/13/2005	1:12	28.68	-91.2	B	1.5E+06	20.8	bottom	20.13
6007c	7/12/2005	17:33	29.12	-91.9	C	1.8E+06	11.2	surface	2.02
6007c	7/12/2005	17:33	29.12	-91.9	C	1.7E+06	11.2	middle	5.54
6007c	7/12/2005	17:33	29.12	-91.9	C	1.9E+06	11.2	bottom	10.57
6008c	7/8/2005	23:53	29.00	-92.0	C	2.3E+06	19.4	surface	1.51
6008c	7/8/2005	23:53	29.00	-92.0	C	0.0E+00	19.4	middle	9.06
6008c	7/8/2005	23:53	29.00	-92.0	C	2.1E+06	19.4	bottom	18.62
6010c	7/9/2005	6:03	28.80	-92.1	C	0.0E+00	31.1	surface	1.51
6010c	7/9/2005	6:03	28.80	-92.1	C	3.4E+05	31.1	middle	15.61
6010c	7/9/2005	6:03	28.80	-92.1	C	8.8E+05	31.1	bottom	30.70
6010d	7/9/2005	23:52	29.63	-93.6	D	5.7E+05	10.3	surface	1.51
6010d	7/9/2005	23:52	29.63	-93.6	D	1.1E+06	10.3	middle	4.53
6010d	7/9/2005	23:52	29.63	-93.6	D	8.9E+05	10.3	bottom	9.06
6013d2	7/10/2005	10:28	29.00	-93.7	D	0.0E+00	19.9	surface	1.01
6013d2	7/10/2005	10:28	29.00	-93.7	D	0.0E+00	19.9	middle	8.56
6013d2	7/10/2005	10:28	29.00	-93.7	D	1.6E+06	19.9	bottom	19.63
6017d	7/10/2005	17:35	29.12	-93.5	D	1.2E+06	20.0	surface	2.01
6017d	7/10/2005	17:35	29.12	-93.5	D	3.8E+05	20.0	middle	9.56
6017d	7/10/2005	17:35	29.12	-93.5	D	1.1E+06	20.0	bottom	18.62
6016d	7/10/2005	19:23	29.30	-93.5	D	2.1E+05	15.2	surface	2.01
6016d	7/10/2005	19:23	29.30	-93.5	D	2.4E+06	15.2	middle	7.55
6016d	7/10/2005	19:23	29.30	-93.501	D	1.1E+06	15.2	bottom	14.58
6029d	7/11/2005	12:01	29.16	-92.899	D	1.6E+06	20.1	surface	1.51
6029d	7/11/2005	12:01	29.16	-92.899	D	9.6E+05	20.1	middle	10.07
6029d	7/11/2005	12:01	29.16	-92.899	D	0.0E+00	20.1	bottom	19.63

Table A.6. Continued

Site	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
6bc2	29.72	33.00	20.32	4.07	79.22	3.70E+00	0.39	1.64
6bc2	28.59	34.28	21.66	3.32	45.25	6.54E-01	0.83	4.90
6bc2	27.48	34.97	22.54	2.56	11.55	3.21E-01	0.79	7.50
6007c	30.07	27.41	16.02	4.17	84.95	1.86E+03	0.49	1.45
6007c	29.52	28.34	16.90	3.77	67.50	6.42E+02	1.49	2.27
6007c	29.56	28.68	17.14	2.93	32.60	6.95E+01	2.18	5.38
6008c	30.22	28.69	16.93	4.78	73.28	7.94E+02	0.63	1.99
6008c	29.23	30.30	18.46	3.85	79.72	2.01E+01	1.44	1.97
6008c	26.48	34.81	22.74	1.50	24.19	1.26E+00	0.88	5.69
6010c	29.66	31.92	19.53	3.91	88.55	7.05E-01	0.12	1.24
6010c	29.03	33.88	21.21	3.91	90.36	3.34E-01	0.12	1.15
6010c	24.61	35.77	24.04	3.26	38.16	3.25E-01	0.74	5.11
6010d	30.62	27.85	16.16	4.70	70.01	5.55E+02	1.25	1.62
6010d	30.21	27.82	16.28	4.33	69.27	1.13E+02	1.89	2.32
6010d	29.94	27.86	16.41	4.00	75.75	1.19E+01	1.27	2.07
6013d2	29.76	31.08	18.87	3.83	90.28	1.10E+00	0.10	1.17
6013d2	29.85	31.18	18.92	3.85	90.68	3.64E-01	0.10	1.18
6013d2	28.05	32.52	20.52	3.20	81.93	3.28E-01	0.58	1.51
6017d	30.17	30.40	18.23	3.84	90.05	1.93E+03	0.09	1.19
6017d	30.11	30.42	18.26	3.84	89.93	6.41E+02	0.12	1.18
6017d	28.16	32.37	20.37	2.97	73.80	1.92E+02	0.54	1.90
6016d	30.50	27.57	16.00	3.86	85.00	1.74E+03	0.24	1.39
6016d	30.47	27.86	16.23	3.82	85.77	3.40E+02	0.32	1.37
6016d	30.13	30.67	18.44	2.63	10.55	38.623	0.73	4.74
6029d	30.19	29.43	17.49	3.74	89.82	75.737	0.13	1.25
6029d	30.31	30.15	17.99	3.78	89.89	14.076	0.15	1.21
6029d	25.86	34.12	22.41	0.49	29.30	4.6306	0.82	7.06

