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Research Article

Reproductive strategy of the invasive green mussel may result in increased competition with native fauna in the southeastern United States

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Abstract

Understanding the population dynamics of invasive species, such as the green mussel *Perna viridis* (Linnaeus, 1758), can aid in explaining the success of newly introduced 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 through histological analysis of mussels collected monthly. This was supported by overall stable energetic reserves as measured through proximal biochemical composition (protein, glycogen and lipid content). However, egg outputs in the summer ($6.4 \times 10^6 \pm 2.6 \times 10^6$ eggs / female) were significantly higher than egg outputs of winter-spawned mussels ($7.7 \times 10^4 \pm 1.4 \times 10^4$ eggs / female). Stability in biochemical composition, suggests 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 44.3–158.5 mg g^{-1} and total lipids from 7.4–13.5 mg g^{-1}. Year-round reproductive capabilities supported by sufficient energy reserves may help explain the rapid colonization and high densities of green mussels along the southeastern United States and suggests the potential for competition with native species, particularly the eastern oyster *Crassostrea virginica* (Gmelin, 1791).

Key words: gametogenesis, protein, glycogen, lipid, induced spawning

Introduction

The green mussel *Perna viridis* (Linnaeus, 1758) is a recent marine invader to southwest Florida, USA. Native to the Indo-Pacific, their introduction to Caribbean waters is believed to have occurred via transport on boat hulls and ballast water through the shipping industry. First detected in Trinidad and Tobago in the early 1990's (Agard et al. 1992), they have aggressively spread throughout 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). Populations now span as far south as Marco Island, Florida on the Gulf Coast and from Palm Beach Gardens, Florida to Charleston, South Carolina on the Atlantic coast (Rajagopal et al. 2006; Baker et al. 2007). Although *P. viridis* are well studied as an aquaculture species and bio-indicator for marine pollutants in its native range, little is known of invasive populations and their potential ecological impact on native bivalve populations in the south-eastern United States.

Perna viridis is a fast growing, biofouling organism, posing a potential threat to native species, specifically the eastern oyster *Crassostrea virginica* (Gmelin, 1791). Similar invasions have resulted in displacement of native bivalves and economic loss due to damage to infrastructure, as exemplified by the Mediterranean mussel *Mytilus galloprovincialis* (Lamark, 1819) in South Africa's coastal waters (Geller 1999; Branch and Nina Steffani 2004) and the zebra mussel *Dreissena polymorpha* (Pallas, 1771) in the Great Lakes (Baker and Hornbach 2000; Connelly et

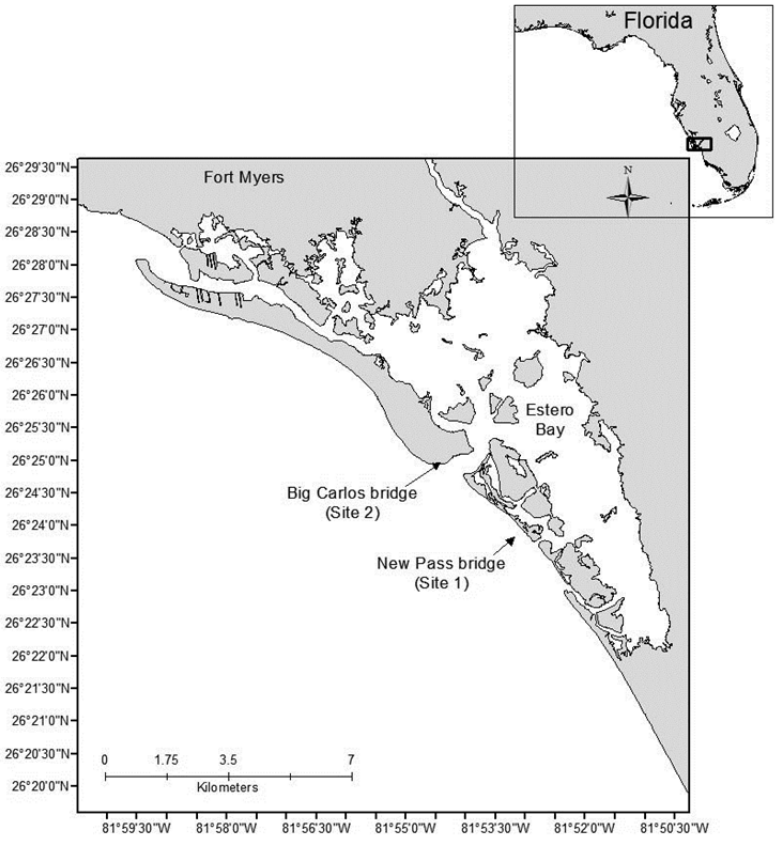


Figure 1. Map of the study site in Fort Myers, Florida, USA. Inset shows a detailed map of Estero bay and collection sites are indicated using arrows.

al. 2007). The rapid colonization of *P. viridis* has been attributed to high reproductive activity and rapid growth rates. Green mussels reach sexual maturity within the first few months of settlement (Rao et al. 1975; Sreenivasan et al. 1989), achieving average growth rates of 6–10 mm month^{-1} (McFarland et al. 2016), and dominate hard substrates at densities as high as 1000–4000 individuals m^{-2} (Fajans and Baker 2005). Thus, concerns abound regarding the future range expansion of the invasive *P. viridis* and potential competition with the native oyster *C. virginica*.

