Population dynamics of the invasive green mussel Perna viridis and their response to the toxic dinoflagellate Karenia brevis: application of Dynamic Energy Budget theory to determine population trends

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To my Grandfathers

Dr. Robert McFarland and Dr. John McCoy

How I wish you were here

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Population dynamics of the invasive green mussel, *Perna viridis*, in southwest Florida and individual energetics through the application of the Dynamic Energy Budget Theory

Table of Contents

Abstract

Worldwide, introductions of exotic species to new regions is of rising concern which can lead to catastrophic ecosystem alterations through competition with native species and disruption in energy flow. *Perna viridis* is a recently introduced bivalve species to US coastal waters and has vigorously spread throughout the southeastern US. However, little information regarding population structure and response to local environmental factors has been reported. Red tide blooms formed by the toxic dinoflagellate *Karenia brevis* are frequent along the Gulf coast of Florida and as a recently introduced species, it is unclear what tolerance *P. viridis* has toward these events and associated brevetoxins (PbTx). Further, as an invasive species ecological concerns have risen regarding potential for spread and competition with native bivalve species, particularly the eastern oyster *Crassostrea virginica*.

This study aimed to characterize the population dynamics of established *P. viridis* populations and their response to naturally occurring *K. brevis* blooms. This was completed through monitoring of growth, mortality, juvenile recruitment, gametogenesis and biochemical composition (protein, glycogen and lipid) throughout a three year monitoring period to evaluate the effects of *K. brevis* blooms. Additionally, tissue PbTx concentrations were analyzed to determine uptake, accumulation and elimination rates. Data collected from the field and information reported in the literature were used to create a functional DEB model to predict individual growth and reproduction of *P. viridis* under environmentally realistic conditions.

Prior to onset of the first *K. brevis* bloom event, *P. viridis* showed rapid growth rates (6 – 11 mm month^{-1}) and high survival (mortality <1%). However, during *K. brevis* blooms growth rate dropped significantly and bioaccumulation of PbTx in the soft tissue was observed. High tissue PbTx concentrations persisted long after bloom dissipation and high rates of mortality ensued, severely reducing population densities. PbTx in mussels nearly doubled that of oysters sampled during the same time and remained above the regulatory limit for significantly longer, 2 ½ weeks and 16 weeks, respectively.

Biochemical composition and reproduction appeared unaffected, exhibiting year round gametogenesis with a partial, intermittent spawning strategy and stability in reserves. A lack of

significant seasonal cycles in biochemical composition suggests sufficient food and energy availability to support the observed year round gametogenesis. While continuous spawning capabilities were evident two major peaks in spawning and recruitment were observed (spring and fall), suggesting reduced fertilization and / or larval development and survival due to the presence of *K. brevis* and associated ichthyotoxins and hemolysins.

These results indicate that while high tissue PbTx concentrations may lead to reduced growth in *P. viridis*, gametogenesis is not inhibited, allowing the population to survive *K. brevis* bloom exposure and reproduce, even while individual mortality was high. Prolonged bioconcentration of PbTx may lead to increased threat of post bloom trophic transfer, resulting in negative impacts on other important fisheries and higher food web implications. While it cannot be conclusively determined that the cause of reduced growth, survival and recruitment is due to red tide events, the parallels observed suggest that *K. brevis* is an important factor in the drastic changes in population structure.

Through the work presented here, population dynamics of locally established *P. viridis* populations were characterized through monthly monitoring and the development of a DEB model to accurately predict the growth and reproduction under dynamic environmental conditions. This work aims to synthesize our knowledge on the individual bioenergetics of *P. viridis* and to aid in understand population dynamics and potential for competition with local *C. virginica* populations

Resumé

Dans le monde entier les introductions d'espèces dans de nouvelles régions constituent une préoccupation écologique croissante ; ces introductions peuvent conduire à des modifications drastiques des écosystèmes, entre autres du fait de la compétition avec des espèces indigènes et également par la modification des réseaux de flux d'énergie dans les écosystèmes. La moule verte *Perna viridis* est une espèce récemment introduite dans les eaux côtières américaines et qui s'est rapidement disséminée le long des côtes du Sud-Est des États-Unis. Cependant, il n'existe pour le moment que très peu d'informations concernant la structure des populations, et leur dynamique en réponse à la variabilité environnementale locale. Les efflorescences de marées rouges formées par le dinoflagellé toxique *Karenia brevis* sont fréquentes le long des côtes de Floride bordant le Golfe du Mexique, et la tolérance de *P. viridis* a l'égard de ces événements et des brévitoxines (PbTx) associées n'est pas connue. En outre, comme *P. viridis* est une espèce invasive, la potentielle concurrence (ressources trophiques, espace, …) avec des bivalves indigènes comme l'huître *Crassostrea virginica*, est une préoccupation majeure dans les systèmes côtiers de Floride.

Cette étude vise à caractériser la dynamique des populations établies de *P. viridis* populations établies et leur réponse aux efflorescences naturelles de *K. brevis*. Les effets des efflorescences à *K. brevis* ont été évalués à partir des résultats d'un suivi de la croissance, de la mortalité, du recrutement, de la gamétogenèse et de la composition biochimique des tissus (protéines, glycogène et lipides) durant trois ans. En outre, les concentrations en PbTx dans les tissus ont été analysées afin de déterminer l'absorption, l'accumulation et des taux d'élimination de ces toxines. Par ailleurs, les données recueillies sur le terrain et des informations de la littérature ont été utilisées pour élaborer un modèle énergétique individuel DEB pour modéliser la croissance et la reproduction de *P. viridis*.

Avant l'apparition de la première efflorescence à *K. brevis*, *P. viridis* présentait des taux de croissance rapide (6-11 mm mois^{-1}) et un taux de survie élevé (mortalité <1%). Au cours des efflorescences à *K. brevis*, le taux de croissance a chuté de façon significative et une bioaccumulation de PbTx dans les tissus mous a été observée. Les concentration élevées en PbTx dans les tissus ont persisté longtemps après la dissipation de l'efflorescence et les taux de

mortalité élevés se sont maintenus, ce qui a réduit fortement l'abondance de *P. viridis*. À la fin de l'efflorescence, la concentration en PbTx dans les moules était presque le double de celle relevée chez l'huître indigène *Crassostrea virginica* pour des individus prélevés à la même période ; chez *P. viridis*, la concentration en PbTx est restée supérieure à la limite réglementaire pour la consommation humaine pendant 16 semaine, alors qu'elle est revenue en dessous de ce seuil en 2 ½ semaines chez *C. virginica*.

La composition biochimique des tissus et la reproduction n'ont pas parues affectées par ces évènements ; *P. viridis* réalise sa gamétogénèse durant toute l'année et a mis en place une stratégie de ponte intermittente partielle ; elle présentait durant toute l'année une grande stabilité de la concentration en composés de réserve. L'absence de cycle saisonnier marqué de la composition biochimique suggère que la ressource trophique est suffisante pour soutenir la gamétogenèse tout au long de l'année. Cependant, la première année du suivi, deux évènement majeurs de ponte et de recrutement ont été observés au printemps et à l'automne. Au cours de la deuxième année de suivi, l'analyse histologique montre que le même patron de ponte massive est observé au printemps ; cependant, à cette période, les efflorescences toxiques ont persisté et le recrutement a été inhibé, ce qui suggère que la fécondation et / ou le développement et la survie des larves ont été affectés par la présence de *K. brevis* et des ichthyotoxines et hémolysines associées.

Ces résultats indiquent que même si des concentrations tissulaires élevées en PbTx peuvent réduire la croissance de *P. viridis*, la gamétogenèse n'est elle pas inhibée, ce qui permet à la population de moule verte de subsister, même si la mortalité individuelle était élevée. Les concentrations élevées et persistantes en PbTx dans les tissus peuvent augmenter le risque de transfert trophique longtemps après les efflorescences, ce qui pourrait avoir des répercussions négatives sur d'autres pêcheries importantes, en plus des conséquences sur la chaîne alimentaire. Même s'il ne peut pas être établi avec certitude que la réduction de la croissance, de la survie et du recrutement sont bien dus aux marées rouges, nos observations suggèrent que *K. brevis* est un facteur important dans les changements radicaux observés de la structure et de la dynamique de la population de *P. viridis*.

Le présent travail a permis, à partir d'un suivi pluri-annuel, de caractériser la dynamique des populations de *P. viridis* établies localement. En outre, le développement d'un modèle

mécaniste de bioénergétique (DEB) a permi d'établir des prévisions sur la croissance et la reproduction de la moule verte à partir d'un forçage par les conditions environnementales. Ce travail a permis de synthétiser nos connaissances sur *P. viridis* et aide à comprendre la dynamique de la population et les potentialités de l'espèce pour entrer en concurrence avec les populations de l'huître locales *C. virginica*.

Abbreviations

PbTx: Brevetoxin

STX: Saxitoxin

NSP: Neurotoxic Shellfish Poisoning

HAB: Harmful Algal Bloom

AUCi: integrated Area-Under-the-Curve

MST: Mean Survival Time

RECON: River, Estuary and Coastal Observing Network

FWRI: Florida Fish and Wildlife Research Institute

SCUBA: Self-Contained Underwater Breathing Apparatus

GSI: GonadoSomatic Index

GI: Gonad Index

CI: Condition Index

ROS: Reactive Oxygen Species

ATP: Adenosine TriPhosphate

DEB: Dynamic Energy Budget

MSX: *Haplosporidium nelsoni*

Dermo: *Perkinsus marinus*

Chapter 1: General Introduction

Chapter 1: General Introduction

1. History, origin and transport of green mussels

The green mussel, *Perna viridis*, is native to the Indo Pacific and is found abundantly in coastal waters of India, Malaysia, Papua New Guinea, Indonesia, China, Japan, and the Philippines (Appukuttan, 1977; Sivalingam, 1977; Siddall, 1980; Haung et al., 1983; Vakily, 1989); reviewed by Baker et al. (2007). High protein content and rapid growth rates, reaching marketable size within 6 – 8 months, make *P. viridis* an ideal aquaculture species (Sivalingam 1977; Vakily 1989) serving a major food source in these regions, harvested through various aquaculture operations and from wild populations. It is however, also a pesky biofouling organism and if left untreated can cause serious damage to infrastructure including the clogging of intake pipes used in various forms of industry and water cooling systems (Rajagopal et al. 1991a,b). Green mussels frequently occur in densities of 1000 – 4000 individuals m^{-2} (Fajans and Baker 2005) and as high as 35,000 individuals m^2 (Huang et al., 1983). This coupled with high growth rates, can result in serious damage to infrastructure and high costs for removal.

Perna viridis made it first appearance in Caribbean waters in the early 1990's along the coast of Trinidad and Tobago (Agard et al., 1992) and has since spread throughout the Caribbean. In 1993 they were recorded along the coast of Venezuela (Rylander et al., 1996; Segnini de Bravo, 1998) and in 1998 populations were observed in Kingston Harbour, Jamaica (Buddo et al., 2003). The first occurrence of *P. viridis* in the United States was observed in 1999 in the water intake pipes of the Tampa Electric Company Power Stations (Gannon and Big Bend) in Tampa Bay, Florida with an estimated initial invasion in early fall of 1998 based on size of individuals collected (Benson et al., 2001; Ingrao et al., 2001). Green mussels have since spread throughout southwest Florida coastal waters via a free swimming larval stage and boat traffic (Baker et al., 2007). Dense populations were recorded in the Estero Bay / Fort Myers, Florida area as early as 2002 and sporadic siting's as far south as Marco Island, Florida in 2003 (Baker et al., 2007), approximately 240 km south of Tampa Bay, indicating an ability for rapid spread and colonization across long distances. Northern spread on the Gulf coast has not been observed likely due to a combination of colder waters and primarily southern flowing currents favoring a southern distribution. A separate Florida invasion has been documented on the Atlantic coast of

Florida in St. Augustine in 2002 in which green mussel populations have spread south to West Palm Beach and as far north as Charleston, South Carolina (Rajagopal et al. 2006; Baker et al. 2007). Northern limits on the Atlantic coast may be more extensive for subtidal offshore reefs due to northern flowing currents and proximity to the Gulf Stream and its prevailing warmer waters (Urian et al. 2010). More recently green mussel populations have been reported throughout Cuba's coastal waters in 2005 (Fernández-Garcés and Rolán 2005), but suffered a mass mortaltiy event in 2012 resulting in reduced densities possibly due in part to high frequency harvesting and low juvenile recruitment (Lopeztegui-Castillo et al., 2014).

Through genetic testing of green mussels from both native and invaded regions, Gilg et al. (2012) suggests a single invasion to the Caribbean, likely from ships traveling from China to Trinidad and Tobago, and a successive stepping stone spreading effect throughout the Caribbean and southeastern United States. Larval transport via ballast water and / or adults colonizing boat hulls are both potential vectors of transport (Benson et al., 2001; Ingaro et al., 2001; Buddo et al., 2003; Rajagopal et al., 2006; Baker et al., 2007). Boat hulls provide hard substrate for settlement and with rapid growth, green mussels reach reproductive maturity within 3 – 4 months (Rao et al., 1975; Parulekar et al., 1982; Walter, 1982) boats traveling from one port to another are likely to carry reproductively active populations providing a brood stock population when they arrive to a new harbor. With a pelagic larvae larval stage lasting 18 – 28 days (Tan, 1975a; Sreenivasan et al., 1988) long distance dispersion via ballast water and / or ocean currents is possible. This allows for accelerated spread of new populations as boat traffic moves along the coasts, between port cities, and across ocean basins. With a history of rapid spread and colonization, concerns abound regarding invasion potential in other US coastal regions and remains to be elucidated.

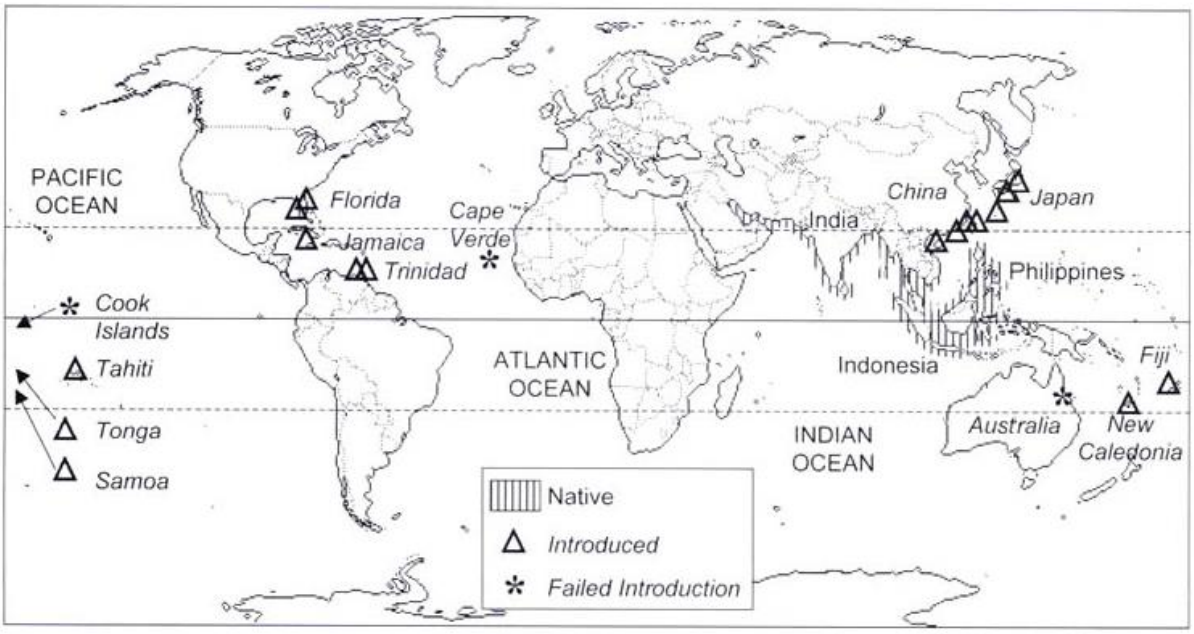

Figure 1: Map of the spread of *P. viridis* throughout the Indo-Pacific and the newly invaded region of the Caribbean and Atlantic (from Baker et al., 2007).

2. Study site: Estero Bay, Florida, US

Classified as an aquatic preserve in 1966, Estero Bay spans 11,000 acres of protected submerged land, creating critical habitat for many commercially and ecologically important species (Florida Department of Environmental Protection, 2015). The bay is protected from the Gulf of Mexico (GOM) by barrier islands and is densely populated by mangrove islands, oyster beds and mud flats (Byrne and Gabaldon, 2008). Six inlets allow for a strong tidal influence and extensive flushing between the estuary and GOM with six major freshwater tributaries from the mainland, resulting in a brackish mixing (Byrne and Gabaldon, 2008). While Estero Bay has a protected status, several of its major tributaries run through urban areas leading to anthropogenic input due to increased development along the rivers amplifying concerns of nutrient loading (Tolley et al., 2006).

Southwest Florida has a unique landscape, dramatically altered by anthropogenic forces and coastal urbanization. Through development, water management, and dredging activities, drainage patterns from the Florida Everglades have been altered from slow meandering streams and creeks to large, heavy flow rivers (Volety et al., 2009). Such hydrological changes have created

inconsistent environmental conditions, especially when coupled with the two very dramatic seasons southwest Florida experiences. In the winter-dry season there is little to no rainfall, and the average salinity in the estuary may become hypersaline ranging from 28-38 ppt while in the summer-wet season, high rainfall and large freshwater flushes result in salinities as low as 0-10 ppt (Barnes et al., 2007; Volety et al., 2009). Many of southwest Florida's estuaries are shallow, allowing for rapid changes in salinity which create an acute and sometimes prolonged exposure to depressed salinities. Besides increased fresh water input, these drainage canals and rivers now flow through areas of increased urban development and agricultural land, accumulating nutrients from fertilizers, pesticides, and increased loads of suspended solids (Volety et al., 2009; Abeels et al., 2012). This high input of nutrients allows for a productive system, however when in excess can initiate and support phytoplankton blooms which may cause sags in dissolved oxygen resulting in benthic mortalities due to anoxic conditions and / or fuel toxic algae blooms, such as *Karenia brevis* (Heisler et al., 2008), which are typically accompanied by a high incidence of marine life mortality and human health concerns.

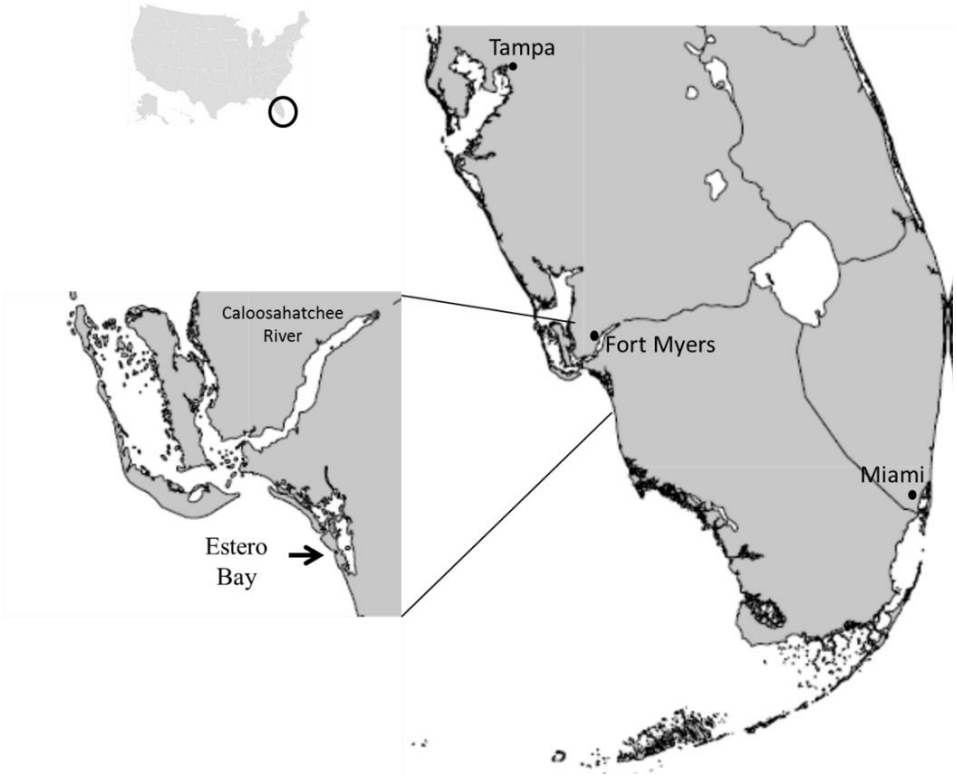

Figure 2: Map of the study site

Estero Bay is a shallow, mud bottom estuary with an average depth of 1 m (Byrne and Gabaldon, 2008) leaving limited natural substrate (mangrove roots and oyster reefs) for biofouling organisms and thus, primarily only artificial substrate (boat hulls, docks, intake pipes, navigational structures, seawalls) available, primarily located in dredged channels, marinas, and bridge passes. The shallow muddy conditions in Estero Bay result in turbid waters when wind or current flow agitates the water column leaving subtital regions hidden from view. To monitor the establishment and spread of invasive species exploratory divers must inspect these regions, which can be costly and the lack of such monitoring is the primary reason *P. viridis* has successfully spread to densely populate new regions and go unseen until they have become fully established.

3. Biology of Perna viridis

Perna viridis is a dioecious species with rare cases of hermaphrodites and while sexual organs are not distinguishable, sex can be determined by tissue color (Lee, 1988). The gonad is dispersed throughout the mantle lobes, mesosoma and intermixed between digestive glands (Rajagopal, 2006) leaving males appearing creamy white in color and females orange or brick red in appearance (Walter, 1982; Sreenivasan et al., 1989; Lee, 1986; Narasimham, 1981). During resting periods of gametogenesis this coloration is not as distinct leading to misidentification without histological confirmation, however in tropical to subtropical regions, reproductive activity frequently persists year round (Parulekar et al., 1982; Walter, 1982). *Perna viridis* are broadcast spawners, releasing egg and sperm into the water column for external fertilization and may be cued not only by environmental factors (temperature, salinity, food), but also from chemical cues (Widdows, 1991; Barber and Blake, 2006) which can be initiated by either sex (Stephen and Shetty, 1981). Larval densities have been recorded from approximately 20,000 larvae m^{-3} (Rajagopal et al., 1998a) to as high as 40,000 larvae m^{-3} (Rajagopal et al., 1998b), similar to that of *Mytilus edulis*, its cold water relative (Schram, 1970).

The life cycle begins with a pelagic larval phase reported to last from as short as 8 – 15 days (Tan, 1975a; Nair and Appukuttan, 2003) to 15-18 days (Sreenivasan et al., 1988; Nair and Appukutan, 2003) and as long as 24-35 days (Nair and Appukuttan, 2003; Laxmilatha et al., 2011) (Table 1). This wide range is due to variations in environmental conditions and substrate

availability, which play a critical role in larval development and settlement (Sreenivasan et al., 1988; Nair and Appukutan, 2003). Marine mussels can delay metamorphosis for several weeks if suitable substrate is not encountered, however this delay results in decreased survival due to the interruption of feeding and growth leading to a prolonged pelagic phase accompanied by increased predation risks (Bayne, 1965; Widdows, 1991).

During the planktonic stage they are at the bottom of the food chain leading to increased predation pressure from plankton grazers and susceptibility to external stressors is at its high (Bayne, 1976). Food and temperature are the two of most important factors affecting growth and development of bivalve larvae (Widdows, 1991), however any environmental altercation outside the threshold will effect growth, development and survival, including temperature, salinity, pH, dissolved oxygen, food availability, HAB's pollutants, etc. (Nair and Appukuttan, 2003; Bayne, 1965; Gilg et al., 2014). Nair and Appukutan (2003) observed significant difference in larval growth reared under different temperatures with a more narrow tolerance range than that of post metamorphosed mussels. Decreased embryogenesis, larval development and survival have been observed during exposure of local species of oysters, clams and scallops to *K. brevis* (Leverone et al., 2006; Rolton et al., 2014).

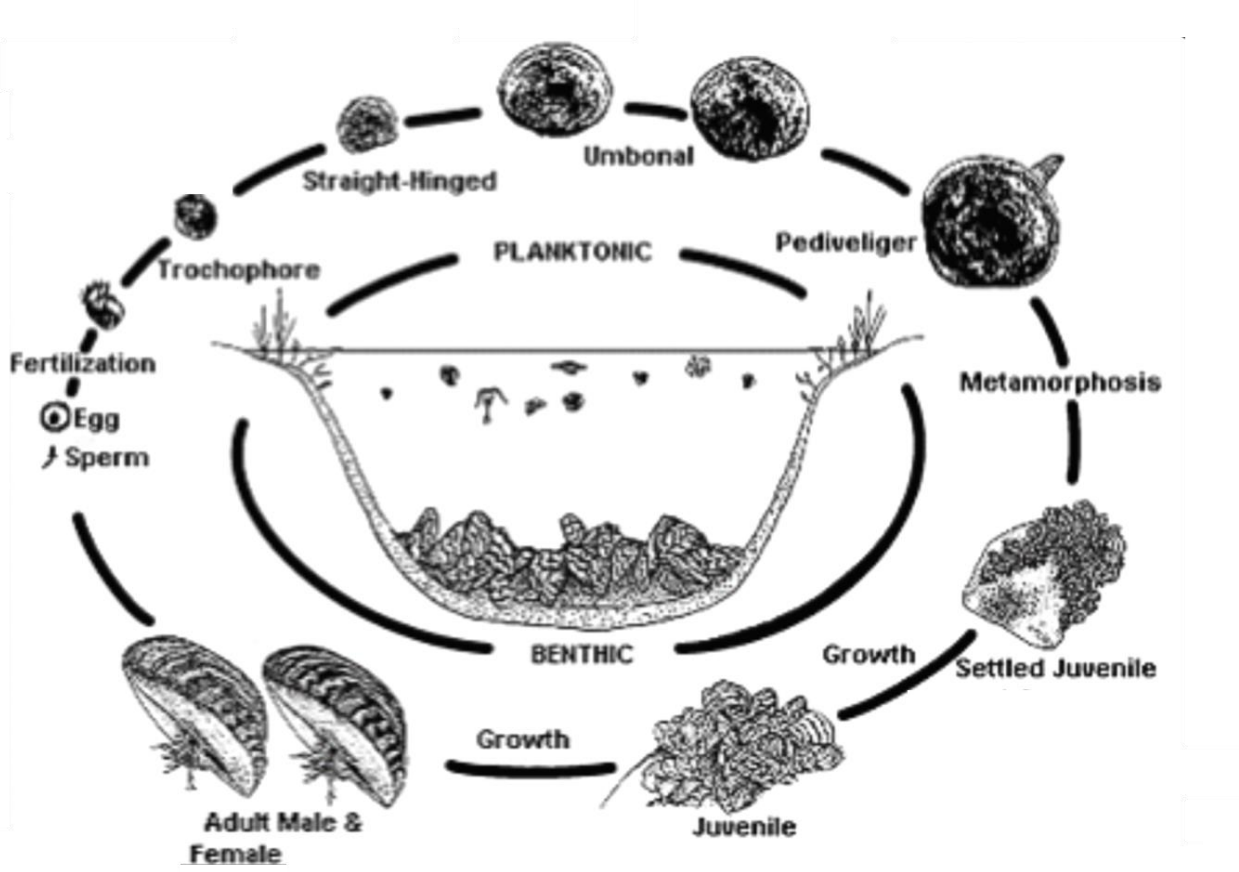

Figure 2: Life stages of *P. viridis* (Adapted from Black, 2015 www.mass.gov)

Table1: Larval growth of *Perna viridis* documented from the literature.

Stage	Size (µm)	Tan (1975)	Sreenivasan et al. (1988)	Nair and Appukuttan (2003)	Laxmilatha et al. (2011)
Trocophore	58	7-8 hours	6-8 hours	5-10 hours	6-8 hours
D- hinge	70 - 90	12-15 hours	20-24 hours	18 hours	20-22 hours
Veliger		18-19 hours		8-30 hours	
Umbo	90 - 260		7-9 days	5-8 days	7 days
Eye Spot	200 – 330			9-20 days	13-14 days
Pediveliger	360 - 380	8 days	11-14 days	11-17 days	16-19 days
Plantigrade	380 – 480		19-23 days		21 days
Spat/Settlement	510 - 910	8-12 days	23-27 days	15-24 days	21-35 days

*Nair and Appkuttan (2003) measured larval growth at a range of temperaures 24-31˚C with the fastest growth rates at 31˚C and lowest growth at 24˚C, 100% larval mortality was observed at 33 and 35˚C. Laxmilatha et al. (2011) had a temperature range of 28-31˚C. Tan (1975) measured growth at 23-25˚C. Sreenivasan et al. (1988) measured growth at 30.1 ˚C.

For the first several days post hatching, early larval stages depend on lipids from the egg as the only source of energy for development before feeding is possible (Bayne et al., 1975; Holland, 1978). Stress in adults during egg production can lead to reduced fecundity and success of progeny due to decreased lipid content and viability in eggs (Bayne, 1972; Bayne et al., 1975; 1978). Thus, larval stages are not only affected by environmental conditions in which they are living, but also the physiological state of the broodstock population.

While larvae are free swimming, mobility is minimal and distribution occurs primarily through current and tidal flow. Depending on the local hydrology, mussel larvae may be transported great distances with currents or may be confined to a more concentrated area (Porri et al., 2006; Gilg et al., 2014). Mussel larvae can secrete specialized byssal threads to aid in floatation and transport, different from those secreted for attachment, within 10 – 12 days of hatching (Siddall, 1980). These long byssal or mucous threads can allow for post-metamorphosis migration via floatation when void of suitable substrate (Widdows, 1991; Rajagopal et al., 2006). Primary settlement onto filamentous drift algae followed by secondary settlement to an established mussel bed is common in Mytilid species (Widdows, 1991; Buchanan and Babcock, 1997; Alfaro et al., 2004). Primary settlement substrate, which commonly includes filamentous drift algae, allows for a prolonged searching period when suitable substrate is not available and transport over greater distances (de Vooys, 1999; Cáceres-Martínez et al., 1993; Buchanan and Babcock, 1997). Following primary settlement, mussels can to move from one substrate to another through the use of the foot (Bayne, 1964). This crawling stage can last for several weeks (Dare and Davis, 1975); 5 – 6 weeks for *M. edulis* (Bayne, 1965) and early settled spat may attach and detach themselves several times before settling in a permanent position (Bayne, 1964, 1965**;** Seed, 1969; Tan, 1975). A prolonged searching phase allows for more selective behavior for substrate and / or environmental conditions prior to settlement and has been observed previously in several species of bivalve larvae (Muus, 1973).

It is not fully understood what induces the larvae to "choose" a substrate and settle, however, as a gregarious species, chemical cues from adults are believed to play a role and increased recruitment is often observed on or near established adult populations (Bayne, 1964; Widdows, 1991; de Vooys et al., 2003; Baker et al., 2006;). Preferential use of filamentous algae for primary settlement also suggests that byssal threads of adult mussels may attract larvae ready

to settle (Eyster and Pechenik, 1987; deVooys et al., 1999; Cáceres-Martínez et al., 1993). By settling within clumps of established adults, new juveniles may find refuge from predation and the presence of adult populations may indicate suitable habitat, increasing survival and proximity giving a reproductive advantage as broadcast spawners (Alfaro, 1994). Once settled, mussels maintain a permanent position through the production of byssal threads allowing them to remain securely fastened to hard substrate and are frequently intertwined with each other forming tight clumps or mats. The byssal gland is in the foot and allows for repositioning of the individual when necessary (Banu et al., 1979). Byssal production is a continuous process allowing for the replacement of broken or damaged threads and have shown increased production and thread thickness as a defense mechanism under predation pressure and / or the presence of damaged conspecifics (Young, 1985; Leonard et al., 1999; Cheung et al., 2004).

Once settled *P. viridis* has shown rapid growth rates averaging 7 – 10 mm month^{-1} (Qasim et al., 1977; Lee, 1986; Haung et al., 1983; Sreenvivasan et al., 1989) and as high as 12 – 15 mm month^{-1} (Rajagopal et al., 1998; Hawkins et al., 1998). Kuriakose and Appukuttan (1980) recorded growth of 15.4 mm in the first month of settlement followed by rates of 11.2 – 13.6 mm in the following four months. These rates are similar to the Mediterranean mussel *Mytilus galloprovincialis* which exhibits growth rates 6.3 – 11 mm month^{-1} in NW Spain (Camacho et al., 1995). However, growth of *P. viridis* far exceeds its cold water relative, *M. edulis*, which has reported growth of 5 cm in 5 years in the cold waters of Plymouth, England (Bayne and Worrall, 1980) and from 11 – 24 months to reach market size depending on the geographical location (Hawkins et al., 1999).

Rapid growth in sessile organisms serves several purposes particularly within the first year of settlement. As smaller mussels will be most vulnerable to predation pressures, fast growth increases the ability to avoid predation. This also allows individuals to quickly reach maturity and reproduce in a short amount of time allowing for rapid colonization of new habitat. Both high growth and reproductive rates may also allow for reduction in competition for food and substrate from similar species allowing for their success as an invasive species.

Growth rate increases with increasing temperature, within the animals threshold and provided food availability is sufficient, thus tropical species typically have prolonged growth periods (Rajagopal et al., 1998a; Lodeiros and Himmelman, 2000). Temperature and food

availability are often interrelated leaving predictions difficult due to the complexity of environmental conditions. Environmental factors play role in eliciting physiological responses and if one or more is above or below the threshold, growth will be hindered. A decrease in growth rate is often observed in the winter months when temperature and food are reduced (Narashiham 1981; Lee 1986; Richardson et al., 1990; Cheung et al. 1991; 1993). For *P. viridis*, Cheung (1991) suggested lower threshold for growth of 20 ˚C and a complete cessation in growth has been documented at 17˚C and below (Lee, 1986). Further, Lee (1985) observed that while growth may occur at lower temperatures, highest growth occurs at temperatures of 24 – 29 ˚C and in regions where temperature remains above this threshold decreases in growth can be attributed to a decrease in food availability.

In areas where food and temperature remain high (ie: power plant facilities), *P. viridis* has been observed to reach 119 mm in the first year, far exceeding the typical values recorded throughout its geographic distribution (Rajagopal et al., 1998b) which typically closer to90 mm in the first year (Roa et al., 1975; Narasimham, 1980; Rivonker et al.,1993). Records of maximal length vary widely with reported values in invaded regions ranging from 161 mm in Trinity Harbour, Austratlia (Stafford et al., 2007) to 172 mm in Tampa Bay, Florida (Baker et al., 2012) and previous work in native regions reporting values from 156 – 230 mm (Roa et al., 1975; Appukuttan, 1977; Narasimham 1981; Cheung, 1990).

