REPRODUCTIVE PATTERNS OF CULTURED AND WILD SUNRAY VENUS CLAMS (*MACROCALLISTA NIMBOSA*) IN FLORIDA WEST COAST WATERS

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PI Laramore and Co-PI Sturmer

Submitted to:
Ms. Serina Rocco
Division of Aquaculture
Department of Agriculture and Consumer Services

Submitted by:
Susan Laramore, Ph.D.
Associate Research Professor
Aquaculture and Stock Enhancement Program
Harbor Branch Oceanographic Institute at Florida Atlantic University
5600 US 1 North
Fort Pierce, FL 34946
772.242.2525
Slaramo1@fau.edu

INTRODUCTION

The shellfish aquaculture industry in Florida was established in the 1990s when federally funded job retraining programs introduced clam culture as an alternative to fisheries along the west coast (Colson and Sturmer 2000). Since then, Florida has seen a dramatic increase in aquacultured shellfish production, from $1.2 million (41 farms) in 1991 to $18.7 million (132 farms) in 2013 (USDA 1992, USDA 2014). However, the industry has relied on a single species, the northern hard clam *Mercenaria mercenaria* (Linnaeus, 1758). Faced with declining market prices and increased production costs, growers have realized that species diversification might increase economic stability as well as enhance the growth of the industry and have responded by examining alternative species, such as blood arks *Anadara ovalis*, ponderous arks *Noetia ponderosa* (Power et al. 2004, 2005, Sturmer et al. 2009), and the sunray venus clam *Macrocallista nimbosa*. The existence of a latent market and potential growth rate of the sunray venus clam, along with it being a native species, made it a logical choice as a candidate culture species.

Research initiated in 2006 concluded that sunray venus clams may be produced using techniques similar to those used for hard clam culture (Scarpa et al. 2008, Sturmer et al. 2009). The sunray venus (SRV) clam was targeted by the commercial fishing industry along the northwest coast of Florida in the 1960s; due to the limited size of fishing grounds the industry never developed (Stokes et al. 1968, Jolley 1972). Experiments conducted at that time indicated that these clams could attain a length of three inches (40 g) in 12 months (Stokes et al. 1968). Over the past six years, through Florida Sea Grant-supported projects, the culture and market potential of these clams has been ascertained (Scarpa et al. 2008, Adams et al. 2009b, Sturmer et al. 2009). These projects have demonstrated that the SRV clam may be produced using techniques similar to those used for hard clam culture. Based on this knowledge, hatchery operators have begun to condition and spawn this clam for production of seed. The SRV clam is considered to be a "fall spawner" with peak spawning occurring in November and December in northwest Florida (Haines 1976). Early work had reported that the SRV clam has a somewhat prolonged spawning season of December to February and clams collected then are assumed to be ripe and ready to spawn (Scarpa et al. 2008). However, hatchery operators have encountered problems in attempting to spawn this species outside its natural spawning cycle. During the 2012-13 winter, a presumptive atypical pattern was seen in which the spawning season extended into April, resulting in sporadic spawning events and lower success rates than anticipated. Whether this represents an anomaly or instead reflects the normal spawning pattern remains unknown. For SRV clam culture to be a successful addition to hard clam operations, it is necessary to answer this question to determine when, how often, and how many SRV clams need to be collected to achieve successful spawning in a hatchery.

Successful aquaculture relies on use of cultured animals and heritable traits, such as growth, meat quality, and age at sexual maturation, are also economically important (Trygve 1983). Natural ("wild") stock has traditionally been more difficult to spawn as compared to cultured organisms due to the variability of conditioning. SRV stock collected from natural populations also tend to be larger and more difficult to condition (Scarpa et al. 2011). In order to move the industry further, it was realized that multi-parental crosses were needed not only to ensure genetic variation but to develop initial founder broodstock lines for the commercial hatchery

sector to use in their genetic selection program. To that end, 1st and 2nd generation SRV stocks have been developed from three geographical lines (Alligator Harbor, Seahorse Key, and Anna Maria Island) (Scarpa et al. 2011, Scarpa and Sturmer 2012). A DVD was produced that reviewed what had been noted as similarities and differences between SRV and hard clams with regards to broodstock maintenance and spawning. However, it is realized that information is still needed to ensure reliable and year-round spawning of SRV broodstock as hatchery personnel have had varying success with spawning attempts. It has been reported that males and females are often not ripe at the same time and therefore do not undergo synchronous spawning. Additionally, it has been difficult to produce spawns outside the "normal" spawning season that occurs in wild populations.