Within its native range, *P. viridis* has shown reproductive strategies ranging between one (Lee 1985) or two spawning peaks per year (Rajagopal et al. 1998) and exhibiting continuous spawning year round (Kripa et al. 2009). This variation in strategy is likely linked to different environmental conditions between Hong Kong and southern India, respectively. In contrast, the native oyster *C. virginica* spawns through the spring, summer, and fall in our study area, but experiences resting periods in gametogenesis during the winter months (approximately December–March; Volety et al. 2009, 2014). Thus, competition for resources (food, space, or both) may arise if introduced populations of green mussels display the extended spawning periods observed in areas of its native range.

Reproduction in marine bivalves is primarily driven by food availability and temperature, while remaining dependent on the amount of energy available, either from reserves or from food intake (Barber and Blake 2006). Seasonal variation in gametogenesis tends to follow variations in energetic reserves, which can be quantified using proximal biochemical composition (protein, lipids, and carbohydrates; Barber and Blake 2006; Vite-García and Saucedo 2008). Uptake, storage, and utilization of energetic reserves are dependent upon species-specific properties that include energetic dynamics, metabolic demands, and reproductive stage (de Zwaan and Zandee 1972; Dare and Edwards 1975; Gabbott 1975), and are driven by seasonal variation of physiological and environmental factors (Bayne and Newell 1983).

The goal of this study was to determine reproductive strategy and energy storage cycles of *P. viridis* in the invaded region of southwest Florida. This was completed through examination of

Table 1. Classification of gonad index rankings (modified from Rajagopal et al. 2006 and Volety et al. 2009).

Rank	Description
1	Empty gonad space with undifferentiated sex, gonad appears thin and transparent
2	New gametes forming in the gonadal wall with small follicles clearly defined
3	Increased area of the gonad producing gametes, beginning to see some gametes in late development
4	Nearly all of the gonad region is packed full of mature / late developing gametes
5	Ripe to spawn and / or spawning, full gonadal area in packed with mature gametes and / or gametes in the gonad ducts in the process of being released

gametogenesis and proximal biochemical composition (glycogen, protein and lipid) over two years in an established green mussel population in Estero Bay, Florida. 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 invasive populations and predicting further spread and potential competition with native oysters.

Methods

Environmental variables

Environmental conditions (temperature, salinity, dissolved oxygen, and chlorophyll *a*) were monitored *in situ* hourly by means of a YSI 6600 data Sondes (YSI Inc., Yellow Springs, OH, USA). Sondes were cleaned, calibrated, and downloaded every two weeks to ensure optimal performance.

Collection of mussels

Green mussels were collected monthly from New Pass and Big Carlos Pass bridges in Estero Bay, Florida (Figure 1) from September 2011 through August 2013. Big Carlos Pass was added as a collection site after a mass mortality event spanning all known collection areas. Mass mortality followed a prolonged red tide event (*Karenia brevis* (C.C. Davis) Gert Hansen and Ø. Moestrup, 2000; McFarland et al. 2015, 2016) which impeded the single site collection at New Pass. Big Carlos is less than 4 km north of New Pass and the nearest location with similar environments and dense populations of green mussels. Both sites are located at the Estero Bay – Gulf of Mexico interface where they experience extensive flushing and high current flow in fully 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.

Histological analysis of seasonal gonad development

Whole, intact tissue of green mussels (N = 15 / month) was dissected (mean maximum linear dimension ± standard error; 65.17 ± 1.95 mm) and tissue cross sections cut for histological analysis. On several occasions, juvenile mussels were collected for analysis of gonad development at small sizes. Tissue sections were cut and processed according to standard histological techniques (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, USA) before being embedded in paraffin wax. Once embedded, tissue sections were cut using a HM 325 Rotary Microtome (Thermo Fisher Scientific™, Waltham, MA, USA) to 7 µm thickness, mounted on slides and stained with Harris' Hematoxylin and Eosin. Gonad index 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 (Enríquez-Díaz et al. 2009).

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

Induced spawning

Spawning behavior and the release of gametes was assessed on two occasions (summer and winter) by inducing mature mussels from the field to spawn in the laboratory (N = 25 / spawn). On 26 September 2013 (L = 50.16 ± 1.22 mm) and 1 January 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 30 September

2013 and 9 January 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 cool-water tank at 18˚C for 20 minutes then back to the warm tank at 30 ˚C, this was repeated until spawning commenced. When spawning began, individuals were immediately removed from the spawning tank and placed into beakers to 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, both males and females, were later killed and sectioned for histological analysis to determine the relative amount of gametes retained following a spawning event. For comparison, 15 mussels collected from both the summer and winter batches that were not subject to spawning induction also were killed and histological sections prepared to represent pre-spawn gonad index. Histological analysis was completed as described above to characterize the spawning potential of both males and females.

