ABSTRACT

Title of thesis: ZOOPLANKTON POPULATION DYNAMICS IN RELATION TO THE RED TIDE DINOFLAGELLATE *KARENIA BREVI*S ON THE WEST FLORIDA SHELF OF THE GULF OF MEXICO

Brianne M. Walsh, Master of Science, 2012

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Blooms of the toxin producing dinoflagellate *Karenia brevis* are common in the Gulf of Mexico, and while several studies have investigated nutrient sources and bloom processes, there has been less research in regards to zooplankton population dynamics within these blooms. Zooplankton community structure and copepod species composition were analyzed from samples collected on the West Florida Shelf during October 2007-2010. Copepods constituted the most important zooplankton group, averaging 60% of total abundance. In 2009 there was a significant difference between the abundance of zooplankton at stations within a *K. brevis* bloom. As the *K. brevis* bloom progressed, total zooplankton abundance decreased. Additionally, the role of zooplankton within *Karenia brevis* blooms was investigated as both grazers of primary productivity and potential sources of regenerated nutrients.

ZOOPLANKTON POPULATION DYNAMICS IN RELATION TO THE RED TIDE DINOFLAGELLATE *KARENIA BREVIS* ON THE WEST FLORIDA SHELF OF THE GULF OF MEXICO

by:

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DEDICATION

"Twenty years from now you will be more disappointed by the things you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream. Discover."

-Quote attributed to Mark Twain

I dedicate this thesis to my mother, Jean Walsh, for her constant support, strength and encouragement. Thank you for all of your love and advice throughout the years, having always provided me with the inspiration to succeed, supporting me in all of my endeavors, and providing the resources to take these chances.

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CHAPTER ONE

INTRODUCTION

Harmful algal bloom events are characterized by the proliferation and occasional dominance of a single species of toxic or harmful algae (Anderson, 2005). Previously referred to as red tides, these events are now grouped under the common title of harmful algal blooms (HABs), and can include both toxin producing and non-toxic forms. All HABs have one important characteristic in common—they cause harm to an existing ecosystem, either through the production of toxins or the accumulated biomass that can negatively impact other populations within the area they exist (Anderson et. al., 2002). Red tides, both toxic and non-toxic, have occurred throughout much of recorded history (Anderson, 1989). However, over the past several decades the global occurrence of harmful algal blooms has increased both spatially and temporally within aquatic environments (Smayda, 1990; Anderson, 1995; Breier and Buskey, 2007). This increase is attributed largely due to anthropogenic influences on coastal waters, as well as an increased awareness and detection of harmful algal blooms (Speekmann et al., 2006). The excessive appearance of blooms may lead to more serious impacts, including the loss of submerged aquatic vegetation (SAV), shifts in ecosystem productivity, low dissolved oxygen concentrations, and mortalities in fish and shellfish populations (Bricker, 2008). In addition, harmful algal blooms have an array of economic impacts—including the cost of conducting and maintaining effective monitoring programs; potential economic losses to the shellfish and commercial fishing industries, and impacts on tourism in coastal regions. Medical treatment costs incurred by exposed coastal populations can also factor

into the economic impact of HABs. When toxic phytoplankton are filtered from the water by shellfish for food, their toxins can accumulate in shellfish biomass to levels that can be dangerous if ingested by humans or other consumers (Anderson, 2005). Despite increased awareness and predictive capabilities, there is still a great deal of uncertainty regarding harmful algal bloom causing species and their potential impacts on ecosystems. Until researchers have a better grasp of the extent of their ecological impacts, management and mitigation efforts will remain ineffectual in either controlling or forecasting bloom events.

The goal of this thesis is to examine the interactions between the toxic dinoflagellate *Karenia brevis* and zooplankton populations on the West Florida Shelf (WFS) of the Gulf of Mexico. While several studies have focused on characterizing the zooplankton assemblage of the Eastern Gulf of Mexico and its associated estuaries, to date, there has only been one *in situ* study characterizing potential perturbations to the zooplankton assemblage of the WFS in relation to *K. brevis* (Lester, 2005).

Karenia brevis

Karenia brevis (Davis) G. Hansen et Moestrup, formerly *Gymnodinium breve* and *Ptychodiscus breve* (Steidinger, 1979), is a toxic dinoflagellate commonly known to form blooms in the Eastern Gulf of Mexico. Blooms of *K. brevis* are not a recent phenomenon, as early Spanish explorers described events suggesting fish kills and aerosol production by blooms of *K. brevis*, and reports of discolored water and the effects of toxins have been recorded in ship's logs dating back to the 1500's (Tester and Steidinger, 1997; Lester, 2005). In many areas of the world, harmful algal blooms are

increasing in both magnitude and frequency largely due to the eutrophication of both freshwater and marine environments. The human population along the Southwest coast of Florida has increased 10-40 fold in the past half century (Brand and Compton, 2007), however, widespread blooms of *K. brevis* are not a new occurrence within this region. Despite the near annual occurrence of *K. brevis* blooms on the WFS, the nutrient sources and dynamics sustaining blooms of such large biomass remain enigmatic to researchers.

K. brevis Physiology

Karenia brevis is an unarmored dinoflagellate, ranging from 18-45μm in diameter (Figure 1.1). Cells are dorsal-ventrally flattened, have two flagella, and are able to move within the water column at swimming speeds up to 1m h^{-1} (McKay et al., 2006). This maximum swimming speed during the day is driven by phototaxis and geotaxis (Kamykowski et al., 1998). *K. brevis* cells migrate vertically on diel cycles, maximizing carbon fixation from photosynthesis in daytime hours and migrating to depth at night to take advantage of dissolved nutrients close to the sediment interface (Van dolah et al., 2009). *K. brevis* is a mixotroph, capable of fixing carbon photosynthetically as a photoautotroph, and also displays heterotrophic capabilities in the uptake of dissolved organic compounds as well as grazing capacity in both field and laboratory studies (Steidinger et al., 2008). *K. brevis* reproduces both asexually through vegetative cell division, and through sexual reproduction via gamete formation (Steidinger et al., 2008), however the actual life cycle of *K. brevis* remains undefined (Fleming et al., 2011). Cell division rates observed both in lab and field populations of *K. brevis* average 0.3 divisions day^{-1}, with a maximum of approximately 0.6 divisions day^{-1} (Van dolah et al.,

2009). In culture, optimum growth is obtained at salinities between 27 and 37, and temperatures between 22°C and 28°C (Wilson, 1966). *K. brevis* does not grow at salinities <17 (Steidinger et al., 2008), and based on these observed temperature and salinity preferences, it can be classified as an oligotrophic shelf, not estuarine, species.

Brevetoxins

Researchers have identified several different Ptychodiscus toxins (PbTxs) produced by *Karenia brevis* in culture. These toxins (commonly referred to as brevetoxins) interfere with sodium channels within nerve cells, causing the depolarization of nerves, which can then lead to muscle paralysis (Huang and Wu, 1989; Speekmann et al., 2006). During Florida red tide blooms the major brevetoxin produced is PbTx-2, along with lesser amounts of PbTx-1 and other analogs (Fleming et al., 2011). Once blooms reach high cell densities they can become toxic, producing PbTxs that can then be transported through the water column and atmosphere. There is evidence that these toxins are then transferred up the food web to higher trophic levels, and the toxins produced by *K. brevis* have been found to accumulate within shellfish tissue. Ingestion of brevetoxin-laden shellfish can result in Neurotoxic Shellfish Poisoning (NSP) in humans (Speekmann et al., 2006). Symptoms of NSP occur one to three hours after the consumption of contaminated shellfish, and include numbness, tingling in the mouth and extremities; incoordination, and gastrointestinal upset. Death is extremely rare in cases of NSP, and recovery usually occurs within two to three days (www.cdc.gov).

Brevetoxins are tasteless, odorless, and heat and acid stable, and therefore cannot be easily detected or removed by food preparation techniques (Fleming et al., 2011). The

closure of shellfish beds along the Florida coast has a clearly defined target of $5x10^3$ cells L^{-1}, however respiratory irritation can occur over a wide range of conditions and fish kills have been reported with concentrations $>5x10^4$ cells L^{-1} (Heil, 2009). When wind and wave action disrupts and lyses the cells, brevetoxins can become aerosolized and cause respiratory distress in humans (Pierce et al., 1990). Brevetoxin aerosols have been documented to travel as much as a mile inland during an active red tide, particularly when there are strong prevailing onshore winds (Kirkpatrick et al., 2010). Exposure to aerosolized brevetoxins has been linked to respiratory distress in humans, and in several cases, marine mammal death (Speekmann, 2006). The economic costs of beach cleanups and decreases in tourism related to HABs can be considerable (Habas and Gilbert, 1974), and this is a major issue in Florida where tourism is one of the largest industries (Steidinger et al., 1998).

K. brevis distribution

To date, *K. brevis* occurs naturally on the Gulf Coast of North America from Mexico to Florida, and also from the Gulf Stream to the mid-Atlantic. The resident population of *K. brevis* is in the Gulf of Mexico, which can be transported via the Loop current, the Florida current, and the Gulf stream, moving north as far as the U.S. South Atlantic Bight (Tester and Steidinger, 1997). Throughout the year, *K. brevis* is found in background concentrations (<1,000 cells $liter^{-1}$) in the Florida current-Gulf stream system, and during a peak bloom phase, cell counts can reach up to 10^6 cells L^{-1} (Speekmann et al., 2006). On rare occasions during bloom conditions, *K. brevis*

populations can become entrained in the Florida current and transported into the Atlantic (Tester and Steidinger, 1997). The extent of the loop current's Northward intrusion onto the West Florida Shelf varies seasonally, and can greatly alter the potential of bloom initiation, transport and retention (Haddad and Carter 1979; Tester and Steidinger, 1997).

Four distinct phases for *K. brevis* blooms have been identified based on cell physiology and growth—initiation, growth, maintenance, and termination (Steidinger et al., 1998). It is hypothesized that blooms originate offshore on the mid shelf region, approximately 18-74 km offshore (Steidinger et al., 1998). Blooms then increase in biomass via reproduction or physical forcing, and are transported inshore via physical conditions (Singh, 2005).

West Florida Shelf dynamics

The inner WFS is a shallow carbonate platform extending approximately 200km west of the Florida coastline (Brand and Compton, 2007). The outermost shelf waters are mainly influenced by variations in the loop current, while inshore waters are affected by wind and land runoff (Brand and Compton, 2007). Inshore coastal currents are strongly wind driven, flowing north in summer months and shifting south in winter (Brand and Compton, 2007). The regions of the Gulf of Mexico that experience *Karenia brevis* blooms have several characteristics in common that make them favorable to the formation of blooms: each area is adjacent to a continental shelf break where it intersects with a permanent seasonal thermocline (Tester and Steidinger, 1997), resulting in temperatures around 20°C. Additionally, the bottom terrain at these locations is likely suitable for overwintering of resting cysts. These areas also experience persistent, or

seasonal slope-shelf upwelling episodes (Tester and Steidinger, 1997). The wet summer season on the WFS is marked by thermal stratification with strong vertical temperature and salinity gradients, which transition to a relatively homogenous water column in the fall due to cold fronts shifting southward and vertical mixing (Vargo et al., 2002). Coastal thermal and salinity fronts are also apparent where the estuarine waters move into coastal zones (Vargo et al., 2008).

Karenia brevis blooms on the WFS

On the West Shelf of the Gulf of Mexico, *K. brevis* blooms occur almost annually, and have resulted in severe economic and environmental impacts in Florida. Although classified as a coastal bloom species (Smayda, 1990), *K. brevis* occurs over a wide range of nutrient regimes, from initiation in offshore oligotrophic waters, to near shore maintenance that can persist over long time scales (Steidinger, 1975). To date, research on *K. brevis* has focused mainly on the factors and conditions that lead to the formation and support of blooms along the WFS. Blooms can occur at any time of the year, but are typical in the late summer or early fall when >70% of the outbreaks have begun (Tester and Steidinger, 1997). Blooms of *K. brevis* are especially frequent from Clearwater to Sanibel Island (Joyce and Roberts, 1975; Tester and Steidinger, 1997). Blooms typically last one to two months, but have been documented to persist up to five months (Speekmann, 2006; Buskey, 2006), with peak booms becoming monospecific (Steidinger and Vargo, 1998). In January 2005, a bloom was observed offshore of St. Petersburg, Florida that lasted through January 2006—over thirteen months in duration (Heil and Steidinger, 2009). This event resulted in massive fish kills; bird, turtle and

manatee deaths; and extensive commercial shellfish closures and benthic mortality in areas of Florida, Mississippi and Alabama (Heil and Steidinger, 2009). The economic impact of red tides in the Gulf of Mexico is estimated to range from $250,000 to $120,000,000 per event (Kusek, 1998). Historically, the occurrence of such widespread HAB events has been correlated to physical processes, large nutrient inputs and anthropogenic sources. However, it is also important to consider the ecosystem interactions within the blooms that could be responsible for the initiation and maintenance of such a bloom event.

The region where *K. brevis* blooms typically occur is characterized by shoreward nutrient gradients as well as latitudinal gradients in nutrient concentrations and fluxes (Heil et al., in review). Potential nutrient sources supporting *K. brevis* blooms on the Gulf Coast of Florida include both external and *in situ* nutrient sources. Allochthonous nutrient sources include estuarine discharge, atmospheric inputs, upwelling, and benthic regeneration. Autochthonous processes include nutrient regeneration via micro- and mesozooplankton grazing and excretion, decay of dead organisms, release from nitrogen fixing organisms, and bacterial remineralization (Figure 1.2).

The initiation of blooms is physically driven and dependent on location, upwelling and nutrient sources. Near shore blooms can be maintained from months to years, however, the specific sources of major nutrients (nitrogen and phosphorus) required to maintain such high biomass, monospecific blooms have yet to be identified (Singh, 2005). The coastal ocean of Southwest Florida receives nutrient inputs from multiple riverine and estuarine sources, as well as atmospheric deposition. In addition, natural deposits of phosphorite along the West Coast of Florida result in an N:P ratio

usually below Redfield, and therefore phytoplankton in this region are usually nitrogen limited (Brand and Compton, 2007). One way that *K. brevis* blooms have been hypothesized to initiate is in response to blooms of the filamentous cyanobacteria *Trichodesmium* spp. *Trichodesmium* is a nitrogen fixer, releasing a significant fraction of newly fixed N in the form of dissolved organic nitrogen (DON) into surface waters, which can potentially provide N to *K. brevis* as a mixotrophic organism (Mulholland et al., 2006). Aeolian deposition of iron during Saharan dust events has been linked to increased growth and N_2 fixation by *Trichodesmium* (Lenes et al., 2001; Walsh and Steidinger, 2001). This contribution from natural blooms of *Trichodesmium* spp. may be sufficient to support moderately dense blooms ($\leq 10^5$ cells L^{-1}) (Glibert et al., 2009). In addition, *K. brevis* is a mixotroph and has been documented to graze on the cyanobacteria *Synechococcus* spp. Grazing by *K. brevis* can contribute up to 40% of the cellular N requirements for cells (Glibert et al., 2009). Additional sources of nutrients supporting *K. brevis* blooms could be coming from decaying fish—the result of fish kills caused by brevetoxins. The excretion and egestion of nutrients by micro- and mesozooplankton are also potential nutrients available for blooms to utilize.

Karenia brevis **and Zooplankton**

Zooplankton Background

Within pelagic food webs, zooplankton play a key role, mediating the transfer of energy produced by unicellular algae through photosynthesis to higher trophic levels (Harris et al., 2000). Zooplankton function as both a sink and source for nutrients, by simultaneous incorporation of prey items into biomass and release of dissolved nutrients

(Wavle and Larsson, 1999) and zooplankton grazing and excretion can also have a large impact the amount and composition of vertical particle flux (Harris et al., 2000). In highly productive regions of the world's oceans, apart from predation, the availability of zooplankton is regarded as the most important environmental factor controlling the year class strength of a number of commercially important fish stocks (Harris et al., 2000).

Zooplankton and HABs

While physical factors determine the distribution and presence or absence of HAB species on a regional scale, nutrient availability and grazing have the ability to affect growth rate, biomass and duration of blooms on a local scale (Vargo, 2009). Several physical, chemical and biological processes drive zooplankton distribution and community dynamics within estuarine ecosystems. While there has been considerable research regarding factors such as temperature, salinity and trophic state and their effects of spatial and temporal distributions of zooplankton, little research exists in regards to zooplankton population dynamics within harmful algal blooms (Badylak and Phlips, 2008). The population dynamics of harmful algal bloom causing dinoflagellate species and the trophic dynamics between dinoflagellates and potential grazer species are not well understood (Breier and Buskey, 2007). Zooplankton have the potential to play two important roles within harmful algal blooms—first as "top down" grazers of primary production, essentially controlling the growth of HABs, as well as fueling "bottom up" productivity, through nutrient regeneration from both sloppy feeding and excretion. It is critical to define the interactions that occur between harmful algal species and their potential grazers in order to understand how HAB species may alter and disrupt marine

food webs, as well as how they are able to form such successful, persistent blooms (Breier and Buskey, 2007).

WFS zooplankton assemblage

To date, little research has focused on potential changes specifically related to zooplankton populations within *K. brevis* blooms in the Gulf of Mexico. While there is considerable knowledge regarding the zooplankton assemblages of estuarine and coastal systems on the West coast of Florida there has been less effort focused on identifying the normal zooplankton assemblage of the WFS (Lester et al., 2008). Previous studies in or near the WFS have been analyses of total biomass variation, quantitative assessments of taxonomic composition at a single station or point in time, or qualitative annual surveys (Lester, 2005). Many of these are limited to the major species and category of abundance and focus primarily on coastal estuaries and lagoons (Dragovich and Kelly, 1967).

Relationships between K. brevis and zooplankton

Grazing of *K. brevis* by zooplankton can result in deleterious effects on grazers, including reduced fecundity and egg production, paralysis and regurgitation. Some copepods are known to directly ingest toxic bloom species, potentially helping control blooms; however others may avoid ingestion or are incapacitated from consumption (Turner and Tester, 1997; Breier and Buskey, 2007). Laboratory studies have shown that when the calanoid copepod *Acartia tonsa* was fed a diet of only *K. brevis*, individuals had lower ingestion rates and offspring production than when feeding on a mixed diet or diet consisting of no *K. brevis* (Breier and Buskey, 2007). However other studies have shown

that *A. tonsa,* and two other copepod species*, Oncaea venustra and Labidocera aestiva* to have no adverse effects after consuming *K. brevis* (Turner and Tester, 1989). Also, copepods that feed on toxic algal species may transfer toxins to fish and up trophic levels (Speekmann et al., 2006). Brevetoxins have been traced through experimental food chains, transferred from dinoflagellates through zooplankton grazers to juvenile fish (Tester et al., 2000; Prince et al., 2006; Landsberg et al., 2006).

Similarly, other dinoflagellates have had mixed effects on zooplankton. In the case of another toxic species, *Pyrodinium bahamense*, Badylak and Phlips (2008) observed variability in zooplankton community responses during a major bloom in the Tampa Bay estuary, with an overall summer decline in key holoplankton species, coinciding with peaks in *P. bahamense* populations (Badylak and Phlips, 2008). However, populations of the larvacean *Oikopleura dioica* increased during peak bloom conditions, potentially benefitting from reduced competition for phytoplankton with other grazers (Badylak and Phlips, 2008).

The overarching goal of this thesis is to understand zooplankton population dynamics within harmful algal blooms, specifically *Karenia brevis* blooms near the WFS in the Gulf of Mexico. Many aspects of HAB dynamics remain unresolved, and due to the large negative environmental and economic impacts that *Karenia brevis* can have, it is important for researchers to understand all aspects of HAB dynamics. This includes the interactions with zooplankton populations in the areas where the blooms are occurring. Specific project objectives aim to extend and confirm the results of Lester (2005), determining the abundance, biomass and composition of the normal zooplankton assemblage of the WFS, and then distinguishing between zooplankton communities

present within bloom concentrations of *Karenia brevis*. Comparison of the zooplankton communities will be assessed both on a spatial scale (distance offshore) and in comparison to a suite of environmental factors, including *Karenia brevis* abundance and bloom phase. Additionally, this study aims to estimate phytoplankton growth rates and mortality due to micro- and mesozooplankton grazing, as well as the potential roles of mesozooplankton as suppliers of regenerated nutrients within *Karenia brevis* blooms.

Study Design

This study was completed within the scope of the NOAA ECOHAB: *Karenia* Nutrient Dynamics in the Eastern Gulf of Mexico project. Lead by a multidisciplinary team of scientists with expertise in nutrients, HABs, *Karenia brevis* physiology and ecology, and the environment of Southwest Florida, the objective of the ECOHAB project was to address two outstanding questions in *Karenia brevis* research: 1) What are the major nutrient sources (nitrogen and phosphorus) fueling the massive, persistent biomass accumulations that occur almost annually on the WFS; and 2) What is the importance of each source during changing bloom physiological state and spatial gradient? Until these two questions are answered, management activities will continue to be ineffectual in either controlling or forecasting *K. brevis* bloom initiation and dynamics. In this study, a "bloom" is defined as an accumulation of *K. brevis* cells with a concentration of 1000 cells L^{-1} or higher. This concentration is based on the protocol used by the Florida Fish and Wildlife Research Institute (FWRI) weekly red tide status reports that are published online. (http://myfwc.com/research/redtide/events/status/).

There are two main hypotheses in regards to *Karenia brevis* nutrient sources

within the ECOHAB project proposal: The "no-smoking gun" hypothesis, that multiple nutrient sources and forms on the West Florida Shelf support *K. brevis* blooms, with the relative contribution and/or importance of each source depending on the physiological state of the bloom, the bloom environment and the location along a spatial gradient. The second coinciding hypothesis is the "physiological plasticity" hypothesis: *K. brevis* is a mixotroph with a flexible metabolism, and therefore, limiting factors and metabolic processes vary with physical, chemical and biological environments. In order to address these hypotheses, research focused on the comparison of the physical, chemical and biological characteristics of *K. brevis* and its surrounding environment during three bloom stages (combined initiation and development, maintenance, and decline) in three different bloom environments (lagoonal, estuarine and coastal). Specific objectives included a comparison of the nutritional physiology (carbon, nitrogen, and phosphorus) of *K. brevis* within various bloom stages, and across environments where blooms occur and in cultures grown under a range of environmentally relevant conditions. The project also sought to evaluate potential sources of new and regenerated nutrients from: a) N_2 fixers (e.g., *Trichodesmium, Lyngbya*, and unicellular diazotrophs) and other microbes, b) zooplankton excretion and assessment of overall contribution to nutrient budgets, c) flux of particulate and dissolved inorganic and organic material from estuaries to coastal waters d) atmospheric deposition, e) benthic fluxes, and d) photochemical reactions.

While there has been a great deal of research centered on global zooplankton population dynamics in terms of spatial and temporal distribution, there is a gap in knowledge focusing on zooplankton community response during harmful algal blooms, specifically in relation to *Karenia brevis*. The overarching goal of this thesis is to

understand zooplankton population dynamics in relation to blooms of the harmful dinoflagellate *Karenia brevis* on the WFS of the Gulf of Mexico. Due to the large negative environmental and economic impacts that *Karenia brevis* can cause for the Florida Gulf Coast, it is important for researchers and policy makers to understand all aspects of HAB dynamic, including the interactions with zooplankton populations in the areas where the blooms are occurring. My research aimed to investigate the relationship between *Karenia brevis*, and the zooplankton community present on the WFS of the Gulf of Mexico and its associated estuaries. Three specific objectives were identified to structure this research:

Objective One: Identify the abundance, biomass and composition of the normal zooplankton assemblage of the WFS and its associated estuaries. Describe inter-annual and spatial variability of zooplankton populations.

Objective Two: Distinguish between, and compare the normal zooplankton assemblage of the WFS and zooplankton communities present within *Karenia brevis* blooms.

Objective Three: Define the role of zooplankton within *Karenia brevis* blooms as both grazers of primary productivity and potential sources of regenerated nutrients fueling production.

Zooplankton community abundance was sampled in October of four years, 2007-2010 during annual ECOHAB cruises aboard the LUMCON Vessel *R/V Pelican*. Prior to indentifying changes in the zooplankton community in relation to bloom populations of *Karenia brevis*, if was first necessary to characterize the ambient zooplankton population of the West Florida Shelf. Chapter 2 will address this analysis, and investigate

environmental conditions driving zooplankton patterns of the WFS, and perturbations to the zooplankton community in relation to *K. brevis*. Chapters 3 and 4 describe the potential role of zooplankton within *K. brevis* blooms, as both "top down" grazers of primary production and "bottom up" suppliers of regenerated nutrients. In order to address the role of zooplankton as grazers on *Karenia brevis*, and as a potential nutrient source fueling blooms, shipboard grazing experiments were completed in all four years of the study, and laboratory experiments investigating zooplankton grazing on *K. brevis* were completed in 2010, 2011 and 2012. The final chapter of this thesis (Chapter 5) provides a synthesis of results found within the confines of this study, describing the population dynamics of zooplankton in relation to *Karenia brevis* on the WFS.

In addressing the goals of the ECOHAB project it is important to consider the ecosystem in its entirety, including the different populations that a bloom will potentially encounter and interact with. Historically, the occurrence of such widespread events have been correlated to large nutrient inputs and anthropogenic sources, but is also important to consider ecosystem interactions within the blooms that could be responsible for the initiation, maintenance and decline of such a bloom. The population dynamics of harmful algal bloom causing dinoflagellate species and the trophic dynamics between potential grazer species are not well understood, and relationships between zooplankton and toxic phytoplankton are complex and species specific (Lester, 2005). It is critical to define the interactions that occur between HAB species and their potential grazers in order to understand how HABs may alter and disrupt marine food webs, as well as how they are able to obtain sufficient that enable the formation of such successful blooms, persisting over long time periods. While there is considerable research on how

temperature and salinity affects the trophic state of zooplankton population distributions of the WFS and its associated estuaries, there has been little research specifically in regards to zooplankton population dynamics within *Karenia brevis* blooms. Further understanding of the grazer-toxic algae relationship is critical for understanding and predicting the effects of harmful algal blooms within marine environments as well as providing key management and mitigation efforts.

Figure 1.1. *Karenia brevis,* the Florida red tide causing dinoflagellate (Source: Provasoli-Guillard National Center for Marine Algae and Microbiota).

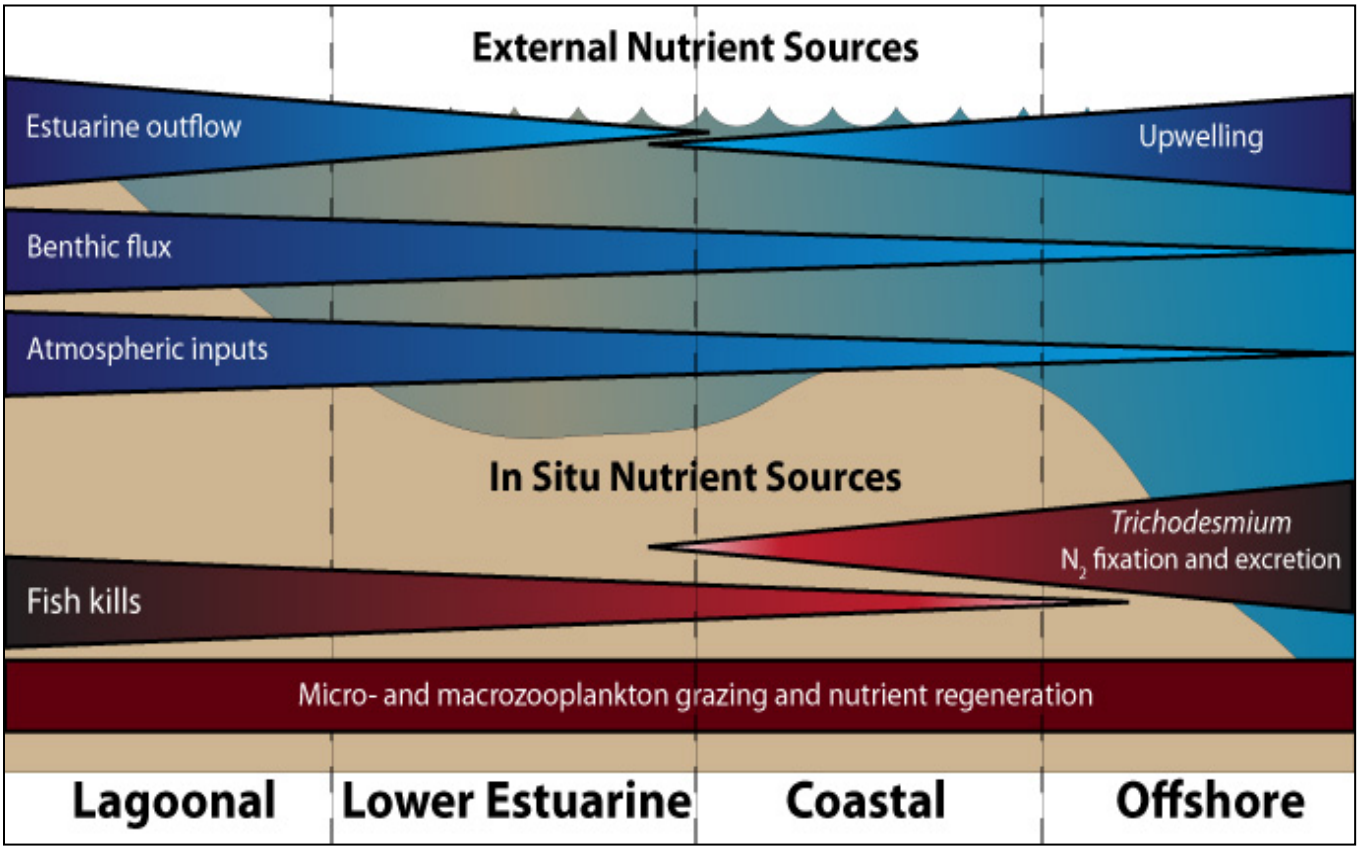

Figure 1.2. External and *in situ* nutrient sources supplying blooms of *Karenia brevis* from a spatial gradient on the West Florida Shelf (adapted from the ECOHAB: *Karenia* Nutrient Dynamics proposal). Length of bar/triangle indicates the importance of each nutrient source over the spatial gradient (Lagoonal, Lower Estuarine, Coastal, Offshore).

CHAPTER TWO

ZOOPLANKTON COMMUNITY ASSEMBLAGES ON THE WEST FLORIDA SHELF IN RELATION TO BLOOMS OF *KARENIA BREVIS*

Abstract

Zooplankton community structure and copepod species composition were analyzed from samples collected on the West Florida Shelf (WFS). Zooplankton abundance ranged from 9,824 individuals m^{-3} at the offshore station in 2008 to 159,499 at the station inside Charlotte Harbor in 2010. In total 18 species of holoplankton were identified, as well as seven groups of meroplankton. Copepods constituted the dominant zooplankton group, averaging 60% of total abundance. *K brevis* bloom populations (>1000 cells L^{-1}) were present in 2007, 2008 and 2009. In 2008, the bloom was in the initiation phase, whereas the 2007 and 2009 blooms were in the maintenance/stationary phase. In 2008 there was no statistical difference in the abundance of zooplankton at bloom and non-bloom stations, however in 2009 there was a statistically significant lower abundance ($p<0.05$) of zooplankton at stations with bloom concentrations of *Karenia brevis* present. During the 2008 and 2009 bloom sampling years, as the *K. brevis* bloom progressed, total zooplankton abundance decreased. The average similarity between bloom stations in 2008 was 64.52%, and in 2009, the average similarity between stations was 80.09%, suggesting that the zooplankton communities present at bloom stations in 2009 were more similar in community composition.

Introduction

Karenia brevis, formerly *Gymnodinium breve* and *Ptychodiscus breve* (Steidinger, 1979), is an unarmored, toxic dinoflagellate that commonly forms blooms along the Western coast of Florida (Speekmann et al., 2006). *K. brevis* occurs on the Gulf Coast from Mexico to Florida, and also from the Gulf Stream to the mid-Atlantic. Non-bloom concentrations of *K. brevis* are often present in the Gulf of Mexico, and range from undetectable to 1000 cells L^{-1}. During peak bloom phase periods, cell counts can reach up to 10^6 cells L^{-1} (Speekmann et al., 2006). On the West Florida Shelf (WFS) of the Gulf of Mexico, *K. brevis* blooms are fairly predictable on an annual basis, and have resulted in severe economic and environmental impacts for the state of Florida (Heil and Steidinger, 2009). Although classified as a coastal bloom species, *K. brevis* occurs over a wide range of nutrient conditions, from initiation in offshore oligotrophic waters (Steidinger, 1975), to near shore maintenance stages that can persist over longer time periods (Steidinger, 1975; Heil and Steidinger, 2009).

