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 southeastern United States

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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 southeastern United States

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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

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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

1. Introduction

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 (Ray & Wilson 1957; Naar et al. 2007) 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. 2004; 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 (Rajagopal

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et al. 2006; Baker et al. 2012). 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 (Fajans & Baker 2005; Baker et al. 2012). Since its invasion to southwest Florida, dense populations of *P. viridis* have thrived in the subtidal marine portions of the estuaries and are well adapted to the high temperatures frequently observed in these shallow coastal bays (McFarland et al., 2014). Visual observations of the populations in Estero Bay have shown high densities with no apparent mortality events in the years prior to this study (Volety et al., unpublished data) and while previous work has shown *P. viridis* to accumulate high levels of toxins produced by *K. brevis* in their tissues (McFarland et al., 2015), this study is the first to look at chronic effects on population structure during natural blooms in established populations.

To further 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

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Green mussels were collected monthly through 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 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

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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, along with monthly shell measurements using Vernier calipers from June 24, 2012, to September 24, 2012. 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.

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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

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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.

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 (1.8 ± 2.0 mm $month^{-1}$) differed from those observed in the summer of 2011 (8.8 ± 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 during 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; $p \leq 0.001$).

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.

3.3 Mortality

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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 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).

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).

4. Discussion

4.1 Growth

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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} (Walter 1982; Lee 1986; Al-Barwani et al. 2007). 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 (Lee 1986; Cheung 1993), growth rates in subsequent monitoring (set 3) did not exceed 3 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 0.1 mm and 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* (Li et al. 2002; May et al. 2010; Leverone et al. 2007), 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 (Shumway & Cucci 1987; Shi et al. 2012). 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,

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several short term studies on marine mussels have shown a reduction in growth during exposure to several HAB species (Nielsen & Strømgren 1991; Bricelj et al. 1993; Li et al., 2002). 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. 2004; 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 (Paster & Abbott 1969; Landsberg 2002; 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 is metabolically demanding, often depleting energy reserves and leading to increased susceptibility to environmental stressors

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(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 (Lee 1986; Rajagopal et al. 1998; Al-Barwani et al. 2007). 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

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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. Indeed, juvenile recruitment has been previously shown to be inhibited during HAB events in wild *P. viridis* populations within their natvive region (Cheung 1993). HAB exposure during early bivalve life stages has resulted in reduced fertilization and survival rates, and delayed embryogenesis and larval development (Granmo et al. 1988; Gallager et al. 1992; Matsuyama et al. 2001 Basti et al. 2012; 2015), 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 (Tester & Steidinger 1997; Steidinger 2009), 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

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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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Figure 1