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 stored at −80 ˚C. Tissues were then vacuum freeze dried at −47 ˚C for approximately 72 hours using a Labconco FreeZone 12 freeze dryer (Labconco©, Kansas City, MO, USA). 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, USA). Dried tissue samples (50 mg) were re-suspended in a homogenizing buffer (50mM phosphate buffer + 1mM EDTA + 0.5mM phenyl methylsulfonyl floride), 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 (as used for protein extraction), 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, USA) at 590 nm. The standard curve was made using glycogen from oyster Type II (Sigma-Aldrich Co., St. Louis, MO, USA).

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.

Statistical analysis

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. Wilcoxon signed tests were used to detect differences in gametogenesis between males and females and Chi square tests were used to test for a relationship between sex and tissue color. Wilcoxon signed tests were also used to assess differences in egg output and gonad occupation between September and January laboratory spawnings. Again, due to non-normality of the data, the Kruskal-Wallis test was used to compare changes in condition index, glycogen, lipid and protein content over time and Wilcoxon signed test used to assess significance between months. Spearman rank correlations were conducted to test for relationships between biological (gonad index, percent gonad occupation, condition index, glycogen, lipid and protein) and environmental variables (chlorophyll *a*, 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 as mean ± standard error.

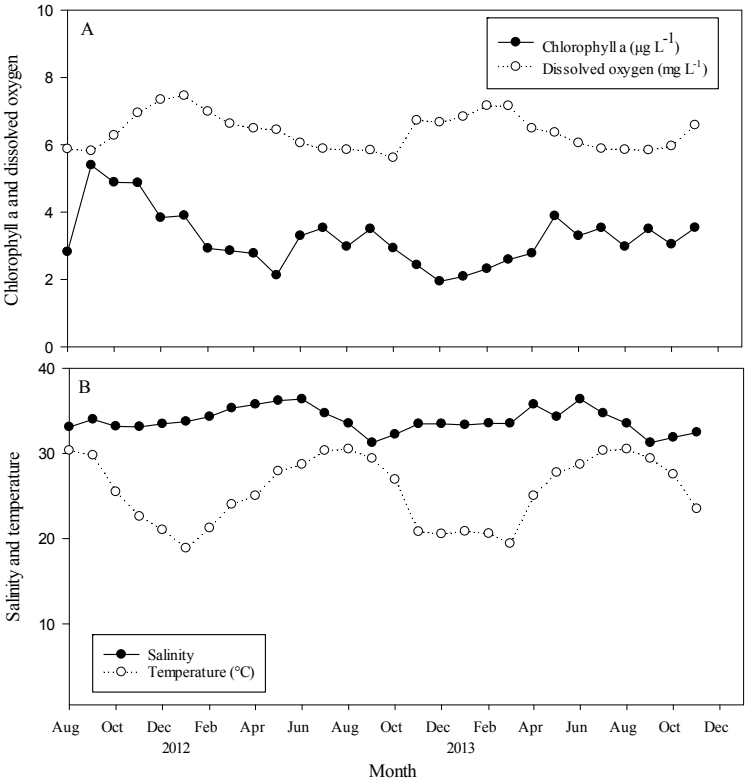


Figure 2. Selected environmental variables measured seasonally, 2012–2013.

Results

Environmental conditions

Although there was seasonal variation in the environmental variables measured during the study period these values remained within the documented tolerance range of *P. viridis* (Vakily 1989). Salinity and dissolved oxygen remained high, ranging from 31–35 and 5.6–7.6 mg L^{-1}, respectively (Figure 2). 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 (Figure 2).

Gametogenesis during field monitoring

Active gametogenesis was observed year round with average gonad index ranging from 3 to 5, representing primarily the late development to spawning phases (Figure 3). Over the course of the study, only four 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 3 (Figure 3). In addition, no true signs of resting or inactive periods were observed; at least a portion of the gonad was always actively producing gametes year round. In fact, it often occurred that individuals showed multiple stages of gonad index ranking with one portion in the regeneration/post spawn phase, while other portions were in the late development to ripe phases. Thus, continuous spawning capability was apparent throughout the year and no significant seasonal trend was detected for either percent gonad occupation or gonad index (Supplementary material Figure S1). However, peaks in gametogenesis and spawning activity were indicated by graphical representation of the percentage of individuals in the ripe stage in the late summer or autumn each year. Gonad index was not significantly different between males and females; however, percent gonad occupation was significantly higher ($Z = -9.413$, $p \leq 0.001$) in males compared to females.