Table A.7. Raw data from August 2005

Site	Date	Time (GMT)	LAT	LONG	Site Depth (m)	Zone	Bacterial Abundance (cells mL^{-1})	Water Column Location	Press.
7014d	8/18/2005	23:35	29.7	-93.5	9.3	D	3.2E+06	surface	1.51
7014d	8/18/2005	23:35	29.7	-93.5	9.3	D	0.0E+00	bottom	8.56
7016d	8/18/2005	19:56	29.3	-93.5	15.5	D	0.0E+00	surface	1.51
7016d	8/18/2005	19:56	29.3	-93.5	15.5	D	2.0E+06	middle	8.06
7016d	8/18/2005	19:56	29.3	-93.5	15.5	D	0.0E+00	bottom	15.09
7017d	8/18/2005	17:16	29.1	-93.5	20.1	D	2.1E+06	surface	1.51
7017d	8/18/2005	17:16	29.1	-93.5	20.1	D	0.0E+00	middle	10.07
7017d	8/18/2005	17:16	29.1	-93.5	20.1	D	1.7E+06	bottom	19.63
7007c	8/20/2005	20:24	29.1	-91.9	10.4	C	2.0E+06	surface	2.01
7007c	8/20/2005	20:24	29.1	-91.9	10.4	C	0.0E+00	middle	5.03
7007c	8/20/2005	20:24	29.1	-91.9	10.4	C	3.3E+06	bottom	9.56
7008c	8/20/2005	18:27	29.0	-92.0	19.6	C	4.8E+06	surface	1.51
7008c	8/20/2005	18:27	29.0	-92.0	19.6	C	1.4E+06	middle	10.57
7008c	8/20/2005	18:27	29.0	-92.0	19.6	C	0.0E+00	bottom	19.13
7010c	8/20/2005	15:53	28.8	-92.1	31.6	C	2.1E+06	surface	1.52
7010c	8/20/2005	15:53	28.8	-92.1	31.6	C	1.7E+06	middle	15.61
7010c	8/20/2005	15:53	28.8	-92.1	31.6	C	2.3E+06	bottom	31.21
7011b	8/22/2005	0:20	29.0	-90.4	12.7	B	3.1E+06	surface	1.51
7011b	8/22/2005	0:20	29.0	-90.4	12.7	B	1.3E+06	middle	7.05
7011b	8/22/2005	0:20	29.0	-90.4	12.7	B	7.0E+06	bottom	12.06
7012b	8/21/2005	20:55	28.9	-90.4	20.7	B	3.0E+06	surface	1.51
7012b	8/21/2005	20:55	28.9	-90.4	20.7	B	0.0E+00	middle	10.07
7012b	8/21/2005	20:55	28.9	-90.4	20.7	B	2.7E+06	bottom	20.64

