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The sunray venus clam, *Macrocallista nimbosa*, exhibits asynchronous spawning

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ABSTRACT

The reproductive cycle of the sunray venus (SRV) clam, *Macrocallista nimbosa,* was initially described over 40 years ago and was labeled as a "fall spawner" based on that study. Interest in the SRV clam as an alternative bivalve species for Florida shellfish aquaculture was established a decade ago but due to it's reputation as an unreliable spawner, production of this clam has stalled. This study was conducted to provide a more thorough description of the reproductive cycle, including detail-oriented reproductive staging in an effort to determine the cause of reported spawning difficulties. Regardless of sex, *M. nimbosa* follicles were observed to be continual spawners. It was not uncommon to observe follicles in four of the six gametogenic stages simultaneously. Spawning was generally protracted with no long period of inactivity. A single hermaphrodite suggested possibility of protandry. Although spawning and gametogenesis were continuous, bimodal spawning peaks were seen; however, these peaks occurred asynchronously. These observations lend credence to reports of unreliable spawning and limited egg production during thermal induction. Continuously collected environmental data indicated that spawning in females followed increased turbidity (used as a phytoplankton proxy). This observed increase in spawning in females reiterates the role of diet in gametogenic production. It may be necessary to adjust currently established hard clam feeding practices during maturation in order to increase egg production and optimize spawning potential in this species. Further research into the optimization of temperature, concentration and types of microalgal species fed during maturation is suggested.

1. Introduction

The sunray venus (SRV) clam *Macrocallista nimbosa* (Lightfoot, 1786) is an indigenous species found from North Carolina to Florida and the Gulf of Mexico (Abbott, 1974). Targeted by commercial harvesters along the northwest coast of Florida in the 1960s, the large 10–18 cm clams were processed for the shucked meat market from 1967 to 1972 (Stokes et al., 1968; Jolley, 1972). Surveys conducted to locate additional populations were not successful and the fishery became inactive. Growth experiments conducted at that time indicated these clams could attain a length of 7.6 cm (40 g) in 12 months (Stokes et al., 1968). With the demise of the fishery, research on SRV clams languished, although Haines (1976) provided a description of the reproductive cycle of *M. nimbosa*.

Shellfish aquaculture was introduced on the west coast of Florida in the 1990's through job retraining programs for fishermen affected by increasing regulations. A successful hard clam (*Mercenaria mercenaria*) culture industry was established (Colson and Sturmer, 2000). Over the past decade, it was recognized that species diversification could stimulate industry growth. A renewed interest in *M. nimbosa* resulted in research endeavors that showed the SRV clam could be produced using spawning and rearing techniques similar to that used for hard clam culture (Scarpa et al., 2008; Sturmer et al., 2009). In spite of these strides, this clam has not been found to be a reliable, year-round spawner, leading to issues in advancing *M. nimbosa* as an alternative bivalve species for Florida shellfish aquaculture.

Haines (1976) described the reproductive cycle of a natural population of SRV clams, establishing *M. nimbosa* as a "fall spawner". However, this research was limited in scope in that sample sizes were small and detailed descriptions of each stage were not included. Reported industry issues concerning reliable spawning necessitates a re-examination of the original work, including a descriptive analysis of male and female gametogenesis, to determine if a one-year study conducted over 40 years ago in the clam's northern range defines a typical reproductive cycle for *M. nimbosa*. In contrast to other commercially reared bivalve species, research conducted and published on this

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species has been limited. Two recent papers (Barber, 2017; Laramore et al., 2017) have offered some insight into the reproductive cycle of this clam; however, the focus was not on identifying gametogenic stages. Barber (2017) focused on the annual relationship between gametogenesis in natural populations and phytoplankton populations and similar to Haines (1976), sample size was small. Laramore et al. (2017) compared two populations (natural, cultured) with regard to fatty acid profile and gametogenesis; although sample size was larger, the study only examined SRV clams for a period of six months during the purported natural spawning season.

Environmental conditions, such as temperature and food availability, are known to affect gametogenesis (Hesselman et al., 1989). Variation in the reproductive cycle of hard clam populations is determined by geography (Manzi et al., 1985). The Haines (1976) study examined *M. nimbosa* populations from north Florida, while Barber (2017) and Laramore et al. (2017) examined more southerly populations. Subtropical species are typically considered protracted spawners (Sastry, 1979; Eversole et al., 1980). It is unclear from previous studies conducted with SRV clams whether this geographic variation could impact their reproductive cycle.

The present study was initiated to revisit the reproductive cycle of *M. nimbosa* as described over 40 years ago. In addition to conducting a detail-oriented description of the various reproductive stages of males and females, this study sought to determine whether evidence exists to define the SRV clam as a "fall spawner" or whether a more protractive subtropical spawning pattern exists that can vary dependent on changing environmental factors.