It is often assumed that stocks acclimated to culture conditions typically perform better (faster growth, faster reproduction) than wild stocks under culture conditions. It is thought, but has not been thoroughly investigated, that gametogenesis and therefore spawning success, may vary between cultured and wild SRV clams. As part of this FL ARC funded project, we compared three geographical lines of cultured stock, along with assemblages of wild stock, histologically to gauge any similarities and differences between these populations. Histological indicators were correlated with continuously collected environmental conditions to identify effects of water temperature, salinity, dissolved oxygen (DO), and turbidity on gametogenesis, in order to lead to an understanding of conditioning requirements necessary to achieve spawning success in hatchery operations.

The specific objectives of this project were to:

1. Determine when spawning occurs in natural and cultured assemblages,
2. Determine the sex ratio of natural and cultured assemblages,
3. Compare differences in spawning and sex ratios of cultured SRV clams from three distinct geographical lines, and
4. Photographically document broodstock gonads and gametes.

MATERIALS AND METHODS

Study Area and Sampling

Samples of cultured *M. nimbosa* in this study were collected monthly from August 2015 to July 2016 and wild *M. nimbosa* were collected from December 2015 to March 2016 (Tables 1 and 2). Wild sunray venus clams (n = 108) were collected from offshore of Seahorse Key in the Gulf of Mexico, while three lines of cultured cohorts (n = 575) were collected from populations held in submerged cages located at the University of Florida experimental lease within the Dog Island Aquaculture Use Zone nearby Cedar Key (Levy County) (Figure 1). Cultured sunray venus clams were 1st and 2nd generation clams that originated from 2012 spawns from wild stock collected from Anna Maria Island (AMI), Seahorse Key (SHK), or mixed parentage from wild SHK stocks crossed with Alligator Harbor cultured stock that had been bred from wild stock in 2006 (F2).

After collection, clams were shipped to HBOI-FAU overnight for subsequent processing. Sunray venus clams were weighed (g) and measured (shell length, height, width; mm). Clams were opened, tissue removed, and soft tissues and empty shell weighed and recorded. A section

of the gonad was first removed for histological processing and the remaining gonadal visceral mass was collected for fatty acid analysis.

Environmental Parameters

Temperature, salinity, turbidity, and dissolved oxygen measurements were continuously measured (30 minute 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 a YSI 6600 multi-parameter sonde.

Histology and Reproductive Staging

A 5-10 mm cross section of the sunray venus 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 a HM microtome, maintaining a minimum separation of 60 μm (the approximate maximum diameter of an oocyte) between sections. Sections were stained with Mayer's hematoxylin and eosin (Luna 1968), mounted on pre-labeled glass slides, and examined at 100-400x with a compound microscope.

Sex was determined and sunray venus clams categorized into one of six qualitative reproductive stages adopted by Walker and Power (2004); that is, inactive (0), early development (3), late development (4), ripe (5), early post spawning (2), and late post spawning (1). When two or more stages occurred simultaneously in a single section, classification was based upon the condition of the majority of follicles present in the section. A 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).

Photo Documentation

Three digital photos were taken of each clam from August to March. Following weights and measurements, each clam was opened with a clam knife and a photo of the intact clam was taken. As clams were processed histologically, a clam cross section was also taken in order that the condition of the gonad could be more clearly seen. A gonadal smear was taken from each clam, placed on a slide, and photographed with a camera mounted on a compound microscope. Two sets of complete photographs of each of the six reproductive stages for both males and females were chosen for inclusion in the guide.