Sexual development in juvenile mussels was clearly distinguishable, with active gametogenesis observed in individuals as small as 10 to 15 mm L. For all mussels sampled, sex was distinguishable before histological analysis by tissue color. When gametogenesis was active, female gonads were bright orange and male gonads were creamy white in color. These

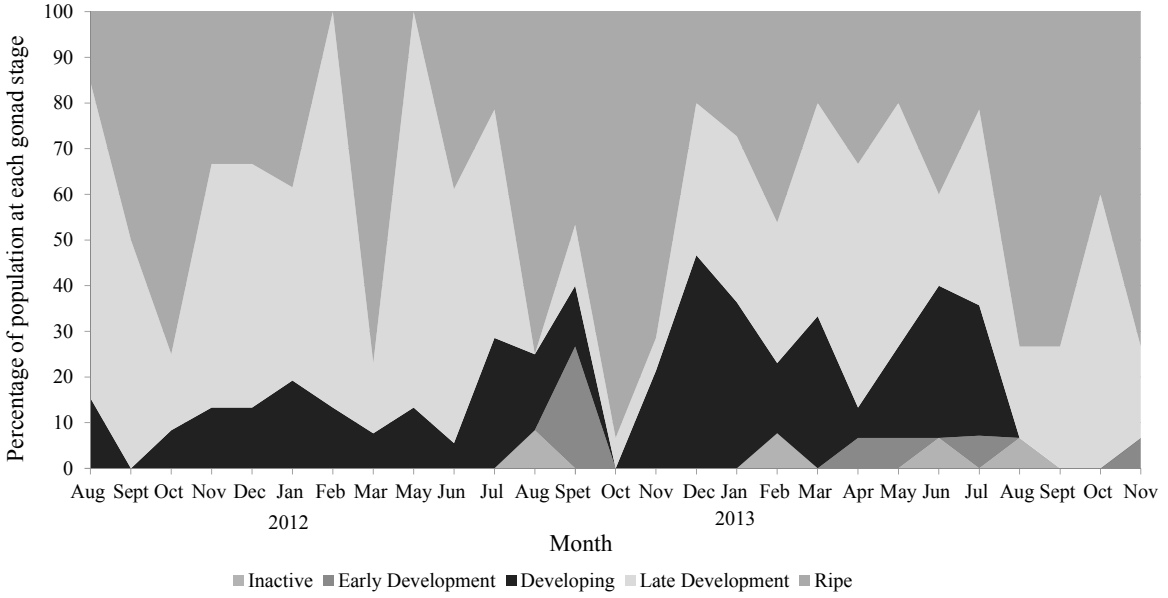


Figure 3. Histogram of gonad index overtime (N = 15 / month). Each stage is presented as a percentage of the total for each month.

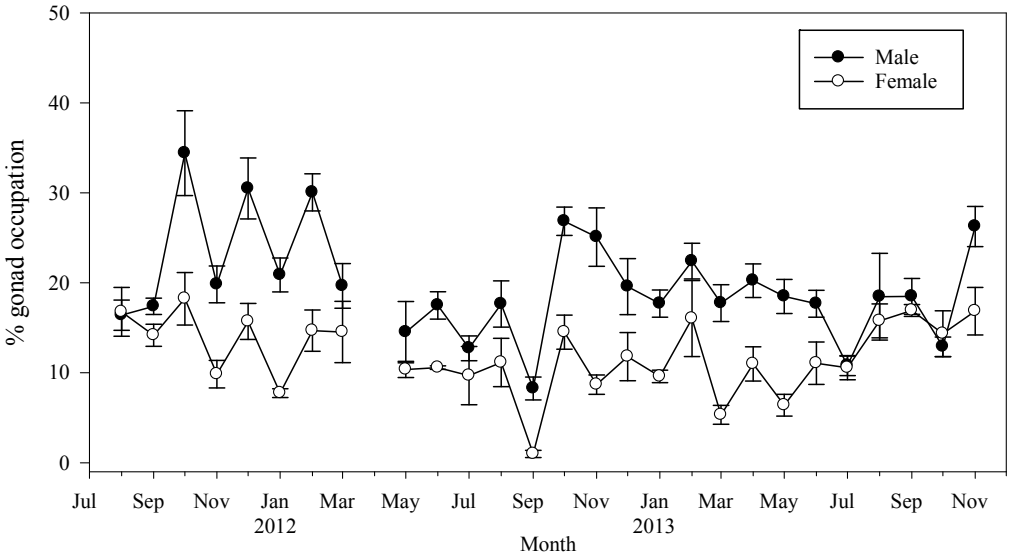


Figure 4. Average (± SE) percent gonad occupation for males and females over the monitoring period (N = 15 individuals per month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts.

Table 2. Results of histological analysis and average egg output (± standard error) of mussels from induced spawning (N = spawned: not spawned individuals).