2. Materials and methods

2.1. Study area and sampling

Samples of *M. nimbosa* were collected monthly (n = 46–48, 570 total) from August 2015 to July 2016 from three separate submerged cages located at the University of Florida experimental lease within the Dog Island Aquaculture Use Zone near Cedar Key (Levy County) on the west coast of Florida (29°08′18.8826″, -83°02′6.4363″). These were first and second generation cultured clams that originated from spawns conducted in 2012 with natural stock collected from Anna Maria Island and Seahorse Key on Florida's west coast.

After collection, SRV clams were shipped to Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU) overnight for subsequent processing. Sunray venus clams from three separate bags were weighed (g) and measured (shell length, height, width; mm). Clams were opened, tissues removed. A gonadal cross section was taken for histological processing.

2.2. Environmental parameters

Temperature, salinity, turbidity, and dissolved oxygen were continuously measured (30 min intervals) from August 2015 to June 2016 at a monitoring station located within the Dog Island Aquaculture Use Zone. The real time station consisted of an YSI 6600 multi-parameter sonde. As the sonde measures turbidity, but not chlorophyll *a*, turbidity was used as a proxy measurement for phytoplankton abundance.

2.3. Histological techniques and reproductive staging

A cross section (5–10 mm) of the SRV clam tissue, encompassing the gonad, was cut transversely with a razor blade (Howard et al., 2004) and placed in Davidson's fixative (Shaw and Battle, 1957) for 48–72 hours before being transferred to 70% ethanol. Histological preparation consisted of dehydrating each sample through a series of ethanol solutions (70–100%) for a minimum of one hour each, followed by clearing with toluene and paraffin embedding (Howard et al., 2004). Multiple 5–8 µm sections were cut from each embedded sample using an HM 355 S rotary microtome (MICROM International GmbH), maintaining a minimum separation of 60 µm (the approximate maximum diameter of an oocyte) between sections. Sections were mounted on pre-labeled glass slides, stained with Mayer's hematoxylin and eosin (Luna, 1968) and examined at 100–400×. Clams were categorized into one of six reproductive stages using a modified classification scheme based on the qualitative criteria from Drummond et al. (2006) but revised to more adequately define stages seen during histological examination of *M. nimbosa*. When two or more reproductive stages were evident within an individual clam, the stage representing the majority of follicles was assigned. In addition, assignment of reproductive staging also followed the methodology of Haines (1975, 1976) so that comparisons to that data set could be made. The main difference between the two methods is that the former assigns the stage based on overall predominant follicular stage in the gonad, while Haines (1975, 1976) reports the proportion of follicles in the various stages of development for each clam rather than assigning a predominant stage. The other difference is that Haines (1975,1976) does not distinguish between early and late post-spawning, which was done here, using both methods. The mean gonadal index was calculated for each sampling month by multiplying the number of individuals from each development stage by the numerical ranking of that stage, and dividing the result by the total number of individuals (Gosling, 2003). A description of the reproductive stages for female and male *M. nimbosa* is given in Table 1. Photomicrographs of gonadal stages are shown in Fig. 1.

3. Results

3.1. Environmental parameters

Mean monthly water temperature, salinity, and turbidity values are depicted in Fig. 2. Monthly dissolved oxygen was within acceptable levels for shellfish survival and growth with an annual average of 7.2 mg/L. The lowest average value (5.81 mg/l) was seen in June and the highest average value (9.43 mg/l) in February 2016. Salinity was relatively constant throughout the year with an average annual salinity of 23.9 ppt and monthly averages ranging from 20.9 ppt in September 2015 to 25.7 ppt in November 2015.

Temperature showed seasonal variation over the course of the 12-month sampling period with the lowest average temperature (13.6 °C) recorded in January 2016 and the highest average temperature (30.0 °C) in August 2015. Nephelometric Turbidity Unit (phytoplankton proxy) daily values varied greatly but monthly averages were generally higher during the fall and spring and lower during the winter and summer with the lowest monthly average (22.6 NTU) in September and the highest monthly average (123.5 NTU) in October 2015. No environmental data was available for the month of July 2016.

3.2. Size

An overall increase in all shell growth measurements: length ($P < 0.0001$), height ($P < 0.0001$), width ($P < 0.0001$) and weight ($P < 0.0001$) was observed over the course of the yearlong study (Table 2). Shell width showed the least variation with an overall annual average of 29.1 mm (range 23.4 to 34.3 mm). Shell length ranged from 45.8 to 92.4 mm with an overall annual average of 75.5 mm, while shell height ranged from 30 to 52.8 mm with an overall annual average of 43.3 mm. Total weight ranged from 27.2 to 103 g with an overall average of 60.8 g (Table 2). There was no difference in size between males and females ($P = 0.126$), or sexually differentiated and undifferentiated ($P = 0.727$) clams.