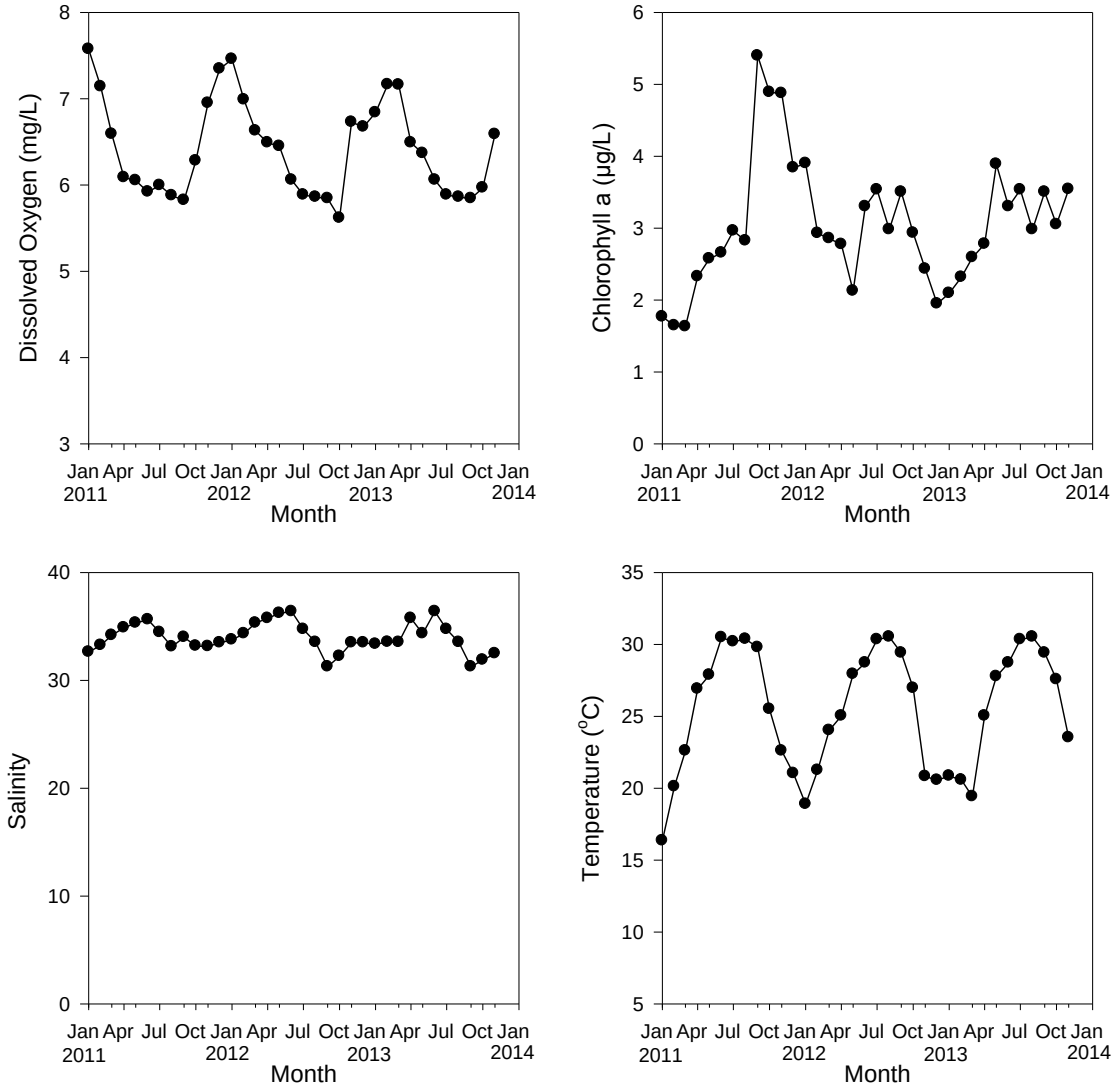

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Figure 2

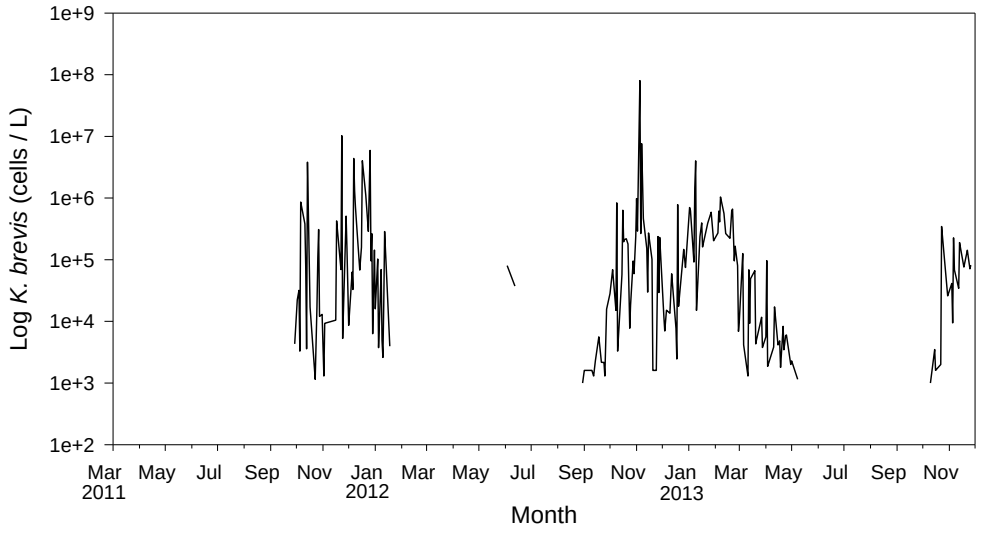

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Figure 3