Karenia brevis produces a suite of neurotoxins referred to as brevetoxins, which are lipid soluble and bind to sodium channels (Shimizu et al., 1986), causing channels to remain open, resulting in the uncontrolled flux of sodium ions into cells (Poli et al., 1986; Trainer and Baden, 1999; Waggett et al., 2012). The presence of *K. brevis* blooms have been associated with fish, bird, and marine mammal mortality, and laboratory studies demonstrate the potential for brevetoxins to accumulate in copepods and fish and be transferred to higher trophic levels (Speekmann et al., 2006). In humans, ingestion of brevetoxin-laden shellfish can result in neurotoxic shellfish poisoning (NSP), whereas aerosolized brevetoxins can result in respiratory distress in humans and even death in

marine mammals (Speekmann et al., 2006).

Blooms typically last one to two months, but have been documented to persist up to five months or longer (Speekmann et al., 2006). In January 2005, a bloom was initially observed offshore St. Petersburg, Florida that lasted through January 2006—over thirteen months in duration (Heil and Steidinger, 2009). This event resulted in massive fish kills; bird, turtle and manatee deaths; and extensive commercial shellfish closures and benthic mortality in areas of Florida, Mississippi and Alabama (Heil and Steidinger, 2009). Historically, the occurrence of such widespread harmful algal bloom (HAB) events have been correlated to physical processes, nutrient inputs and anthropogenic sources (Vargo, 2009). Physical factors determine the distribution, and presence or absence of harmful algal bloom species on a regional scale, whereas nutrient availability and grazing can affect blooms on a local scale (Vargo, 2009). It is important to consider these biological processes occurring within bloom environments that could be responsible for the initiation, maintenance and termination of such a bloom.

Zooplankton populations play a critical role in the marine food web, linking energy from photosynthetic phytoplankton to higher trophic levels. Physical, chemical and biological processes drive zooplankton distribution and community dynamics within coastal and estuarine ecosystems. While there has been considerable research focused on the effects of temperature (Austin and Jones, 1974), salinity (Silva et al., 2009; Zervoudaki, 2009), and trophic state (Leising et al., 2005), as well as spatial and temporal distributions (Kelly and Dragovich, 1967; Badylak and Phlips, 2008) of zooplankton; there has been less research in regards to zooplankton population dynamics within HABs (Badylak and Phlips, 2008; Lester et al., 2008) and their relationships with potential

grazer species (Breier and Buskey, 2007). Previous studies have shown several species of toxic algae to negatively impact the behavior, reproduction and survival of certain zooplankton species (Gill and Harris, 1987; Turner and Tester, 1989; Badylak and Phlips, 2008), however, there is still uncertainty about how the zooplankton community as a whole may respond to the presence of HAB species (Badylak and Phlips, 2008).

Numerous studies have aimed to characterize the zooplankton assemblages of estuaries and river systems of the Eastern Gulf of Mexico, however less effort has focused on identifying the ambient zooplankton assemblage of the WFS (Hopkins, 1977; Lester et al., 2008). Previous studies in or near the WFS have consisted of analyses of total biomass variation, quantitative assessments of taxonomic composition at a single station or point in time, or qualitative annual surveys (Lester, 2005). Many of these are limited to the major species and category of abundance and focus primarily on coastal estuaries and lagoons (Kelly and Dragovich, 1967; Lester et al., 2008). Gulf of Mexico zooplankton communities are predominately dominated by a diverse assemblage of copepods, as well as other species such as larvaceans and meroplanktonic larvae (Elliot et al., 2012). The continental shelf of the Northern Gulf of Mexico is broad and physically precludes many deep-water species of zooplankton from appearing on the shelf (Ortner et. al., 1989). Offshore epizooplankton are often found in shelf waters, as there are a number of physical mechanisms through which central Gulf of Mexico water can cross over the WFS (Ortner et. al., 1989; Lester, 2005). Considerable overlap between estuarine, coastal and offshore zooplankton populations is expected due to the hydrological dynamics on the WFS, however previous research has shown that zooplankton populations on the shelf are different than estuarine and offshore

assemblages (Minello, 1980; Hopkins et. al., 1981; Ortner et. al., 1989; Sutton, 2001; Lester, 2005).

The overarching goal of this project was to understand zooplankton community dynamics in relation to *Karenia brevis* blooms on the West Florida shelf of the Gulf of Mexico. Specific objectives included first determining the non-bloom ambient zooplankton assemblage of the WFS and its associated tributaries, and then distinguishing between the zooplankton community and populations present during *K. brevis* bloom and non-bloom periods, by quantifying changes in abundance and species diversity. These analyses were then used to estimate the impact of zooplankton as both regulators of bloom biomass through the preferential grazing on non-toxic species, and as a source of regenerated nutrients available to *K. brevis*.

Material and Methods

Study Design

Sampling of the West Florida Shelf zooplankton assemblage took place during four annual ECOHAB: *Karenia Nutrient Dynamics* cruises in October 2007-2010 aboard the *R/V Pelican*. Zooplankton samples were collected at all main stations along the cruise track (Figure 2.1). A CTD profile was conducted at every station for a vertical profile of temperature and salinity, and water samples were collected to determine chlorophyll *a* (Chl *a*) concentrations and *K. brevis* cell counts (cells L^{-1}). For the purpose of this study, temperature, salinity and Chl *a* were averaged over the whole water column. Nutrient concentrations for dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), dissolved ammonium (DNH_4^+) and total dissolved nitrogen

(TDN) and phosphorus (TDP) were analyzed using standard methods by Virginia Institute for Marine Sciences (VIMS) (Bronk et al. in review) and Mote Marine Laboratory (MOTE) (Dixon et al. in review).

Zooplankton Collection

Integrated water column samples of zooplankton were taken using a 0.25m diameter 64μm mesh paired bongo net with an oblique tow from bottom to surface. The volume of water filtered through each of the nets was recorded with a General Oceanics model 2030R flow meter and all zooplankton counts were corrected to abundance m^3. The average volume of water filtered per tow was $2.06m^3$.

Once onboard, live samples were concentrated through a 64μm mesh sieve and any large gelatinous zooplankton were removed and measured via displacement volume and recorded for later inclusion into biomass calculations. Samples were preserved in 5% buffered formalin and stored in the dark at ≤5°C until identification and enumeration at Horn Point Laboratory in Cambridge, Maryland. Whole sample biomass was measured as wet displacement volume (Harris et al., 2000). Zooplankton were identified and counted using a Nikon SMZ800 stereomicroscope. For most samples, zooplankton were too abundant for whole sample counting, and therefore representative subsamples were obtained with a Stempel pipette (Harris et al., 2000). Samples were counted so that at least 100 individuals of the most abundant species were identified. Whenever possible, holoplankton were identified to species level, and meroplankton identified to group. Counts from replicate samples (tows) for each station were averaged.

Statistical Analysis

Statistical significance between bloom and non-bloom station abundance and biomass was quantified using a two-sample (independent) sample t-test (p=0.05). Analysis was completed with the MATLAB® Version 7.12.0 (The MathWorks Inc, Natick MA) statistical package. Prior to any statistical analyses, data were tested for normality with the Kolmogorov-Smirnov test (p =0.05). Pearson correlations were completed comparing both zooplankton biomass and abundance to the environmental factors: temperature, salinity and Chl *a*, and dissolved nutrient concentrations: dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), dissolved organic ammonium (DONH4), and total dissolved nitrogen and phosphorus (DTN and DTP).

The Shannon-Wiener diversity index (H') was calculated for all stations sampled. When calculated, the Shannon Index will result in a value between 0 and 4. A higher diversity index value indicates greater complexity (number of species present) within a community:

$$H' = -\sum_{i=1}^{OS} pi(\ln pi)$$

Where OS is the observed number of species, and pi is the proportion of individuals of species i in the community (Harris et al., 2000). Statistical significance between diversity index values was determined using a two-sample (independent) sample t-test (p=0.05).

To further assess relationships between community compositions in relation to *K. brevis*, several statistical techniques were carried out using the Plymouth Routines in Multivariate Ecological Research (Primer v6) for Windows program (PRIMER-E Ltd., Plymouth, UK). Hierarchal cluster analysis was used to compare trends in community

abundance between stations. Data were not standardized since all stations were already on the same scale of abundance (m^3), however data were log transformed to minimize variations in abundance (Clarke and Warwick, 1994). Similarity matrices using multi-dimensional scaling (MDS) plots were created to provide a visual representation of relationships between zooplankton communities in relation to both distance from shore, as well as absence or presence of *K. brevis*. The BIO-ENV routine of PRIMER calculates Spearman rank coefficients (ρ) between species based resemblance matrices (Bray-Curtis Similarity) and environmental variables (Euclidian distance), and was used to explore the set of environmental variables that best explained the patterns in community composition among sample years. Prior to running the BIO-ENV test, environmental parameters (Temperature, salinity, Chl a, DON, DOP, DTN, DTP, DNH_4^+, Durea, *K. brevis* concentration) were standardized to the same scale. A one-way SIMPER routine using Bray-Curtis was performed on the normalized abundance data to analyze the relative contributions of each species to overall variation within and between sampling sites.

Results

Physical/Environmental Parameters

In 2007, average water temperatures at the stations sampled for zooplankton ranged from 26.9°C at station KB07-11 to 28.4°C at KB07-9 (Figure 2.2). During the 2008 cruise, temperatures were lowest at station CH-In (26.2°C) and highest at the offshore station (28.1°C). In 2009 water temperatures were higher than the three other sampling years, with a low of 28.7°C at station CH-In and highest at station KB09-9

(30.6°C). In 2010, water temperature averaged 25.1°C (Table 2.1). Stations were sampled in the morning, and therefore water temperature is likely an underestimate of average temperatures on each sampling date. In each of the sampling years, the water column was well mixed, as fall storms and cooling result in a breakdown of thermal stratification along the WFS (Austin, 1974).

Average salinity ranged from 28 within the Tampa Bay and Charlotte Harbor estuaries in 2009 to 37 at the coastal stations in 2007 (Figure 2.3). In 2009, an estuarine signal characterized the coastal stations, as both estuarine and coastal salinities were lower in comparison to the three other years. In 2009, river discharge ($ft^3\ s^{-1}$) at USGS gauge stations in the Manatee River (Figure 2.4) and Hillsborough River (Figure 2.5), both major tributaries of Tampa Bay, recorded higher mean discharge in the months prior to sampling. The same pattern was true for tributaries of Charlotte Harbor—the Myakka River (Figure 2.6) and Peace River (Figure 2.7), where mean river discharge was an order of magnitude larger in September of 2009 than other study years. Chlorophyll *a* concentrations ranged from 0.29 to 7.19 $\mu g\ L^{-1}$ and values were higher in 2007 and 2009 than in other years (Figure 2.8).

Non-bloom Zooplankton Community

Zooplankton abundance ranged from 9,824 individuals m^{-3} at the offshore station in 2008 to 156,499 individuals m^{-3} at station CH-In in 2010 (Table 2.2). Biomass ranged from 1.43 mL m^{-3} at the offshore station in 2007 and 91.04 mL m^{-3} at CR-Out in 2008 (Figure 2.10). During the 2007 cruise, no stations were sampled for zooplankton that did not have bloom concentrations (>1000 cells L^{-1}) of *Karenia brevis* present.

In total 18 species of holoplankton were identified, as well as seven groups of meroplankton (Figure 2.11). Copepods constituted the most important zooplankton group, averaging 60% of total abundance. In comparison to the coastal and offshore stations, total zooplankton community biomass was higher within Tampa Bay and Charlotte Harbor, as well as at station CR-Out, which is located at the mouth of the Caloosahatchee River.

Results from the one-way SIMPER routine highlighted the degree of similarity in community composition between site geographic location (offshore, coastal or estuarine). The SIMPER analysis could not be run on the offshore station because in each of the sampling years, only one offshore station was sampled. The most abundant zooplankton species at the offshore station in 2008 were *Temora turbinata*, *Parvocalanus crassirostris*, and cirriped larvae. During the 2009 sampling, the dominant offshore species were *Oithona spp.*, pelecypod larvae, and cirriped larvae. Coastal zooplankton populations were dominated in 2008 by *Oikopleura dioica*, the cladoceran *Penilia avirostris* and *Paracalanus quasimodo* with a 60.07% average similarity in community composition between the four coastal stations (Table 2.3). Four species, *Oithona spp.*, *O. dioica*, cirriped larvae, *C. americanus* were characteristic of these stations, and contributed to fifty percent similarity between coastal stations. In 2009, *P. crassirostris* and *Oithona spp.* were also abundant in coastal stations, with six species accounting for 61.67% similarity between coastal stations (Table 2.4). In all years, *Acartia tonsa*, a dominant coastal and estuarine species of the Gulf of Mexico was most abundant within Charlotte Harbor and Tampa Bay, as well as the coastal stations at the mouths of estuaries. *C. velificatus* was present at the mouths of estuaries, and at the offshore

station. *C. americanus* was present at all stations except 09CH-In. This poecilostomatoid was more abundant at coastal stations than in estuaries, however it was not a major contributor in Tampa Bay and absent from Charlotte Harbor. *Euterpina acutifrons* is the only common near shore harpacticoid on the West Florida Shelf, typically found at the mouths of estuaries (Johnson and Allen, 2005). *E. acutifrons* was present at all stations, with decreasing abundances as distance offshore increased. Two species of cladoceran were present during the study period, *Evadne tergestina* and *Penilia avirostris*. *E. tergestina* was present within estuaries in all years, and at coastal stations in 2009. Highest concentrations of *P. avirostris* occurred at coastal stations, and this species was not found at the offshore station. *O. dioica*, the most abundant appendicularian in coastal and estuarine areas of the North West Florida Shelf (Johnson and Allen, 2005) was present at all stations, with highest abundance occurring in estuarine and coastal stations, and decreasing with distance offshore. *Oithona* spp. is a primary dominant species in WFS estuaries, and was present at all stations, with lower offshore abundances in 2008 than in 2009. *Parvocalanus crassirostris* was most abundant within estuaries, and at coastal stations at the mouths of estuaries. *Paracalanus quasimodo* populations peaked at the mouths of estuaries, however was also present at the offshore stations in low concentrations in 2008, and higher in 2009. Several species of meroplankton were present at stations. Cirriped and polychaete larvae were present at all non-bloom stations. Several other larval species were present within estuaries and at the coastal regions, with decreasing abundance as distance offshore increased.

MDS plots from each of the four years show limited overlap between offshore, coastal and estuarine communities, indicating that samples differ based on spatial

distribution on a spatial scale. In 2008, there was a clear distinction between coastal and estuarine groups, whereas in 2009, coastal and estuarine zooplankton populations were more similar (Figure 2.12). Comparison of the community compositions to the environmental data allowed the investigation of community variability across the entire sample set, and the determination of which measured factors contributed most to community composition. The BIOENV routine in Primer was utilized to determine the combination of environmental parameters best correlated with community composition. During non-bloom BIOENV analysis, *Karenia brevis* concentrations were excluded from the variables, as the value was zero at each of these stations. In all non-bloom stations sampled, three factors correlated best with the zooplankton community: temperature, Chl *a*, and DON (ρ=0.240). The stations sampled in 2008 and 2009 were also compared individually by year (Table 2.5). In 2008, temperature and Chl *a* correlated best with zooplankton abundance between stations (ρ=0.648). In 2009, four combined factors, salinity, Chl *a*, DNH_4, DTP and dissolved urea described the variability within zooplankton populations (ρ=0.503).

K. brevis Bloom Zooplankton Assemblage

Bloom concentrations of *Karenia brevis*, which are technically defined by a concentration greater than 1000 cells L^{-1} (FWRI) were present during the 2007, 2008, and 2009 cruises. All three blooms were first detected and sampled in the area to the West/Southwest of Sanibel Island, but each bloom displayed varying cell concentrations, phase and areal extent (Heil et al., in review).

In 2007, bloom concentrations of *Karenia brevis* were present at all stations sampled for zooplankton. The *K. brevis* bloom in 2007 was high biomass, and the highest cell concentrations during sampling (414 $x10^3$ cells L^{-1}) were recorded at station KB07-8. Based on cell physiological state and duration, this bloom was classified as the maintenance/stationary phase, and had resulted in large fish kills within the area. Zooplankton abundance during the 2007 sampling period ranged from 30,162 individuals m^{-3} at station TB-Out to 64,545 individuals m^{-3} at Station KB07-9 that was located to the East of the Caloosahatchee River outflow station sampled in other years. The zooplankton community was similar to the normal zooplankton assemblage of the WFS at non-bloom, coastal stations sampled in other study years. Dominant zooplankton species at the 2007 stations were *O.* spp., *T. turbinata*, and *Corycaeus americanus*.

The *K. brevis* bloom present in 2008 was in the initiation phase. This bloom was not detected prior to the cruise, and in the first week of sampling was monitored moving from offshore to the coastal regions. The 2009 bloom was an older, offshore bloom in the maintenance/stationary phase. *K. brevis* cells within this bloom contained high numbers of lipid bodies, indicative of older cells in the stationary phase (Steidinger, 1979).

Acartia tonsa, a dominant coastal and estuarine calanoid copepod species of the Gulf of Mexico showed increased abundance (in comparison to non-bloom stations) at several stations in 2007 and 2008, however was not present during the 2009 bloom sampling. Nine species were present at all of the bloom stations sampled within the three years (Table 2.6). The only species that had been present at non-bloom stations and absent in all bloom communities was *Oithona plumifera*.

An unpaired T-test was completed to determine if there was a statistical difference between bloom and non-bloom zooplankton abundance in 2008 and 2009. Data was tested for normality using the Kolmogorov-Smirnov test (α=0.05), and log transformed prior to analysis. In 2008 there was no statistical difference in the abundance of bloom and non-bloom stations, however in 2009 there was a statistically significant difference ($p<0.05$) between zooplankton abundance at non-bloom stations and stations with *K. brevis* present. Multidimensional scaling (MDS) plots created for 2008 and 2009 illustrate this difference in bloom vs. non-bloom stations (Figure 2.13). SIMPER results determined the average similarity between bloom stations was 64.52% in 2008 (Table 2.7), and in 2009, the average similarity between stations was 80.09%, suggesting that the zooplankton communities present at bloom stations in 2009 were more similar in community composition (Table 2.8).

A Shannon-Wiener diversity index was calculated for all samples in each of the sampling years (Table 2.2). Higher diversity at non-bloom sampling sites was found within estuaries, as well as at stations sampled at the mouths of Tampa Bay, Sarasota Bay, Charlotte Harbor, and the mouth of the Caloosahatchee River. In 2008, the mean species richness (number of species present at each station) was highest at the estuarine stations (mean=17), and lowest at the coastal stations (mean=14). In 2009, the mean species richness was equal at the estuarine and coastal stations (mean=16), and decreased at the offshore station. In 2010, 17 species were present at station CH-In, while 15 species were present at CR-Out.

Shannon-Wiener diversity indices were also calculated for each of the bloom station samples (Table 2.6). In 2007, diversity ranged from 2.11 at KB07-11 to 2.40 at

KB07-8. Similar diversity indices occurred in 2008, and in 2009, the SW index ranged from 2.07 at KB09-8F to 1.90 at KB09-10. The mean species richness values were 16, 16, and 11 respectively for each of the sampling years. The lowest number of species from all years present at the bloom site KB09-10—9 species, compared to 16 at estuarine and coastal stations in same year. A t-test for significance was calculated to determine whether there was a significant difference between diversity in bloom and non-bloom samples in 2008 and 2009. No statistically significant differences between diversity index at bloom and non-bloom stations was found in either year.

Discussion

While physical properties are important to the distribution of zooplankton on the WFS, it is important to also consider the biotic factors that could be impacting zooplankton community composition. Reduced abundance of some zooplankton species could be a result of patchy distribution; a direct response to the presence of toxic algae, or an indirect response such as availability of preferred food items.

In a similar study comparing non-bloom and bloom zooplankton assemblages (Lester et al. 2005), a statistically significant difference in abundance or community composition was observed at all stations but one. In our study, a significant difference was observed only during the 2009 sampling period, however, *K. brevis* cell concentrations during the previous study were considerably higher than those experienced during our four sampling efforts. Lester et al. (2005) documented *K. brevis* cell concentrations ranging from 8 $x10^3$ cells L^{-1} to 5270 $x10^3$ cells L^{-1}. The highest cell

concentrations observed during this study were and order of magnitude lower than the Lester (2005) values (414 x10^3 cells L^{-1} at station KB08 in 2007).

In 2008, the *K. brevis* bloom was in the initiation phase, and background concentrations of *K. brevis* were present. As the bloom progressed, *K. brevis* cell concentrations increased, and zooplankton abundance decreased. With lower concentrations of *K. brevis* present, it is likely that the abundance of other, more palatable and nutritionally adequate species of phytoplankton are available for zooplankton to graze on.

Several species of zooplankton present on the WFS, including *A. tonsa* have been shown to preferentially graze on other species when in the presence of *K. brevis* (Collumb and Buskey, 2004; Lester, 2005; Speekmann et al., 2006; Waggett et al., 2012). Waggett et al. (2012) fed both toxin and non-toxin strains of *K. brevis* to *A. tonsa*, and noted that with toxic algae there was depressed egg production relative to what would be expected with a nutritionally adequate diet. When fed non-toxin producing *K. brevis*, there was also lower egg production, suggesting that toxins are not the only factor affecting reproductive ability, and *K. brevis* is a nutritionally inadequate diet for copepods. If copepods are selectively grazing on *K. brevis* competitors, this could allow *K. brevis* to outcompete other phytoplankton species (Waggett, 2012).

Lester et al. (2008), observed variations in species abundance between non-bloom and bloom stations, with decreased abundance of three important zooplankton contributors when bloom concentrations of *K. brevis* were present: *C. americanus*, *P. avirostris* and *E. acutifrons*. When comparing the *K. brevis* stations to the coastal stations in each of the four years of study, we observed decreases in abundance of several

important zooplankton species, including *P. avirostris*, *P. quasimodo* and *O. dioica* in 2008, and in 2009, the cladocerans *P. avirostris*, and *E. tergestina* as well as several important copepod species—*A. tonsa*, *C. velificatus*, *C. americanus*, and *E. acutifrons*.

A. tonsa and *C. velificatus*, are both calanoid copepods, and with the exception of *A. tonsa* which has previously been classified as omnivorous, *C. velificatus* prefer phytoplankton >12µm in size (Jonsson and Tiselius, 1990; Johnson and Allen, 2005). In previous work characterizing the zooplankton assemblage of the WFS, *C. velificatus* was most prevalent between the 5m and 25m isobaths (Minello, 1980, Lester, 2005), a result that was confirmed within our study. There has been no previous research indicating a relationship between *C. velificatus* and *K. brevis* (Lester, 2005), however, during a rare bloom of *K. brevis* bloom on the Eastern coast of the United States, the congener *Centropages typicus* was collected and laboratory experiments were completed to see if this species would ingest the toxic dinoflagellate. Turner and Tester, 1989, found that *C. typicus* ingested *K. brevis* at very low rates, or not at all. Cohen et al. (2007), observed C. typicus to readily graze on *K. brevis* cells, and noted sub lethal effects on swimming and photo behaviors. Given the biogeographic range of *C. typicus*, these ecological conclusions may not be applicable to species co-occurring with *K. brevis*. However, the congener *C. velificatus* is a common species of the Gulf of Mexico, and was recorded in high numbers in *K. brevis* blooms. If these copepods elicit a response similar to *C. typicus* when exposed to *K. brevis*, this could impact predator-prey relationships of the WFS (Cohen et al., 2007). In studies utilizing *Centropages yamadai* feeding on the brevetoxin compound producing raphidophyte *Chattonella antiqua*, it is suggested that

other copepods of this genus may have the ability to ingest brevetoxin-producing species (Uye, 1986; Kleppel, 1996; Paffenhoffer and Knowles, 1980; Lester, 2005).

High abundances of *Temora turbinata* have been previously reported in the Northern Gulf of Mexico (Dagg, 1995; Lester, 2005). The omnivorous calanoid copepod, *Temora* creates strong feeding currents (Paffenhoffer and Knowles, 1980), and are potentially important contributors to copepod grazing within this region (Dagg, 1995). During toxin vector studies, *T. turbinata* was found to ingest *K. brevis* at an average of 72 *K. brevis* cells copepod^{-1} hour^{-1} in the absence of other food types (Tester et al., 2000; Lester, 2005). Cohen et al., 2007 found that *T. turbinata* ingested *K. brevis* at relatively low levels, and in mortality experiments *K. brevis* was only toxic within 24 h of exposure to 1 x10^{7} cells L^{-1}. This suggests that exposure to low to moderate blooms may not be fatal, although behavioral changes were observed. No species of *Oithona* have been examined in respect to toxic phytoplankton, however, *Oithona* spp. do not create feeding currents, but instead encounter food either through the movement of the prey, or repeated jumps that land them into a food patch (Paffenhoffer, 1993).

Species of some freshwater diatoms that grow best in low nutrient regimes produce lipids that are essential for zooplankton reproduction, and in conditions of nutrient over-enrichment, these species are replaced by species that produce low, or negligible quantities of these lipids—such as harmful dinoflagellates (Kilham et al., 1997; Glibert et al., 2011). Culture studies have identified the inability of *K. brevis* to produce sterols necessary for copepod growth and reproduction. *K. brevis* lacks the 27-methyl group needed for their sterols to be converted into cholesterol by copepods, and cholesterol is the dominant sterol found in calanoid copepods (Waggett et al., 2012). In

regions where harmful algal blooms have been increasing due to eutrophication, a shift in zooplankton communities' dominance from calanoid to cyclopoid species was identified in San Francisco Bay (Glibert et al., 2011).

In all years of this study there was a decrease in abundance of the cladoceran *P. avirostris* with increasing concentrations of *K. brevis*. *P. avirostris* is a filter feeder that grazes on particles <20µm in diameter, particularly those <5µm (Johnson et al., 2005).

Despite decreased abundance in 2008, *O. dioica* was present at all bloom stations. This species has been overlooked as an abundant grazer of *K. brevis* on the WFS, however Sutton et al. 2001 calculated that larvaceans had a significant impact on *K. brevis* in offshore mixed layer and near shore salinity gradients. High numbers of larvaceans have been noted when *K. brevis* was abundant, and this suggests that *O. dioica* is a potentially important grazer of *K. brevis* because of their high clearance rates (Lester, 2005).

During the 2008 and 2009 bloom sampling years, as the *K. brevis* bloom progressed, total zooplankton abundance decreased. This decrease did not always coincide with increasing cell concentrations of *K. brevis*. It is unclear if the declines in copepod abundance during bloom conditions are based on a direct response to the presence of toxic algae, such as the harmful effects of non-selective grazing, or if it is an indirect response to reduced availability of preferred food items (Badylak and Phlips, 2007).

It has also been suggested that zooplankton populations with prolonged exposure to harmful algal species may gain resistance to toxins. Colin and Dam (2004) exposed both native and naïve copepods to the toxic dinoflagellate *Alexandrium fundyense*. When

naïve copepods were fed a diet containing the toxic species, they exhibited lower somatic growth, size at maturity egg production and survival than those species that lived in an area that has experienced *A. fundyense* blooms for decades. *K. brevis* blooms have been documented to occur on the West Florida shelf almost annually. Although it is not clear whether *K. brevis* is toxic or nutritionally inadequate to zooplankton, there is potential that certain zooplankton species have been able to develop resistance to the impacts of brevetoxins within the food chain.

Conclusions

The objective of this study was to distinguish between the ambient zooplankton assemblage of the West Florida shelf, and potential shift in zooplankton communities within *Karenia brevis* blooms. The normal zooplankton assemblage identified within this study was similar to previous work characterizing zooplankton communities of the WFS and it's associated estuaries (Table 2.9). In 2009, a *K. brevis* bloom in the maintenance phase was well established, and there was a significant difference in zooplankton abundance between non-bloom and bloom samples. In other sample years, when the bloom was in the initiation phase or when there was either insufficient data to conclude, there was not a significant difference between bloom and non-bloom stations. BIOENV results show zooplankton community composition correlate best with temperature, salinity, Chl *a* and dissolved nutrients. This signifies that zooplankton populations within the study period were being driven by physical characteristics rather than biotic factors. Therefore, it is suggested that a threshold in *K. brevis* abundance must be reached before significant shifts in zooplankton abundance are observed. Previous studies have found

similar results, indicating that zooplankton assemblages on the West Florida Shelf are altered in the presence of the harmful dinoflagellate *K. brevis*.

Zooplankton are primary grazers of oceanic primary productivity, regulating phytoplankton populations through top down control of abundance. By altering the abundance and species assemblage of the West Florida shelf, harmful algal bloom forming species, such as *K. brevis*, may be released from top down grazing pressure and able to increase their ability to form proliferating blooms (Sunda et al., 2006; Waggett et al., 2012). Zooplankton mediated nutrient regeneration is also an important contributor to nutrient pools, and a reduction in nutrient conditions could allow for nutrient adapted species such as *K. brevis* to survive.

Table 2.1. Environmental data for each of the ECOHAB: *Karenia* stations sampled for zooplankton in all cruise years (2007-2010).

Station	*Date*	*Temperature (°C)*	*Salinity*	*Chl a ($\mu g\ L^{-1}$)*	*DON ($\mu M\ L^{-1}$)*	*DOP ($\mu M\ L^{-1}$)*	*DNH_4^+ ($\mu M\ L^{-1}$)*	*DTN ($\mu M\ L^{-1}$)*	*DTP ($\mu M\ L^{-1}$)*	*Durea ($\mu M\ L^{-1}$)*
2007										
TB-Out	Oct 22	28.2	37.1	6.03	12.81	0.19	0.22	13.12	0.19	0.35
KB07-8	Oct 23	28.3	36.8	6.26	13.31	0.18	0.26	13.64	0.18	0.64
KB07-9	Oct 24	28.3	36.7	7.19	13.95	0.21	0.35	14.37	0.21	0.28
KB07-11	Oct 26	26.9	36.2	3.84	22.47	---	2.27	25.36	0.78	1.90
2008										
Off	Oct 2	28.1	36.3	0.55	6.75	0.41	0.35	7.20	0.42	0.48
CH-Out	Oct 3	27.0	35.5	2.19	13.63	0.56	0.75	14.77	0.78	0.40
CR-Out	Oct 4	27.6	36.2	2.07	12.62	0.52	0.12	12.86	0.52	0.29
CH-In	Oct 5	26.2	32.8	2.08	18.92	0.67	1.71	22.27	1.87	0.56
SB-Out	Oct 6	26.6	36.1	1.62	12.70	0.55	0.60	13.52	0.61	0.33
TB-Out	Oct 7	26.3	35.9	1.17	13.58	0.57	0.25	13.96	0.68	0.29
TB-In	Oct 8	26.3	33.5	1.10	16.90	0.50	0.46	17.78	1.91	0.29
KB08-9	Oct 10	28.1	34.3	2.54	---	---	---	---	---	0.24
KB08-10	Oct 11	28.1	34.2	3.04	17.34	0.39	1.25	18.67	0.56	0.49
KB08-11	Oct 12	27.7	36.3	4.60	14.12	0.42	1.37	15.81	0.56	0.78
2009										
Off	Oct 2	29.4	35.9	0.29	6.02	0.09	0.41	6.46	0.44	0.22
CH-Out	Oct 3	29.0	34.4	2.68	13.78	0.22	0.43	14.95	0.87	0.18
CR-Out	Oct 4	30.4	36.4	4.53	10.85	0.11	0.33	11.40	0.11	0.21
CH-In	Oct 5	28.7	28.3	4.84	24.46	0.33	0.63	25.34	2.42	0.56
SB-Out	Oct 6	29.0	35.5	2.62	13.03	0.21	0.26	13.32	0.34	0.19
TB-Out	Oct 7	29.1	34.6	5.75	12.77	0.16	0.32	13.96	0.44	0.19
TB-In	Oct 8	28.8	28.8	4.31	19.97	0.26	0.20	17.78	3.12	0.43
KB09-9	Oct 10	30.6	36.0	1.49	8.19	0.19	0.29	8.52	0.26	0.60
KB09-9A	Oct 10	30.5	35.9	1.36	10.11	0.20	0.34	10.50	0.27	0.58
KB09-10	Oct 11	30.5	35.8	2.92	9.57	0.26	0.07	9.71	0.42	0.67
2010										
CR-Out	Oct 14	26.4	35.0	0.72	---	---	---	---	---	---
TB-In	Oct 18	23.9	31.7	1.71	---	---	---	---	---	---

Table 2.2. Zooplankton abundance, biomass and species diversity for all stations sampled on NOAA: ECOHAB cruise track where *Karenia brevis* was not present in bloom concentrations (>1000 cells L^{-1}).