3.3. Histology

3.3.1. Sex ratio

Of the 570 cultured clams collected from cages located at Dog

Table 1
(A) Detailed description of reproductive stages of the female *Macrocallista nimbosa*. (B) Detailed description of reproductive stages of the male *Macrocallista nimbosa*.

(A)

Stage 0 Resting/Inactive	Follicles are few, appear compressed, and contain numerous undifferentiated cells. In this stage, gametogonia are not recognizable, therefore sex is undetermined.
Stage 1 Early Development	Follicles are larger and more numerous than in the inactive stage. Increasing amounts of follicle cells and oogonia are detected in the follicle walls. Follicle wall is visibly well defined with primary oocytes attached to the wall and center lumen is empty. *Note that while primary oocytes are the predominant cells in the follicle, old oocytes remain. Oocytes are differentiated from oogonia as evidenced by a large nucleus containing a prominent nucleolus. Nutritive cells are observed surrounding gonadal follicles within the peri-follicular sinuses. As the follicles mature, the nutritive cells become less prominent.*
Stage 2 Late Development	Oogonia are present and oocytes continue to develop. Some oocytes are free in the lumen and others are attached to the follicle walls via peduncles. The follicles are approximately half full with oogonia and oocytes.
Stage 3 Ripe	Follicles are expanded and full of oocytes and there is no free space in the lumen. Large oocytes are free and nucleus and nucleolus are visible in some. Follicle walls are thin and follicle cells and oogonia are minimal. Peduncles are rarely observed and the number of nutritive cells has decreased.
Stage 4 Early Post Spawn	The remaining number of follicles are reduced. Breaking down of the follicle wall is noticeable. Few residual eggs are undergoing resorption and phagocytes are present. *Note that a key characteristic observed in this stage is the development of the gonadal duct. The gonadal duct grows to extend from the follicle wall to the periphery of the gonad as oocytes are ready for release.*
Stage 5 Late Post Spawn	Follicles are empty of most oocytes as the walls are collapsed and being resorbed. Egg resorption continues and numerous hemocytes and phagocytes are present, few ripe oocytes remain. The remaining number of follicles in the gonad are greatly reduced.

(B)

Stage 0 Resting/Inactive	Follicles are few, appear compressed, and contain numerous undifferentiated cells. Gametogonia are not recognizable, therefore sex is undetermined.
Stage 1 Early Development	Numerous spermatogonia are present in the periphery of the follicle. The spermatogonia are circular follicle cells that attach to the basal membrane of the follicle wall. Spermatocytes are beginning to proliferate towards the lumen. There is a minimal presence of spermatids or spermatozoa in this stage.
Stage 2 Late Development	All cell types can be seen in this stage. Spermatogonia and spermatocytes are present near the periphery of the follicle. Spermatids and spermatozoa can be observed arranging into organized bands, aligning towards the center of the lumen.
Stage 3 Ripe	Follicles are filled with dense, organized radial bands of spermatozoa. The bands of spermatozoa are arranged with the tails towards the lumen and the heads facing the follicle wall. Spermatogonia and spermatocytes continue to be observed around the periphery of the follicle walls.
Stage 4 Early Post Spawn	Spermatozoa are arranged in swirling patterns in the follicles in preparation for release. In some follicles, a portion of the follicle wall has opened up a passageway, connecting the follicle to the gonadal duct. During active spawning, spermatozoa are visible at the periphery of the gonad, at the end of the gonadal duct. A thin layer of spermatogonia and spermatocytes are still present along the basal membrane of the follicle wall.
Stage 5 Late Post Spawn	In this stage, follicles are relatively empty and the walls appear thin and broken. Numerous hemocytes and phagocytes are present and remaining spermatozoa are undergoing resorption.

Island, 230 were males and 334 were females, 5 were sexually undifferentiated, and 1 was a hermaphrodite (Fig. 3). The overall male:female sex ratio of 0.689:1 differed significantly ($P < 0.0001$) from the expected 1:1 ratio in favor of females. Sexually undifferentiated clams were found in September 2015, February 2016, and June 2016. A single hermaphrodite was found in the March 2016 sample.

3.3.2. Reproductive cycle

The annual reproductive cycle is shown in Fig. 4 with Fig. 4A illustrating the typical "majority rules" method of staging and Fig. 4B illustrating the staging methodology of Haines (1975,1976). Both methods show similarity with respect to the predominant stage, however that clams are undergoing multiple stages of reproduction simultaneously can be more clearly visualized using Haines methodology. For example, using the majority rules method, all males would be considered to be in the same stage (late post spawning), in August 2015; with Haines methodology although 68% of male follicles are in the late post spawning stage, three other stages can also be seen. The majority of SRV clams from both sexes were spawned out in August. Males underwent gametogenesis from September to December prior to spawning from January to March. Following another developmental peak in April, males spawned continuously from May to July. Limited gametogenesis was seen in females in October and November with peak development occurring from January to March followed by spawning in April and May. A second period of gametogenic activity occurred from May through July.