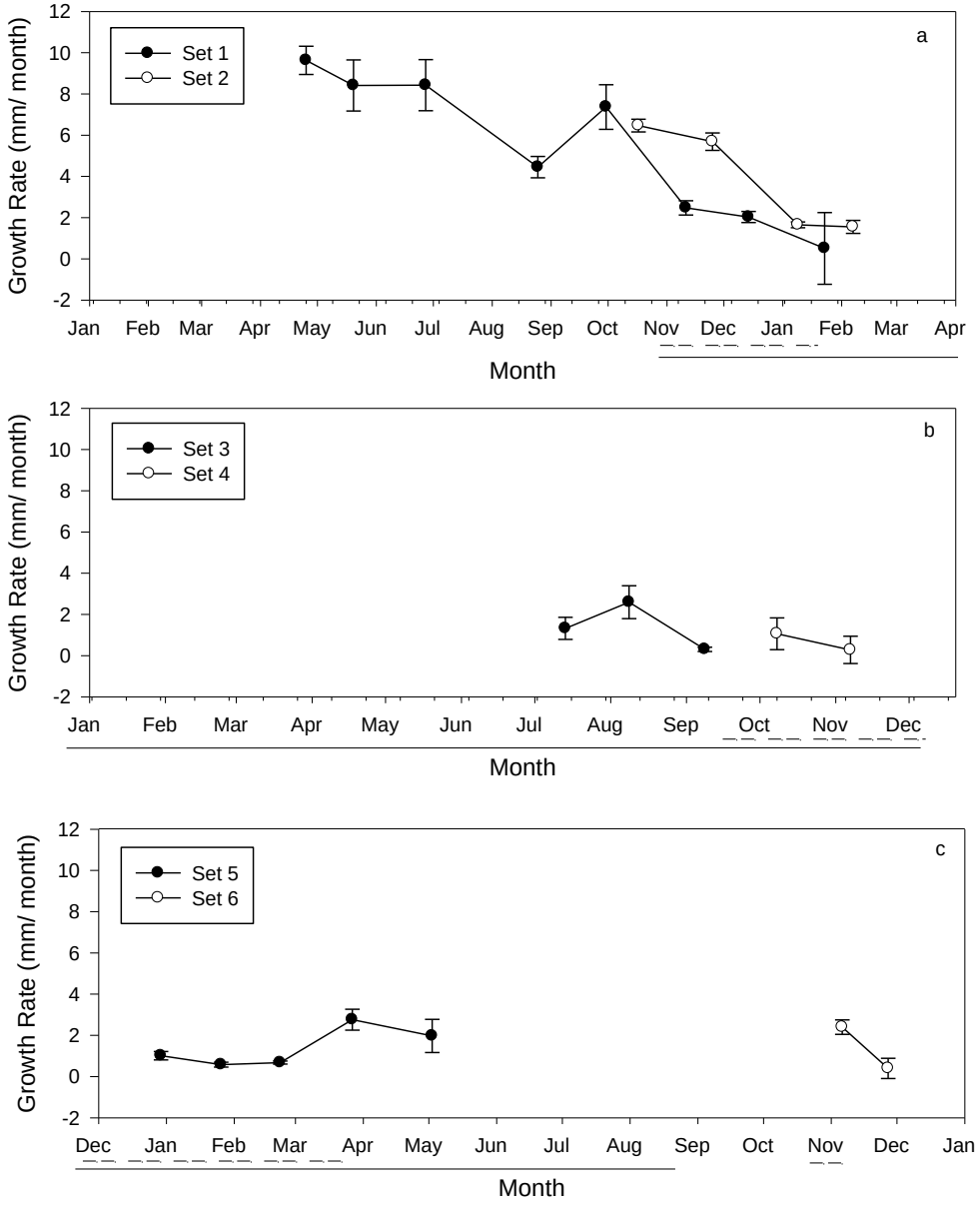

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Figure 4

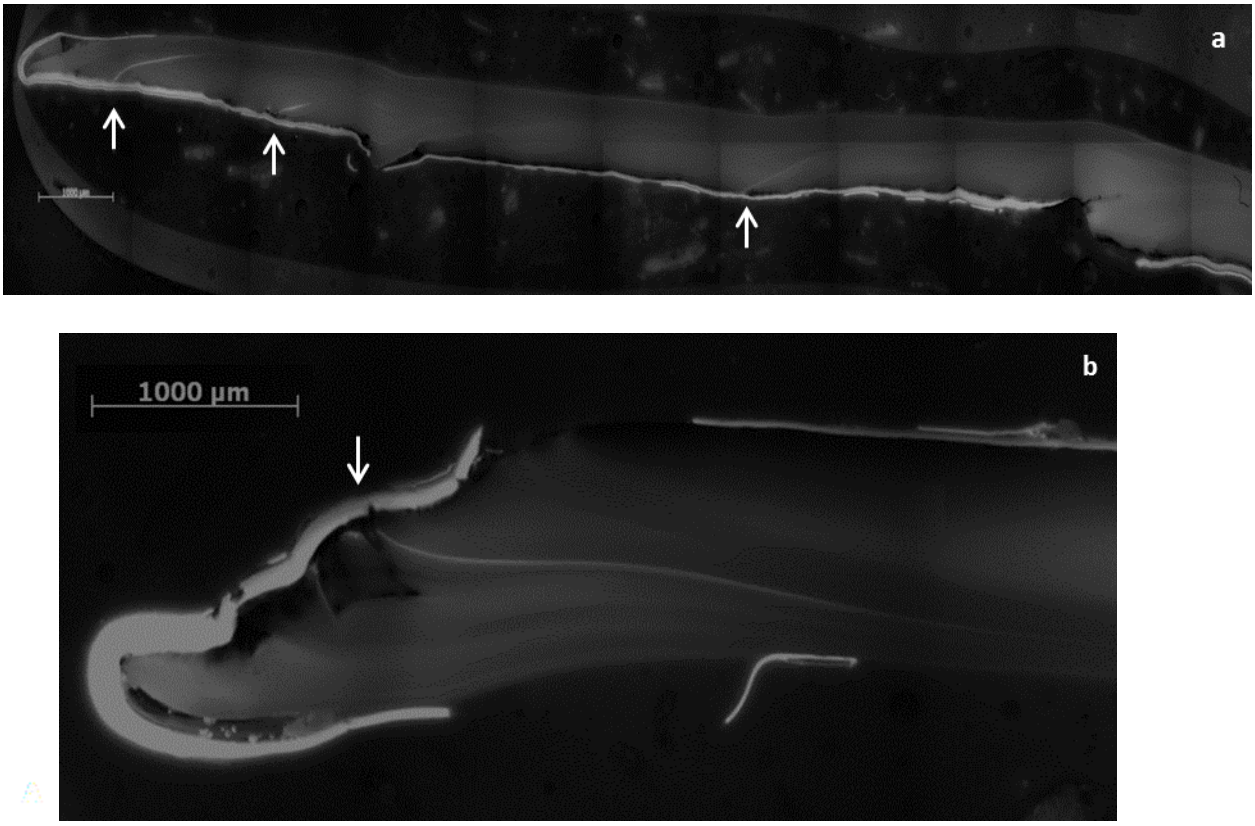