	October 2008							October 2009							October 2010	
Station	**Off**	**CH-Out**	**CR-Out**	**CH-In**	**SB-Out**	**TB-Out**	**TB-In**	**Off**	**CH-Out**	**CR-Out**	**CH-In**	**SB-Out**	**TB-Out**	**TB-In**	**CH-In**	**CR-Out**
***Karenia brevis* (x10^{3}cells L^{-1})**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Appendicularian	---	---	---	267	637	---	6536	---	1809	---	10890	5880	---	7559	13735	---
Oikopleura dioica	248	5473	45394	18950	1848	5016	3985	3130	6119	17927	8522	2514	2116	3075	1686	8874
Calanoid	1637	1321	10957	2669	2837	6131	4942	2484	3502	3130	---	6676	4794	377	9011	11832
Acartia tonsa	---	---	---	267	637	---	6536	---	1809	---	10890	5880	---	7559	13735	---
Centropages velificatus	298	566	3131	1068	---	---	---	1580	385	1502	---	4741	4088	---	808	13804
Labidocera aestiva	---	---	---	---	---	---	---	---	---	---	---	---	---	---	562	---
Parvocalanus crassirostris	1240	944	15653	5071	1452	3066	6855	4064	14394	8213	5880	97	---	3710	20111	21692
Paracalanus quasimodo	99	---	21914	1868	1056	---	---	3756	2155	3654	250	2937	---	4206	562	2958
Temora turbinata	3870	1699	3131	267	1980	2508	2551	4700	1616	3546	1748	7462	5354	3710	8290	11832
Cladoceran	---	---	---	---	---	---	---	2196	616	199	---	121	---	3392	---	---
Evadne tergestina	---	---	---	801	---	---	2710	---	---	1429	1661	3532	10148	1131	---	---
Penilia avirostris	---	---	10957	1334	858	14213	2710	---	---	995	1765	2427	15520	377	10802	---
Cyclopoid	1240	944	15653	5071	1452	3066	6855	4064	14394	8213	5880	97	---	3710	20111	21692
Oithona spp.	99	3397	3131	3203	3365	3623	10841	10066	1886	7905	27366	3516	32000	64300	48688	31552
Oithona plumifera	---	---	---	---	---	---	---	2196	616	199	---	121	---	3392	---	---
Harpacticoid	3870	1699	3131	267	1980	2508	2551	4700	1616	3546	1748	7462	5354	3710	8290	11832
Euterpina acutifrons	99	1510	4696	2402	1122	279	1275	1570	3271	1013	2910	11417	2677	9087	1370	7888
Ostracod	347	377	---	534	66	---	---	---	705	1294	---	885	1013	1899	966	---
Euconchoecia chierchiae	---	---	---	---	---	---	---	---	---	---	---	---	597	---	---	---
Poecilostomatoid	---	377	---	6672	0	557	797	---	---	---	---	---	4841	---	2898	---
Corycaeus americanus	1637	1321	10957	2669	2837	6131	4942	2484	3502	3130	---	6676	4794	377	9011	11832
Other	198	189	---	1068	396	836	1275	---	705	436	---	---	---	---	15615	986
Bryozoa larvae	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Chaetognath	347	377	---	534	66	---	---	---	705	1294	---	885	1013	1899	966	---
Cirriped larvae	50	5473	3131	8808	2441	5295	2710	6865	4088	2807	15340	4849	4088	14862	19865	14790
Crab zoea	---	377	---	6672	0	557	797	---	---	---	---	---	4841	---	2898	---
Echinoderm larvae	595	---	---	2402	0	279	---	---	---	193	250	---	307	318	---	986
Mysid	198	189	---	1068	396	836	1275	---	705	436	---	---	---	---	15615	986
Pelecypod larvae	347	---	---	267	5213	2508	2710	---	385	---	250	15930	705	7915	---	19720
Polychaete larvae	347	3397	7827	2936	330	1115	1116	---	2348	2117	14152	3492	4088	5397	1124	3944
Snail veliger	347	377	---	534	528	---	---	---	---	---	---	290	---	---	---	---
Total Abundance (m^{-3})	9824	25100	129921	61119	24326	45705	51014	44783	44221	56664	107322	76199	88293	128957	156499	152830
Biomass (mL m^{-3})	1.43	15.94	91.04	10.65	6.39	21.66	19.63	12.68	7.97	34.98	15.64	7.54	35.18	57.25	79.84	17.24
Shannon Index	1.84	1.40	1.67	2.51	2.54	1.97	2.15	2.13	2.18	2.17	2.13	2.51	2.03	1.96	2.15	2.39

Table 2.3. Results of SIMPER analysis showing determinant species for non-bloom coastal stations during EK2008. Community abundance data was square root transformed, n=4. Average similarity is 60.07%.

Species	Av.Abund	Contrib%	Cum.%
Oithona spp.	58.11	13.88	13.88
O. dioica	100.21	13.67	27.55
Cirriped larvae	63.03	13.5	41.05
C. americanus	68.15	11.45	52.51
T. turbinata	47.93	10.53	63.04
P. crassirostris	62.32	8.8	71.84
Polychaete larvae	49.58	6.99	78.84
E. acutifrons	39.39	6.27	85.1
P. avirostris	63.3	5.57	90.67

Table 2.4. Results of SIMPER analysis showing determinant species for non-bloom coastal stations during EK2009. Community abundance data was square root transformed, n=4. Average similarity is 61.67%.

Species	Av.Abund	Contrib%	Cum.%
O. dioica	79.43	10.11	10.11
Oithona spp.	111.3	9.69	19.8
Cirriped larvae	64.39	9.69	29.49
P. quasimodo	56.48	8.93	38.42
T. turbinata	61.76	8.63	47.06
E. acutifrons	72.8	8.59	55.65
Polychaete larvae	56.75	8.53	64.18
P. crassirostris	70.34	7.5	71.67
C. americanus	54.06	7.01	78.68
A. tonsa	51.54	4.34	83.02
Pelecypod larvae	58.7	3.28	86.3
Chaetognath	24.39	2.91	89.21
E. tergestina	32.71	2.81	92.03

Table 2.5. Results of BIOENV procedure comparing non-bloom zooplankton to environmental variables. Abundance data was log transformed and environmental variables were adjusted so that they were all on the same scale.

Correlation	***Variables***
2008 (non-bloom)	
0.648	Temperature, Chl *a*
0.644	Temperature
0.639	Temperature, Chl *a*, DON
0.599	Temperature, DON
2009 (non-bloom)	
0.503	Salinity, Chl *a*, DNH4, DTP, Durea
0.495	Salinity, Chl *a*, DOP, DTP, Durea
0.490	Salinity, Chl *a*
0.482	Temperature, salinity Chl *a*, DOP, Durea
All non-bloom (2008, 2009)	
0.240	Temperature, Chl *a*, DON
0.233	Temperature, Chl *a*
0.228	Temperature, Chl *a*, DON, Durea
0.223	Chl *a*, DON

Table 2.6. Zooplankton abundance, biomass and species diversity and *Karenia brevis* cell concentrations for all stations sampled on NOAA: ECOHAB cruise track where *Karenia brevis* present in bloom concentrations (>1000 cells L^{-1}).

Station	TB-Out	KB07-8	KB07-9	KB07-11	KB08-9	KB08-10	KB08-11	KB09-8F	KB09-9	KB09-9A	KB09-10
***Karenia brevis* (x10^3cells L^{-1})**	74	414	176	1.7	4.0	91	321	91	35	127	n/c
Appendicularian											
Oikopleura dioica	505	2110	2420	1513	6418	723	7169	14105	4817	2859	5825
Calanoid											
Acartia tonsa	379	2813	4034	2836	12034	10120	---	---	---	---	---
Centropages velificatus	126	703	807	945	4011	3614	13262	1614	963	150	752
Labidocera aestiva	---	---	---	---	---	---	---	---	---	---	---
Parvocalanus crassirostris	2903	3095	3227	5861	24871	3976	358	4589	2312	978	1503
Paracalanus quasimodo	1388	2391	2743	1324	5616	361	---	4163	---	---	---
Temora turbinata	4165	5064	5809	3782	16046	2169	1792	510	1541	752	2255
Cladoceran											
Evadne tergestina	4669	---	---	756	3209	4699	3226	---	---	---	---
Penilia avirostris	0	1829	2098	1702	7221	11205	2151	---	---	---	---
Cyclopoid											
Oithona spp.	9717	15755	25818	22878	15243	3253	3226	14960	7515	8652	9583
Oithona plumifera	---	---	---	---	---	---	---	---	---	---	---
Harpacticoid											
Euterpina acutifrons	1136	3235	3711	2458	10430	1446	1792	2211	2312	1354	1127
Ostracod											
Euconchoecia chierchiae	---	---	---	---	---	---	---	---	---	---	---
Poecilostomatoid											
Corycaeus americanus	2145	1829	2098	6618	28080	4337	8961	3995	3661	2859	3382
Other											
Bryozoa larvae	---	---	---	---	---	---	---	---	---	---	---
Chaetognath	---	985	1130	189	802	1084	358	2125	289	---	---
Cirriped larvae	505	2110	4357	5105	21662	2891	7527	4335	3468	1354	5261
Crab zoea	505	422	484	189	802	---	358	---	---	---	---
Echinoderm larvae	---	---	---	---	---	---	717	---	---	---	---
Mysid	631	1407	1614	1134	---	2530	717	---	---	---	---
Pelecypod larvae	---	2954	---	---	---	---	---	---	---	---	---
Polychaete larvae	379	563	645	945	4011	5422	1792	5269	963	828	1315
Snail veliger	---	---	---	---	802	---	---	---	---	301	---
Total Abundance (m^{-3})	30162	48952	64545	58236	161259	57830	54842	68591	30252	19786	31003
Biomass (mL m^{-3})	10.33	82.76	20.05	28.38	40.59	26.25	10.75	18.16	5.92	2.49	---
Shannon Index	2.14	2.40	2.19	2.11	2.41	2.43	2.27	2.07	2.04	1.73	1.90

Table 2.7. Results of SIMPER analysis showing determinant species for all *Karenia brevis* stations sampled in 2008. Community abundance data was square root transformed, n=3. Average similarity is 64.52%.

Species	Av.Abund	Contrib%	Cum.%
C. americanus	109.36	11.35	11.35
Cirriped larvae	95.9	9.7	21.05
C. velificatus	79.54	9.3	30.35
P. avirostris	79.07	8.79	39.15
Oithona spp.	79.1	8.66	47.81
E. tergestina	60.66	8.64	56.45
Polychaete larvae	59.77	7.39	63.84
T. turbinata	71.86	6.64	70.47
O. dioica	63.89	6.53	77.01
E. acutifrons	60.83	5.99	82.99
P. crassirostris	79.9	4.86	87.86
A. tonsa	70.1	4.51	92.37

Table 2.8. Results of SIMPER analysis showing determinant species for all Karenia brevis stations sampled in 2009. Community abundance data was square root transformed, n=4. Average similarity is 80.09%.

Species	**Av. Abund**	**Contrib%**	**Cum.%**
Oithona spp.	99.98	22.16	22.16
O. dioica	79.49	15.1	37.26
C. americanus	58.83	13.7	50.96
Cirriped larvae	58.52	11.7	62.66
E. acutifrons	41.37	8.96	71.62
P. crassirostris	46.47	8.81	80.43
Polychaete larvae	42.17	7.48	87.9
T. turbinata	34.19	6.71	94.61

Table 2.9. Comparison of zooplankton abundance results found within this study to previous studies in the Gulf of Mexico and its associated estuaries. (Adapted from Lester, 2005).

	Mesh Size (μm)	*Location*	*Time of Year*	*Bottom Depth (M)*	*Abundance (# m^{-3})*
Estuarine					
This study, averaged	64	Tampa Bay	October	5	85014
Hopkins, 1977	---	Tampa Bay	Year average	4 (average)	80782
This study, averaged	64	Charlotte Harbor	October	2	108313
Squires, 1984	---	Charlotte Harbor	Year average	4 (average)	156958
5-Meter Isobath					
This study, averaged	64	WFS	October	5	73157
Lester, 2005	153	WFS	December	5	12227
Ortner, 1989	333	NGOMX		5	1298
Lester, 2005	153	WFS	Year average	5	6915
Minello, 1980	200	NWFS		8	3412
25-Meter Isobath					
Lester, 2005	153	WFS	December	25	2066
Ortner, 1989	333	NWGOMX		30	484
This study, 2008	64	WFS	October	31	9824
This study, 2009	64	WFS	October	31	44783
Lester, 2005	153	WFS	April	25	212
Ortner, 1989	333	NGOMX		35	212
Ortner, 1989	333	CGOMX		38	76
Lester, 2005	153	WFS	Year average	25	1289

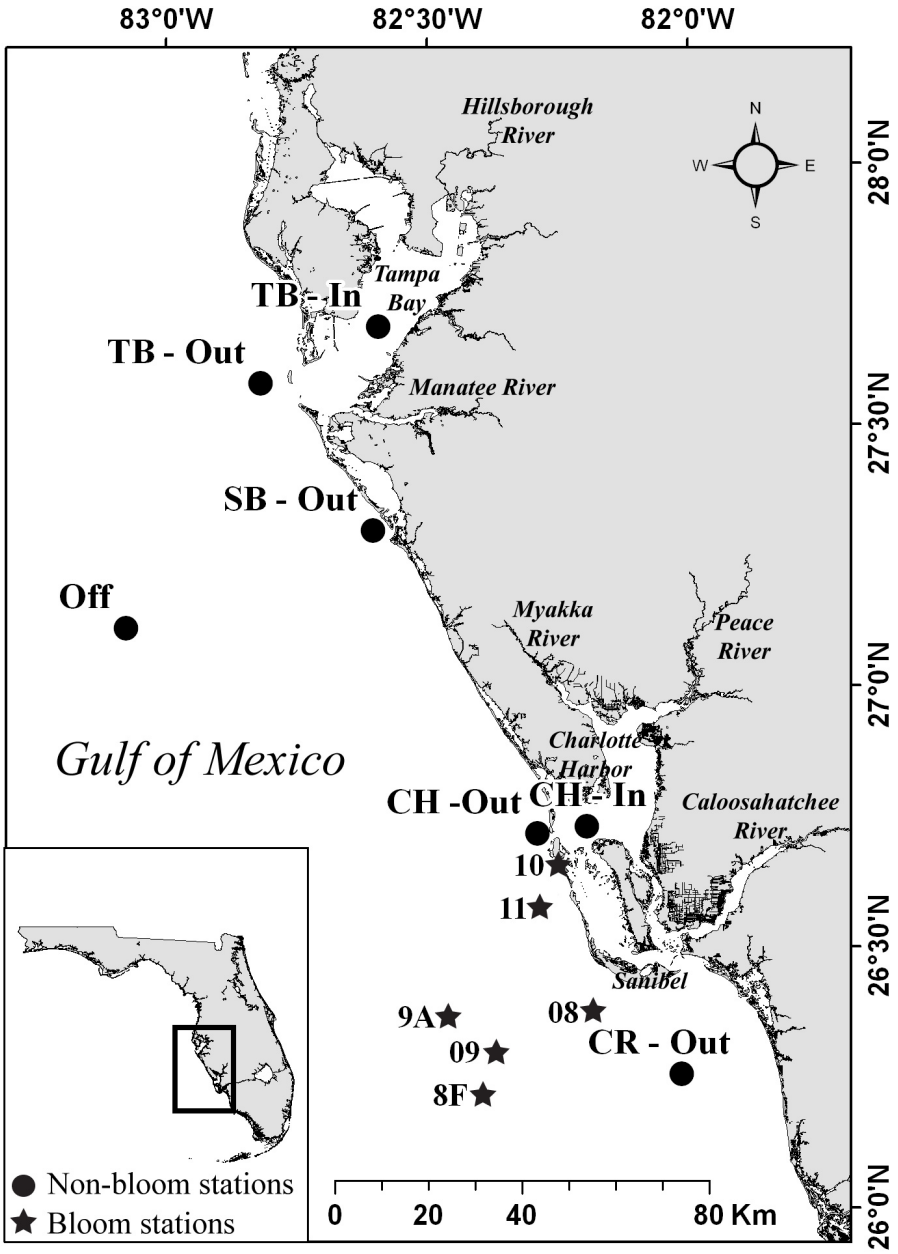

Figure 2.1. ECOHAB: *Karenia* station map.

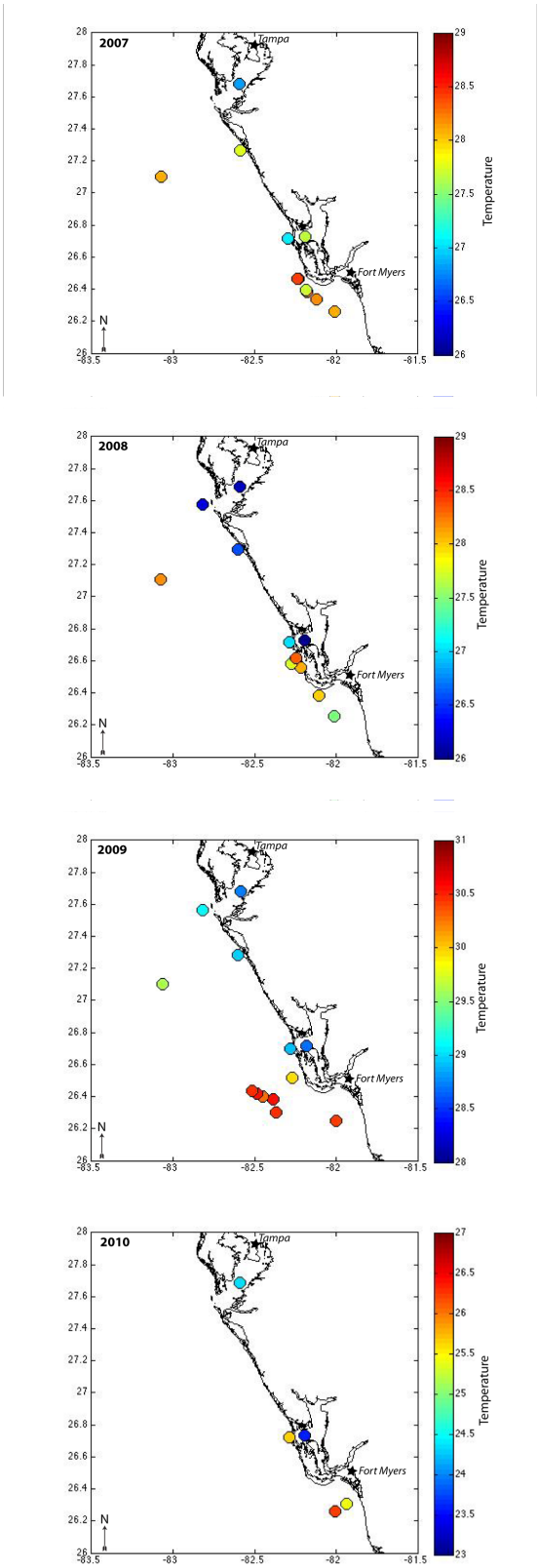

Figure 2.2 Water temperature (°C) at each of the stations sampled for zooplankton in 2007-2010.

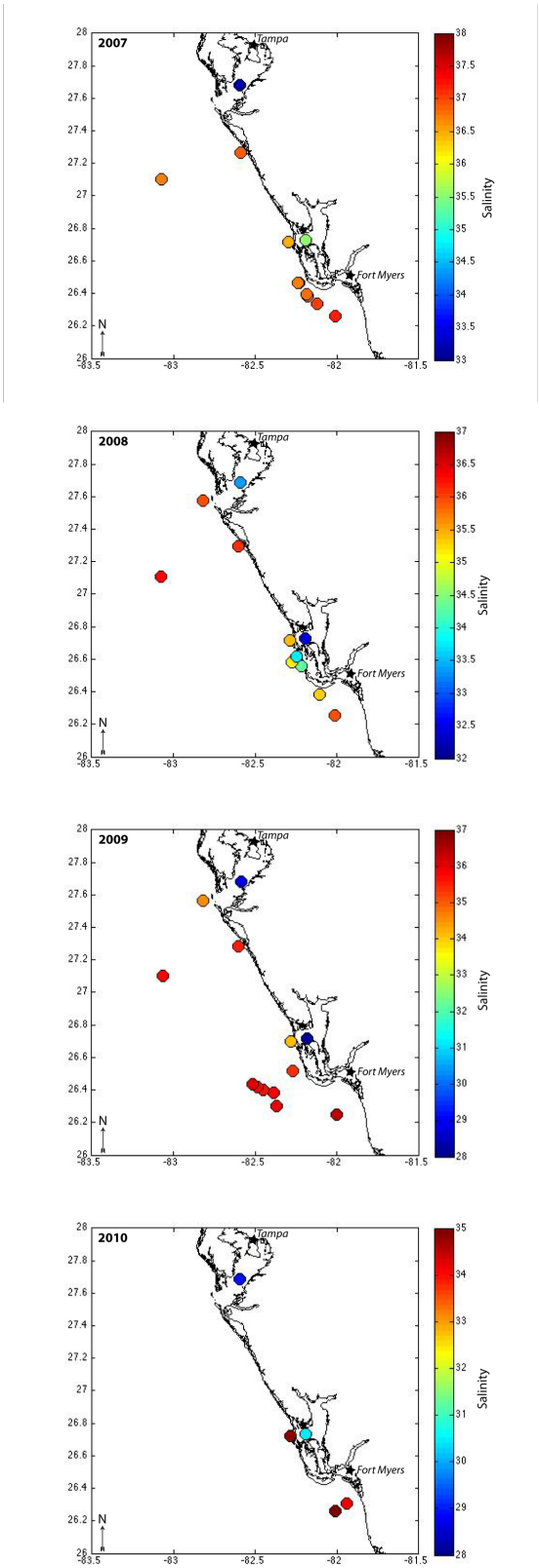

Figure 2.3. Salinity at each of the stations sampled for zooplankton in 2007-2010.

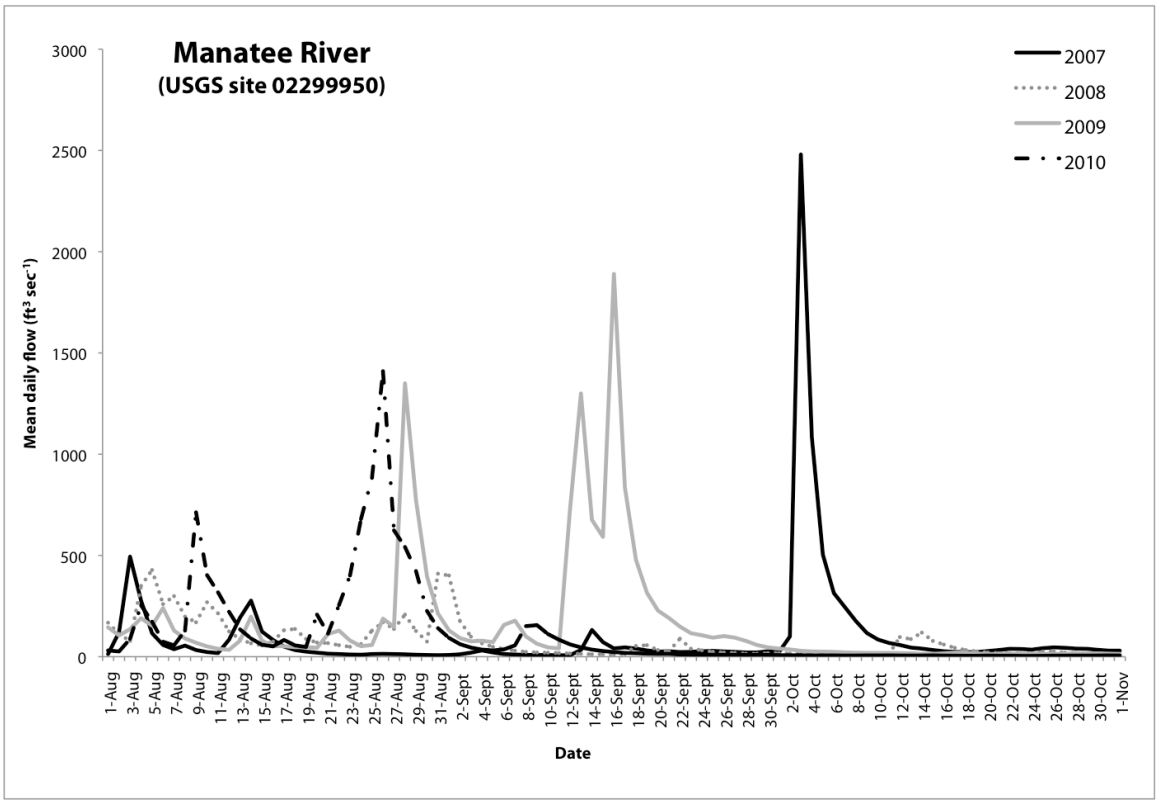

Figure 2.4. Mean daily flow for Manatee River from August 1 to November 1 for all four sampling years (2007-2010). Data from USGS monitoring site #02299950.

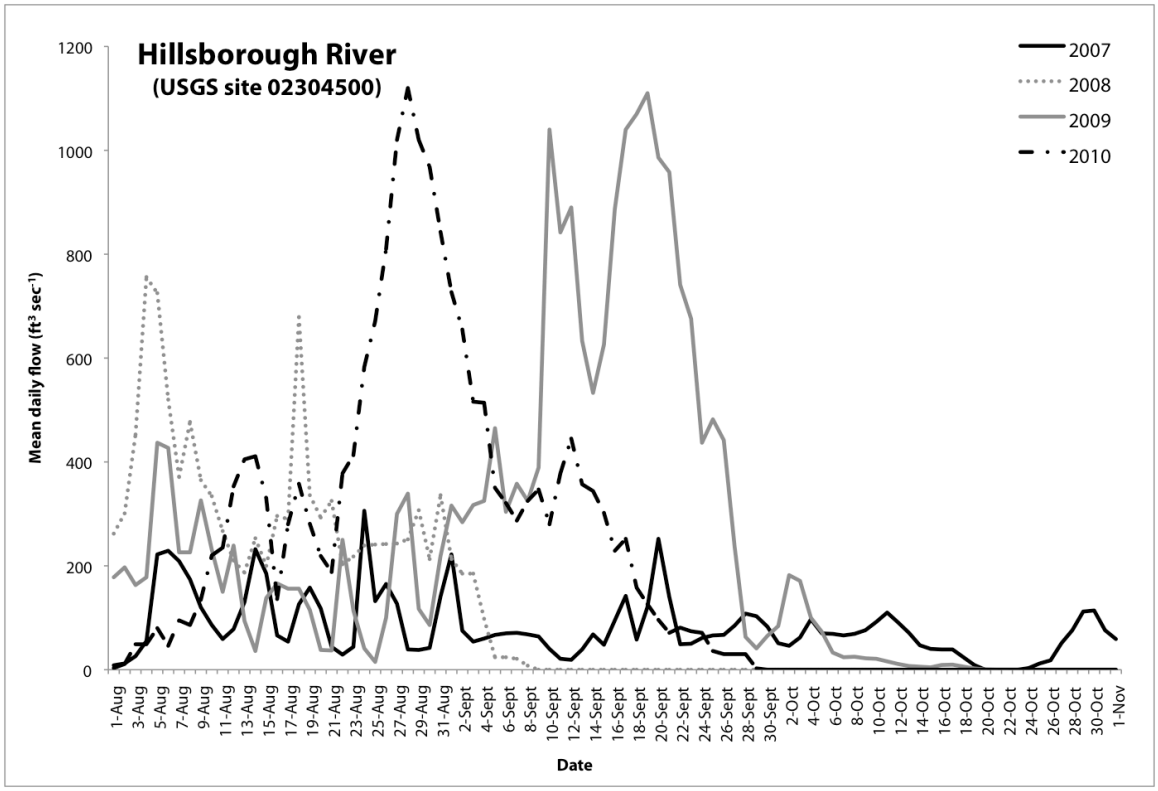

Figure 2.5. Mean daily flow for Hillsborough River from August 1-November 1 for all four sampling years (2007-2010). Data from USGS monitoring site #002304500.

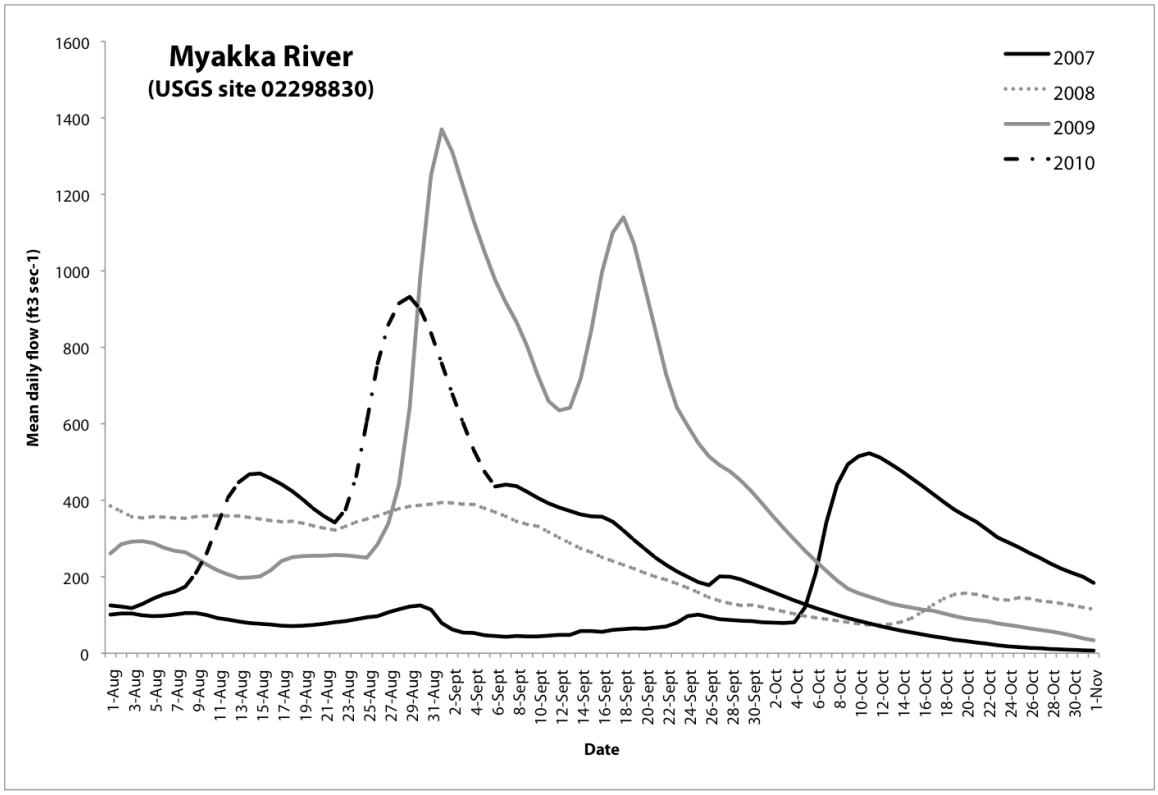

Figure 2.6. Mean daily flow for Myakka River from August 1-November 1 for all four sampling years (2007-2010). Data from USGS monitoring site #02298830.