		Eggs per female	Pre spawn		Post spawn	
			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

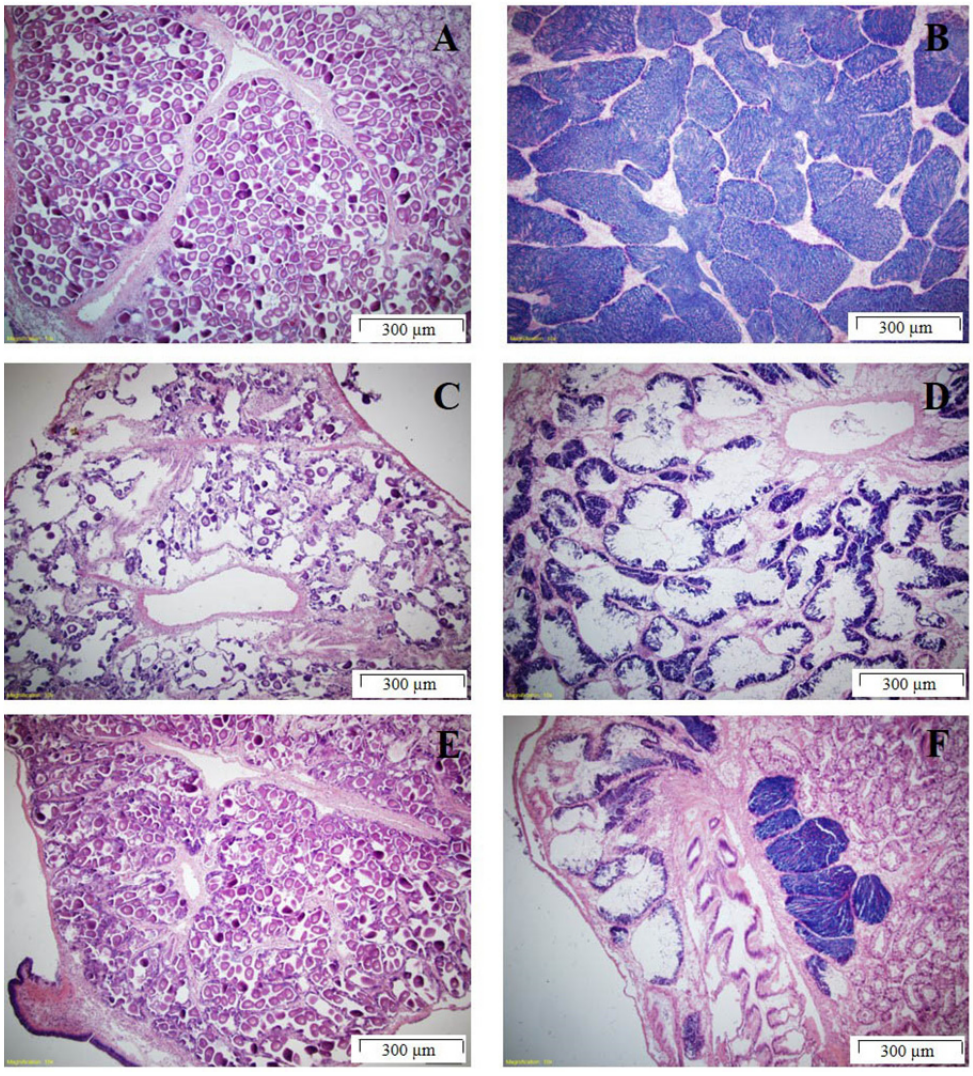


Figure 5. Micrographs of histological sections taken from mussels used during induced spawnings. Gonad from unspawned female (**A**) and male (**B**) mussels showing densely packed gametes. Some mussels released a majority of their gametes (female **C** and male **D**), resulting in large empty spaces within the gonad, while many only released a portion of their gametes during spawning (female **E** and male **F**) leaving part of the gonad void of gametes with other portions still in the ripe stage.

observations were noted and later confirmed through histological analysis and a significant relationship detected between tissue color and sex ($\chi^2_{(6)} = 43.320$, $p \leq 0.001$, phi = 0.321). This method allowed for the estimation of sex from tissue color for individuals that were collected for biochemical analysis.

Gametogenesis during induced spawning

Successful spawning was observed 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. The average number of eggs released per female in September ($6.4 \times 10^6 \pm 2.6 \times 10^6$) was significantly higher ($Z = -2.023$; $p = 0.045$) than that in January ($7.7 \times 10^4 \pm 1.4 \times 10^4$). 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).

One female from January 2014 released 5.4×10^4 eggs while maintaining a large portion of gametes post spawning. In September, only one female released the entire gonad (19×10^6 eggs) while all others released only a portion of their gonad releasing 3 to 6×10^6 eggs.

