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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