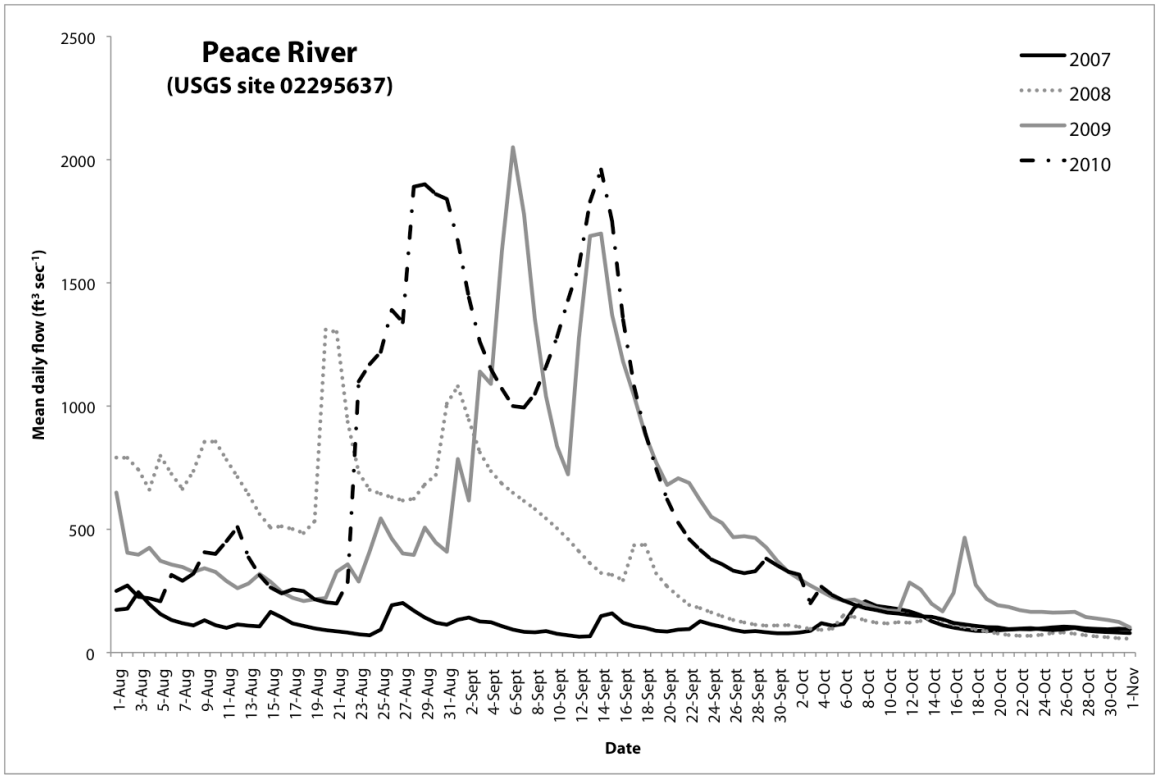

Figure 2.7. Mean daily flow for Peace River from August 1-November 1 for all four sampling years (2007-2010). Data from USGS monitoring site #02295637.

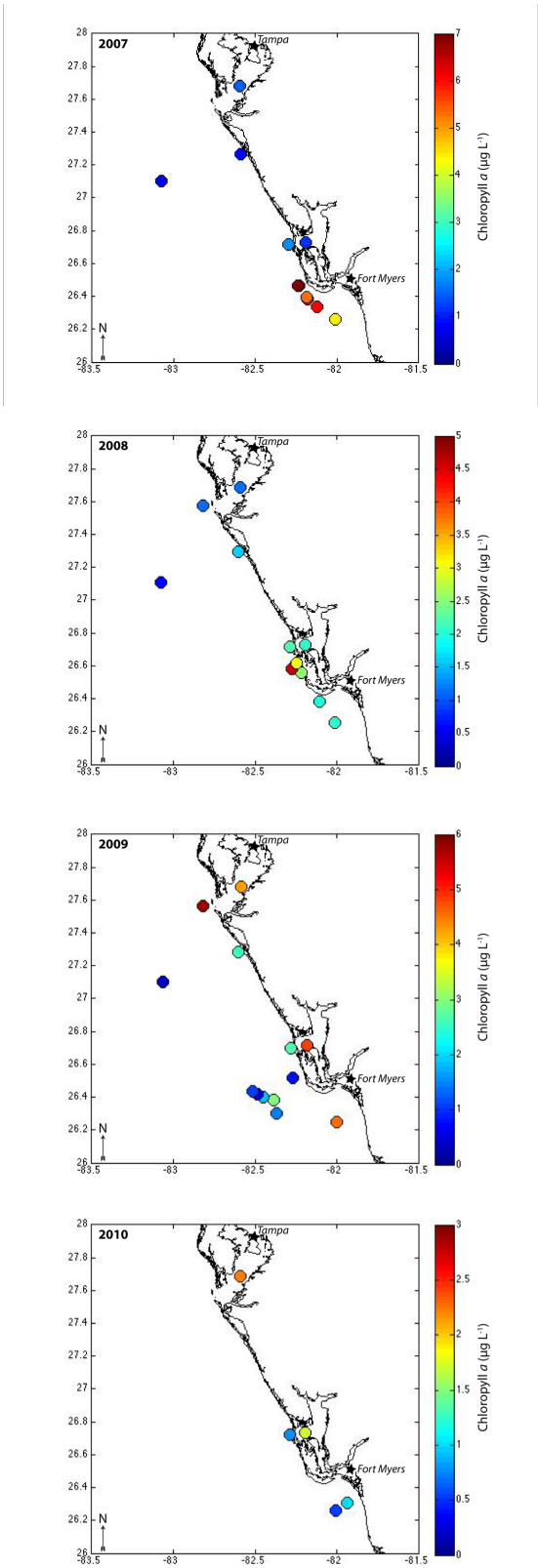

Figure 2.8. Chlorophyll *a* (µg L^{-1}) at each of the stations sampled for zooplankton in 2007-2010.

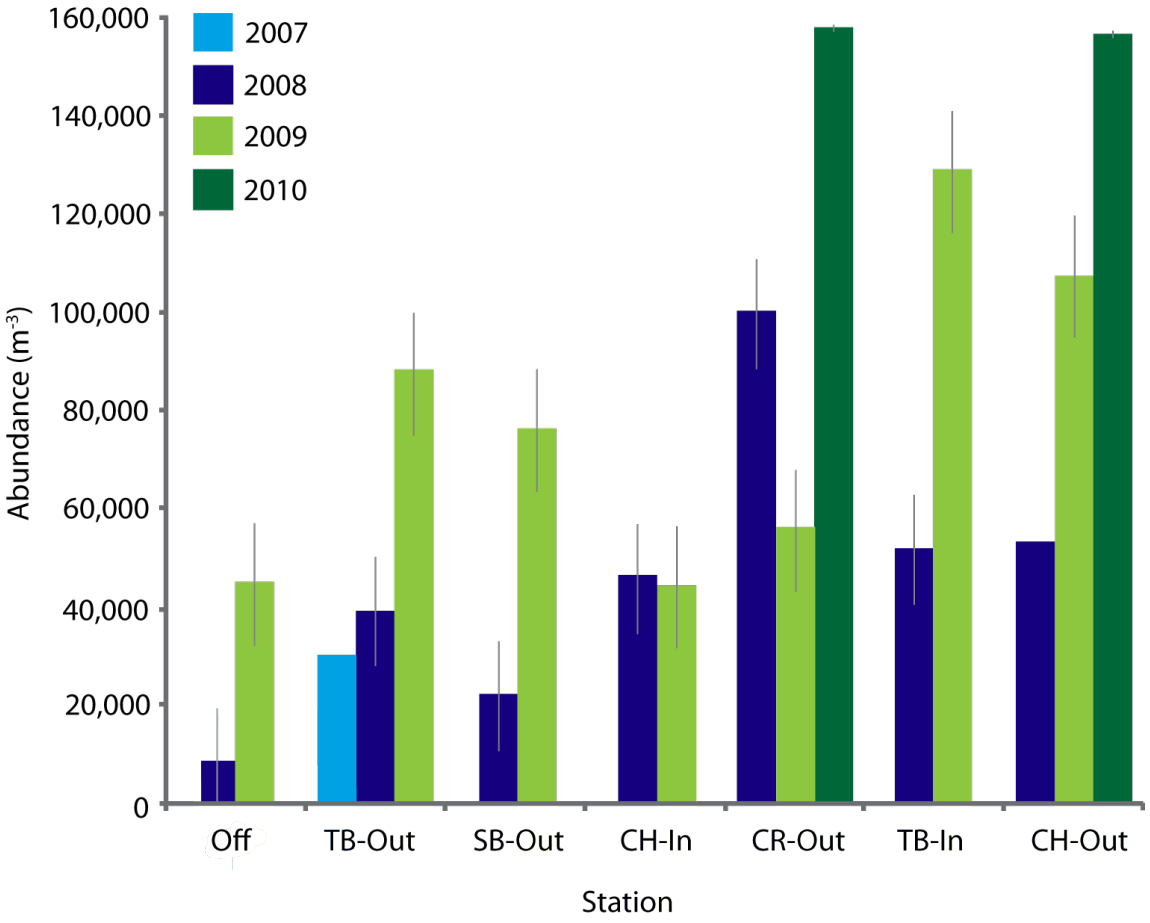

Figure 2.9. Zooplankton abundance for all non-bloom stations on the ECOHAB: *Karenia* cruise track October 2007-2010. Error bars represent ± 1 S.E. from mean.

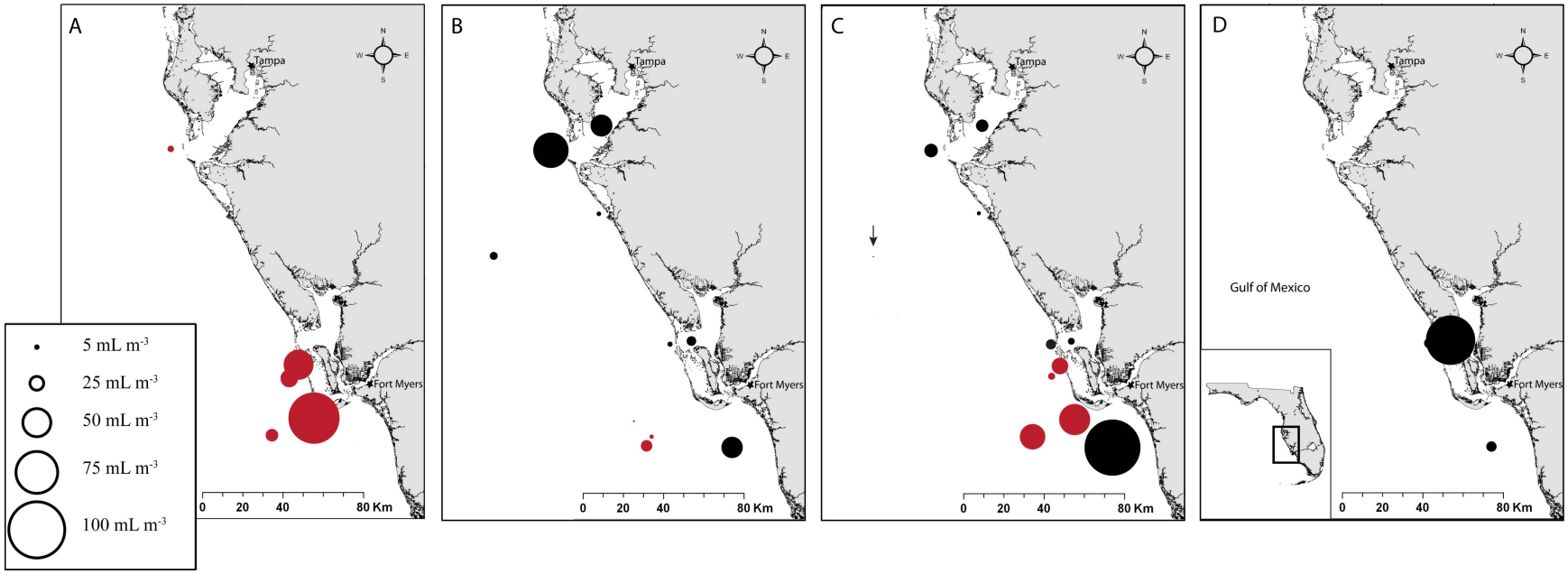

Figure 2.10. Comparative zooplankton biomass (mL m^{-3}) at all stations sampled for zooplankton in October 2007 (A), 2008 (B), 2009 (C), and 2010 (D). Black circles represent stations where bloom concentrations of *K. brevis* were not present. Red circles indicate presence of bloom concentrations of *K. brevis* during sampling.

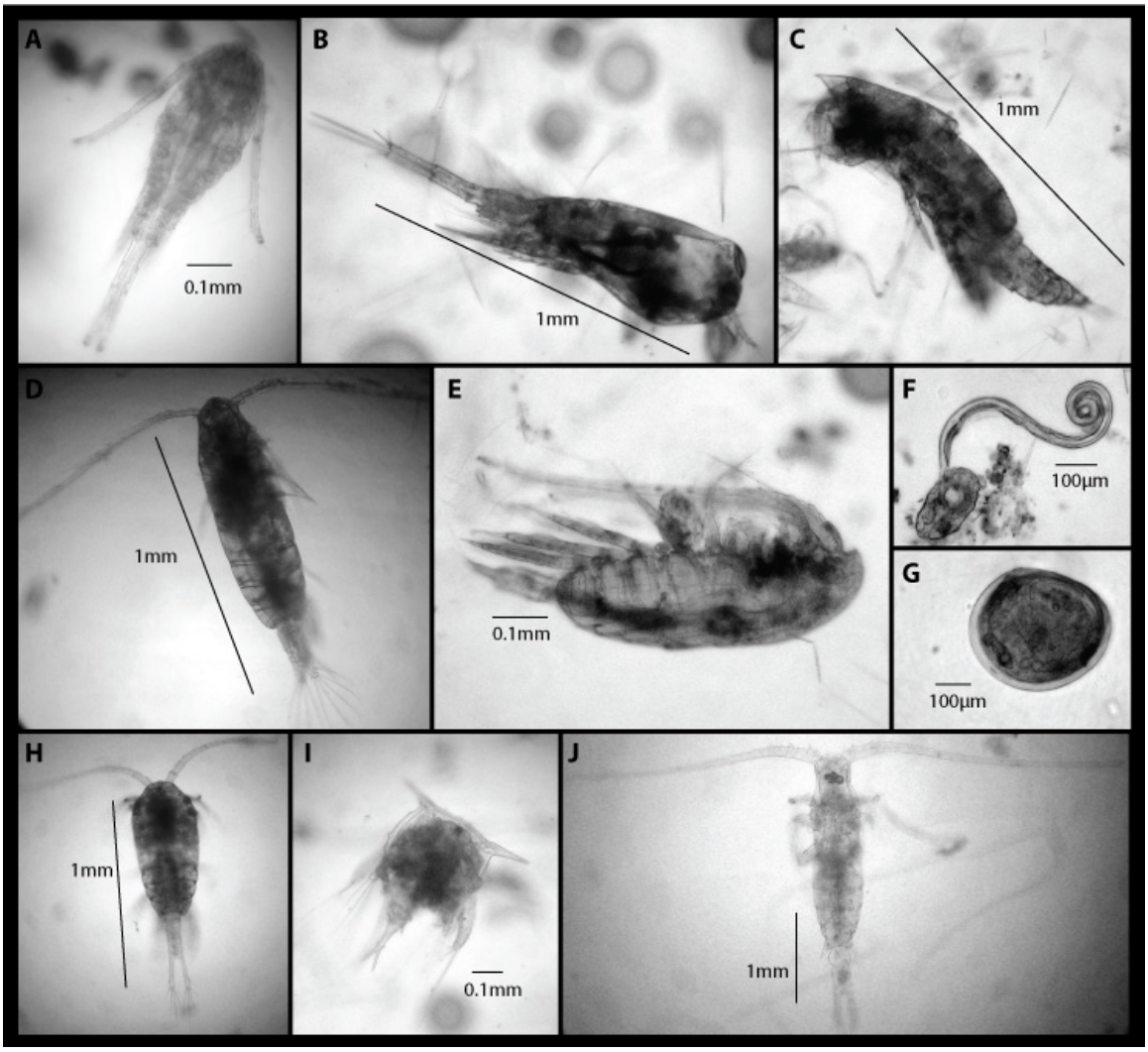

Figure 2.11. Photographs of several of the dominant zooplankton species present within study samples. A) *Oithona* spp., B) *C. americanus*, C) *E. acutifrons*, D) *A. tonsa*, E) *P. crassirostris*, F) *O. dioica*, G) pelecypod larvae, H) *T. turbinata*, I) Cirriped larvae, J) *C. velificatus.*

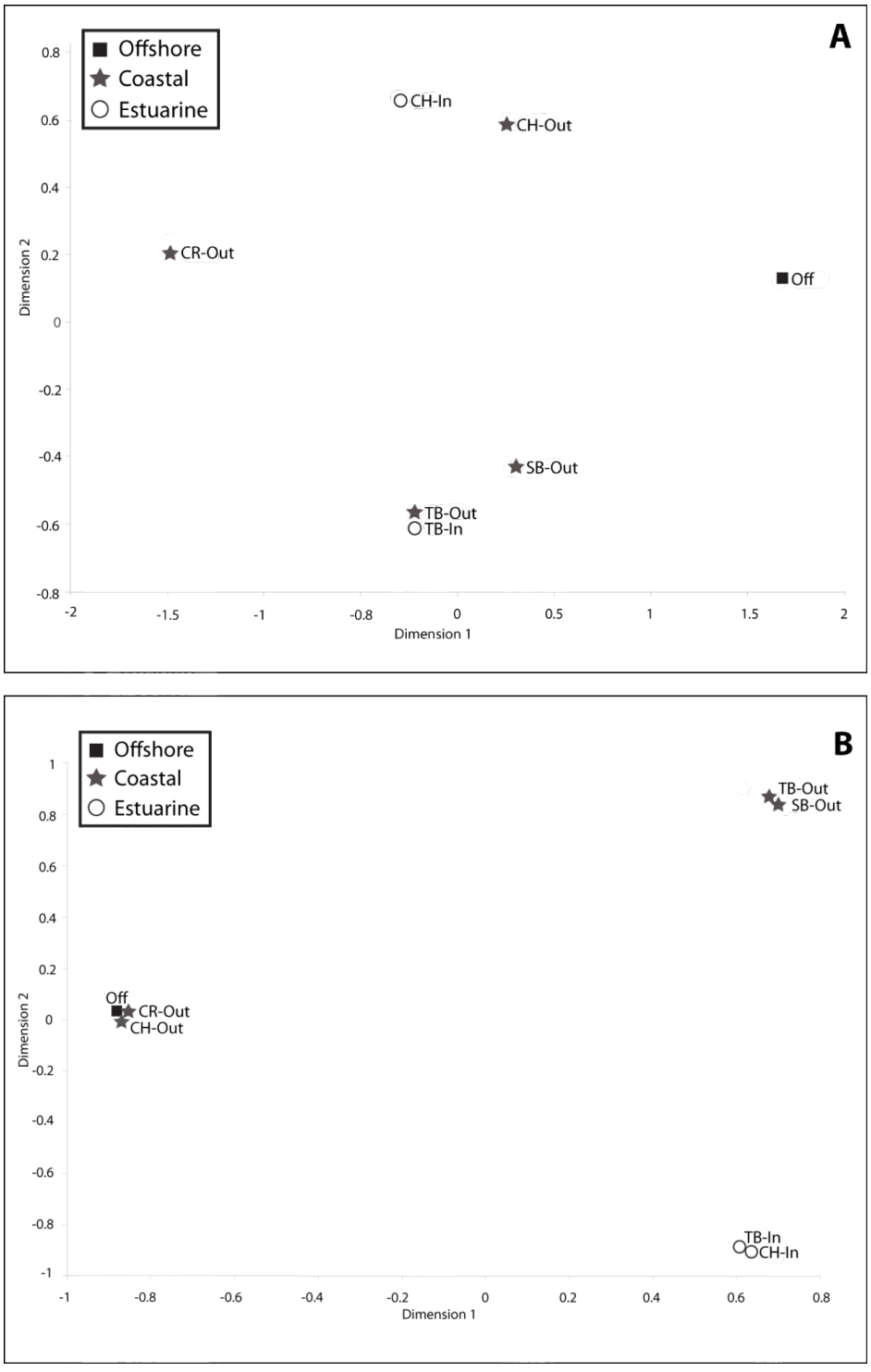

Figure 2.12. Multiple dimensional scaling (MDS) diagram of percent similarities for non bloom samples in years 2008 (A) and 2009 (B). Zooplankton communities were characterized into three groups based on distance offshore (estuarine, coastal, offshore). Stress is 0 and 0.1 respectively.

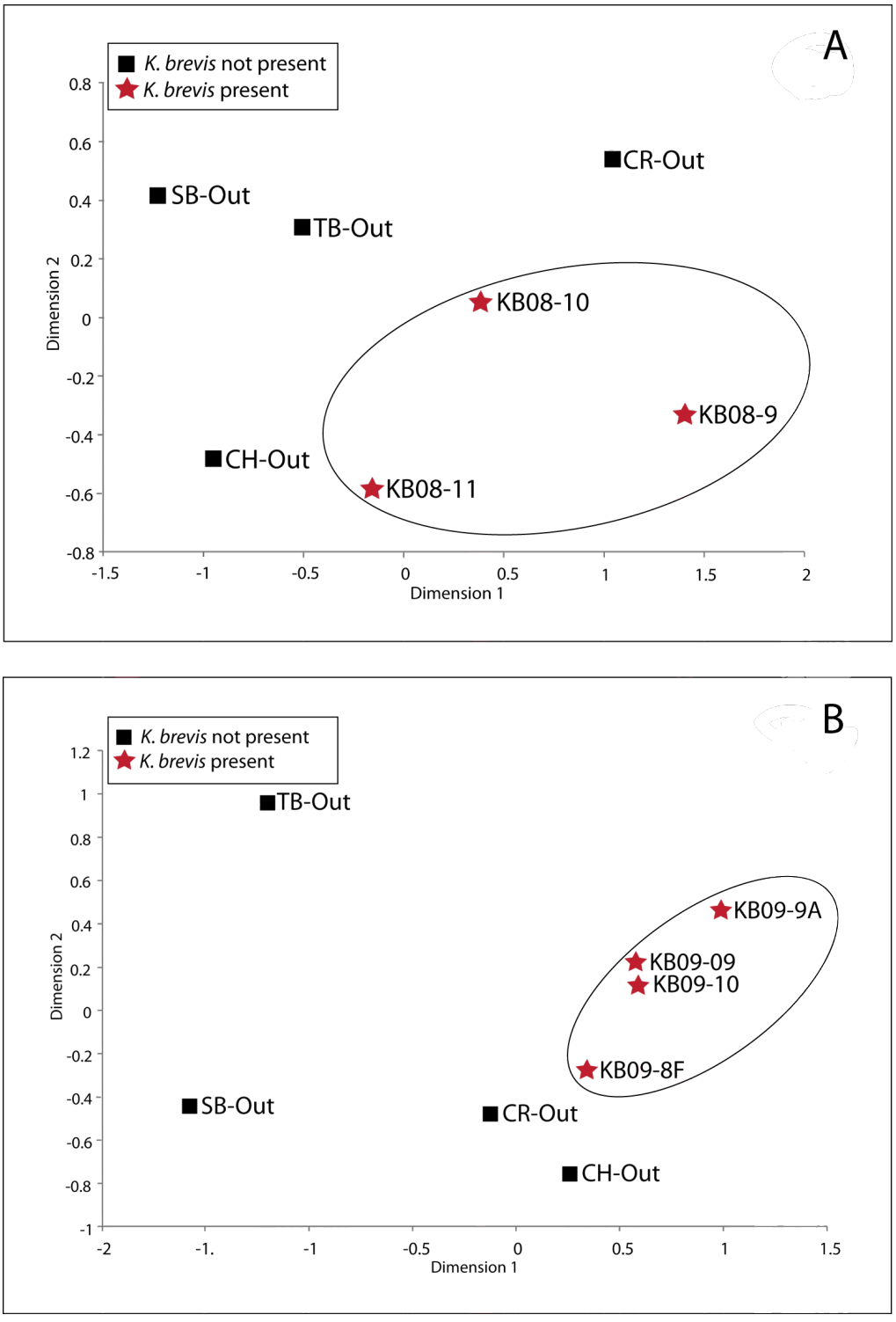

Figure 2.13. Multiple dimensional scaling (MDS) diagram of percent similarities for 2008 (A) and 2009 (B). Zooplankton communities were characterized into three groups based on presence or absence of *Karenia brevis*. Stress is 0.05 and 0.01 respectively.

CHAPTER THREE

ASSESSMENT OF GRAZING IN RELATION TO *KARENIA BREVIS*

Abstract

Blooms of the toxin producing dinoflagellate *Karenia brevis* occur routinely on the West Florida Shelf of the Gulf of Mexico. Nutrient supplies are thought to play a large role in the formation and maintenance of these blooms. The role of top-down control has been less well studied, but grazing, or the lack thereof, on these toxic species may also enhance the formation of large biomass blooms in this region. Using the seawater dilution technique (Landry and Hasset, 1982), we measured phytoplankton growth and microzooplankton grazing rates within and outside of *Karenia brevis* blooms present on the West Florida Shelf in sampling years 2007-2010. Growth rates ranged from 0.013 day^{-1} at the offshore station in 2009 to 2.08 day^{-1} at the mouth of Tampa Bay in the same year. Grazing rate was lowest at the mouth of Tampa Bay in 2009 (0.19 day^{-1}) and highest at station 2F in 2007 (2.78 day^{-1}). In general, phytoplankton growth rates were lowest at the offshore stations in all four years, and higher within estuaries, although a one-way ANOVA ($p=0.05$) showed no significant difference between phytoplankton growth rates (day^{-1}) at the offshore, estuarine and coastal stations. There was not a significant difference between phytoplankton growth rates or microzooplankton grazing rates between bloom and non-bloom in any of the years when *K. brevis* bloom concentrations were present. In order to investigate copepod ingestion rates in relation to *K. brevis*, shipboard and laboratory experiments of the single label method of ^{14}C labeled phytoplankton culture, and time course ingestion experiments with isolated copepods

were performed. Calculated ingestion rates suggest that the copepod species *Centropages velificatus*, and *Acartia tonsa* ingested *K. brevis*, however rates were variable among collection sites and *K. brevis* strains. *Parvocalanus crassirostris* did not ingest *K. brevis* in any of the experiments.

Introduction

Marine food webs depend on a balance between bottom-up availability of nutrients and top-down control via grazing (Harris et al., 2000). Blooms of phytoplankton occur as a result of net biomass production in response to favorable conditions and the uncoupling of losses to a population, and although physical conditions set the stage for blooms, physiological responses to local conditions and trophic interactions can control the persistence and impacts of harmful algal blooms (HABs) (Donaghay, 1997).

Within planktonic food webs, grazing by microzooplankton (<200μm fraction) and mesozooplankton (0.2-20mm size range) are believed to play an important role in the regulation of primary productivity. Heterotrophic dinoflagellates and ciliates are generally the most important grazers of small photosynthetic dinoflagellates, however rotifers and copepod nauplii can also be important grazers of phytoplankton communities (Stoecker et al., 2008). Unlike copepods and larger mesozooplankton that have longer generation times (on the order of weeks to months), protistan microzooplankton have generation times on the order of hours to days, and therefore are tightly coupled to their prey populations (Stoecker et al., 2008). In addition to the ability to control phytoplankton primary productivity, in most environments studied, grazing by microzooplankton appears to dominate all other sources of nutrient regeneration, and dissolved organic carbon excreted by phytoplankton and scavenged by bacteria may be transferred to larger metazoan grazers (Ducklow et al., 1986; Sherr and Sherr, 1987).

The importance of micro- and mesozooplankton in controlling and terminating HABs is highly site and species specific (Vaque et al., 2006). Net growth of planktonic

algal populations can only occur when growth coefficients (μ) exceed mortality due to grazing (g), unless new cells are being added to the population from outside of the system (Stoecker et al., 2008). Goleski et. al (2010) observed the absence of, or a decrease in micro- and mesozooplankton grazing during cyanobacteria blooms in Florida Bay, citing that decreased grazing pressure may have played a role in the success of cyanobacteria blooms within the region. Several factors could have accounted for this: the inability of grazer populations to increase at the same pace as the cyanobacteria; toxins produced by cyanobacteria, or poor nutritional quality (O'Neil, 1999) discouraging zooplankton grazing, or a possible shift in mesozooplankton trophic structure. Predators can be impacted (e.g. either damaged or killed) by toxic or otherwise undesirable cells, and the selection of non-toxic species can favor the proliferation of blooms (Vaque et al., 2006).

Laboratory investigations have indicated a strong link between protozoa and nano- and picoplankton, suggesting that marine protozoan grazers may play a key role in the recycling of organic material (Verity et al., 1993). Evidence from field and laboratory studies also suggest that protistan grazing has the ability to suppress or control dinoflagellate blooms, especially in the initiation stage (Stoecker et al., 2000). During a prolonged bloom of *Aureoumbra lagunensis* in the Laguna Madre (Texas), it was determined that the collapse of grazer populations prior to the initiation of the bloom, coupled with the ability of *A. lagunensis* to grow at maximum growth rates under saline conditions, were likely factors allowing bloom initiation (Buskey et al., 2001). Field and laboratory studies have also shown *A. lagunesis* to be nutritionally inadequate food for planktonic grazers, reducing grazing and increasing the ability of the bloom to persist (Buskey et al., 2001).

When investigating HAB dynamics it may be important to consider zooplankton grazers as important contributors to the control of phytoplankton standing stock. On the West Florida Shelf (WFS), zooplankton grazing has the potential to account for a large loss of the phytoplankton standing stock. Sutton et al. (2001) determined that the zooplankton assemblage present grazed an average of 7.9% of primary production on the shelf during September. In the Northern Gulf of Mexico, ingestion by zooplankton had the potential to remove 15-62% of the phytoplankton biomass within the Mississippi River plume (Dagg, 1995). However, the effect of grazing on algal populations is likely species specific, and the presence and ingestion of toxic algae could result in major implications for zooplankton grazers, altering their ability to graze on phytoplankton populations (Teegarden et al., 2001).

Copepods are discriminating feeders, with the ability to capture, handle and reject (or ingest) particles as a function of nutritional quality (Huntley et al., 1986). Brier and Buskey (2007) have previously identified three possible roles for copepods within *K. brevis* blooms: 1) Bloom initiators, with decreased grazing at the initiation of a bloom due to selective feeding on non-HAB species; 2) Bloom suppressors, as copepod grazing on *K. brevis* decreases phytoplankton cell concentrations; or 3) No impact, as copepods experience a toxic effect from *K. brevis* and are unable to graze. For mesozooplankton with relatively slow response times (weeks) in comparison to phytoplankton (days), their impact on HABs seems to be mainly constrained to the initiation phase of a bloom, when the phytoplankton population is still developing (Vargo et al, 2006).

Some copepods are known to directly ingest toxic bloom species, potentially controlling bloom biomass; while others may avoid ingestion or are incapacitated from

consumption (Turner and Tester, 1997; Breier and Buskey, 2007). When a dinoflagellate is introduced into the water column, its presence can inhibit grazing, and this rejected dinoflagellate can then increase its own biomass (Huntley et al., 1986). The preferential grazing of non-HAB species may aid in the initiation and maintenance of blooms. In addition, the exclusion of zooplankton predators may be due to the zooplankters "learned" behavior to avoid the region after sampling food particles, or the result of a chemical compound (Fielder, 1982). Several species of herbivorous copepods were observed to avoid a dense layer of *Gymnodinium splendens* off the coast of Southern California (Fielder, 1982).

To date, only two *in situ* studies have investigated whether grazing by zooplankton species can contribute significantly to *K. brevis* bloom dynamics (Turner and Tester, 1989; Lester, 2005). Several studies investigating ingestion rates of zooplankton populations have been carried out on natural non-toxic populations, or from naive copepods not previously exposed feeding on *K. brevis* (Huntley et al., 1986; Turner and Tester, 1989; Cohen et al., 2007). However, it is difficult to extrapolate these rates to the native Gulf of Mexico zooplankton population, specifically in regards to *K. brevis* (Turner and Tester, 1989). The calculation of zooplankton ingestion rates is necessary to determine whether zooplankton grazing, or lack of, is involved in the initiation and/or termination of *K. brevis* blooms.

