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Tidal Influences on Bacterial and Phytoplankton Abundances and the Resulting Effects on Patterns of Dissolved Oxygen in the Skidaway River Estuary

Casey Colleen Brazell

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TIDAL INFLUENCES ON BACTERIAL AND PHYTOPLANKTON ABUNDANCES
AND THE RESULTING EFFECTS ON PATTERNS OF DISSOLVED OXYGEN IN
THE SKIDAWAY RIVER ESTUARY

by

CASEY COLLEEN BRAZELL

(Under the Direction of STEPHEN P. VIVES)

ABSTRACT

Two studies were conducted to investigate the physical and biological processes contributing to the dissolved oxygen (DO) availability in the Skidaway River Estuary (SRE), Savannah, GA during the summer of 2005. A temporal study looked at changes in DO concentrations, Chlorophyll *a* concentrations, bacterial abundance, water depth, and salinity, every hour, for 26 hours, over both a neap and a spring tide. A spatial study looked at changes in the above variables at 5 sites along the SRE while following the tide inland during a neap high and low tide, and a spring high and low tide. DO concentrations varied between 3.82 and 5.98 mg O₂/L during both studies. Statistical analysis results showed, temporally, physical variables had a stronger ability to predict behavior in DO than biological variables, and, spatially, there was a significant difference in DO between sites and between tides.

INDEX WORDS: Dissolved Oxygen, Chlorophyll *a*, Bacterial Abundance, Tides, Estuary, Eutrophication, Hypoxia, Anoxia

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MASTER OF SCIENCE

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DEDICATION

“When one tugs at a single thing in nature, he finds it attached to the rest of the world.” John Muir

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CHAPTER 1

INTRODUCTION

Estuaries are defined as semi-enclosed areas where freshwater from rivers and streams meet and mix with salt water from the ocean (Lalli and Parsons 1997). Estuaries have many interacting biological, chemical, and physical processes, each with their own temporal cycles (Sanford *et al.* 1990; Lalli and Parsons 1997; D’Autilia *et al.* 2004; Chen *et al.* 2007; Hull *et al.* 2007). Biological cycles of production and consumption of dissolved oxygen are integral to the health of an estuary. These processes are influenced by the seasonal and diel patterns of the physical environmental forces of sunlight, wind, rain, and the tidal cycle (D’Autilia *et al.* 2004). Therefore each estuary is unique in the combination of these processes at any one time (Sanford *et al.* 1990; D’Autilia *et al.* 2004; Chen *et al.* 2007; Hull *et al.* 2007).

Changes in tidal action and strength in river flow control the mixing of the water column in estuaries (Lalli and Parsons 1997). Changes in water depth (tidal energy) in estuaries are caused by the combination of the sun and the moon’s gravitational force on the earth, specifically on the oceans (Lalli and Parsons 1997). The earth rotates on its axis producing our 24 hour day, as the moon revolves around the earth producing our lunar month, and as both the earth and the moon revolve as one unit around the sun producing our astronomical year (Lalli and Parsons 1997). As tidal energy increases, mixing of the water column increases, and as tidal energy decreases, mixing of the water column decreases (Lalli and Parsons 1997). The degree of mixing of the water column in an estuary has a significant impact on the distribution of dissolved oxygen in the water column.

Estuarine dissolved oxygen (DO) is primarily produced through photosynthesis, a process carried out by phytoplankton at the surface of the water column (Stanley and Nixon 1992; Bergondo *et al.* 2005; Lin *et al.* 2006; Chen *et al.* 2007). Production of DO by phytoplankton is limited by availability of sun light and nutrients, primarily in forms of nitrogen, phosphorous, and silica. Exchange of DO between the water surface and the atmosphere can be either a source or a sink of DO for the water column, depending on how saturated the water column is with DO.

The level of mixing in an estuary affects the distribution of DO in the water column. DO produced at the water column surface is made available for respiration by other organisms, in the water column and in the benthos, during periods of mixing from strong tidal energy and/or strong river flow (Stanley and Nixon 1992; Bergondo *et al.* 2005; Lin *et al.* 2006; Chen *et al.* 2007). Winds at the water surface aid in mixing the water column, particularly in shallow estuaries (Verity *et al.* 1998; Bergondo *et al.* 2005). During periods of low tidal energy and/or low river flow, thermohaline stratification of the water column can develop and prevent DO produced at the surface from diffusing to lower water layers (Stanley and Nixon 1992; Bergondo *et al.* 2005; Lin *et al.* 2006; Chen *et al.* 2007). As the supply of DO is cut off from the lower water column, the DO in the lower water column becomes depleted. Mixing of the water column also contributes to DO demand by resuspending sediments that were deposited during the slower neap tide currents (Nelson *et al.* 1994). These sediments both increase turbidity of the water column, lowering the photosynthetic production of DO, and increase surface area for bacterial films that take up DO during respiration (Nelson *et al.* 1994). Dissolved oxygen

concentrations have an inverse relationship with temperature, for this reason estuaries experience their lowest DO concentrations during the summer months.

Effects of Hypoxia

With few exceptions, most organisms require dissolved oxygen to live (Hull *et al.* 2007). Miller *et al.* (2002) reported LC₅₀ levels of DO for 22 species of temperate, saltwater organisms representative of those found along the eastern coast of the US, including fish, crustaceans, and a bivalve, to be 3 mg/L, or lower. Organisms are directly and immediately affected in a negative way when concentrations of DO fall below these acceptable levels (Hull *et al.* 2007). When hypoxia (DO concentrations fall below 3 mg O₂/L), or anoxia (DO ≤ 1 mg O₂/L), develop, organisms that are able to leave the area move, and those unable to leave, such as benthic and attached organisms can die (Breitburg *et al.* 1994; Verity *et al.* 2006).

Eutrophication can cause hypoxia, and is defined as an increase in the rate of supply of nutrients into an ecosystem (Bricker *et al.* 1999; Pinckney *et al.* 2001). Nutrients enter an estuary through wet and dry atmospheric deposition, run off, sewage, and primarily river and streams (Galloway *et al.* 2003). Physical mixing in estuaries of freshwater from rivers and saltwater from the oceans, combined with tidal action and winds, helps estuaries retain nutrients carried in by rivers (Lalli and Parsons 1997). Anthropogenic nutrient supplies have doubled the nitrogen input and tripled the phosphorous input into coastal areas (Selman *et al.* 2008).

The relationship of eutrophication, stratification, and reduced levels of dissolved oxygen in estuaries is well established (Bricker *et al.* 1999; Cloern 2001; Pinckney *et al.* 2001; Verity 2002a, b; Verity *et al.* 2006). Increases in concentrations of nutrients

entering the water support exponential phytoplankton growth at the water surface (Bricker *et al.* 1999; Bergondo *et al.* 2005; Verity *et al.* 2006). The high organic matter load, produced when large numbers of phytoplankton die and sink to the sediments, quickly depletes the lower water column DO during stratified water column conditions (Nelson *et al.* 1994). However, symptoms of eutrophication are now being reported for estuaries experiencing periods of well-mixed water column conditions (Bricker *et al.* 1999).

Development of hypoxia/anoxia in an estuary is determined by combinations of biological and physical processes particular to that estuary, and in partially-mixed to well-mixed estuaries the physical forcing of the tides can alter the biological processes of production/consumption of DO (Lin *et al.* 2006; Hull *et al.* 2007). Summer neap tides can set up conditions that allow the formation of hypoxia, and summer spring tide vertical mixing of the water column can, in certain cases, terminate, and in other cases intensify hypoxia (Sanford *et al.* 1990; Stanley and Nixon 1992; Nelson *et al.* 1994; Parker *et al.* 1994; Verity *et al.* 1998; D'Autilia *et al.* 2004; Bergondo *et al.* 2005; Chen *et al.* 2007; Hull *et al.* 2007).

Partially-mixed estuaries, such as Narragansett Bay and Chesapeake Bay, experience alternating periods of water column stratification (neap tides), which produce conditions that allow for hypoxia in the summer months, with periods of well-mixed water column conditions (spring tides) that end the hypoxia (Sanford *et al.* 1990; Stanley and Nixon 1992; Bergondo *et al.* 2005). Higher spring-time freshwater flows bring in more nutrients into estuaries that, together with longer periods of daylight, allow an increase in phytoplankton biomass which increases the supply of both DO and organic

matter to the water column (Sanford *et al.* 1990; Bergondo *et al.* 2005). Stronger river flow also increases water column stratification when less dense freshwater flows over the incoming heavier saltwater during neap tides preventing the DO produced at the surface from reaching the bottom waters (Sanford *et al.* 1990; Bergondo *et al.* 2005). During the larger tidal ranges of spring tides, turbulent mixing of the water column breaks up the phytoplankton bloom and water column stratification, distributing DO throughout the water column (Sanford *et al.* 1990; Bergondo *et al.* 2005). Sanford *et al.* (1990) presented data that showed that in the Chesapeake Bay movement of layers of water of differing DO concentrations and salinities over sampling sites was the primary source of variation of DO over time. Bergondo *et al.* (2005) observed hypoxic events during the summers of 2002 and 2003, and sighted the importance of tides on temporal variation of DO, in the Narragansett Bay. Stanley and Nixon (1992) reported that in the Pamlico River Estuary both water column stratification and warm water temperatures (water temperatures $\geq 15^{\circ}\text{C}$) must occur simultaneously for hypoxia to develop. Spring tides generated vertical mixing of the water column strong enough to restore the estuary to healthier DO levels in Narragansett Bay, Chesapeake Bay, and Pamlico River Estuary following episodes of neap tidal hypoxia (Sanford *et al.* 1990; Stanley and Nixon 1992; Bergondo *et al.* 2005).

In estuaries such as the Selangor and Klang River estuaries, in Malaysia, hypoxia develops in the well-mixed water column of the spring tides of the summer months due to resuspension of sediments with high biological oxygen demand (BOD) that are deposited on the bed during slow moving neap tides (Nelson *et al.* 1994). Both the Klang and Selangor estuaries experience hypoxic conditions during summer spring tides (Nelson *et al.* 1994). BOD created by resuspended sediments was found to be the source of

hypoxia/anoxia in the Usk River Estuary (Parker *et al.* 1994). Hyperconcentrated benthic layers are the sediments that are deposited in highly turbid estuaries during neap tides when water currents are slower due to decreased tidal energy and then resuspended during the stronger tidal energy of spring tides (Parker *et al.* 1994). DO levels in the Usk River Estuary were lowered from an average of summer neap tide 8 mg O₂/L to 5 to 3 mg O₂/L during summer spring tides (Parker *et al.* 1994). The decrease in DO occurred when oxygen depleted bacteria of the hyperconcentrated benthic layers were resuspended and distributed through the water column by the increase in tidal energy (Parker *et al.* 1994).

Estuaries are nursing grounds to ecologically and economically important species. Periods of low DO, especially during the nursing season, could have a dramatic affect on organisms that inhabit estuaries during key developmental periods. Hypoxia can have consequences, other than respiratory problems, for the environment such as altering the flow of carbon through changes in predator-prey (Breitburg *et al.* 1997). Breitburg *et al.* (1997) showed affects of low DO on predator/prey interactions between sea nettles and their prey (anchovy fish eggs, zooplankton, and naked goby fish larvae), and juvenile striped bass and their prey (naked goby fish larvae). Breitburg *et al.* (1994) concluded that DO levels below 3 mg O₂/L lowered the ability of adult naked and juvenile striped bass to capture prey, but lowered the escape swimming speed of naked goby larvae thereby making them easier prey for sea nettles. These results demonstrate that low DO concentrations, even small changes in DO, could create shifts in predator-prey relationships that significantly alter energy flow through food webs (Breitburg *et al.* 1994, 1997).