Table A.7. Continued

Site	Depth (m)	Temp (°C)	Sal. (PSS)	Sig-Th (kg m^{-3})	O_2 (mL L^{-1})	%Tran	PAR (mE m^{-2} s^{-1})	FL. (µg L^{-1})	OBS (mg L^{-1})
7014d	1.5	31.67	27.76	15.74	3.85	69.73	1.67E+02	0.64	2.32
7014d	8.5	30.85	28.15	16.32	2.34	40.53	8.02E+00	1.11	5.13
7016d	1.5	31.25	28.93	16.76	3.97	83.52	2.85E+03	0.39	1.37
7016d	8	31.22	30.10	17.64	4.03	84.28	5.58E+02	0.75	1.30
7016d	15	28.07	33.78	21.46	0.19	70.20	9.28E+01	1.27	2.41
7017d	1.5	31.32	30.23	17.70	3.97	86.40	2.38E+03	0.25	1.28
7017d	10	31.21	31.82	18.93	3.31	87.21	4.84E+02	0.35	1.23
7017d	19.5	29.82	34.84	21.66	2.17	4.94	5.94E+01	0.71	8.40
7007c	2	32.18	26.02	14.27	3.96	72.74	1.46E+03	0.75	1.82
7007c	5	31.76	28.80	16.49	4.00	83.13	2.86E+02	0.65	1.36
7007c	9.5	30.48	32.41	19.62	0.62	38.20	6.92E+01	0.99	4.56
7008c	1.5	31.98	27.51	15.45	4.26	76.83	1.78E+03	0.68	1.61
7008c	10.5	28.81	33.95	21.34	1.47	86.83	1.19E+02	0.80	1.23
7008c	19	27.15	35.25	22.86	0.19	24.95	2.82E+01	0.50	6.80
7010c	1.5	31.54	30.70	17.98	3.76	88.87	2.47E+03	0.10	1.16
7010c	15.5	30.78	33.30	20.19	3.77	89.93	3.35E+02	0.16	1.14
7010c	31	24.82	35.89	24.07	1.58	50.12	3.42E+01	0.70	4.07
7011b	1.5	33.14	24.23	12.61	4.07	62.86	1.73E+01	1.00	2.42
7011b	7	30.98	32.06	19.19	1.16	82.79	1.05E+00	0.72	1.50
7011b	12	29.40	34.36	21.45	0.23	48.82	4.90E-01	0.30	4.32
7012b	1.5	32.33	30.67	17.68	3.86	86.07	4.21E+02	0.29	1.28
7012b	10	31.19	33.85	20.46	3.81	88.27	5.79E+01	0.42	1.19
7012b	20.5	28.28	35.34	22.56	0.62	66.99	1.14E+01	0.89	3.27