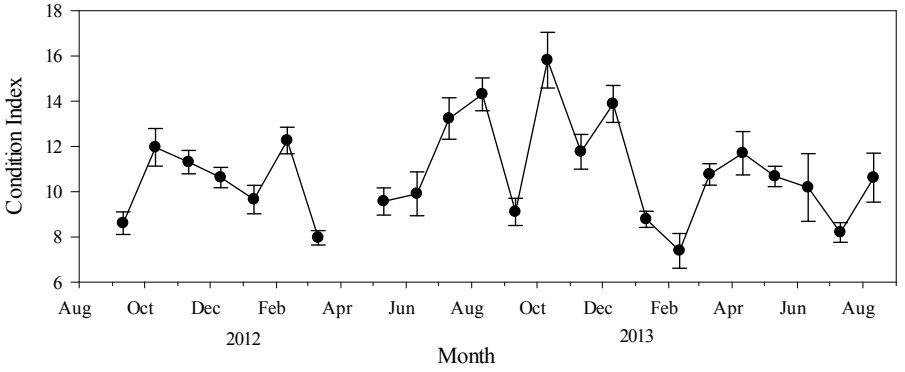


Figure 6. Average (± SE) condition index (dry visceral mass / dry shell weight) averaged by month (N = 10 / month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts.

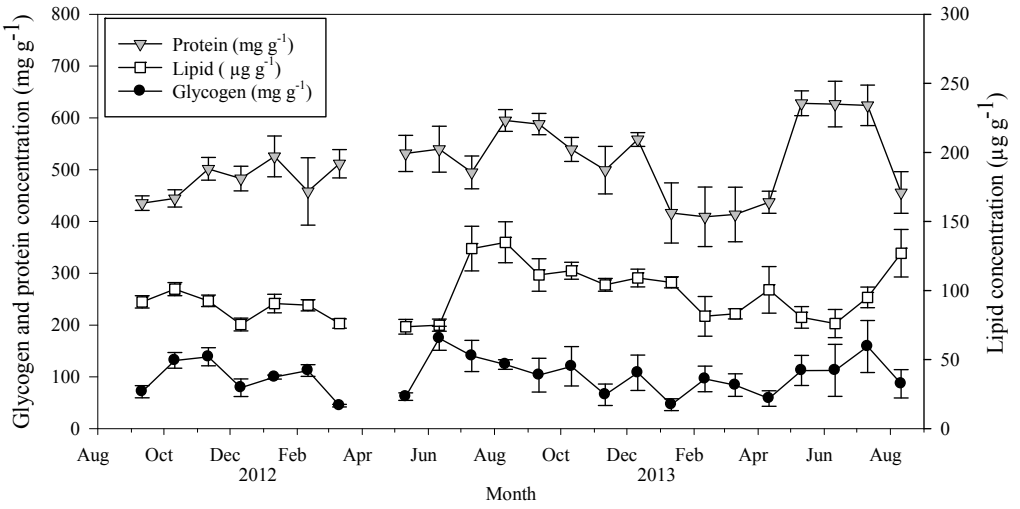


Figure 7. Average (± SE) proximal biochemical composition by month (N = 10 / month). Collections were not possible in April 2012 due to low densities following a mortality event in March 2012 and low visibility during collection attempts.

Males maintained a high percent gonad occupation even when a high density of sperm was released. Most individuals that spawned released only a portion of their gametes, leaving a portion of the gonad emptied and a portion densely packed with gametes (Figure 5e, f). However, a few individuals did release nearly all gametes (Figure 5c, d), leaving minimal gonadal area and a gonad index ranking of 1.

Condition index and proximal biochemical composition

Condition index showed high variation among individuals and between months, ranging from 8 to 16 (Figure 6); however, no significant seasonal trend was observed (Figure S2A). Spearman rank correlations showed a significant relationship with condition index for both glycogen (r_s = 0.348; $P \leq 0.001$) and total lipids (r_s = 0.340; $P \leq 0.001$). Glycogen and lipids peaked in the summer and early fall each year with annual ranges of 44.3 to 158.5 mg g^{-1} dry tissue weight, and 7.4 to 13.5 mg g^{-1} dry tissue weight, respectively. Protein did not vary significantly between months and ranged from 409.0 to 628.0 mg g^{-1} dry tissue weight (Figure 7; S2). Glycogen comprised 4.0 to 15.8% of total dry tissue weight, while protein and lipids accounted for 39.3 to 60.7% and 7.3 to 13.5% total dry tissue weight, respectively. During onset of the mortality event in March 2012, glycogen declined to a low of 2.4%, while protein and lipid remained in the expected ranges of 45.7% and 7.6%, respectively. Glycogen was the only variable measured in which significant correlations with environmental

variables were detected; chlorophyll *a* (r_s = 0.183; p = 0.005) and temperature (r_s = 0.212; p = 0.01) (Supplementary material Table S1). Although no significant seasonal trend was observed (Figure S2), both glycogen and lipids reached maxima during the summer months (Figure 7). Further, lipids were significantly correlated with gonad index (r_s = 0.158; p = 0.015), and glycogen and lipid levels were significantly correlated with each other (r_s = 0.144; p = 0.031) (Table S1), although both relationships were weak.