Georgia's Estuaries

Georgia's estuaries are well-mixed estuaries that are generating concern due to trends in nutrient concentrations, related to increasing coastal human populations, and DO levels in recent years (Cai *et al.* 1999; Alber *et al.* 2005; Verity *et al.* 2006). The Coastal Georgia Regional Development Center has reported that Georgia's coastal population increased by 62% between 1970 and 2000 (Ross and Barringer 2006). Based on research by Georgia Institute of Technology's Center for Quality Growth and Regional Development, Georgia's coastal population will increase by 51% from 2000 to 2030 (Ross and Barringer 2006). Increasing anthropogenic pressure on Georgia's coastal environment creates a need to better understand the effects of physical forces acting on the biological processes that support life in these environments (Peterson *et al.* 1998; Lauria *et al.* 1999; Bergondo *et al.* 2005).

Major rivers of Georgia have shown increasing nutrient and declining DO concentrations over the last decade (Cai *et al.* 1999; Verity *et al.* 2006). Alber *et al.* (2005) reported a concern for Cumberland Sound, on the southern coast of Georgia, due to low surface and bottom DO levels recorded from 2000 to 2004. Data collected from twenty stations in Cumberland Sound showed that both surface and bottom DO concentrations fell below the Environmental Protection Division's minimum DO level of 4 mg O₂/L for 18% of the four year sample period, and 44% of the dissolved inorganic nitrogen levels were considered high (Alber *et al.* 2005). Nutrient data collected from the Skidaway River Estuary, GA, showed a trend of steadily increasing nutrient and decreasing DO concentrations, both associated with increasing local human populations (Verity *et al.* 2006).

Georgia's Skidaway River Estuary (SRE) is a shallow, well-mixed estuary that is causing concern due to decreasing DO concentrations (Verity 2002a, b; Alber *et al.* 2005). A weekly dock sample collection, to investigate changes in bacterial abundance, chlorophyll *a* and DO concentrations, and water quality parameters has been conducted in the SRE since 1986 (Verity 2002a, b; Verity *et al.* 2006). The data collected from 1986 to 1996 shows that the annual mean, maximum, and minimum concentrations, averaged over the ten year period, of every nutrient (NO_3 , NH_4 , PO_4 , Si(OH)_4 , and dissolved organic nitrogen) and particulate organic matter (particulate organic carbon, particulate organic nitrogen, and chlorophyll *a*) tested for in the sampling program continuously increased over the sampling period (Verity, 2002a). Accompanying these increases in nutrients and particulate organic matter were steadily declining levels of dissolved oxygen in the Skidaway River Estuary, with the DO minimum falling below 3 mg O_2 / L (Verity *et al.* 2006).

Research Goals

The goal of my research was to observe and describe changes in dissolved oxygen, water depth, salinity, water temperature, chlorophyll *a* concentration, and bacterial abundance in the Skidaway River Estuary (SRE) over time and space. Much of the data available on dissolved oxygen processes in estuaries focuses on long time scales of months and years; there is very little data on dissolved oxygen processes in estuaries on short time scales of days and hours. Most of the processes affecting dissolved oxygen in estuaries change over such short time scales. Bacteria and phytoplankton populations can change within hours due to their short life spans, and the production of dissolved oxygen is limited to daylight hours. The biological processes contributing to the DO

availability in estuaries are directly influenced by strong tidal forcing that continuously mixes the fresh water input from rivers and the salt water intrusion from the ocean and resuspends the sediments.

Recent data (Cai *et al.* 1999; Alber *et al.* 2005; Verity *et al.* 2006) show that there are concerns about Georgia's estuaries with regard to DO availability. By looking at changes in DO, and the processes that contribute to DO availability over time and space in the SRE, we can better understand how the Skidaway River Estuary might respond to future changes in factors such as increased nutrient loading.

Temporal Study

The temporal neap and spring tide data were compared to each other to address the following hypotheses.

1) If tidal energy was high, i.e. the range in depth between high/low or low/high tide was large, as experienced during a spring tide, then DO concentrations were expected to be low, due to increased BOD of bacteria in sediments that are resuspended by mixing of water column generated by high tidal energy.

2) If water temperatures were high, then DO concentrations were expected to be low due to the decreasing solubility of DO as water temperatures increase.

3) If phytoplankton abundance (chlorophyll *a* concentration) was high, then DO concentrations were expected to be high due to high phytoplankton photosynthetic production of DO.

4) If bacterial abundance was high, then DO concentrations were expected to be low due to BOD of bacteria.

Based on these hypotheses lower DO concentrations were expected over time during the stronger mixing of the water column during the spring tide. Lower DO concentrations were also expected when lower chlorophyll *a* concentrations, higher bacterial abundance, and warmer water temperatures occurred.

Spatial Study

The spatial sampling data was used to address the following hypotheses.

1) If there was a difference in water depth between sites, therefore a difference in tidal energy between sites, then a difference in DO concentrations among sites was expected.

2) If there was a difference in tidal energy between tides, then a difference in DO concentrations among tides was expected.

CHAPTER 2

MATERIALS AND METHODS

Study Site

The Skidaway River Estuary (SRE) is a tidally dominated estuary experiencing two high tides, and two low tides, each day. The SRE is located on the eastern coast of Georgia, USA, (Figure 1) and receives freshwater from the Savannah River to the north, and the Ogeechee River to the south (Verity 2002a).

Temporal Study Sampling Schedule and Details

The dock for the temporal sampling was located at the Skidaway Institute of Oceanography on Skidaway Island (SkIO), Georgia, 9 km from Wassaw Sound (Verity 2002a). The location of the temporal sampling site is collection site number three in Figure 1. The temporal neap tide sampling was conducted on June 14, 2005, and the spring tide sampling was conducted on June 22, 2005. Each hour, for 26 hours, DO meter readings, for DO concentration, salinity, and water temperature data, were recorded with an YSI 556 DO meter at the surface, each 1 m of depth, and at the bed. For each depth measurement, DO, salinity, and water temperature, were averaged together to produce depth-averaged values for DO, salinity, and water temperature for each hour. Water depth was measured each hour using 1 m markings on the DO meter cable, and the range of change in water depth over the sampling period was used as proxy for tidal energy. Water samples, for chlorophyll and bacterial analyses, were collected from the water column surface, for each water depth profile made with the DO meter. Temporal wind speed data was obtained from the web site *www.weatherunderground.com*.

Spatial Study Sampling Schedule and Details

DO meter readings were recorded and surface water samples collected at each of five stations in the SRE on both the low and following high tide on July 13, 2005 (Neap tide) and July 20, 2005 (Spring tide), respectively. The locations of the sample sites are shown in Figure 1. The cruise for each spatial sampling began at the mouth of Wassaw Sound (site 1), followed the tide into the Skidaway Estuary stopping at each site to make water sample collections and meter recordings, and ended at the Skidaway Narrows (site 5). For each spatial water sample collected meter readings were recorded at the water column surface, at each middle depth of the water column, and at the bed, and the data for each depth were averaged to produce depth-averaged values for DO, salinity, and water temperature at each site. Spatial surface water sampling followed the temporal sampling protocol.

Water Sample Collection and Analysis Protocols

Water samples for chlorophyll *a* concentration and bacterial abundance analysis were collected from the water column surface in 250 ml Nalgene bottles. Water samples were prepared for analysis for chlorophyll *a* concentrations on return to the lab when two 10 ml samples were removed from a Nalgene bottle, and each sample vacuum filtered through 22 mm ϕ Whatman GF/F glass microfiber filters (Parsons *et al.* 1984; Grasshoff *et al.* 1999). Each filter was then wrapped in aluminum foil and stored in the dark at -20 °C until analysis (Parsons *et al.* 1984; Grasshoff *et al.* 1999).

Water samples were analyzed for chlorophyll *a* concentrations within three to five days following sampling by first placing the chlorophyll *a* filters in 10 ml of 90%

acetone. Using forceps, the filters were torn and each separate (torn) filter was placed into a falcon tube. Each sample was extracted by sonication for 15 s using a Fisher Sonic Dismembrator model 300. The samples were placed in the freezer for 1.5 hrs, then removed and analyzed using a 10 AU Fluorometer.

Preparation for analysis of the water samples for bacterial abundance began with 1.0 ml of sample water removed from a Nalgene bottle and placed in an autoclaved 1.5 ml micro-centrifuge tube (mct). Three replicates for analysis for bacterial abundance analysis were prepared by adding 100 μ l of DAPI, for staining, to each sample and allowing each sample to sit in the dark, at room temperature, for three minutes to allow for staining. Each sample was then vacuum filtered using a Poretics, Polycarbonate, Black, 0.22 micron filter for the bacterial filter. After filtering, each Poretics filter was placed on a glass slide, with a drop of immersion oil placed on top, covered with a glass coverslip, and then placed in a freezer until analysis. Skipper software for image analysis was used to quantify the bacterial abundance of each slide prepared (Shopov *et al.* 2000).

Statistical Analysis

For statistical analysis of the temporal data, independent variables were divided into two groups, physical variables of water depth, salinity, water temperature and wind speed, and biological variables of chlorophyll *a* concentrations, and bacterial abundance. SigmaStat v.2 was used to test all data sets for normality. JMP v.8 was used to run step-wise linear regression models on each individual independent variable, and then step-wise multiple linear regression models on all possible permutations of independent variables, for the dependant variable DO concentration. An Akaike Information Criteria, AIC, was calculated for each regression model produced, and the regression models with

the lowest AIC were used as final predictors of DO concentrations in the SRE. Microsoft Office Excel 2007 was used to calculate ANOVA for the DO concentration spatial data set, and to plot all graphs.

CHAPTER 3

RESULTS

Temporal Study

Descriptive statistics for the temporal data set are presented in Table 1. Neap tide depth-averaged DO concentrations (4.59 – 5.70 mg O₂/L) were higher than spring tide depth-averaged DO concentrations (3.82 – 5.20 mg O₂/L) (Figure 2a). Tidal energy, as shown by changes in water depth and salinity, was lower during the temporal neap tide than during the temporal spring tide (Figures 2b and c). The ranges in water depth and salinity during the temporal neap tide sampling period were less than that of the temporal spring tide sampling period (Figures 2b and c). Depth-averaged water temperatures during the temporal neap tide sampling period were higher than those of the temporal spring tide sampling period; however, there was very little change in water temperatures during either temporal sampling period (Figure 3a). Neap tide wind speeds (0.0 – 7.1 m/s) were higher than spring tide wind speeds (0.0 – 4.1 m/s) (Figure 3b). From time 14:00 to 18:00, neap wind speeds were three to four times the speeds experienced at any time during the spring tide sampling period (Figure 3b).

The neap tidal period experienced a stratified water column, with respect to salinity, during both flood tides, and a well-mixed water column, with respect to salinity, during the ebb tide periods (time 10:00 - 16:00 and 23:00 – 4:00) (Figure 4a). The neap tide also experienced water column stratification, with respect to DO and water temperature, during the first flood tide, time 10:00 – 16:00, but was well-mixed before and after the first flood tide (Figures 4b and c). The spring tide produced a well-mixed

water column for salinity, DO, and water temperature, for the entire sampling period (Figures 5a, b, and c).