High fecundity has been reported in both native and invaded ranges. Many studies within tropical regions report year round gametogenesis with one or two major peaks in spawning that could be linked with environmental variables (Roa et al., 1975; Walter, 1982; Fatima et al., 1985). Even in more temperate regions where mussels undergo more distinct cycles, highly productive spawning seasons are observed with two major peaks and intermittent spawning throughout the season (Lee, 1986; Rajagopal et al., 1998a, b; Barber et al., 2005). *Perna viridis* is capable of reaching reproductive maturity within the first 2 – 3 months of settlement at lengths of 15 – 20 mm (Parulekar et al., 1982; Roa et al., 1975; Sreenivasan et al., 1989; Siddall, 1980), potentially allowing for cohorts to spawn within the first year. Populations in Tampa Bay, Florida were found to have a long and productive spawning season, but experienced a resting phase in December and January (Barber et al., 2005) when local water temperatures drop as low as 13 ˚C (Badylak et al., 2007). However, on the Atlantic coast in St. Augustine, Florida where

observed winter lows averaged 16 ˚C, *P. viridis* was observed to maintain high reproductive activity year round with no observed resting stage (Urian, 2009).

High growth and reproductive activity comes at a high energetic cost. The ability to maintain year round gametogenesis and allocation of energy towards growth requires that the individual receives enough energy (food) to support these processes. Green mussels populate eutrophic estuaries and bays with high nutrient availability (Vakily, 1989; Wong and Cheung, 2001) and have exhibited high clearance rates when compared to other bivalves (Hawkins, 1998; McFarland et al., 2013). This high efficiency feeding is needed to supply energy required to fuel the high metabolic demand of rapid growth and year round gametogenesis. Fast growing bivalves have been shown to have high feeding rates and metabolic efficiency (Bayne, 2000). *Perna viridis* has been reported to have clearance rates ranging from 2.62 – 4.21 L $h^{-1}g^{-1}$ (Wang et al., 2005) to as high as 8.06 – 9.68 L $h^{-1}g^{-1}$ (Blackmore and Wang, 2003) with a maximum clearance rate recorded at 15 L $g^{-1}h$ (Hawkins et al., 1998). In the invaded region of southwest Florida *P. viridis* has been shown to exhibit clearance rates 2 – 3 times greater than the native oyster *C. virginica* (0.08 – 1.2 and 0.09 – 0.43 L $h^{-1}g^{-1}$, respectively) at salinities within its optimal range and maintained rates similar to that of the oyster at depressed salinities as low as 10 ppt (McFarland et al., 2013). Hawkins et al. (1998) compared the clearance rate of *P. viridis* and four other oyster species (*Crassostrea belcheri, Crassostrea iradelei, Saccostrea cucculata and Pinctada margarifera*) from its native range and found *P. viridis* to have both higher feeding efficiencies and clearance rates; 7.2 ± 3.1 L h^{-1} compared to 4.1 - 5.5 L h^{-1} for the oysters. In comparing feeding rates and efficiencies, Hawkins et al. (1998) also observed higher retention efficiency (63 +/- 3%), ingestion rate (24.8 +/- 3.6 mg $g^{-1}h^{-1}$) and absorption rate (21.5 +/- 3.4 mg $g^{-1}h^{-1}$) in *P. viridis* compared to all species of oysters (4 – 11%; 2.0 – 9.5 mg $g^{-1}h^{-1}$; 1.3 – 6.0 mg $g^{-1}h^{-1}$ respectively). Indicating that high clearance rates observed for *P. viridis* are accompanied by a high retention efficiency and energy assimilation rate fueling the increased metabolic demands of high growth and reproductive activity.

When compared to clearance rates of other Mytilids, *P. viridis* exceeds that of *M. edulis* which ranges from 1.66 – 4.12 L h^{-1} under different diets (Bayne et al., 1987) and from different field populations ranging from 1.12 – 2.55 L h^{-1} (Okumuş and Stirling, 1994), but is similar to the more closely related green-lipped mussel *Perna canaliculus* which has clearance rates of 6.8

L h^{-1} (Hawkins et al., 1999) to 8.6 L h^{-1} (James et al., 2001). While others report a clearance rate higher than that of oysters when compared directly (Hawkins et al., 1998; McFarland et al., 2013), the clearance rate of *C. virginica* has been reported to range from 1.6 – 8 L h^{-1} (Riisgård, 1988; Grizzel et al., 2008) which is more comparable to that reported for *P. viridis.* While comparison between studies is difficult due to differences in methodologies and environmental conditions, it is clear that *P. viridis* can rapidly clear food particles from the water column with high assimilation efficiencies and are likely to compete with other bivalves.

Feeding behavior and pseudofeces production of *P. viridis*, and many other bivalves, has shown to vary significantly depending on the food quality and quantity (Hawkins et al., 1998; Wong and Cheung, 1999; Wong and Cheung, 2001). Ward et al. (1998) used endoscopy to show selective ability in the labial palps of several species and adaptive capabilities for rapidly and efficiently sorting, allowing for the maintenance of increased filtration rates typically observed (Ward et al., 1998; Ward et al., 2003). Production of pseudofeces aids in the selective ability of bivalves which allows for the filtration of all particles in the water column and selectivity in choosing particles to be ingested and those to be discarded as pseudofeces based on size and organic content (Ward et al., 1998; Baker et al., 1998; Hawkins et al., 1999; Ward et al., 2003). This is especially helpful in shallow estuaries, common in southwest Florida, which may have large amounts of suspended solids (ie: silt) in the water column that must be sorted from food particles prior to ingestion (Morton, 1987; Seed and Richardson, 1999). During periods of low food (either quality or quantity) or conditions of starvation mussels may also increase food gut time to increase nutrient extraction efficiency (Hawkins and Bayne, 1984; Bayne et al., 1987). These behaviors may allow for a greater increase in energy acquisition in the winter when food is low allowing for the maintenance of year round gametogenesis.

While the commonly accepted life span of *P. viridis* is 3 years (Lee et al., 1985; García et al., 2005) others have documented at least 4 years with a growth rate of 4 mm $year^{-1}$ in the fourth year (Narasimham, 1981). Other *Perna* species have been documented to have a higher longevity. McQuaid and Lindsay (2000) determined an average lifespan of 6.7 years with a maximum of 9 years for *Perna perna* in sheltered areas. *Perna perna*, however, have a lower estimated ultimate length of 65 – 117 mm (McQuaid and Lindsay, 2000) compared to *P. viridis* (156 – 230 mm; Roa et al., 1975; Appukuttan, 1977; Narasimham 1981; Cheung, 1990).

Mortality rates vary between geographical locations, Al-Barwani et al. (2007) reported approximately 1.69 / year and Choo and Speiser (1979) report mortality rates of 3.66 (~93%) for mussels ≤15 mm and 0.52 (~41%) for mussels ≥15 mm. In comparing densities between caged and rope cultured *P. viridis*, Lee (1986) attributed the 20 – 40% loss in rope culture mussels to predation and / or dislodgement rather than natural mortality. Studies which report high mortality rates often occur during periods of environmental stress such as extreme variations in temperature (Power et al., 2004; Urian et al., 2010; Baker et al., 2012), HAB events (Gacutan et al., 1985; Tracey, 1988; Baker et al., 2012), pollution (Lee, 1986), temperature (Power et al., 2004; Baker et al., 2012), salinity (Gilg et al., 2014) and / or a combination of stressors (Cheung, 1993).

4. Environmental boundaries

The niche of a new species is defined by several factors. Not only must the habitat fit the species regarding to physical attributes (substrate type and location, salinity, temperature, food availability), but the population must be able to successfully compete with local species for these resources and avoid predation. Green mussel populations are primarily found in habitats ranging from oceanic and high salinity estuarine waters favoring areas with high phytoplankton and / organic matter (Morton, 1987; Vakily, 1989; Wong and Cheung, 2001) and high current flow / flushing aiding in the removal of waste and continuous food supply (Rajagopal et al., 1998b; Buddo et al., 2003; Rajagopal et al., 2006). Green mussels commonly dominate biofouling communities on and near power plants where water temperatures remain high and flushing is extensive (Rajagopal et al, 1991a,b). High density settlement has caused damage to power plants in their native range due to extensive fouling of water intake pipes (Rajagopal et al, 1991a) and the water cooling systems of power plants in Tampa Bay are believed to be the first point of invasion creating a broodstock population allowing for the further spread throughout the bay (Benson et al., 2001).

As an important aquaculture species, much attention has been given to production and environmental tolerances within their native range. In both native an invaded regions they are aggressive biofoulers and have been found coating hard substrate in densities as high as 4,000 – 35,000 individuals m^{-2} (Haung et al., 1983; Baker and Benson, 2002; Fajans and Baker, 2005)

and clogging water intake pipes at biomasses exceeding 200 g m^{-2} (Rajagopal et al., 1991a). As a gregarious species, high growth rates and fecundity have allowed for the rapid colonization of new habitat and decreased native fauna through resource competition. Thriving in a range of tropical to subtropical waters, conditions frequently allow for year round gametogenesis and high food availability in productive bays and estuaries allows for continuous input to energy reserves and ample food supply for developing larvae.

Perna viridis populations are most commonly found on hard substrate including mangrove prop roots and submerged rock and shell, and other bivalves including oyster reefs, but also frequent artificial substrate such as pilings, piers, and floating objects such as buoys (Vakily, 1989; Buddo et al., 2003; Baker et al., 2007; 2012). Locally, Estero Bay comprises of soft bottom sediments with little hard substrate available leaving intertidal oyster reefs and artificial structure as the only available substrate. However, green mussel populations have been observed on the soft bottom sediments within seagrass beds in Tampa Bay and Hillsborough Bay Florida covering areas as large as 2300 m^2 (Johansson and Avery, 2004) and in Kingston Harbor Jamaica (Buddo et al., 2003) often attaching to shell fragments, filamentous root structure, and / or other bivalves (Ingrao et al., 2001).

Although often referred to as an intertidal species (Shafee, 1978; Vakily, 1989) *P. viridis* is primarily found on subtidal substrate or areas with minimal emersion time (Tan, 1975b; Rajagopal et al., 1998b). Juvenile recruitment is often observed in the intertidal regions, however these individuals rarely survive to maturity (Baker et al., 2012; personal observation) and dense populations are primarily found subtidally (Nair and Appukuttan 2003) with the highest growth observed at depths below the mean low tide mark (Sivalingam, 1977). Tan (1975b) found the highest population densities at depths of 1.5 – 11.7 m below the high water spring tide mark and no living mussels were observed above the high tide mark. This distribution is likely due to desiccation stress from aerial exposure in which normal physiological requirements, (feeding, exchange of gases, and removal of waste) are inhibited. Green mussels found in the intertidal zone have been shown to be very sensitive to even short term extremes in air temperature (Urian et al., 2010; Baker et al., 2012; McFarland et al., 2014). Both field and lab observations suggest that *P. viridis* cannot tolerate aerial exposure at temperatures below 14˚C (Baker et al. 2012; Firth et al., 2011; Power et al., 2004) and Urian et al. (2010) observed 100% mortality at 3 ˚C

within the first 24 hours of exposure. Likewise green mussels have shown an inability to cope with aerial exposure under high temperature stress with mortality rates (≥97%) at air temperatures as low as 25 ˚C in which surface temperature often exceed 30 ˚C when under direct sunlight (McFarland et al., 2014).

As a tropical species, *P. viridis* thrives in warm waters with an optimal range of 26 – 32 ˚C and lower and upper limits showing 50% survival at 10˚C and 35˚C, respectfully (Silvalingam, 1977). Populations in invaded regions, however, may adapt to changing environments and develop localized tolerances. Segnini de Bravo et al. (1998) reported lethal lower and upper temperatures of 6 ˚C and 37.5 ˚C in the invaded region of Venezuela while Ueda et al. (2013) reported a lower tolerance of 12 ˚C in Japan. Locally, southwest Florida populations have an observed tolerance of 13- 30 ˚C determined through both laboratory exposures and field observations (Baker et al., 2012; McFarland et al., 2014). Likewise, populations on the Atlantic coast of Florida do not survive extreme winter water temperatures (Power et al., 2004; Spinuzzi et al., 2013) experiencing 93.8% mortality after 30 days at 14 ˚C and 100% mortality at 10 ˚C within 13 days (Urian et al., 2010). Likewise an intolerance to high temperature extremes is reported. Nicholson (2002) observed 30% and 70% mortality at 31 ˚C and 34 ˚C, respectively. Similarly, McFarland et al. (2014) observed 100% mortality within 12 days at 35 ˚C, indicating reduced tolerance with rising temperatures. Reduced filtration rates, byssal production and gonad development have been observed at temperatures ≥ 35 ˚C (Rajagopal et al., 1995; Sreedevi et al., 2014).

However, wide ranges reported from short term laboratory studies may overstate the boundaries for long term growth and survival. Urian et al. (2010) found a significant increase in heat shock proteins (Hsp70) after only 2 hours of exposure to 10 ˚C suggesting that stress from short term exposures may have prolonged metabolic effects especially if other stressors are involved or repeat exposures occur. The various ranges reported in the literature are likely due to methodologies including the rate of change and exposure duration. Temperature range is also dependent upon salinity in which low salinity conditions reduced the temperature range at which *P. viridis* can survive (Rajagopal et al., 1995; unpublished data cited by Spinuzzi et al., 2013).

A wide range of salinity tolerances have been reported for *P. viridis* throughout both native and invaded regions. The optimal salinity in its native range has been reported as 27 – 33

ppt with a tolerance of 19 – 44 ppt and is reflected accordingly in population distribution within bays and estuaries (Silvalingam, 1977; Sundaram and Shafee, 1973; Huang et al., 1983; Vakily, 1989), however, wider tolerances have been reported through laboratory studies. Silvalingam (1977) observed 50% survival at salinities of 24 and 80 ppt and Sengini de Bravo et al. (1998) determined a range from 0 – 64 ppt by decreasing the salinity by 1 ppt / day in the laboratory. These were however, short term exposures and physiological changes such as increased valve closure, inability to osmoregulate, and reduced clearance rates suggest long term survival at these extremes is unlikely (McFarland et al., 2013). In Tampa Bay, established populations are found in areas which remain above 14 ppt and the highest densities are found in regions where the salinity ranges from 20 – 28 ppt (Baker et al., 2012). Through both acute and gradual salinity decreases, southwest Florida populations have an observed lower threshold of 12 ppt (McFarland et al., 2014), however at salinities of 15 ppt and below osmotic stress may reduce long term survival (McFarland et al., 2013). Likewise, upper salinity tolerance may be more limited in natural populations versus laboratory experimentation. Buddo et al. (2003) found dense populations in regions with average salinities of 27.7 ppt, but no mussels were found near salt ponds where salinities averaged 33.8 ppt.

Harmful algal blooms may impose additional environmental constraints. Blooms of the toxic dinoflagellate, *K. brevis*, are a common occurrence in southwest Florida and the response of *P. viridis* to these events is unknown. *Karenia brevis* produces a suite of potent neurotoxins known as brevetoxins (PbTx) and blooms are commonly accompanied by massive fish kills (Ray & Wilson 1957; McFarren et al. 1965; Naar et al. 2007) and in some cases marine mammal, sea turtle and seabird strandings and mortalities (Adams et al. 1968; Forrester et al. 1977; Flewelling et al., 2005). While local bivalves are tolerant to these bloom events, they accumulate PbTx in their soft tissue body parts posing a threat to predators through trophic transfer and human consumption (Plakas et al., 2002; 2004; Pierce and Henry, 2008; Rolton et al., 2014). Locally, clams *Mercenaria mercenaria* and oysters *C. virginica* are monitored routinely during these events as a human health precaution. PbTx levels $\geq$800 ng g^{-1} PbTx-3 equivalent in shellfish soft tissue and / or *K. brevis* cell counts $\geq$5,000 cells L^{-1} result in mandatory closure of shellfish harvesting (Tester & Steidinger, 1997; Steidinger, 2009; Plakas et al., 2008). Clams and oysters have been shown to depurate the toxin within 2 – 8 weeks post bloom when tissue concentrations ranged from 1,500 – 28,000 ng g^{-1} PbTx-3 equivalent (Morton and Burklew, 1969; Steidinger

and Ingle, 1972; Plakas et al., 2002; Plakas et al., 2004; Plakas et al., 2008; Bricelj et al., 2012; Griffith et al., 2013), however PbTx accumulation and depuration rates are unknown for *P. viridis*. Previous work addressing the effects of toxic dinoflagellates on bivalves has shown mussel species to accumulate high toxin levels and increased sensitivity to HAB exposure when compared with species of oysters and clams (Ingham et al., 1986; Shumway and Cucci, 1987; Shumway et al., 1988, 1990, 1995; Bricelj and Shumway, 1998; Lesser and Shumway, 1993).

Through observations of established populations of *P. viridis* in Tampa Bay, Baker et al. (2012) reported >90% mortality in regions of the bay exposed to a natural *K. brevis* bloom, while populations in portions further removed from the bloom appeared unaffected. However, this mortality event was followed by a rapid repopulation of juvenile green mussels in the following year post bloom dissipation (Leverone, 2007) Although a population rebound was observed, green mussels have yet to rebound and consistently maintain the high densities observed in Tampa Bay upon their arrival (Dr. S. Baker, personal communication).

In their native range, *P. viridis* has shown tolerance to several HAB species including *Karenia mikimotoi* (Robin et al., 2013) and *Gymnodinium nagasakiense* (Karunasagar and Karunasaga, 1992), but has resulted in high mortality rates during exposure to *Alexandrium monilatum* (Hégaret et al., 2008) and both increased mortality and prolonged harvesting bans due to accumulated tissue toxins were observed during and following a natural *Pyrodinium bahamense* var. *compressa* bloom in the Philippines (Gacutan et al., 1984). Cheung et al. (1993) documented high spring mortalities affecting 30 and 70% of adult *P. viridis* in Hong Kong during May – September of 1987 and 1988, respectively and, though not directly attributed, these mortality events occurred during periods of high red tide frequency. Juveniles from cohorts spawned during this period (June – July) had low survival. Similar mortality events have been documented in other bivalve species sensitive to toxic algae blooms. Tracey (1988) monitored *M. edulis* populations during and after a brown tide bloom caused by *Aureococcus anophagefferens* in the summer of 1985 which caused nearly 100% mortality and inhibited juvenile recruitment. Following this event, recruitment did not resume until the fall of 1986 at which time only sporadic spat were observed. This reduction in larval recruitment was attributed to the severe decline in density of spawning adults and poor quality of eggs and larvae produced due to stress and reduced feeding in the adults during the bloom (Tracey, 1988). While an

intolerance to *K. brevis* in south Florida populations is a positive for limiting population spread, it may pose trophic transfer and human health concerns. *Perna viridis* is not harvested for human consumption, however illegal harvesting occurs and several species of fish, crab and seabirds may be at risk for toxin accumulation if mussels harbor the toxins for periods of time following exposure without succumbing to death. Tolerance of *P. viridis* to *K. brevis* blooms is currently unclear and the extent to which they accumulate associated toxins in their tissue is unknown.

5. Problems with invasive species: Biofouling and potential harm to oysters and ecosystem disruption

Introducing non-native species to new environments is a rising problem world-wide. Increased boat traffic and international shipping have increased vectors for spread over wide geographic distances and increased urbanization and tourism in coastal areas has created new substrate for species including navigational structures, piers, and break walls (Bax et al., 2003). While Florida coastal waters already have a significant biofouling problem, consisting mainly of barnacles, tunicates and other native bivalves, none reach the same sizes and densities observed in *P. viridis* populations (Benson et al., 2001). Increased biofouling communities lead to increased costs for removal and can halt industrial production in water cooling systems. Pipes and pumps may become clogged leaving a reduced flow causing pumps to overheat and burn out, thereby increasing cost of both removal of the species and replacement of damaged infrastructure. Rajagopal et al. (1997) found dense populations of *P. viridis* coating water cooling systems of power plants in densities reaching 211 kg m^{-2} causing damage to equipment and clogging of pipes reducing water flow for the cooling system. Clogging is caused not only by dense settlement of mussels but also from byssal mats which can get sucked into the pumps and block protecting screens (Ingrao et al., 2001). While green mussels are only contributing to an existing biofouling problem not creating one, their fast growth and reproduction may increase this problem and frequency of treatment.

From an ecosystem perspective, invasive species may cause alterations in ecosystem services including nutrient cycling; habitat modification (or loss); competition and displacement of native species, which may include aquaculture facilities and fisheries; act as vectors for disease, viruses, parasites, bacteria, and harmful algae (Bax et al., 2003; Hégaret et al., 2008).

Bivalves may transport and introduce parasites or diseases from their native range not normally found in the new ecosystem or may be immune to parasites in the new ecosystem leaving themselves free of parasites giving them a leg up on the competition (Branch and Steffani, 2004). Competition for resources, such as food and settling space, can lead to displacement and reduction in numbers of native species (Dulvy et al., 2003; Karatayev et al., 2007). As ecosystem engineers, bivalve invasions have been shown to drastically alter ecosystems. For example the zebra mussel *Dreissena polymorphia* in the Great Lakes resulted in alterations of the phytoplankton community, reduction of native species and an estimated cost of $1 billion per year in biofouling damage and control (Pimentel et al., 2005; Karatayev et al., 2007). Similarly, the Mediterranean mussel, *Mytilys galloprovincialis* reduced native bivalves and limpets by out competing for space on the coast of South Africa (Branch and Steffani, 2004) and *P. viridis* invasion in Venezuela has resulted in heavy competition and a population decrease of the brown mussel *Perna Perna* due to substrate competition (Segnini de Bravo et al., 1998; Rylander et al., 1996). In Tampa Bay Florida, dead oyster shell was found under green mussel populations on bridge pilings and the subtidal, outer crest of a reef system (Baker et al., 2007).

Through rapid growth and vigorous reproductive activity, *P. viridis* is an aggressive marine invader rapidly reaching high densities allowing for heavy competition with other bivalves for hard substrate. The primary concern surrounding green mussel population spread in the southeastern United States is competition with the native oyster *Crassostrea virginica* (Fig. 3). Oysters are a keystone species in the soft bottom bays of south Florida where they form permanent three-dimensional habitat which creates a refuge and / or foraging grounds for over 300 species of fish and crab (Wells, 1961) including several recreational and commercially important species (Henderson and O'Neil, 2003; Tolley et al., 2005). Many estuarine species spend their entire lives on the oyster reefs while others utilize the intricate reef system as a safe hiding place to lay their eggs and nursery for juveniles (Tolley and Volety, 2005). Many offshore fish and crab species enter the estuary for breeding where their young use oyster reefs as a refuge (Beck et al., 2011). This refuge for early life stages and small organisms creates an extensive feeding grounds for larger species, both estuarine and oceanic (Peterson et al., 2003; Grabowski et al., 2005). Peterson et al. (2003) found that oyster reefs enhanced growth and increased the abundance of 19 species of fish and large mobile crustaceans, with an estimated contribution to fish production of 2.57 kg $10m^{-2}$ yr^{-1}.

Figure 3: Dense settlement of green mussels on top of oysters on Gandy Bridge, Tampa Bay Florida (Photos by Dr. Patrick Baker, University of Florida)

Oyster reefs have the most dramatic effect on increasing fish production in mud bottom estuaries, such as Estero Bay, where they provide essential habitat in bays which would otherwise be barren (Grabowski et al., 2005). Abeels et al. (2012) identified oyster reefs in southwest Florida as an in important source for trophic transfer of carbon and biomass from reef to resident species to pelagic transients leading to a cascading effect on higher trophic levels influencing both coastal and pelagic systems. Many juvenile pelagic fishes forage on oyster reefs and, as they mature and leave the estuary, they transport nutrients and energy from the estuary into the marine food web through biomass (Beck et al., 2011). Many oceanic species depend on the productivity of estuarine systems to produce a stable food source (Beever et al., 2009). For example, four species of crustaceans, almost exclusively found on oyster reefs in southwest Florida, comprised at least 44% of the relative importance index of the diet of 12 species of fish

(Wasno et al., 2009). As a major contributor to the food web, a decrease in oyster reefs could lead to a cascading effect on the food chain, potentially hindering coastal fish production.

The permanent reef structure created by oysters aids in preventing and slowing erosion of the shoreline by stabilizing bottom sediments and creating a natural break wall to take the brunt of wind, wave energy, current action and boat wakes (Henderson and O'Neil, 2003; Coen et al., 2007). Reef systems form along the mangrove fringes and toward water currents creating habitat barriers, protection and increased sedimentation to support root growth for estuarine vegetation (sea grass beds, salt marshes and mangroves) (Henderson and O'Neil, 2003; Grabowski and Peterson, 2007). In protecting and enhancing grass beds, oyster reefs indirectly provide increased habitat for coastal fish and crabs (Grabowski and Peterson, 2007).

Through natural filter feeding activities, dense oyster populations may reduce the eutrophication of estuaries by speeding up the process of denitrification (Newell et al., 2002) and by removing suspended particles including organic matter, phytoplankton, microbial biomass and detritus from the water column (Dame, 1996; Dame et al., 2001). This behavior also aids in controlling and reducing phytoplankton blooms within estuarine systems, improving water quality and reducing turbidity leading to increased light attenuation (Baker et al., 1998; Barnes et al., 2007; Coen and Grizzle, 2007; Coen et al., 2007; Grabowski and Peterson, 2007). Through rapid filtration of organic matter from the water column and deposition to the seafloor as feces and pseudofeces oysters are key players in benthic pelagic coupling and nutrient cycling leading to an overall increase in productivity of the ecosystem (Dame, 1996). These processes enhance transfer of energy and material between the water column and benthic community (Coen and Grizzle, 2007) creating a direct link from primary producers to higher tropic levels (Barnes et al., 2007; Coen and Grizzle, 2007).

While green mussels can fill some of these ecological functions with similar filter feeding and benthic-pelagic coupling capabilities, they do not form permanent reefs, as oysters do; they instead attach to each other and hard surface areas through the production of byssal threads and, unlike oysters, create little three-dimensional habitat. If green mussels were to displace oysters they would not provide the same habitat services vital for the survival of many estuarine species (Baker et al., 2006). Additionally, byssal threads disintegrate upon death leaving the empty shell to wash away destroying what little habitat once existed as opposed to oysters which remain

cemented to the hard substrate or reef systems even after death. If mussels were to become the dominant bivalve just one mortality event could wipe out a substantial amount habitat essential to the survival of many estuarine and pelagic species.

In Estero Bay while juvenile green mussels may settle in intertidal regions and isolated mussels have been observed on oyster reefs, they do not appear to withstand extended periods of desiccation which may keep the two species in separate niches. However, if green mussels can invade to the estuarine portions of the bays and estuaries in southwest Florida and out-compete oysters for food and substrate, it could have impacts on the ecosystem functioning and recreational and commercial fisheries. Loss of oyster reefs will cause a loss of habitat for reef-resident species and those species which use the reef for nurseries and/or feeding grounds. Commercial and recreational fishing are economically important for southwest Florida and production is enhanced by the extensive oyster reef systems (Peterson et al., 2003; Grabowski et al., 2005). It has been estimated that approximately one in every three tourists visits Florida to fish, and in 2007 commercial fishing brought in $174 million of fish to the docks in Lee County alone (Beever et al., 2009).

6. Understanding population dynamics through applying the Dynamic Energy Budget Theory

Coastal regions of south Florida are highly urbanized and the dredging of channels and implementation of bridges, docks and markers for structural and navigational purposes has altered the watershed and created increased subtidal, hard substrate in an otherwise shallow, muddy bay (Antonio et al., 2002; DEP, 2013). This increase in artificial substrate adds to the difficulty of monitoring and invasions often go unnoticed until populations are well established. While many power plants use forms of mitigation such as chlorination and heat treatments (Rajagopal et al., 1991a; 1995; 1997) these practices are not suitable for wide eradication in estuarine waters and require continuous treatment or repeat dosing. Other forms of removal can be costly and require divers to search out locations and manually scrape the substrate to remove dense populations. These practices are retrospective and typically not put into effect until populations are already established, adding to the difficulty in control and mitigation. Biological modeling can serve as an aid to predict the spread of new species into new habitats and identify regions vulnerable to an invasion, allowing for a proactive mitigation plan.

Several studies have used scope for growth (SFG) or allometry to model individual growth of *P. viridis* (Shafee, 1978; Cheung, 1993; Wang et al., 2005; Hemachandra and Thippeswamy, 2008; Wang et al., 2011). These estimates however do not utilize a dynamic approach to a constantly changing environment and many different values for allometric growth equations are reported in the literature (Table 2), showing inadequacy in using allometry to characterize populations in a changing environment or compare between geographic ranges. When using allometry to model growth many authors report high seasonal variation in *b* values relating physical length to weight using the equation $W = aL^b$ where *a* and *b* are constants and W = dry weight and L = Length (Shafee, 1978; Cheung, 1993; Hemachandra and Thippeswamy, 2008). These values vary widely between geographic regions (Table 2) as they are dependent on the environmental conditions, leading to population specific estimations. Significant variations in parameters for *P. viridis* have been observed throughout the year and between sites within Tolo Harbor, Hong Kong (Cheung, 1990) highlighting the sensitivity of these parameters and the need for calibration between populations and over seasonal cycles. Reproductive state can lead to increased variations in allometric values with seasonal variations in gametogentic cycles (Cheung, 1993; Hemachandra and Thippeswamy, 2008) as well as differences between mature and immature individuals (Shafee, 1979; Mohan, 1980; Cheung, 1990). Production of gametes adds to the total mass of an individual, which is not accounted for in the simple length weight equation, as has been observed through seasonal variation in condition index which can often be linked to spawning and gametogenic cycles (Bernard et al., 2011). Thus, allometric models are species and site specific leaving them unable to predict outcomes between locations without a new calibration, even within the same species. Further, these predictions typically apply to a specific age group or life stage, which neglects changes in metabolism and bioenergetics that occur between life events; for example birth, metamorphosis and puberty all represent stages in the bivalve life cycle which represent major changes in energy dynamics and resource allocation (Kooijman, 2010).

Table 2: Summary of allometric exponents reported in the literature (*a* and *b*) and maximum length (L_∞). Several studies were completed from an aquaculture with monitoring stopped at the time of harvest (60 – 70 mm), thus maximum length was not reported.

Location	*a*	*b*	L_∞ (mm)	Reference
Malacca, Malasia	0.00024	2.60	102	Al-Barwani et al., 2007
Hong Kong	0.00112	2.37	101.1	Lee, 1986
Vengurla, India	0. 00066	2.24	150	Rao et al., 1975
Goa, India (total wt.)	0. 000109	2.26	-	Parulekar et al., 1982
Goa, India	0.000039	2.57[1]	-	Qasim et al., 1982
Goa, India	0.000033	2.66[1]	-	Qasim et al., 1982
Kakinada Bay, India	0.0000696	2.75	184.6	Narasimham, 1981
Penang, Malaysia	0.0000222	2.76	89.4	Choo and Speiser, 1979
Madras, India	0. 00277	3.03	159.5	Shafee, 1979
Tolo Harbor, Hong Kong	0.0263	0.76[1]		Cheung, 1990
Tolo Harbor, Hong Kong	0.000000044	3.9 [1]	100 – 223[1]	Cheung, 1990*
Singapore	0.0981	2.79[2]	-	Cheong and Chen, 1980
Chachoengsao, Thailand	0. 07067	2.78[2]	-	Chonchuenchob et al., 1980
Udupi, India	0.005	2.93[2]	-	Hemachandra and Thippeswamy, 2008

*high variation in values between months and populations
[1] variation between sites / populations
[2] measurements in cm, all others in mm

These discrepancies between studies are likely a result of site specific environmental factors affecting physiological condition of the individual including food availability, temperature, presence of stressors, etc. Shell shape, which more accurately explains the size of the organism, is largely influenced by these environmental variables (Hemachandra and Thippeswamy, 2008). Individuals from different populations with similar dry weights, but variations in shape may result in differences in length weight relationships, thus partially explaining variation in allometric constants. Indeed, Mohan (1980) argued that the allometric equation $W = aL^b$ cannot accurately be applied across the lifespan of *P. viridis*.

Incorporating environmental parameters into biological modeling is essential to compare predicted outcomes with natural observations and between populations of different geographical regions. The environment is a dynamic system, continuously changing regarding one or more parameters which makes predictions from laboratory studies and simulations difficult. Further, energy allocation is a complex process of metabolic requirements of growth, maturation and reproduction each with their own overhead and maintenance costs. These metabolic process are sensitive to environmental factors complicating predictions and are often left unaccounted for in basic bioenegertic models leaving comparisons between populations difficult.

The Dynamic Energy Budget (DEB) theory for metabolic organization (Kooijman, 2010) offers a framework that can be applied to any species. DEB uses food and temperature as forcing variables to predict changes in metabolic responses and the cascading effects on growth and maturation of the organism. This allows for a more accurate representation of the dynamics of the mass and energy budgets under realistic environmental conditions and removes the problem observed in SFG models in which parameters are measured under constant environmental conditions making the comparison between populations or even seasons difficult.

The DEB theory characterizes the dynamics of metabolism and energetics throughout the lifespan of an individual based on a basic set of data regarding physical attributes that describe the organism (ie: lengths, weights, and ages at different maturation time points) in relation to temperature and food availability (from Lika et al., 2014). The standard DEB model assumes three basic life stages; embryo, juvenile and adult, and three state variables; structural volume (V), reserve energy (E), and maturity / reproduction buffer (E_H). The functional response, f, depends on food availability and is categorized on a scale of 0 – 1, with 0 being starvation conditions and 1 being *ad libitum*. The basics of energy flow within an organism can be explained by figure 4. For bivalves, food is taken in via filter feeding activities ($\dot{p}_X$) and assimilated ($\dot{p}_A$) into reserves with a portion of energy from the food lost to heat dissipation and feces production. Food uptake is proportional to surface area and depends on the food density or availability (f). Energy is mobilized from the reserves ($\dot{p}_C$) and a fix fraction (κ) goes towards somatic maintenance ($\dot{p}_S$) and growth ($\dot{p}_G$) with the remainder (1-κ) used for maturation in juveniles or production of gametes in adults ($\dot{p}_R$) and maturity maintenance ($\dot{p}_J$). All above mentioned fluxes have the units of energy over time ($e\ t^{-1}$) and f and κ are unitless ratios. Somatic maintenance is given priority over growth and allocation to reproduction / gametes only occurs once maturation is complete.