Discussion

Monthly monitoring of established *P. viridis* populations revealed year round reproductive activity with newly settled juveniles reaching reproductive maturity within 2–3 months. The observed year round reproduction was fueled by an ability to maintain adequate energy reserves with minimal seasonal variation, which allowed for continuous gametogenesis. This capacity for potential year round spawning may be a contributing factor to the aggressive invasion and rapid colonization by this species throughout the Caribbean and southeastern United States.

Gametogenesis

During this study, *P. viridis* exhibited year-round gametogenesis and winter spawning. 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 contents during spawning, leaving a portion of the gonad in spawning condition. Continuous gametogenesis, with incomplete spawning, leaves follicles within the same gonad at different stages of development. This strategy is common for tropical bivalves (Gaspar et al. 1999; Pouvreau et al. 2000) and is well documented for *P. viridis* (Walter 1982; Lee 1986; Cheung 1991; Rajagopal et al. 1998). Kripa et al. (2009) observed year-round gametogenesis in *P. viridis* sampled in southwest India where all individuals were at least partially ripe and no true resting period was observed. In contrast, *P. viridis* populations in Tampa Bay, just 160 km north of Estero Bay, experience a resting period during winter months (Barber et al. 2005). The differences between studies may be explained in part by variations in temperature regime, a primary factor in *P. viridis* reproduction cycles (Cheung 1991; Lee 1988). Barber et al. (2005) observed water temperatures as low as 13˚C during winter in Tampa Bay, while the lowest temperature observed in Estero Bay during this study was 18.9 ˚C. Lee (1988) suggested a minimum of 24 ˚C was required for spawning in Hong Kong, however, *P. viridis* has demonstrated year round gametogenesis and winter spawning potential at temperatures as low as 13 to 16 ˚C on the Atlantic coast of Florida (Urian 2009; Gilg et al. 2014). While gametogenesis was not significantly related to seasonal variation in temperature, spawning is likely to be stimulated by spring warming of waters, as is evident in pulses in juvenile recruitment of local populations (McFarland et al. 2016). Gilg et al. (2014) suggested that spawning in *P. viridis* was not induced by a temperature threshold; but rather by a warming from the winter low. Although spawning events in the winter are likely to be minimal, this reproductive strategy permits spawning as soon as conditions are ideal (even when only temporarily). Low density spawning between peaks may allow for early juvenile recruitment and increases the chance for at least some batches to experience ideal conditions for survival to adulthood (Barber and Blake 2006). This strategy provides a competitive advantage over local bivalves (e.g., *C. virginica*) that experience resting periods during the winter months (Volety et al. 2009, 2014).

Although no significant seasonal trend in gametogenesis was observed during field monitoring, egg output in the laboratory was significantly higher in the summer than in winter. This finding suggests that while gametogenesis occurs throughout the winter, higher gamete output occurs in the summer months when conditions are more suitable for larval growth and development. However, both summer and winter laboratory spawnings produced viable gametes with high fertilization rates (>93%), confirming field observations of a continuous, partial, spawning strategy, allowing for early spring spawning that continues throughout the summer and fall. Similarly, Cheung (1991) found the proportion body mass occupied by the gonad varied seasonally, individuals remained in an active state year round. This suggests that a fixed fraction of assimilated energy was allocated towards reproduction, which allows for continuous spawning with gamete abundance being dependent upon energy assimilation. This reproductive strategy is not uncommon as bivalves may reduce energy allocated to gametogenesis when food availability is low to maintain reserves, leading to different outputs and efficiencies throughout the year (Chipperfield 1953; Shafee and Lucas 1980; Bayne and Newell 1983). This strategy helps explain the lower egg output in the winter months when both temperature and chlorophyll *a* were at seasonal lows—two factors known to drive gametogenesis cycles in bivalves (Bayne and Newell 1983; Sreedevi et al. 2014).

Similar to Urian (2009), males consistently maintained a higher gonad volume fraction than

females, and several others have reported that males spawn more frequently and regenerate faster than females following spawning events (Walter 1982; Lee 1988). This was confirmed during laboratory spawnings in which males remained in a ripe state even after releasing large amounts of sperm, consistent with a partial spawning strategy and allowing for continuous spawning capabilities.

Proximal biochemical composition

Condition index is often linked to spawning through the loss of mass in the form of gametes, serving as an indicator of spawning events (Narasimham 1981; Lambert and Dutil 1997; Hemachandra and Thippeswamy 2008). However, high individual variation, continuous gametogenesis, and the partial spawning strategy of *P. viridis* may explain the lack of significant between-month differences for both condition and gonad indices leading to a "dilution" of a possible trend (Flye-Sainte-Marie et al. 2007). Since no significant trend was observed in percent gonad occupation or gonad index, it is not surprising that a clear trend is not observed for condition index. Condition index was, however, correlated with both glycogen and lipid levels. Further, glycogen was correlated with chlorophyll *a* and temperature, and lipids with gonad index, suggesting some linkage between reproduction and biochemical parameters as they relate to seasonal cycles.