During both temporal sampling periods mean surface chlorophyll *a* (Chl *a*) concentrations followed levels of irradiance, with higher Chl *a* concentrations during the day, lower concentrations during the night, and the temporal neap tide Chl *a* concentrations being lower than those of the temporal spring tide (Figures 6a and b). The temporal neap tide mean surface bacterial abundance was greater than that of the temporal spring tide (Figure 6c).

Of the physical independent variables tested for, individually, water temperature had the greatest R^2 value with DO, having the lowest AIC for a linear regression model compared to the AIC values of the regression models for the other three physical variables (Tables 2, 3, and 4). Water depth, salinity and wind speeds, individually, had similar strength in predicting the behavior of DO. However, when taken together the physical variables had an even greater R^2 value with DO concentrations, but the specific combinations of the physical variables that had the greatest R^2 value with DO concentrations varied between neap and spring tides. During the neap tide sampling period water depth, salinity, water temperature, and wind speed together had the lowest AIC value with DO ($R^2 = 0.77$, $DF = 25$, $p < 0.0001$). During the spring tide sampling period, water depth and water temperature combined had the lowest AIC value with DO ($R^2 = 0.78$, $DF = 25$, $p < 0.0001$).

Bacterial abundance, as a proxy for respiration, had larger R^2 values with DO concentrations in the SRE, for both temporal neap and spring tide sampling periods, than Chl *a*, as a proxy for production of DO (Tables 5 and 6). All biological independent

variables, together or combined, had a much smaller R^2 value with DO concentrations over time in the SRE than did the physical independent variables tested (Table 2).

Spatial Study

There was a statistically significant difference ($F_{(3, 4)} = 4.806$ $DF = 4$, $p = 0.015$, $F_{critical\ value} = 3.259$, Table 7, Figure 7a) in DO concentrations among sites along the SRE. Depth-averaged DO concentrations decreased inland during the spatial neap and spring low tides, and decreased from site 1 to site 4, then increased to site 5 during the spring high tide (Figure 7a). The water depth data from the spatial sampling period gives a general description of the topography of the SRE (Figure 7b). Sample sites 1 (Wassaw Sound) and 2 were the deepest of the collections sites, where the Skidaway River connects to the ocean. As the tide moved inland site 3, near the SkIO dock, site of the temporal sampling, was shallower than both site 2 and 4, and site 5, was the shallowest. During each spatial sampling, depth-averaged salinity decreased inland, with the greatest change in salinity a decrease after site 2 (Figure 7c). Depth-averaged water temperature results show there was very little change in water temperature among sites (Figure 8).

There was a statistically significant difference ($F_{(4, 3)} = 10.773$, $DF = 3$, $p = 0.001$, $F_{critical\ value} = 3.490$, Table 7, Figures 7a) in DO concentration in the SRE between tides. The neap high tide had the highest DO values of the four tides sampled, and showed the most variation with DO being highest at site 1 and 5, and site 2-3 having similar DO concentrations (Figure 7a). The spatial spring high tide produced the deepest water depths and the widest range in water depth between sites, compared to the neap high tide, and neap and spring low tide (Figure 7b). Both neap tides and the spring low tide produced similar water depths at each site, and similar ranges in water depth (Figure 7b).

Each tide produced similar values and ranges in depth-averaged salinity, with the spring high tide producing the maximum salinities (Figure 7c). There was very little change in depth-averaged water temperature both among sites, and between tides, during the spatial sampling (Figure 8).

The neap low tide and spring high tides produced a well-mixed water column, with respect to DO, salinity, and water temperature, for all sites (Figures 9 and 10). The neap high tide produced a stratified water column, with respect to DO and water temperature, for all sites, and a well-mixed water column, with respect to salinity, for all sites (Figure 11). The spring low tide produced a combination of stratified and well-mixed water column along the estuary. Sites one and three experienced stratification of the water column with respect to DO and water temperature, and was well-mixed with respect to salinity, while sites two, four, and five were well-mixed for all physical variables during the spring low tide (Figure 12).

Mean surface chlorophyll *a* concentrations increased inland on both neap and spring high tides (Figure 13a). On both neap and spring low tides there was a pattern of increase inland, with a slight decrease after site 3, site 3 having the maximum Chl *a* concentration during low tides, and site 5 having the maximum during high tides. The ranges of mean surface bacterial abundance were similar for each spatial tidal period (Figure 13b).

CHAPTER 4

DISCUSSION

Temporal Study

Changes in DO levels in the Skidaway River Estuary during the temporal neap and spring tide sampling periods were associated with changes in the physical forces of water depth, water temperature, and salinity, and primarily with water temperature. Temporal hypothesis 1, if tidal energy was high, i.e. the range between high/low or low/high tide was large (spring tide), then DO concentrations were expected to be low, due to increased BOD by bacteria in sediments that are resuspended by mixing of water column generated by high tidal energy, was accepted. The higher tidal energy of the spring tide (range in water depth and salinity) produced lower DO concentrations than the lower tidal energy of the neap tide. Temporal hypothesis 2, if water temperatures were high, then DO concentrations were expected to be low due to the decreasing solubility of DO as water temperatures increase, was rejected. Higher DO concentrations occurred during warmer water temperatures of the neap tide, than the cooler water temperatures of the spring tide. A period of stratification during the first flood tide of the neap tide period showed a 2 m deep surface layer of warmer, more oxygenated water moving over the cooler, less oxygenated lower water column. This positive relationship between DO and water temperature was unexpected. Gao and Song (2008) reported similar results of a positive linear relationship between water temperature and DO from a study in the Changjiang Estuary. In their study conducted in May 2003, DO variation with time had a strong positive correlation with water temperature (Gao and Song 2008).

Temporal hypothesis 3, if phytoplankton abundance (chlorophyll *a* concentration) was high, then DO concentrations were expected to be high due to high phytoplankton photosynthetic production of DO, was rejected. Lower DO concentrations occurred during the higher Chl *a* concentrations of the spring tide. Temporal hypothesis 4, if bacterial abundance was high, then DO concentrations were expected to be low due to BOD by bacteria, was rejected. Higher DO concentrations occurred during the higher bacterial abundance of the neap tide.

Low irradiance levels inhibit DO production (Chl *a*), and warmer water temperatures allow for higher respiration (bacterial abundance) and lower DO solubility, all contributing to lowering water column DO concentrations. With lower DO production, higher DO consumption, and less DO solubility, compared to spring tide, neap tide DO concentrations were expected to be lower. However, the reverse was true during the temporal sampling period. Higher DO values occurred during neap tide than spring tide. This reversal of expected DO levels may be due to previous processes, and/or increased wind speeds.

Effects of Previous Tides on Current Temporal DO Dynamics

The neap tide Chl *a* concentrations, bacterial abundance, water temperature, and tidal energy (water depth), seemed more appropriate for producing DO levels similar to the spring tide DO levels, and vice versa. This may not be the case here, but Parker *et al.* (1994) suggest that high/low, neap/spring tides can create effects that are observed on following tides. Their investigation of suspended sediments and DO in the Usk Estuary showed that increases in suspended sediments and decreases in DO levels coincided (Parker *et al.* 1994). Changes in the suspended sediments, which contribute the bulk of

DO consumption in the form of bacteria which are often oxygen-deprived and attached to sediment grains, and DO levels lagged behind the changes in water level (Parker *et al.* 1994). The Usk estuary is a hypertidal estuary with water depth range of 6 m, neap tide, to 12 m, spring tide (Parker *et al.* 1994). The Skidaway Estuary is a mesotidal estuary, with water depth ranges of 3.5 to 4 m, neap to spring tide, respectively. Even though SRE's tidal range is lower than that of the Usk, the lag in effects seen in water quality parameters in the SRE from one tide to the next should be shorter than those seen in the Usk, but still present. The DO levels found in the SRE during the neap and spring tides may not be due to the previous spring and neap tides, but to biological and physical processes that occurred earlier, such as the previous day, or even hours, earlier. A longer study period, every hour for 1-2 weeks, may present better insights into this scenario; however, the required personal and supplies for such a study are costly. The maximum difference in the DO levels, of 1 mg O₂/L between the neap and spring tide in the SRE, were, according to Parker *et al.* (1994), as expected.

Effect of Wind on Current Temporal DO Dynamics

Wind speeds may have contributed to producing higher DO levels during the neap tide sampling period compared to that of the spring tide sampling period. Increasing winds along the water surface increase the rate of gaseous O₂ exchange between the atmosphere and the water surface (Verity *et al.* 1998; Harrison *et al.* 2008). In waters that are under-saturated, this re-aeration of the water column supports higher DO levels during low DO water column levels (Verity *et al.* 1998). Wind speeds during the neap tide period were on average twice those of the spring tide period, with maximum wind speeds during the neap low tide period. Maximum wind speeds during the low tide had

two affects on the DO water column budget – increasing atmospheric supply of O₂ to the water column, and distributing the DO through the water column by mixing the water column (Verity *et al.* 1998; Lin *et al.* 2006). Verity *et al.* (1998) reported that winds supply kinetic energy through the water column that, due to low water depth, mixes the water column. The affect of wind on mixing the water column increases with decreasing water depth (Verity *et al.* 1998). The wind induced mixing during the ebb tide combined with both the river current and tidal forcing to thoroughly mix the water column, with respect to salinity, water temperature, and DO. The resulting well-mixed water column with respect to DO supported the increase in DO levels during the night. The increase in water temperature during this period resulted from advection of water seaward that was warmed in the late afternoon upriver. This increase in water temperature coincided with an increase in DO, not a decrease as expected (Conrads *et al.* 2002; Gao and Song 2008). As the wind speed decreased during the predawn hours, the DO levels also decreased, as did water temperature. The variation in DO over time followed that of water temperature.

Wind speeds during the spring tide sampling period were nearly half those of neap tide, and remained consistent for the entire sampling period. The process that best explains the low spring tide DO levels with the accompanying DO production/consumption processes, compared to that of the neap tide, is the lack of strong wind speeds to re-aerate the water column.

Spatial Study

Spatial hypothesis 1, if there was a difference in water depth between sites, then a difference in DO concentrations between sites was expected, was accepted. The difference in DO among sites may have been due to water depth, which is strongly influenced by estuarine bed topography, the physical form of the estuarine bed. Sharp changes in topography, creating changes in water depth, and rough topography contribute to mixing of the water column. Differences in topography, from one area to another, alter the degree of mixing of the water column along an estuary. As the tide moves inland during the flood tide and seaward during the ebb tide, moving from shallow (with depths between 2 and 5 m, such as site 3) to deeper (with depths between 8 and 12 m, such as sites 1, 2, and 4) areas, changes in how the water column is mixed, from one location to another, can create the differences in DO between the sites that was observed in the data.

Spatial hypothesis 2, if there was difference in tidal energy between tides, then a difference in DO concentrations between tides was expected. The difference in tidal energy (mixing) between tides created the difference in DO between tides. The shallow water depth of the neap low tide allowed for friction between the water and the bed to have a greater force on the water column, producing a well-mixed water column, with respect to DO, salinity, and water temperature, for all sites. The stronger tidal current of the spring high tide also produced a well-mixed water column, with respect to DO, salinity, and water temperature, for all sites. The spring low tide produced a combination of stratified and well-mixed water column along the estuary, lending support to the differences in DO seen among sites. The differences in the mixing of the water column between sites during the spring high tide shows the degree to which the water column is

mixed can change along the estuary, and, as presented here in this study, the mixing of the water column strongly influences DO availability along the estuary.