Recent work has indicated that *K. brevis* may not be directly toxic to copepod species, but rather nutritionally inadequate to support maximum egg production (Breier and Buskey, 2007). Laboratory studies have shown that when *Acartia tonsa* was fed a diet of only *K. brevis*, copepods exhibited lower ingestion rates and offspring production

than when fed a mixed diet or no *K. brevis* (Breier and Buskey, 2007). However, there are other studies with conflicting results showing that copepod species such as *A. tonsa*, *Oncaea venustra* and *Labidocera aestiva* have no adverse effects after consuming *K. brevis* (Turner and Tester, 1989). There is also evidence that copepod species that were historically exposed to toxic dinoflagellates such as *K. brevis*, can evolve resistance to toxins (Colin and Dam, 2004). When two species of copepods collected from La Jolla, California were fed *K. brevis*, *S. trochoidea* and *C. pacificus* fed at 46.9% and 29.4% respectively of the clearance rate when given a non-toxic control species. Ingestion of *K. brevis* caused either regurgitation or elevated heart rate and loss of motor control, and in both cases, normal feeding resumed when copepods were placed in a suspension of *Gymnodinium resplendens* (Fiedler, 1982).

It is important to determine ingestion rates of co-occurring zooplankton species to determine if grazing of *K. brevis* by zooplankton can potentially have an impact on bloom dynamics (Lester, 2005). Turner and Tester (1989) experimentally derived ingestion rates for several abundant copepod species within a *K. brevis* bloom off the coast of North Carolina. They found that several species of copepods sympatric with *K. brevis* blooms did indeed ingest the dinoflagellate, however rates of ingestion tended to be variable and low. In the presence of a mixed diet, three copepod species preferentially grazed on the diatom *Skeltonema costatum* over *K. brevis* (Turner and Tester, 1989). In similar ingestion experiments, Lester (2005) derived ingestion rates from three naturally occurring copepod species from the Gulf of Mexico—*Acartia tonsa*, *Paracalanus quasimodo*, and *Labidocera aestiva*. All three experimental copepod species ingested *K. brevis*, with ingestion rates increasing with cell concentration, a result similar to those

found by Turner and Tester (1989). Highest ingestion rates were found for *A. tonsa*. Based on calculated ingestion rates and assuming an average *K. brevis* growth rate of 0.2 divisions day^{-1}, the zooplankton community present at one station in the Lester (2005) study, zooplankton grazing pressure could have been sufficient to reduce the *K. brevis* population to background levels within seven days. For the remaining bloom stations sampled, zooplankton grazing was negligible (Lester, 2005).

The objective of this study was to investigate the role zooplankton grazing on phytoplankton communities of the West Florida Shelf. This was completed by examining the impact of whole water community dilution experiment grazing and growth rates, and also by investigating ^{14}C-labeled *K. brevis* ingestion rates of several dominant West Florida Shelf copepods. Prior to determining the importance of zooplankton in terms of both losses and gains for the phytoplankton community both in and out of *Karenia brevis* blooms, it was first necessary to characterize the zooplankton community present on the West Florida Shelf. The ambient zooplankton assemblage of the WFS, and zooplankton communities present during blooms of *K. brevis* are described in Chapter Two of this thesis.

Methods

Dilution Experiments

Whole community grazing rates were determined using the dilution method (Landry and Hassett, 1982; Landry, 1993). Dilution grazing experiments are the most widely used method for estimating microzooplankton community grazing on the whole phytoplankton community, and have been used globally to estimate phytoplankton growth rates and mortality. Dilution experiments include all grazers: small heterotrophic

and mixotrophic flagellates, as well as larger ciliates and dinoflagellates. Whole seawater (WSW) containing natural assemblages of phytoplankton and microzooplankton is diluted with 0.2μm filtered, "particle free" seawater (FSW) from the same sample. This dilution creates a gradient in predator-prey encounter frequency, and allows a) an estimate of instantaneous rates of growth for the phytoplankton and b) an estimate of grazing mortality of phytoplankton based on the change in chlorophyll along the gradient over a given time period.

The dilution method is based on four assumptions as discussed in detail by Landry and Hassett (1982): (1) specific growth rate of prey is not density dependent, (2) predation is a direct linear function of prey abundance, (3) prey growth can be adequately represented by the exponential growth equation and (4) phytoplankton growth is not limited by nutrient availability.

A dilution series of 0% (whole seawater), 20%, 40%, 60% and 100% (particle free water) was set up at each station in 1L cubitainers. Seawater was collected with 20L carboys from a CTD cast with 20L Niskin bottles. Dilution water, or filtered seawater (FSW) was prepared using a 0.2μm cellulose membrane filter on a Geotech filter rig. Cubitainers (1 L) were incubated in on-deck flow-through incubators for 24 h under shade cloth to mimic ambient conditions. Three replicates from each dilution series received nutrient amendments of 10μM PO_4^{-2}; 20μM NH_4^+; and 20μM NO_3^-; and the other three replicates in each dilution series were maintained at ambient nutrient conditions. These nutrient concentrations were chosen based on observed nutrient levels of coastal West Florida Shelf and its associated estuaries. The coastal region of the WFS is a nitrogen limited system, and nutrient amendments were made to ensure that

phytoplankton communities within the bottles were not nitrogen limited.

One cubitainer from each series was sampled at time zero (t_0) to determine initial values for Chl *a*, and microzooplankton abundance. At the end of the twenty-four hour incubation period, the cubitainers were again sampled for parameters and 500 mL of liquid from each was filtered onto a GF/F filter using gentle vacuum filtration for chlorophyll analysis. Filters were stored frozen in the dark, until extracted in acetone and analyzed on a Turner AU-10 Fluorometer (EPA-Method-445). A 50mL sample for microzooplankton community enumeration was taken from each cubitainer and preserved in 10% (final concentration) acid Lugol's solution and returned to the Horn Point Laboratory where abundance, biomass, size distribution and composition of larger microzooplankton was determined. By comparing the difference between t_0 and t_{24} values in the above parameters, both with and without nutrients, insight can be gained into both grazing and growth processes.

Statistical Analysis

Statistical significance between bloom and non-bloom phytoplankton growth and microzooplankton grazing in 2008 and 2009 were quantified using a paired sample T-test ($p=0.05$). Significance in factors comparing all years were completed using one-way analysis of variance (ANOVA). Analysis was completed with the MATLAB® Version 7.12.0 (The MathWorks, Inc., Natick, MA) statistical package. Prior to any statistical analyses, data were tested for normality with the Kolmogorow-Smirnov test ($p=0.05$). Multiple linear regression was completed to compare both phytoplankton growth and microzooplankton grazing to a suite of environmental factors: temperature, salinity and Chl *a*, *Karenia brevis* cell concentration, and dissolved nutrient concentrations: dissolved

organic nitrogen (DON), dissolved organic phosphorus (DOP), dissolved ammonium (DNH_4^+), and total dissolved nitrogen (DTN) and phosphorus (DTP).

Ingestion Experiments

Investigation of copepod ingestion rates in relation to *K. brevis* was completed through shipboard and laboratory experiments of the single label method of ^{14}C labeled phytoplankton culture, and time course ingestion experiments with isolated copepods were performed. Experimentally derived ingestion rates of several dominant copepod species on *K. brevis* were compared to ingestion rates on other phytoplankton species, as well as the ingestion rates of copepods not native to the Gulf of Mexico.

2010 Shipboard Experiments

Zooplankton samples were collected during an ECOHAB: *Karenia* Nutrient Dynamics cruise aboard the LUMCON Vessel *R/V Pelican* in October, 2010 (Figure 3.1). Copepod samples were collected using paired 64μm mesh bongo net towed at the surface for five minutes. Adult copepods were picked from the tows with a wide-bore plastic pipette and placed into GF/F filtered seawater. Copepods were then sorted under a microscope for identification and individuals of similar size and species were selected. The predominant copepod species within the net tow community was *Centropages velificatus*, which has also been identified as a predominant member of the zooplankton assemblage of the WFS (This study, Chapter 2), and therefore this species was selected for use in ingestion experiments. There is no research to date detailing ingestion of *K. brevis* by *C. velificatus*, however several studies have investigated ingestion by one of its congeners, *Centropages typicus*.

Cultured *K. brevis* samples were obtained from the culture collection at Florida Fish and Wildlife Research Institute (FWRI) in St. Petersburg, Florida. A sample of culture (1mL) was preserved in 10% (final concentration) Lugol's solution for *K. brevis* cell counts. A 100 mL sample of *K. brevis* culture was transferred into a polycarbonate bottle, and 5 mL of 10 μCi mL $H^{14}CO_3$ was added. This sample was wrapped in 2-3 layers of neutral-density screening and placed in an on-deck flow through incubator for 5 h. At the end of the incubation period, 10 mL of 'hot' ^{14}C-labeled *K. brevis* culture were then transferred to 70 mL polycarbonate Nalgene® bottles containing 50mL of filtered seawater. Copepods remained in particle free seawater for 24 h to allow for gut clearance. After 24 h, three adult copepods were added to the 70 mL Nalgene® bottles to which 10mL of previously labeled *K. brevis* had been added. Ingestion of the ^{14}C-labeled *K. brevis* into the copepods though grazing was determined over time course incubations of 0, 30 and 60 minutes, with 3 replicates for each time point.

Experiments were terminated by filtering samples onto pre-weighed 12μm Nuclepore™ filters. Samples were rinsed three times with an isotonic solution of 6% (w/v) ammonium formate to remove salt (Omori and Ikeda, 1984) and rinsed once with 10% HCl to remove excess label. Copepods were removed from the filters containing *K. brevis* using a hypodermic needle, and placed onto clean pre-weighed 12μm filters. Both copepod and *K. brevis* samples were dried at 60°C and weighed using an electronic microbalance (± 0.001 mg). Filters were then placed in 5mL Fisher Scintisafe™ Econo 2 LSC cocktail and disintegrations per minute (d.p.m.) were determined using a Packard (Tri-carb 2200CA) Liquid Scintillation Analyzer (O'Neil and Roman, 1992).

2012 Laboratory Experiments

Zooplankton samples were collected on February 14, 2012 from Tampa Bay using paired 64μm mesh bongos net towed from the Sunshine Skyway fishing pier in St. Petersburg, Florida (Figure 3.2). Adult *Acartia tonsa* were picked from the tows with a wide-bore plastic pipette and placed into GF/F filtered seawater. Copepods were then sorted under a microscope for identification and individuals of similar size and species were selected. Upon collection and sorting, copepods were then flown same-day to the Horn Point Laboratory in Cambridge, MD.

A. tonsa individuals were also obtained from culture at the Horn Point Laboratory that was originally collected from the mouth of the Choptank River a tributary of the Chesapeake Bay (Figure 3.3).

Additionally, *A. tonsa* and *Parvocalanus crassirostris* were obtained from AlgaGen LLC., a biotechnology company specializing in the culture of microalgae based in Vero Beach, Florida. Original collection sites for these copepods as reported by AlgaGen LLC. were the Gulf of Mexico for *A. tonsa* and Hawaii for *P. crassirostris*.

A. tonsa was selected as the experimental species because it is an abundant species within zooplankton communities of the WFS, and is found in areas where *K. brevis* is known to commonly form blooms. It has also been used in several studies investigating the ingestion of *K. brevis* and potential toxic effects on the copepods. Additional copepods collected from the Chesapeake Bay were included in the experiment to investigate the potential differences in ingestion between allopatric and sympatric species. *P. crassirostris* is an additional copepod species common to the Gulf of Mexico, however copepods used in this experiment were from the Pacific Ocean.

Cultured *K. brevis* (database number CCMP718)*, K. mikimotoi* and *P. minimum* samples were obtained from the culture collection at Florida Fish and Wildlife Research Institute (FWRI) in St. Petersburg, Florida. An additional culture of *K. brevis* (Wilson) was provided by MOTE Marine Laboratory in Sarasota, Florida. A sample of the *K. brevis* culture (100 mL) was transferred into a polycarbonate bottle, and 5 mL 10.5 μCi mL $H^{14}CO_3$ was added. This sample was then placed in an environmental chamber for 5h. After the 5h incubation period, experiments were run as outlined in the above section (*2010 Shipboard Experiments*). For experiments with *P. crassirostris*, size fractionation filtration was used. Samples were first filtered onto 20μm filters and copepods were picked off using a hypodermic needle onto clean, pre-weighed 12μm filters, and then filtrate was re-filtered onto GF/Fs.

Ingestion rates (cells copepod^{-1} hour^{-1}) were determined by first calculating clearance rate (F) in mL copepod^{-1} hour^{-1}, and then converting to ingestion rate based on phytoplankton concentrations during each of the experiments. Clearance rate is defined as the volume of water cleared per consumer for a given unit of time (Harris et al., 2001). Control sample readings were subtracted from experimental samples to correct for any isotope background . Clearance Rate (F) in mL animal^{-1} hr^{-1} was calculated as:

$$F = \frac{(dpm_{animal} \times v)}{(dpm_{algae} \times t)}$$

Where dpm_{animal} is the radioactivity of one copepod, dpm_{algae} is the radioactivity of v mL of phytoplankton culture, and t is the incubation time in hours (Bamstedt et al., 2000).

Ingestion rates (cells copepod^{-1} hour^{-1}) were calculated by multiplying the clearance rate (F) by phytoplankton concentration (cells mL^{-1}) (Bamstedt et al., 2000):

$$Ingestion = F \times \left[phytoplankton \right]$$

Short-term incubations correct for isotopic recycling and excretion/egestion by assuming that these are negligible over the experiment duration (1 h). Additionally, it is assumed that the radioisotope is neither absorbed through the surfaces of grazer cells nor taken up osmotically by the grazers, and the rinsing of grazers with HCl corrects for any unincorporated isotope (Harris et al., 2000).

Results

Dilution Series

Community growth and grazing rates were calculated from the regression equation between net growth rate for each dilution bottle and the dilution factor. The intercept of the equation is an estimation of the gross growth rate without predators and the slope coefficient corresponds to the community grazing rate.

Phytoplankton growth rates ranged from 0.013 d^{-1} at the offshore station (OFF) in 2009 to 2.08 d^{-1} at the mouth of Tampa Bay (TB-Out) in the same year. Grazing rate was lowest at that same station in 2009 (TB-Out) at 0.19 d^{-1} and highest at station 2F in 2007 (2.78 d^{-1}). In general, phytoplankton growth rates (d^{-1}) were lowest at the offshore stations in all four years, and higher within estuaries, although a one-way ANOVA ($p=0.05$) showed no significant difference between phytoplankton growth rates (d^{-1}) at the offshore, estuarine and coastal stations, due to the large variability obtained in rates (Table 3.1).

In each of the three sampling years when *K. brevis* was present, a student's T-test was used to determine whether there was a significant difference in phytoplankton growth rate or grazing rate between coastal stations and bloom stations. There was not a significant difference between phytoplankton growth rates or whole community grazing rates between bloom and non-bloom in any of the years when *K. brevis* bloom concentrations were present. Additionally, t-test analysis ($p=0.05$) was completed to compare mean phytoplankton growth and grazing between ambient nutrient concentration and nutrient amended bottles in 2008 and 2009. There was a significant difference between –nutrient and +nutrient dilution series in 2008 [$t(8)=0.0084$; $p<0.05$], but no significant difference was found in 2009 [$t(7)=0.0084$; $p<0.05$].

In all years, total nitrogen was significantly correlated with both phytoplankton growth and grazing.

Phytoplankton Community

The phytoplankton community present in each of the four sampling years was characterized based on samples taken during dilution experiments. Phytoplankton individuals were identified to group level whenever possible. In 2007, the *K. brevis* bloom was in the maintenance phase, and *K. brevis* was the dominant phytoplankton species in samples. In lesser abundance were the congener *Karenia mikimotoi*, as well as several chain diatoms, and the centric diatom *Coscinodiscus* spp. The dinoflagellates *Protoperidinium* spp., *Prorocentrum* spp., *Scripsiella* spp., *Dinophysis* spp. were also abundant within 2007 samples. In 2008, a mixed phytoplankton assemblage was present within bloom stations. Based on cell physiological state and growth rates, the bloom sampled in 2008 was characterized as in the initiation phase. In conjunction with

phytoplankton counts from Florida Wildlife Research Institute, twenty-eight species were recorded in the phytoplankton community, with the predominant members being diatom and dinoflagellate species. In comparison, the 2009 bloom was similar to the bloom sampled in 2007, in the maintenance phase, and seventeen species were present in bloom samples, with *K. brevis* being the dominant phytoplankton species.

Ingestion Experiments

Calculated ingestion rates suggest that the copepod species *Centropages velificatus*, and *Acartia tonsa* all ingested *K. brevis*, however rates were variable among collection sites and *K. brevis* strains (Table 3.2). *Parvocalanus crassirostris* did not ingest *K. brevis* in any of the experiments (Figure 3.4). Ingestion rates for *C. velificatus* feeding on *K. brevis* SARA (FWRI) isolates ranged from 261.4 to 467.0 cells copepod^{-1} h^{-1}, with the highest ingestion rates occurring at the t_{30} time point (Figure 3.5). For *Acartia tonsa* collected from the Tampa Bay estuary, highest ingestion rates were calculated when copepods were feeding on *K. brevi*s WILSON culture from FWRI (Figure 3.6) that also had the highest cell concentration (96250 cells L^{-1}). Naive *A. tonsa* collected from Chesapeake Bay also ingested *K. brevis* (Figure 3.7) although at a lower rate than copepods that may co-occur with *K. brevis* collected from Tampa Bay (Average= 272.1 cells copepod^{-1} h^{-1}). A one-way ANOVA ($p=0.05$) indicated that there was a significant difference between the ingestion rate of *A. tonsa* on *K. brevis* Wilson (FWRI) isolates and the ingestion rate of *A. tonsa* on other *K. brevis* cultures. AlgaGen LLC. *A. tonsa*, isolated from the Gulf of Mexico, also ingested *K. brevis*, but at a lower ingestion rate (Figure 3.8). In all experiments except one, ingestion rates were highest at the t_{30} time-point and decreased at one hour. This could be due to a saturation of

ingestion rates after the twenty-four hour starvation period. The only experiment where ingestion rates increased past the t_{30} time-point was the *A. tonsa* (Tampa Bay) *K. brevis* MOTE experiment (Figure 3.9). When comparing experiments in which *A. tonsa* was presented with *K. brevis* or the congener *K. mikimotoi* (Figure 3.10), ingestion rates increased with increasing *Karenia* spp. cell concentrations (Figure 3.11)

K. brevis cultures used in the experiment ranged in cell concentrations from $2.7x10^4$ cells L^{-1} to $9.6x10^4$ cells L^{-1}. Toxin analysis was not completed at the time of the experiments, however previous LCMS/MS analysis was completed on the cultures by Florida Wildlife Research Institute (St. Petersburg, Fl.) and MOTE Marine Laboratory (Sarasota, Fl.). PbTx-1 (pg $cell^{-1}$) and PbTx-2 (pg $cell^{-1}$) levels were averaged in both analyses (Table 3.3). Toxicity among clones can vary (as much as an order of magnitude) according to conditions and growth stage. Therefore, these results only provide a baseline comparative measurement between strains, and may not reflect the exact toxicity levels at the time of the experiment (L. Flewelling, personal communication). When plotted, maximum ingestion rates appear to be negatively correlated to average toxicity (Figure 3.12), however, the relationship was not statistically significant (R^2=0.23).

Discussion

While there were no significant differences between non-bloom and bloom whole water grazing rates in the three years where bloom concentrations were present, there do appear to be growth and grazing relationships based on environmental factors and bloom phase. In 2008, there was a significant difference in phytoplankton growth between ambient and nutrient amended samples, a result that was not observed the following year.

This suggests that the phytoplankton community present in 2008 was nutrient limited in comparison to 2009. Due to large natural deposits of phosphorite along the West Florida Shelf, N:P ratios are usually observed well below Redfield Ratios (16:1), resulting in a generally nitrogen limited phytoplankton community (Brand and Compton, 2007). In 2009, an estuarine signal characterized the coastal stations, as both estuarine and coastal salinities were lower in comparison to the three other years. In 2009, river discharge (ft^3 s^{-1}) at USGS gauge stations in the Manatee River and Hillsborough River, both major tributaries of Tampa Bay, recorded higher mean discharge in the months prior to sampling. The same pattern was true for tributaries of Charlotte Harbor (i.e. the Myakka River and Peace River), where mean river discharge was an order of magnitude larger in September of 2009 than other study years. The higher river flow in 2009 also likely contributed to a large influx of nutrients to the system, accounting for the lack of difference in the nutrient addition experiments during that cruise.

From October 2007 to 2009, three *Karenia brevis* blooms occurred on the West Florida shelf, all of which were sampled within the confines of this study. In 2010, no bloom concentrations of *K. brevis* (>1000 cells L^{-1}) were observed. Based on cell physiology and concentrations, the blooms were characterized as being in one of three stages: initiation, maintenance, or termination. The 2007 bloom was well-established and high biomass, and had resulted in fish kills along the Gulf coast of Florida. The 2009 bloom was also well established and characterized as in the maintenance phase. In 2008, the bloom sampled was in the initiation phase. Comparatively, average whole community growth and grazing rates were highest in 2008, when the *K. brevis* bloom was in the initiation phase. In 2009, as the bloom progressed, grazing rate decreased from

1.52 day^{-1} to 0.23 day^{-1}. This coincides with a decrease in total zooplankton abundance that was identified in Chapter 2; which suggests that as the bloom progressed, grazing pressure on the *K. brevis* population may have also been reduced. The variability in grazing rates may reflect past feeding history, physiological state and toxicity of *K. brevis* (O'Neil, 1999). To further investigate the changes in grazing rate noted during whole water dilution experiments, and to explore the impact of mesozooplankton on community grazing, ingestion rates of dominant copepod species within *K. brevis* blooms were analyzed in the second part of this study.

Regardless of collection site and culture strain, *Acartia tonsa* ingested *K. brevis* in all of the experiments, but with varying rates. Highest ingestion rates were observed for *A. tonsa* that may have previously been exposed to *K. brevis*, collected from Tampa Bay, with lower rates for naïve or those with less potential for past *K. brevis* exposure, copepods collected in Chesapeake Bay, and cultured by AlgaGen LLC. Comparatively higher ingestion rates were also observed by *C. velificatus* collected from the Gulf of Mexico.

One species, *Parvocalanus crassirostris* did not show appreciable ingestion of *K. brevis* or ingestion rates were negligible (Figure 3.13). *P. crassirostris* is a small (1.0-1.5mm) calanoid copepod abundant year round in high-salinity estuaries of the United States and the Gulf of Mexico (Johnson and Allen, 2005). Calbet et al., (2000) conducted studies investigating ingestion rates of *P. crassirostris* on a mixed pico- and nanoplankton community in Kaneohe Bay, Hawaii, and recorded ingestion rates of 1.5 cells second^{-1}. This corresponds to 5400 cells h^{-1}, in comparison to the negligible rates documented in this study. While *P. crassirostris* does naturally co-occur with *K. brevis*

on the WFS, and was an important contributor to bloom populations within this study (Chapter 2), copepods used within this experiment were collected from Hawaii, and they may not be a comparable indicator of those from the Gulf of Mexico. Additionally, the *K. brevis* cells introduced as a food source for *P. crassirostris* could have potentially been outside of the copepod's preferred food size. *P. crassirostris* are mainly herbivorous and prefer phytoplankton >5 μm, however its diet is not well studied (Johnson and Allen, 2005). *K. brevis* cells range from 18-45μm in size.

Although the results of several recent studies differ in the extent to which *A. tonsa* grazes on *K. brevis*, it is generally accepted that *K. brevis* is a low quality food for this copepod which is evidenced by experimental results for egg production and hatching rates similar to those of starvation (Collumb and Buskey, 2004; Prince et al., 2006, Cohen et al., 2007). The short-term ingestion rates for *Acartia tonsa* feeding on *K. brevis* culture found in this study fell between the two ingestion rates for *A. tonsa* found in previous research (Turner and Tester,1989; Lester, 2005). Adult *Acartia tonsa* are a dominant coastal and estuarine species throughout the Gulf of Mexico, ranging from 1.0-1.5mm in size (Johnson and Allen, 2005). Lester (2005) observed *A. tonsa* ingestion rates at the 10^4 cell concentration of *K. brevis* of 210 cells copepod^{-1} L^{-1}. This was an order of magnitude less than ingestion rates observed within this study at a *K. brevis* concentration of $9.6x10^3$ cells L^{-1}. Turner and Tester (1989) observed ingestion rates for *A. tonsa* ranging from 2000 cells copepod^{-1} hr^{-1} at a cell concentration of 4000 cells mL^{-1} to 16000 cells copepod^{-1} h^{-1} at a concentration of 20000 cells mL^{-1}. These differences in ingestion rates were likely due to differences in methodologies between the three studies, as well as *K. brevis* cell concentrations used. Additionally, the physiological state and toxicity of

the varying *K. brevis* strains could account for variability in ingestion rates.

Both of these previous studies looked at changes in ingestion rate in comparison to *K. brevis* concentration, whereas this study investigated the change in ingestion rates over a 1 h time period with varying *K. brevis* cell concentrations. Lester (2005) used a similar ^{14}C-label methodology, whereas Turner and Tester (1989) used *K. brevis* cell disappearance to calculate ingestion rates. Both studies found that with increasing concentrations of *K. brevis*, copepod ingestion rates also increased. To investigate this result, all of the *A. tonsa* ingestion rates were plotted against *Karenia* spp. culture cell concentration, and a positive, direct relationship resulted (r^2=0.27). Therefore, this study supported previous results that ingestion of *K. brevis* by *A. tonsa* increases with cell concentration.

Turner and Tester (1989) completed a similar study investigating ingestion rates of copepod species on *K. brevis* during an expatriate red tide on the coast of North Carolina. During this particular event, *K. brevis* bloom concentrations were entrained in the Gulf Stream and carried North to coastal North Carolina. *C. typicus*, a congener of *C. velificatus* was tested for ingestion of *K. brevis*, however no significant ingestion occurred (Turner and Tester, 1989). This was puzzling based on the preferential food size for *C. typicus*, and because in the same study it ingested substantial amounts of *Skeletonema costatum*, a non-toxic dinoflagellate. Researchers accounted the differences in biogeography in the two species for the negligible ingestion rates observed.

These previous results, coupled with those found within the confines of this study, suggest that copepods that may be exposed to natural assemblages of *K. brevis* are more likely to ingest the toxic dinoflagellate, or populations may have evolved resistance to the

toxins. Geographically separated populations of *Acartia hudsonica* that had been historically exposed to the toxic dinoflagellate *Alexandrium* spp. showed significantly better survival and reproduction than naïve copepods in the presence of the dinoflagellate (Avery, 2007). The copepods used by Turner and Tester (1989) were collected from a region experiencing *K. brevis* blooms. Whereas copepods in this study and in Lester (2005) were collected from waters free of brevetoxins (Lester, 2005). Colin and Dam (2004) exposed both native and naïve copepods to the toxic dinoflagellate *Alexandrium fundyense*. When naïve copepods were fed a diet containing the toxic species, they exhibited lower somatic growth, size at maturity, egg production and survival than those species that lived in an area that as experienced *A. fundyense* blooms for decades. *K. brevis* blooms have been documented to occur on the WFS almost annually. Although it is not clear whether *K. brevis* is toxic or nutritionally inadequate to zooplankton, there is potential that certain zooplankton species have been able to develop resistance to the impacts of brevetoxins within the food chain.

Conclusions

The results from this assessment did not find a significant difference between whole community growth and grazing rates between bloom and non-bloom conditions. However, there was a significant difference between the ambient nutrient and nutrient amended dilution series in 2008, supporting the idea that phytoplankton communities on the West Florida Shelf may be nutrient limited, however this is likely based on a suite of physical conditions.

Several of the abundant copepods observed within *K. brevis* blooms are capable of ingesting *K. brevis*, and whole community grazing rates show there may be a decrease

in grazing pressure on *K. brevis* as blooms progress and increase biomass. While it is uncertain whether brevetoxins are toxic or nutritionally inadequate to zooplankton grazers, the uncoupling of grazing on a bloom population could result in the successful recruitment and survival of a bloom population.

The results of the grazing assessment by Lester (2005), indicated that grazing pressure by the zooplankton assemblage at one station could have decreased the *K. brevis* population to background concentrations within one week. Based on observed grazing rates within this study, coupled with previously published ingestion rates (Lester, 2005), the ingestion capability of the zooplankton community on chlorophyll present at bloom stations was calculated. All ingestion rates were converted to ng chl ind^{-1} d^{-1} and prorated to a 12-h day. Additionally, *K. brevis* cell concentrations were converted to ng chl L^{-1} based on the average chlorophyll *a* concentration of 8.5 pg chl *a* cell (Evens et al., 2001).

Of the eleven stations sampled for zooplankton within *K. brevis* blooms, at five of these stations, calculated zooplankton ingestion rates (ng chl ind^{-1} d^{-1}) could be sufficient to clear the water column of all *K. brevis* cells present within the bloom. At each of these stations, there were high abundances of smaller copepods, *O. colcarva* and *P. crassirostris*, as well as the appendicularian *O. dioica*, which likely contribute to these high ingestion rates. At the remainder of the stations, including KB07-8 where highest *K. brevis* cell concentrations were observed, total community rates were negligible and grazing could only account for a fraction of the chlorophyll *a* present within the bloom.

There are several assumptions that must be considered within this calculation (Lester, 2005). While it is unclear whether *K. brevis* is toxic or nutritionally inadequate

to zooplankton, several feedings studies have indicated that when given the opportunity to feed on a phytoplankton species other than *K. brevis*, zooplankton will preferentially graze on the non-toxic species. Based on ChemTax analysis data provided by MOTE Marine Laboratory and phytoplankton counts by FWRI, none of the *K. brevis* blooms within this study were monospecific blooms. Therefore, because the ingestion rates within this study were based on a diet solely of *K. brevis*, they are likely an overestimation of natural ingestion rates on *K. brevis* that would have been occurring during blooms. If other species of phytoplankton are present, grazing on *K. brevis* should be assumed negligible.

Additionally, carnivory by these copepods and diel variation in feeding rates were not considered, which could result in an overestimation of feeding rates. Zooplankton and *K. brevis* both exhibit diel vertical migration, however stations sampled within this study were relatively shallow, and there was a breakdown in stratification, resulting in a well mixed water column.

The results of the grazing assessment indicate that it is unlikely that the zooplankton community present during *K. brevis* bloom sampled were sufficient to terminate bloom formation during the conditions sampled. However, the results of both whole community grazing rates and zooplankton ingestion experiments do suggest an uncoupling of growth and grazing as a bloom progresses, so further examination of this relationship is necessary. While we did assess the microzooplankton community grazing capacity, a complete analysis of microzooplankton abundance would be beneficial to quantify specific changes in community abundance and species specific grazing ability.

Table 3.1. Whole water phytoplankton growth rates and grazing rates calculated from Landry and Hasset (1982) dilution series experiments for both ambient (- nutrients) and nutrient amended (+ nutrients) samples.