APPENDIX B

BOTTOM WATER DISSOLVED OXYGEN CONCENTRATIONS

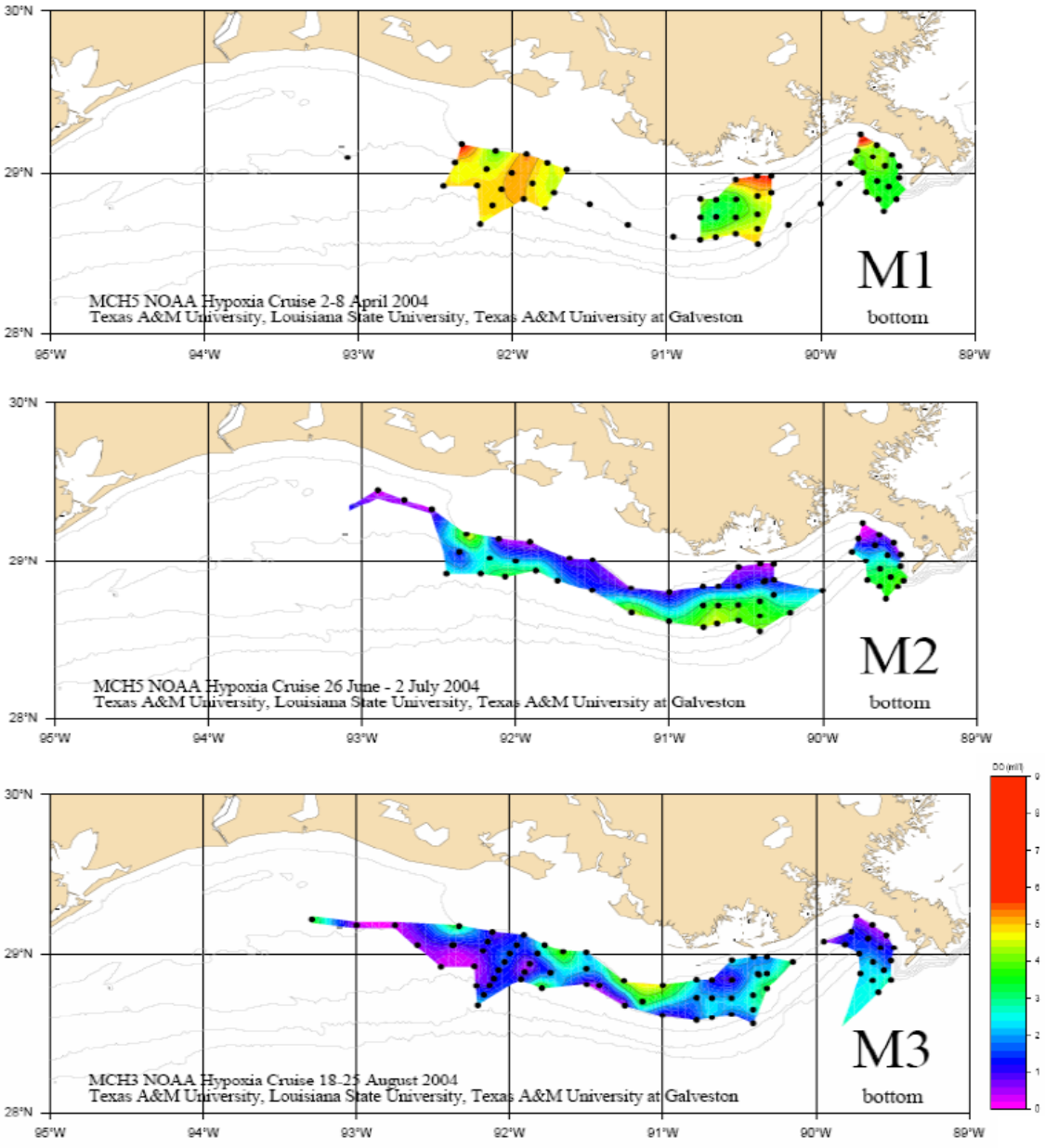

Fig B.1. Bottom water Dissolved Oxygen Concentrations from 2004. Measurements were taken during (M1) April, (M2) June and (M3) August of 2004 from bottom water approximately 0.5 m above the seafloor. Dissolved Oxygen concentrations were obtained through automated potentiometric titrations using a titroprocessor and then calibrated by Winkler titration of discrete bottom water samples collected by specially fashioned Niskin bottles triggered by contact with the seafloor. Contour plots were generated using Generic Mapping Tools (Wessel and Smith 1991). Points without surrounding contours lines had insufficient references to generate contour lines. (Plots courtesy, S. DiMarco, TAMU).