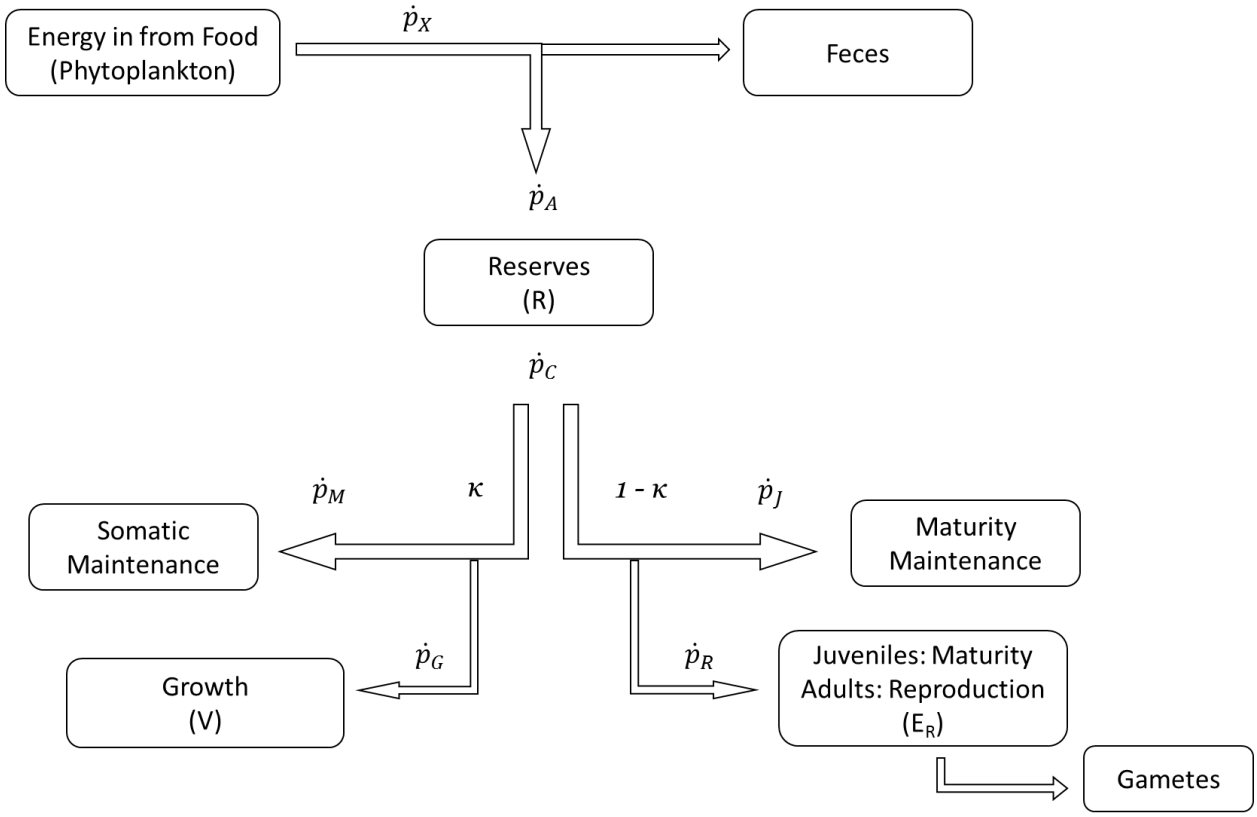

Figure 4: Flow of energy through the organisms in a DEB context.

Reserves are continuously and simultaneously used and replaced. The rate at which energy is mobilized from reserves, energy conductance ($\dot{v}$), remains constant. Somatic maintenance refers to the continual energy input to maintain the continuous building / re-building of structure (ie: protein turnover), while maturity maintenance includes overhead costs of maturation and cleaning up of gonads post spawning to prepare for a new cycle. Because of strong homeostasis, while the reserve relative to structure may change, the chemical composition of reserves and structure remains constant (van der Meer, 2006). Life stages are linked to maturity and with bivalves include, birth, metamorphosis, and puberty representing periods of change in energy flux and metabolism. Embryos grow, but do not feed; juveniles feed, but do not reproduce; adults feed and reproduce leading to a variation in physiological rates and allocation priorities between life stages, which is controlled in the model by a maturity variable for each stage.

A primary set of parameters are estimated based on physical attributes at different life stages which are inherently linked to physiological rates and influenced by temperature and food (energy). Parameters will change in a changing environment as physiological rates are affected by environmental conditions. To account for these changes the species specific Arrhenius temperature is incorporated using the equation:

$$\dot{k}(T) = \dot{k}_1 exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\}$$

Where $\dot{k}$ is the selected physiological rate, T is the ambient temperature, T_1 is the reference temperature, $\dot{k}_1$ is the physiological rate measured at T_1, and T_A is the Arrhenius temperature. DEB theory assumes that temperature affects all physiological rates in the same manner and the incorporation of the Arrhenius relationship allows for accurate predictions of the physiological response to changing temperatures. Knowing the upper and lower thermal limits allows for restrictions to be placed on the organism to control changes in metabolic rates with changes in temperature. Temperature is especially important for bivalves which, as sessile ectotherms, are subject to environmental fluctuations in temperature.

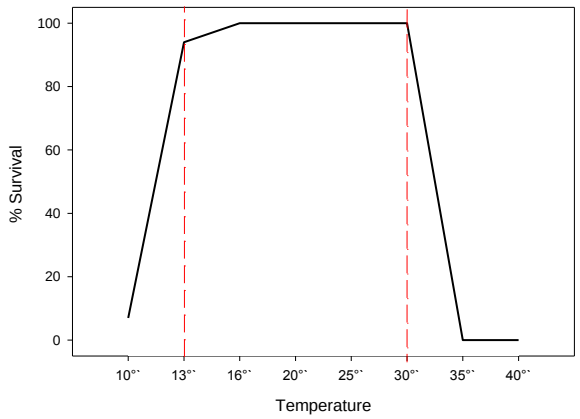

Figure 5: Temperature tolerance range as defined by McFarland et al. (2014) for local *P. viridis* populations.

DEB models have been successfully applied to many bivalves, primarily as a tool for aquaculture (Ren and Ross, 2005; Ren and Schiel, 2008; Rico-Villa et al., 2010) and assessment of natural populations / culture sites (Pouvreau et al., 2006; Rosland et al., 2009; Sará et al., 2012; Lavaud et al., 2013). Utilization of such models can also aid in understanding of

population dynamics of a new species. This information may further be used to predict the growth and reproduction under different environmental conditions, which may help identify regions vulnerable to species invasions based on local environmental conditions. Through this work we aim to characterize locally established populations of *P. viridis* in southwest Florida through monthly field monitoring of growth, recruitment, gametogenesis, and energy storage cycles. This information and data previously reported in the literature will be used to 1.) Determine the primary parameter set required for the standard DEB model, 2.) Develop a working model that accurately predicts growth and reproduction of *P. viridis*, 3.) Validate the model by plotting growth and reproduction from other regions by only changing environmental parameters (temperature and food). For all three steps, separate data set are used to assure accurate calibration and validation.

Objectives of the Study

The main objective of this study was to characterize the population dynamics of the invasive green mussel, *Perna viridis*, through field monitoring of established populations and application of the Dynamic Energy Budget (DEB) Theory. Data from the literature was utilized and a two year study of growth, reproduction, larval recruitment and proximal biochemical composition of a local population to aid in the parameterization and calibration of the model predicting growth and reproduction of *P. viridis* under local environmental conditions. Further, we aimed to describe the uptake and elimination brevetoxins produced by the toxic algae *K. brevis* and the effects on *P. viridis* population structure during natural exposures in the field.

- **Paper I:** Analyze the natural uptake, accumulation and depuration of brevetoxin, a neurotoxin produced by the red tide forming dinoflagellate *Karenia brevis*, during and following two consecutive natural blooms.
- **Paper II:** Investigate effects of *K. brevis* on growth, survival and juvenile recruitment in existing population of green mussels through field monitoring during natural *Karenia brevis* blooms in Estero Bay, Florida.
- **Paper III:** Investigate seasonal patterns in reproduction and energy storage in established populations of green mussels in Estero Bay, Florida.

- **Paper IV:** Apply what we have learned from both the literature and field and lab studies to create a working model to predict individual growth and reproduction of *P. viridis* utilizing the Dynamic Energy Budget Theory.

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Chapter 2: Uptake and elimination of brevetoxins

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Uptake and elimination of brevetoxin in the invasive green mussel, *Perna viridis*, during natural *Karenia brevis* blooms in southwest Florida

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ABSTRACT

Perna viridis is a recently introduced species to US coastal waters and have vigorously spread throughout the southeastern seaboard since their invasion. Little information regarding their response to local environmental factors has been reported including responses to the local HAB species, *Karenia brevis*. This study monitored the tissue toxin concentration of brevetoxins in *P. viridis* from existing populations throughout two consecutive natural *K. brevis* blooms. The results showed *P. viridis* to rapidly accumulate PbTx upon exposure to the bloom, far exceeding the peak tissue concentrations of oysters, *Crassostrea virginica*, sampled during the same period, 57,653 ± 15,937 and 33,462 ± 10,391 ng g^{-1} PbTx-3 equivalent, respectively. Further, *P. viridis* retained high PbTx concentrations in their tissues post bloom remaining above the regulatory limit for human consumption for 4–5 months, significantly longer than the depuration time of 2–8 weeks for native oyster and clam species. In the second year, the bloom persisted at high cell concentrations resulting in prolonged exposure and higher PbTx tissue concentrations indicating increased bioaccumulation in green mussels. While this species is not currently harvested for human consumption, the threat for post bloom trophic transfer could pose negative impacts on other important fisheries and higher food web implications.

1. Introduction

Red tide events occur regularly in the Gulf of Mexico, with the first recorded event in 1844 (Lasker and Smith, 1954) and identified as *Karenia brevis* in 1946 (Davis, 1948). This unarmored dinoflagellate produces a suite of neurotoxins known as brevetoxins (PbTx), which cause massive fish kills, marine mammal and seabird mortalities and neurotoxic shellfish poisoning (NSP) in humans through consumption of toxic shellfish (Ray and Wilson, 1957; McFarren et al., 1965; Adams et al., 1968; Forrester et al., 1977; Baden, 1989; Steidinger et al., 1973; Flewelling et al., 2005; Naar et al., 2007; Landsberg et al., 2009). Over the past several decades outbreaks of *K. brevis* blooms have increased in frequency and duration due to eutrophication of coastal waters through anthropogenic input (Brand and Compton, 2007).

Bivalve molluscs bioaccumulate PbTx through normal filter feeding behavior with little to no mortality and, as a consequence, pose a threat to both human consumers and natural marine predators including whelks, crab, and fish (Tester et al., 2000; Brand et al., 2012). Several studies have demonstrated the transfer of PbTx from bivalves to whelks and fish (Ingham et al., 1986; Pierce et al., 2002; Naar et al., 2007; Bricelj et al., 2012). Shellfish beds are monitored regularly for both *K. brevis* cell counts and tissue toxin concentration, particularly during and following bloom events. Closure of shellfish harvesting is required if *K. brevis* cell counts exceed 5000 cells L^{-1} or shellfish tissue concentrations exceed 800 ng g^{-1} PbTx-3 equivalent (Plakas et al., 2008).

Abbreviations: PbTx, brevetoxin; HAB, harmful algal bloom; STX, saxitoxin; NSP, neurotoxic shellfish poisoning.

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The green mussel, *Perna viridis*, is a recently introduced species to southwest Florida. The extent to which PbTx accumulates and persists in their tissues remains to be elucidated and is not monitored. If PbTx remains sequestered in tissues after bloom dissipation this poses a threat to predators including commercially important crab and fish, subsequently transferring toxins through the trophic web even long after a red tide event has subsided. Stomach content analyses in post bloom fish and dolphin mortalities have revealed fish with high levels of PbTx (Flewelling et al., 2005) and seabirds with toxic clams in the gut (Forrester et al., 1977) indicating a lethal dose through trophic transfer (Landsberg et al., 2009). Likewise, dead burrfish (*Chilomycterus schoepfi*) collected during a red tide bloom had the remains of toxic bivalves in their stomachs (Naar et al., 2007). While green mussels are not harvested locally for human consumption, they are an edible species and may pose potential human health concerns.

Accumulation of PbTx has been reported in the green lipped mussel, *Perna canaliculus*, during exposure to *Karenia selliformis* blooms (Morohashi et al., 1995; Ishida et al., 2004a, 2004b), however no published information directly addresses the accumulation of PbTx from *K. brevis* blooms in *P. viridis*. Therefore, the goal of this study was to assess the rate of PbTx uptake and elimination in *P. viridis* during a naturally occurring *K. brevis* bloom. This was accomplished through monthly collections from a population, which had become established in southwest Florida, before, during and after a red tide event in Estero Bay, Florida over a two year study period.

2. Material and methods

2.1. Collection of Perna viridis

Perna viridis of an average length of 74.3 ± 7.3 mm were collected by SCUBA diving from Estero Bay, Florida at New Pass Bridge (26°22′40.89″N; 81°51′39.69″W) or Big Carlos Bridge (26°24′15.44″N; 81°52′49.64″W) once a month from October 2011 through October 2013 (N = 5/month). Immediately following collections mussels were cleaned of epiphytic growth, soft tissue was dissected from the shell and individually frozen at −80 °C. Whole individuals were homogenized cryogenically to a powder using a MixerMill 400 (Retsch® Solutions in Milling and Sieving, Hann, Germany) under liquid nitrogen and one gram wet tissue was weighed for PbTx extraction. For samples from October 2011 through December 2012 archived freeze-dried tissue was used following the same extraction method using wet to dry weight ratios to determine weight for each individual (Dr. Leanne Flewelling, personal communication).

2.2. Bloom formation

Karina brevis cell counts were obtained from Florida Wildlife Research Institute monitoring program. More frequent sampling was conducted in the Sanibel/Captiva area than Estero Bay due to the presence of several commercial shellfish beds. Because it is located less than 30 km north of the study site, monitoring data from Sanibel/Captiva was used to supplement data from the study site.

Onset of the bloom in local waters first occurred in the Sanibel/Captiva area in September 2011 and was observed in Estero Bay (study site) in November 2011 accompanied by a large fish kill consisting primarily of mullet, *Mugil cephalus*. The bloom persisted into January 2012, but cell counts diminished by the end of the month. This cycle was observed again in 2012–2013 with an increase in cell concentrations in the Sanibel/Captiva area in late September 2012 and Estero Bay by mid-October 2012. The boom persisted into February 2013 at which time a fish kill comprising several species was observed on Ft. Myers Beach. The bloom peaked in February and diminished by the end of April 2013.

2.3. Analysis of PbTx in tissues

All samples were extracted in methanol according to Naar et al. (2002); briefly the protocol is as follows. One gram homogenized wet tissue (or its equivalent in dry tissue) was vortexed well in 80% methanol, heated in a water bath at 60 °C for 20 min, then transferred to an ice bath for 10 min and centrifuged. The supernatant was poured off and stored on ice and these steps repeated on the remaining precipitate. The combined supernatants were brought to a total volume of 10 mL 80% methanol, washed with hexane and stored at −20 °C until further analysis.

Brevetoxin ELISA assays were run within seven days of the tissue extraction using a competitive ELISA kit prepared at the University of North Carolina Wilmington according to Griffith et al. (2013). The assay was completed in a 96-well microplate (Nunc-Immunoplate with Maxisorp surface, Thermo Scientific™, Waltham, Massachusetts). The plate was first coated with 100 μL PbTx-3-bovine serum albumin conjugate and incubated on a plate shaker for one hour. The plate was then washed with Phosphate Buffered Saline (PBS) followed by adding a blocking buffer (Superblock™ dry blend) (200 μL) and incubated for 30 min on a plate shaker. Following the incubation period the plate was washed with PBS-Tween™ (buffered detergent). Samples and standards were loaded (100 μL) onto the plate in duplicates with the addition of a primary antibody (100 μL) and incubated on a plate shaker for 1 h. The plate was washed again with PBS-Tween™, then a secondary antibody was loaded into the wells (100 μL) and again incubated for 1 h. Following this incubation the plate was washed with PBS-Tween™ and a final PBS wash. Tetramethylbenzidine (TMB) was added as an indicator and the reaction stopped after approximately three minutes using 0.5 M H_2SO_4. The absorbance was measured at 450 nm on a TECAN Genois Pro® plate reader (TECAN Group Ltd., Männedorf, Switzerland). The antibody used detects type-2 brevetoxins which make up 90–95% of the toxins produced during blooms and include PbTx-2, PbTx-3 and PbTx-9 (Naar et al., 2002). Results are presented as PbTx-3 equivalent to reflect the standard curve used in calculations of PbTx concentrations.

2.4. Statistical analysis

To assess PbTx concentrations throughout each bloom, paired t-tests were performed to assess differences across time within each collection group. Due to non-normality of the data, the intensity of post-bloom tissue PbTx accumulation was assessed through integrated trapezoidal area-under-the-curves regarding increase from baseline (AUCi) according to the previously published formula by Pruessner et al. (2003). AUCi was calculated each month separately to compare cumulative increase in PbTx in relation to onset of the bloom between years. The differences in the intensity of PbTx accumulation (AUCi) during corresponding months following *K. brevis* blooms (2012 and 2013) were evaluated using independent t-tests. All data are presented as means ± S.D. with statistical significance being defined as $p \leq 0.05$.

3. Results

Green mussels accumulated high levels of PbTx in their tissues during a natural exposure to *K. brevis*. Tissue PbTx concentrations reached a peak in February 2013 (137,000 ± 40,000 ng g^{-1} PbTx-3 equivalent) following a bloom phase which lasted approximately four months. Peak tissue PbTx concentrations corresponded with

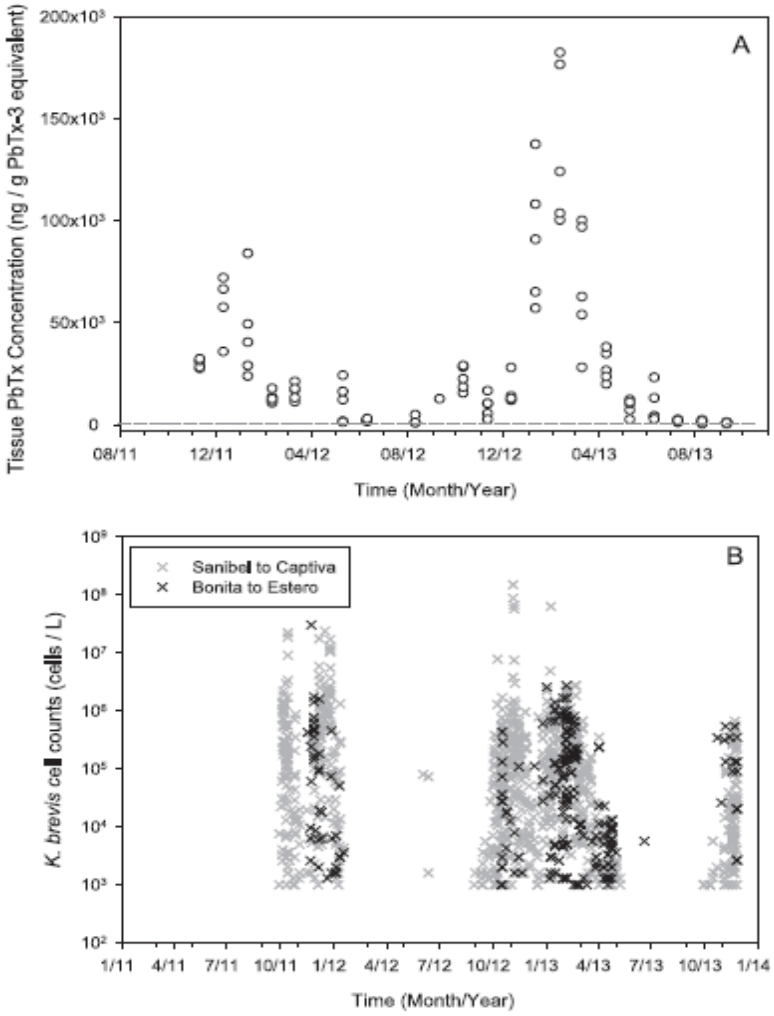

Fig. 1. Brevetoxin concentration in individual mussels plotted over time (A). Dashed line indicates the regulatory limit (800 ng g $^{-1}$ PbTx-3 equivalent). *Karenia brevis* cell counts (log scale) by location over time (B).

peaks in cell counts and duration of bloom (Fig. 1). Tissue toxin concentration remained above the regulatory limit (800 ng g^{-1} PbTx-3 equivalent) four to five months post bloom dissipation (Fig. 1A).

The second year of exposure showed a higher peak in toxin concentration compared to the first year (Fig. 1A), coinciding with a more intense and prolonged bloom during the second year (Fig. 1B). Intensity of PbTx accumulation (AUCi) was significantly different between the two bloom years ($p \leq 0.001$) in which a slower accumulation of PbTx was observed during the second bloom with a peak in PbTx tissue concentrations at the end of the bloom after four months of exposure (Fig. 2). During both bloom years, mussels eliminated PbTx while *K. brevis* was present in the water column, during the waning phase of the bloom in late January 2012 and March–April 2013 (Fig. 1), however this process was slow; PbTx concentration in tissue remained above the regulatory limit until September 2013 and never fell below regulatory limit in 2012.

In March 2012, a population-wide mass mortality event was observed in the green mussels that decimated the population, leaving only sporadic individuals. During the time of the mortality event the average PbTx tissue concentration in the green mussels was 15,694 ± 3885 ng g^{-1} PbTx-3 equivalent. This mortality event made the collection of adults impossible in April and July 2012 and difficult throughout the summer months. Smaller mortality events continued to be observed throughout the remainder of the monitoring period with the highest mortality observed in the fall of 2012 and 2013 with the return of bloom status in *K. brevis*.

4. Discussion

Through monthly monitoring of PbTx concentrations in the soft tissue of *Perna viridis* established in southwest Florida before, during and after two consecutive *Karenia brevis* blooms, we were able to characterize the uptake and elimination under natural

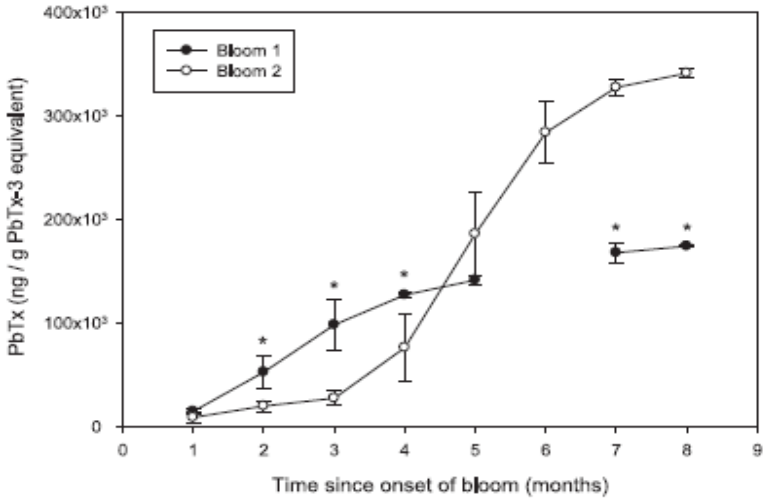

Fig. 2. Relative concentrations of PbTx in green mussel tissues in relation to onset of the bloom for comparison between the two bloom cycles as determined by AUC analyses. Error bars represent standard deviation and * indicate significant differences between blooms.

conditions. The data presented here demonstrate that *P. viridis* rapidly accumulated large quantities of PbTx in their tissues and concentrations remained above the regulatory limit (800 ng g^{-1} PbTx-3 equivalent) for an extended period even after bloom dissipation, suggesting the potential for trophic transfer up to five months post bloom.

PbTx tissue concentrations corresponded well with the *K. brevis* cell counts showing peaks in tissue concentrations during and following peaks in water column cell concentrations, suggesting that rapid uptake likely occurred through filtering of whole *K. brevis* cells. Free PbTx in the water column may have contributed to high tissue concentrations. Water column PbTx concentrations range from 0.4 to 0.12 μg L^{-1} when cell counts are $\leq 2.4 \times 10^3$ cells L^{-1} (Pierce et al., 2005) and frequently exceed 24 μg L^{-1} during bloom concentrations of 10^6 cells L^{-1} (Pierce et al., 2008). Likewise, Rolton (2015) detected PbTx in *K. brevis* culture filtrate to range from 5 to 51 μg L^{-1}. The peak in tissue concentration occurred near the end of the bloom indicating continued bioaccumulation of the toxin in mussels, which did not die, leading to increased tissue concentrations through continuous exposure. Tissue PbTx concentrations were higher in the second year and persisted at elevated levels for an extended period due to the prolonged bloom conditions in 2013 versus 2012 as both elevated exposure intensities and duration affect the accumulation and depuration rate (Morton and Burklew, 1969; Bricelj and Shumway, 1998). However, this peak in tissue concentration occurred late in the bloom cycle after four months of exposure suggesting bioaccumulation and an inability to eliminate toxins during prolonged exposure. Previous exposure history is also a contributing factor to the increased toxin levels in 2013 as tissue toxin concentrations never fell below the regulatory limit following the 2011–2012 bloom. In June 2012, *K. brevis* cell counts reached bloom concentrations for several days at which time it appears the uptake rate exceeded the elimination rate resulting in a small spike in tissue PbTx concentration, prolonging the elimination period.

Table 1
Southwest Florida bivalve species and the reported time required to depurate PbTx to levels below the regulatory (800 ng g^{-1} PbTx-3 equivalent) limit following exposure to *K. brevis*.

Species	Depuration time	Exposure	Source
Crassostrea virginica	2 weeks	lab	Plakas et al., 2002; Griffith et al., 2013
Crassostrea virginica	2 weeks	field	Plakas et al., 2008
Crassostrea virginica	2–8 weeks	field	Morton and Burklew, 1969
Crassostrea virginica	10 weeks	field	Dickey et al., 1999
Crassostrea virginica	6 weeks	field	Pierce et al., 2002
Mercenaria mercenaria	6 weeks	field	Pierce et al., 2002
Mercenaria mercenaria	2 weeks	lab	Griffith et al., 2013
Perna viridis	16 – 20 weeks	field	This Study

While PbTx accumulation and elimination has not previously been studied in *P. viridis*, evidence for metabolism/biotransformation of PbTx has been found in several bivalve species including the closely related New Zealand green-lipped mussel *Perna canaliculus* (Ishida et al., 2004a, 1995, 2004b; Morohashi et al., 1995; Murata et al., 1998; Nozawa et al., 2003). In both field and controlled laboratory exposures, *P. canaliculus*, showed both an accumulation of PbTx and metabolism from PbTx-2 (most prevalent in the *K. brevis* cell) to PbTx-3 and BTX-5 at rates dependent upon the intensity and duration of exposure (Ishida et al., 2004a, 2004b).

When compared with native Florida bivalves, oysters and clams, *Crassostrea virginica* and *Mercenaria mercenaria*, *P. viridis* appear to accumulate higher PbTx concentrations and demonstrate slower elimination rates. These local bivalves have been shown to accumulate PbTx with high survival and rapid depuration rates of 2–8 weeks (Table 1) (Morton and Burklew, 1969; Steidinger and Ingle, 1972; Plakas et al., 2002, 2004, 2008; Bricelj et al., 2012; Griffith et al., 2013). *Crassostrea virginica* sampled from Pine Island Sound, just 30 km north of Estero Bay, during the bloom of 2011–2012 were shown to reach a peak in PbTx levels averaging approximately 35,000 ng g^{-1} PbTx-3 equivalent but exhibited a rapid initial elimination rate, decreasing to 7500 ng g^{-1} PbTx-3 equivalent just two weeks after bloom dissipation and dropped below the regulatory limit within 2 ½ months (Volety et al., unpublished results). *Perna viridis* sampled from Estero Bay during this period exhibited nearly double (~58,000 ng g^{-1} PbTx-3 equivalent) the PbTx concentrations of *C. virginica* (Table 2) and did not fall below the regulatory limit before the return of bloom conditions in October 2012 and a second exposure period.

Field monitoring of bivalves during red tide events in Tampa Bay, Florida have reported tissue concentrations in *C. virginica* ranging from 2800 to 16,500 ng g^{-1} (Pierce et al., 2002; Plakas et al., 2008) to as high as 28,660–80,000 ng g^{-1} (Dickey et al., 1999; Weidner et al., 2002) and *M. mercenaria* ranging from 1800 to 6600 ng g^{-1} (Poli et al., 2000; Pierce et al., 2002; Weidner et al., 2002). In controlled exposures, Griffith et al. (2013) found *C. virginica* and *M. mercenaria* to reach 2000 and 1000 ng g^{-1}, respectively after eight days of exposure with acute daily dosing of 5×10^5 cells L^{-1}, whereas Plakas et al. (2004) reported tissue concentrations in *C. virginica* of 740 ng g^{-1} after only two doses of 10^4 cells L^{-1} over 48 h. Concentrations observed in *P. viridis* tissues are higher than those observed in local species. This is likely due to a combination of several factors including differences in metabolic activity (uptake, ingestion, and assimilation rates), growth rates, energy turnover, and/or reduced mechanism for "detoxifying"/metabolizing PbTx which could lead to increased tissue damage and physiological impairment. Additionally, PbTx's are lipophilic, thus both lipid composition and lipid turnover rate will play a role in the accumulation and elimination rates and will vary between species and within species at different times of the year or reproductive cycle (Svensson and Förlin, 2004).

Previous work has shown several mussel species to rapidly accumulate high concentrations of toxins produced by HAB's compared to many oyster and clam species (Ingham et al., 1986; Shumway and Cucci, 1987; Shumway et al., 1988, 1990, 1995; Bricelj and Shumway, 1998; Lesser and Shumway, 1993). In the Philippines, *P. viridis* was found to be highly toxic with low survival following a *Pyrodinium bahamense* var. *compressa* red tide event in 1983 reaching 9620 MU 100 g^{-1} saxitoxin (STX) (~18,000 ng g^{-1}) leading to an eight month shellfish ban due to prolonged toxicity (Gacutan et al., 1984) and in more recent blooms, *P. viridis* reached concentrations as high as 90,000 ng g^{-1} STX, far exceeding the regulatory limit while other local bivalves showed minimal toxicity (Montojo et al., 2010, 2012). Jaafar et al. (1989) reported high tissue STX concentrations in *P. viridis* during a *P. bahamense* bloom in Malaysia reaching 50, 354 ng g^{-1}, but contrary to blooms in the Philippines showed rapid elimination rates.

Species specific metabolism of PbTx has been reported widely throughout the literature (Cummins et al., 1971; Steidinger et al., 1998; Nozawa et al., 2003; Ishida et al., 2004a, 2004b; Abraham et al., 2012; Echevarria et al., 2012) offering a likely explanation to observable differences between *P. viridis* in this study and those reported for *C. virginica* and *M. mercenaria*. Indeed, Ishida et al. (2004b) found a significant difference in PbTx metabolism in *P. canaliculus* and *Crassostrea gigas*, two similar species to those compared in this study, with different concentrations of metabolites found in each indicating similar pathways, but different reaction rates. *C. gigas* showed a more rapid transformation of PbTx-2 to PbTx-3 compared to *P. canaliculus*. While both species had PbTx-3, BTX-B1 and BTX-B5, *P. canaliculus* also produced BTX-B2, BTX-B3 and BTX-B4, but *C. gigas* did not (Ishida et al., 2004b; Morohashi et al., 1999; Nozawa et al., 2003). Concentrations of metabolites present may effect tissue retention time between species as different derivatives may be stored differently and in various tissue compartments, thus effecting elimination rates (Echevarria et al., 2012). Detoxification is partially dependent upon the peak tissue concentration (Bricelj and Shumway, 1998) and previous exposure history can affect the uptake kinetics of biotoxins (Shumway and Cucci, 1987). Feeding behavior and metabolic demands play a significant role in differences in accumulation rates between species. *P. viridis* has been shown to have high clearance rates compared to *C. virginica* (McFarland et al., 2013) suggesting increased exposure due to increased filtration of *K. brevis* cells. Additionally, *P. viridis* has high growth and reproductive rates leading to increased metabolic demands (Rajagopal et al., 2006; Siddall, 1980; Vakily, 1989) and increased nutrient requirements which may increase filtration behavior causing increased exposure to the toxin.

As a recently introduced species, *P. viridis* may lack the needed adaptions for rapid PbTx elimination observed in *C. virginica* and *M. mercenaria*, which have a long history of *K. brevis* exposure during which PbTx tolerance and metabolism may have developed, existing in coastal waters of the eastern US seaboard since the 1800's (NOAA, 2005; Volety et al., 2014). Previously exposed bivalves have shown increased resistance and reduced sensitivity to HAB exposure compared to unexposed populations (Shumway and Gainey, 1992). While *P. viridis* experiences harmful algal blooms in their native range (Gacutan et al., 1984, 1985; Choi et al., 2003; Li et al., 2005; Montojo et al., 2012), exposure to a new toxin may cause reduced physiological function and could cause the widespread mortality observed in southwest Florida. Leverone et al. (2007) observed decreased clearance rates in juvenile *P. viridis* during short term exposure to *K. brevis* indicating reduced feeding, which may turn lethal if conditions persist. Baker et al. (2012) documented a population-wide mortality event in Tampa Bay following a red tide bloom from which green mussel populations never fully recovered and Gacutan et al. (1984) reported >90% *P. viridis* mortality following a red tide event caused by *P. bahamense* in the Philippines. In the current study, a similar mortality event was observed two months post bloom dissipation, however tissue toxin concentrations were still relatively high (15,700 ng g^{-1} PbTx-3 equivalent). The lag time between peak tissue PbTx concentration and the first mortality event indicates

Table 2
Comparison of green mussel and local oyster average tissue brevetoxin concentrations during the 2011–2012 *K. brevis* bloom. PbTx concentrations expressed as ng g^{-1} PbTx-3 equivalent.

Month	*Perna viridis* (this study)		*Crassostrea virginica* (Volety et al., unpublished results)		Red tide status (FWRI)
	PbTx	SD	PbTx	SD	
Oct. 2011	N.D.				
Nov. 2011	29,696	2051			bloom
Dec. 2011	57,653	15,937	33,462	10,391	bloom
Jan. 2012	44,982	23,786	7583	2516	waning
Feb. 2012	12,913	2709	6406	1010	gone
Mar. 2012	15,694	3885	4159	309	
April 2012	no sampling[a]		810	317	
May 2012	10,806	9815	no sampling		

N.D. indicates that brevetoxin levels were below the detection limit.
[a] April collections could not be completed for *P. viridis* due to a mass mortality event in March 2012 severely reducing the population in addition to poor visibility during SCUBA diving making collections impossible.

that other factors are likely involved. Indeed, it coincided with a major spawning period which may have inflicted additional stress on the animal. Galimany et al. (2008) showed a decreased ability to resume normal tissue function, including paralysis of the adductor muscle and prolonged histopathology in *Mytilus edulis* which had spawned during a laboratory exposure to *Alexandrium fundyense*. While the mortality events observed in this study cannot be directly related to *K. brevis* exposure, *P. viridis* had high levels of accumulated toxins in their tissues during the observed mortality indicating the potential for prolonged cumulative effects of high tissue toxin burden and physiological stress. Allelopathic, hemolytic, and other ichthyotoxic compounds produced by *K. brevis* may also contribute to the negative effects observed in marine organisms (Kubanek et al., 2005; Marshall et al., 2005). While prolonged elevated tissue toxin levels are of concern for trophic transfer, if the mussels die the risk is reduced and invasive species kept at bay.

5. Conclusion

To date, the green mussel populations have not recovered from mortalities observed during red tide events in Estero or Tampa Bay, Florida (Baker et al., 2012; this study). However, not all green mussels died during *K. brevis* exposure as collections of adults were made in the months following the mortality event. Thus, seemingly normal mussels may contain high levels of PbTx for long periods post bloom posing a threat to both natural marine predators and human consumption. Landsberg et al. (2009) attributed post bloom mortality in several fish species and blue crab to trophic transfer from feeding on benthic fauna and post bloom dolphin mortalities in 2004 to trophic transfer from toxic fish. Brand and Compton (2007) noted an increase in the presence of sublethal or background concentrations of *K. brevis* which may be contributing to prolonged tissue toxins post bloom and several studies have noted the persistence of PbTx in marine sediments and seagrass epiphytes (Shumway and Cucci, 1987; Mendoza et al., 2008). Additionally, whole cell and temporary encystment of toxic dinoflagellates have been observed in bivalve biodeposits following exposure leading to increased risk for transport and increased exposure to benthic organisms via deposition into the sediments (Hégaret et al., 2008). Even at low tissue concentrations, this persistence allows for long term exposure to higher trophic levels through biomagnification post bloom (Rounsefell and Nelson, 1966; Landsberg, 2002; Flewelling et al., 2005). Animals that prey directly on benthic filter feeders including gastropods, crab, fish and water birds are at risk of exposure and may pose a threat to higher trophic levels through trophic transfer of the toxins by themselves becoming prey (Shumway et al., 1995; Pierce et al., 2002; Flewelling et al., 2005; Hégaret et al., 2008; Bricelj et al., 2012).