	- Nutrients			***+ Nutrients***		
Station	*Growth (day^{-1})*	*Grazing (day^{-1})*	*Grazing:Growth*	*Growth (day^{-1})*	*Grazing (day^{-1})*	*Grazing:Growth*
2007						
CH-Out	0.43	2.51	5.82	---	---	---
2F	0.64	2.78	4.34	---	---	---
CR-Out	0.20	2.54	12.4	---	---	---
CH-In	0.51	2.36	4.65	---	---	---
SB-Out	0.04	0.26	5.97	---	---	---
2008						
Off	0.03	0.77	28.37	0.04	0.91	22.64
CH-Out	0.11	1.88	17.51	0.06	0.20	3.58
CR-Out	1.40	1.81	1.29	2.91	1.86	0.64
CH-In	1.39	2.40	1.73	2.93	2.88	0.98
SB-Out	0.83	0.63	0.76	2.81	2.43	0.86
TB-In	0.83	1.23	1.48	2.90	2.42	0.83
TB-Out	0.62	0.91	1.45	1.76	1.52	0.86
St. 8	0.29	1.82	6.24	5.70	1.93	0.34
St. 9	0.81	2.27	2.80	2.03	1.60	0.79
St. 10	0.72	0.82	1.15	1.71	1.42	0.83
2009						
Off	0.01	0.43	33.37	0.42	0.42	1.00
CH-Out	0.20	1.50	7.38	16.72	16.72	1.00
CR-Out	0.26	1.75	6.67	6.22	8.14	1.31
CH-In	4.35	2.07	0.47	3.90	2.71	0.70
SB-Out	0.58	2.13	3.66	4.19	3.39	0.81
TB-Out	2.08	0.19	0.09	0.22	---	1.31
St. 8F	0.10	1.52	15.14	1.44	---	0.23
2010						
CH-Out	0.03	1.14	41.11	---	---	---
St. 12	1.98	1.35	0.68	---	---	---
St. 14b	2.00	1.10	0.55	---	---	---

Table 3.2. Copepod species collection site and *K. brevis* strain and toxicity data for species used for ^{14}C-label ingestion experiments.

Copepod Species	*Copepod Collection Site*	*Culture Strain*	*Culture Collection*	*Toxic (Y/N?)*	*Average toxicity (pg cell^{-1})*
C. velificatus	Gulf of Mexico	*K. brevis* (Sara)	FWRI	Y	25.0
A. tonsa	Tampa Bay	*K. brevis* (Wilson)	FWRI	Y	2.0
A. tonsa	Tampa Bay	*K. mikimotoi*	FWRI	N	n/a
A. tonsa	Tampa Bay	*K. brevis* (Wilson)	MOTE	Y	0.002
A. tonsa	Chesapeake Bay	*K. brevis* (Sara)	FWRI	Y	25.0
A. tonsa	AlgaGen- Gulf of Mexico	*K. brevis* (Sara)	FWRI	Y	25.0
P. crassirostris	AlgaGen- Hawaii	*K. brevis* (Sara)	FWRI	Y	25.0

Table 3.3. Minimum and maximum ingestion rates (cells copepod^{-1} h^{-1}) calculated for each of the seven ^{14}C-label ingestion experiments.

Species	*Collection Site*	*Phytoplankton Culture*	*Culture (cells mL^{-1})*	*Min Ingestion Rate (cells copepod^{-1} h^{-1})*	*Max Ingestion Rate (cells copepod^{-1} h^{-1})*
C. velificatus	Gulf of Mexico	*K. brevis* Sara (FWRI)	4374	0	467
A. tonsa	Tampa Bay	*K. brevis* Wilson (FWRI)	9625	0	1276
A. tonsa	Tampa Bay	*K. mikimotoi* (FWRI)	4843	0	639
A. tonsa	Tampa Bay	*K. brevis* Wilson (MOTE)	2302	0	169
A. tonsa	Chesapeake Bay	*K. brevis* Sara (FWRI)	2697	0	272
A. tonsa	Gulf of Mexico	*K. brevis* Sara (FWRI)	2697	0	152
P. crassirostris	Hawaii	*K. brevis* Sara (FWRI)	2697	0	2

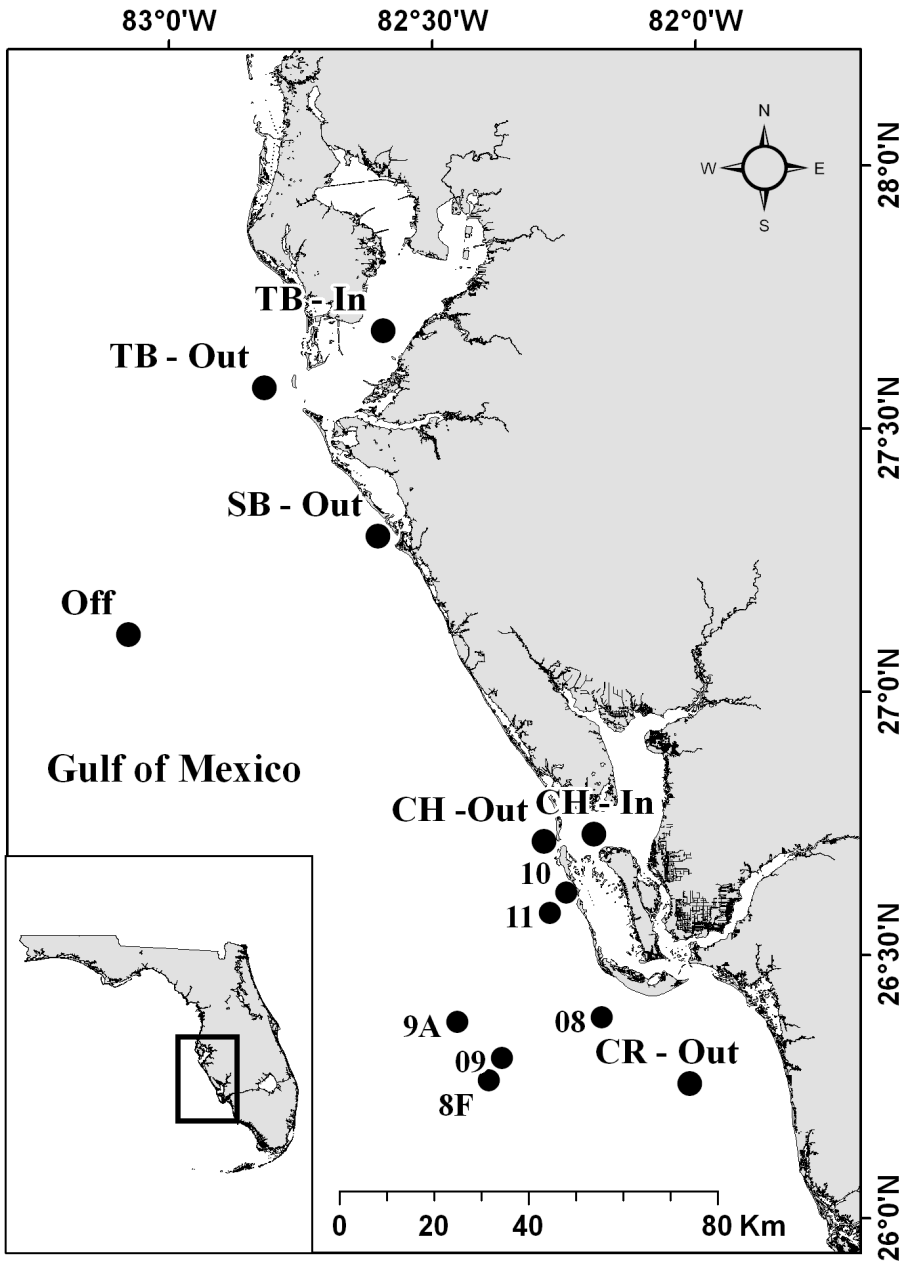

Figure 3.1. ECOHAB: *Karenia* station map. *Centropages velificatus* individuals used for ^{14}C-label ingestion experiments were collected at Stations CH-Out.

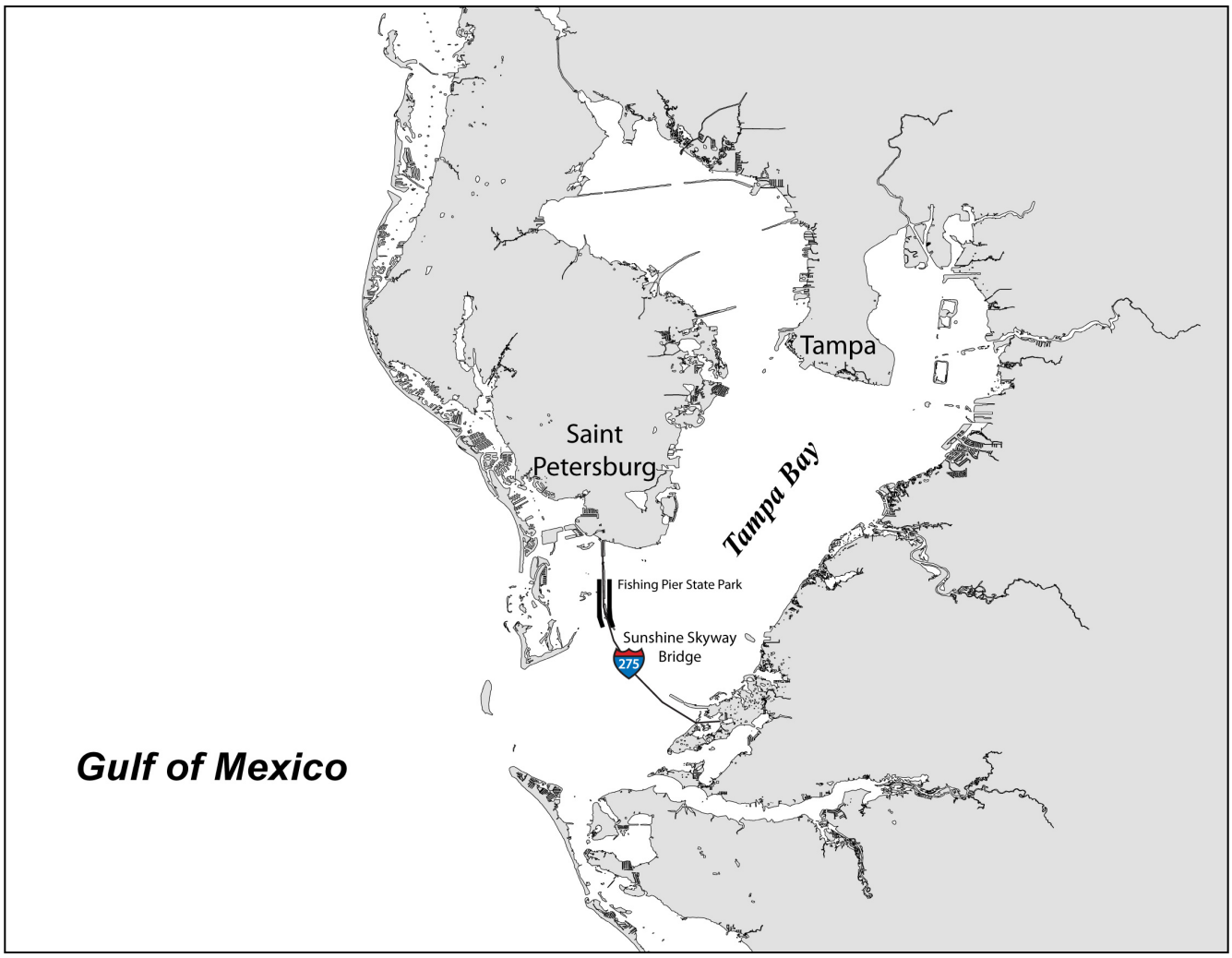

Figure 3.2. Map of Tampa Bay. *A. tonsa* individuals used for ^{14}C-label ingestion experiments were collected on from Fishing Pier State Park.

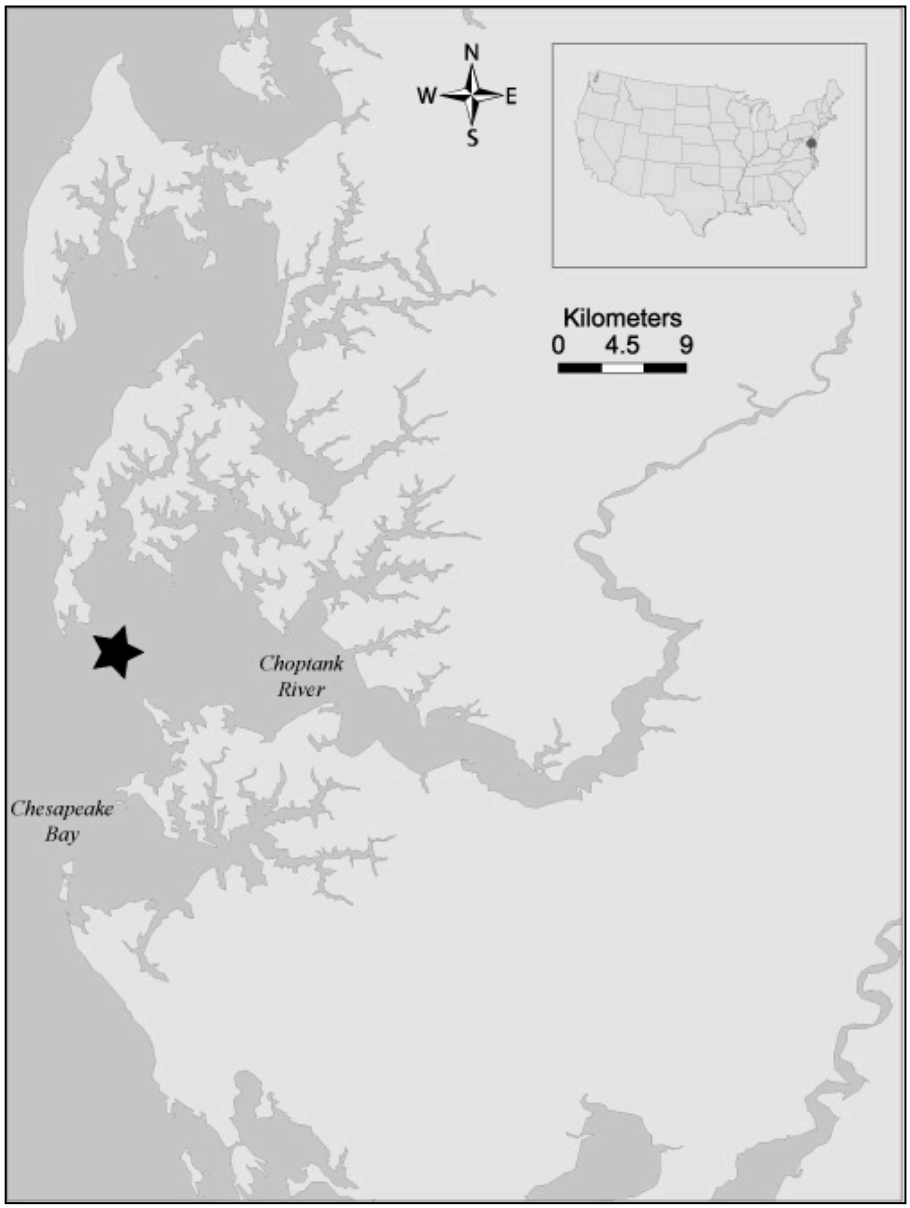

Figure 3.3. Map of Choptank River, a tributary of the Chesapeake Bay. *A. tonsa* individuals used for ^{14}C ingestion experiments were sampled from the mouth of the Choptank River (Indicated by star). Map courtesy of Tracey Saxby and Kate Boicourt, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

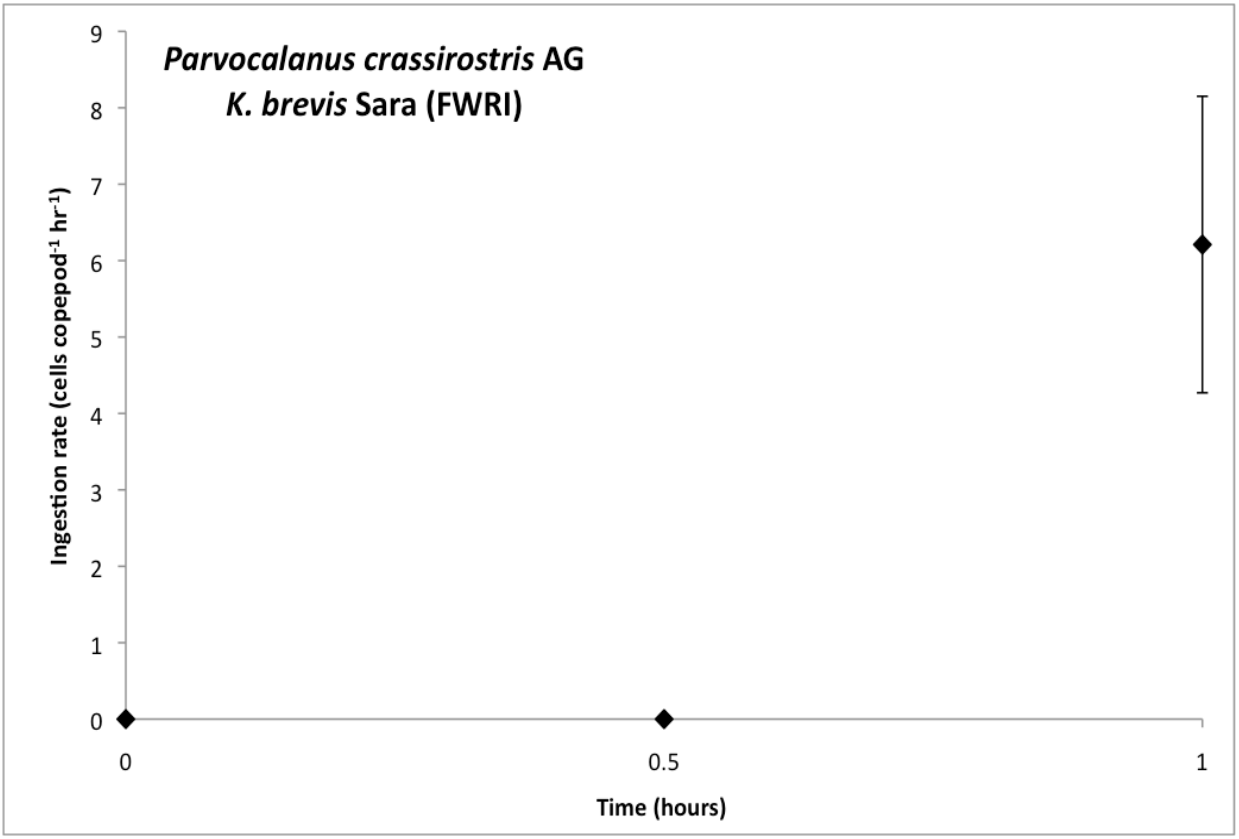

Figure 3.4. Grazing rates of *P. crassirostris* from AlgaGen LLC. (Originally collected from Hawaii) on *K. brevis* Sara (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

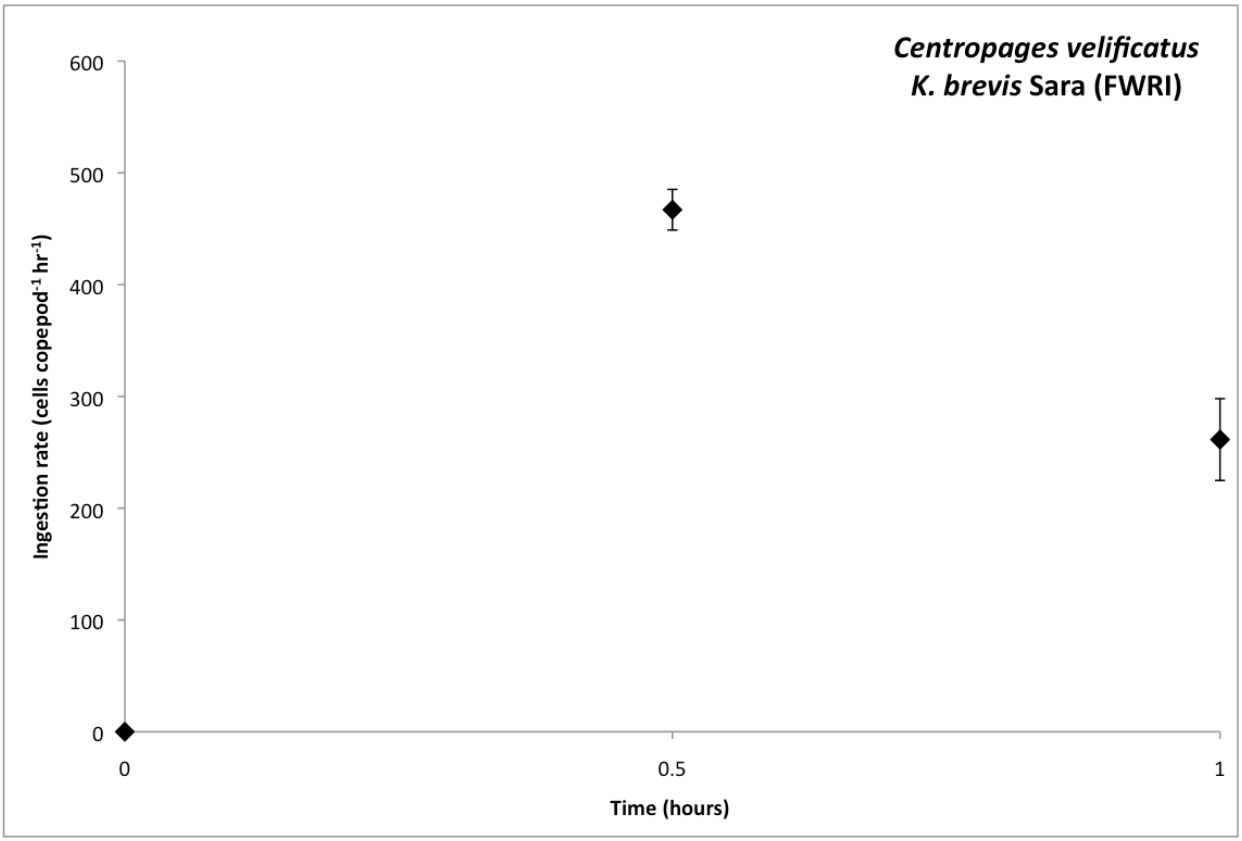

Figure 3.5. Grazing rates of *Centropages velificatus* collected from the Gulf of Mexico on *K. brevis* Sara (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

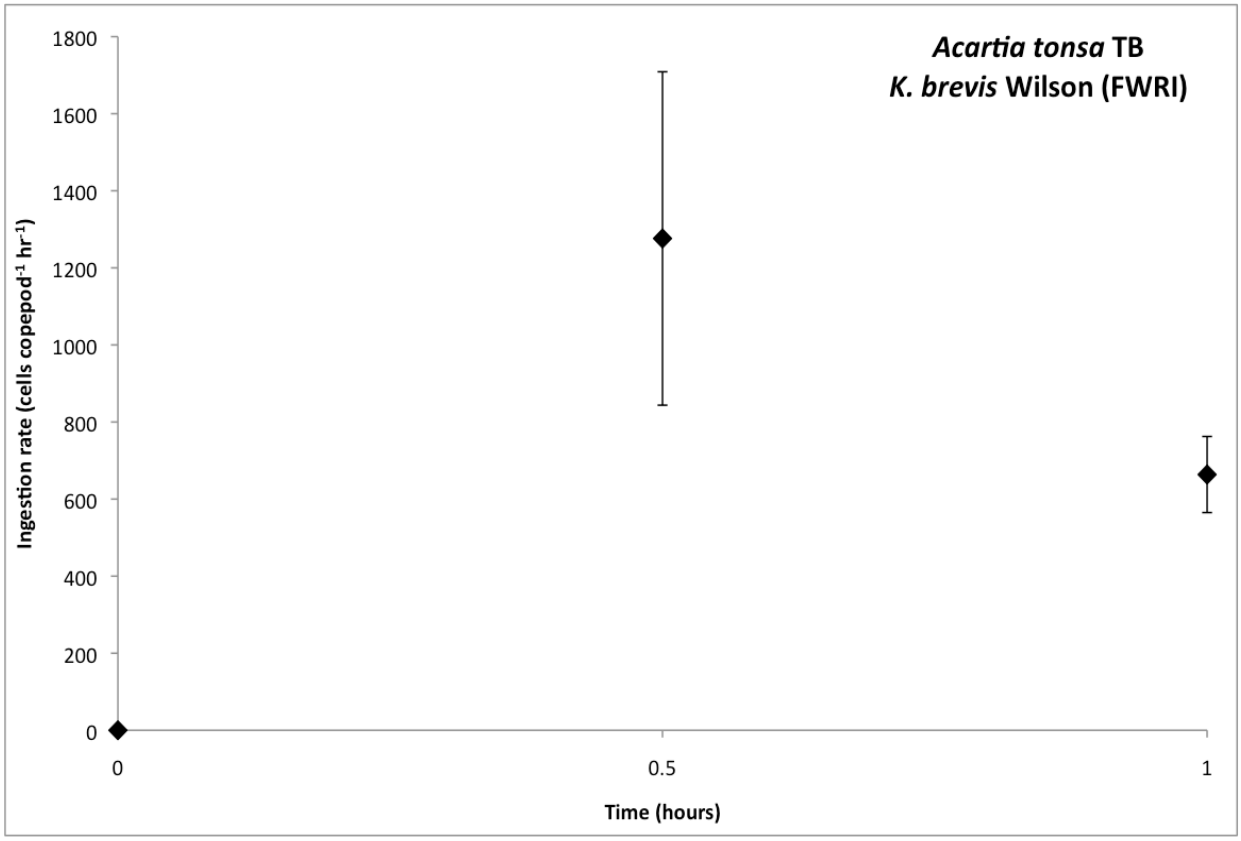

Figure 3.6. Grazing rates of *Acartia tonsa* collected from Tampa Bay on *K. brevis* Wilson (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

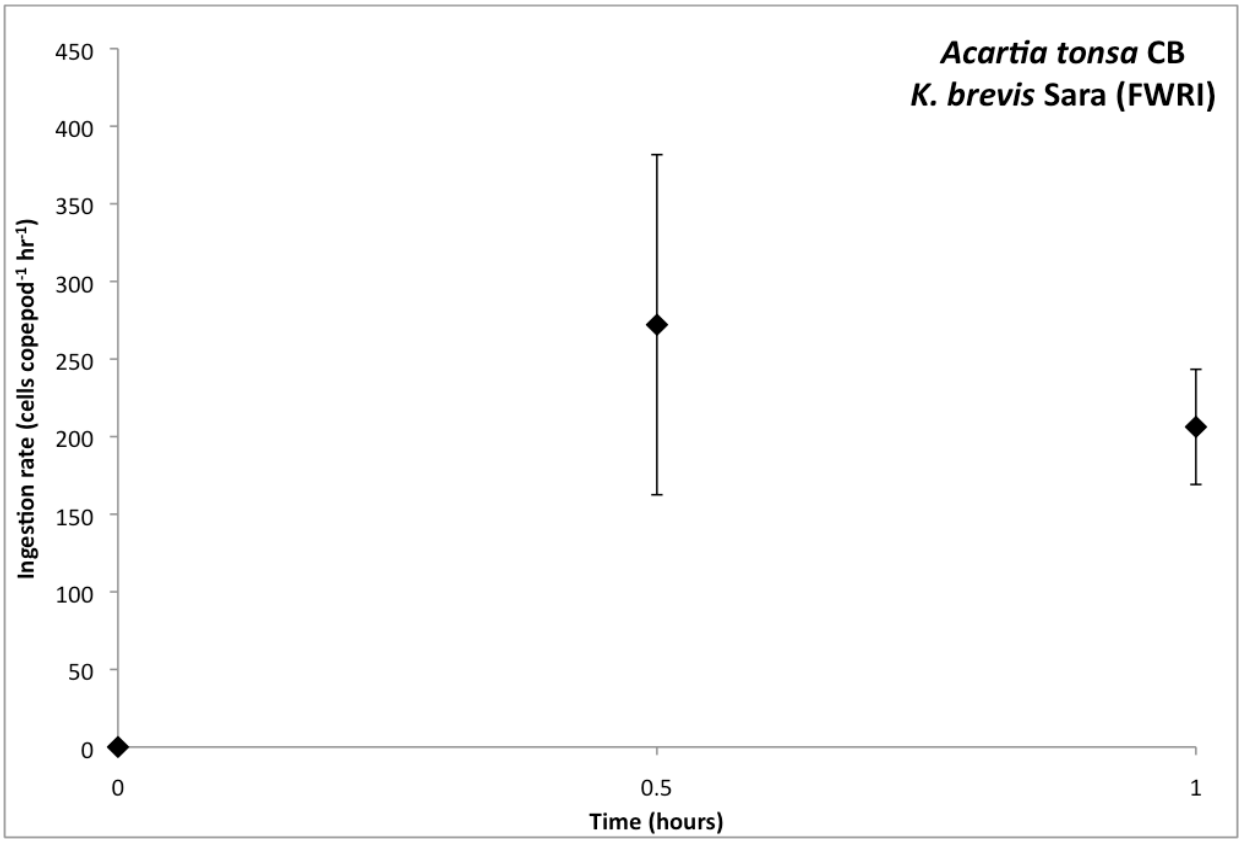

Figure 3.7. Grazing rates of *Acartia tonsa* collected from Chesapeake Bay on *K. brevis* Sara (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

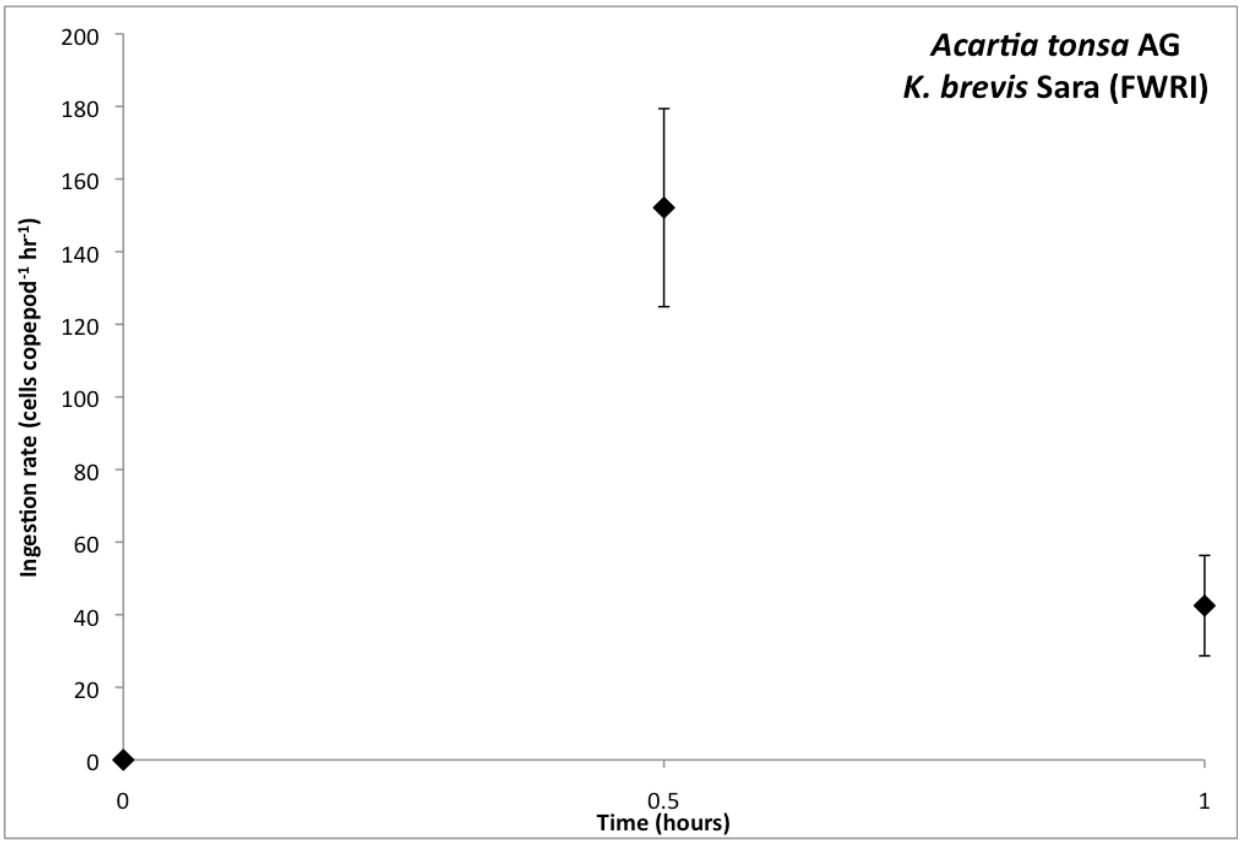

Figure 3.8. Grazing rates of *Acartia tonsa* from AlgaGen LLC. (Originally collected from the Gulf of Mexico) on *K. brevis* Sara (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