4. Discussion

4.1. Histology

The gonadal region of *M. nimbosa*, regardless of sex, contains follicles in various simultaneous stages throughout the year and although spawning peaks were seen, spawning was generally protracted. It was not uncommon to observe follicles in four of the six gametogenic stages simultaneously. Gametogenesis was protracted and no long periods of inactivity were seen. Only 1% of the SRV clams could not be distinguished as to sex regardless of time of year. Protandry was suggested by both Haines (1976) and Barber (2017) as the sex ratio was skewed towards males in smaller clams and females in larger clams. This study lends support to those by the finding of a hermaphrodite clam.

4.2. Size

Shell length is one of the typical size parameters used to delineate growth in clams (Crisp, 1984; Malouf and Bricelj, 1989; Grizzle et al., 2001). Haines (1975) collected clams with an average shell length of 117–134 mm, while Barber (2017) collected both small (35–79 mm; 60 mm average length) and large (94–152 mm; 129 mm average length) clams. The average length of SRV clams used in the present study was smaller at 75.5 mm, and as they were cultured the age (~ three years) is known. Barber (2017) estimated that the smaller clams collected in that study were between six and eight months old based on an assertion by Stokes et al. (1968) that *M. nimbosa* can reach 73 mm in one year. Cultured SRV clams have been shown to reach 60 mm in 19–21 months (Scarpa et al., 2008; Sturmer et al., 2009). However, as it is known that environmental variation (i.e., temperature, food availability) can affect growth, size may not always be the best indicator of age or reproductive capability.

4.3. Sex ratio

In this study, SRV clams were skewed towards females (0.68:1). This ratio is similar to that reported previously (0.8:1) in SRV clams collected from the same cohort (Laramore et al., 2017). Haines (1975) reported a similar ratio (0.72:1) in SRV clams collected from Saint

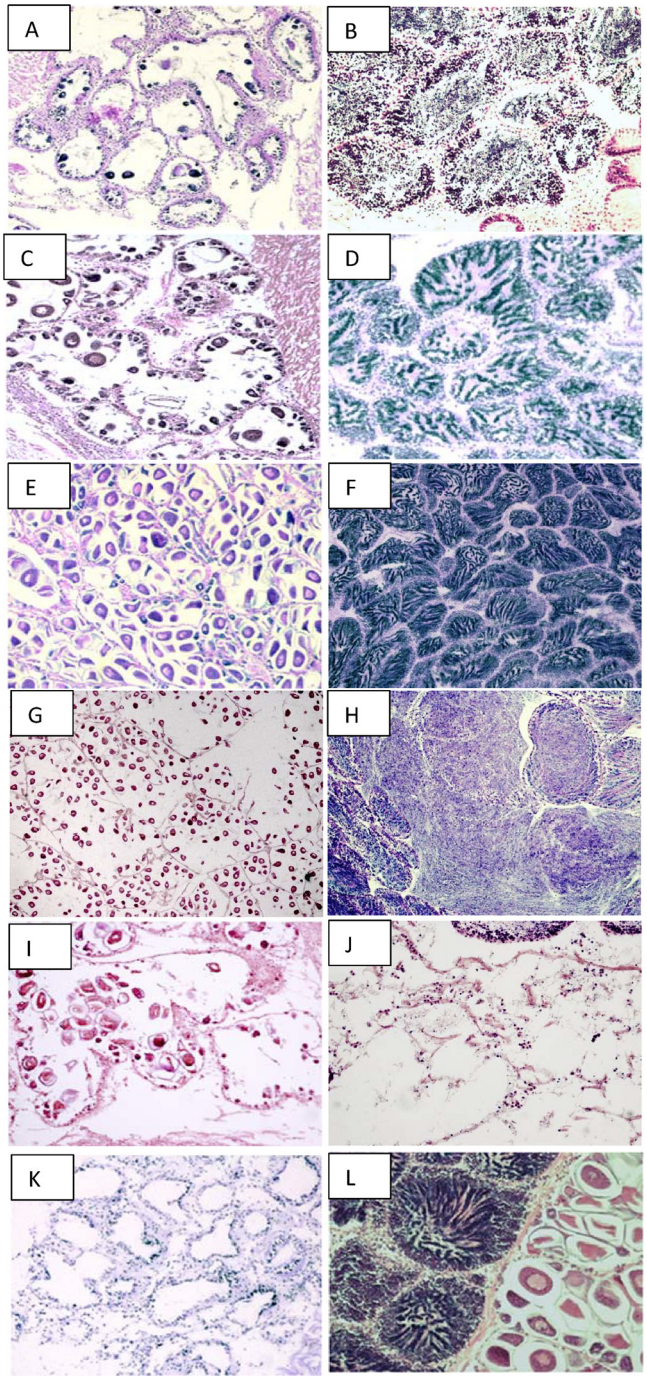

Fig. 1. Photomicrographs of gonadal stages for female and male *Macrocallista nimbosa* stained by H&E. A–B. Stage 1: Early development. C–D. Stage 2: Late development. E–F. Stage 3: Ripe. G–H. Stage 4: Early post-spawning. I-J: Stage 5: Late post-spawning. K. Inactive follicle. L. Hermaphrodite. A–F, H-K: 10X objective, G: 4X objective, L: 20X objective.