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Figure 5

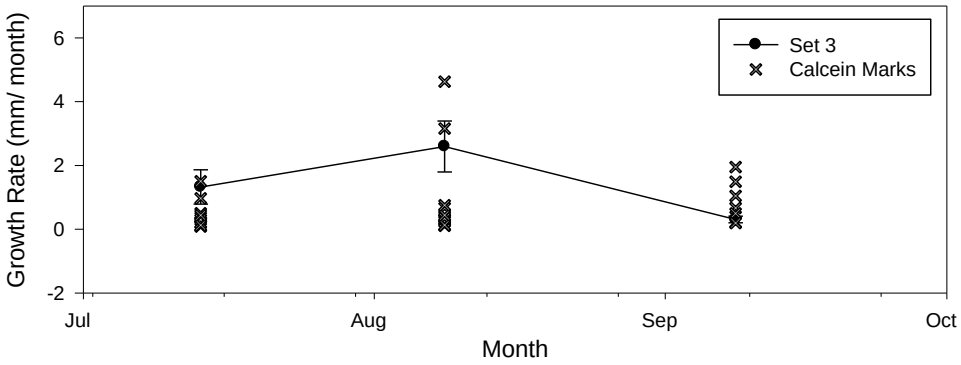

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Figure 6

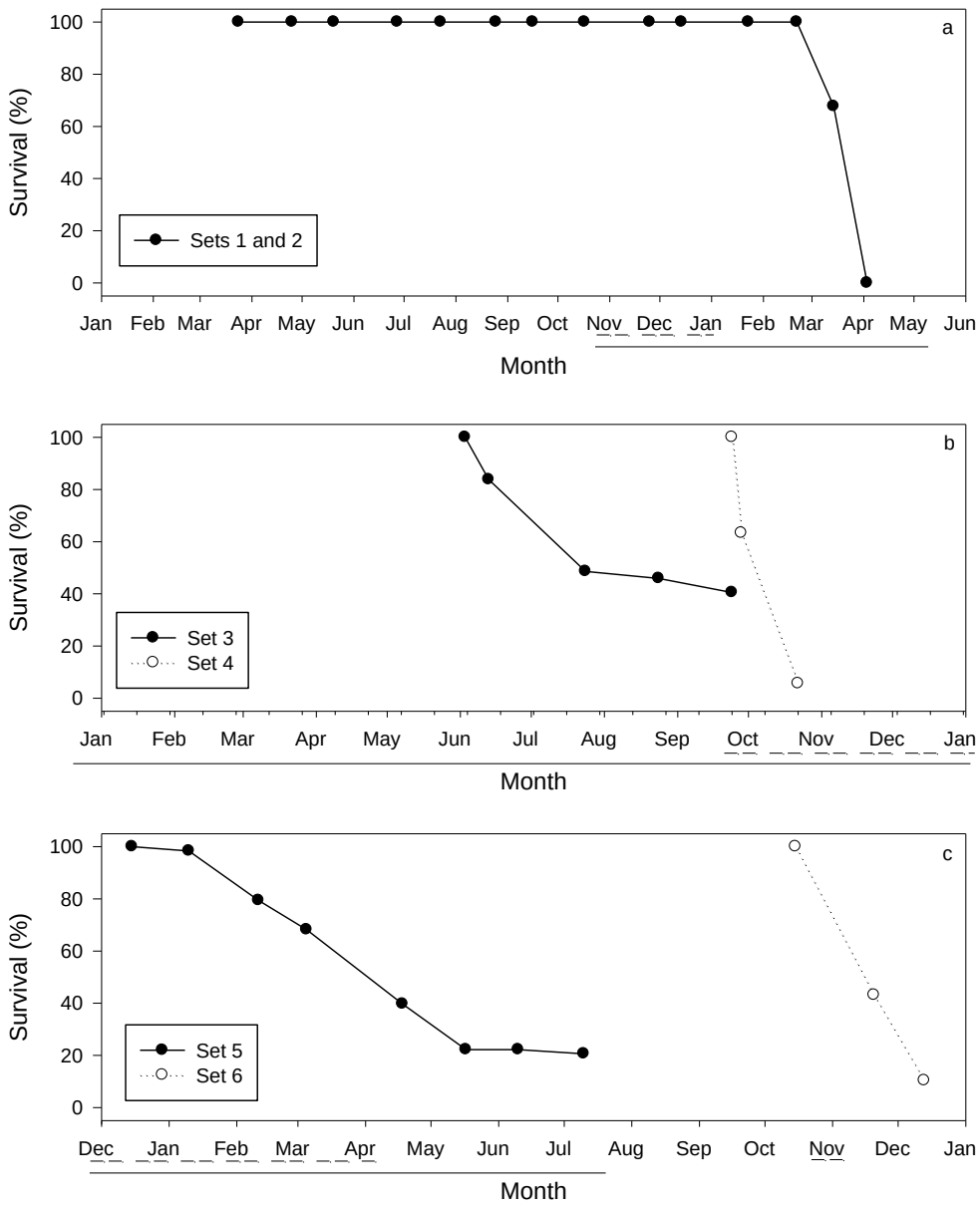