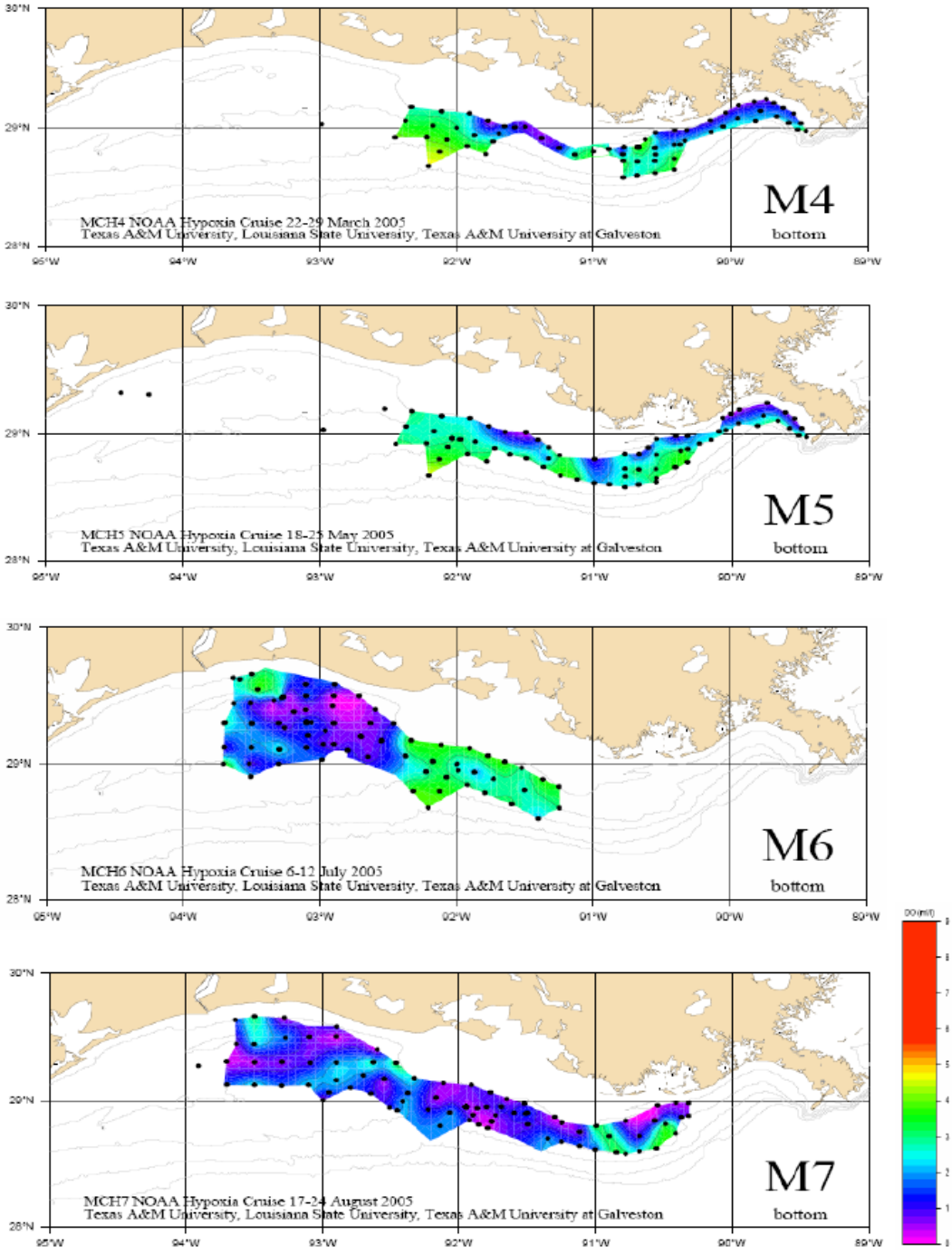

Fig B.2. Bottom water Dissolved Oxygen Concentrations from 2005. Measurements were taken during (M4) March, (M5) May, (M6) July and (M7) August of 2005 from bottom water approximately 0.5 m above the seafloor. Dissolved Oxygen concentrations were obtained through automated potentiometric titrations using a titroprocessor and then calibrated by Winkler titration of discrete bottom water samples collected by specially fashioned Niskin bottles triggered by contact with the seafloor. Contour plots were generated using Generic Mapping Tools (Wessel and Smith 1991). Points without surrounding contours lines had insufficient references to generate contour lines. (Plots courtesy, S. DiMarco, TAMU).