Joseph Bay for histological examination even though collected clams were much larger. Large SRV clams collected by Barber (2017) were predominantly female (0.56:1), while small clams exhibited the expected 1:1 ratio. Size appears to be more a factor in determining sex of these clams in the wild than in cultured counterparts. Haines (1975) reported that SRV clams less than 70 mm were predominately male. Barber (2017) reported that more small than large clams were males. No correlation between size and sex ratio was seen in the present study.

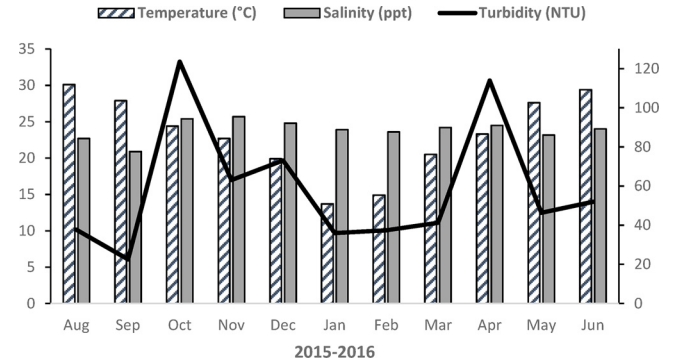

Fig. 2. Average monthly water temperature, salinity, and turbidity at the Dog Island Lease Area, Cedar Key, Florida water quality monitoring station from August 2015-June 2016. Data from July 2016 were not available.

Table 2
Monthly averages ± standard deviations of shell measurements (width, length, height) and total weight for *Macrocallista nimbosa* collected from August 2015 to July 2016.

Month	Number (*n*)	Width (*mm*)	Length (*mm*)	Height (*mm*)	Weight (*g*)
August	48	27.8 ± 0.4	72.9 ± 4.3	41.6 ± 2.6	53.4 ± 6.6
September	48	28.0 ± 0.4	72.4 ± 3.8	40.9 ± 2.4	52.5 ± 6.1
October	48	28.4 ± 1.0	74.3 ± 4.6	42.0 ± 2.8	57.4 ± 7.1
November	47	29.1 ± 0.3	75.4 ± 2.9	43.1 ± 1.7	60.5 ± 5.2
December	46	28.8 ± 0.8	74.9 ± 5.6	42.5 ± 3.3	60.0 ± 8.4
January	47	29.3 ± 1.3	74.5 ± 6.4	43.9 ± 2.9	61.5 ± 8.7
February	47	29.5 ± 1.7	76.2 ± 2.9	43.5 ± 1.6	62.8 ± 3.9
March	48	29.6 ± 1.1	76.5 ± 2.9	43.5 ± 1.9	63.1 ± 5.7
April	48	29.8 ± 0.7	77.0 ± 3.0	43.7 ± 2.0	64.6 ± 5.3
May	48	29.5 ± 1.2	77.5 ± 1.6	45.8 ± 1.0	65.6 ± 2.1
June	48	30.0 ± 0.9	77.5 ± 4.2	44.3 ± 2.6	65.8 ± 7.6
July	48	29.5 ± 0.7	77.4 ± 2.9	45.0 ± 1.6	63.8 ± 4.5

4.4. Reproductive stage

This study was initiated in response to reports of asynchronous spawning of SRV adults by Florida shellfish hatchery personal. In light of these reports, it was felt that the annual reproductive cycle of *M. nimbosa* first described by Haines (1976) needed to be reexamined to determine whether the initial study describing this clam as a fall spawner was representative of a typical SRV clam reproductive cycle. In addition, this study sought to provide a detailed description of the gametogenic stages of *M. nimbosa* since clams in general are sometimes difficult to stage. Both Manzi et al. (1985) and Eversole et al. (1980) found staging of *M. mercenaria* difficult due to variability of stages found within a single gonad. In contrast to published descriptions of SRV clam reproductive stages, Haines did not differentiate between early (EPS) and late post spawning (LPS) phases, using the categories partially spawned and spent. The spent phase includes both LPS and inactive follicles.