This study has demonstrated the natural accumulation and depuration rates of local *P. viridis* populations with PbTx concentrations and depuration periods exceeding that of native shellfish in southwest Florida. Further research on the potential trophic transfer from contaminated mussels to commercially important fish and crab is vital to monitoring the bloom and post bloom risks. Additionally, as *P. viridis* is an edible species, both monitoring and public awareness are essential to prevent cases of NSP during post bloom periods in which mussels may still be toxic.

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Chapter 3: Growth, mortality and juvenile recruitment

Potential impacts of blooms of the toxic dinoflagellate *Karenia brevis* on the growth, survival and juvenile recruitment of the non-native green mussel *Perna viridis* in southwest Florida

Abstract

Red tide blooms formed by *Karenia brevis* are frequent along the Gulf coast of Florida and it is unclear what tolerance the green mussel *Perna viridis*, a recently introduced species to coastal waters, has toward these events. Established populations of *P. viridis* were monitored along the coastal waters of Estero Bay, Florida before, during and following two consecutive red tide blooms to assess the potential effects on growth, survival and juvenile recruitment. Upon onset of the bloom, growth rates fell from 6 – 10 mm month^{-1} (March 2011 – November 2011) to less than 3 mm month^{-1}. In the succeeding years, *K. brevis* blooms were present, and average growth of individually tagged mussels remained below 3 mm month^{-1}. During growth monitoring the use of calcein as an internal marker was tested with positive staining results and no observed effect on growth or survival. In March 2012, following the first red tide bloom, a population-wide mortality event was observed. Following this event, increased mortality rates were observed with peaks during onset of the bloom in the fall of 2012 and 2013. Juvenile recruitment was also limited during years in which blooms persisted into the spring spawning period suggesting gamete and / or larval sensitivity to *K. brevis*. Although it cannot be conclusively determined that the cause of reduced growth and survival is due to red tide events, the parallels observed suggest that *K. brevis* is a factor in the observed changes in population structure.

Key Words: invasive species, larval development, red tide, growth rate, brevetoxin

Worldwide, harmful algal blooms (HABs) have been increasing in frequency, contributing to great economic loss (Anderson et al. 2000). Red tide blooms formed by the toxic dinoflagellate *Karenia brevis* are a regular occurrence in the Gulf of Mexico, especially along the coast of Florida, resulting in high fish mortality rates (Naar et al. 2007; Ray & Wilson 1957) and marine

mammal, sea turtle, and seabird casualties (Flewelling et al. 2005, Landsberg et al. 2009). Conversely, local bivalves have been documented to have high survival rates during red tide blooms (Pierce et al. 2002; Plakas et al. 2008); however, sublethal effects during short term laboratory studies have demonstrated reduced clearance rates (Leverone et al. 2007), loss of muscle control (Roberts et al. 1979), increased susceptibility to disease and infection (Landsberg 2002), and subcellular effects including lysosomal disruption and alterations in lipid peroxidation (Keppler et al. 2006). Sessile organisms, such as marine bivalves, may be exposed to sublethal concentrations that persist for months, potentially amplifying adverse effects observed in laboratory studies and presenting greater consequences to overall health, growth, and survival of the organism due to chronic effects of prolonged exposure (Griffith et al. 2013; McFarland et al. 2015).

The green mussel *Perna viridis* is a recently introduced species, and responses to local environmental conditions, including *K. brevis* blooms, are understudied. Understanding the environmental boundaries and potential for spread of this aggressive biofouling species are essential from an economic and ecological standpoint. High densities of *P. viridis* have been found clogging water intake pipes and coating boat hulls and navigational structures (Baker et al. 2012; Rajagopal et al. 2006). This behavior poses a threat to native oyster reefs, which have been displaced in some regions of Tampa Bay by dense settlement of green mussels (Baker et al. 2012; Fajans & Baker 2005).

Growth and recruitment are good indicators to monitor the success of new populations, providing valuable insight to population dynamics. To assess green mussel tolerance to *K. brevis* blooms and the response of local populations, growth, survival and juvenile recruitment were monitored before, during and following two consecutive red tide blooms in Estero Bay, Florida from March 2011 – May 2014. Because mortalities have been observed in green mussel populations in Tampa Bay following red tide events (Baker et al. 2012), this study examined potential sublethal and lethal effects observed during two prolonged *K. brevis* blooms in the Gulf of Mexico, allowing for the evaluation of the effects of *K. brevis* blooms on established *P. viridis* populations.

2. Methods

2.1 Environmental parameters

Environmental parameters, temperature, salinity, dissolved oxygen, and chlorophyll a, were monitored on site through the maintenance of a YSI 6600 data Sonde (YSI Inc., Yellow Springs, OH) with recordings once every hour and from continuous data monitoring Sondes courtesy of Sanibel-Captiva Conservation Foundation through the River, Estuary and Coastal Observing Network (RECON) during the monitoring period (March 2011 – May 2014). Red tide events were documented and cell counts provided by Florida Fish and Wildlife Research Institution (FWRI).

2.2 Monthly growth rates and mortality

Green mussels were collected monthly using SCUBA from New Pass and Big Carlos Pass bridges in Estero Bay, Florida from March 2011 through October 2013. Mussels of average shell lengths 35.3 ± 10.9 mm (set 1) and 43.5 ± 9.8 mm (set 2) were collected in March 2011 and September 2011, respectively. All mussels were cleaned of epiphytic growth, individually tagged using numbered shellfish tags from Hallprint Pty. Ltd. (Hindmarsh Valley, Australia) and initial shell length recorded before being returned to the field. For each set, three replicate rigid cages with mesh size 25 mm^2 were constructed to hold mussels (N=50 / cage) in the field for mark and recovery and to protect them from predation. Cages were securely fastened to the fender system at New Pass Bridge at approximately 1.0 m below mean low tide mark, and mussels were switched into new clean cages every two weeks to prevent excessive fouling. Shell length was measured to the nearest 0.1 mm once a month using Vernier calipers until March 2012 when monitoring was halted due to a population wide mortality event. During the onset of the mortality event, one piling was specifically marked and carefully inspected for density of live mussels to compare with survival in cages and rule out caging as a cause of death.

Following this mortality event the experiment was restarted frequently but continued to be disrupted by a high occurrence of mortality. In June 2012 (set 3; 60.1 ± 5.2 mm), September 2012 (set 4; 46.9 ± 18.5 mm), December 2012 (set 5; 47.7 ± 9.1 mm) and October 2013 (set 6; 47.7 ± 9.1 mm) new mussels were collected and individually tagged, and cages were maintained as noted above with monthly measurements of length and survival recorded. Due to the scarcity of mussels following the mortality event, mussels were collected from all known locations and

replicates were limited by mussel availability; June – September 2012 (2 rep; N=22 / cage), September – November 2012 (3 rep; N=30 / cage), December 2012 – May 2013 (2 rep; N=33 / cage); October – December 2013 (2 rep; N= 28 / cage).

2.3 Calcein staining and shell analysis

Additional growth analysis was completed for mussels collected in June 2012 using calcein as an internal marker. The calcein staining solution was made according to Thébault et al. (2006) at a concentration of 150 mg L^{-1} using the following protocol. Calcein (1.5 g) was incorporated into $NaHCO_3$ (10.5 g) in 90 mL deionized water through gentle stirring overnight and in the dark. This solution was then added to 10 L of aerated filtered seawater for animal exposure. A new mixture was made for each staining period to assure the integrity of the fluorochrome and avoid fluorescence decay. Mussels were immersed in the calcein solution for 2 hours just prior to returning to the field.

This staining procedure was completed three times, once per month, and monthly measurements using Vernier calipers were recorded from June 24, 2012, to September 24, 2012. Preliminary studies showed green mussels to be unaffected by this staining protocol exhibiting high growth rates and <1% mortality. At the end of the field monitoring in September 2012, cages were recovered, mussels were sacrificed, and the shells left to air dry in the dark. Shells were mounted in a metal epoxy resin to prevent fracture and cut along the axis of maximal growth from umbo to ventral margin using a diamond wafering blade on an Isomet low speed saw (Buehler, Lake Bluff, IL, USA) to a thickness of 600 µm. Shell sections were then glued to microscope slides, ground down to a thickness of approximately 350 – 400 µm using 800 and 1200 grit paper and polished with Al_2O_3 powder of 1 µm and 0.33 µm grain size to increase clarity and remove scratching from cutting and grinding. Slides were analyzed on a Zeiss Lumar V12 stereomicroscope equipped with an Osram 50-W high-pressure Hg lamp (OSRAM GmbH©, Munich Germany) and an I2/3 filter block (excitation filter BP450-490, dichroic mirror RKP510 and emission filter LP515). A Zeiss AxioCam MRc 5 color digital camera (Carl Zeiss Microscopy, LCC Thornwood, NY) was used for visualization and measurement of growth between successive calcein marks.

This staining protocol was tested to assess effects of calcein on survival and growth following staining of sets 1 and 2. Set 1 was stained at time zero and after six months. Set 2 was stained only at time zero. Shells from these sets were not analyzed for marking and were used only to test effects on growth and survival.

2.4 Recruitment

Recruitment was monitored from May 2012 to April 2014 through visual observation of spat densities and through measurements and counts of bycatch during monthly collections of adults from a parallel monitoring study (McFarland et al. unpublished data). Bycatch was defined as juveniles attached to the clumps of adults and inadvertently brought onboard. From May 2013 to April 2014 spat collectors were deployed to monitor minor peaks in recruitment. Collectors were deployed in pairs with one set examined after one month and one set after two months of deployment with new, clean collectors replacing the old ones. Each set comprised two unglazed clay tiles (20 by 10 cm) strung with wire and fastened to the bridge pilings approximately 1.0 m below mean low tide mark, where the collection of adult mussels took place. Each month spat collectors were carefully retrieved, and all spat present were counted and measured. Although collectors were only used for one year of monitoring, patterns were recorded through bycatch throughout the entire monitoring period to allow for comparison of results between methods.

2.5 Statistical analyses

Growth was assessed using independent *t*-tests to detect differences in mean length between months, and linear regressions were used to detect relationships between growth rate and environmental parameters. Spearman Rank Correlations were used to detect changes in growth during months with *K. brevis* blooms present and months with tissue concentrations above the regulatory limit (data from McFarland et al. 2015). Mortality was analyzed graphically and Kaplan-Meier survival analysis used to calculate mean survival time for comparison between seasons. Due to variations in sampling methods, recruitment was treated as qualitative data and analyzed graphically for assessment of seasonal peaks among years. All statistical analysis was completed using SPPSS 22, and results are presented as means ± standard error with a significance level of $p \leq 0.05$.

3. Results

3.1 Environmental parameters

Salinity and dissolved oxygen remained stable with average salinities remaining above 30 and dissolved oxygen ranging from 5.6 – 7.6 mg L^{-1} (Fig. 1). Chlorophyll a remained above 2 µg L^{-1} throughout the year, and temperature averaged 17 – 20˚C in the winter and as high as 28 – 31˚C in the summer months (Fig. 1). In November 2011, onset of the first red tide event was detected in Estero Bay. This bloom persisted through December and dissipated by mid-January (Fig. 2). For approximately one week in June 2012 high concentrations (10^5 cells L^{-1}) were detected, but conditions did not persist. In October 2012, a second bloom was detected ($\geq 10^5$ cells L^{-1}) and persisted through February 2013, dissipating in early May 2013 (Fig. 2). In November 2013, bloom conditions were again detected for several weeks but did not persist.

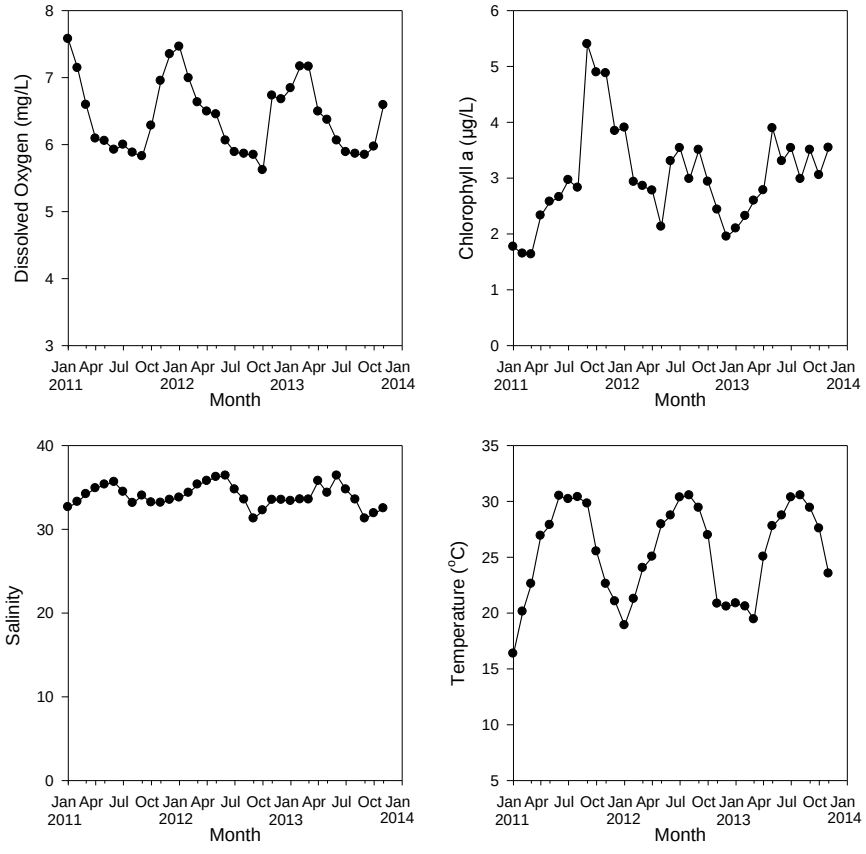

Figure 1. Monthly mean water quality parameters during the monitoring period.

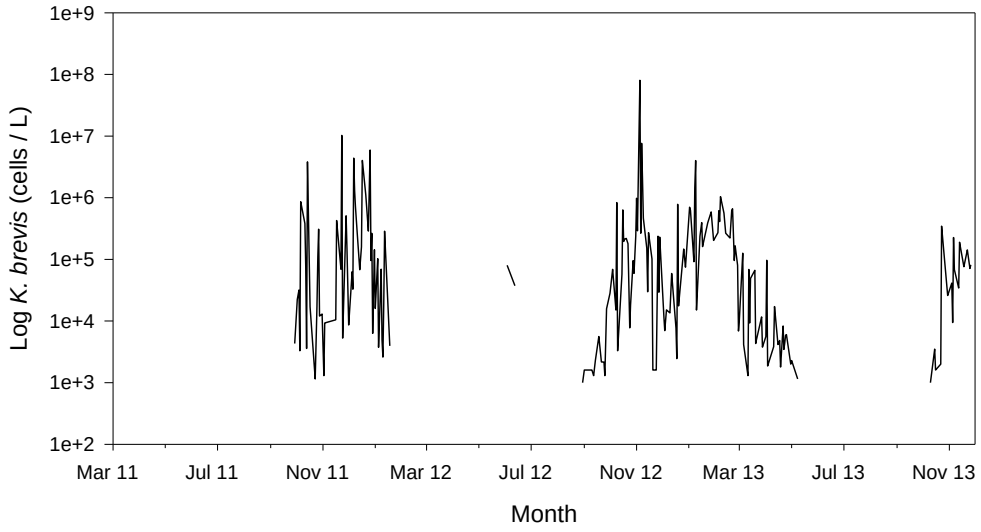

Figure 2. Mean daily *Karenia brevis* cell counts throughout the monitoring period. Cell counts are presented on a log scale.

3.2 Growth

Significant growth was observed between months from March 2011 through November 2011 (set 1) and December 2011 (set 2) ($p \leq 0.05$) with average monthly growth rates ranging from 5 to 11 mm month^{-1} and several individuals exceeding 13 mm month^{-1} (Fig. 3A). From December 2011 through February 2012 average growth rates fell to 1.5 – 3.5 mm month^{-1}, and no significant increase in length between months was observed (Fig. 3A). During subsequent growth monitoring from June 2012 – October 2013 (sets 3 – 6) no significant growth was observed between months, and average growth rates rarely exceeded 3 mm month^{-1} (Fig. 3B, C). The growth rates observed in the summer of 2012 (8.8 ± 2.0 mm month^{-1}) differed from those observed in the summer of 2011 (1.8 ± 1.3 mm month^{-1}) and were accompanied by high mortality causing the succeeding experiments (sets 3 – 6) to come to a halt within 2 – 4 months of initiation. Linear regressions showed no significant relationships with environmental factors; rather, environmental conditions (temperature, salinity, chlorophyll, dissolved oxygen) were well within the optimal range for *P. viridis*. The decline in growth was, however, observed at the first onset of *K. brevis* bloom formation and bloom conditions persisted on and off through the duration of this field study. Growth was negatively related to *K. brevis* bloom presence ($R^2 = -0.677$; $p \leq 0.001$) and elevated tissue toxin levels ($R^2 = -0.798$; $p \leq 0.001$).

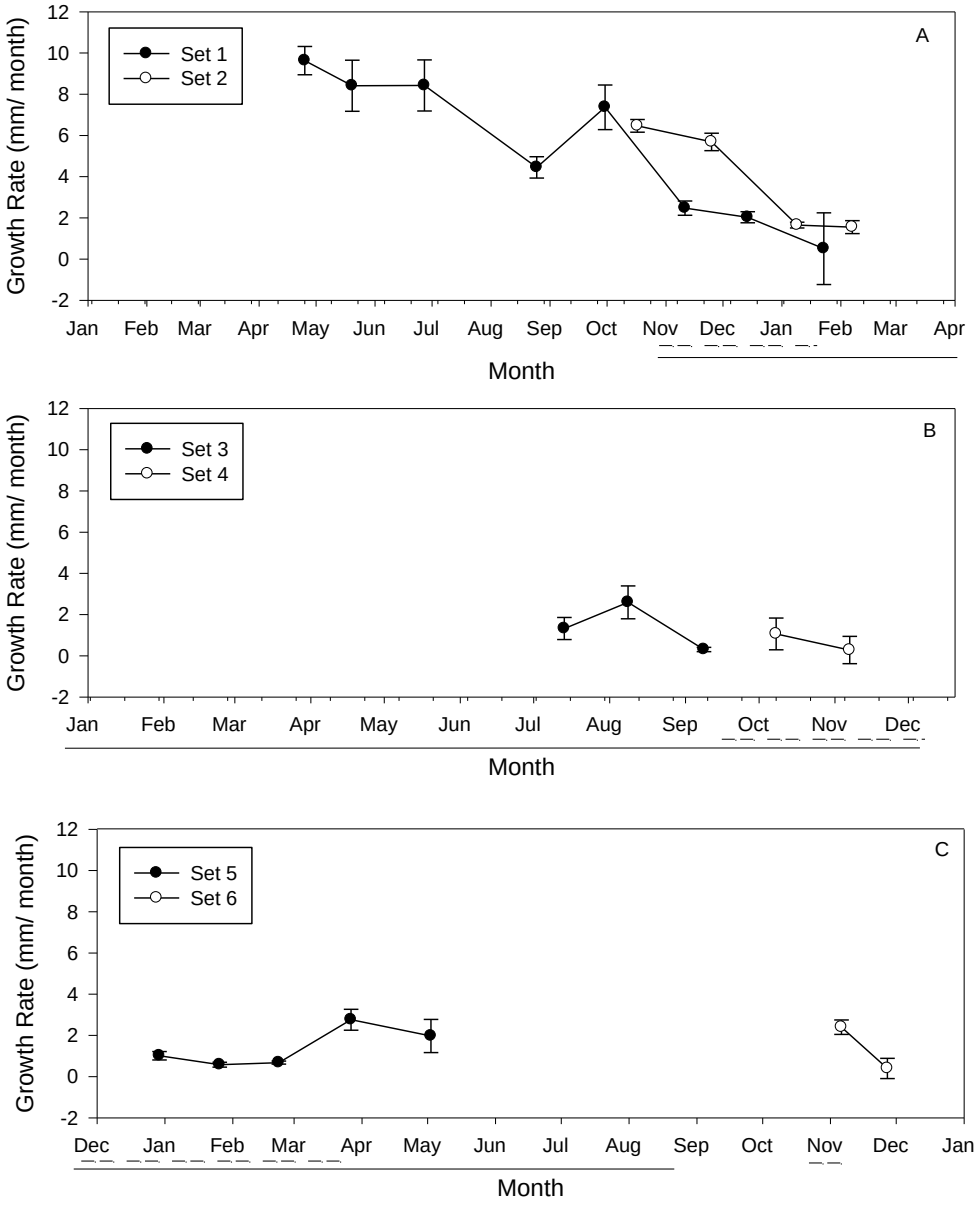

Figure 3. Growth rate of *P. viridis* by month for the first two sets of growth cages from March 2011 – March 2012 (A), sets 3 and 4 from June 2012 – December 2012 (B), and sets 5 and 6 from January 2013 – December 2013 (C). Growth rates are standardized to a 30 day growth period for each month with bars representing standard error. Dashed underlines indicate months when *K. brevis* blooms were present, and solid underlines indicate months when tissue PbTx concentrations were above the regulatory limit (data from McFarland et al. 2015).

No significant mortality and high growth were observed following calcein staining for sets 1 and 2. Calcein marks were observed in shells cut from the summer 2012 (set 3) field growth monitoring (Fig. 4), and measurements from the marks to ventral margin corresponded

well with the growth measurements taken by hand (Fig. 5). Due to reduced growth in the summer of 2012, individual or daily growth bands were not distinguished, and therefore periodicity of deposits were not established.

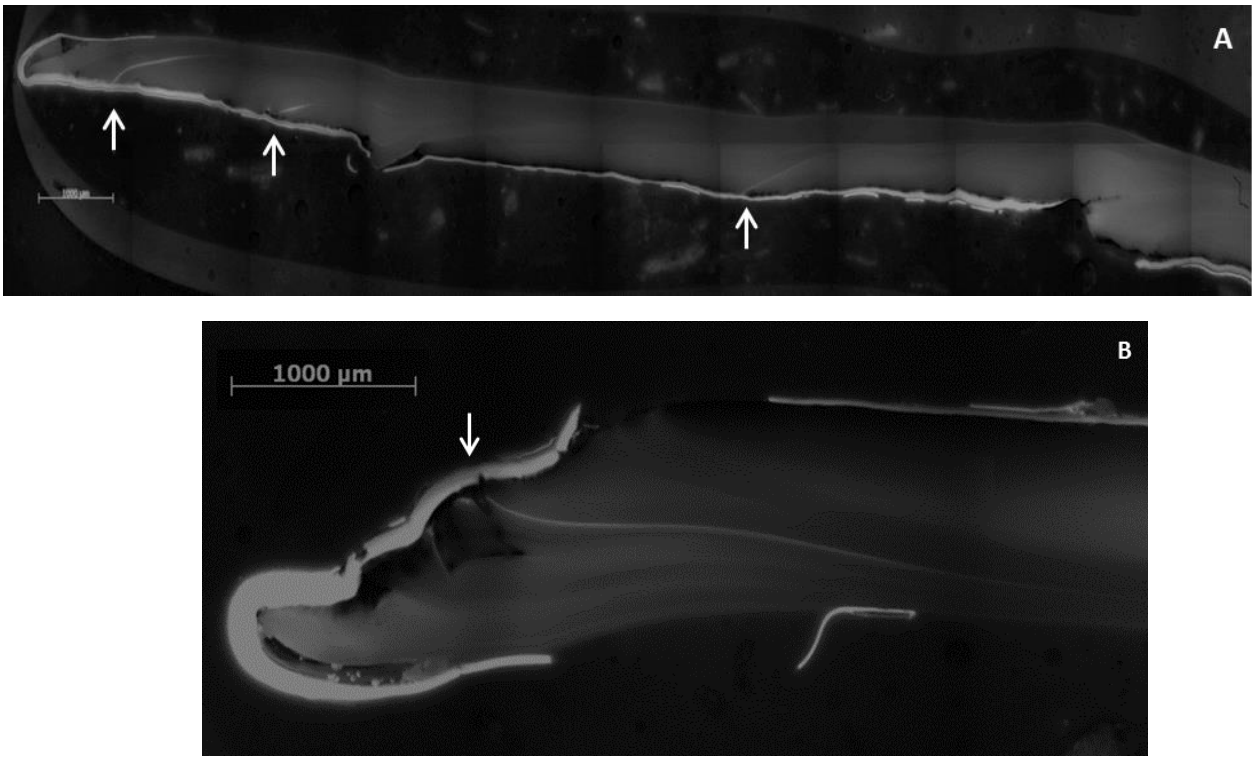

Figure 4. Micrographs indicating distinct marks from the calcein stain (arrows) with bright lines marking shell formation during the staining process.

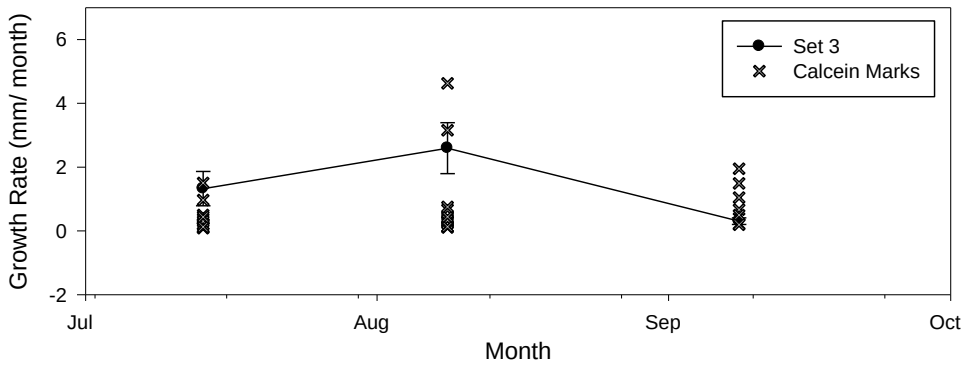

Figure 5. Growth rates measured during the summer of 2012 (set 3) by hand (black circles) and microscopically by calcein marks (gray x's).

3.3 Mortality

From March 2011 through February 2012 mussels showed high survival in field cages with no significant mortality observed (<1%). On March 14, 2012, two cages showed 100% mortality (Fig. 6A). Upon careful inspection of the marked piling, dense coatings of mussels were found with only a few isolated dead individuals still attached to the clumps. On April 3, 2012, 100% mortality was observed in the three remaining cages and on the inspected piling.

Summer growth cages, June – September 2012 (set 3), showed high individual mortality with 3 – 6 dead mussels per cage during each visit (every two weeks), and for those initiated on September 26, 2012 (set 4), over a third were dead within two weeks and 94% mortality was observed by October 22, 2012 (Fig. 6B). On October 22, 2012, a search of the seafloor was completed under high visibility conditions, and many dead mussel shells (fully intact, but no tissue remaining) were found, including mussels of all sizes with the largest observed at 123 mm. Growth cages from December 2012 – July 2013 (Set 5) had lower mortality rates until April 2013 when 60% cumulative mortality was observed (Fig. 6C). Growth cages initiated on October 25, 2013 resulted in 94% mortality by December 6, 2013 (Fig. 6C), during which time bloom concentrations of *K. brevis* were reported in the area for approximately one week in mid to late November 2013. Lowest mean survival time was observed during bloom onset in the fall of each year (Table 1).

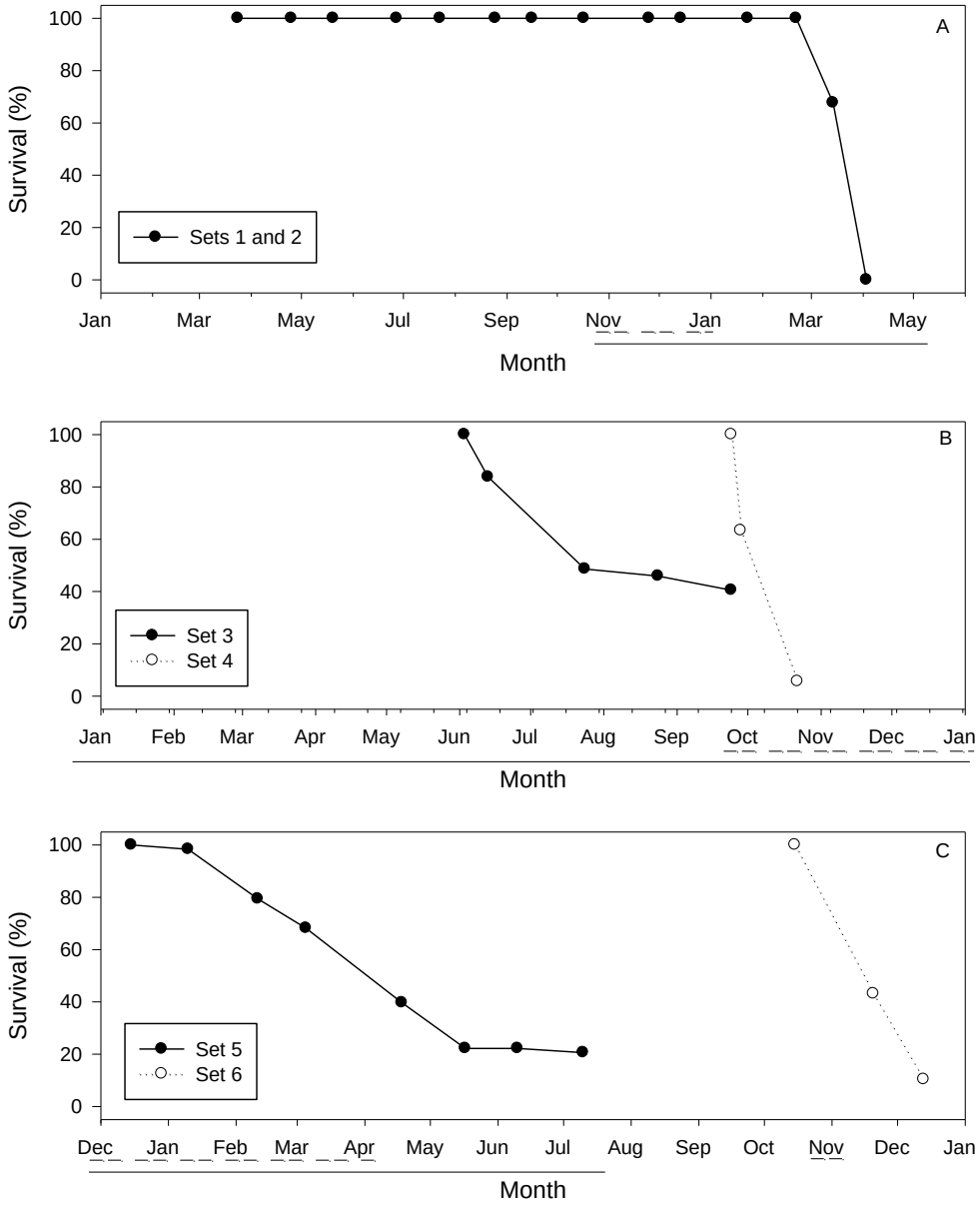

Figure 6. Survival of *P. viridis* over time. Dashed underlines indicate months when *K. brevis* blooms were present and solid underlines indicate months when tissue PbTx concentrations were above the regulatory limit (data from McFarland et al. 2015). Tissue toxin levels during and following the red tide event in November 2013 are unknown.

Table 1: Kaplan-Meier Survival Analysis (Mean Survival Time) of caged green mussels during growth monitoring

Year	Spring	Fall	Annual
2011 – 2012			365.2 days
2012	73 days*	41.2 days	
2013	103.5 days	31.8 days	

* Monitoring was intentionally stopped and mussels were sacrificed

3.4 Juvenile recruitment

Field observations documented through bycatch in 2012 – 2013 indicated two peaks in recruitment in the spring and fall with high numbers of spat in May – June 2012 (≤ 15 mm) and again in December 2012 (6 – 15 mm) (Fig. 7A). During monitoring using recruitment tiles (2013 – 2014), only one major peak in juvenile recruitment was observed in October – November 2013 with a high density of spat ranging from 3 – 17 mm over a 2-month settlement period and no observed spring peak (Fig. 7B).

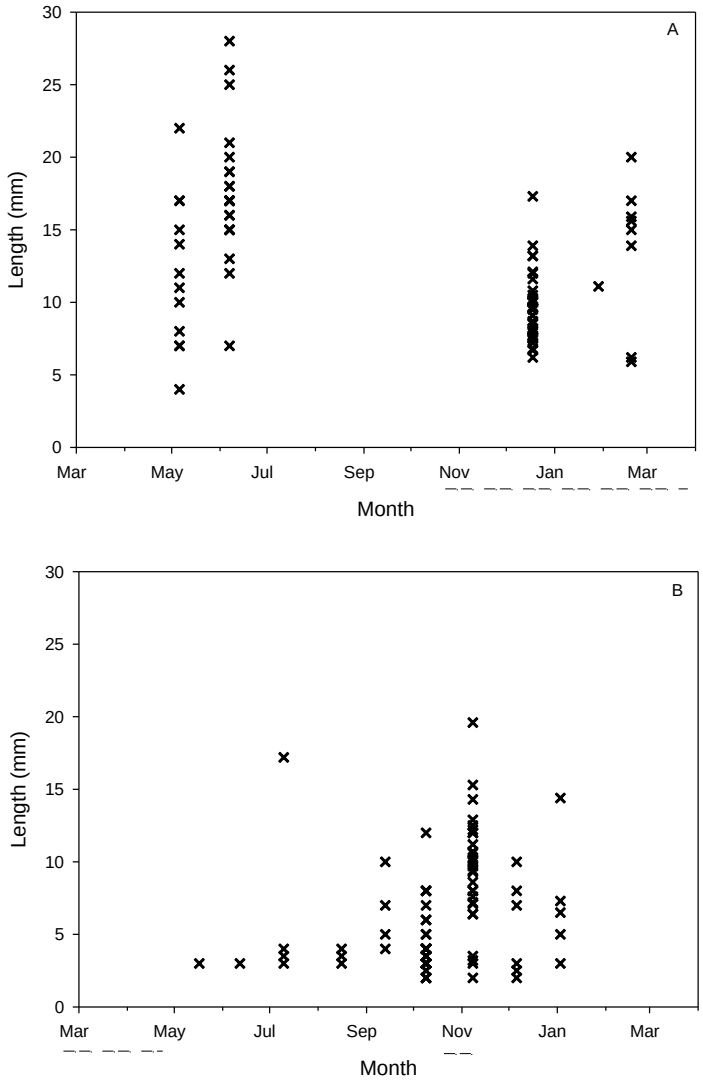

Figure 7. Size frequency of *P. viridis* spat observed through bycatch during 2012 – 2013 (A) and spat settlement on collectors during 2013 – 2014 (B). Dashed underlines indicate months in which red tide blooms were present.