CHAPTER 5

CONCLUSION

The Skidaway River Estuary is a shallow, well-mixed estuary on the eastern coast of the US that has been under investigation for changes in biological processes affecting the availability of DO for the last two decades (Verity 2002a, b). The study presented here investigated changes in DO concentrations and the biological and physical processes affecting DO availability over time (2 - 26 hour, hourly sampling periods) and space (4 spatial sampling cruises, each sampling 5 sites) in the SRE in the summer of 2005.

Dissolved oxygen concentrations observed in the Skidaway River Estuary during this study, 3.82 to 5.98 mg O₂/L, were above what is considered hypoxic, i.e. above 3 mg O₂/L. This study was conducted during the summer months to capture the lowest annual DO concentrations. Based on the criteria set by Miller *et al.* (2002), and the data from this study, the dissolved oxygen concentrations in the Skidaway River Estuary were at an acceptable level for the health of the organisms in the estuary.

The temporal neap tide DO concentrations were higher than those of the temporal spring tide, and based on the literature this is as expected in well-mixed estuaries with high nutrient loading (Nelson *et al.* 1994; Parker *et al.* 1994). Turbulence generated by large spring tidal changes in water depth resuspends sediments, which can contain bacterial concentrations that have a high BOD, deposited by the previous neap tide (Nelson *et al.* 1994; Parker *et al.* 1994). During each sampling period of this study water in the estuary was observed to be highly turbid due to the resuspension of sediments. However, suspended sediment concentrations were not measured during this study. In this study bacterial abundance measured at the surface was a proxy for DO consumption,

and mean surface Chl *a* concentrations were a proxy for DO production. The temporal sampling data provided conflicting patterns of DO production/consumption processes and DO levels. The temporal neap tide period also experienced higher wind speeds (wind stress) than the temporal spring tide, and strong winds at the water surface could have increased water-air O₂ exchange producing the pattern found in the data (Verity *et al.* 1998; Harrison *et al.* 2008). Biological and physical processes occurring hours or days preceding the study could also have contributed to the measurements recorded during this study (Parker *et al.* 1994).

Regression analysis of the temporal data revealed that the physical variables, water depth, salinity, water temperature, and wind speed had a strong relationship with DO. Of the physical factors, taken individually, water temperature had the strongest relationship with DO levels, with water depth, salinity, and wind speed having less, but statistically similar, effects on DO. Combinations of these physical factors had an even greater effect on DO, and the combination of physical factors that had the greatest influence on DO varied over both neap and spring tidal periods. These results support the highly dynamic nature of the estuary in that the primary process, or processes, affecting DO change(s) with time.

The spatial sampling data revealed that there was a statistically significant difference in DO between spatial sampling sites along the SRE and between the tides. The primary forcing factors on DO in the SRE are physical processes, and the combination of these processes that have the strongest influence on DO in the SRE changes with time, even over small time scales of hours, influencing both temporal and spatial patterns of DO in the SRE.

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Table 1 Temporal descriptive data collected on June 14th (neap tide) and June 22nd (spring tide), 2005, from Skidaway River Estuary, GA

Tide	Variable	Units	Range	Mean	SD	Min	Max
Neap	Water Depth	m	3.5	4.7	0.8	2.0	5.5
Neap	Salinity	ppt	2.92	19.66	0.87	18.63	21.56
Neap	Water Temperature	°C	1.2	28.3	0.3	27.8	28.9
Neap	Wind Speed ¹	m/s	7.2	3.8	2.0	0.0	7.2
Neap	Chl <i>a</i> Concentration	µg Chl <i>a</i> /L	17.26	9.34	3.81	5.45	22.71
Neap	Bacterial Abundance	# of cells/ml	2.61E+07	1.86E+07	7.52E+06	9.54E+06	3.57E+07
Neap	DO Concentration	mg O ₂ /L	1.12	5.06	0.31	4.59	5.70
Spring	Water Depth	m	4.0	4.8	1.2	3.0	7.0
Spring	Salinity	ppt	6.84	19.82	1.78	17.30	24.14
Spring	Water Temperature	°C	1.1	27.4	0.3	27.0	28.1
Spring	Wind Speed ¹	m/s	0.6	2.2	0.8	0.0	4.1
Spring	Chl <i>a</i> Concentration	µg Chl <i>a</i> /L	15.48	10.54	4.52	5.79	21.00
Spring	Bacterial Abundance	# of cells/ml	2.29E+07	1.07E+07	5.35E+06	9.54E+06	3.57E+07
Spring	DO Concentration	mg O ₂ /L	1.37	4.38	0.43	3.82	5.20

¹Wind speed data was obtained from www.weatherunderground.com.

Table 2 Multiple linear regression results for temporal dissolved oxygen data collected on June 14th (neap tide) and June 22nd (spring tide), 2005, from Skidaway River Estuary, GA

Tide	Dependant Variable	Depth	Salinity	Temp	Wind Speed	Bacteria	R²	DF	<i>p</i>	AIC
Neap tide	DO Concentration	Depth	Salinity	Temp	Wind Speed		0.77	25	<0.0001	-10.23
Neap tide	DO Concentration					Bacteria	0.26	25	0.0080	10.97
Spring tide	DO Concentration	Depth		Temp			0.78	25	<0.0001	-1.27
Spring tide	DO Concentration					Bacteria	0.24	25	0.0108	28.11

Table 3 AIC values for the physical variables used to predict temporal neap tide dissolved oxygen concentrations from data collected on June 14th, 2005, from Skidaway River Estuary, GA^{1,2}

Dependant Variable	Water Depth	Salinity	Water Temperature	Wind Speed	AIC
DO Concentration	Water Depth				17.24
DO Concentration		Salinity			18.42
DO Concentration			Water Temperature		-1.74
DO Concentration				Wind Speed	17.96
DO Concentration	Water Depth	Salinity			20.00
DO Concentration	Water Depth		Water Temperature		-10.11
DO Concentration	Water Depth			Wind Speed	19.49
DO Concentration		Salinity	Water Temperature		-3.71
DO Concentration		Salinity		Wind Speed	20.45
DO Concentration			Water Temperature	Wind Speed	-0.56
DO Concentration	Water Depth	Salinity	Water Temperature		-8.06
DO Concentration	Water Depth	Salinity		Wind Speed	22.55
DO Concentration		Salinity	Water Temperature	Wind Speed	-2.77
DO Concentration	Water Depth	Salinity	Water Temperature	Wind Speed	-10.23

¹Akaike's information criterion (AIC) for each combination of physical variables used, to generate multiple linear regression models, to predict Temporal Neap tide Dissolved Oxygen Concentration.

²Bold AIC is the minimum AIC and therefore the variables are used as the independent variables in the multiple linear regression model and regression results are presented in Table 2.

Table 4 AIC values for the physical variables used to predict temporal spring tide dissolved oxygen concentrations from data collected on June 22nd, 2005, from Skidaway River Estuary, GA ^{1,2}

Dependant Variable	Water Depth	Salinity	Water Temperature	Wind Speed	AIC
DO Concentration	Water Depth				36.25
DO Concentration		Salinity			31.94
DO Concentration			Water Temperature		23.03
DO Concentration				Wind Speed	35.30
DO Concentration	Water Depth	Salinity			34.60
DO Concentration	Water Depth		Water Temperature		-1.27
DO Concentration	Water Depth			Wind Speed	35.38
DO Concentration		Salinity	Water Temperature		9.96
DO Concentration		Salinity		Wind Speed	34.43
DO Concentration			Water Temperature	Wind Speed	25.48
DO Concentration	Water Depth	Salinity	Water Temperature		0.47
DO Concentration	Water Depth	Salinity		Wind Speed	37.40
DO Concentration		Salinity	Water Temperature	Wind Speed	13.02
DO Concentration	Water Depth	Salinity	Water Temperature	Wind Speed	3.71

¹Akaike's information criterion AIC, for each combination of physical variables used, to generate multiple linear regression models, to predict temporal spring tide dissolved oxygen concentration.

²Bold AIC is the minimum AIC and therefore the variables are used as the independent variables in the multiple linear regression model and regression results are presented in Table 2.

Table 5 AIC values for the biological variables used to predict temporal neap tide dissolved oxygen concentrations from data collected on June 14th, 2005, from Skidaway River Estuary, GA ^{1,2}

Dependant Variable	Chl <i>a</i>	Bacterial Abundance	AIC
DO Concentration	Chl <i>a</i>		16.11
DO Concentration		Bacterial Abundance	10.97
DO Concentration	Chl <i>a</i>	Bacterial Abundance	12.28

¹Akaike's information criterion AIC, for each combination of biological variables used, to generate multiple linear regression models, to predict temporal neap tide dissolved oxygen concentration.

²Bold AIC is the minimum AIC and therefore the variable is used as the independent variable in the linear regression model and regression results are presented in Table 2.

Table 6 AIC values for the biological variables used to predict temporal spring tide dissolved oxygen concentrations from data collected on June 22nd, 2005, from Skidaway River Estuary, GA ^{1,2}

Dependant Variable	Chl <i>a</i>	Bacterial Abundance	AIC
DO Concentration	Chl <i>a</i>		30.42
DO Concentration		Bacterial Abundance	28.11
DO Concentration	Chl <i>a</i>	Bacterial Abundance	28.41

¹Akaike's information criterion AIC, for each combination of biological variables used, to generate multiple linear regression models, to predict temporal spring tide dissolved oxygen concentration.

²Bold AIC is the minimum AIC and therefore the variables are used as the independent variables in the multiple linear regression model and regression results are presented in Table 2.

Table 7 ANOVA results for spatial dissolved oxygen concentration data collected on July 13th (neap tide) and July 20th (spring tide), 2005 from the Skidaway River Estuary, GA

Source of Variation	DF	SS	MS	F	<i>p</i>	<i>F critical value</i>
Between Subjects (Site)	4	1.939	0.485	4.806	0.015	3.259
Between Treatment (Tide)	3	3.260	1.087	10.773	0.001	3.490
Error	12	1.210	0.101			
Total	19	6.409				

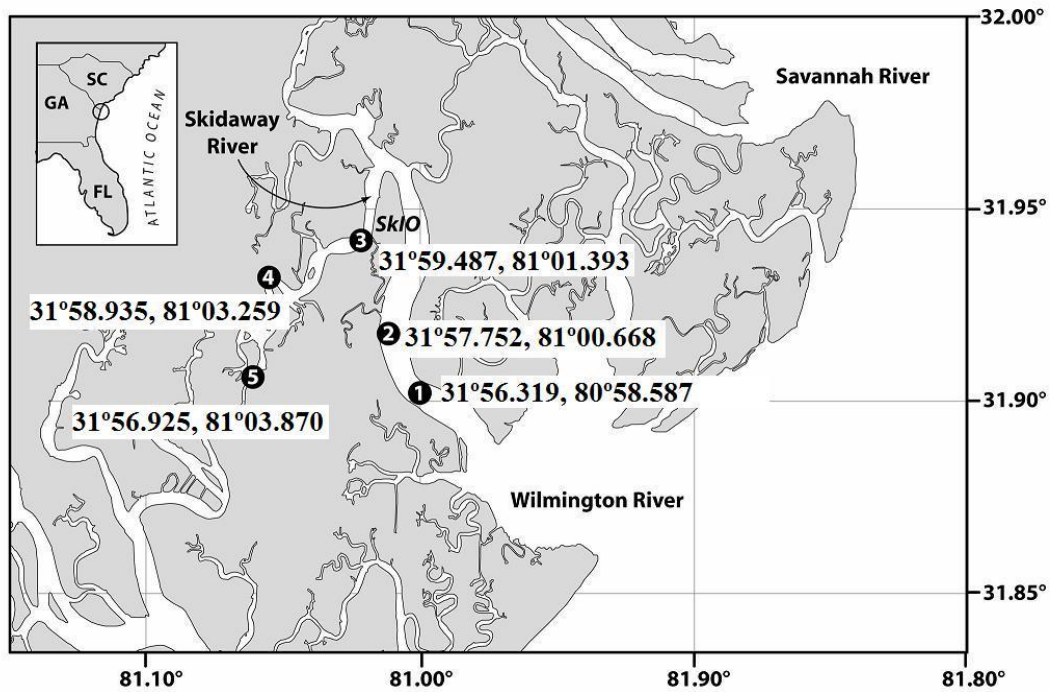


Figure 1 Locations of study sites in the Skidaway River Estuary, GA
The temporal sampling was conducted at site 3. Each spatial sampling began at site 1 and proceeded upstream to conclude at site 5.