APPENDIX C

ATCHAFALAYA RIVER DISCHARGE

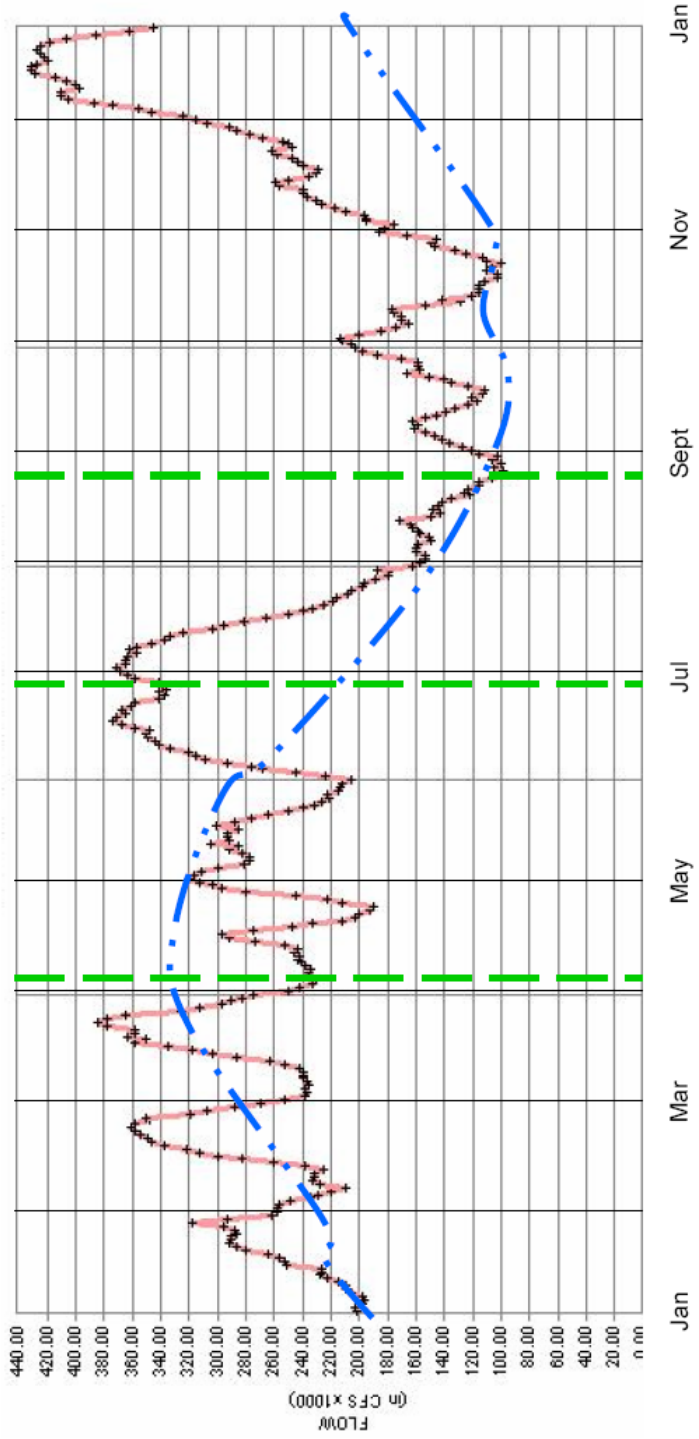

Fig. C.1. Atchafalaya 2004 Discharge Hydrograph. The flow rate (pink line), in cubic feet of water per second (CFS)x1000, of the Atchafalaya River is monitored by the New Orleans District U.S. Army Corps of Engineers (USACE) 110 miles from the coast at Simmesport, LA. The blue dashed line shows the average flow rate throughout the course of the year. Vertical green lines mark when samples were taken on the coast off of the Atchafalaya River. *Modified from U.S. Army Corps of Engineers database*