4. Discussion

4.1 Growth

The results of growth monitoring from March 2011 through November 2011 (5 − 11 mm month^{-1}) compare well with previously reported growth rates of 7 − 13 mm month^{-1} (Al-Barwani et al. 2007; Lee 1986; Walter 1982). Observed growth rates dropped from 8.8 ± 1.3 to 3.5 ± 0.5 mm month^{-1} (set 1) in November 2011 and from 7.4 ± 0.5 to 2.4 ± 0.2 mm month^{-1} (set 2) in

December 2011. Although decreasing water temperatures during the winter months have been reported to hinder *P. viridis* growth (Cheung 1993; Lee 1986), growth rates in subsequent monitoring (set 3) did not exceed 2 mm month^{-1} in the summer of 2012 when conditions were expected to support high growth. However, red tide events ensued throughout the remainder of the growth study and may have contributed to abnormal growth rates due to bioaccumulation of PbTx in the soft tissue of green mussels. During field monitoring, utilization of calcein showed positive results with high growth and survival rates and clearly marked growth rings when analyzed microscopically. Calcein measurements were comparable to hand measurements but offer more precision allowing for short term growth studies yielding detailed results. Indeed, Vernier caliper measurements give a precision of ca. 0.1 mm but can vary for a single specimen depending on the way the caliper is put in place on the shell. On the other hand, digital measurement of distances between two successive calcein marks or between a given calcein mark and the ventral margin provide accuracy close to a few tens of microns.

Our study is unique given that changes in bivalve growth in response to HAB exposure is understudied due to the difficulty in maintaining bloom conditions in a laboratory setting and the unpredictable nature of blooms during field monitoring. Previous work has shown *P. viridis* to exhibit reduced clearance rates when exposed to toxic dinoflagellates, including *K. brevis* (Leverone et al. 2007; Li et al. 2002; May et al. 2010), which may affect growth in the long term due to a reduction in food intake and energy acquisition. During *K. brevis* blooms this toxic dinoflagellate often dominates the phytoplankton population, leaving a harmful food source which can cause tissue damage, especially in the gills and digestive tract, leading to increased avoidance behavior through valve closure and reduced feeding efficiency (Shi et al. 2012; Shumway & Cucci 1987). Reduction in feeding leads to a disruption in energy assimilation that, in turn, may limit growth in response to increased cost of somatic maintenance as energy allocation to maintenance and tissue repair will take priority over growth (Bayne and Newell 1983). Indeed, several short term studies on marine mussels have shown a reduction in growth during exposure to several HAB species (Bricelj et al. 1993; Li et al., 2002; Nielsen & Strømgren 1991). With such effects observed after short term exposures, those observed in the field are likely to be amplified, and prolonged bloom conditions could lead to chronic effects disrupting growth for extended periods even post bloom.

4.2 Mortality

The first mortality event was observed two months post-bloom dissipation (March 2012), during which time PbTx tissue concentrations were nearly 20 times the shellfish regulatory limit (McFarland et al. 2015). Previous studies have indicated that local bivalves typically tolerate *K. brevis* blooms and toxin accumulation with high survival rates during natural exposures (Pierce et al. 2002; Plakas et al. 2008). However, physiological effects of toxic algae can turn lethal as prolonged *K. brevis* exposure is associated with potent neurotoxins (PbTx) and hemolytic compounds, potentially exacerbating mortality due to sublethal accumulation of tissue toxins contributing to increased immune-suppression and disease susceptibility (Landsberg 2002; Paster & Abbott 1969; Tatters et al. 2010) and muscle, cardiac and respiratory impairment (Wu et al. 1985).

In its native range of the Indio-Pacific, *P. viridis* shows high tolerance to blooms of *Karenia mikimotoi* (Robin et al. 2013) and *Gymnodinium nagasakiense* (Karunasagar & Karunasagar 1992). Conversely, Gacutan et al. (1984) documented a *Pyrodinium bahamense* var. *compressa* red tide event in the Philippines that nearly decimated *P. viridis* populations. Likewise, *P. viridis* showed high mortality rates compared with other bivalves during laboratory exposures to *Alexandrium monilatum* (Hégaret et al. 2008) and Baker et al. (2012) observed >90% *P. viridis* morality following a *K. brevis* bloom in Tampa Bay, Florida. As a recently introduced species, *P. viridis* may lack the adaptations to tolerate and eliminate PbTx, increasing vulnerability and physiological stress (McFarland et al., 2015).

The mortality event reported in this study also coincided with peaks in spring spawning activity (McFarland et al. unpublished data). Spawning requires significant metabolic demand, often depleting energy reserves and leading to increased susceptibility to environmental stressors (Emmett et al. 1987; Myrand et al. 2000). Galimany et al. (2008) observed adductor muscle paralysis preventing valve closure in *Mytilus edulis* that had spawned during exposure to *Alexandrium fundyense,* resulting in increased mortality and incidence of pathological changes. Although *P. viridis* maintained high survival rates despite elevated accumulation of tissue toxins during the bloom, the stress of spawning may have contributed to the observed post-bloom mortality.

4.3 Juvenile Recruitment

Two major peaks (fall 2011 and spring 2012) in juvenile recruitment were observed during the first year of monitoring with only one major peak (fall 2012) observed during year two. Although year round gametogenesis is common in *P. viridis*, two major peaks in spawning and juvenile recruitment (spring and fall) are typically observed (Al-Barwani et al. 2007; Lee 1986; Rajagopal et al. 1998). Likewise, gametogenic cycles of local *P. viridis* populations indicate peaks in spawning activity in the spring and fall each year (McFarland et al. unpublished data). Recruitment patterns in the first year of monitoring supports these two peaks; however, during the second year only one recruitment peak was observed, suggesting that although adults are actively spawning, secondary factors may impede fertilization and/or growth and survival during the pelagic phase.

Juvenile recruitment is dependent on the fecundity and productivity of the adult brood stock population (Dickie et al. 1984). Without a sufficient supply of gametes and close proximity of spawning adults, fertilization and larval production is limited. Mortality peaked each year October – November, leaving the spring brood stock population sparse. Low density and reduced proximity of spawning adults may also reduce fertilization success and alter chemical cues used in the induction of spawning and synchronicity between males and females (Stephen and Shetty 1981). Stress following HAB exposure in adults has been shown to decrease egg quality by reducing lipid content required for growth and survival during early larval stages (Bayne 1975; Holland 1978). Rolton (2015) observed decreased gamete viability and larval development following laboratory spawning of adult *Crassostrea virginica* exposed to a *K. brevis* bloom in the field, and others have reported decreased sperm viability following short term laboratory exposure of adults (Haberkorn et al. 2011; Le Goïc et al. 2013). Although *P. viridis* were still producing gametes and actively spawning, stress related reductions in energy allocation may result in reduced gamete quality.

Karenia brevis blooms may have also directly affected larval survival. Bloom concentrations were detected in October 2012 and persisted through April 2013. This regime would allow for larval development and settlement from August and September 2012 spawnings, supporting the recruitment peaks observed in October and November 2012; however, presence of *K. brevis* may have inhibited the development of larvae produced during the early spring 2013

bloom resulting in failed juvenile recruitment. Juvenile recruitment has been previously shown to be inhibited during HAB events in wild *P. viridis* populations (Cheung 1993). HAB exposure during early bivalve life stages has resulted in reduced fertilization and survival rates, and delayed embryogenesis and larval development (Basti et al. 2012; Gallager et al. 1992; Granmo et al. 1988; Matsuyama et al. 2001), results that have been observed in local species of oysters (*C. virginica*), clams (*Mercenaria mercenaria*), and scallops (*Argopecten irradians*) during exposure to *K. brevis* (Leverone et al. 2006; Rolton et al. 2014). As red tide blooms were frequent throughout much of the monitoring period, it is possible that reduced larval survival and recruitment can be attributed to *K. brevis* and associated ichthyotoxins and allelochemicals in the water column (Paster & Abbott 1969; Tatters et al. 2010). Additionally, due to both the increased duration and intensity of bloom exposure in the wild (Steidinger 2009; Tester & Steidinger 1997), deleterious effects are likely amplified during natural exposures leaving laboratory exposures as underestimations of the actual effects on larvae.

5. Conclusions

Results presented here suggest that *P. viridis* populations may be inhibited by *K. brevis* blooms resulting in decreased growth, survival and juvenile recruitment, leading to significant reductions in population densities. Although a reduction in green mussel densities can be viewed as positive for the ecosystem, these populations should not go unmonitored. Several adult green mussels have been found to survive these events, and the pulse in recruitment each fall indicates there are enough mature adults in the area to repopulate given the right conditions. Previous exposure history plays an important role in tolerance and sensitivity (Shumway et al. 1985; Shumway & Cucci 1987), thus the possibility that *P. viridis* may adapt to *K. brevis* blooms and associated toxic compounds overtime should not be disregarded. Future studies should include laboratory exposures to directly assess physiological and behavioral changes that may inhibit growth and survival at several life stages to better explain field observations. Long term experiments to assess the effect of chronic exposure and the recovery period that follows bloom dissipation and continued monitoring of local populations would greatly benefit the overall understanding of the effects observed during this study. These results will also aid in the prediction of population response of green mussels to Florida red tides and further define environmental limitations of this newly established species.

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Chapter 4: Reproduction and biochemical composition

Seasonal variation in gametogenesis and proximal biochemical composition of the invasive green mussel, *Perna viridis*, in southwest Florida

Abstract

Understanding the population dynamics of invasive species, such as the green mussel *Perna viridis*, can aid in explaining the success of new populations and help predict the potential for spread. During a two year field study of established populations in the invaded region of southwest Florida, year round gametogenesis and continuous spawning capabilities were observed. Laboratory induced spawning of mussels naturally conditioned in the field under both summer (September 2013) and winter (January 2014) conditions further supported these results. However, egg outputs in the summer ($6.4 \times 10^6 \pm 2.6 \times 10^6$ eggs / female) were significantly higher ($p = 0.045$) than egg outputs of winter spawned mussels ($7.7 \times 10^4 \pm 1.4 \times 10^4$ eggs / female). Monthly variation in biochemical composition (protein, glycogen and lipid) also remained high with no significant variation between months suggesting temperature and food availability were sufficient year round, allowing for the maintenance of reserves and active gametogenesis. Protein ranged from 409.0 – 628.0 mg g^{-1}, glycogen from 44.3 – 158.5mg g^{-1} and total lipids from 7.4 – 13.5 mg g^{-1} with peaks in the summer months. These findings help explain the rapid colonization and high densities of green mussels along artificial substrate in the southeastern United States and suggests the potential for competition with native oysters *Crassostrea virginica*.

Key Words: Reproductive strategy, protein, glycogen, lipid, induced spawning

1. Introduction

The green mussel *Perna viridis* is a recent marine invader to southwest Florida. Native to the Indo-Pacific they are wide spread throughout coastal waters and harvested as a food source from both natural populations and aquaculture practices (Sivalingam 1977; Vakily 1989). They are believed to have been introduced to Caribbean waters via transport on boat hulls and / or ballast water through the shipping industry to Trinidad and Tobago in the early 1990's (Agard et

al. 1992). Since this invasion they have aggressively spread throughout coastal waters of the Caribbean and southeastern United States (Baker et al. 2007; Benson et al. 2001; Buddo et al. 2003; Ingrao et al. 2001; Rylander et al. 1996). Although it is well studied as an aquaculture species and bio-indicator for marine pollutants in its native range, little is known of invasive populations in the Caribbean and southeastern United States. Green mussels are a fast growing, biofouling organism making them a potential threat to both native species, specifically the eastern oyster *Crassostrea virginica*, and infrastructure, including damage to water intake pipes, navigation structures and boat hulls.

Since their invasion into Tampa Bay, Florida in 1999 (Benson et al. 2001; Ingrao et al. 2001) green mussels have spread throughout coastal regions of the southeastern United States spanning as far south as Marco Island on the Gulf Coast and from Palm Beach Gardens, Florida to Charleston, South Carolina on the Atlantic coast (Baker et al. 2007; Rajagopal et al. 2006). This rapid colonization has been attributed to high reproductive activity and rapid growth rates. For example, green mussels reach sexual maturity within the first few months of settlement (Rao et al. 1975; Sreenivasan et al. 1989) and dominate hard substrates at densities as high as 1000 – 4000 individuals m^{-2} (Fajans and Baker 2005).

The success of an invasive species is dependent upon its ability to meet energetic demands for growth, maintenance and reproduction, with both gametogenic and energy storage cycles serving as valid indicators of overall health and condition. Specifically, protein, glycogen and lipids serving as the main substrates of reserve in bivalves (Holland 1978). Glycogen is the precursor for lipogenesis and both tend to vary seasonally in bivalves (Gabbott 1975). Glycogen is typically accumulated and stored in the summer months when food is plentiful and utilized to fuel gametogenesis in the winter when food is sparse (Ansell 1972; Holland 1978; Mohan and Kalyani 1989), while lipid and protein are the main substrates of egg reserves (Holland 1978). Previous work has shown that early developing larvae are completely dependent on these egg reserves, which are proportional to energetic reserves of the female at the time of egg production (Gabbott 1975). Thus, sufficient reserves in adults are essential to producing successful progeny.

Seasonal cycles in proximal biochemical content and gametogenesis are dependent upon species-specific properties including energetic dynamics, metabolic demands and reproductive stage (Dare and Edwards 1975; de Zwaan and Zandee 1972; Gabbott 1975). Additionally, the

rate of energy storage and utilization is dependent on food availability and assimilation and allocation rates, determined by seasonal variation of physiological and environmental factors (Bayne and Newell 1983). The goal of this study was to monitor seasonal variation in gametogenesis and proximal biochemical composition (glycogen, protein and lipid) in an established green mussel population in Estero Bay, Florida over a two-year period. Field observations were further verified by induced spawning in the laboratory of summer and winter acclimated mussels collected in September 2013 and January 2014, respectively, to quantify gonad output. This information is essential to understanding the success of these populations and predicting further spread and potential threat to native species.

2. Methods

2.1 Environmental Parameters

Environmental parameters, temperature, salinity, dissolved oxygen and chlorophyll a, were monitored on site through the maintenance of a YSI 6600 data Sonde (YSI Inc., Yellow Springs, OH) with recordings once every hour and from Sondes courtesy of Sanibel-Captiva Conservation Foundation through their River, Estuary and Coastal Observing Network (RECON). Sondes were cleaned, calibrated and data extracted every two weeks to assure performance.

2.2 Collection of Mussels

Green mussels were collected from New Pass and Big Carlos Pass bridges in Estero Bay, Florida from August 2011 through November 2013. Big Carlos Pass was added as a collection site after a mass mortality event spanning all known collection areas impeded a single site collection at New Pass. Both sites are located at the Estero Bay – Gulf of Mexico interface where they experience extensive flushing and high current flow in marine conditions. Collection of mussels, both numbers and size range, was dictated by availability following the mortality event. All mussels were cleaned of epiphytic growth and processed the day of collection.

2.3 Histological analysis of seasonal gonad development

Whole, intact tissue of green mussels (N = 15 / month) was carefully dissected out of the shell (L = 65.17 ± 1.95 mm) with a total of 450 mussels analyzed over the duration of the field monitoring. Tissue sections were cut and processed according to standard histological techniques in order to assess gonad development (Howard et al. 2004). Sections were immersed in Davidson's fixative (Shaw and Battle 1957) for one week, rinsed with 70% ethanol for 24 hours and run through a ThermoShandon Citadel 1000 automatic processor (Global Medical Instruments Inc., Ramsey, MN) before being embedded in paraffin wax. Once embedded, tissue sections were cut using a HM 325 Rotary Microtome (Thermo Fisher Scientific ™, Waltham, MA) to 7µm thickness, mounted on slides and stained with Harris' Hematoxylin and Eosin. Gonad development was scored with reference to Rajagopal et al. (2006) on a scale of 1 – 5 (Volety et al. 2009) (Table 1). Gametogenesis was also converted to percent gonad occupation. Scanned images of the whole tissue sections were analyzed using ImageJ™ image analysis software. The area of active gonad divided by total tissue area (excluding gills) was used to calculate percent gonad occupation.

$$\%\ \text{Gonad Occupation} = \frac{\text{gonad area}}{\text{total body area}} \times 100\%$$

2.4 Induced spawning

In order to better explain observations in the gametogenic cycle, spawning behavior and the release of gametes was assessed on two occasions by inducing mature mussels from the field to spawn in the laboratory (N = 25 / spawn). On September 26, 2013 (L = 50.16 ± 1.22 mm) and January 1, 2014 (L = 55.36 ± 1.84 mm) green mussels were collected from New Pass bridge in Estero Bay, Florida, cleaned of epiphytic growth and kept in tanks with recirculating seawater under chilled conditions (18˚C) to prevent spontaneous spawning. On September 30, 2013 and January 9, 2014 respectively, mussels were induced to spawn using temperature adjustments (from 18˚C to 30˚C). Mussels were placed in a tank at room temperature (22˚C) with a gradual increase to 30˚C using aquarium heaters. If they did not spawn after 30 minutes they were then

transferred to a cold tank at 18˚C for 20 minutes then back to the warm tank at 30˚C, this was repeated until spawning commenced. As spawning began, individuals were immediately removed from the spawning tank and placed into beakers to finish spawning and allow for the collection of gametes. Eggs were counted for each individual female to quantify output (N = 7 in September 2013 and N = 5 in January 2014).

Spawned individuals were sacrificed and cut for histology in order to analyze the amount of gametes retained following a spawn. For comparison, 15 mussels from each batch were not subject to spawning induction and were sacrificed for histology to represent "pre-spawn" gonad. Histological analysis was completed as mentioned previously for monthly collections using both gonad index and percent gonad occupation to describe the spawning event.

2.5 Analysis of proximal biochemical composition

On the day of collection, whole tissue of individual mussels (N = 10 / month) was dissected out of the shell (L = 72.5 ± 1.0 mm) and frozen at -80˚C. Once the tissue was completely frozen, samples were vacuum freeze dried at -47 ˚C for approximately 72 hours using a Labconco FreeZone 12 freeze dryer (Labconco©, Kansas City, MO). Shells were dried for 48 hours at 60˚C and dry shell weight and freeze dried tissue weight were used to calculate condition index (CI) according to Emmet et al. (1987). The dried tissue was then individually homogenized to a fine powder using a MixerMill 400© (Retsch® Solutions in Milling and Sieving, Hann, Germany). Subsamples were then taken for subsequent analysis of protein, glycogen and lipid content for each individual with a total of 270 individuals analyzed over the study period.

$$\mathrm{CI} = \frac{\text{dry tissue weight}}{\text{dry shell weight}} \times 100\%$$

Protein analysis was completed according to Lowry's method for soluble proteins (Lowry et al. 1951) using a DC Bio-Rad™ protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA). Dried tissue samples (50 mg) were re-suspended in a homogenizing buffer, extracted with 1M NaOH and boiled for 10 minutes. Samples were loaded into a 96-well plate with reagents (Alkaline copper tartrate and Folin reagent) from the Bio-Rad kit and incubated for 15 minutes.

All samples were run in triplicates and read on a TECAN Genois Pro® microplate reader (TECAN Group Ltd., Männedorf, Switzerland) at an absorbance of 690 nm. The standard curve was calculated using the protein standard provided in the Bio-Rad kit.

Glycogen analysis was completed using the anthrone method (Baturo et al. 1995). Dried tissue samples (200 mg) were re-suspended in a homogenizing buffer, diluted with 30% KOH and boiled for 20 minutes. Glycogen extract was purified using saturated Na_2SO_4 and 100% ethanol, and the precipitate was dried overnight at 60˚C. Samples were then re-suspended in deionized water, anthrone reagent was added and incubated at 90˚C for 20 minutes. All samples were run in triplicates in a Revelation® microplate reader (DYNEX Technologies, Inc., Chantilly, VA) at 590 nm. Standard curve was made using glycogen from oyster Type II (Sigma-Aldrich Co., St. Louis, MO).

Lipid extractions from freeze dried samples (50 mg) were completed according to Bligh and Dyer (1959) using chloroform, methanol and water (2:1:1 by volume) and purified by running the extract through a Na_2SO_4 column. Total lipids were determined gravimetrically after drying under a gentle stream of nitrogen.

2.5 Statistical analysis

Wilcoxon signed test was used to assess differences in egg output and gonad occupation between September and January spawning events. Chi- Square tests were used to test for significant changes in the sex ratio of males to females. Due to non-normality of the data the nonparametric Kruskal-Wallis test was used to compare changes in gonad index and percent gonad occupation over time. Likewise, the Kruskal-Wallis test was used to compare changes in protein, glycogen and lipid content over time. Analysis was completed by month and, due to high individual variability, months were also grouped by season (spring, summer, winter, fall). Stepwise multiple linear regressions were used in order to detect significance between biological (gonad index, percent gonad occupation, protein, lipid, glycogen, and condition index) and environmental variables (chlorophyll, temperature, salinity, and dissolved oxygen). All statistical analyses were completed using IBM SPSS Statistics 22. Significance was reported as $p \leq 0.05$ and data presented using ± standard error.

3. Results

3.1 Environmental parameters

Environmental variables measured during the study period remained within the tolerance range of *P. viridis*, with optimal salinity range of 27 – 33 and an optimal temperature of 26 – 32˚C (Vakily, 1989). While seasonal variation was observed, no significant relationship was detected between biological parameters (gametogenesis, protein, glycogen and lipid content) and environmental variables (temperature, salinity, chlorophyll and dissolved oxygen). Salinity and dissolved oxygen remained high, ranging from 31 – 35 ppt and 5.6 – 7.6 mg L^{-1}, respectively (Fig. 1). Temperature and chlorophyll a showed distinct seasonal variation reaching lows in the winter and peaks in the summer with monthly averages ranging from 18.9 – 30.5˚C and 2.0 – 5.5 µg L^{-1}, respectively (Fig. 1).

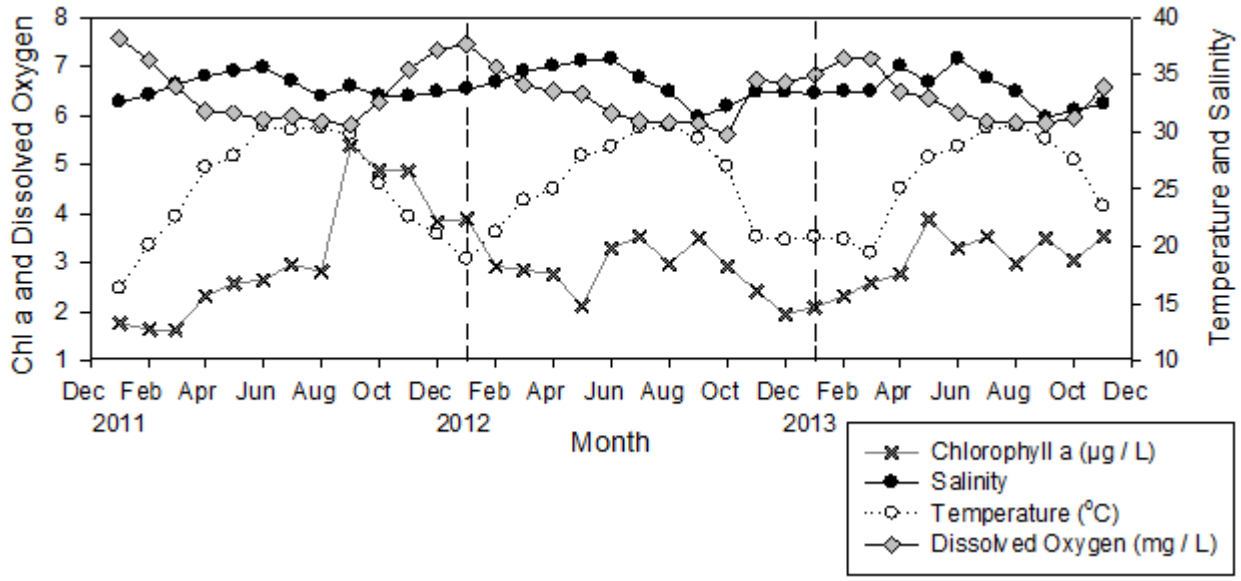

Figure 1: Environmental parameters over time during the study period.

3.2 Gametogenesis during field monitoring

Active gametogenesis was observed year round with average gonad index ranging from 3 – 5 representing primarily the late development to spawning phases (Fig. 2). Over the course of the study, only four of 450 individuals were given a rank of 1 due to an inability to distinguish sex, and in September 2012 all females analyzed (N = 4) received a ranking of 2. However, these cases were not common and gonad rankings rarely fell below a 3 (Fig. 2). In addition, no true signs of resting or inactive periods were observed, but rather, at least a portion of the gonad was actively producing gametes year round. In fact, it often occurred that individuals showed multiple stages of gonad index in different regions of the gonad with one portion in the regeneration / post spawn phase, while the other portion was in the late development / ripe phase. Thus, a continuous spawning capability was apparent throughout the year with portions of the gonad reabsorbing gametes following a partial spawn and other portions in the late development or ripe stages. While graphically, the gonad occupation showed more seasonality than gonad index (Fig. 3), no significant relationship was detected. However, two major peaks in gametogenesis per year were observed, in the early spring and late fall.

Sex ratios were often skewed, although not significantly, from the typical 1:1 ratio and only 4 hermaphrodites were observed out of the 450 individuals analyzed. Juvenile mussels showed distinguishable sexes with active gametogenesis as small as 10 - 15 mm and sex was distinguishable before histological analysis by tissue color. When gametogenesis is active, females are bright orange and males are creamy white in color (Lee 1986; Narasimham 1981; Sreenivasan et al. 1989; Walter 1982). These observations were noted and later confirmed through histological analysis. Pearson's correlation between sex and tissue color showed significance at $p \leq 0.001$. This method allowed for the estimation of sex from tissue color for individuals collected for biochemical analysis.

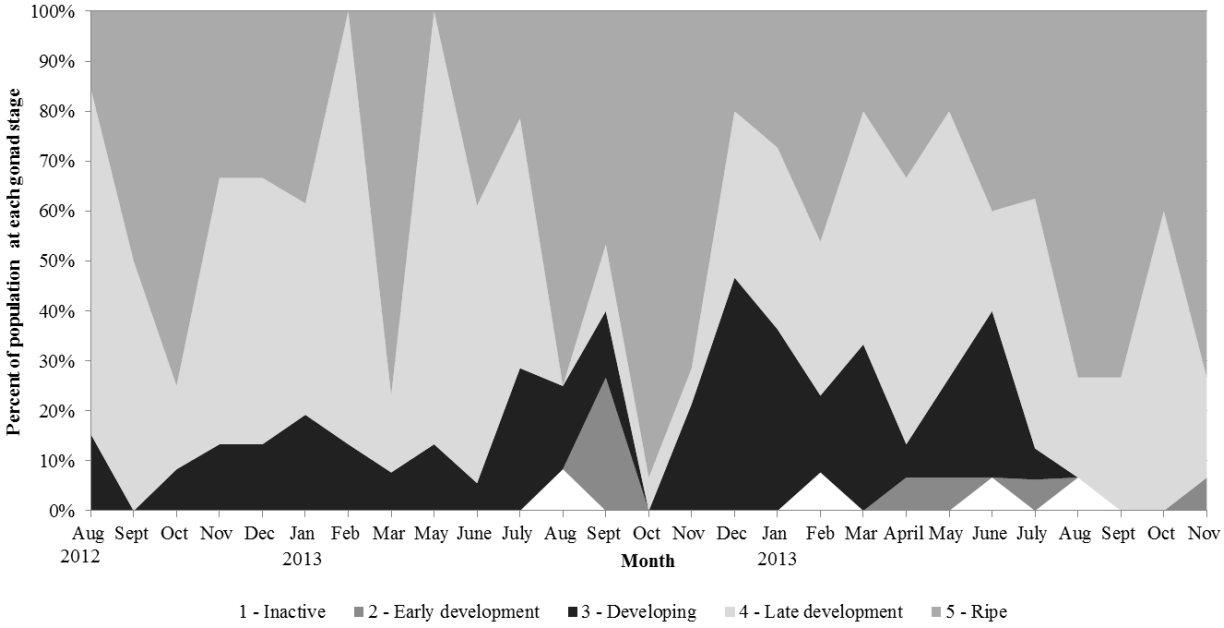

Figure 2: Histogram of gonadal index overtime (N = 15 / month). Each stage is presented as a percentage of the total for each month.

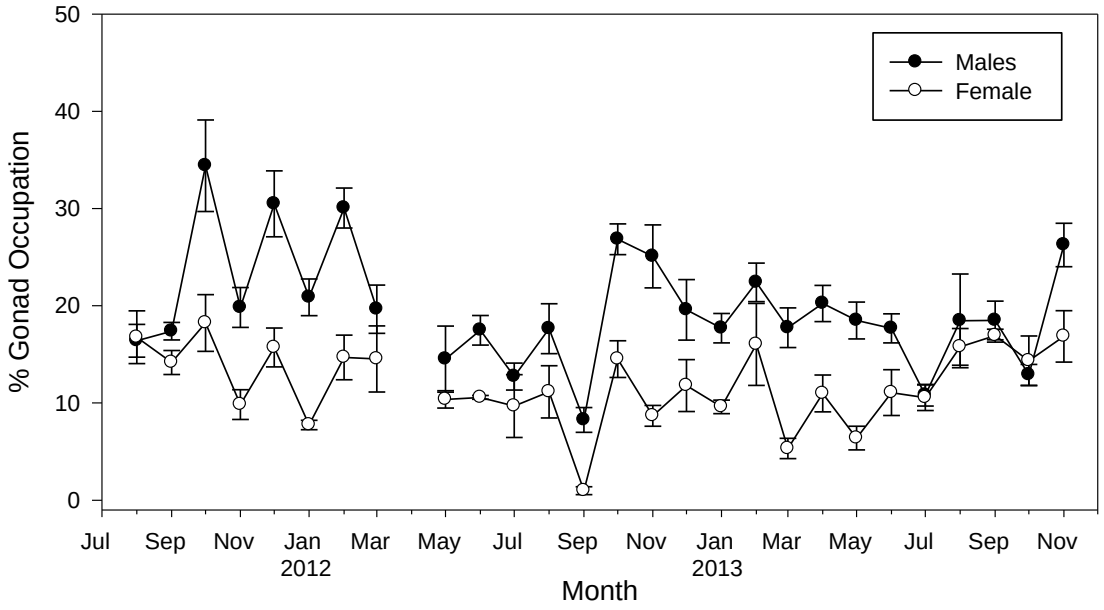

Figure 3: Percent gonad occupation for males and females over the monitoring period (N = 15 / month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts. Error bars represent standard error.

3.3 Gametogenesis during induced spawning

Successful spawning was accomplished in mussels collected in both late summer and winter with high fertilization rates (93.9% and 93.7% respectively) and viable larvae produced from both batches. During the September 2013 trial, spawning commenced within 20 minutes of the third cycle in the warm water tank and during the January 2014 trial, spawning commenced in the first warm cycle once the temperature reached 30˚C. The average number of eggs released per female in September was significantly higher ($6.4 \times 10^6 \pm 2.6 \times 10^6$) than that in January ($7.7 \times 10^4 \pm 1.4 \times 10^4$), ($Z = -2.023$; $p = 0.045$). The "pre-spawn" percent gonad for females was higher in September versus January ($Z = -3.783$; $p < 0.001$), but the gonad index for both indicated active gametogenesis (Table 2).

Males maintained a high percent gonad occupation after spawning, even when a high density of sperm was released. Most individuals that spawned only released a portion of their gametes leaving a portion emptied and a portion that remained densely packed with gametes (Fig 4e, f), however a few individuals did release nearly all gametes (Fig 4c, d) leaving minimal gonadal area and a gonad index ranking of 1. One female from January 2014 released 5.4×10^4 eggs and maintained a large portion of gametes post spawning. In September, only one female released the entire gonad releasing 19×10^6 eggs, orders of magnitude higher. Other females in the September spawn only released a portion of their gonad resulting of $3 – 6 \times 10^6$ eggs released.

Table 2: Results of egg output and histological analysis of mussels from induced spawning (n = spawned; not spawned individuals)

			Pre Spawn		Post Spawn	
		Eggs per female	Gonad Index	% Gonad	Gonad Index	% Gonad
September, 2013	Females (n = 7; 5)	6.35×10^6	4	14.6 ± 1.3	2.9	5.5 ± 1.0
	Male (n = 8; 3)		4.5	15.6 ± 0.5	3.6	14.5 ± 2.7
January, 2014	Females (n = 5; 5)	7.77×10^4	3	8.5 ± 1.7	2.1	7.9 ± 0.8
	Male (n = 10; 5)		4.2	18 ± 1.2	2.75	11 ± 1.7

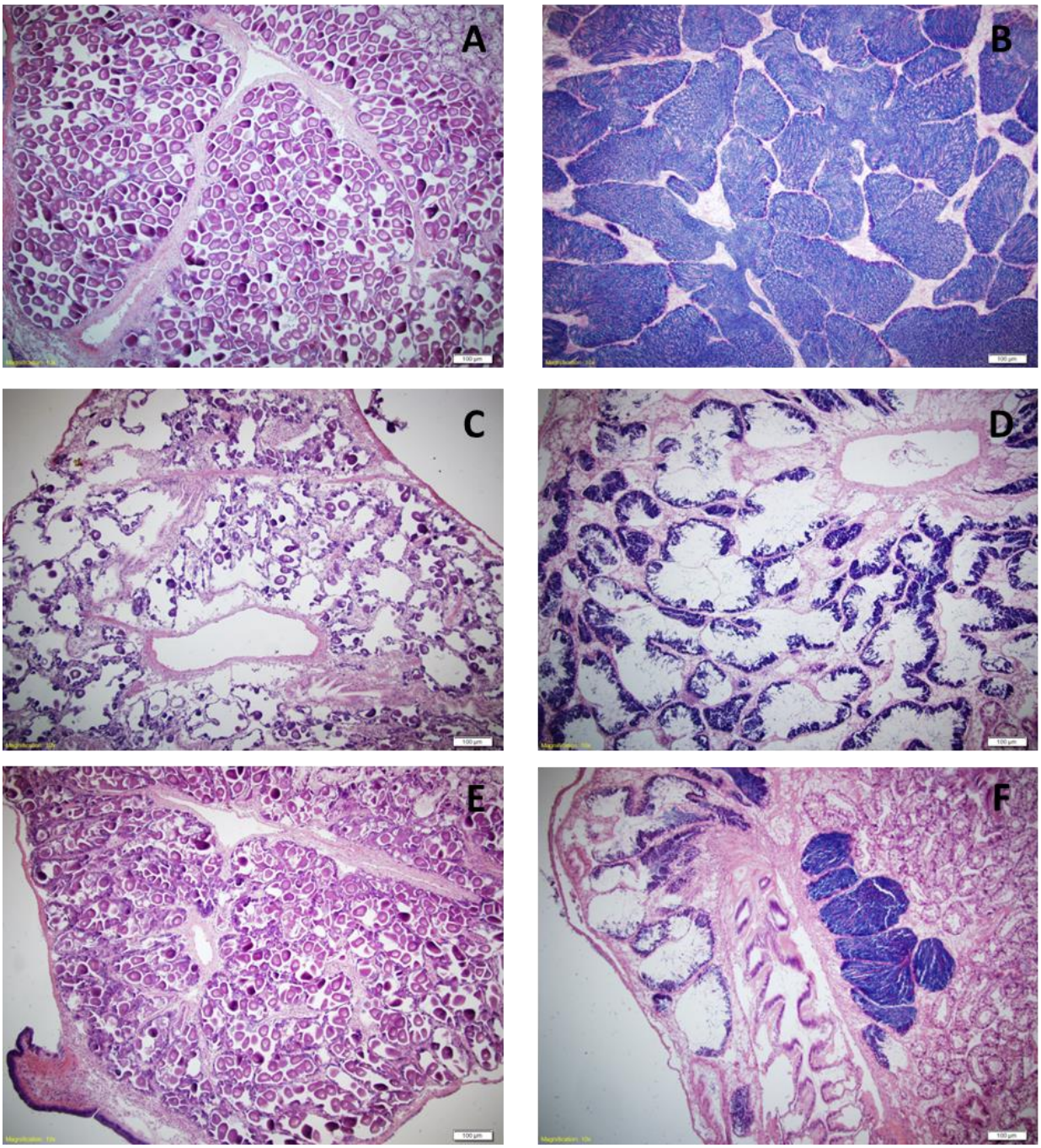

Figure 4: Micrographs of histological sections taken from mussels used during induced spawnings. Gonad from unspawned female (A) and male (B) mussels. Some mussels released a majority of their gametes (C and D) while many only released a portion of their gametes during spawning (E and F).