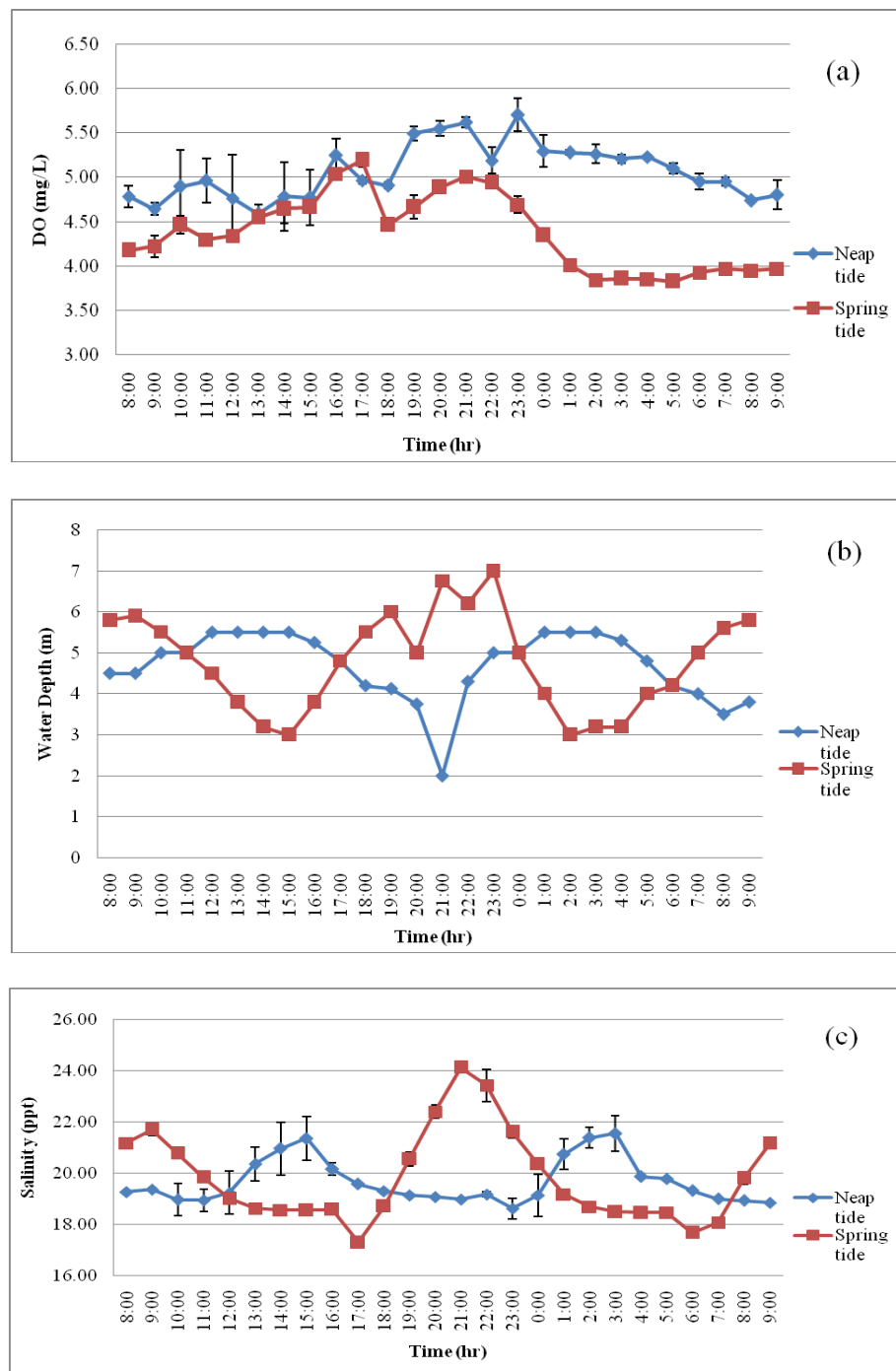


Figure 2 Dissolved oxygen, water depth, and salinity data from the temporal studies on June 14th (neap tide) and June 22nd (spring tide), 2005, in the Skidaway River Estuary, GA
 DO and salinity values are depth-averaged. Error = 1 SD.

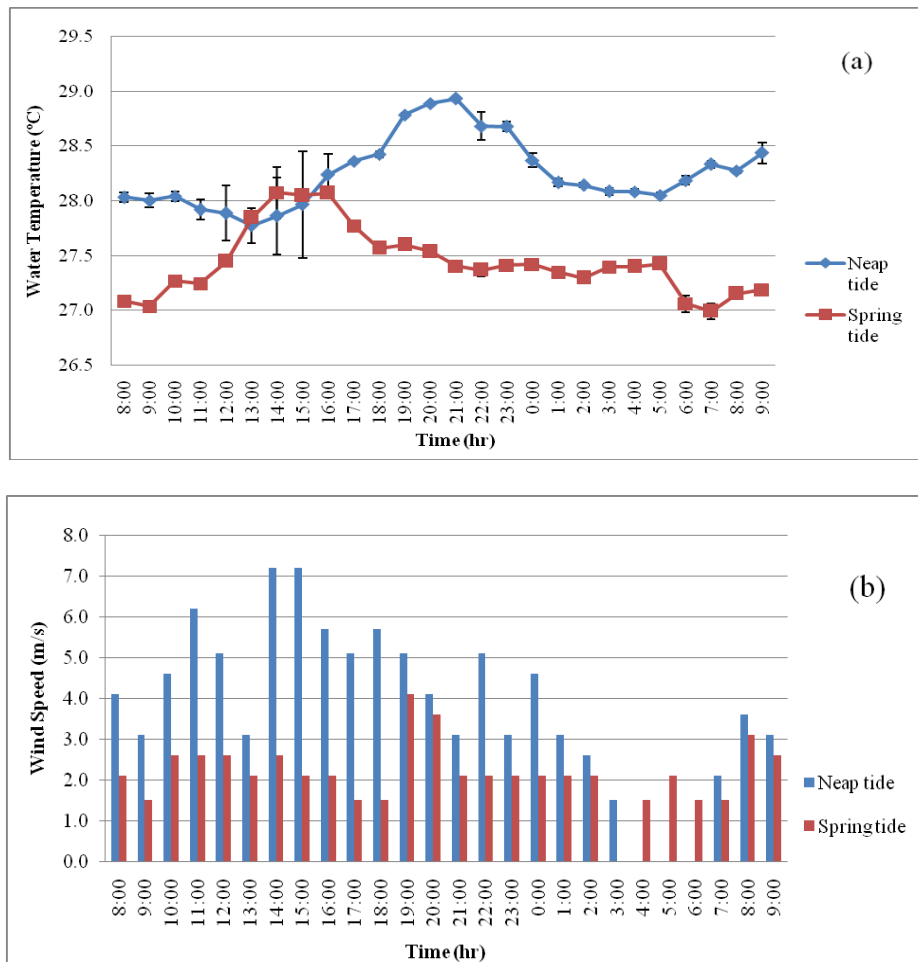


Figure 3 Water temperature and wind speed data from the temporal studies on June 14th (neap tide) and June 22nd (spring tide) of 2005, in the Skidaway River Estuary, GA
 Water temperature values are depth-averaged. Error = 1 SD.
 Wind speed data found at www.weatherunderground.com.

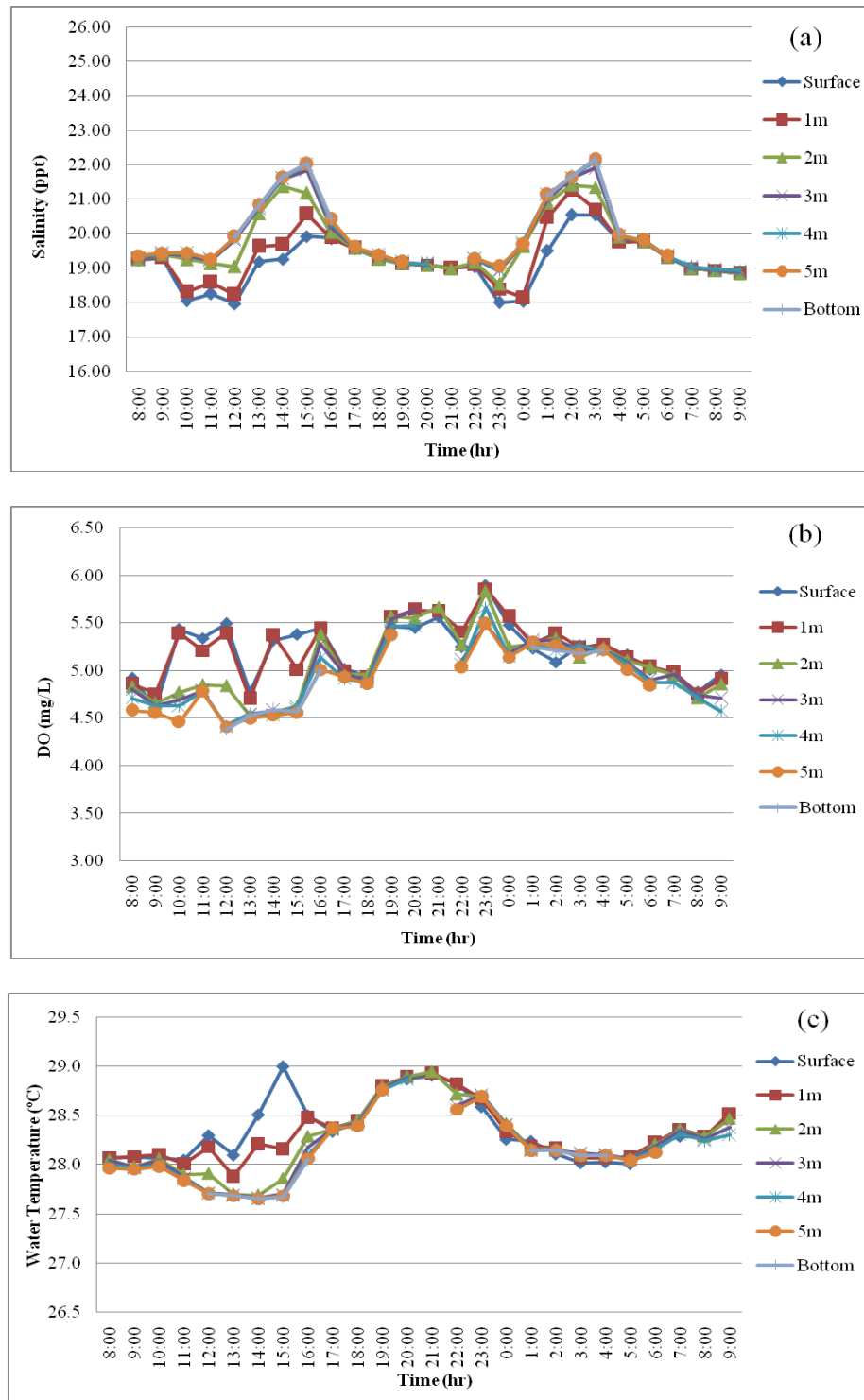


Figure 4 Salinity, dissolved oxygen, and water temperature data, by depth, from the temporal neap tide study on June 14th, 2005, in the Skidaway River Estuary, GA