The reproductive stage of a clam is typically defined by the stage in which the majority of follicles are found (Manzi et al., 1985; Drummond et al., 2006; Laramore et al., 2017). Haines (1976) suggested that to assign each animal to just one phase would mask other phases present due to his observation that individual SRV clams contain follicles in several different phases. Therefore, Haines (1976) reported the proportion of follicles in each stage for each SRV clam. Both methodologies were compared in the present study to see if, as suggested by Haines (1976), one was more appropriate to use for *M. nimbosa* due to the presence of multiple follicular stages observed simultaneously. This study confirms that either method may be used for depicting gametogenic stages as both yield similar results with regards to the overall reproductive stage in both males and females. However,

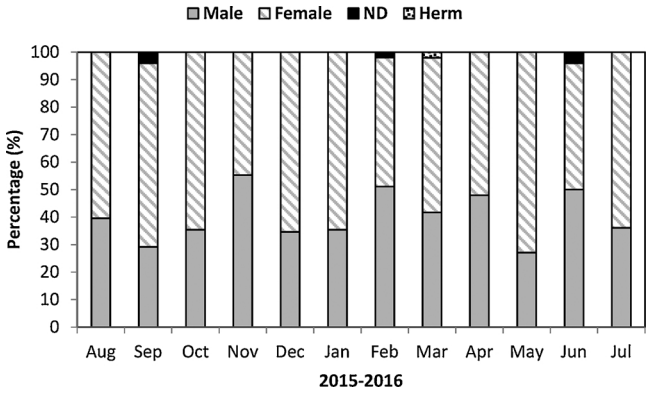

Fig. 3. Average monthly sex ratio of *Macrocallista nimbosa* collected from the Dog Island Lease Area in Cedar Key, Florida from August 2015-July 2016. ND = not differentiated, Herm = Hermaphrodite.

Haines's methodology does have the advantage in revealing the extent of follicular variation encountered in this clam species.

The annual reproductive cycle of bivalves in temperate climates is defined by distinct periods of inactivity with one or two spawning peaks (Loosanoff, 1937a,b; Eversole et al., 1980). Barber (2017) commented on the distinct lack of synchronicity in females. The few studies that have assessed the reproductive cycle of *M. nimbosa* concur with that observation and agree that follicles are continually in multiple stages of development making it difficult to define a clear reproductive stage. These observations provide confirmation of hatchery personnel designating this clam a "sputter spawner" due to difficulties encountered in getting males and females to simultaneously release sperm and eggs under thermal induction. There was no clearly defined period of inactivity in these clams. In the present study, the percentage of SRV clams with inactive follicles reached 32% in females and 24% in males concurrent with fall spawning peaks. Haines reported similar values during the fall spawning peak (40% in females, 25% in males). Barber (2017) noted a high period of inactivity (40–50%) in females in November and, again, in the spring following spawning.

In the present study, a clear difference is seen with regards to male and female reproduction. Males became ripe earlier than females and underwent gametogenesis continually from September through July with distinct spawning peaks noted in May and August. Females exhibited a protracted period of spawning and inactivity (August–November) with gametogenic activity occurring from December through March, followed by spawning in April and May. Spawning activity occurred simultaneously with gametogenesis during June and July. Thus, males were found to be continuously developing with peak spawning occurring in late spring and late summer.

Haines (1976) also reported that males exhibited continuous gametogenesis with the maximum amount of ripe follicles in June. Males began spawning in July and August with peaks in November and December. Females contained partially spawned and inactive follicles throughout the year with the highest proportion of spawning and