RESULTS and DISCUSSION

Size

Wild versus Cultured Stocks

Sunray venus clams collected from natural populations (wild) off Seahorse Key were significantly larger than cultured cohorts collected at the UF lease (P>0.0001) with regards to all measurements (Table 1). Cultured clams were approximately three years in age, while age of collected wild clams was unknown. The size of wild clams was more variable than that of

cultured clams and little variation was seen from month to month, while cultured clams showed increases in growth over the four-month collection period.

Cultured Lines

Size of the cultured lines increased over time (Table 2). The F2 hybrid cultured line was significantly larger than the SHK cultured line with regards to weight, height and length, but not width. The AMI cultured line was not significantly different from either the F2 or the SHK cultured line.

Environmental Parameters

Mean monthly water quality parameters with minimum and maximum values recorded at the Dog Island sample location are shown in Table 3. Water temperature and salinity for the Dog Island sample location are also shown in Figure 2 and turbidity is also shown in Figure 3. Monthly dissolved oxygen was within acceptable parameters for shellfish survival and growth. Salinity was relatively constant and monthly averages ranged from 20.89 to 25.75.

Temperature showed seasonal variation with lowest temperatures in January and February and highest temperatures from June to August. Turbidity is thought to be a proxy for the determination of food (plankton) in the water. Plankton appeared to be highest during the fall and spring and lowest during the winter and summer.

Histology

Wild versus Cultured Stocks

Sex Ratio of Wild and Cultured Stocks

For wild collected clams the average monthly sex ratio was close to the expected 50:50 sex ratio (Figure 4). For cultured lines the ratio was close to the expected 50:50 or skewed towards females. It has been suggested that asynchronous spawning events might be explained by an excess of males.

Results of this study show that as long as adequate numbers (n = 23-48) are used in spawning attempts problems with spawning do not appear to be due to an overabundance of males in populations. The sex of one of the collected culture clams could not be determined in the month of February. In February (wild) and March (cultured) hermaphrodites were found. This points to the suggestion that sunray venus clams are protandric, similar to other clams, and that the size of clams collected in this study were adequate to ensure similar numbers of males and females.

Histological Comparison of Wild and Cultured Stocks

Results of this study show that the gametogenic cycle of wild and cultured clams collected at the same time of the year from a similar geographic area are comparable (Figures 4 and 5). This study depicts slight differences between males and females. In December, females are in the process of spawning, while males appeared to have spawned earlier as the majority of males were in development stages. The majority of males and females were ripe in the winter, with females showing evidence of early stages of spawning in March. Haines (1976) considered sunray venus clams “fall spawners” based on histological evidence of wild clams conducted at that time. More recent studies (Scarpa et al. 2008) and anecdotal evidence from hatchery managers have suggested that these clams are ripe and can be induced to spawn during the

winter. Histological evidence in this study supports those claims. The difference in timing of spawning between those studies and reports is likely a function of temperature and food availability. The availability of food in October, as evidenced by increased turbidity (Table 3, Figure 3) likely resulted in clams ripening in the fall and subsequently spawning when the temperature decreased. There appears to be more variability in cultured clams with respect to gametogenic stage. This might be explained by variability between the three lines of cultured clams as these graphs depict the average stage of all three lines and variability (see section below) was apparent in some months between the three cultured lines.

Figure 6 represents the typical way of describing gametogenesis in which the stage reported represents the stage that the majority of follicles within each individual are in. In northern bivalve species, such as the hard clam *Mercenaria mercenaria*, most follicles are in the same stage as reproduction occurs in response to changes in temperature. Reproduction in tropical species, such as the sunray venus clam, is less dependent on temperature cues and occurs in response to other environmental stimuli, such the availability of food. Therefore, reproduction can occur year round, but rather than releasing all gametes at once, eggs and sperm are released asynchronously, resulting in describing these species as "dribble spawners". Figure 5 more adequately represents the various stages seen within each individual and is based on the methodology used by Haines (1976) to describe this phenomenon. Although the trends are similar, reporting the gametogenic stage based on the stage of the majority of follicles can be misleading.