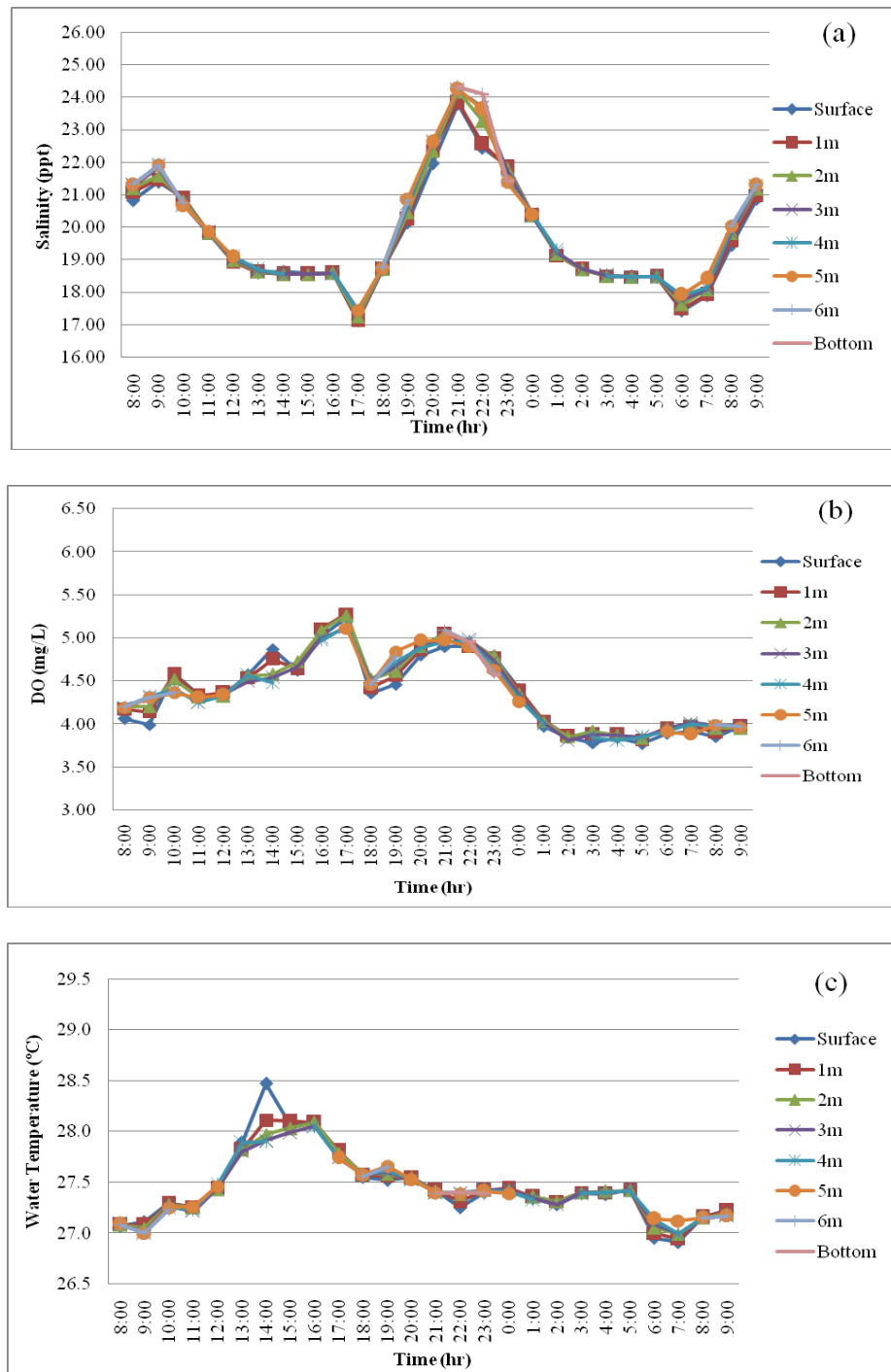


Figure 5 Salinity, dissolved oxygen, and water temperature data, by depth, from the temporal spring tide study on June 22th, 2005, in the Skidaway River Estuary, GA

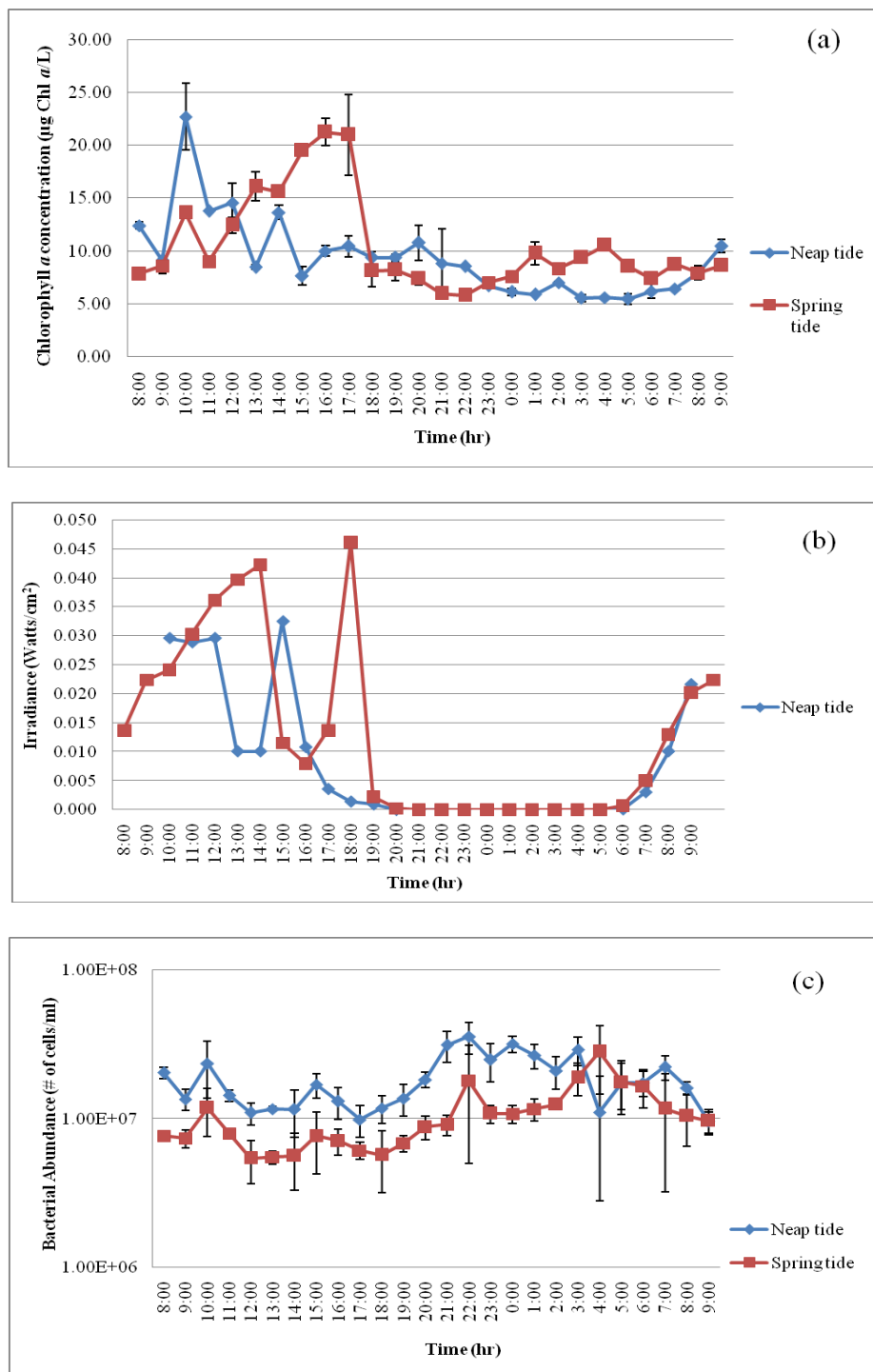


Figure 6 Chlorophyll *a*, irradiance, and bacterial abundance data from the temporal studies on June 14th (neap tide) and June 22nd (spring tide) of 2005, in the Skidaway River Estuary, GA
 Chlorophyll *a* and bacterial abundance values are mean surface values. Irradiance data was measured 5 cm below water surface. Error = 1 SD.

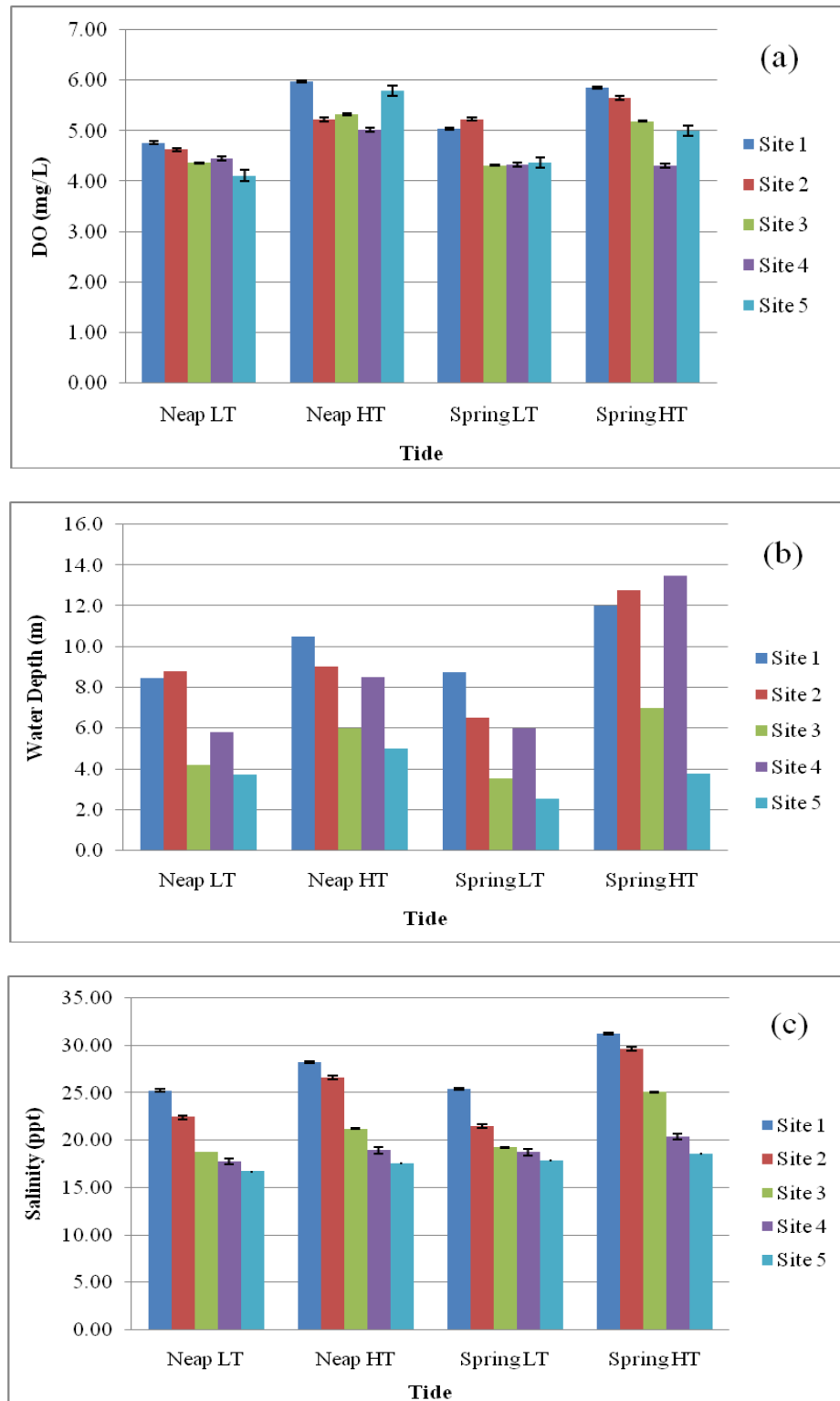


Figure 7 Dissolved oxygen, water depth, and salinity data from the spatial cruises on July 13th (neap tide) and 20th (spring tide), 2005, in the Skidaway River Estuary, GA
 DO and salinity values are depth-averaged. Error = 1 SD.