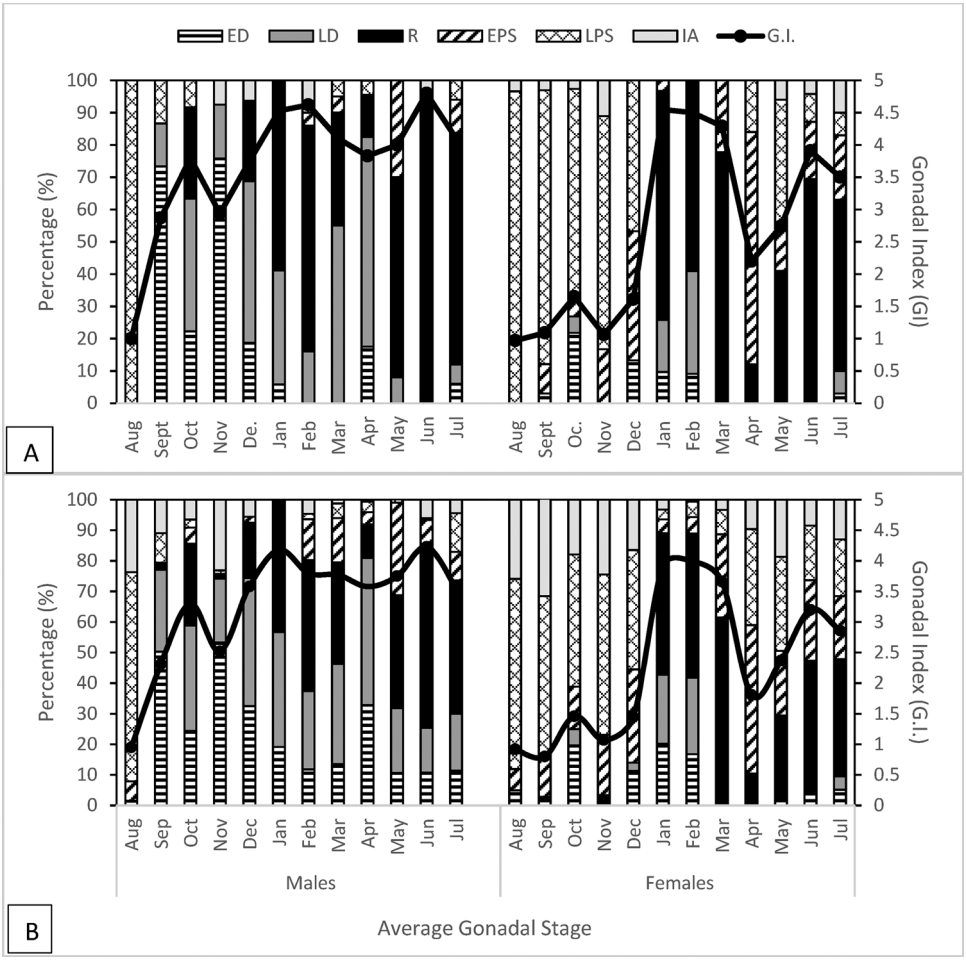

Fig. 4. (A,B) Annual average gametogenic cycle for *Macrocallista nimbosa* males and females collected from the Dog Island Lease Area in Cedar Key, Florida, as determined by (A) stage of majority of follicles in each clam and (B) as per Haines (1976), showing all follicular stages occurring in each clam. ED = early development, LD = late development, R = ripe, EPS = early post spawning, LPS = late post spawning, and IA = inactive. Monthly gonadal index (G.I.) values were determined by averaging the number of sunray venus clams assigned to each category (ED = 3, LD = 4, R = 5, EPS = 2, LPS = 1, IA = 0).

inactivity occurring August through November peaking in October. Females followed the same general pattern as males but had a shorter spawning period. Haines (1976) concluded that the SRV clam was a fall spawner. Female SRV clams surveyed by Barber (2017) in 2014–2015 exhibited two major periods of gametogenic activity, August to October and December and May to July. Spawning was observed in November, January, and February with peak inactivity occurring in March and April. No attempt was made to define a monthly reproductive stage for males, instead the percentage of the gonadal area occupied by spermatozoa was reported with spermatozoa observed throughout the year. A decline in the gonadal area in May and July indicated spawning activity in the spring and summer (Barber, 2017). Laramore et al. (2017) compared the reproductive cycle of two populations (wild, cultured) during the presumptive "fall spawning" period in 2014 and 2015. Males exhibited spawning activity in November or December and, again, in February. Wild females displayed a similar pattern to males, while cultured females presented a more protracted spawning period. Even though wild females collected by both Barber (2017) and Laramore et al. (2017) showed similar stages in November and January, Laramore et al. (2017) observed the highest percentage of ripe females in the spring, while Barber (2017) reported a high percentage of inactive females at that time.

4.5. Reproductive comparison

Although *M. nimbosa* follicles are perpetually in multiple stages of development, spawning peaks occur, yet the reported timing of these peaks varies between studies. Spawning peaks for males have been reported in spring, summer and fall. The present study along with Barber (2017) observed spawning peaks for males in spring and summer, while Haines (1976) observed spawning peaks in summer and fall. Laramore et al. (2017) observed spawning peaks in spring and fall. Peak spawning activity between males and females does not necessarily correspond. Haines (1976) described only one spawning peak for females (fall). Barber (2017) reported a fall and winter into spring peak. Laramore et al. (2017) observed a winter and spring peak, and the present study found a fall and spring peak. Disparities in the timing of spawns may be explained by a multitude of factors, including methodology used to determine reproductive stage, sample size, or environmental factors. Haines (1976) sampled 203 clams over the course of the year, collecting 10–23 clams per month, examining 20 randomly chosen follicles to determine the proportion of follicles in each reproductive stage. Barber (2017) sampled 226 clams, 20 per month. However, follicles were examined and females were assigned a stage, males were not; instead the size of gonadal area containing spermatozoa was measured. Laramore et al. (2017) examined 345 clams from two populations over a period of six months. In the present study, 570 clams (~ 48/month) were collected. The entire gonadal region (average of 814 follicles) was examined and the percentage of follicles in each stage determined and reported as either the stage that the majority of follicles were in as well proportionally, following Haines (1976).