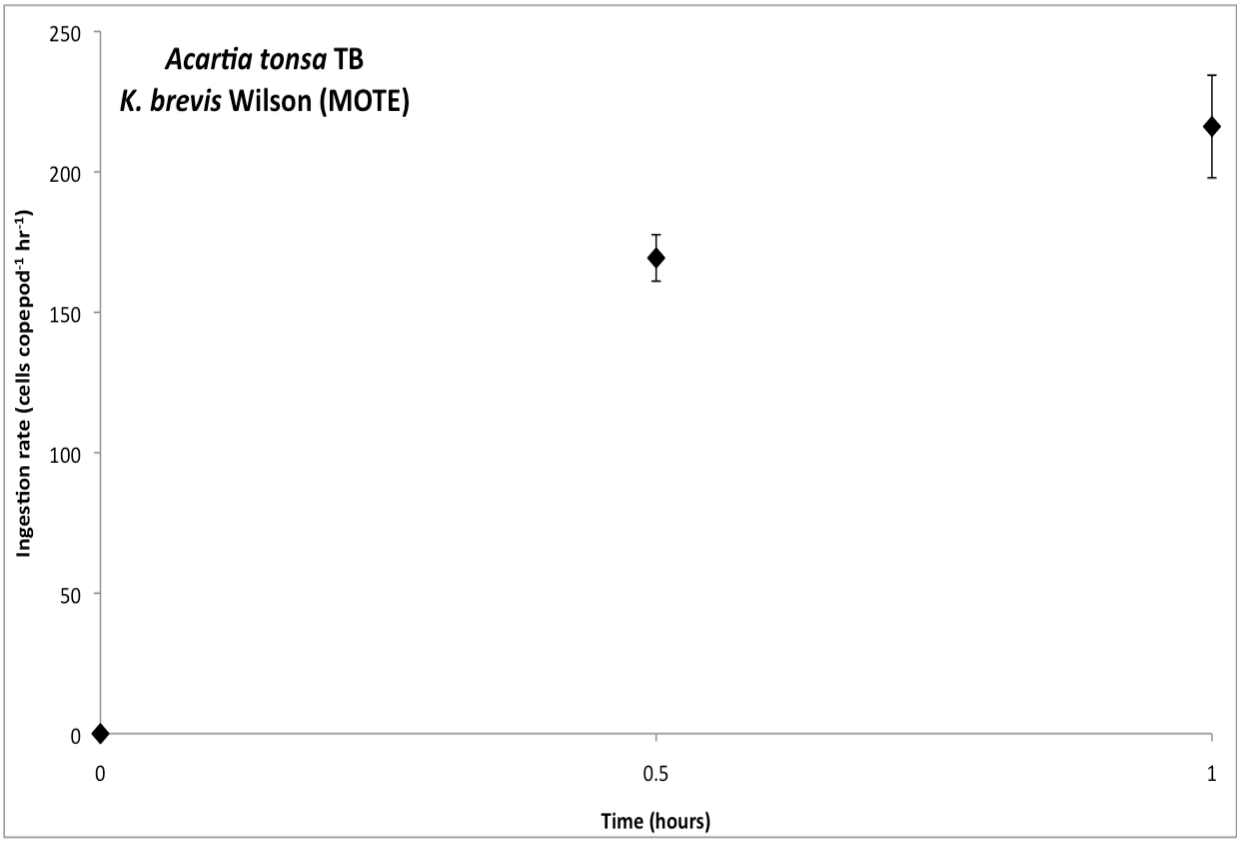

Figure 3.9. Grazing rates of *Acartia tonsa* collected from Tampa Bay on *K. brevis* (MOTE). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

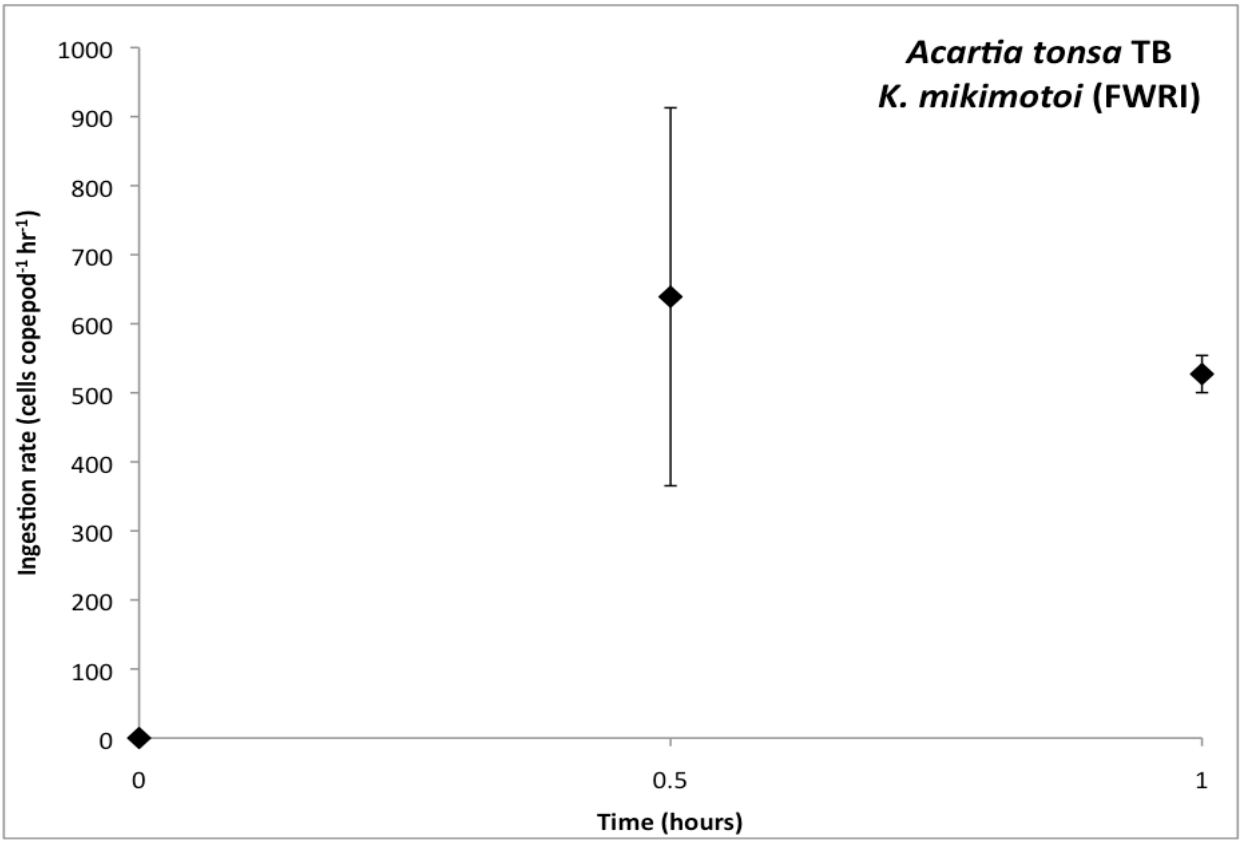

Figure 3.10. Grazing rates of *Acartia tonsa* collected from Tampa Bay on *K. mikimotoi* (FWRI). Points represent the average of three replicates. Error bars represent ± 1 S.E. from mean.

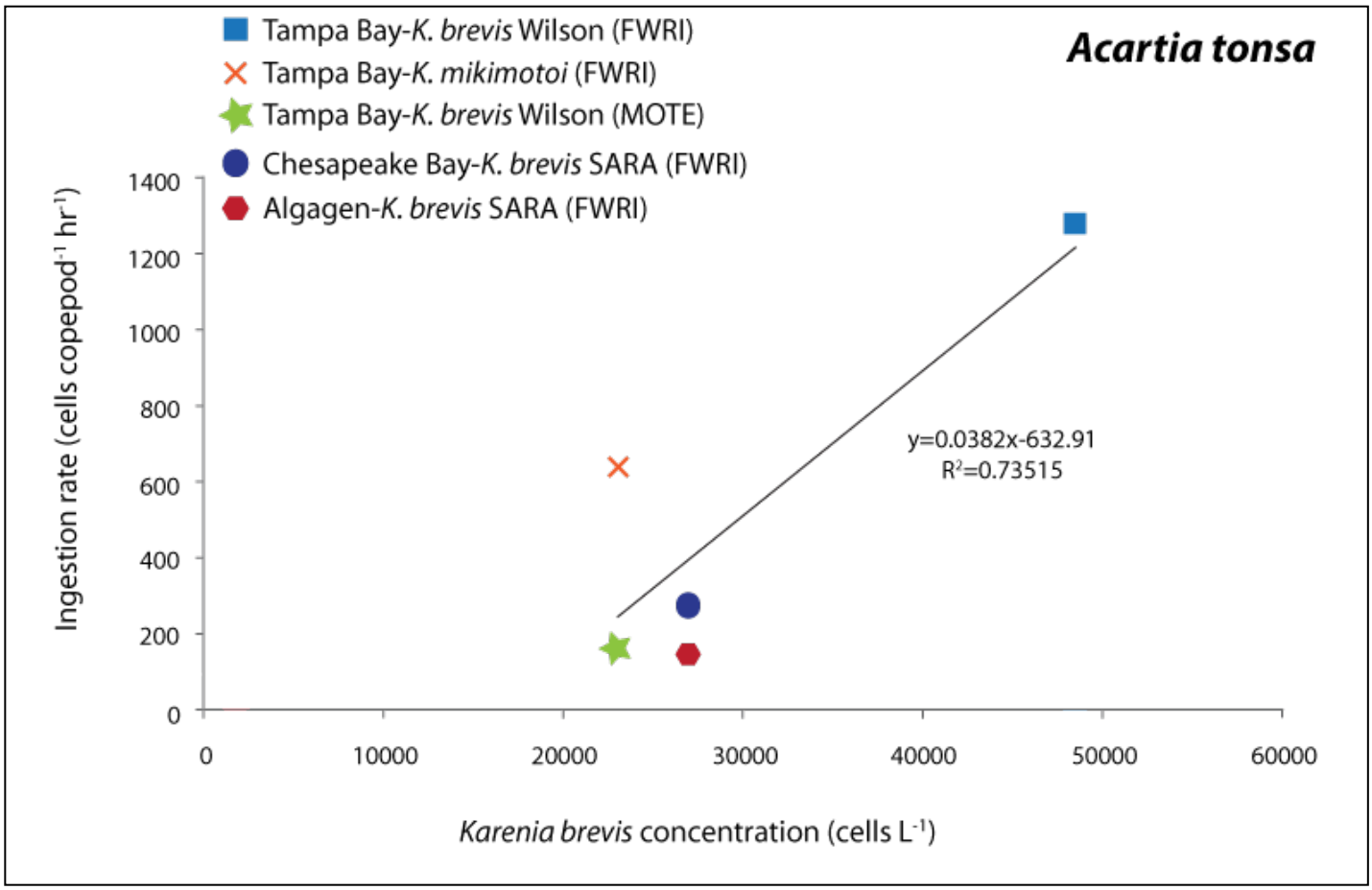

Figure 3.11. Correlation of ingestion rates for *Acartia tonsa* feeding on *Karenia* spp. in comparison to cell concentration.

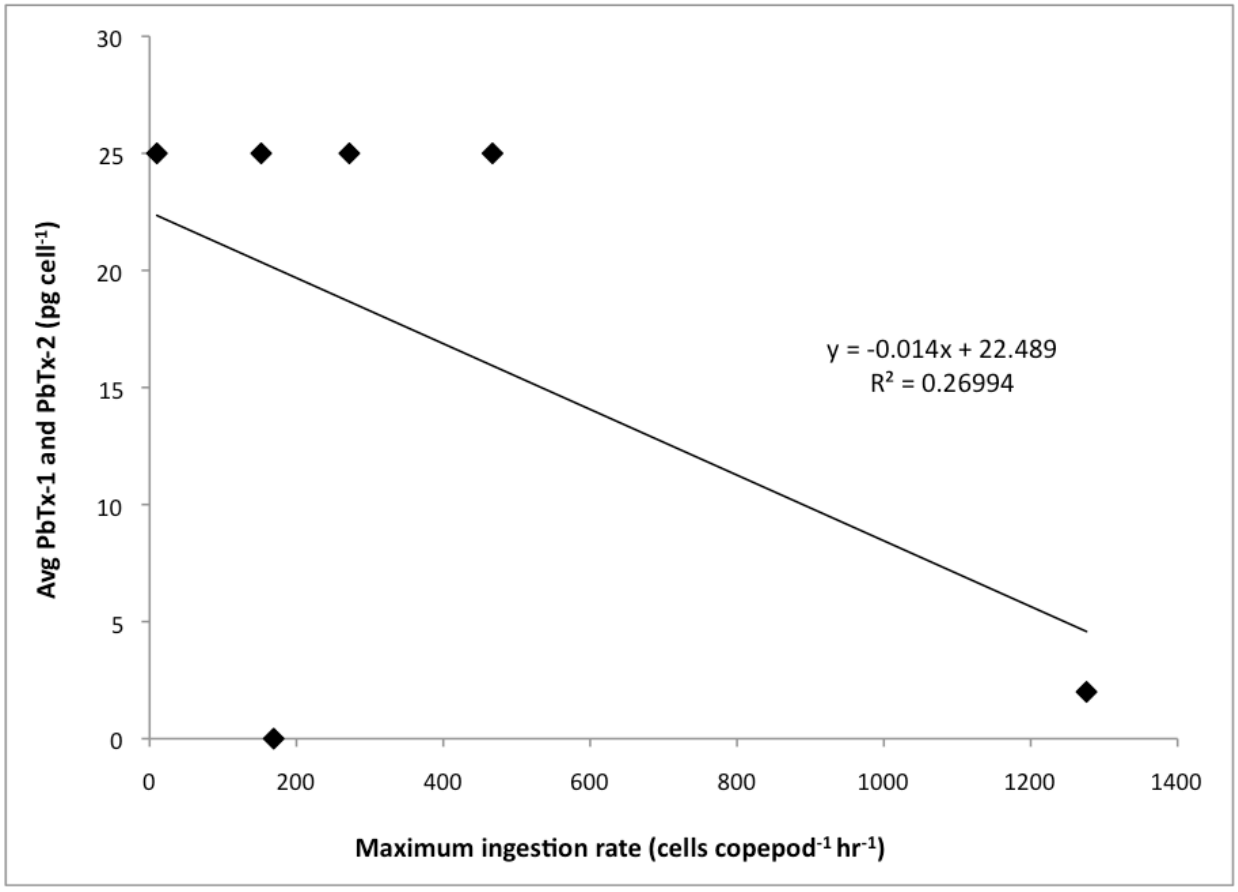

Figure 3.12. Correlation of copepod ingestion rates (cells copepod^{-1} hr^{-1}) in comparison to *K. brevis* culture toxicity (pg cell^{-1}).

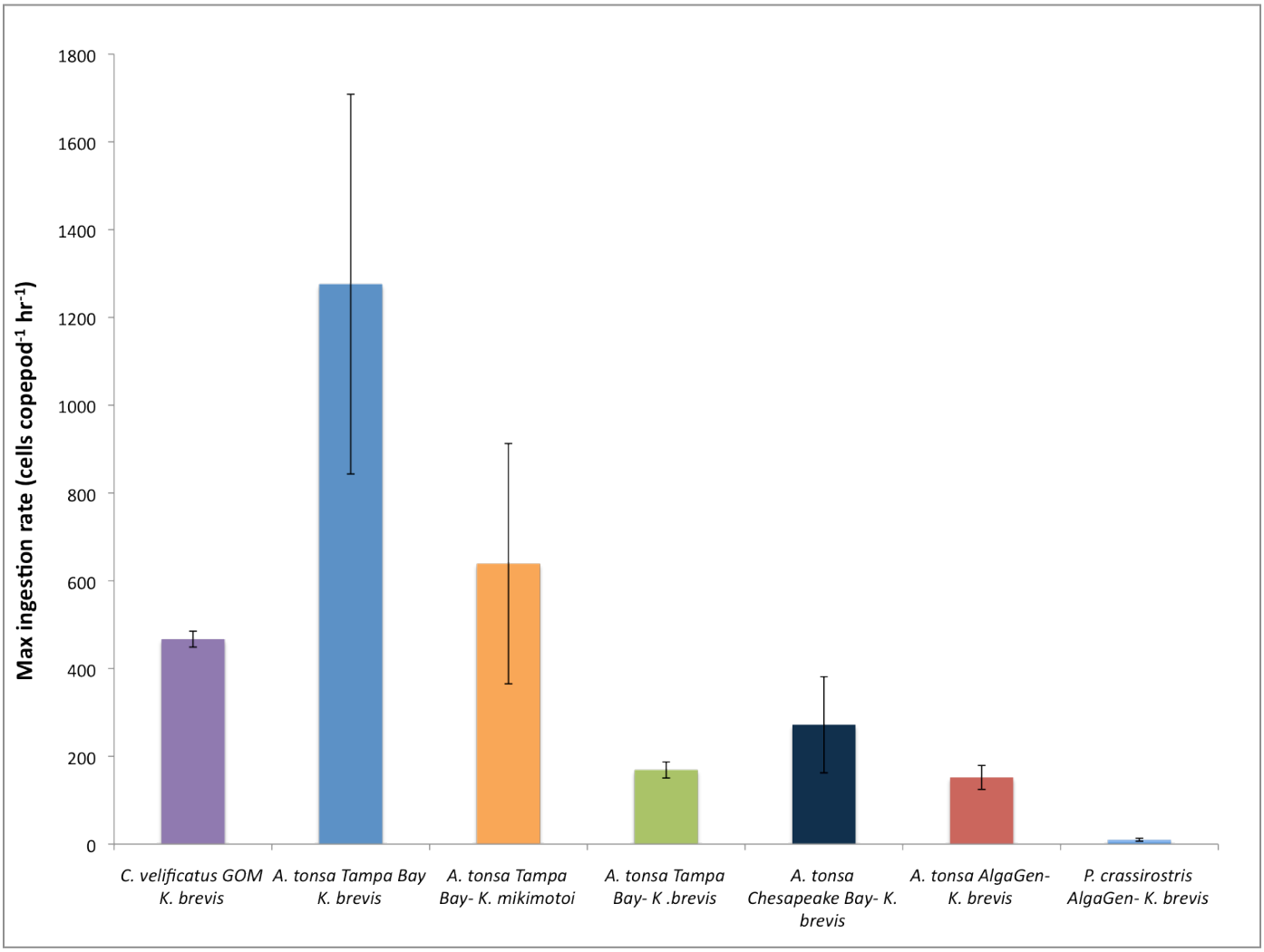

Figure 3.13. Maximum ingestion rates (cells copepod^{-1} hr^{-1}) for each of the seven ^{14}C-label ingestion experiments. Highest ingestion rates were observed for *A. tonsa* from Tampa Bay feeding on *K. brevis* Wilson (FWRI). Lowest ingestion rates were observed for *P. crassirostris* collected in Hawaii by AlgaGen LLC. feeding on *K. brevis* Sara (FWRI). Error bars represent ± 1 S.E. from the mean.

CHAPTER 4

CONCLUSIONS

The purpose of this study was to examine the interactions between the toxic dinoflagellate *Karenia brevis* and zooplankton populations on the West Florida Shelf of the Gulf of Mexico. While there has been a great deal of research centered on global zooplankton population dynamics in terms of spatial and temporal distribution, there is a gap in knowledge focusing on zooplankton community response during harmful algal blooms, specifically in relation to *Karenia brevis*. Several studies have focused on characterizing the zooplankton assemblage of the Eastern Gulf of Mexico and its associated estuaries yet to date, there has only been one *in situ* study characterizing potential perturbations to the zooplankton assemblage of the WFS in relation to *K. brevis*.

Prior to defining the potential role of zooplankton within *K. brevis* blooms, it was first necessary to comprehensively define the normal zooplankton assemblage where *K. brevis* blooms commonly occur. In general, total zooplankton abundance was higher within Tampa Bay and Charlotte Harbor and at the mouths of estuaries, decreasing with increased distance from shore. Peak zooplankton abundances were observed at the coastal and estuarine stations, and abundance correlated most closely with temperature, salinity, and Chl *a* concentrations—the relative importance of each dependent on physical dynamics in each year.

The zooplankton assemblage of the WFS and its associated estuaries observed within this study was consistent with previous work characterizing the zooplankton community of the Florida Shelf, Tampa Bay and Charlotte Harbor (Table 2.9) (King,

1949; Hopkins, 1966; Dragovich and Kelly, 1976; Ortner et al., 1989; Lester 2005; Badylak and Phlips, 2008). Observed zooplankton abundances were greater than previous studies of the Eastern Gulf of Mexico. Comparison among studies can often be difficult due to differences caused by sampling mesh size and seasonality of zooplankton populations (Ortner, 1989; Lester, 2005). Previous studies quantifying the zooplankton community of the Eastern Gulf of Mexico all report lower zooplankton abundances, however those studies used larger mesh sizes-- 153μm (Lester et al., 2008), 333μm (Ortner et al., 1989) and 200μm (Minello, 1980)—compared to the 64μm mesh net used in our study. There is also inherent error associated with patchiness and variability of zooplankton populations; however, it can be useful to compare studies of abundance and community composition in an effort to validate sampling methods (Lester, 2005).

Statistical analysis of non-bloom and *K. brevis* coastal stations showed a significant difference between zooplankton abundance only during the 2009 sampling period. The 2009 *K. brevis* bloom was in the maintenance phase, and as sampling progressed, total zooplankton abundance (m^{-3}) decreased. These results suggest that there may be concentration thresholds of *K. brevis* in terms of their effects on zooplankton. Lester et al. (2008) compared the natural zooplankton assemblage at four sites on the WFS to communities present during a *K. brevis* bloom, and observed that at low *K. brevis* concentrations ($7.5x10^3$ to $16x10^3$ cells $liter^{-1}$) the typical zooplankton assemblage was present; whereas at stations where *K. brevis* exceeded $5x10^6$ cells L^{-1}, typical zooplankton assemblages were either absent, or significantly reduced in abundance. Lester et al. (2005) documented *K. brevis* cell concentrations ranging from $8x10^3$ cells L^{-1} to

$5270x10^3$ cells L^{-1}. The highest cell concentrations observed during this study were $414x10^3$ cells L^{-1} at station KB08 in 2007.

Several of the abundant copepods observed within *K. brevis* blooms are capable of ingesting *K. brevis*, and whole community grazing rates show there may be a decrease in grazing pressure on *K. brevis* as blooms progress and increase biomass. While it is uncertain whether brevetoxins are toxic or nutritionally inadequate to zooplankton grazers, the uncoupling of grazing on a bloom population could result in the successful recruitment and survival of a bloom population. In addition, in the presence of non-toxic and toxic dinoflagellate species, copepods will preferentially graze on non-toxic food choices. There were no significant differences between non-bloom and bloom whole community phytoplankton growth rates or grazing rates in any of the sampling years when *K. brevis* was present. However, there was a significant difference in phytoplankton growth rates between ambient nutrient concentrations and nutrient amended samples in one year, suggesting that the phytoplankton community was nutrient limited during the 2009 sampling dates.

The bulk values calculated here for ammonium and phosphate excretion for the zooplankton community observed in Appendix 1 of the study suggest that the *K. brevis* blooms present during the sampling periods (2007-2009) could theoretically be obtaining their total NH_4^+ and P requirements from zooplankton excretion. If this is indeed the case, it may be more important to ask the question, why do we not always have wall-to-wall *K. brevis* blooming on the WFS? The zooplankton excretion rates (both N and P) that I calculated were all based on literature values for both *K. brevis* requirements and zooplankton excretion rates. While the regeneration of nutrients by the zooplankton

community could be a potentially important source of nutrients for *K. brevis* blooms, *in situ* rate measurements are necessary to fully understand this pathway of nutrient regeneration.

In 2010, no *K. brevis* populations were sampled within the cruise-track, and barely even background concentrations (>1000 cells L^{-1}) were present at a handful of sample sites. Although blooms have occurred almost annually along the West Coast of Florida, retrospective analysis of non-bloom years (Weisberg et al., in press) have stressed the important of physical parameters on the West Florida Shelf in structuring bloom dynamics. This year (2010) illustrates the importance of defining *Karenia brevis* bloom dynamics within the West Florida Shelf in an effort to create effective ocean observing and modeling products.

The combined findings from theses studies reinforce the importance of defining the interactions between harmful algal bloom causing dinoflagellate species and the trophic dynamics between potential grazer species. Blooms of *Karenia brevis* are not a new occurrence on the West Florida Shelf of the Gulf of Mexico, however with the increased development of the region, the ecological and economic impacts of such blooms continue to grow. Further understanding of the grazer-toxic algae relationship is critical in order to provide effective management and mitigation of harmful algal blooms within marine environments.

APPENDICES

APPENDIX A. ZOOPLANKTON NUTRIENT REGENERATION: A POTENTIAL SOURCE OF NITROGEN AND PHOSPHORUS FOR *K. BREVIS* BLOOMS

Abstract

On the West Florida Shelf of the Gulf of Mexico, the nutrient sources (nitrogen and phosphorus) fueling the massive, persistent blooms of Karenia brevis continue to puzzle researchers. One potentially large source to the pool of nutrients available to K. brevis is the regeneration of nutrients by zooplankton. To test this hypothesis, previously published zooplankton excretion rates were applied to mesozooplankton community abundance numbers observed in October of 2007, 2008 and 2009 to determine if the zooplankton population present on the WFS during blooms could be supplying a significant amount of the daily ammonium and phosphorus needs of the blooms that were present within these study years. The values calculated here for ammonium and phosphate regeneration for the total zooplankton communities present on the West Florida Shelf indicate that the *K. brevis* blooms observed during our study could be obtaining all of their nitrogen and phosphate from zooplankton nutrient regeneration.

Introduction

Zooplankton play a key role within the pelagic food web, mediating the transfer of organic energy produced by unicellular algae through photosynthesis to higher trophic levels (Harris et al., 2000). Zooplankton function as both a sink and a source for nutrients, through the simultaneous incorporation into biomass and release of dissolved nutrients (Walve and Larsson, 1999), and zooplankton grazing and excretion largely determines the amount and composition of vertical particle flux (Harris et al., 2000). In highly productive regions of the world's oceans, apart from predation, the availability of zooplankton is regarded as the most important environmental factor controlling the year class strength of a number of commercially important fish stocks (Harris et al., 2000). The function of marine food webs depends greatly on the balance between "bottom-up" and "top-down" control of resources. "Bottom up" control is resource driven, primarily influenced by the supply of nutrients determining the amount of primary production; whereas "top down" control is the ability of herbivores to control primary productivity through grazing (Harris et al., 2000).

Nutrient Regeneration

Within the world's oceans, the regeneration of nutrients is a significant interaction between higher and lower trophic levels; through the interactions of bacteria and phytoplankton within the microbial loop, coupled with the classic food chain (phytoplankton⇒zooplankton⇒fish); dissolved organic matter (DOM) is cycled within the system (Lalli and Parsons, 1993). Zooplankton contribute to the pool of DOM through several pathways, including excretion and egestion, sloppy feeding, as well as

cell lysis by viruses. Excreta of zooplankton can include both liquid—dissolved nitrogen and phosphorus—and solid forms—fecal pellets (Omori and Ikeda, 1984). During the grazing process of zooplankton, DOM can also be introduced into the water column as a result of "sloppy feeding". In most ocean regions, micro- and mesozooplankton are the dominant grazers on primary production, and it is through this process that DOM is leaked into the water column. Dissolved nitrogen excreted by zooplankton is in the form of total N, amino-N, urea-N and ammonia-N, with ammonium making up the major form of DIN (Omori and Ikeda, 1984). Dissolved phosphorus in the form of total P, organic-P and inorganic-P is also excreted (Omori and Ikeda, 1984). Pomeroy et al (1963) determined that 33-50% of the total phosphate excreted by a mixed zooplankton community is in organic forms (Omori and Ikeda, 1984). These nutrients can then be taken up by organisms, or converted to inorganic material via the microbial loop. Active grazing by herbivores leads to the sinking of fecal pellets, and this flux is believed to be one of the greatest contributors of vertical transport within the deep ocean (Kiorboe, 2001).

Zooplankton as potential source of nutrients

Harmful algal blooms (HABs) have complex interactions with zooplankton populations, and can affect grazing and reproduction, potentially also causing changes to nutrient regeneration (Saba et al., 2011). Zooplankton grazers can structure phytoplankton communities through selective feeding, and the remineralization of nitrogen, phosphorus and silicon by grazers can impact nutrient availability to phytoplankton (Strom and Strom, 1996). Trophic cascades and food chain interactions

result from predator-prey relationships, and the release of organisms from predation pressure can propagate changes in the availability of nutrient substrates (Glibert et. al., 2011). Zooplankton release N and P directly, but the amount and form can depend on what was eaten, and the lag time in consumption (Glibert et al., 2011). Release rates and the chemical composition of nutrients excreted by zooplankton populations can be affected by the ingestion rate, coupled with the type and quality of food consumed (Saba, 2009). Consumption of unpalatable HAB species can reduce zooplankton grazing and result in decreased nutrient regeneration. This reduced input of nutrients could increase bloom formation of species adapted to nutrient-limited environments (Sunda et. al., 2006). Little is known about how a diet of HAB causing dinoflagellates can affect zooplankton metabolic processes, and while much research has focused on grazer-mediated control of HABs, less effort has considered the effects of harmful algae on zooplankton regeneration of nutrients (Saba et al., 2011).

A characteristic behavioral feature of zooplankton is vertical migration, usually marked by the upward migration of individuals towards the surface at night, and downward during the daytime hours. Mesozooplankton populations feed at night in the surface waters and migrate down to depth during the daytime hours to respire, excrete and egest. This could potentially be a major input of nutrients within the system. *K. brevis* does not elicit a directed downward migration pattern like other dinoflagellates in order to access higher nutrient levels (Vargo, 2009), but is has shown pronounced negative geotactic and phototactic (Steidinger 1975; Heil, 1986, Tester, 1997; Kamykowski, 1998) behavior. When combined with random swimming movements during the dark period, *K. brevis* has access to the entire water column for nutrient

acquisition. Because the role of nutrients within HAB dynamics is such a largely contributing factor to understanding and potentially managing future blooms, this is further indication of the importance of focusing research on the population dynamics of zooplankton while trying to better understand processes occurring within HABs.

On the West Florida Shelf (WFS) of the Gulf of Mexico, nutrient sources supporting high biomass, long-lived blooms of *Karenia brevis* continue to puzzle researchers. *K. brevis* appears to possess a flexible metabolism, and is capable of utilizing both inorganic and organic nutrient sources, however the major nutrients required for growth and reproduction are nitrogen and phosphorus (Steidinger et al., 1998; Lester, 2005). *K. brevis* blooms have been hypothesized to initiate in response to blooms of the filamentous cyanobacteria *Trichodesmium* spp. *Trichodesmium* is a nitrogen fixer, and may release a significant fraction of newly fixed N in the form of dissolved inorganic nitrogen (DON) into surface waters. Aeolian deposition of iron during Saharan dust events has been linked to increased growth and N_2 fixation (Walsh and Steidinger, 2001; Lenes et al., 2001). This contribution from natural blooms of *Trichodesmium* spp. may be sufficient to support moderately dense blooms (≤10^5 cells L-1) (Glibert et al., 2009). In addition, the mixotrophic abilities of *K. brevi*s, including it's ability to graze on the cyanobacteria *Synechococcus* spp. Grazing by *K. brevis* can contribute up to 40% of the cellular N requirements for cells (Glibert et al., 2009).

Vargo et al., determined that zooplankton excretion rates could supply all of the N and P required to support *K. brevis* populations $>10^6$ cells L^{-1}(Lester, 2005). However, these excretion rates were obtained from only two species common to the WFS—*Acartia tonsa* and *Centropages velificatus* (Lester, 2005). In calculations based on observed

zooplankton abundance and *Karenia brevis* cell concentrations, Lester (2005) experimentally derived the total ammonium and total phosphate excretion of four WFS copepods, *A. tonsa*, *Paracalanus quasimodo*, *Labidocera aestiva* and *Temora turbinata*. Excretion rates were prorated to a twenty-four hour day, based on previous work by Checkley et al. (1992), determining that excretion rates are two times greater during daytime than at night (Lester, 2005). These prorated excretion rates were then extrapolated to other important contributors to the zooplankton community of the WFS and Lester (2005) determined whether the observed zooplankton populations we sufficient to support blooms. Based on observed bloom concentrations, *K. brevis* blooms could be obtaining their phosphate needs from zooplankton excretion, however ammonium excretion rates could only account for the required needs of a bloom up to 10^4 cells L^{-1} in size (Lester, 2005).