3.4 Condition index and proximal biochemical composition

Condition index showed high variation among individuals and between months ranging from 8 – 16 (Fig. 5). Overall no significant seasonal trend in energy storage was observed. The results of the stepwise multiple linear regression showed condition index to be partially explained by glycogen and lipids ($R^2 = 0.171$; $p \leq 0.001$), however this effect is small and only explains 17% of the variance observed.

Glycogen, protein and lipids all peaked in the summer months with annual ranges of 44.3 – 158.5 mg g^{-1} dry tissue weight, 409.0 – 628.0 mg g^{-1} dry tissue weight, and 7.4 – 13.5 mg g^{-1} dry tissue weight respectively (Fig. 6). Relatively, glycogen comprised 4.0 – 15.8% of total dry tissue weight, while protein and lipids accounted for 39.3 – 60.7% and 7.3 – 13.5% total dry tissue weight, respectively. During a mortality event in March 2012, glycogen reached a low of 2.4%, while protein and lipid were 45.7% and 7.6% respectively. No significant correlations were observed between biochemical composition, month or environmental conditions. Additionally, no significant relationships were detected between biochemical energy cycles and sex or reproductive state. However, when grouped by seasons (winter, spring, summer, fall) a significant change in glycogen content was observed ($\chi^2_3 = 46.72$; $p \leq 0.0001$; Kruskal-Wallis), with peaks in the summer (2012 and 2013). Although not significant, both glycogen and lipids reached highest levels prior to spawning, particularly in July and August each year (Fig. 2 and 6).

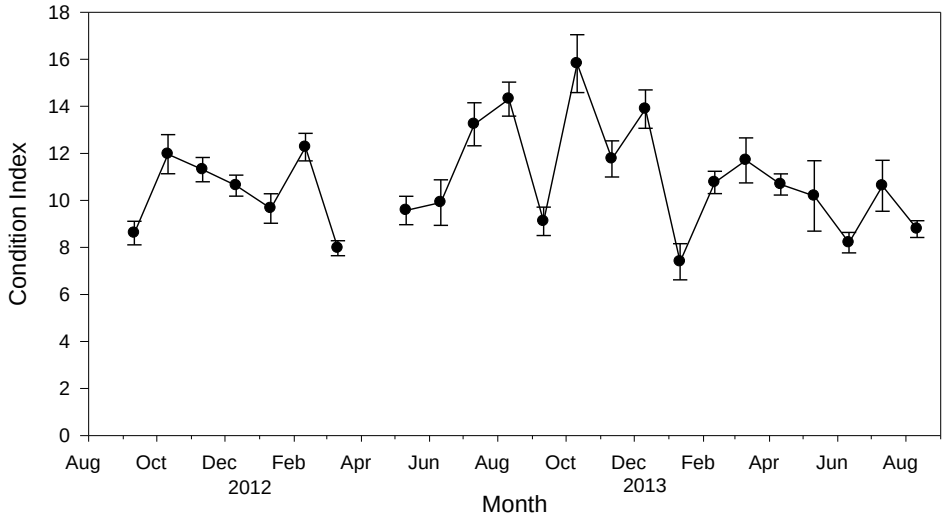

Figure 5: Condition index averaged by month (N = 10 / month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts. Error bars represent standard error.

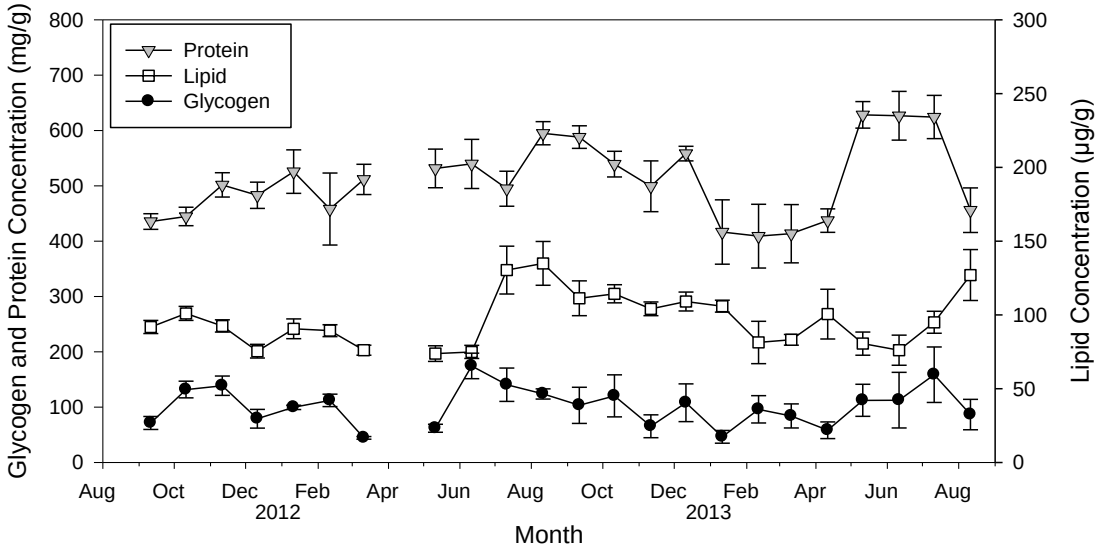

Figure 6: Proximal biochemical composition averaged by month (N = 10 / month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts. Error bars represent standard error.

4. Discussion

Monthly monitoring of established populations revealed year round reproductive activity with two seasonal peaks and newly settled juveniles reaching reproductive maturity within 2 – 3 months. The observed year round reproduction is fueled by an ability to maintain adequate energy reserves with minimal seasonal variation of proximal biochemical composition, allowing for the continuous energetic contribution to gametogenesis. This strategy allows for year round spawning and the ability to reproduce at the first sign of suitable environmental conditions and helps to explain the aggressive invasion and rapid colonization observed throughout the southeastern United States and Caribbean.

4.1 Gametogenesis (field and laboratory analysis)

During this study, *P. viridis* exhibited year round gametogenesis and winter spawning capabilities, as observed through monthly histological analysis and the successful laboratory spawning of winter acclimated mussels. Histological analysis of laboratory spawned mussels confirmed the partial spawning strategy observed in the field in which the majority of mussels released only a portion of their gonad during spawning, leaving a portion in a ripe / spawning condition. Winter spawned mussels produced less eggs per female, however both summer and winter spawnings produced viable gametes with high fertilization rates (>93%). Continuous gametogenesis, with incomplete spawning leaving follicles within the same gonad at different stages of development is common for tropical bivalves (Gaspar et al. 1999; Pouvreau et al. 2000) and is well documented for *P. viridis* (Cheung 1991; Lee 1986; Rajagopal et al. 1998; Walter 1982). Kripa et al. (2009) observed year round gametogenesis in *P. viridis* sampled in southwest India in which all individuals were at least partially ripe and no fully spent mussels were observed. However, these findings are in contrast to that observed in *P. viridis* populations in Tampa Bay, just 160 km north of Estero Bay, which experienced a resting period in the winter months (Barber et al. 2005). Differences between studies may be explained in part by variations in temperature, which has shown to be the primary forcing factor in *P. viridis* reproduction cycles (Cheung 1991; Lee 1988). Tampa Bay experiences colder winter temperatures than observed in Estero Bay, 12.9˚C (Badylak et al. 2007) and 18.9˚C (this study), respectively. Lee (1988) suggested a minimum of 24 ˚C was required for spawning in Hong Kong, however on the

Atlantic coast of Florida, *P. viridis* has shown year round gametogenesis and winter spawning capabilities at temperatures as low as 13 - 16˚C (Gilg et al. 2014; Urian 2009).

Gonadal development was not significantly different between males and females; however, males remained in a continuous state of late development or ripeness and maintained a higher gonad occupation compared to females, which demonstrated greater variation in reproductive state. Similarly, Urian (2009) observed males to consistently have a higher gonad volume fraction than females and several others have reported males to spawn more frequently and regenerate faster than females following spawning events (Lee 1988; Walter 1982).

No significant relationship between seasonality and gametogenesis was observed in field monitoring, however egg output of females spawned in the summer was significantly ($p \leq 0.05$) higher than that of winter spawned females (Table 2). This may be due in part to decreased water temperatures, which may slow the rate of gametogenesis (Sreedevi et al. 2014), and / or decreased food availability (as estimated by chlorophyll a), which may result in less energy available for gametogenesis. Cheung (1991) found while individuals remained in an active state year round, the amount of gonad occupied varied seasonally, suggesting that a fixed fraction of assimilated energy was allocated towards reproduction, allowing for continuous reproduction with gamete density being dependent upon available energy. This reproductive strategy is not uncommon as bivalves may reduce energy allocated to gametogenesis when food availability is reduced to maintain reserves, leading to different outputs and efficiencies throughout the year (Bayne and Newell 1983; Chipperfield 1953; Shafee and Lucas 1980). While spawning events in the winter are likely to be minimal, this reproductive strategy allows for the ability to spawn as soon as conditions are ideal (even when only temporarily) and continuous low density spawning between peaks may allow for early juvenile recruitment and increase the chance for at least some batches to experience ideal conditions for survival to adulthood (Barber and Blake 2006). This strategy may allow for a competitive advantage over local bivalves.

Condition index is often linked to spawning events through the loss of mass in the form of gametes and can be used as an indicator of spawning events (Hemachandra and Thippeswamy 2008; Lambert and Dutil 1997; Narashihim 1981). Although no significant relationship between reproductive cycles and condition index was detected, when analyzed graphically (Fig. 2 and 5) some synchronicity in spawning activity is observed. Most pronounced, in the fall of 2012,

histological analysis indicated a major peak in spawning activity accompanied by a distinct decline in condition index.

High individual variation and continuous gametogenesis may explain the lack of significance in seasonal cycles. Only 4 out of 450 individuals received a gonad rank of 1 indicating no active gametogenesis. This, however, occurred during peak spawning periods (except one individual in February 2013), as indicated by others sampled, suggesting that these individuals may have recently released all of their gametes in a spawning event. This is further supported by the results of the spawning trials in which few individuals released nearly all of their gametes in one spawn while most only released a portion of their gametes. Walter (1982) observed variation in the duration of spawning periods in different size classes of *P. viridis* and suggested reduced synchronicity among individuals to explain the lack of distinct cycles in gametogenesis in tropical and subtropical regions. It appears that local Florida populations are synchronized in the peak spawning events (spring and fall), but show little sign of synchronization during minor, intermittent spawning events between peaks, allowing for increased chance for recruitment success.

4.2 Biochemical composition

Lipid and protein measurements remained fairly stable ranging from 7.3 – 13.5% and 41.3 – 60.7% of total dry tissue weight, respectively. These values compare well with those previously reported in the literature for *P. viridis* of 4.6 – 18.2% lipid and 45.8 – 68.6% protein with observed peaks in lipids just prior to spawning events (Li et al. 2007; Rivonker and Pararulekar 1995; Shafee 1978). Peak lipid values observed during this study (12.3 – 13.5% total dry tissue weight) were observed each year in July – August, just prior to peaks in fall spawning activity. It is typical in bivalves to observe a peak in lipids during the late development / ripe stages and a rapid decrease following spawning events, corresponding with the release of lipid rich oocytes (Barber and Blake 1981; Beukema 1997; Emmet et al. 1987; Mohan and Kalyani 1989). Although no significant relationship between energetic content and gametogenesis was detected, as illustrated in Fig. 2 and 6, the cycles appear to follow similar trends, with peaks in

glycogen, followed by a peak in total lipid going into the months with peaks in spawning activity. This is especially evident during late summer and fall each year.

Glycogen content showed the greatest variation during this study period ranging from 2.4 – 15.8% of total dry tissue weight. This is similar to that previously reported in the literature for *P. viridis* from 3 – 5% (Kuriakose and Appukattam 1980) to as high as 25.6% (Fatima et al. 1986). Low glycogen stores can lead to increased susceptibility to natural stressors as well as negative impacts on growth and reproduction (Patterson et al. 1999). Although an interruption in gametogenesis was not observed, low glycogen content (2.4%) was observed during onset of a mass mortality event in March 2012. Typically, glycogen is stored when food is in excess (summer) for utilization during periods of low food availability (winter) (Ansell 1972; Emmett et al. 1987; Mohan and Kalyani 1989). In times of food shortage, glycogen is mobilized as a precursor for lipids which are an essential component of gamete production (Gabbott 1975), thus explaining significance in glycogen variation when grouped by season (low in winter, high in summer) while lipids and proteins remained fairly stable.

Previous work has shown tropical *P. viridis* to have high individual variability in proximal biochemical composition (Mohan and Kalyani 1989) and decreased environmental variation at lower latitudes allowing for more stability in reserves and less drastic "storage periods" (Hawkins et al. 1987). Nutrient availability drives primary production which, in turn, affects energy storage cycles and gametogenesis (Ansell 1972; Newell et al. 1982). Consistency in reserves suggests that food availability is sufficient for *P. viridis* to maintain energy reserves while contributing to reproduction with little seasonal effect. Reports have shown mean annual chlorophyll concentrations of 5.7 ± 0.35 µg L^{-1} in Estero Bay (Ott et al. 2006) and monitoring during this study showed monthly chlorophyll concentrations ≥ 2 µg L^{-1}. Further, the absence of drastic decreases in biochemical composition or gametogenesis suggests that the food source (ie: phytoplankton) is sufficient year around.

During the monitoring period of the present study two consecutive red tide blooms occurred leading to an accumulation of brevetoxins in *P. viridis* tissues (McFarland et al. 2015) and several mortality events (McFarland et al. unpublished data). While these events appeared to impede growth (McFarland et al. unpublished data), no significant affect was observed in

gametogenesis and spawning cycles. Although environmental stress can lead to a reduction in energy allocated to reproduction, it is typical for bivalves to give priority to reproduction over growth during periods of environmental stress (Browne and Russel-Hunter 1978; Lodeiros et al. 2001). However, a reduction in reserves to meet the high metabolic demand of spawning may cause additional physiological stress, partially explaining the observed post bloom mortality event. The lowest glycogen level (42.9 mg g^{-1}; 2.4%) was detected in the month in which the first mass mortality event occurred (March 2012) following the first major bloom exposure. Additionally, protein hit a prolonged 4 month low (409 – 437 mg g^{-1}; 39 – 44%) during the winter 2013 bloom and spiked (628 mg g^{-1}; 60%) following bloom dissipation in June 2013.

5. Conclusions

Although currently *P. viridis* and *C. virginica* occupy separate niches within Estero Bay (McFarland et al. 2014) the reproductive strategy observed during this study suggests the potential for increased competition. Locally, oysters spawn from April through October and experience a resting period in the winter months (December and January) in which gametogenesis is inactive (Volety et al. 2009; 2014). This difference in reproductive strategy may allow for green mussels to get a head start in the spring spawning season, giving an advantage to newly settled juveniles. If warm spring waters come early, oysters may be at a disadvantage allowing mussels to outcompete for substrate availability. Currently, green mussels in Estero Bay are limited to subtidal substrate on the fringes of the estuary where salinity is more stable (McFarland et al. 2014) and are sensitive to red tide blooms (*Karenia brevis*) (Baker et al. 2012; McFarland et al. 2015), which may limit population densities. Oysters on the other hand, are well adapted to local conditions with dense reefs in intertidal regions of the bay where temperature and salinity variations may be extreme (Barnes et al. 2007). With the threat of sea level rise, however, these populations should not go unmonitored. High fecundity and reproductive rates suggest the potential, given the right conditions, to rapidly invade and overtake existing oyster reefs.

To further explain what is observed in the field, it would be interesting to condition *P. viridis* under a range of temperatures and different nutritional diets to observe changes in both

the reproductive output and reserve maintenance, storage and utilization. Modeling of the energetics will help to explore and understand dynamic links between environmental variability, growth and reproduction. Additionally, biochemical composition of individual tissue compartments may better identify seasonal trends within the organism.

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Chapter 5: Application of the Dynamic Energy Budget theory to model growth and reproduction of established population of the non-native green mussel *Perna viridis* in southwest Florida coastal waters

Application of the Dynamic Energy Budget theory to model growth and reproduction of established population of the non-native green mussel *Perna viridis* in southwest Florida coastal waters

Abstract

As an invasive species ecological concerns have risen with regards to the spread and success of the green mussel *Perna viridis* throughout southeastern United States and Caribbean coastal regions. As a subtidal marine and estuarine species the arrival to new locations often goes unnoticed until the populations have become established. Application of the Dynamic Energy Budget (DEB) theory allows for the inclusion of a changing environment and a systemic response from the organism. Through the application of the DEB theory a set of parameters was estimated to accurately model the growth and reproduction of green mussels forced by varying environmental conditions. Environmental variables, particularly food and temperature, are driving forces of both growth and reproduction and thus play an essential role in the foundation of the DEB predictions. The model was calibrated using data from field monitoring of growth and reproduction throughout a two year monitoring period to ensure realistic outputs and validated using an external data set to assure the ability to use this information for populations across a wide geographic span simply by changing the environmental parameters.

1. Introduction

The green mussel, *Perna viridis*, is an invasive bivalve to southwest Florida and due to its aggressive biofouling behavior, concerns abound regarding ecological and economic impacts of this invasion (Benson et al., 2001; Ingrao et al., 2001; Baker et al., 2007). Native to the Indo-Pacific, they were first observed in Trinidad and Tobago in the early 1990's (Agard et al., 1992) and have since rapidly spread at high densities throughout the coastal waters of the Caribbean and southeastern United States (Rylander et al., 1996; Benson et al., 2001; Ingrao et al., 2001; Buddo et al., 2003; Baker et al., 2007). As a recently introduced species, little is known about

local population dynamics and their potential for spread and competition with native fauna. While currently northern spread is limited by cold winter temperatures, the threat of climate change and warming ocean temperatures (IPCC, 2013) has increased concern regarding tropical invasions. Growth and reproduction are major factors involved in population dynamics and success of a species and are useful predictors of population success. *Perna viridis* reaches reproductive maturity within the first 2 – 3 months of settlement and has shown year round gametogenesis and spawning capabilities (Parulekar et al., 1982; Rao, 1975; Sreenivasan et al., 1989; Chapter 4). High fecundity coupled with rapid growth rates, $\geq$10 mm month^{-1} (Lee, 1986; Sreenivasan et al., 1989), make them a competitive marine invader and raises concern for competition with native bivalves.

The Dynamic Energy Budget (DEB) theory for metabolic organization (Kooijman, 2010) allows for a dynamic approach to modeling bioenergetics of individuals and has been successfully applied to bivalves for several species particularly from an aquaculture and stock enhancement point of view (ie: Ren and Ross, 2005; Bacher and Gangney, 2006; Sará et al., 2012). DEB theory provides a generalized framework for bioenergetics at the individual level with linkages between life stages allowing for a more complete approach to modeling physiological processes in an individual throughout a life cycle. DEB is unique in that is relies on generic assumptions about energetics and a basic set of parameters that are used to define energy fluxes. These rules and parameter sets are universal to all species with the only difference being the numerical value for the parameters which are species specific.

This study is the first to estimate a set of parameters and apply DEB theory to model growth and reproduction of *P. viridis* as a function of the environment for local populations. Basic data sets and information on life stages are pooled from the literature and model parameters calibrated using field monitoring from local populations (Ch. 3 and 4). The resulting model was validated for other geographic populations using data from the literature.

2. Methods

2.1 Model formulation

The model used in this study was based on the principles of the DEB theory (Kooijman, 2010) which allows for a dynamic implementation of physiological response to a changing environment. According to this theory biomass can be divided in three compounds which are described as state variables; structure (V), reserves (E) and a reproduction buffer (E_R) to describe the bioenergetics of an individual organism throughout a lifespan. The standard DEB model with an extension for metabolic acceleration was applied which assumes one food source, one type reserve, and one type of structure. A brief explanation of the energy flow through an organism is given by Figure 1. The allocation of energy from reserves follows the kappa (κ) rule in which a fixed fraction of energy from reserves (κ) is allocated towards maintenance and growth with a priority given to maintenance and overhead costs of growth. Once maintenance is paid, energy may be allocated to growth and if maintenance costs are higher than κ, growth will cease. The remainder of the energy mobilized from reserves (1-κ) is allocated to maturation in juveniles and reproduction (gametes) in adults, with a priority given to maturity maintenance costs, which include maintaining the level of maturity, overheads of gamete production and metabolic costs of spawning. While these two metabolic processes are separated in the model, they share similarities and are both strongly influenced by temperature and functional response. Under conditions of severe nutrient limitation or starvation, energy may be taken from the reproduction buffer to meet somatic maintenance costs, and during extreme starvation, the complete depletion of reserves can lead to the utilization / or lysis of structure in a survival attempt to pay the costs of maintenance, a process referred to as “shrinking” (Bernard et al., 2011).

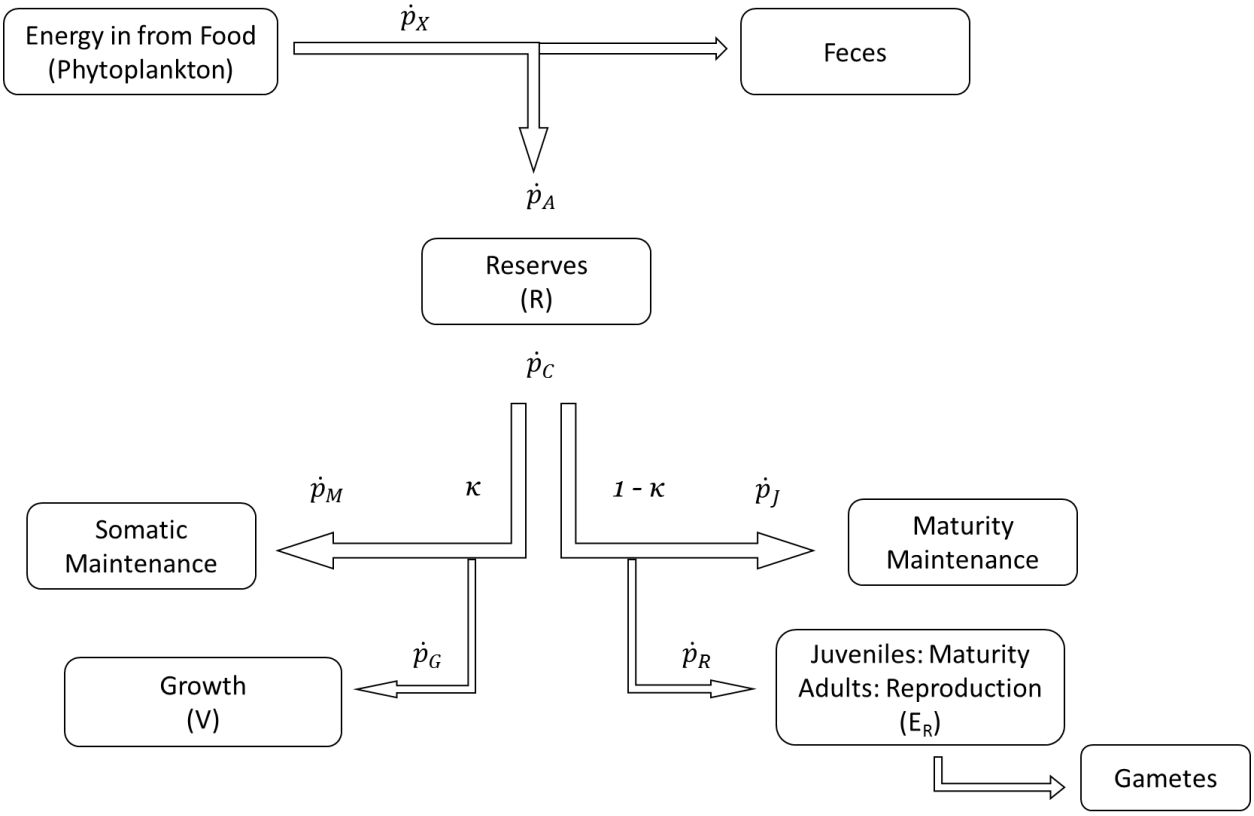

Figure 1: Flow of energy through the organism according to the DEB theory. Food is acquired through feeding activities ($\dot{p_X}$) and is assimilated as energy into reserves ($\dot{p_A}$) with a portion of energy being lost to heat dissipation and feces production. Energy is then mobilized from the reserves ($\dot{p_C}$) and a fixed fraction (κ) goes towards somatic maintenance ($\dot{p_S}$) and growth ($\dot{p_G}$) with the remainder (1-κ) used for maturation in juveniles and production of gametes in adults ($\dot{p_R}$) and maturity maintenance ($\dot{p_J}$).

The standard DEB model only includes the embryo, juvenile and adults stages, however, bivalves experience a fourth key developmental stage known as metamorphosis which follows a pelagic larval stage. Metamorphosis represents an important metabolic switch in which distinct changes in anatomy, growth and energy allocation occur between the pelagic larval stage and the sessile post metamorphosis life stage. Juveniles and adults are considered to be isomorphs (no change in shape during growth) while larvae exhibit a V-1 morph growth pattern (increase in surface area proportional to structural volume). The variation in growth patterns between larval and adult stages was addressed by the inclusion of a metabolic acceleration factor and the estimation of two shape coefficients (δ_M) which define growth with respect to shell length and

volume; one describing growth in the larval phase as a V-1 morph (δ_{M_b}) and a second describing growth after metamorphosis as an isomorph (δ_{M_j}) (Kooijman, 2014; Lika et al., 2014).

Physiological rates are dependent on temperature and show significant fluctuation with environmental variation. Within the range of tolerance, physiological rates tend to increase, while outside the tolerance, rates tend to decrease (Bourlès et al., 2009). To account for this fluctuation DEB theory makes use of the Arrhenius relationship to predict physiological changes based on the species specific upper and lower temperature threshold and the Arrhenius temperature correction factor (described in detail by Bourlès et al., 2009).

2.3 Parameter estimation

Parameters were estimated using the covariation method described by Lika et al. (2011) through the add_my_pet and DEBtool routines (http://www.bio.vu.nl/thb/deb/deblab/debtool/) using Matlab R2012a. Parameter estimates were determined using the `nmregr` routine (in the DEBtool package) in order to reduce variation between predictions and observations through the weighted least-squares (WLS) estimation method. This method allows for the simultaneous estimation of all parameters in one step based on empirical data from the literature and observations of locally established populations. This data includes basic information on physical measurements (zero-variate data) including age, length and weight at birth (Laxmilatha et al., 2011), metamorphosis (Tan, 1975), puberty (Rao et al., 1975; Ch. 4), and adulthood (Rajagopal et al., 2006) (Table 1). Additionally, time series data including physiological rates (univariate data) were included: length versus age data (Sreenivasan et al., 1989), weight versus age data (Sreenivasan et al., 1989), and length versus wet weight data (Mohan, 1980). Weight coefficients are used to increase focus on data in which there is more confidence or certainty in the values. For example, more weight was given to length and weight data as they are observable data for which we have more confidence. Conversely, we reduced the weight given to ages at birth and metamorphosis as life stages are defined by maturation rather than definitive age.

Following this procedure, a set of primary parameters was estimated (Table 2). The `mre` routine in the DEBtool software was used to calculate the mean relative error expressed as a

mark of goodness of fit between predictions and real observations. Fit is defined as Fit = 10(1 – mean relative error) in which a fit of 10 would indicate that predictions and observations line up perfectly (Lika et al., 2011).

Table 1: Zero-variate data gathered from the literature (observed values) and provided to the estimation procedure. The predicted values are obtained with the set of estimated parameters.

Symbol		Dimension	Value		Reference
			Observed	Predicted	
a_b	Age at birth	days	0.9	1.063	Laxmilatha et al., 2011
a_p	Age at metamorphosis	days	12	11.69	Tan, 1975
a_j	Age at puberty	days	60	53.63	Rao et al., 1975; field data
L_b	Physical Length at birth	cm	0.008	0.00907	Laxmilatha et al., 2011
L_j	Physical Length at metamorphosis	cm	0.04	0.00397	Laxmilatha et al., 2011
L_p	Physical Length at puberty	cm	2.0	1.635	Rao et al., 1975
L_i	Ultimate Physical Length	cm	18	18.79	Rajagopal et al., 2006; field data
W_b	Dry Weight at birth	g	3×10^{-8}	3.1×10^{-7}	estimated from *Mytilids*
W_j	Dry Weight at metamorphosis	g	3×10^{-6}	2.6×10^{-5}	estimated from *Mytilids*
W_p	Dry Weight at puberty	g	0.03	0.02633	estimated from field data
W_i	Ultimate Dry Weight	g	28	27.79	estimated from literature
R_m	Maximum Reproduction Rate	# / day	1.59×10^5	1.854×10^5	estimated from lab studies
t_m	Life Span	days	1200	1200	estimated from literature

2.3 Study site, forcing variables and calibration

The model was calibrated using growth and reproduction data from monitoring of local populations in southwest Florida. The study site was located at the Gulf of Mexico and Estero Bay interface (Fig. 2) where dense populations of *P. viridis* cover the subtidal bridge pilings and fender system. This site has a strong marine influence maintaining high salinities and extensive tidal flushing. Seasonal cycles in growth and reproduction were determined through monthly analysis over a two year monitoring period. Specifically, growth rates were measured monthly using individually tagged and caged juvenile green mussels (Ch. 3) and gametogenesis was determined through histological analysis on mussels collected from the same established populations (Ch. 4). Reproduction was quantified through standard histological analysis (Howard

et al., 2004) to characterize cycles in gametogenesis and spawning. The gonad of *P. viridis* is dispersed throughout the mantle lobes, mesoma, and interspersed between the digestive glands (Rajagopal et al., 2006), thus a distinct dissection of the gonad from the body is not possible for the measurement of gonadosomatic index (ratio of gonad mass to whole body mass; GSI). To handle this issue, a ratio of gonad material to whole body was determined using image analysis according to Kang et al. (2003). Scanned images of the whole tissue sections from histology slides were analyzed using ImageJ™ image analysis software to quantify the area of active gonad and total tissue (excluding gills). These areas were then converted to volume using the measured physical length for each individual and converted to weights using the measurement for total wet weight measured for each individual. Wet weights were converted to dry weights using the ratio for wet to dry weight determined through monthly analysis of condition (Ch. 4).

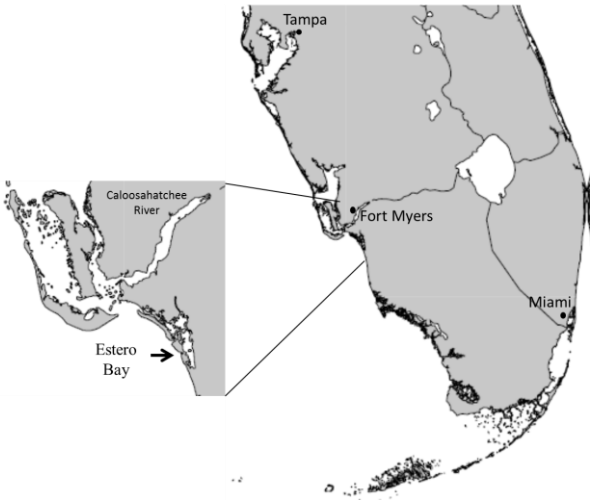

Figure 2: Map of the study site

Environmental conditions (temperature, salinity, dissolved oxygen and chlorophyll a) were monitored through continuous hourly data collection using YSI Sondes maintained on site and from Sondes courtesy of Sanibel-Captiva Conservation Foundation (SCCF) through the River, Estuary and Coastal Observing Network (RECON). In May – August 2011, the equipment for the SCCF sonde was damaged and unable to be immediately replaced. To correct

for this missing data, daily averages were taken over the previous 5 years to fill in the data gap with an estimation of the approximate environmental conditions.

2.4 Model simulation

Reproduction in *P. viridis* has been shown to be synchronous in seasonal peaks (spring and fall), but asynchronous, intermittent spawning occurs between these peaks (Rao et al., 1975; Walter, 1982; Fatima et al., 1985). Intermittent spawning between peaks, is likely an adapted reproductive strategy to maximize the potential for fertilization contributing to a continuous flux of juvenile recruitment (Newell et al, 1982; Barber and Blake, 2006). Spawning events were estimated through the inclusion of thresholds for the forcing variables within minimum requirements for temperature (degree days), food and GSI. As *P. viridis* are partial spawners, it was assumed that the reproduction buffer was not completely emptied at each spawning event, and estimated that only 30% of the gametes were released. In this way, the individual would continuously maintain a minimum requirement for GSI allowing for a rapid recovery and continuous / partial spawning capabilities as observed in local populations (Ch. 4). GSI was modeled using weight to describe the building of the reproduction buffer (E_R) and weight gain and loss in the form of gametes (Lavaud et al., 2013). Once the minimum required GSI is reached the model simulates a spawn, provided the prerequisites of spawning are met (temperature and food minimums).

$$W = V + \left((E + E_R) \times \frac{\omega_E}{\mu_E}\right)$$

$$W_E = E \times \frac{\omega_E}{\mu_E}$$

$$W_{ER} = E_R \times \frac{\omega_E}{\mu_E}$$

$$\mathrm{GSI} = \frac{W_{ER}}{Ls^{1/3}} \times 1000$$

Table 2: Calculations for intital values of state varaibles

Equation	Variable description
L_i	Initial length
W_i	Initial dry weight
$V_i = (L_i \cdot \delta_M)^3$	Initital structure
$E_i = \frac{\{\dot{p}_{Am}\}}{\dot{v}} V_i$	Initital reserve
$E_{Ri} = \left[W_i - V_i d_{Vd} - \left(E_i \frac{\omega_E}{\mu_E}\right)\right]\left(\frac{\mu_E}{\omega_E}\right)$	Intial repoduction buffer
$E_{Hi} = E_H^p$	Intial maturity

2.5 Validation of the model

Growth and reproduction data from Rajagopal et al. (1998) was used to validate the model. This validation was completed first by adjusting the initial conditions to match the data set which included changing the initial length, weight and age measured to estimate the initial contribution of each state variable; structure, reserve, and reproduction buffer (Table 2). Additionally, the forcing factors, temperature and chlorophyll, were updated to the conditions reported by Rajagopal et al. (1998) during monitoring. Growth was fit by adjusting the half-saturation coefficient (X_K), as explained by Alunno-Bruscia et al. (2011) which controls for ingestion through the functional response:

$$f = \frac{X}{(X + X_K)}$$

Spawning was more complicated to fit as variations in spawning strategy occur between geographic regions and invasive populations can react differently to new or unpredictable environments leading to increased variation between populations (Newell et al., 1982; Richard et al., 2006). Secondly, gametogenesis was reported in a different manner than calculated for the calibration data set and thus needed to be taken into consideration when simulating spawning events. For these reasons adjustments were also made to the rules of spawning including changing the temperature and minimum GSI required for spawning.