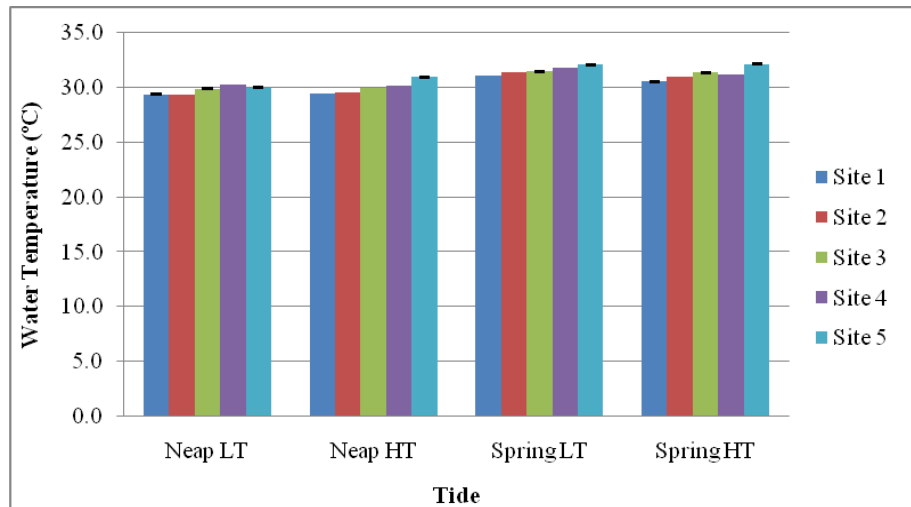


Figure 8 Water temperature data from the spatial cruises on July 13th (neap tide) and July 20th (spring tide), 2005, in the Skidaway River Estuary, GA

All water temperature values are depth-averaged. Error = 1 SD.

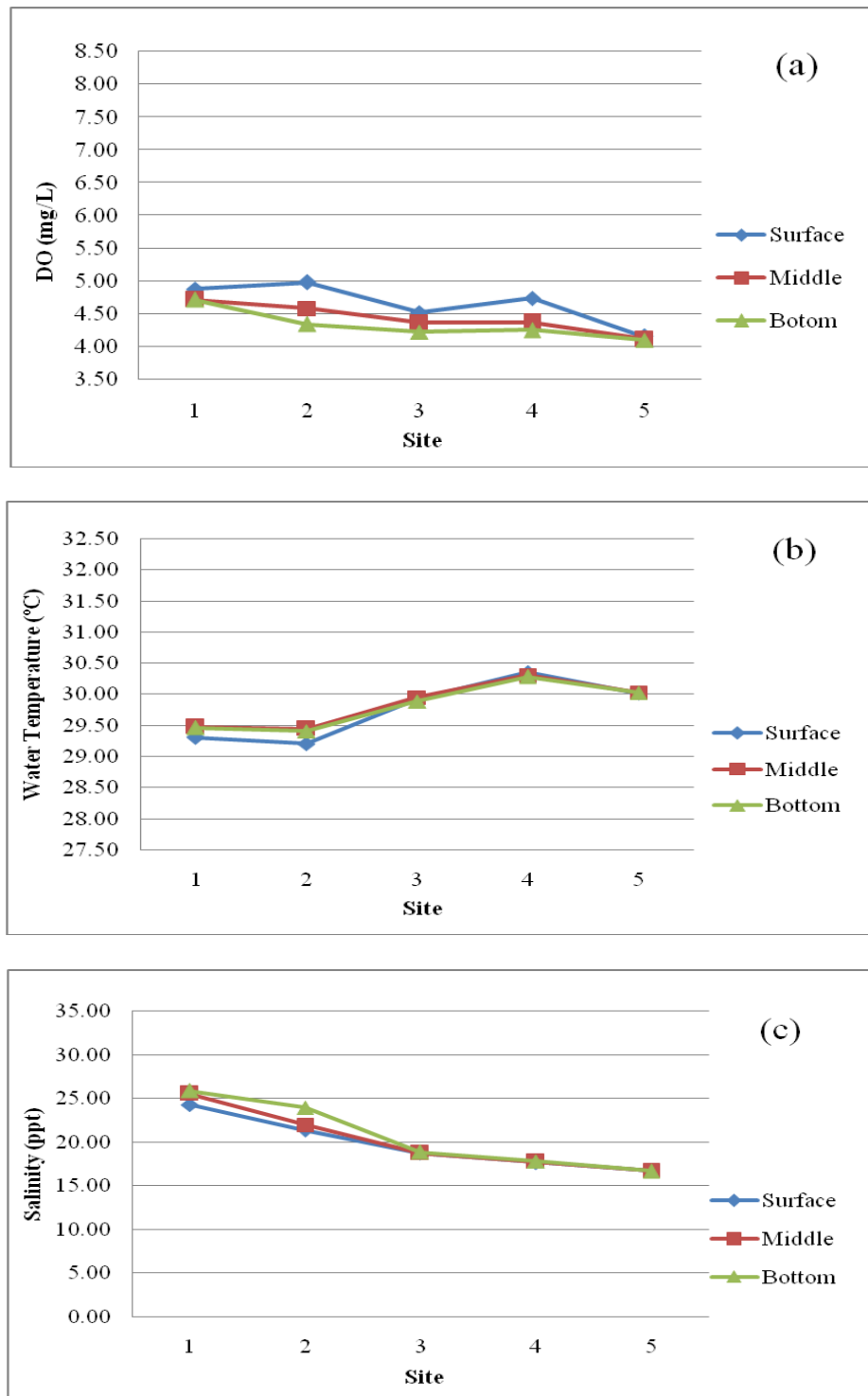


Figure 9 Dissolved oxygen, water temperature, and salinity data, by depth, from the spatial neap low tide cruise on July 13th, 2005, in the Skidaway River Estuary, GA

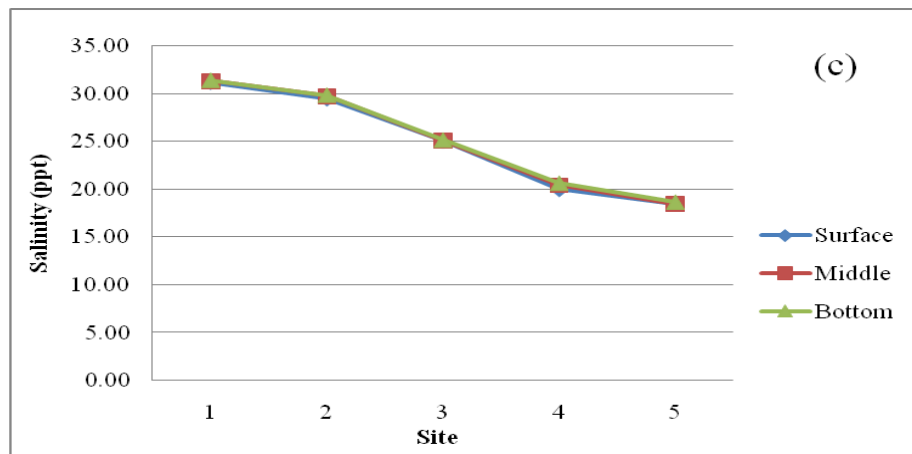
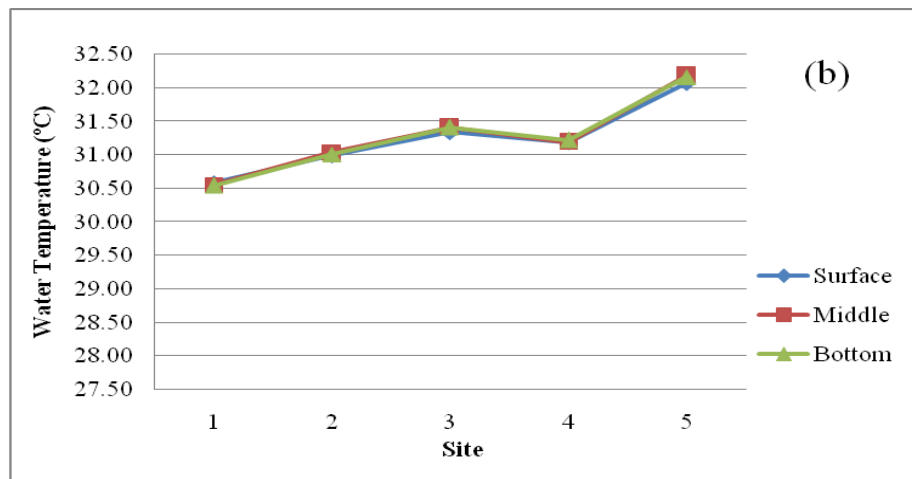
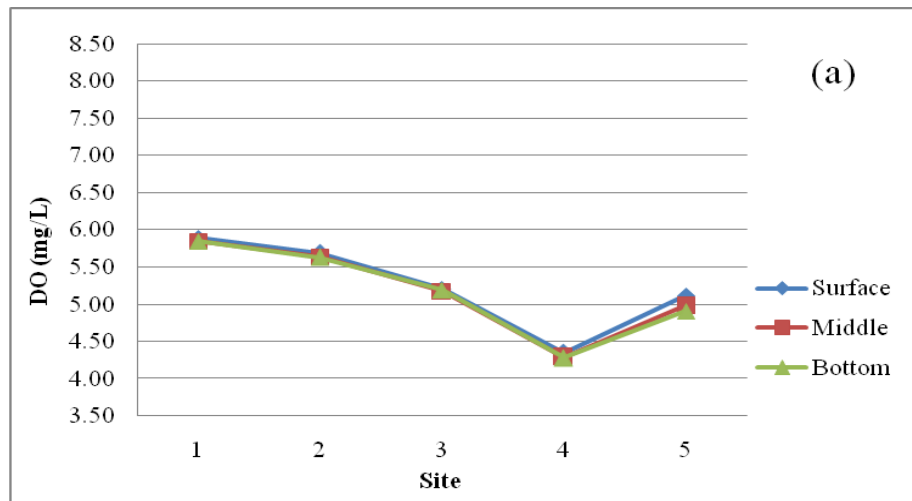