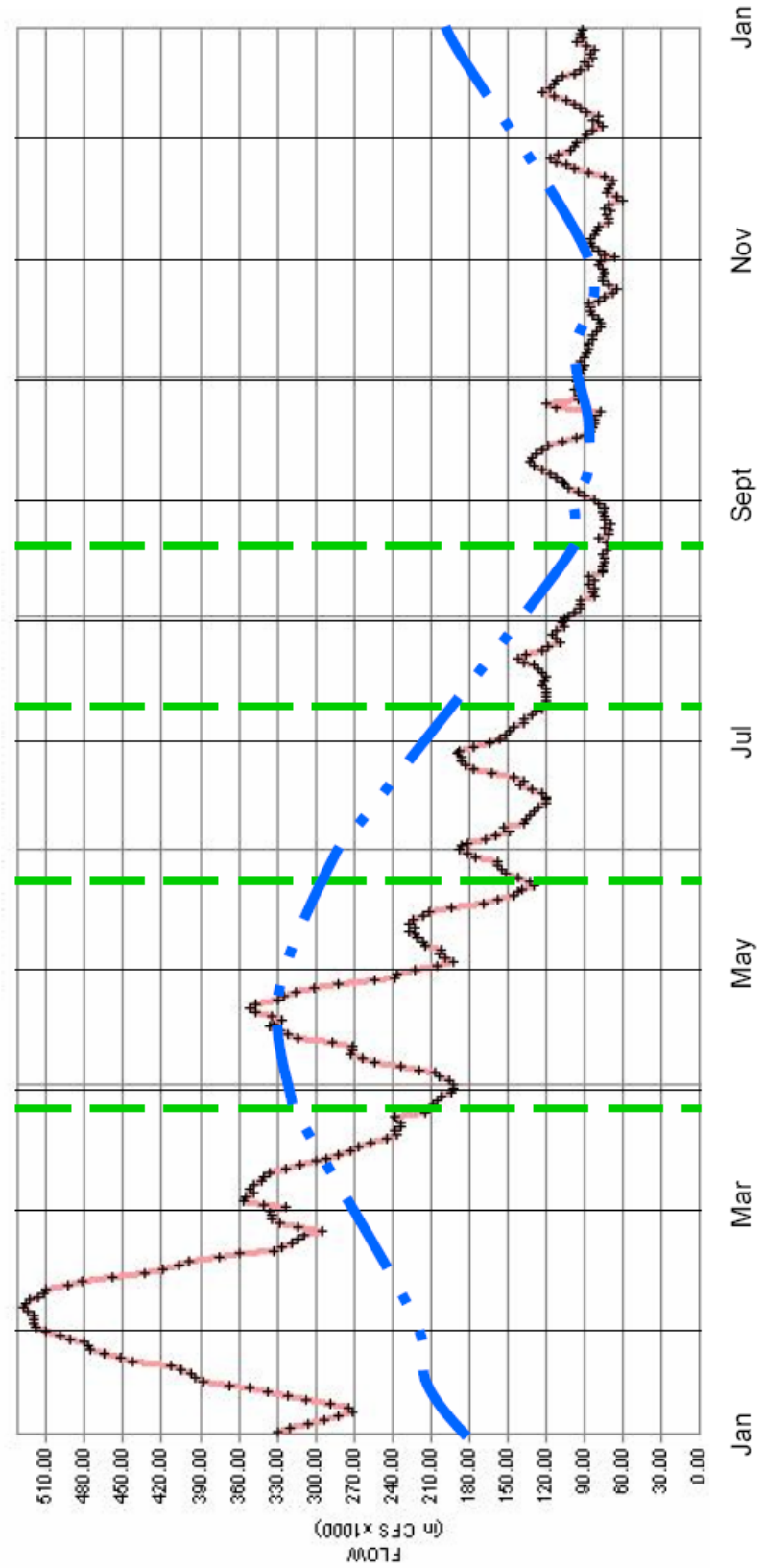

Fig. C.2. Atchafalaya 2005 Discharge Hydrograph. The flow rate (pink line) in cubic feet of water per second (CFS)x1000, of the Atchafalaya River is monitored by the New Orleans District U.S. Army Corps of Engineers (USACE) 110 miles from the coast at Simmesport, LA. The blue dashed line shows the average flow rate throughout the course of the year. Vertical green lines mark when samples were taken on the coast off of the Atchafalaya River. *Modified from U.S. Army Corps of Engineers database*

APPENDIX D

CYANOBACTERIA ABUNDANCES

Table D.1. Cyanobacteria abundances and percentage of the total bacterial population on the Texas-Louisiana Shelf. Due to the high frequency of Cyanobacteria affiliations from the clone libraries in Chapters 3 and 4, Cyanobacteria abundances, of sites from the center of each zone, were directly counted from prepared slides (as described in Chapter 3) using the Zeiss Axioplan Imaging 2 universal microscope (filter set 31). ND denotes no data present.