3. Results

3.1 Parameter estimates

The parameter estimates (Table 3) resulted in a fit of 8.52 out of 10. All estimates for zero-variate data matched up well with the expected values reported in the literature (Table 1) with the exception of weight at birth and metamorphosis. These are, however, very difficult time points to obtain accurate measurements of weight due to the size of the individuals and thus estimations were used for these values.

Table 3: Primary parameters.

Symbol	Unit	Description	Value
T_{Ref}	K	Temperature at which parameter rates are given	293
T_A	K	Arrhenius Temperature	7416
f	-	Scaled functional response	0.6
$\{\dot{p}_{Am}\}$	J/day/ cm^2	Maximum surface-area specific assimilation rate	49.556
δ_{M_b}	-	Shape coefficient before metamorphosis	1.418
δ_{M_j}	-	Shape coefficient after metamorphosis	0.2752
F_m	1/day/ cm^2	Maximum specific searching rate	6.5
κ_X	-	Digestion efficiency	0.7
$\dot{v}$	cm/day	Energy conductance	0.03144
κ	-	Allocation fraction to soma	0.4418
κ_R	-	Allocation fraction to reproduction	0.95
$[\dot{p}_M]$	J / day / cm^3	Volume specific maintenance cost for structure	11.14
$\dot{k}_j$	day^{-1}	Maturity maintenance coefficient	0.002
$[E_G]$	J cm^{-3}	Volume specific cost for structure	4338
E_H^b	J	Maturity at birth	0.00846
E_H^b	J	Maturity metamorphosis	0.7171
E_H^p	J	Maturity at puberty	732.5
$\dot{h}_a$	day^{-2}	Weibull aging acceleration	2.8e-08
s_G	-	Gompertz stress coefficient	1e-04

The predictions for growth in shell length over time and length weight relationships line up well with observed values (Fig. 3) during the parameter estimation procedure. In the length versus weight predictions (Fig. 3C), the predicted curve (red line) is at the bottom and even slightly below the expected values (blue crosses), but this is expected for two reasons. First, the weight of structure only is estimated through simulation, while the measured weight includes structure, reserve and reproduction buffer which cannot be physically separated for measuring each on its own. Second, *P. viridis* contributes to gamete production year round so the reproduction buffer will always have a significant contribution to measured physical weight.

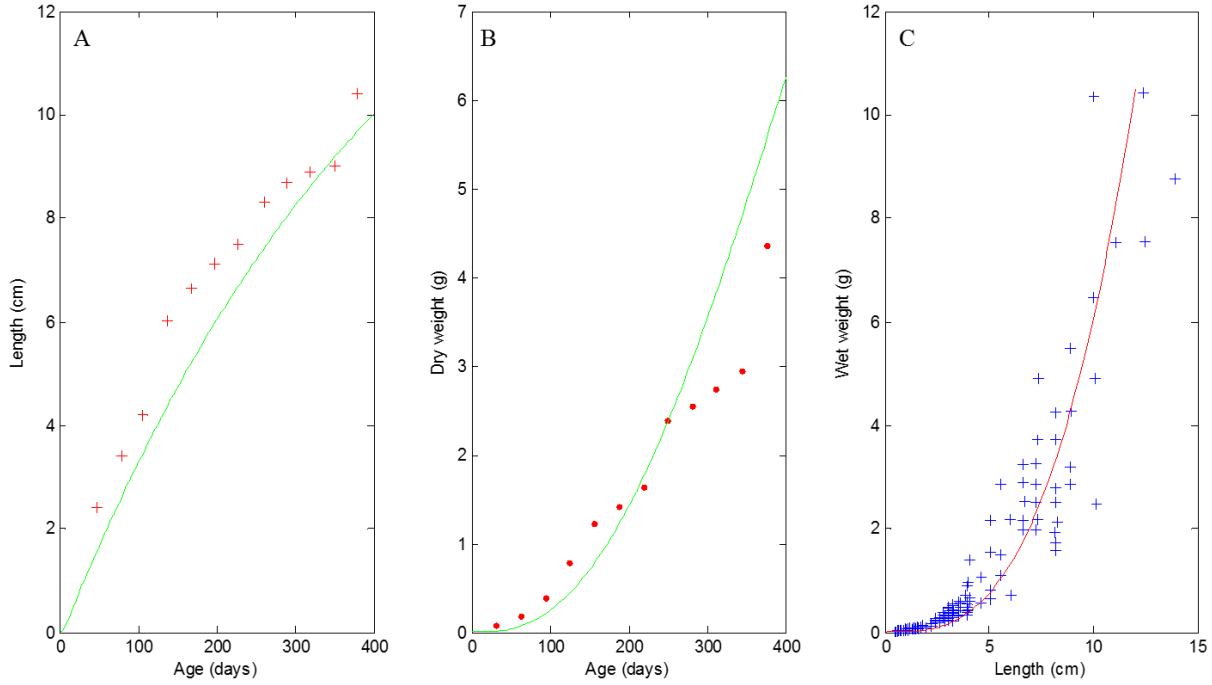

Figure 3: Model predictions (solid lines) plotted against observed data (crosses) for length versus age (A), weight versus age (B) and weight versus length (C).

3.2 Environmental forcing factors

Temperature shows a fairly consistent pattern in annual cycles with monthly averages of 17 – 20˚C in the winter and as high as 28 – 31˚C in the summer (Fig. 4). However, some inter-annual variation was detected in the duration of the annual low. In the winter of 2013 temperatures remained low for a longer period, but the monthly averages were relatively high, 19.4 – 20.8 ˚C. In contrast, the winters of 2011 and 2012 were shorter, but lowest monthly averages were 16.3 ˚C and 18.8 ˚C, respectively, suggesting a shorter, but more intense winter. Chlorophyll a showed seasonal variation and more inter-annual variation, with monthly averages ranging from 2.0 – 5.5 µg L^{-1} which has been suggested to be sufficient to support growth (Chapter 3) and gametogenesis (Chapter 4) in local populations.

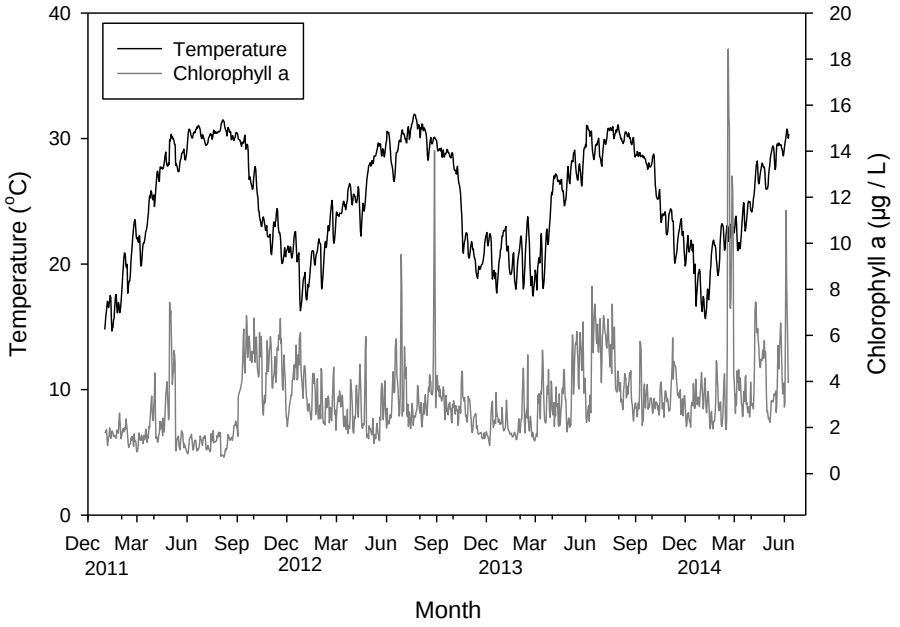

Figure 4: Chlorophyll a and temperature averaged daily during the monitoring period.

3.3 Model simulation

Growth was nicely simulated in the model to fit the observed growth during field monitoring of local populations (Fig. 5) with the estimated parameter set. Growth was further adjusted within the model by adjusting X_K which controls for ingestion and allows for calibration with the new dynamic food condition simulated in the model. The intermittent and continuous spawning strategy of *P. viridis* was captured by adjusting the spawning efficiency. This allowed for a partial release of the gonad once the threshold GSI was reached in which only 30% of the gonad was released upon each spawning event, assuring that the reproduction buffer was never fully emptied and a rapid regeneration to the next spawning event was achieved. The DEB model follows an individual over a lifetime, thus the simulated spawning represents the spawning events of one individual over a lifetime. This information is impossible to collect from natural populations as individuals must be sacrificed in order to collect information on gametogenesis and spawning. Further, the data collected were point samples taken once a month. This type of sampling does not allow for the detection of every detail of the gonad cycle to fully characterize the details of reproduction. Thus, while predicted and observed spawning events do not always line up perfectly in frequency and magnitude, they do follow similar cycles and represent a

realistic description of spawning in natural conditions throughout the life cycle of an individual (Fig. 6). The two wide gaps between spawning at 325 – 400 days and 675 – 800 days represent the winter months (approximately November – February) during which time the reproduction buffer is being built-up in preparation for the spring spawning season. The first peak in spring spawning is observed in the model prediction with a peak in spawning mid-February to March following the period of building the buffer. Everything between represents continuous, intermittent spawning through the spring, summer and fall.

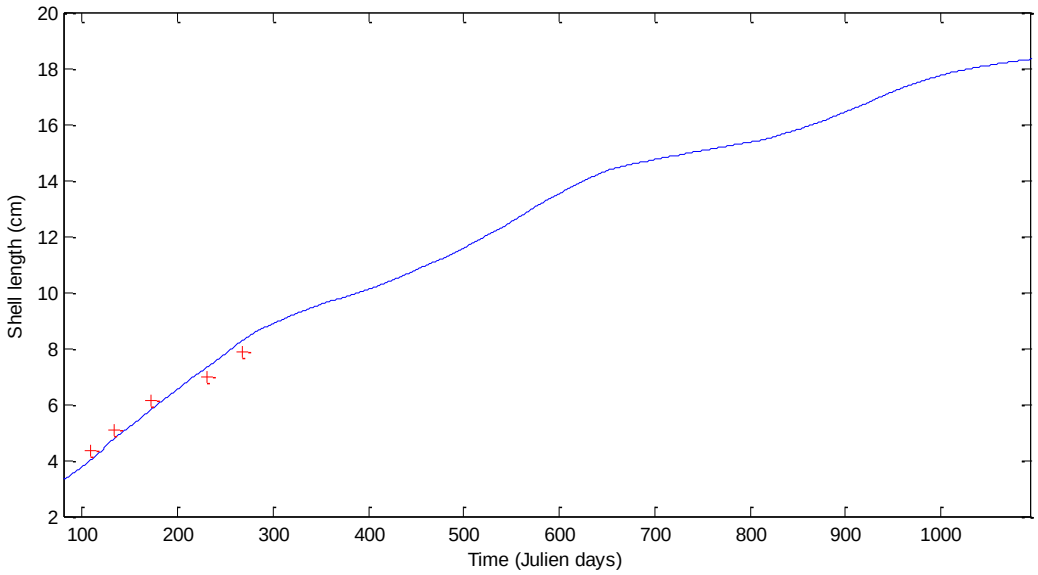

Figure 5. Model output for growth in shell length using the estimated parameters to compare field monitoring data from local populations (red) to calibrate model predictions (blue).

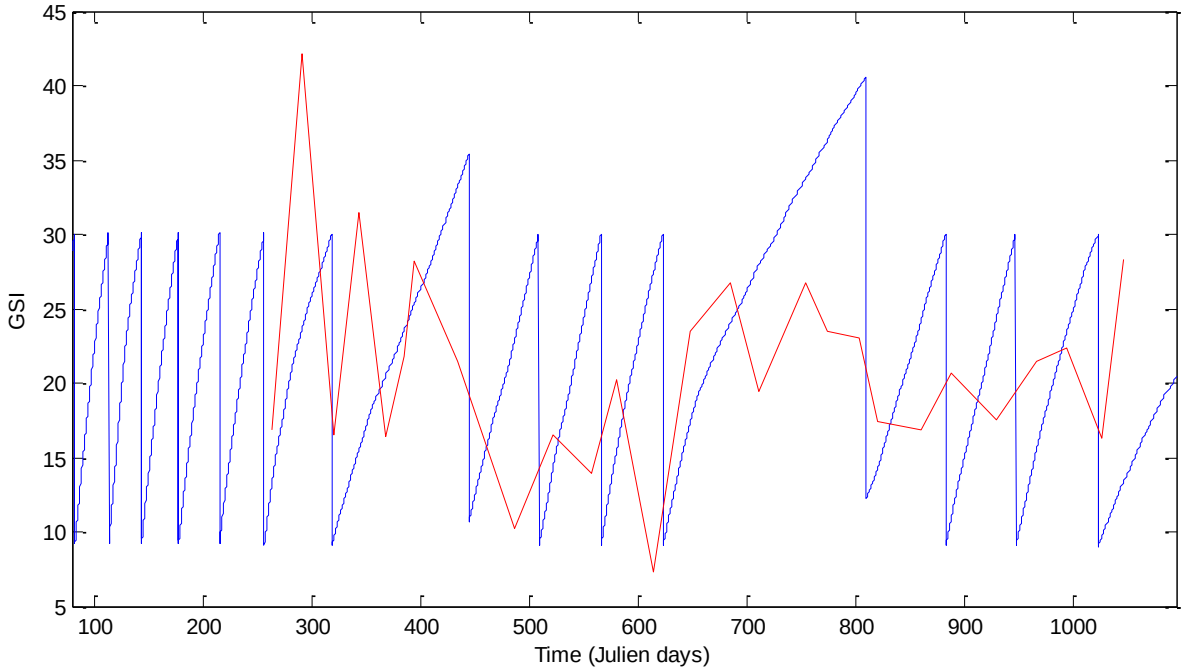

Figure 6. Spawning cycle observed in local *P. viridis* populations (red) and that predicted by the model (blue) as described by GSI over time.

3.4 Validation

The model was validated using data from Rajagopal et al. (1998) which monitored growth and reproduction in natural *P. viridis* populations within the native range of southeast India. The model predictions for growth in length (Fig. 7) fit for all three cohorts monitored by simply adjusting X_K to fit the new environmental conditions, primarily food condition. However, spawning for this data set was not so easily fit, particularly in the winter months when the model predicts a building of the buffer, but the data shows a spawning event (Fig. 8). The native populations show a more synchronized and less frequent spawning pattern than those in Florida. This pattern was partially captured by the model and although the timing of the spring peak is off, the model does represent the spawning strategy in the summer months.

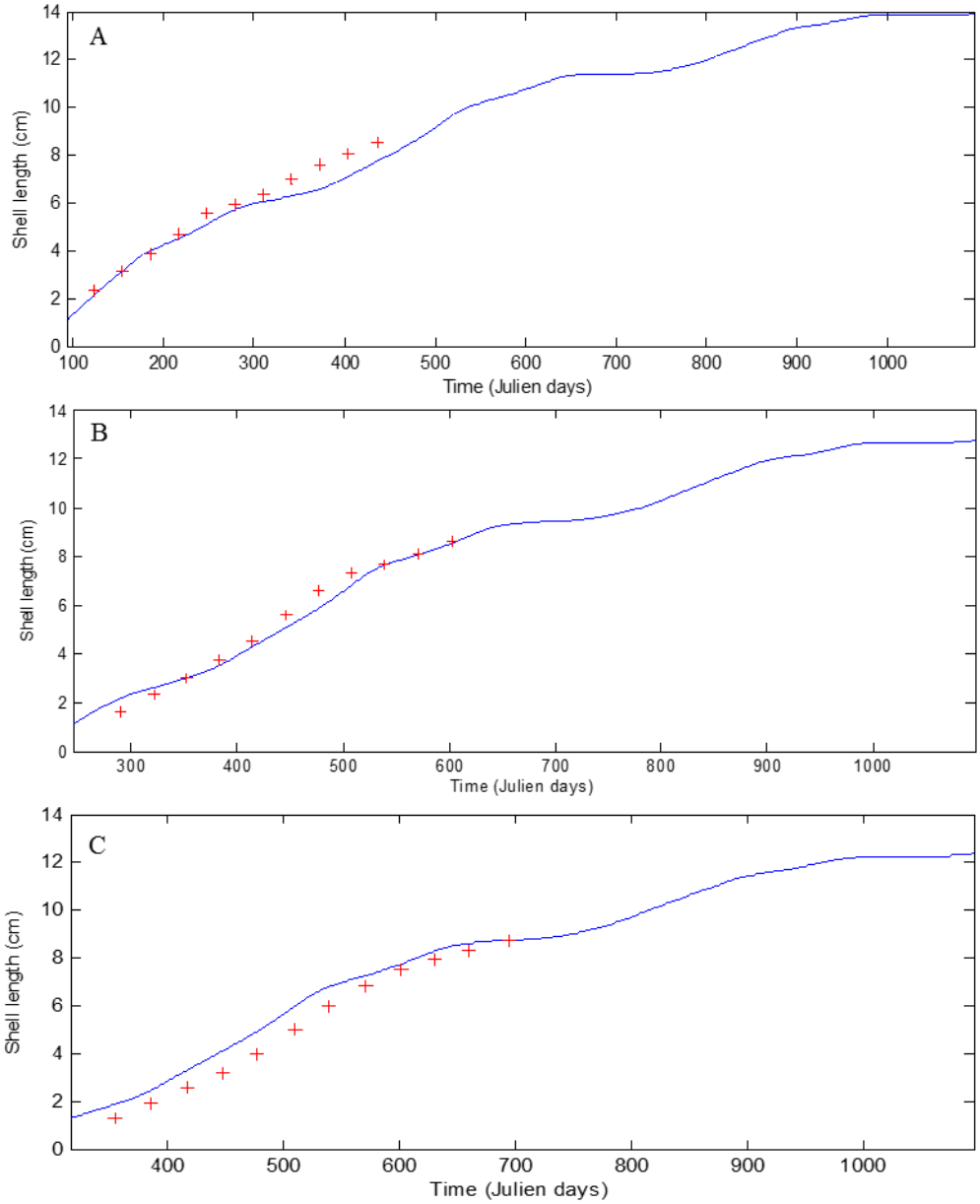

Figure 7: Results of the estimation for Rajagopal et al. (1998) growth in shell length. Three different cohorts were followed; beginning in April (A), September (B) and November (C) and followed for the first year of growth. Red crosses represent observed data and blue line represents model estimation.

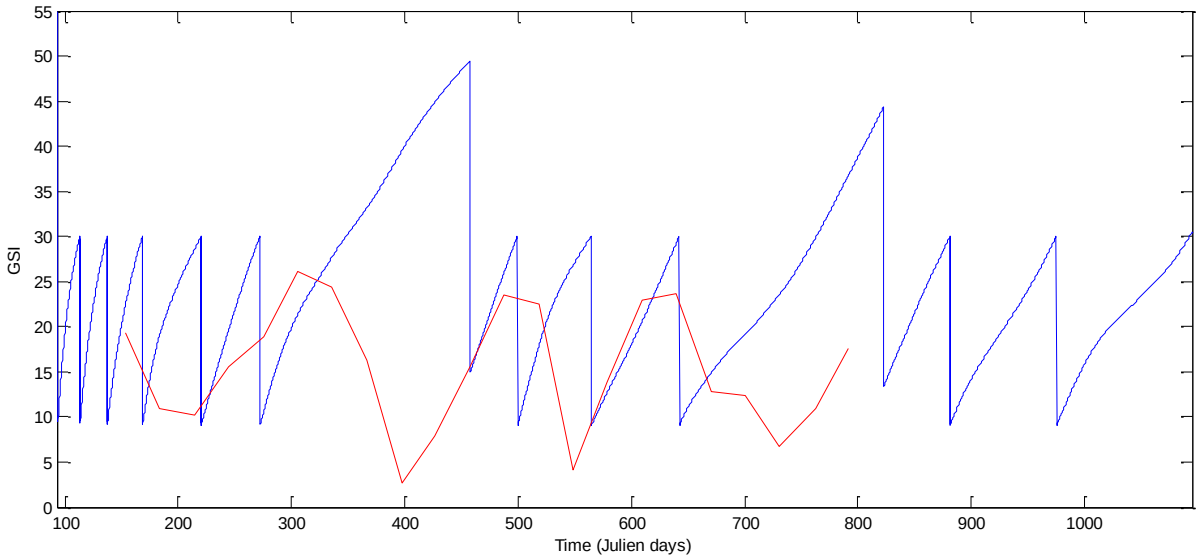

Figure 8: Results from the spawning simulation for the Rajagopal et al. (1998) data, the blue line represents the model simulation and the red line represents field observations.

4. Discussion

This study aimed to model growth and reproduction of *P. viridis* in a dynamic environment through the application of the DEB theory. This was accomplished by including metabolic acceleration in the standard DEB model with one food source, one type of structure and one type of reserve. Through the DEBtool routines a set of primary parameters describing maturation and energy flux through time were estimated for an individual mussel. Overall, these estimates resulted in a good fit of 8.52 between observed and predicted values (Table 1). However, despite adding an acceleration factor and a shape coefficient specific to the larval stage, weight at birth and metamorphosis were slightly overestimated. Physical weights at these early stages are difficult to measure and in the case of *P. viridis*, observed values were estimated from those reported for several other Mytilid species in the add_my_pet collection. While the species used for estimates (*Mytilus edulis, Mytilus galloprovinalis, Mytilus californianus*) have similar larval life stages, it is possible that using larval data from these species resulted in an underestimation of weight for *P. viridis* at these stages. Indeed, values reported for these species show high variability (Table 4). Moreover, age at metamorphosis for all bivalves varies between

geographical regions depending on environmental factors and delaying metamorphosis to prolong the searching phase is common in Mytilid species (Bayne, 1965). Nevertheless, the focus of this model was on the post metamorphosis life stage and thus, these discrepancies in larval weight do not appear to significantly affect the output.

Table 4: Comparison of values between Mytilid mussels from the add_my_pet collection. Age (a) is given in days, length (L) in cm and weight (W) in grams.

Species	a_b	L_b	W_b	a_j	L_j	W_j	a_i	L_i	W_i	δ_M
M. edulis	0.3	0.003	2.9e-09	0.3	0.003	2.9e-09	10119	4.7	17.1	0.2942
M. galloprovinalis	1.2	0.004	1.6e-08	2.7	0.008	1.2e-07	5503	4.8	19.8	0.1989
M. californianus	1.1	0.006	3.5e-08	4.3	0.013	2.9e-07	7300	4.4	12.0	0.2741
P. viridis	0.9	0.008	3.0e-08	18	0.04	3.0e-06	1200	18.8	28.8	0.2752

Overall, the model fit field observations of *P. viridis* populations in southwest Florida, however, is somewhat limited by a lack of growth data across the entire lifespan. During this study, field growth monitoring was forced to a halt after one year, due to a mass mortality event attributed to red tide blooms in the area (Chapter 3) and growth data in the literature is collected from an aquaculture perspective in which the interest lies in the first 6 – 12 months (ie: Mohan, 1980; Sreenivasan et al., 1989; Masilamoni et al., 2011). Those studies which do report growth past the first year are predicted values from scope for growth models as opposed to measured values (ie: Al-Barwani et al., 2007). The realistic representation of ultimate length, however, suggests that the estimates represent reasonable expectations for growth and reproduction from 12 months onward. The observed red tide events during this study period may also impact the observed GSI and spawning activity, however when compared with qualitative data from the *P. viridis* populations on the Atlantic coast (Urian, 2009) the general pattern in spawning is captured.

Growth was accurately predicted during both the calibration and validation procedures, yielding realistic growth curves for two distinctly different environments in different geographic regions. These growth curves were obtained by changing the environmental conditions to those associated with the data sets for growth and the adjustment of X_K which allows for the correction of ingestion rates due to variations in food quality between environments. Adjustment for X_K is common when comparing populations between geographical regions, as described in detail by

Alunno-Briscia et al. (2011).While chlorophyll a reported in Rajagopal et al. (1998) was consistently higher than that reported for Florida populations, a higher X_K was required to fit the model for the Rajagopal data (1.1 and 3.4, respectfully), suggesting that while phytoplankton was plentiful, the quality was poor (Saraiva et al., 2011a). However, the ability to fit the model to both native and invaded regions confirms the applicability of the model to *P. viridis* populations across a wide range of geographic regions.

While spawning was realistically predicted for local populations, it was not as closely fit in the model predictions for the Rajagopal et al. (1998) data. This is likely due to several factors. The method of recording gametogenesis was different between the two regions. Rajagopal et al. (1998) used a method of ranking stages of gametogenesis on a development scale rather than GSI, as was done for local Florida populations, which may result in differences in interpretation of spawning behavior and gonad occupation. Further, comparison of X_K suggests that the chlorophyll data does not accurately reflect the available food source in the Rajagopal et al. (1998) data which could be due to frequency of sampling (bi-monthly) or low nutritional quality of phytoplankton. Feeding behavior of *P. viridis*, and many other bivalves, has shown to vary significantly depending on the food quality and quantity (Hawkins et al., 1998; Wong and Cheung, 1999; 2001) and elevated suspended solid levels can result in reduced ingestion efficiency even when chlorophyll is high due to increased need for particle selection (Kooijman, 2006). Thus, the need for adjusting X_K when modeling populations under different food environments (Alunno-Bruscia et al. 2011). This could be corrected by incorporating a second food source and synthesizing units to further characterize feeding, as in Lavaud et al. (2013). Chlorophyll does not necessarily represent the total diet available to bivalves as it does not include dissolved and particulate organic matter or microzooplankton which also contribute to the diverse diet of bivalves (Ren and Ross, 2005; Saraiva et al., 2011b), nor do chlorophyll measurements give any information on the quality of food source (Bourlès et al., 2009), both of which can have significant effects on digestion and assimilation efficiencies (Ren and Ross, 2005; Ren et al., 2006). However, this was the only food measurement available and has been widely used as a proxy for food availability in a DEB context (Ren and Ross, 2005; Pouvreau et al., 2006; Flye-Sainte-Marie et al., 2007; Ren and Schiel, 2008). Use of chlorophyll as a proxy works nicely for local conditions, however, chlorophyll data in the Rajagopal et al. (1998) data set was only collected bi-monthly as point samples, which may not capture the true cycle in food

availability, compared to continuous hourly data points collected during monitoring of Florida populations, leading to discrepancies in the model between observed and predicted cycles for Indian populations.

Reproductive cycles are complex physiological processes which rely on the metabolic state of the organism and environmental factors, primarily food and temperature (Dare and Edwards, 1975; Bayne and Newell, 1983), leading to high variation between populations and individuals within a population. Spawning was simulated in the model under the assumption that once the reproduction buffer reached a certain threshold, as identified by GSI, and a given temperature requirement was met, then spawning would occur. To simulate the continuous intermittent spawning behavior of *P. viridis*, only a portion of the gonad (30%) was released upon each event within the model, thus allowing for a rapid regeneration and more frequent events, as described by Lavaud et al. (2013). Kappa also played a major role in simulating the frequency of spawning events (explained briefly in van der Veer et al., 2006). The kappa value used in this study is relatively low (0.44), indicating a large allocation of energy to reproduction, when compared with the general consensus in the value of species in the add_my_pet collection. However, it still within the range documented for other bivalves with a value of 0.45 reported for both *M. edulis* (Rosland et al., 2009) and *Crassostrea gigas* (van der Veer et al., 2006). A lower kappa reduces the energy allocation to growth to allow for increased allocation to reproduction resulting in the maintenance of a high GSI ratio and a more rapid recovery between spawning events and maintenance of the reproduction buffer (Saraiva et al., 2011a). However, even with a low kappa, high growth rates were still attained suggesting this to be a suitable estimation.

While temperature showed fairly consistent seasonal cycles some variation was observed in the duration and intensity of the winter low between years (Fig. 3). This inter-annual variation aids in the explanation of the differences in winter build-up and the timing of spring peaks between years. Thus, the use of degree days acts as an important stimulator for spawning. Gilg et al. (2014) described spawning in *P. viridis* on the Atlantic coast of Florida to be more closely linked with the rising of water temperatures following the coldest winter lows, leading to a difference in the timing of the first spring peak between years and spawning capabilities at temperatures as low as 16˚C. This was observed in the model in which the first peak is observed in February 2012 at approximately 20˚C, following a low of 18 ˚C in January compared to the

spring peak in March 2013, which was preceded by four months of winter lows averaging 20˚C. Thus, the dramatic differences in environment when comparing invasive and native populations, subtropical and tropical respectively, may require more detail in the spawning rules to more accurately predict spawning events. Temperature, an important forcing factor in the model, showed a very different pattern in the native region, remaining above 25˚C throughout the year, compared to local conditions which fell to 20˚C and below for several months in the winter. Further, differences in spawning between the two populations could also be partially explained by salinity regimes. Populations on the coast of India experience dramatic decreases in salinity (as low as 5) during the monsoon season (Rajagopal et al., 1998), which may trigger spawning, partially explaining the early spring peak when compared to the model predictions. In comparison, *P viridis* populations studied in Florida are established in areas in which salinity never fell below 25.

To further complicate the prediction of spawning events, DEB follows a single individual over a lifetime, while data used to calibrate spawning represents the average of several individuals during single time point sampling. Field studies documenting reproduction by nature cannot follow an individual, leading to increased error in the characterization of detailed spawning cycles in wild populations. The spawning strategy of *P. viridis* in which year round intermittent, partial, and asynchronous spawning occurs between peaks, leaves the estimation of an individual's activity difficult. Flye-Sainte-Marie et al. (2007) explains in detail how using the average of a population of asynchronous spawners can "flatten" out the trajectory leading to the average observation being a diluted trend of what the actual observed seasonal variation in an individual would be. This could partially explain the smoother data set observed in the Rajagopal et al. (1998) expectations, particularly because their sample size was double that of the calibration data set, possibly leading to a dilution of the partial spawning events resulting in a smooth curve.

Reproduction may also be more specifically defined by adding a gonad compartment allowing for the characterization of energy allocated specifically to gametes and reproduction buffer as separate compartments (Bernard et al., 2011). This may allow for an improved characterization and fit for comparisons between populations and capture a more detailed story of frequency and duration of spawning. However, while the model predictions do not always line

up perfectly with each event, the continuous spawning nature throughout the spring, summer and fall suggests a realistic estimation of reproductive activity in southwest Florida populations. Spawning between peaks may also be better represented if an estimation of gametes released per spawn could be quantified throughout seasonal time points.

5. Conclusions and perspectives

The model output showed a successful prediction of growth and reproductive output based on environmental parameters and can be used across geographic regions to predict population growth. While not within the scope of this paper, the next step for this model could be to simulate effects of global climate change in order to predict subleathal effects (growth and reproduction) and changes in energy allocation in response to environmental change and stress. Taking this step can put DEB modeling to use for the contribution to mitigation efforts in predicting the potential for future spread of *P. viridis* and aid in the prediction of areas which may be vulnerable to invasions serving as a proactive mitigation plan.

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Chapter 6: General Discussion

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Worldwide, introductions of exotic species to new regions is of rising concern (Bax et al., 2003). The potential for a new species to invade and compete with native fauna can lead to catastrophic ecosystem alterations and is typically accompanied by high costs of removal (Rajagopal et al., 1991). Environmental tolerances and species plasticity and adaptability play a role in the ability to populate new regions, thus an understanding of physiological responses to seasonal variation and local stressors is essential to understanding population dynamics and potential for spread. Through the work presented here, population dynamics of locally established green mussel (*Perna viridis*) populations were characterized through monthly monitoring of growth, mortality, recruitment, gametogenesis, and biochemical composition. Information reported in the literature and data collected during field monitoring was used for the development of a Dynamic Energy Budget (DEB) model to accurately predict the growth and reproduction under environmentally realistic conditions.

1. Field observations

1.1. Effects on juvenile and adult mussels

Monthly monitoring of established *P. viridis* populations in Estero Bay, Florida revealed rapid growth rates (6 – 11 mm month^{-1}) and high reproductive activity through year round gametogenesis and spawning capabilities. Rapid growth and high rates of juvenile recruitment have allowed *P. viridis* to rapidly spread throughout coastal waters of southwest Florida in high densities (Baker et al., 2007; 2012). In Estero Bay, dense *P. viridis* populations cover artificial hard substrate and several isolated adults have been observed on intertidal oyster reefs (Volety et al., unpublished), suggesting the potential for competition with native oyster populations, *Crassostrea virginica*. However, in March 2012 green mussel populations suffered a mass mortality event nearly decimating the population with > 90 % mortality. This event occurred during a peak in spring spawning activity, as identified by monthly histological analysis of gametogenesis, and was preceded by the first major red tide event in the area since monitoring

began in 2009. Florida red tides are formed by the toxic dinoflagellate *Karenia brevis* which produces a suite of potent neurotoxins known as brevetoxins (PbTx) (Baden and Mende, 1982) and are the cause of massive fish mortality events (Ray and Wilson, 1957; Naar et al., 2007; Landsberg et al., 2009). Leading up to and during the mortality event in March 2012, *P. viridis* had accumulated high concentrations of PbTx and were nearly 20 times the regulatory limit. Additionally, upon onset of the bloom in November 2011, growth rates of juvenile and adult *P. viridis* were significantly reduced (Chapter 3). Prior to bloom exposure, in the summer and fall of 2011, growth rates averaged 6 – 11 mm month^{-1} and several individuals frequently exceeded 13 mm month^{-1}. Rapid growth is a common strategy in juveniles to avoid predation pressures (Seed and Brown, 1978). Furthermore, when conditions are suitable rapid growth rates can be observed even in mature adults, as was observed during the first year of monitoring in which growth rates in mussels with average lengths of 72.6 mm averaged 7.4 mm month^{-1} in September 2011 during a peak in spawning activity, as identified through histological analysis. However, during and following red tide exposure growth was significantly reduced (< 3 mm month^{-1}), even in the summer months when conditions are expected to support high growth. During this period of reduced growth, two consecutive red tide blooms occurred in the area and tissue PbTx concentrations remained above the regulatory limit (800 ng g^{-1} PbTx-3 equivalent). High tissue toxin burden may lead to increased somatic maintenance and energy allocated to "detoxifying" leading to reduced energy allocated towards growth.