Figure 10 Dissolved oxygen, water temperature, and salinity data, by depth, from the spatial spring high tide cruise on July 20th, 2005, in the Skidaway River Estuary, GA

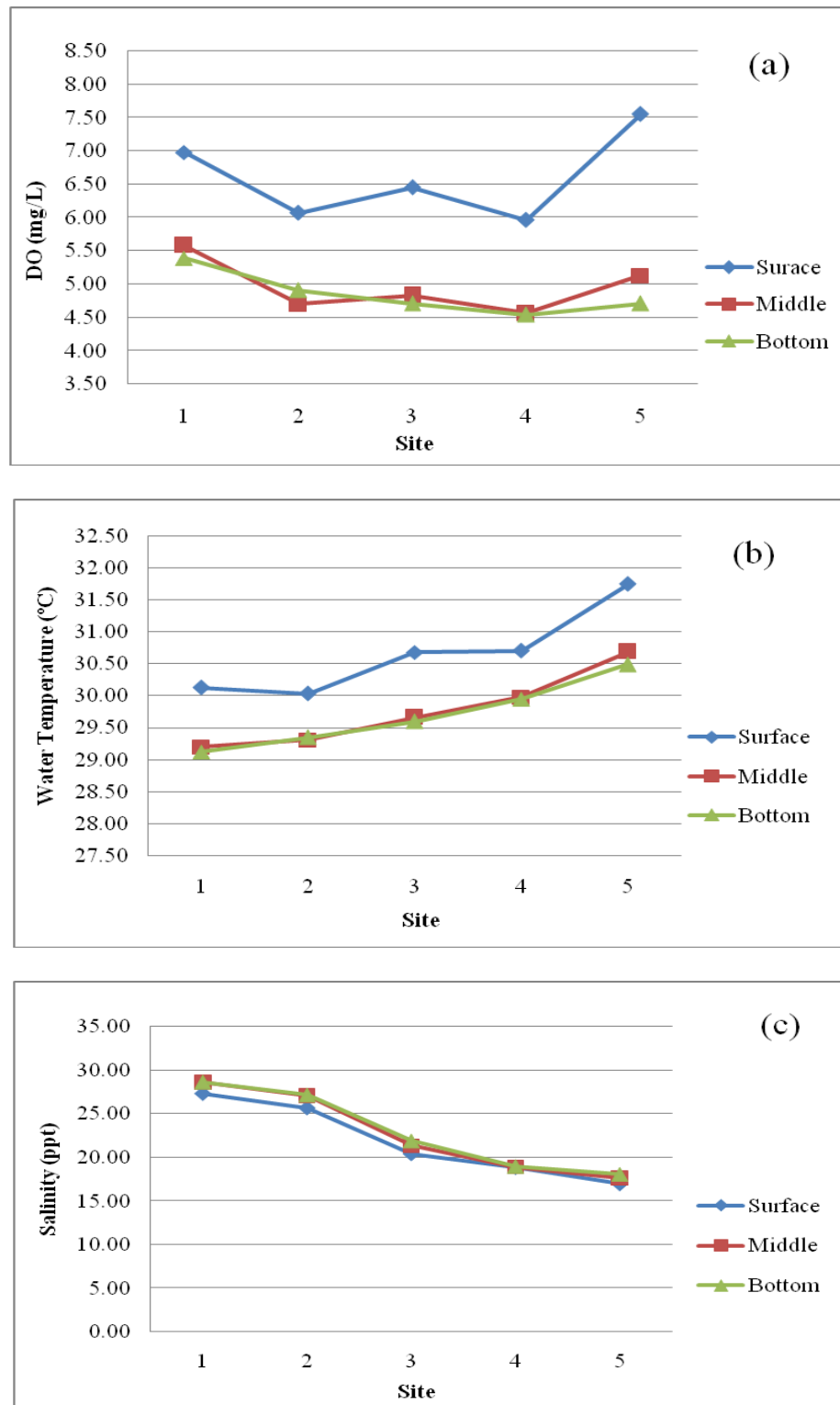


Figure 11 Dissolved oxygen, water temperature, and salinity data, by depth, from the spatial neap high tide cruise on July 13th, 2005, in the Skidaway River Estuary, GA

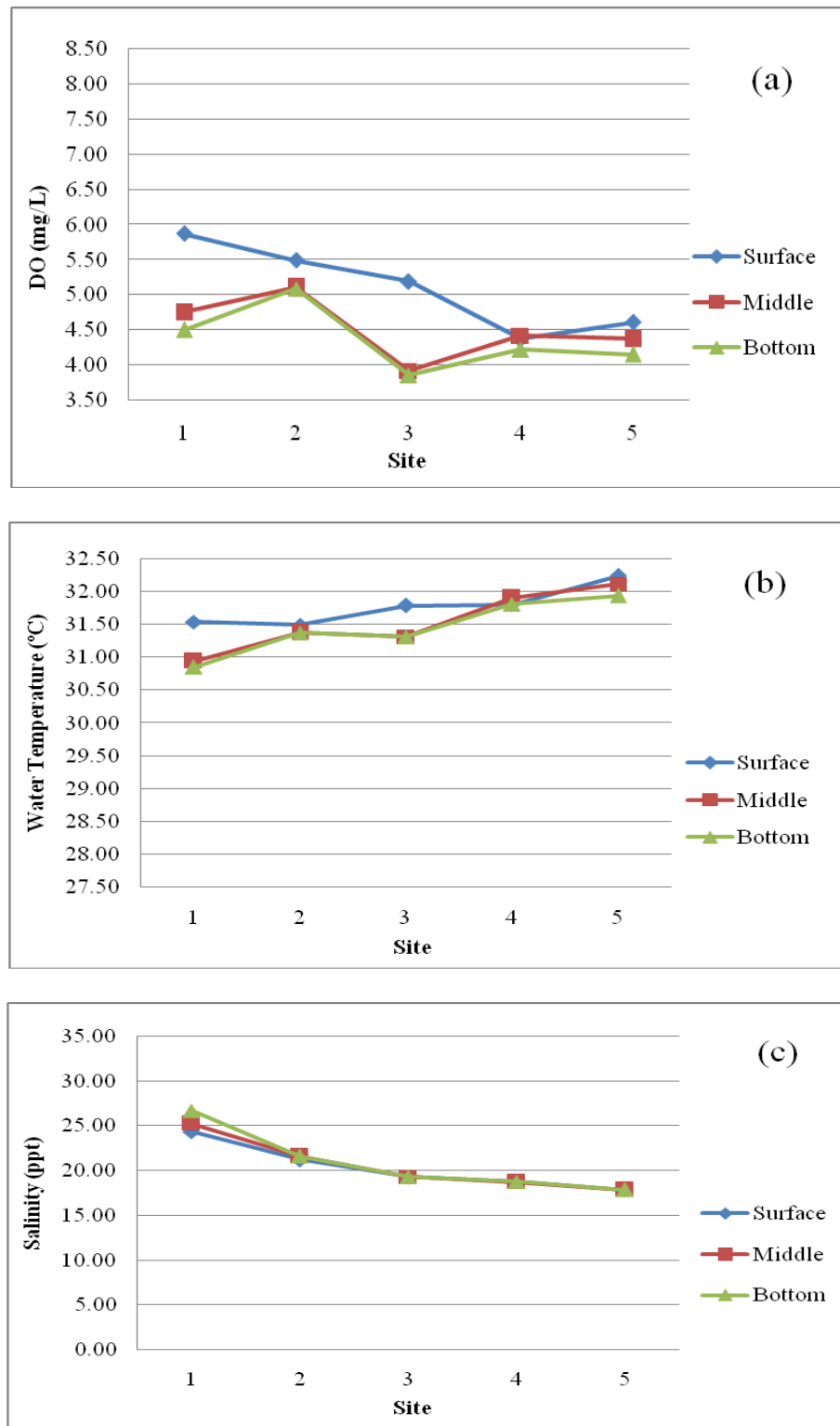


Figure 12 Dissolved oxygen, water temperature, and salinity data, by depth, from the spatial spring low tide cruise on July 20th, 2005, in the Skidaway River Estuary, GA

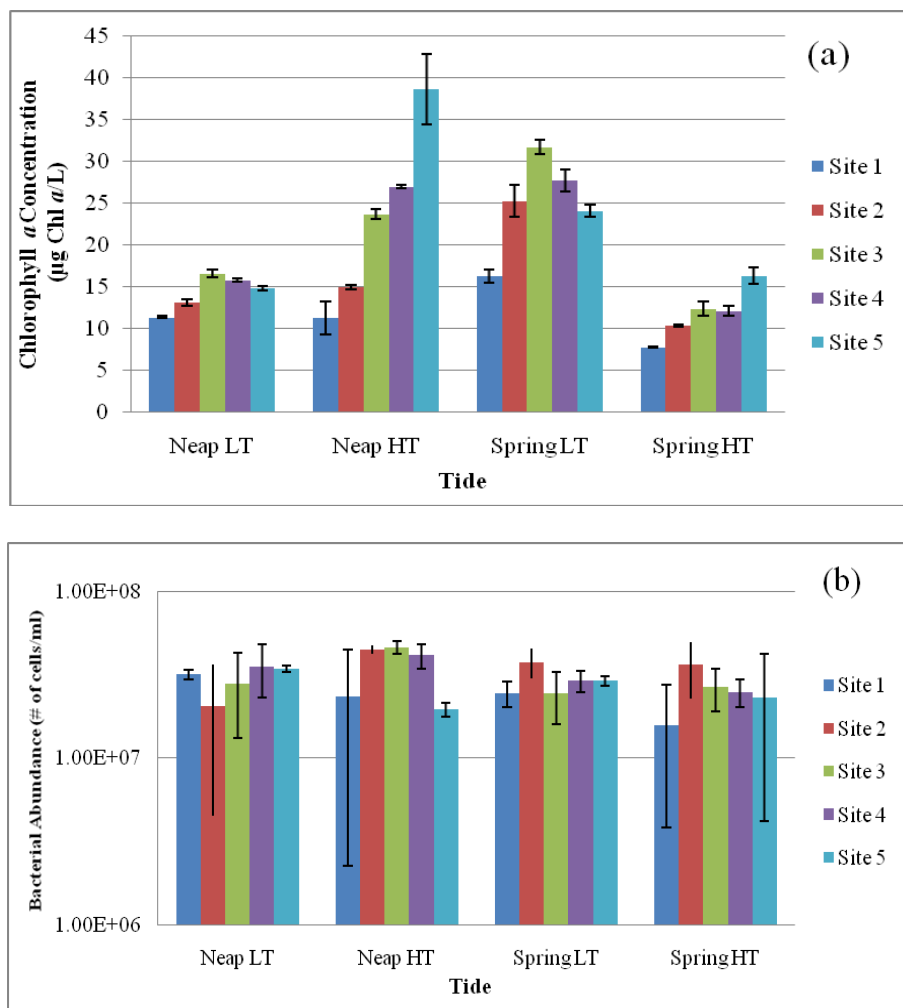


Figure 13 Chlorophyll *a* concentration and bacterial abundance data from the spatial cruises on July 13th (neap tide) and July 20th (spring tide), 2005, in the Skidaway River Estuary, GA
 Error = 1 SD.