Although the three published studies with *M. nimbosa* were conducted on Florida's west coast, locations of collected SRV clams differed. Haines (1976), collected SRV clams in Saint Joseph Bay in northwest Florida, while Barber (2017) gathered SRV clams approximately 300 km south in Tampa Bay. SRV clams examined in the present study as well as those collected by Laramore et al. (2017) were from the Cedar Key area, located mid-way between these two locations.

A number of studies have investigated gonadal development and spawning patterns of *M. mercenaria* along the Atlantic coast from Long Island Sound to Florida. Eversole et al. (1980) observed that the breeding season of *M. mercenaria* changed with latitude, where the spawning season of hard clams in more southerly locations was prolonged. Early studies by Loosanoff (1937b) determined that temperature was the most important environmental factor regulating gametogenesis in hard clams. Loosanoff (1937b) observed a unimodal or annual breeding cycle in *Venus mercenaria* with a peak spawn between August and September in Long Island Sound. Although many of these clams were ripe, they remained dormant until water temperatures became favorable for spawning. Keck et al. (1975) compared the developmental stages of hard clams from two different areas in Delaware Bay. Although both groups were in the same stage, the mechanisms of development differed. Females from one area (Henlopen) rapidly developed large numbers of oocytes that increased slowly in size during winter and spring, while clams from another (Delaware Bay) developed slowly both in size and number over a long period of time, even though both exhibited a unimodal breeding pattern from June to October with a peak in August and September. Keck et al. (1975) concluded that the two divergent patterns of development were due to the Henlopen clams being in close proximity to cooler oceanic water in the summer and warmer water in the fall and winter.

Porter (1964) observed a bimodal breeding pattern for hard clams in North Carolina with peak spawning in June, followed by a lighter spawn from September to October. Eversole et al. (1980) reported a similar bimodal breeding pattern in *M. mercenaria* in South Carolina with spawning peaks in May and June and in September and October. Manzi et al. (1985) observed that the spawning cycle of *M. mercenaria* in South Carolina was continuous from April-May through September-October with two spawning peaks occurring during the summer months. Further south in the Indian River Lagoon in Florida, Hesselman et al. (1989) observed that *M. mercenaria* exhibited continuous spawning from February to June followed by a second spawning event that lasted from September to December.

With reported variation in the timing of spawning in *M. mercenaria* in southerly locations extending from the Carolinas to Florida, it is not surprising that some deviation is seen with respect to *M. nimbosa* reproductive patterns from three Florida locations conducted in multiple years. Haines (1975) reported a single annual spawning period for *M. nimbosa*; however, it was protracted over a five-month period from late summer to late fall. Although temperature data was not reported, lower annual temperatures are typically seen in northwest Florida compared to mid-coastal areas. Barber (2017) reported temperatures ranging from 14.5 °C in February 2014 to 30 °C from May to August 2015 in Tampa Bay, which is approximately 300 km south of the Saint Joseph Bay site sampled by Haines (1976). Still, it was concluded that food supply played a greater role in determination of the reproductive cycle than did temperature. Although abundance of phytoplankton was not assessed, diatom diversity varied seasonally with the greatest diversity proceeding gametogenic activity (Barber, 2017). Laramore et al. (2017) reported similar temperature ranges within the same time period. Concurrent with the rise in temperature in March, mean turbidity increased followed by increased gametogenic development (Laramore et al., 2017). In the present study, temperature ranges were similar to the prior year. Turbidity peaked twice, in October as temperatures decreased and in April as temperatures rose. Increased gametogenic activity followed increased turbidity in both instances.

In conclusion, this study clearly defines the reproductive stages that occur in *M. nimbosa* and provides evidence for this clam being considered a protandric species. This study also verifies the reputation of the SRV clam being a "sputter spawner", which may help to explain the difficulties hatchery personnel encounter in attempting to spawn this clam. The reproductive pattern observed is not dissimilar to reports of bimodal and continuous spawning in other bivalves, such as *M. mercenaria*, that are found at southerly latitudes. The few reported studies that have examined the reproductive pattern of the SRV clam show variation in timing of spawning. This is likely due to changes in annual weather patterns that cause temperature shifts, which, in turn, may affect phytoplankton diversity and abundance. Rather than label *M. nimbosa* as a fall spawner, the term protracted spawner appears to apply in that spawning can occur year round with bimodal rather than unimodal spawning peaks, as described by Haines (1976), being the norm.

This implies that spawning can be induced year-round, and that while similar maturation protocols established for hard clam operations can be applied, feeding strategies and holding temperatures during maturation may need to be optimized for success to be achieved. Established spawning procedures (i.e., number of temperature cycles) may also need to be adjusted for successful fertilization and larval production to be achieved.

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