In this chapter, previously published zooplankton excretion rates were applied to zooplankton community abundance numbers observed in October of 2007, 2008 and 2009 to determine if the zooplankton population present on the WFS during blooms could be supplying a significant amount of the daily ammonium and phosphorus needs of the blooms that were present within these study years.

Methods

Using mesozooplankton abundance numbers and *Karenia brevis* bloom concentrations previously reported in Chapter 2 of this thesis (Table A), we tested the hypothesis that nutrient regeneration from zooplankton excretion could be fueling production by *K. brevis* blooms.

Integrated water column samples of zooplankton were taken using a 0.25m diameter 64μm mesh paired bongo net with an oblique tow from bottom to surface. The volume of water filtered through each of the nets was recorded with a General Oceanics model 2030R flow meter and all zooplankton counts were corrected to abundance m^{-3}. The average volume of water filtered per tow was $2.06m^3$.

Once onboard, live samples were concentrated through a 64μm mesh sieve and any large gelatinous zooplankton were removed and measured via displacement volume and recorded for later inclusion into biomass calculations. Samples were preserved in 5% buffered formalin and stored in the dark at ≤5°C until identification and enumeration at Horn Point Laboratory in Cambridge, Maryland. Whole sample biomass was measured as wet displacement volume (Harris et al., 2000). Zooplankton were counted and identified using a Nikon SMZ800 stereomicroscope. For most samples, zooplankton was too abundant for whole sample counting, and therefore representative subsamples were obtained with a Stempel pipette (Harris et al., 2000). Samples were counted so that at least 100 individuals of the most abundant species were identified. Whenever possible, holoplankton were identified to species level, and meroplankton identified to group. Replicate samples (tows) for each station were averaged.

Based on an N and P content determined by Heil (1986) of $1.08x10^{-5}$ μmoles per cell and $4.88x10^{-7}$ μmoles per cell respectively, and an assumed growth rate of 0.2 divisions day^{-1} (Shanley, 1985; Van Dolah and Leighfield, 1999) nutrient criteria for each of the bloom cell concentrations sampled during this study were determined. Literature determined values of WFS zooplankton excretion rates (Lester, 2005) were used to calculate community excretion rates for populations present within *K. brevis* blooms

(Table #). In addition, to calculate a potential range of nutrient needs for K. brevis blooms, the maximum growth rate of 0.6 divisions day^{-1} that has been observed in laboratory culture was also used (Shanley, 1985; Van Dolah and Leighfield, 1999).

Results

In the four sampling years, eleven stations were sampled that had bloom concentrations of *K. brevis* (>1000 cells L-1) present (Table A). *K. brevis* cell concentrations ranged from $1.7x10^3$ cells L^{-1} at $KB07^{-11}$ to $414x10^3$ cells L^{-1} at station KB07-8. In 2008 there was no statistical difference in zooplankton abundance between non-bloom and bloom stations. In 2009 there was a statistically significant difference ($P<0.05$) between the abundance of zooplankton at stations with *K. brevis* present. During the 2008 and 2009 bloom sampling years, as the *K. brevis* bloom progressed, total zooplankton abundance decreased.

Ammonium and phosphate excretion rates were determined for the zooplankton community present at each of the bloom stations using previously published experimentally derived values for ammonium (Table B) and phosphate (Table C). Values were prorated to a 24-hour day due to findings by Checkley et al. (1992) indicating zooplankton excretion rates are two times greater during the day than at night (Lester, 2005). At all of the *K. brevis* bloom stations sampled, zooplankton regeneration could account for greater than 100% of all ammonium and phosphate bloom requirements (Table D).

K. brevis nutrient requirements were also calculated based on the maximum growth rate of 0.6 divisions day^{-1}. Based on observed zooplankton abundances,

zooplankton regeneration could also account for greater than 100% of all ammonium and phosphate bloom requirements if the *K. brevis* bloom was growing at maximum growth rates. Additionally, during the 2007 ECOHAB: Karenia sampling, Sipler et al., (in press) calculated a maximum growth rate of *K. brevis* of 1.0 divisions day^{-1}. Based on observed *K. brevis* cell concentrations and zooplankton abundance during the 2007 cruise sampling, the zooplankton community could supply of the ammonium for a *K. brevis* community growing at a rate of 1.0 divisions day-1 (Figure A). At this same growth rate, the observed zooplankton community could supply all of the phosphate necessary for the *K. brevis* blooms at all of the stations except KB07-8 (Figure B) where it would only be supplying 60.5% of the required phosphate..

Discussion

Using literature values of excretion rates in combination with zooplankton community data obtained in this study, I tested the hypothesis that nutrient regeneration from zooplankton excretion could be fueling production of *Karenia brevis* blooms. Based on these extrapolations, at all of the *K. brevis* bloom stations sampled, zooplankton regeneration could account for greater than 100% of all ammonium and phosphate required.

In a previous study quantifying the importance of zooplankton nutrient regeneration to *K. brevis* blooms, Lester (2005) found NH_4^+ excretion rates inadequate to support $>10^4$ cells L^{-1}. Phosphate excretion rates were adequate to support 10^6 cells L^{-1}. In comparison to the zooplankton abundance numbers found within our study, Lester (2005) had lower abundances at all stations sampled. These differences could be

attributed to differences in sampling techniques, or due to higher cell concentrations of *K. brevis* present.

Several environmental factors can affect the amount of N excretion by zooplankton. Temperature and excretion rates are directly related, and the amount of nitrogen excreted can change seasonally and based on life stage (Miller, 1992). Zooplankton species migrate vertically in the water column, avoiding predation during the day by staying at depth, and coming up to the surface to feed at night. If species are in a heavily stratified water column, zooplankton must osmoregulate, and therefore adjust amino acid concentrations within the hemolymph (Miller, 1992). During osmoregulation, amino acids are catabolized to ammonium, and therefore ammonium excretion should increase as animals adjust to fresher water (Farmer and Reeve, 1978; Miller, 1992). Additionally, it is commonly believed that there is a lag between ingestion and excretion (Checkley et al., 1992) during diel vertical migration—individuals are at the surface feeding at night, and returning to depth during the day where they are excreting. Therefore, regeneration must take into the account that individuals are likely only excreting nutrients for 12 h day^{-1}.

Within bloom environments, the nutrient quality of food can be an important factor in excretion rates, as well as the various nutrients excreted. Nitrogen excretion may be highly variable, both with the time of day and other factors related to physiological and nutritional state (Miller and Roman, 2008). The amount of nitrogen excreted by copepods depends largely on the quality of N ingestion, the biochemical composition of ingested N compounds, and the N/C ratio of food relative to the copepod and it's C and N assimilation and growth efficiencies (Landry, 1993; Tang and Dam,

1999; Miller and Roman, 2008).

Based on these factors, nutrient regeneration numbers calculated are likely an overestimation, as they are based on ideal conditions, and several species are estimated from excretion rates of similar species not feeding on *K. brevis*.

Conclusions

The values calculated here for ammonium and phosphate regeneration for the total zooplankton community present on the West Florida Shelf indicate that the *K. brevis* blooms observed during our study could be obtaining all of their nitrogen and phosphate from zooplankton nutrient regeneration.

Table A. Zooplankton abundance, biomass and species diversity and *Karenia brevis* cell concentrations for all stations sampled on NOAA: ECOHAB cruise track where *Karenia brevis* present in bloom concentrations (>1000 cells L^{-1}).

Station	TB-Out	KB07-8	KB07-9	KB07-11	KB08-9	KB08-10	KB08-11	KB09-8F	KB09-9	KB09-9A	KB09-10
***Karenia brevis* (x10^3cells L^{-1})**	74	414	176	1.7	4.0	91	321	91	35	127	n/c
Appendicularian											
Oikopleura dioica	505	2110	2420	1513	6418	723	7169	14105	4817	2859	5825
Calanoid											
Acartia tonsa	379	2813	4034	2836	12034	10120	---	---	---	---	---
Centropages velificatus	126	703	807	945	4011	3614	13262	1614	963	150	752
Labidocera aestiva	---	---	---	---	---	---	---	---	---	---	---
Parvocalanus crassirostris	2903	3095	3227	5861	24871	3976	358	4589	2312	978	1503
Paracalanus quasimodo	1388	2391	2743	1324	5616	361	---	4163	---	---	---
Temora turbinata	4165	5064	5809	3782	16046	2169	1792	510	1541	752	2255
Cladoceran											
Evadne tergestina	4669	---	---	756	3209	4699	3226	---	---	---	---
Penilia avirostris	0	1829	2098	1702	7221	11205	2151	---	---	---	---
Cyclopoid											
Oithona spp.	9717	15755	25818	22878	15243	3253	3226	14960	7515	8652	9583
Oithona plumifera	---	---	---	---	---	---	---	---	---	---	---
Harpacticoid											
Euterpina acutifrons	1136	3235	3711	2458	10430	1446	1792	2211	2312	1354	1127
Ostracod											
Euconchoecia chierchiae	---	---	---	---	---	---	---	---	---	---	---
Poecilostomatoid											
Corycaeus americanus	2145	1829	2098	6618	28080	4337	8961	3995	3661	2859	3382
Other											
Bryozoa larvae	---	---	---	---	---	---	---	---	---	---	---
Chaetognath	---	985	1130	189	802	1084	358	2125	289	---	---
Cirriped larvae	505	2110	4357	5105	21662	2891	7527	4335	3468	1354	5261
Crab zoea	505	422	484	189	802	---	358	---	---	---	---
Echinoderm larvae	---	---	---	---	---	---	717	---	---	---	---
Mysid	631	1407	1614	1134	---	2530	717	---	---	---	---
Pelecypod larvae	---	2954	---	---	---	---	---	---	---	---	---
Polychaete larvae	379	563	645	945	4011	5422	1792	5269	963	828	1315
Snail veliger	---	---	---	---	802	---	---	---	---	301	---
Total Abundance (m^{-3})	30162	48952	64545	58236	161259	57830	54842	68591	30252	19786	31003
Biomass (mL m^{-3})	10.33	82.76	20.05	28.38	40.59	26.25	10.75	18.16	5.92	2.49	---
Shannon Index	2.14	2.40	2.19	2.11	2.41	2.43	2.27	2.07	2.04	1.73	1.90

Table B. Zooplankton ammonium excretion rates used in K. brevis bloom nutrient calculations. Table adapted from Lester, 2005.

Species	*NH_4^+ Excretion rate ($\mu M\ animal^{-1}\ day^{-1}$)*	*Based on*	*Source*
Acartia tonsa	0.318	Actual	Lester, 2005
Centropages velificatus	0.039	Actual	Lester, 2005
Corycaeus americanus	0.115	*T. turbinata*	Checkley et al., 1992
Euterpina acutifrons	0.115	*T. turbinata*	Lester, 2005
Evadne tergestina	0.048	Daphnia spp.	Martinez and Gulati, 1999
Labidocera aestiva	1.963	Actual	Lester, 2005
Oikopleura dioica	0.026	*Mnemiopsis ledyii*	Nemazie et al., 1993
Oithona colcarva	0.115	*T. turbinata*	Lester, 2005
Penilia avirostris	0.048	Daphnia spp.	Martinez and Gulati, 1999
Parvocalanus crassirostris	0.059	½ *P. quasimodo*	Lester, 2005
Paracalanus Quasimodo	0.118	Actual	Lester, 2005
Temora turbinata	0.115	Actual	Lester, 2005
Decapod larvae	0.003	Actual	Schmitt and Santos, 1998
Pelecypod larvae	0.010	Actual	Yantian et al., 1999

Table C. Zooplankton phosphate excretion rates used in K. brevis bloom nutrient calculations. Table adapted from Lester, 2005.

Species	*P Excretion rate* ($\mu M\ animal^{-1}\ day^{-1}\ x10^{3}$)	*Based on*	*Source*
Acartia tonsa	1.82685	Actual	Lester, 2005
Centropages velificatus	1.82685	*L. aestiva*	Lester, 2005
Corycaeus americanus	3.59312	*T. turbinata*	Lester, 2005
Euterpina acutifrons	3.59312	*T. turbinata*	Lester, 2005
Evadne tergestina	0.20000	Daphnia spp.	Martinez and Gulati, 1999
Labidocera aestiva	71.80433	Actual	Lester, 2005
Oikopleura dioica	1.82685	½ *A. tonsa*	Lester, 2005
Oithona colcarva	3.59312	*T. turbinata*	Lester, 2005
Penilia avirostris	0.20000	Daphnia spp.	Martinez and Gulati, 1999
Parvocalanus crassirostris	5.42666	½ *P. quasimodo*	Lester, 2005
Paracalanus quasimodo	10.85332	Actual	Lester, 2005
Temora turbinata	3.59312	Actual	Lester, 2005
Decapod larvae	0.03593	0.01**T. turbinata*	Lester, 2005
Pelecypod larvae	0.03593	0.01**T. turbinata*	Lester, 2005

Table D. Average K. brevis cell concentrations at bloom stations during ECOHAB sampling, with calculated N and P requirement for each of the bloom based on Heil (..) N and P requirements, with an assumed growth rate of 0.2 divisions d^{-1} and percentage of daily requirements provided via zooplankton regeneration based on calculations from Lester, 2005

Station	Date	K. brevis abundance ($x10^3$cells L^{-1})	N requirement ($\mu M\ day^{-1}$)	N provided by zooplankton regeneration ($\mu M\ day^{-1}$)	Percentage of daily requirement	P requirement ($\mu M\ day^{-1}$)	P provided by zooplankton regeneration ($\mu M\ day^{-1}$)	Percentage of daily requirement
07TB-Out	22-Oct-07	74.0	0.1598	2.6714	>100	0.0072	0.0999	>100
KB07-8	23-Oct-07	414.0	0.8942	4.5356	>100	0.0404	0.1920	>100
KB07-9	24-Oct-07	176.0	0.3802	6.2972	>100	0.0172	0.2477	>100
KB07-11	26-Oct-07	1.7	0.0037	5.7077	>100	0.0001	0.2388	>100
KB08-9	10-Oct-08	4.0	0.0086	14.8078	>100	0.0004	0.0004	>100
KB08-10	12-Oct-08	91.0	0.1966	5.7071	>100	0.0089	0.0089	>100
KB08-11	12-Oct-08	321.0	0.6934	2.7966	>100	0.0313	0.0313	>100
KB09-8F	9-Oct-09	91.0	0.1966	3.6845	>100	0.0089	0.2825	>100
KB09-9	10-Oct-09	35.0	0.0756	2.0276	>100	0.0034	0.1379	>100
KB09-9A	10-Oct-09	127.0	0.2743	1.7039	>100	0.0124	0.0651	>100
KB09-10	10-Oct-09	n/c						

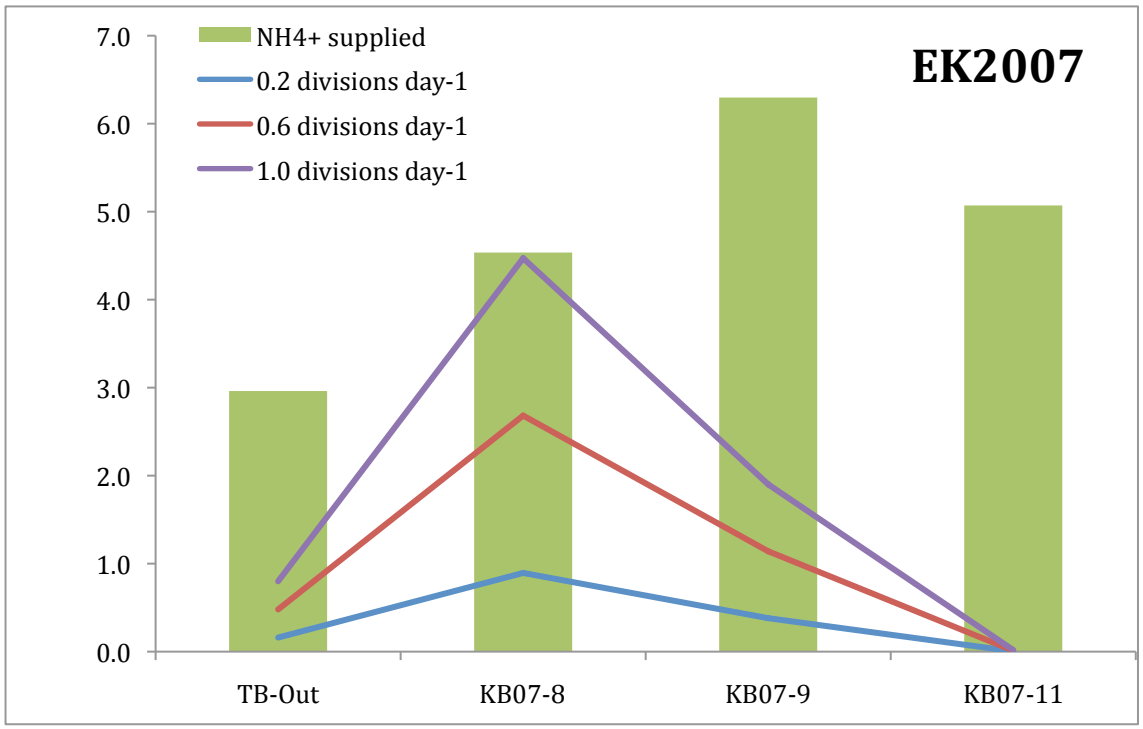

Figure A. Ammonium supplied by zooplankton community present at each of the bloom stations sampled during the 2007 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates. Additionally, a maximum growth rate of 1.0 divisions day^{-1}as observed by Sipler et al., (in press) during the 2007 cruise.

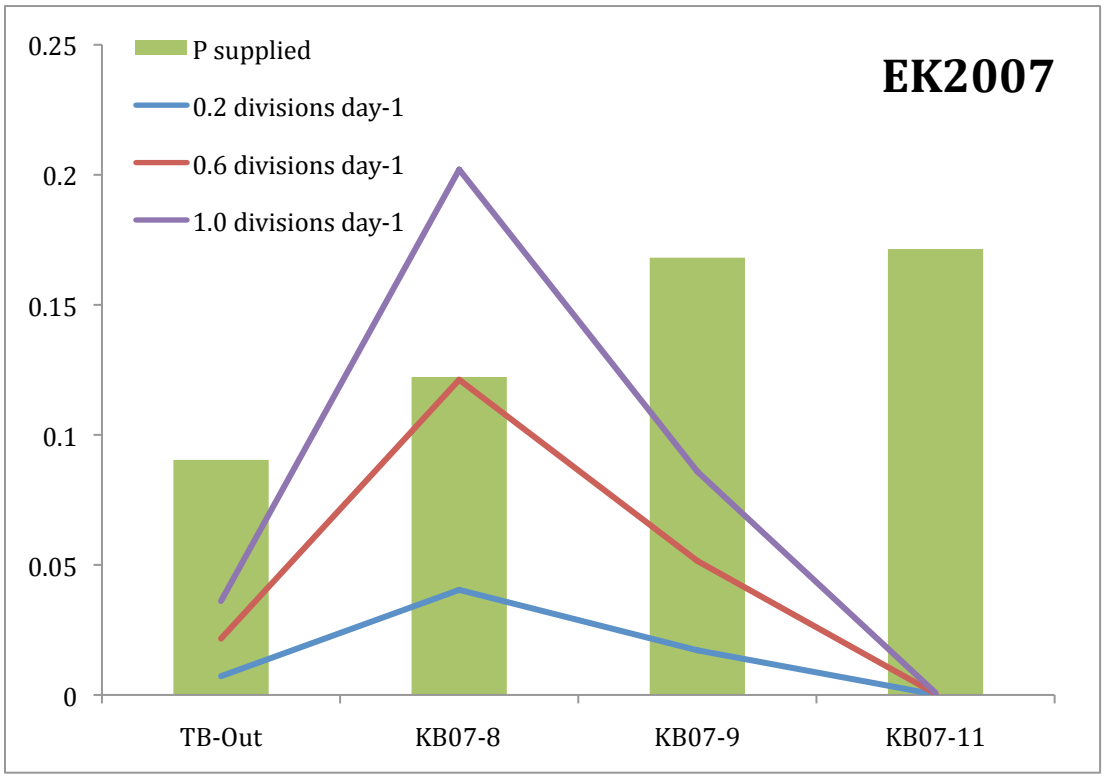

Figure B. Phosphate supplied by zooplankton community present at each of the bloom stations sampled during the 2007 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates. Additionally, a maximum growth rate of 1.0 divisions day^{-1}as observed by Sipler (source) during the 2007 cruise.

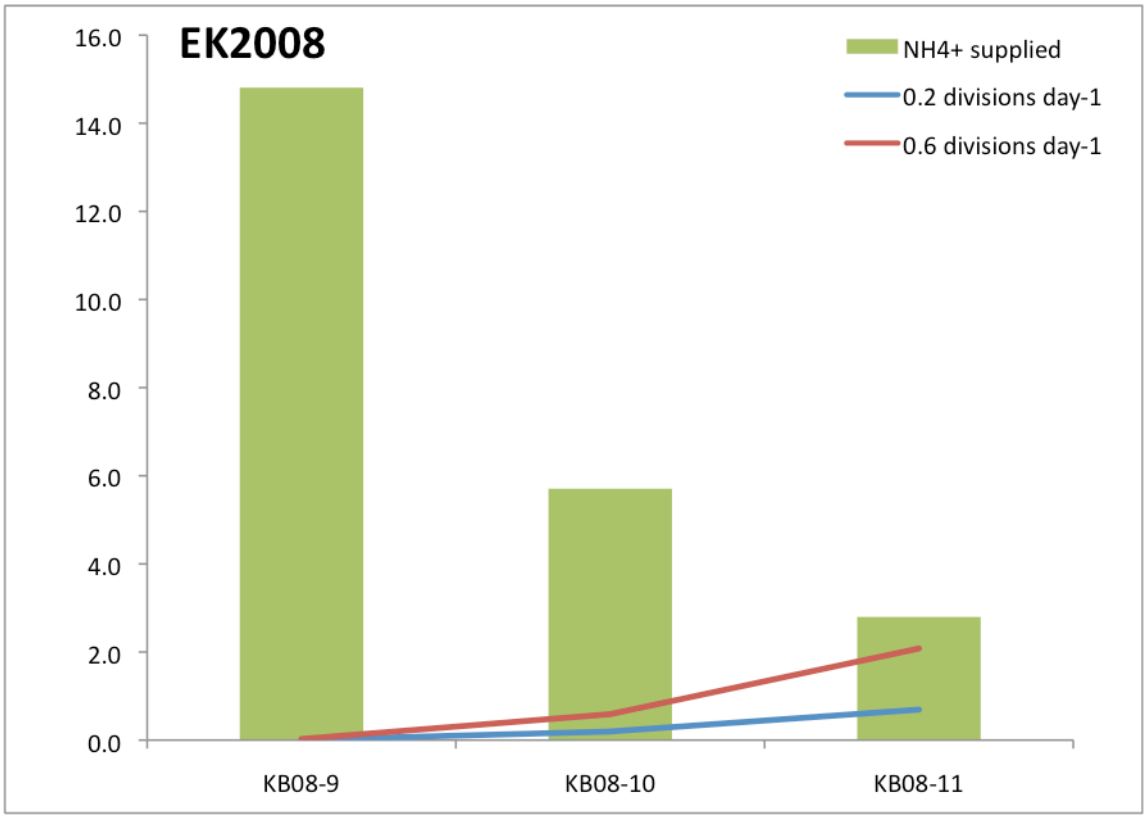

Figure C. Ammonium supplied by zooplankton community present at each of the bloom stations sampled during the 2008 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates.

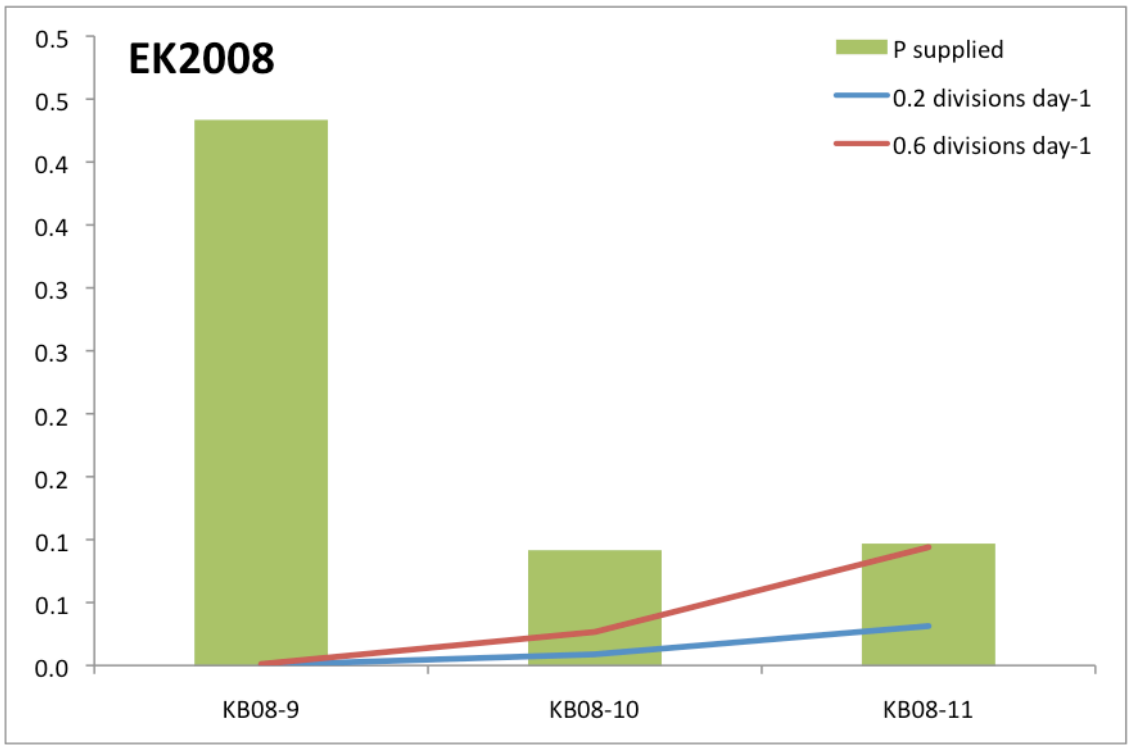

Figure D. Phosphate supplied by zooplankton community present at each of the bloom stations sampled during the 2008 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates.

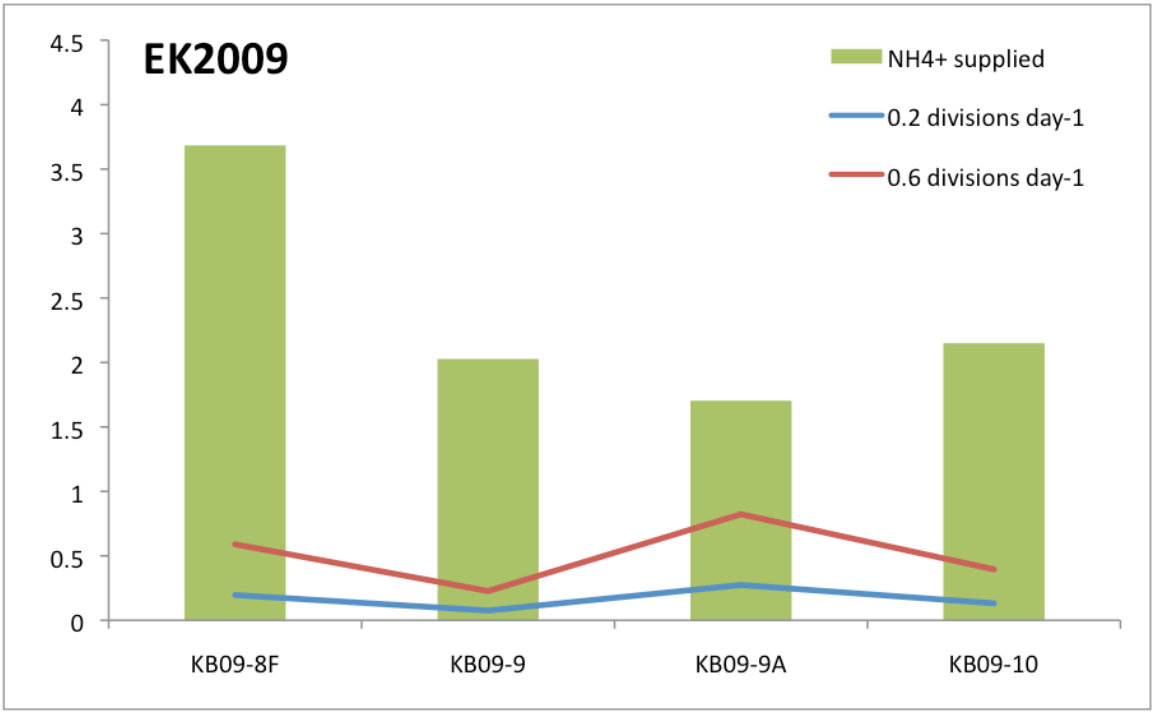

Figure E. Ammonium supplied by zooplankton community present at each of the bloom stations sampled during the 2009 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates

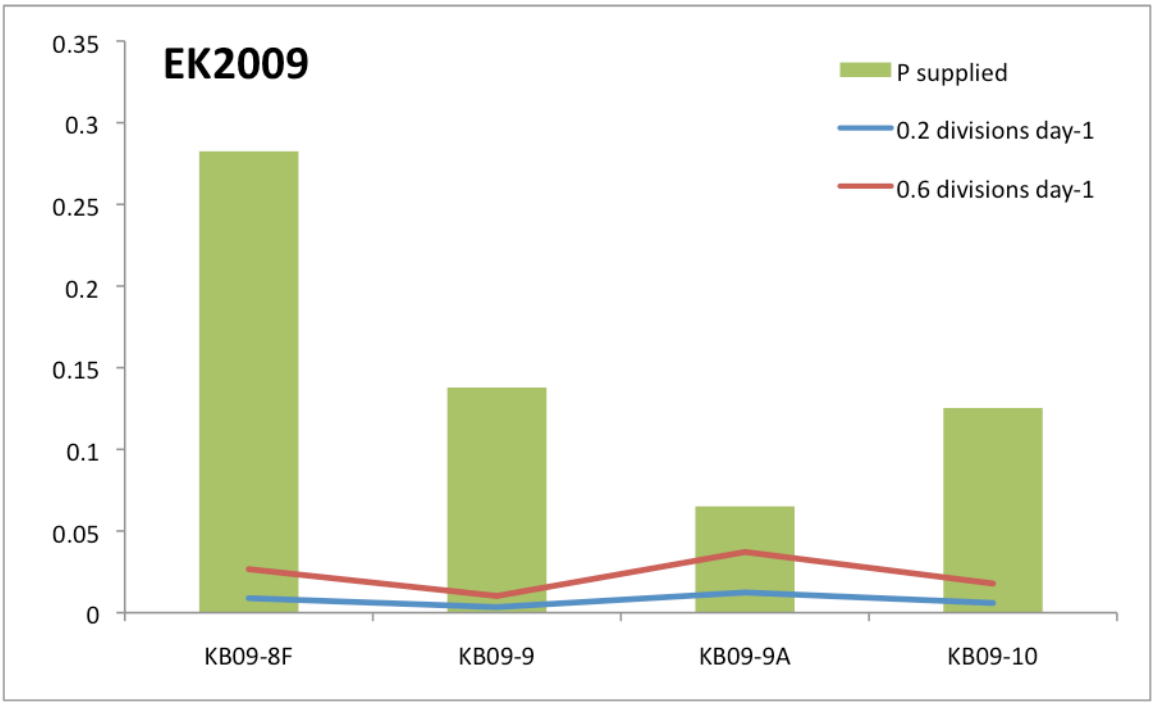

Figure F. Phosphate supplied by zooplankton community present at each of the bloom stations sampled during the 2009 cruise. Lines represent nutrient requirements of *K. brevis* blooms at minimum (0.2 divisions day^{-1}) and maximum (0.6 divisions day^{-1}) growth rates.

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