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Figure 7

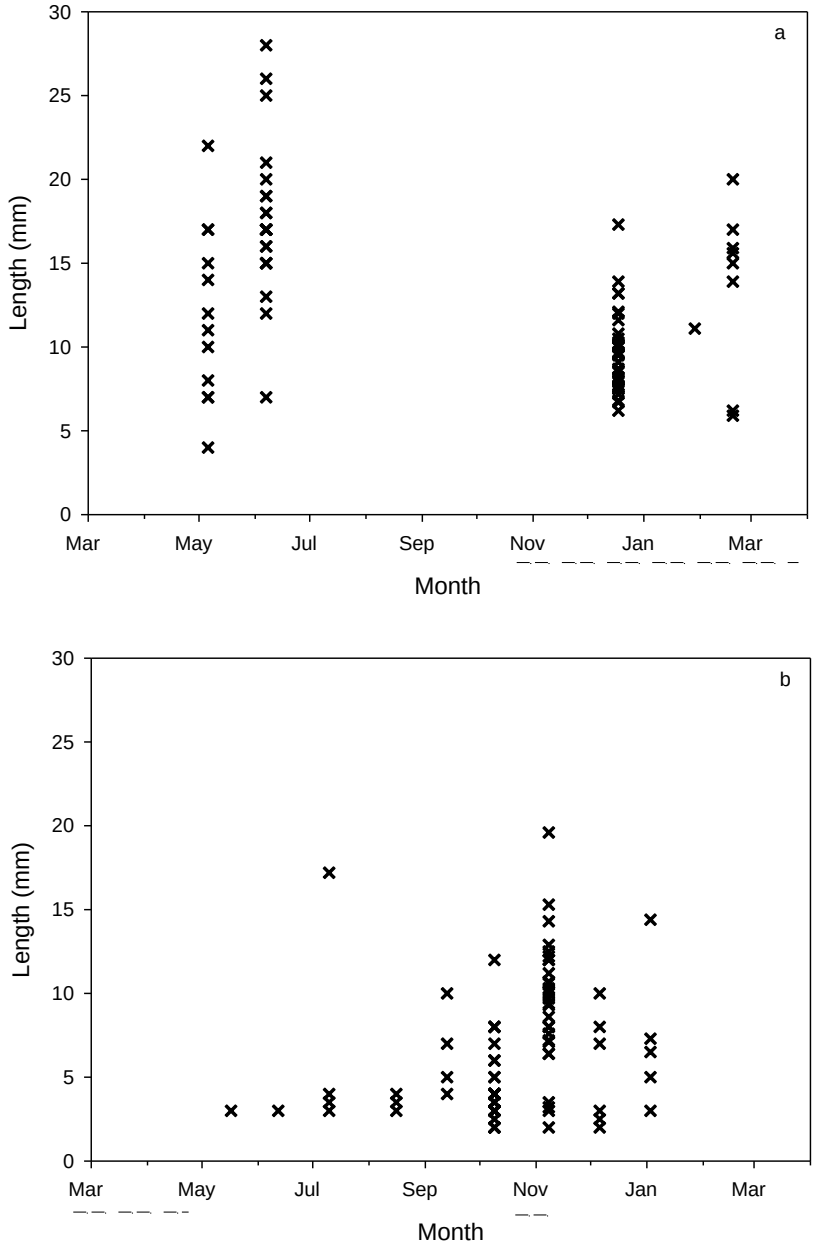

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Table 1: Kaplan-Meier Survival Analysis (Mean Survival Time) of caged green mussels during growth monitoring

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 for calcein analysis

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