The stability in proximal biochemical composition observed during this study is not uncommon to tropical bivalves and measurements were within the range of those previously reported for *P. viridis* in native regions (Kuriakose and Appukuttan 1980; Fatima et al. 1986). Peak glycogen content was observed in the summer and early fall each year, coinciding with elevated temperature and chlorophyll *a* levels, suggesting glycogen storage increased while food supply was high. This summer storage allows for use during periods of low food availability (Ansell 1961; Emmet et al. 1987; Mohan and Kalyani 1989), partially explaining the ability to maintain stable lipid levels throughout the year. Low glycogen reserves can lead to increased susceptibility to natural stressors as well as negative effects on growth and reproduction (Patterson et al. 1999). Although an interruption in gametogenesis was not observed, low glycogen content was observed during onset of a mass mortality event in March 2012, during which growth was slowed greatly (McFarland et al. 2016).

Similarly, lipid and protein content remained fairly stable and were comparable to published values *P. viridis* (Shafee 1978; Rivonker and Parulekar 1995; Li et al. 2007). Although not significant, apparent peak lipid values were observed each year between July–August, just prior to peaks in fall spawning activity and consistent with observations in the native range (Shafee 1978; Rivonker and Parulekar 1995; Li et al. 2007). The mobilization of glycogen as a precursor for lipids is an essential component of gamete production and commonly observed in bivalves (Gabbott 1975). Specifically, total lipids peak during the late development and fully ripe stages and rapidly decrease during spawning events due to the release of lipid rich oocytes (Barber and Blake 1981; Emmet et al. 1987; Mohan and Kalyani 1989; Beukema 1997). When examined graphically, glycogen and lipid peaks were accompanied by a peak in gonad index. However, this effect was only observed during fall spawning events and no relationship was observed during spring and summer spawning events. This may be in part due to reduced food availability (chlorophyll *a*) during winter months, which apparently was sufficient to provide energy for gametogenesis, but not the building of energetic reserves (glycogen and lipid). To corroborate this finding, glycogen and lipid peaks in the early fall occurred after several months of high chlorophyll *a* levels allowing for active gametogenesis and continuous spawning, as well as the allocation of assimilated energy to reserves. No significant decrease in lipid or glycogen was observed following spawning events, which is consistent with the partial spawning strategy. Further, the lack of significant trend in biochemical composition suggests that the seasonal variability of food and temperature are sufficient for *P. viridis* to maintain energy reserves while contributing to reproduction.

During this study, two consecutive red tide blooms (*K. brevis*) occurred resulting in an accumulation of brevetoxins in the soft tissues of *P. viridis* and several mortality events (McFarland et al. 2015, 2016). While these events appeared to impede growth (McFarland et al. 2016), no significant affect was observed in gametogenesis and spawning. 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 was detected during onset of the first mass mortality event (March 2012) following the first major red-tide bloom. Additionally, protein levels were low for four-months during the winter 2013 bloom and spiked following bloom dissipation in June 2013.

Conclusions

Although *P. viridis* and *C. virginica* currently occupy separate niches within Estero Bay (McFarland et al. 2014), the reproductive strategy observed during this study suggests the potential for competition. Locally, oysters spawn April–October and experience a resting period in the winter months (December–March) in which gametogenesis is inactive (Volety et al. 2009, 2014). This difference in reproductive strategy may allow for *P. viridis* to get a head start in the spring spawning season, giving an advantage to newly settled juveniles. If warm spring waters come early, mussels may get a head start on juvenile recruitment allowing them to outcompete oysters for substrate and may be further exacerbated by high growth rates observed in local *P. viridis* populations (McFarland et al. 2016). Currently, green mussels in Estero Bay are limited to subtidal substrates on the fringes of the estuary where salinity is more stable (McFarland et al. 2014), and are sensitive to red tide blooms (Baker et al. 2012; McFarland et al. 2015, 2016), which may limit population densities. Locally, oysters are more tolerant of red tide events with high survival (Pierce et al. 2004; Plakas et al. 2008) and accumulate comparatively less toxins than green mussels (McFarland et al. 2016), which may further keep *P. viridis* from overtaking oyster reefs, however the potential for adaptation should not be neglected and continued monitoring is essential.

To better explain what is observed in the field, it would be interesting to rear *P. viridis* under a range of temperatures and different nutritional diets to observe changes in reproductive output as well as maintenance, storage, and use of energetic reserves. Modeling the energetics will help to explore and understand dynamic links between environmental variability, growth, and reproduction of this species. Additionally, proximal biochemical composition of individual tissue compartments may better identify seasonal trends within the organism.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Results of the spearman rank correlations between biological measurements and environmental variables.

Figure S1. Box and whisker plots for gonad index and percent gonad occupation over time.

Figure S2. Box and whisker plots for proximal biochemical measurements of *P. viridis* over time for condition index, glycogen, lipid and protein.

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