Reproduction appeared uninhibited by these bloom events, as observed by year round gametogenesis and successful laboratory spawning and fertilization during periods of exposure, suggesting a priority is given to reproduction over growth during periods of increased environmental stress. Although reproduction is metabolically costly (Bayne, 1976) it is common strategy for bivalves to maintain reproduction over growth when under environmental stress (Browne and Russel-Hunter, 1978; Lodeiros and Himmelman, 2000; Lodeiros et al., 2001). Growth only occurs when "energy acquisition is in excess of energy expenditure" (Bayne and Newell, 1983; Kooijman, 2010). Part of this energy expenditure includes the "maintenance ration" which is dependent on both endogenous and exogenous factors, including environmental stress and reproduction, further reducing the amount of energy available for allocation towards growth (Bayne and Newell, 1983). In other species of mussels, growth has shown to be inhibited during periods of intense spawning or gametogenesis due to the increased stress and metabolic

demands (Coe and Fox, 1942; Seed and Brown, 1978). *Mytilus edulis* conditioned and spawned in the laboratory under low food ration and / or temperature stress maintained gametogenesis, but exhibited reduced growth (SFG) (Bayne, 1975; Bayne et al., 1975). Indeed, energy priority to gonadal growth over somatic growth has been observed during periods of food limitation (Bayne, 1975; Lodeiros et al., 2001).

Under natural conditions, Cheung (1991) observed a decrease in *P. viridis* growth in regions of Tolo Harbor, Hong Kong under high pollution stress, while gametogenesis remained active year round. Additionally, while condition index showed distinct seasonal cycles, the gonadosomatic index (GSI) remained high throughout the year suggesting a fixed fraction of energy allocated to reproduction even at times of reduced food supply (Cheung, 1991). Likewise, several others report reduced growth rates in *P. viridis* when water temperatures decrease in the winter, while gametogenesis remains active (Walter, 1982; Lee, 1988; Rajagopal et al., 1998), further supporting a priority given to reproduction over growth. It is thus plausible that under stress of *K. brevis* exposure and elevated tissue PbTx concentrations, growth was reduced in order to maintain physiological state and energetic contribution to gametogenesis.

During this monitoring period mussels of a size range 30 – 50 mm were easily collected on a monthly basis suggesting that even under stress, newly settled mussels are capable of growth to maturity relatively quickly, particularly those which settle when bloom conditions are not present. Throughout the monitoring period *P. viridis* maintained active gametogenesis and exhibited a partial spawning strategy. This was further confirmed through histological analysis of laboratory spawned mussels in which most individuals released only a portion of their gametes with a portion of the gonad remaining in the ripe or late development stage. This behavior of continuous gametogenesis with incomplete, successive spawning and follicles within the same gonad at different stages of development is common for tropical bivalves (Gasper et al., 1999; Pouvreau et al., 2000). Barber and Blake (2006) suggest incomplete or partial spawning as a reproductive strategy to maximize overall larval survival and recruitment, assuring that at least some batches will encounter environmental conditions conducive for growth, survival and settlement.

However, this reproduction strategy may lead to increased mortality following spawning in a stressful environment due to further increased energetic demands. Increased tissue PbTx

concentrations may have caused additional physiological stress and an increased maintenance ration for tissue repair and detoxification, leading to a reduction in the energy allocated to growth. This additional stress and maintenance cost during a period of high metabolic demand (spawning) may partially explain the delayed post bloom mortality. A reduction in reserves during spawning while under the stress could lead to reduced fitness and an inability to keep up with metabolic demands for survival. Indeed, while reserves were not completely depleted, the lowest glycogen levels were observed during the mass mortality event in March 2012; 2.4% total dry tissue weight compared to 5.8 – 15% throughout the rest of the year, thus indicating a disruption in energy balance. Similar summer mortality events have been documented in bivalves due to a reduction in carbohydrates during spawning events coupled with environmental stress including elevated water temperatures (Mallet et al., 1990; Shpigel et al., 1992) and food shortage (Barber and Blake, 1981; Tremblay et al., 1998). Myrand et al., (2000) observed a complete cessation in growth and a peak in mortality of *M. edulis* when glycogen was at a low and increased mortality following spawning events. During a prolonged *K. brevis* bloom exposure in the winter of 2013 (December – April), protein levels also hit a low (39 - 43%) during which tissue PbTx levels were 29,000 – 110,000 ng g^{-1} PbTx-3 equivalent and spiked to 60% following bloom dissipation when tissue PbTx concentrations dropped to 9,071 ± 523 ng g^{-1} PbTx-3 equivalent in June 2013. While no significant relationship was detected, when examined graphically it appears that prolonged environmental stress from HAB exposure and accumulated tissue PbTx, coupled with the increased metabolic demands of spawning may partially explain the observed temporary reductions in reserves.

PbTx persisted in *P. viridis* tissues for prolonged periods, remaining above the regulatory limit for 4 – 5 months following bloom dissipation, however, the decrease immediately following bloom dissipation was rapid. This dramatic decrease may be partially explained by increased morality following peaks in accumulation in which those mussels that accumulated higher tissue toxin levels died, while those with lower tissue levels survived. This is supported by the increase in standard deviation when average PbTx levels are ≥ 30,000 ng g^{-1} PbTx-3 equivalent, ranging from 7,000 – 55,000 ng g^{-1} PbTx-3 equivalent, orders of magnitude higher than when levels are ≤ 10,000 ng g^{-1} PbTx-3 equivalent in which standard deviation ranged from 300 – 8,000 ng g^{-1} PbTx-3 equivalent. Further, following bloom dissipation, the elimination of PbTx from ~10,000 ng g^{-1} PbTx-3 equivalent to below regulatory limits (800 ng g^{-1} PbTx-3

equivalent) took 4 months, compared to the initial elimination rate of >20,000 ng g^{-1} PbTx-3 equivalent in first month. Li et al. (2005) found elimination rates to be dependent on body compartment and could range from 3 – 15% elimination per day depending on the species and initial toxicity (Bricelj and Shumway, 1998). This may also be due in part to metabolic pathways of eliminating PbTx and high clearance rates of *P. viridis* leading to faster accumulation rates compared to elimination rates during exposure.

1.2. Effects on early life stages (gametes and larvae)

While growth remained low, the surviving mussels maintained year round gametogenesis and peaks in recruitment were observed in the spring and fall indicating that, given the right conditions, *P. viridis* populations have the ability to rebound. However, the second bloom event persisted from October 2012 through April 2013 inhibiting juvenile recruitment during the spring spawning season. While a direct cause cannot be confirmed through field studies, the absence of a spring peak in juvenile recruitment indicates that the presence of *K. brevis* and its associated PbTx's and hemolysins inhibited fertilization and / or larval growth and survival. Exposure of early life stages of local species of clams (*Mercenaria mercenaria*), oysters (*Crassostrea virginica*) and scallops (*Argopecten irradians*) to *K. brevis* has been shown to inhibit gametogenesis and larval development (Leverone et al., 2006; Rolton et al., 2014) and in native regions reductions in *P. viridis* larval abundances and juvenile recruitment have been documented during harmful algal blooms (Cheung, 1993).

Gametes produced under stressful environmental conditions are often of lower quality, even when gametogenesis in the adult appears normal, leading to reduced larval survival (Bayne, 1972; 1975). Decreases in weight, organic matter and lipid content in eggs produced by females under stress has been documented in laboratory studies, leading to reduced fertilization success and ecological fitness of larvae, due to a reduction in energetic reserves essential for early larval development (Bayne et al., 1975; 1978). Rolton (2015) observed a decrease in gamete viability and fertilization success after adult *C. virginica* had been exposed to a natural *K. brevis* bloom in the field and induced to spawn in the laboratory. Further, several studies have shown negative effects in sperm following short term laboratory exposure of adult *Crassostrea gigas* to HAB's

including decreased motility, reductions in ATP, alterations in DNA and reduced mitochondrial membrane potential (Haberkorn et al., 2010; Le Goïc et al., 2013) as well as decreased ROS production in eggs (Le Goïc et al., 2014). Thus, while *P. viridis* was still producing gametes and actively spawning, stress may have caused a reduction in gamete quality and reduced energetic reserves, leading to reduced survival due to a lack of sufficient nutrients in the early life stages.

Although stress in the adult can lead to decreased gamete quality, gametogenesis remained active and successful spawning was achieved in January 2014 in individuals collected during a *K. brevis* bloom when tissue PbTx concentrations of adults averaged 91,399 ± 14,616 ng g^{-1} PbTx-3 equivalent. High fertilization rates (93.7%) and hatching rates (66.3 %) were observed during this spawning, suggesting that inhibition of juvenile recruitment during the bloom is likely due to external exposure to *K. brevis* and associated icthyotoxins (PbTx) and hemolysins. Early pilot work has indicated decreased growth and survival of D-stage *P. viridis* larvae when exposed to *K. brevis* (Volety et al., unpublished) and decreased embryogenesis, larval development and survival have been observed during exposure to *K. brevis* in local species of oysters, clams and scallops (Leverone et al., 2006; Rolton et al., 2014). Early life stage sensitivity to HAB's is well documented for bivalves, resulting in reduced fertilization, hatching and larval successes upon embryonic and early larval exposures at concentrations as low as 100 cells mL^{-1} (Granmo et al., 1988; Matsuyama et al., 2001; Basti et al., 2011; 2013; Mu and Li, 2013). Due to both longer duration and greater intensity of exposure in the wild, blooms frequently exceed 10^6 cells mL^{-1} and can persist for up to 18 months (Rounsefell & Nelson 1967; Tester & Steidinger 1997; Steidinger 2009), it is likely that the deleterious effect on larvae is amplified during natural exposures leaving laboratory exposures as underestimations of the effects. While gametes, embryos and early stages of larvae are not capable of ingesting *K. brevis* cells, they may be more significantly affected by physical contact of water column PbTx and allelochemicals produced by *K. brevis* (Kubanek et al. 2005; Marshall et al. 2005).

As elevated *K. brevis* concentrations persisted through much of the time larval recruitment was monitored it is likely that reduced larval survival and thus recruitment can be attributed to the presence of toxic algae and associated ichthyotoxins and allelochemicals in the water column. Additionally, due to the stress of high tissue toxin burden on the adults, gametes

produced during this study may have been of poor quality, putting larvae at a disadvantage due to reduced energy reserves resulting in high mortality during the pelagic phase.

2. DEB model

Through the application of the Dynamic Energy Budget (DEB) theory (Kooijman, 2010) we were able to accurately predict individual growth and reproduction of *P. viridis* under local conditions. Parameter estimates were completed using several data sets from the literature and calibrated using field data from local populations. As a final step the model was validated using a third external data set from the literature. By using separate data sources from different geographic regions we are able to say with confidence that the parameters fit the species in general and the model for growth and reproduction can be applied to other populations by changing environmental variables, specifically, food and temperature and adjusting only the half saturation coefficient (X_K) which adjusts for changes in ingestion and functional response based on local food conditions, while all other parameters remain constant.

Differences in growth and reproduction between individuals of the same species can be explained by environmental conditions and life history exposure and can be further complicated by environmental stress. However, the general growth patterns were able to be reproduced for three separate data sets during parameterization, calibration and validation steps.

2.1 Metabolic acceleration

Bivalves go through a life stage transition referred to as metamorphosis, when a transformation occurs from the pelagic larval phase to the sessile juvenile stage at which they remain through adulthood. Metamorphosis represents a life stage transition of intense metabolic demands and major changes in bioenergetics and energy requirements. By including an acceleration factor we can more accurately capture the metabolic changes that occur at this stage with accurate predictions of both larval and adult growth. During these two stages they undergo different growth patterns in which as larvae they grow as V-1 morphs and post-metamorphosis become isomorphs (Kooijman, 2010). These two different growth strategies require the incorporation and estimation of energy allocation and utilization parameters for the two life

stages including differences in energy conductance ($\dot{v}$) and surface area specific assimilation rate ($\{\dot{p_{Am}}\}$). To account for these changes, a metabolic acceleration factor was included and separate shape coefficients (δ_M) for these stages were estimated, that describe the relationship between length and volume during growth. Utilizing δ_M to describe growth allows for a more realistic estimation across a life span and reduces the error by characterizing growth according to shape in relation to volume, rather than one dimensional length or weight measurements which tells less about how the individual is growing as a whole. This is especially helpful in determining structural volume which is not easily measured, but allows for more detail in estimating and explaining the flow of energy through an individual.

$$V = (\delta_M L)^3$$

Through these procedures individual growth was successfully modeled across the full life span under realistic and dynamic environmental conditions using locally collected growth data and growth data previously reported in the literature using the same set of parameters.

2.2 Spawning in tropical bivalves

Food and temperature are among the two most influential factors that determine gametogenic and spawning cycles (Lee, 1988; Cheung, 1991; Sreedevi et al., 2014) and are included as forcing variables in DEB models. We thus included in the model for reproduction temperature, in the form of degree days, and food, chlorophyll a concentrations, as two threshold components to stimulate spawning in the model predictions.

Perna viridis maintained year round gametogenesis and while two major peaks were observed each year (spring and fall), intermittent year round spawning was evident (Chapter 4). Gilg et al. (2014) suggested that rather than a threshold temperature, *P. viridis* spawning is cued by the gradual warming that follows the winter low leading to spawning events in temperatures as low as 16˚C. Indeed, the successful laboratory spawning in winter acclimated mussels in January 2014 (Chapter 4) indicates that just a short exposure to increased temperatures can induce a spawning event in the field even in the winter when resting periods are expected. For

these reasons degree days were kept low so as to allow a spawning event after even just a short warming of the water temperature.

To account for year round spawning capabilities, a low spawning efficiency was used (30%). This allowed for the simulation of only a partial release of the gonad so long as a specified threshold for GSI was reached. With only a partial release of the gonad, mussels were able to show rapid regeneration for several spawning events between the two seasonal peaks observed in the spring and fall each year. While each small peak indicating intermittent spawning does not match up perfectly, the sporadic nature is indicative of what is observed in the field. DEB follows an individual mussel over a lifetime whereas our data is an average of several asynchronous individuals from a population sampled from a single point in time (N = 15 / month), thus leaving the precise timing of peaks between observed and predicted values difficult.

Spawning in bivalves is a complex process that is highly dependent on environmental variables and internal chemical cues (Seed, 1976; Stephen and Shetty, 1981; Widdows, 1991; Barber and Blake, 2006) adding to the complexity of successfully modeling and predicting an already elaborate process. During the validation procedure, spawning in the Rajagopal et al. (1998) data was unable to be replicated with simple adjustments and requires more population and site specific details. Reproduction can vary between populations of the same species due to biological factors including genetic makeup, life history exposure, phenotypic plasticity (Ren and Schiel, 2008) and environmental factors including food quality and quantity, seasonal variation (or lack of) in the environment and pollution (Lowe et al., 1982; Lee, 1985; 1986). Site specific environmental data is essential to accurately predicting physiological responses, particularly reproduction. The information on food (chlorophyll a) and temperature used in the validation step (Rajagopal et al., 1998) was taken as bi-monthly point samples which leaves room for error in the interpolation procedures between points. The inclusion of more continuous data and / or one or more food source would improve the estimates for spawning in the Rajagopal et al. (1998) predictions. To further complicate the comparison of spawning between studies, differences in methodologies commonly occur. This is particularly important to consider for *P. viridis* as the gonad is dispersed throughout the mesoma and is not easily dissected from the rest of the body leaving direct measurements impossible. For example, during field monitoring gonad material was converted to mass using ratios of gonad to soma via image

analysis while many others, including Rajagopal et al. (1998), use some metric of gonad ranking which describes the stage of gametogenesis more so than it does the amount of gonad material. Because of these discrepancies, it is essential to calibrate the spawning rules used within the model separately for the different populations.

3. Ecological implications and concerns for P. viridis spread

During this study period, several concerns have been brought to attention regarding local *P. viridis* populations; i) Extended period of PbTx contamination in the soft tissue and potential for trophic transfer; ii) Concern for northern spread with the continued rising of sea surface temperatures; iii) Year round reproduction giving a competitive edge against native oysters, particularly if sea level rise brings increased subtidal habitat.

3.1 Trophic transfer of brevetoxins

Perna viridis have shown heavy competition with native oysters (Baker et al., 2007) putting reef systems at risk, thus a reduction in *P. viridis* densities, as observed during the mortality event in March 2012, would benefit oyster reefs and existing restoration efforts. Locally, oysters are tolerant of *K. brevis* exposure and associated toxins exhibiting high survival during natural blooms (Pierce et al., 2002; Plakas et al., 2008) and during examination of the seafloor under good visibility conditions, *P. viridis* was the only observed bivalve mortality present in high numbers. However, while *K. brevis* exposure may keep green mussels at bay and reduce competition with oysters, increased bioaccumulation of PbTx poses a threat for post bloom trophic transfer. When compared with *C. virginica* sampled during the same bloom period, *P. viridis* tissue PbTx concentrations were nearly double that of *C. virginica* and over 70 times the regulatory limit of 800 ng g^{-1} PbTx-3 equivalent. Several species have been observed to feed on *P. viridis* including several species of crab, whelk and fish (Cheung et al., 2004; Mitchem et al., 2007; Volety et al., unpublished data; personal observations). Feeding on benthic invertebrates has resulted in trophic transfer of PbTx in several species and can lead to acute lethal dosing (Ingham et al., 1986; Shumway et al., 1995; Pierce et al., 2002; Flewelling et al., 2005; Naar et al., 2007; Bricelj et al., 2012). Indeed, post bloom fish mortalities have been

attributed to lethal dosing of PbTx through feeding on benthic invertebrates (Landsberg et al., 2009). This may be further amplified if filter feeders retain PbTx in the tissue for long periods post bloom when conditions would otherwise suggest reduced threat. *Perna viridis* retained PbTx in their soft tissue at elevated concentrations for 4 – 5 months post bloom dissipation, much longer than typically observed in local bivalves of 2 – 8 weeks (Morton and Burklew, 1969; Steidinger and Ingle, 1972; Plakas et al., 2002, 2004, 2008; Bricelj et al., 2012; Griffith et al., 2013). This raises concern for crab and fish species which may prey directly on green mussels and could, in turn, pose threat to higher trophic levels by themselves becoming prey, leading to cascading effects through the food web (Shumway et al., 1995; Pierce et al., 2002; Flewelling et al., 2005; Hégaret et al., 2008; Bricelj et al., 2012). Analysis of a post bloom mortality event of striped burrfish (*Chilomycterus schoepfi*) revealed elevated tissue PbTx levels and stomach contents containing toxic bivalves in the gut, suggesting post bloom trophic transfer via filter feeders (Landsberg et al., 2009) and during a post bloom bottlenose dolphin (*Tursiops truncates*) mortality, Flewelling et al. (2005) found elevated tissue PbTx concentrations in all of dolphin tested (N = 36) many of which also had high numbers of toxic menhaden (*Brevoortia* spp.) in their stomachs. Thus confirming the potential for cascading food web effects, particularly given the long depuration period required for *P. viridis* in southwest Florida.

Additionally, although *P. viridis* is not currently harvested for human consumption, it is an edible species and thus may pose a threat to human health, resulting in increased cases of neurotoxic shellfish poisoning (NSP) if harvested. Local oyster reefs and clam beds are monitored for PbTx accumulation, however based on depuration times documented in this study, re-opening for oysters and clams would not indicate safety for green mussel harvesting due to prolonged bioconcentration. This work has identified the need for public awareness and increased monitoring of *P. viridis* populations during and following bloom events.

3.2 Effects of climate change on habitat expansion

Worldwide, rising water temperatures have allowed for increased range expansion and increased biological invasions, particularly into many temperate or cold water regions (Stachowicz et al.,2002; Reise and Beusekom, 2008). *Perna viridis* is a tropical to sub-tropical species, thriving in warm coastal waters in subtidal habitat spanning from southern Florida to as

far north as Charleston, South Carolina (Rajagopal et al., 2006; Baker et al., 2007). The extent of the northern spread on the Atlantic coast is largely due to the prevailing warm waters of the Gulf Stream as they are limited by cold water temperatures (Power et al., 2004; Urian et al., 2010; Baker et al., 2012; McFarland et al., 2014). However, spread may continue to push north as the effects of climate change continue, and warmer seas provide more tolerable conditions along northern coastlines. Through hydrodynamic modeling and field monitoring of larval recruitment patterns, Gilg et al. (2014) suggested that, given the right conditions, *P. viridis* larvae are capable of traveling as far as 100 km with the currents in a northern expansion during the pelagic stage. However, even short term cold snaps or winter extremes, particularly in shallow coastal waters and intertidal regions, may limit long term survival with northern spread (Canning-Clode et al., 2011).

To complicate this scenario, invasive bivalves are often largely free of parasites and diseases that impact native fauna allowing for a more rapid proliferation of the invasive bivalve while the native species suffers (Krakau et al., 2006; Troost, 2010). Increased disease in native bivalves with rising temperatures could lead to reduced physiological function and survival, giving the green mussel yet another advantage over the oyster. Warm water temperatures tend to increase the rate and spread of disease (Reise and Beusekom, 2008). Two oyster parasites, *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX), exhibit increased infection rates and intensities at elevated water temperatures in marine portions of estuaries across the eastern United States (Levinton et al., 2011; 2013). Locally, oysters tend to have the highest prevalence and intensity of Dermo infection in January when salinities are high and in August when temperatures are high, however salinity and temperature act antagonistically to keep overall infection intensity low (Volety et al., 2003; 2009). High rates of infection in the winter months (Volety et al., 2009; Volety and Haynes, 2012) suggest that a rise in water temperatures will only exacerbate an existing problem and while Dermo related mortality is currently low (Volety and Haynes, 2012), increased winter water temperatures could allow infections to increase in intensity resulting in increased mortality rates.

During this study period *P. viridis* were analyzed for Dermo and histopathology, neither of which revealed significant disease or parasite infections (McFarland et al., unpublished data). Local oysters, on the other hand, experience high rates of Dermo in the summer months and

typically harbor a suite of parasites (Winstead et al., 2004; Volety and Haynes, 2012; Volety et al., 2014). Currently, oysters across southwest Florida habitats are typically given a Dermo intensity rating of low to moderate with 67 – 82% infected (Volety et al., 2014), however, in the Caloosahatchee Estuary oysters have increased prevalence with monthly averages ranging from 80 – 100% infected year round (Volety and Haynes, 2012). Low intensity of infection is attributed to decreased salinity during the summer months when temperature is elevated due to fresh water flow (Volety et al., 2014).

If oyster populations experience increased rates of disease induced physiological impairment and mortality in the summer months, which normally support increased growth and reproduction, negative impacts on the population structure may be exacerbated, allowing disease free mussels to thrive. Further, while fresh water releases from Lake Okeechobee may lead to too much freshwater flow for oysters, many coastal regions will experience an overall increase in salinity due to salt loading from increased ground water input to oceans and rivers (Zekster and Loaiciga, 1993). Decreased salinities near the rivers may push new oyster recruits further downstream and an overall increase in salinity throughout the bay could result in a localized expansion of green mussel habitat where an overall increase or stabilization in salinity may occur. This could result in a squeezing of the niches between oysters and mussels, resulting in increased competition pressures on the oyster.

A second consequence of climate change is sea level rise turning intertidal estuaries into coastal lagoons, creating more subtidal habitat for mussels within the estuaries (Reise and Beusekom, 2008; Troost, 2010). Further, the rate of sea level rise could result in an inability for growth of estuarine plants to keep up, leading to a loss of estuarine habitat and species diversity (Roessig et al., 2005; Reise and Beusekom, 2008). By 2100 it is predicted that the top 100 m of the ocean surface water temperature will rise 0.6 – 2˚C with the sea-level rising between 0.26 – 0.82 m (IPCC, 2013). In shallow bays such as Estero Bay, where the average depth is only 1 meter (Byrne and Gabaldon, 2008), this could lead to dramatic ecosystem changes and completely submerge existing intertidal oyster reefs. Currently, an intolerance to aerial exposure in green mussels has prevented their spread into the estuary leaving oysters with a refuge, safe from invasion (McFarland et al., 2014). The submersion of intertidal reefs would lead to increased competition in which mussels may utilize oyster reefs as hard substrate for dense

population settlement. This type of competition has been observed in Tampa Bay in which submerged portions of oyster reefs have been smothered by green mussel settlement (Baker et al., 2007).

3.2 Competition for space with oysters

Energetic priority is given to reproduction in *P. viridis*, as identified through year round gametogenesis and winter spawning capabilities (Tan, 1975; Urian, 2009; Ch 4), allowing for a competitive advantage against native oysters which remain in a resting phase during the winter months (Volety et al., 2009; 2014). Oysters spawn April – October in southwest Florida (Volety et al., 2009; Volety and Haynes 2012; Volety et al., 2014) and are limited to June – September in the northeastern United States (Shumway, 1996). Locally, oysters undergo a resting period from December – March in which gametogenesis is inactive and in some portions of the estuary this inactive period extends from November – March (Volety and Haynes 2012). *Perna viridis* maintains continuous gametogenesis throughout the winter months, giving them a reproductive advantage over oysters with the ability to spawn at the first sign of warm spring waters. This strategy could result in young of the year for *P. viridis* being several weeks or even months ahead of *C. virginica*. Further, *P. viridis* exhibit rapid growth, particularly in their first six months (Lee, 1986; Al-Barwani et al., 2007) allowing them to dominate hard substrate. High density populations of *P. viridis* have been observed coating infrastructure and in some cases smothering oyster reefs below (Rajagopal et al., 1991; Baker et al., 2007). This gregarious nature coupled with rapid growth rates may allow for green mussels to out-compete oysters for substrate, leading to dramatic changes in ecosystem structure.

Oyster reefs are already on the decline and in many regions restoration efforts are in place in order to maintain and increase population densities (Beck et al., 2011). The current reduction in oyster reef densities and spatial coverage in southwest Florida estuaries has been attributed to the timing and duration of fresh water releases from Lake Okeechobee and the Caloosahatchee River (Volety et al., 2014). Thus, while fresh water release may reduce disease intensity and prevalence, negative effects on oyster larvae could have devastating effects on population structure and existing reef systems. If increased fresh water flows continue to push

oysters towards the outer fringes of the bay to more marine waters, mussel and oyster territory will begin to overlap. While local populations of green mussel do not appear to tolerate salinities < 15 ppt they will remain competitive at intermediate and marine salinities, 15 – 30 ppt (McFarland et al., 2013; 2014).

Table 1: Comparison of biological parameters between oysters and mussels to illustrate potential for competition.

	Green mussels	Oysters
Clearance Rates (L g_{dry}^{-1} hr^{-1})	1.2[2]	0.43[2]
	2.6 – 4.2 [3]	1.2 – 2.2 [5]
	8.6 – 9.7 [4]	6.4[6]
Growth Rates (mm $month^{-1}$)	6 – 10 [*]	5 – 7 [1]
Maximum observed	15[*]	11[1]
Condition Index	8 - 16[*]	2.5 - 47[8]
Reproduction		
Spawning Season	Feb – Nov. [*]	April – Oct.[7]
Resting Period	None[*]	Dec. – Mar.[7]
Infection Rate		
Perkinsus	None[*]	Moderate – high[1,7]
Red Tide		
Mortality	High[9]	Non-significant
PbTx accumulation (ng g^{-1})	50,000 – 100,000[9]	35,000[9] – 80,000[10]
Depuration	4 – 5 months[9]	2 – 8 weeks[11]

[1]Volety et al., 2003; [2]McFarland et al. 2013; [3]Wang et al., 2005; [4]Chong and Wang, 2003; [5]Riisgård 1988; Grizzle et al. 2008; [6]Newell and Koch 2004; [7]Volety et al., 2009; [8]Volety and Haynes 2012; [9]McFarland et al. 2015; [10]Weidner et al., 2002; [11]Morton and Burklew, 1969; [*]McFarland et al., unpublished data

4. Future studies

Observations documented during these field studies should be further explained through laboratory exposures of green mussels to *K. brevis*. While the parallels observed during this study suggest negative effects on growth, survival and larval recruitment, field exposures are complicated by the interacting effects of environmental parameters and the physiological responses of the individual. Although field studies can allow for a more accurate prediction of long term population responses to environmental stressors, laboratory studies can control for

specific stressors allowing for a more accurate description of the cause and effect relationship by controlling variables. Controlled laboratory exposures would allow for a more detailed characterization of uptake and elimination kinetics which could then be incorporated into the DEB model, allowing for a risk assessment of the threat of trophic transfer and human NSP post bloom based on the extent of the exposure in the field.

The next step for the DEB model of *P. viridis* should be to couple the model with predictions for climate change induced rise in sea surface temperatures and sea level rise to predict potential habitat changes and ability of *P. viridis* to thrive and spread under these predicted changes. DEB coupled with hydrodynamic modeling (ie: Gilg et al., 2014) would greatly benefit the knowledge on population dynamics and allow for more accurate predictions on potential competition with oysters. A DEB model for *C. virginica* is in the works (La Peyre et al., personal communication) and thus coupling models for both species and predicted environmental changes would aid in creating proactive mitigation plans for resource managers.

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Publications and Communications

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SCIENTIFIC MANUSCRIPTS IN PROGRESS

McFarland K., F. Jean, J. Thébault and A.K. Volety. Potential impacts on growth, survival and juvenile recruitment of the green mussel *Perna viridis* during blooms of the toxic dinoflagellate *Karenia brevis* in southwest Florida. In Review: Toxicon

McFarland K., F. Jean, P. Soudant and A.K. Volety. Seasonal variation in gametogenesis and energy storage of the invasive green mussel, *Perna viridis*, in southwest Florida. In Review: Estuaries and Coasts

McFarland K., F. Jean and A.K. Volety. Parameter estimation and application of the Dynamic Energy Budget Theory to model growth and reproduction to non-native populations of *Perna viridis* in southwest Florida.

PRESENTATIONS

McFarland K., F. Jean, R. Lavaud, A.K. Volety, 2015. Application of the Dynamic Energy Budget theory to *Perna viridis* to model growth and reproduction under various environmental conditions. Dynamic Energy Budget theory Symposium. Marseille, France. April 28 - 30, 2015.

McFarland K., F. Jean, R. Lavaud, A.K. Volety, 2015. Application of the Dynamic Energy Budget theory to *Perna viridis* to model growth and reproduction under various environmental conditions. National Shellfisheries Association Annual Meeting. Monterey, CA March 22 - 27, 2015.

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understand their potential spread using Dynamic Energy Budget theory. Research Day at Florida Gulf Coast University. April 18, 2014.

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AWARDS/HONORS

2014 Dean's Award for best Graduate Student Poster
McFarland K., F. Jean, P. Soudant, J. Thébault, A.K. Volety, 2014. Monitoring seasonal cycles in growth, reproduction and energy storage of the invasive green mussel, *Perna viridis*, to understand their potential spread using Dynamic Energy Budget theory. Research Day at Florida Gulf Coast University. April 18, 2014.

2014 Gordon Gunter Award for outstanding student poster presentation
McFarland K., F. Jean, P. Soudant, J. Thébault, A.K. Volety, 2014. Monitoring seasonal cycles in growth, reproduction and energy storage of the invasive green mussel, *Perna viridis*, to understand their potential spread using Dynamic Energy Budget theory. National Shellfisheries Association Annual Meeting. Jacksonville, FL March 29 - April 2, 2014. Poster Presentation.

2013 2nd Place Overall Poster Winner
McFarland, K. F. Jean, P. Soudant, J. Thébault, A.K. Volety, 2013. No limit for green mussel invasion in Estero Bay? Monitoring seasonal cycles in growth, reproduction and energy storage to understand their potential spread. Université de Bretagne Occidentale Third Year Doctoral Student Forum. Plouzane France, October 24-25. Poster presentation

2012 Graduate Student Poster Winner
McFarland, K., J. Devine, A.K. Volety, 2011. Influence of acute and gradual salinity change on the hemolymph osmolality and survival in the green mussel, *Perna viridis*, and the eastern oyster, *Crassostrea virginica*. Florida Gulf Coast University Research Day. April 2012

Abstract

Worldwide, introductions of exotic species to new regions is of rising concern which can lead to catastrophic ecosystem alterations through competition with native species and disruption in energy flow. *Perna viridis* is a recently introduced bivalve species to US coastal waters and has vigorously spread throughout the southeastern US. However, little information regarding population structure and response to local environmental factors has been reported. Red tide blooms formed by the toxic dinoflagellate *Karenia brevis* are frequent along the Gulf coast of Florida and as a recently introduced species, it is unclear what tolerance *P. viridis* has toward these events and associated brevetoxins (PbTx). Further, as an invasive species ecological concerns have risen regarding potential for spread and competition with native bivalve species, particularly the eastern oyster *Crassostrea virginica*.

This study aimed to characterize the population dynamics of established *P. viridis* populations and their response to naturally occurring *K. brevis* blooms. This was completed through monitoring of growth, mortality, juvenile recruitment, gametogenesis and biochemical composition (protein, glycogen and lipid) throughout a three year monitoring period to evaluate the effects of *K. brevis* blooms. Additionally, tissue PbTx concentrations were analyzed to determine uptake, accumulation and elimination rates. Data collected from the field and information reported in the literature were used to create a functional DEB model to predict individual growth and reproduction of *P. viridis* under environmentally realistic conditions.

Prior to onset of the first *K. brevis* bloom event, *P. viridis* showed rapid growth rates (6 – 11 mm month^{-1}) and high survival (mortality <1%). However, during *K. brevis* blooms growth rate dropped significantly and bioaccumulation of PbTx in the soft tissue was observed. High tissue PbTx concentrations persisted long after bloom dissipation and high rates of mortality ensued, severely reducing population densities. PbTx in mussels nearly doubled that of oysters sampled during the same time and remained above the regulatory limit for significantly longer, 2 ½ weeks and 16 weeks, respectively.

Biochemical composition and reproduction appeared unaffected, exhibiting year round gametogenesis with a partial, intermittent spawning strategy and stability in reserves. A lack of

significant seasonal cycles in biochemical composition suggests sufficient food and energy availability to support the observed year round gametogenesis. While continuous spawning capabilities were evident two major peaks in spawning and recruitment were observed (spring and fall), suggesting reduced fertilization and / or larval development and survival due to the presence of *K. brevis* and associated ichthyotoxins and hemolysins.

These results indicate that while high tissue PbTx concentrations may lead to reduced growth in *P. viridis*, gametogenesis is not inhibited, allowing the population to survive *K. brevis* bloom exposure and reproduce, even while individual mortality was high. Prolonged bioconcentration of PbTx may lead to increased threat of post bloom trophic transfer, resulting in negative impacts on other important fisheries and higher food web implications. While it cannot be conclusively determined that the cause of reduced growth, survival and recruitment is due to red tide events, the parallels observed suggest that *K. brevis* is an important factor in the drastic changes in population structure.

Through the work presented here, population dynamics of locally established *P. viridis* populations were characterized through monthly monitoring and the development of a DEB model to accurately predict the growth and reproduction under dynamic environmental conditions. This work aims to synthesize our knowledge on the individual bioenergetics of *P. viridis* and to aid in understand population dynamics and potential for competition with local *C. virginica* populations