SITE	Depth	Cyanobacteria (cells mL^{-1})	% of Total Bacterial Population
1008c	SURFACE	2.6E+03	0.97
1008c	MIDDLE	1.5E+04	1.22
1008c	BOTTOM	9.1E+03	0.49
1012a	SURFACE	3.3E+02	0.04
1012a	MIDDLE	ND	ND
1012a	BOTTOM	ND	ND
1012b	SURFACE	1.7E+03	0.14
1012b	MIDDLE	2.0E+03	0.20
1012b	BOTTOM	7.1E+02	0.07
1016a	SURFACE	1.2E+03	0.22
1016a	MIDDLE	2.2E+03	0.51
1016a	BOTTOM	ND	ND
2002b	SURFACE	1.2E+04	ND
2002b	MIDDLE	3.8E+03	ND
2002b	BOTTOM	7.7E+02	ND
2007c	SURFACE	2.7E+04	1.49
2007c	MIDDLE	ND	ND
2007c	BOTTOM	1.3E+04	1.06
2010a	SURFACE	1.4E+05	4.58
2010a	MIDDLE	3.0E+03	0.45
2010a	BOTTOM	1.0E+03	0.13
3007a	SURFACE	1.4E+05	nd
3007a	MIDDLE	4.0E+04	2.13
3007a	BOTTOM	8.6E+03	nd
3010b	SURFACE	2.9E+04	nd
3010b	MIDDLE	5.5E+04	7.35
3010b	BOTTOM	2.0E+04	2.88
3010c	SURFACE	6.6E+03	0.43
3010c	MIDDLE	1.2E+04	0.75
3010c	BOTTOM	3.3E+03	ND
4007c	SURFACE	7.4E+02	0.05
4007c	MIDDLE	4.3E+03	0.26
4007c	BOTTOM	2.2E+03	0.13
4011b	SURFACE	ND	ND
4011b	MIDDLE	ND	ND
4011b	BOTTOM	1.8E+04	0.57
4012a	SURFACE	1.9E+04	0.39
4012a	MIDDLE	ND	ND
4012a	BOTTOM	ND	ND
4012b	SURFACE	1.1E+04	0.39
4012b	MIDDLE	1.0E+03	ND
4012b	BOTTOM	3.3E+02	ND
5007c	SURFACE	1.8E+05	3.91
5007c	MIDDLE	1.6E+05	4.33
5007c	BOTTOM	7.2E+04	2.26
5011a	SURFACE	5.2E+03	0.23
5011a	MIDDLE	7.4E+02	0.04
5011a	BOTTOM	2.7E+03	0.09
5011b	SURFACE	3.6E+04	0.71
5011b	MIDDLE	1.9E+04	0.90
5011b	BOTTOM	5.5E+04	1.53
5012a	SURFACE	1.0E+05	3.81
5012a	MIDDLE	2.3E+04	1.16
5012a	BOTTOM	6.3E+03	0.24
5012b	SURFACE	1.9E+04	0.76
5012b	MIDDLE	1.5E+04	0.79
5012b	BOTTOM	1.1E+04	0.38
6007c	SURFACE	3.1E+04	1.74
6007c	MIDDLE	4.6E+04	2.78
6007c	BOTTOM	4.0E+04	2.13
6016d	SURFACE	1.2E+04	5.91
6016d	MIDDLE	3.1E+04	1.31
6016d	BOTTOM	1.7E+04	1.58
7008c	SURFACE	1.9E+04	0.40
7008c	MIDDLE	3.5E+04	2.55
7008c	BOTTOM	1.8E+04	ND
7012b	SURFACE	2.6E+04	0.87
7012b	MIDDLE	ND	ND
7012b	BOTTOM	3.6E+04	1.31
7017d	SURFACE	ND	ND
7017d	MIDDLE	6.9E+04	4.16
7017d	BOTTOM	1.3E+05	6.41

APPENDIX E

SIMILARITY OF ACTIVE AND TOTAL SITE COMMUNITIES

Table E.1. Similarity of Total and BrdU-labeled communities at each site. The pair-wise Jaccard similarity coefficient was calculated from the presence of OTUs of Total and BrdU-labeled communities at each site using NTSYSpc.

MARCH 2005	
SITE	COEFFICENT
4007am	0.23
4012am	0.33
4012ab	0.02
4011bs	0.23
4012bm	0.18
4014bs	0.10
4014bm	0.12
4014bb	0.21
4007cs	0.14
4008cm	0.35
4008cb	0.41

MAY 2005	
SITE	COEFFICENT
5012ab	0.22
5011bm	0.12
5011bb	0.07
5012bs	0.24
5012bm	0.11
5014bs	0.07
5014bm	0.09
5014bb	0.07
5007cb	0.06
5008cs	0.13
5008cm	0.22
5008cb	0.12
5010cs	0.08
5010cm	0.21

JULY 2005	
SITE	COEFFICENT
6008cs	0.38
6008cm	0.19
6008cb	0.32
6010cs	0.23
6010cm	0.11
6010cb	0.05
6010ds	0.38
6010dm	0.31
6010db	0.23
6013d2s	0.21
6013d2m	0.16
6013d2b	0.29
6017ds	0.32
6017dm	0.43
6017db	0.27
6016ds	0.20
6016dm	0.29
6016db	0.35
6029ds	0.33
6029dm	0.40
6029db	0.15

AUGUST 2005	
SITE	COEFFICENT
7014ds	0.07
7014db	0.12
7016ds	0.12
7017dm	0.03
7008cs	0.23
7008cm	0.33
7008cb	0.25
7010cs	0.21
7010cm	0.48
7010cb	0.23
7011bs	0.43
7011bm	0.32
7011bb	0.31
7012bs	0.39
7012bm	0.29
7012bb	0.47

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