Cultured Lines

Sex Ratio of Cultured Lines

For the three cultured lines of sunray venus clams, the male to female sex ratio was close to the expected 50:50 ratio or was skewed towards females (Figure 7). Clams from the F2 line tended to exhibit the expected 50:50 ratio in the majority of months, while the AMI and SHK lines appeared to consist of more females than males. This skew towards females is not explained by size as clams from the F2 line were larger (Table 2). The results seen are likely due to the small sample size (n =15) collected monthly from each line. Five clams during the course of this study were in a completely inactive reproductive state and sex could not be determined: two from the SHK line in September, one from SHK line in February, and two from the F2 line in March. One hermaphrodite was detected in the AMI line in March.

Histological Comparisons of Cultured Lines

The annual gametogenic cycle of the three cultured lines is shown in Figures 8-11 and reflects the variability in the various follicular stages using the methodology of Haines (1976). Figure 8 is an average of the gametogenic cycle of all three lines. A clear difference is seen with regards to male and female reproductive patterns. Although both males and females show the greatest period of activity from late summer to early fall, males tend to become ripe earlier. With the exception of April, males are in a ripe or spawning stage from December to July. Females have a much longer period (August-November) in which follicles are inactive or they are spawned out (LPS stage). Females tend to be ripe predominately during winter (January-March), with spawning occurring predominately from April to July.

A similar trend is seen in all three lines, with the AMI line (Figure 9) being most representative of the average, particularly with regards to female reproductive patterns. This is not particularly surprising as collected clams from both the AMI and SHK cultured lines tended to be predominately female in six of the 12 months.

Clams collected from the F2 line (Figure 10) were most likely to have an equal number of males and females. It is therefore not surprising that both the male and female reproductive patterns of this line are very similar to the average (Figure 8). The major differences between the three lines are seen in males in September and July. In both months, collections were skewed towards females for both the AMI and SHK lines, and in females in May, which is more difficult to explain as collections were skewed towards females in that month as well.

DISCUSSION AND CONCLUSIONS

It is interesting that Haines (1976) referred to sunray venus clams as fall spawners. The Haines study indicated that spawning activity for both sexes began in late summer and peaked in November and December, with male spawning activity beginning one month earlier and lasting one month longer. Haines did report an increase in both developmental and ripe stages from January to May. One of the differences between this study and the Haines study is sample size. The Haines sample size varied between 7 and 20 clams dependent on month. Sample size in this study was much more consistent from month to month. Haines collected clams in Alligator Harbor in the Panhandle while clams in this study were collected in the Cedar Key area. Temperature may have had more of an impact in the Haines study. In a related six-month study conducted with wild and cultured clams (F2 line) from November 2014 to April 2015, we noted some similarities as well as some differences with this current study. Clams were collected from the same area and sample size was similar with 30 wild and 30 cultured clams collected monthly. Unlike this study and similar to Haines, we saw spawning in males in November and December. Similar to this study, males were predominately ripe from January to April. Wild females showed similar reproductive patterns to males, but cultured females did not. Cultured females were in developmental stages in November, spawned in December and January, and were ripe from February to April. The main difference between the two years was temperature and turbidity. In 2014-2015, the temperature was cooler in the fall and plankton blooms, as evidenced by turbidity, did not occur in the fall as was noted in this study. This likely impacted the development of gametes and therefore timing of spawning. Egg production is known to be more affected by type and quantity of food than is sperm. Taken together, these three studies point to the importance of temperature and food in controlling the gametogenic cycle. Further research is needed in order to determine how these stimuli impact development of gametes, particularly eggs, in order to improve broodstock conditioning and to determine cues for synchronous gamete production and spawning, realizing that sunray venus clams may not rely on the same environmental cues as the hard clam.

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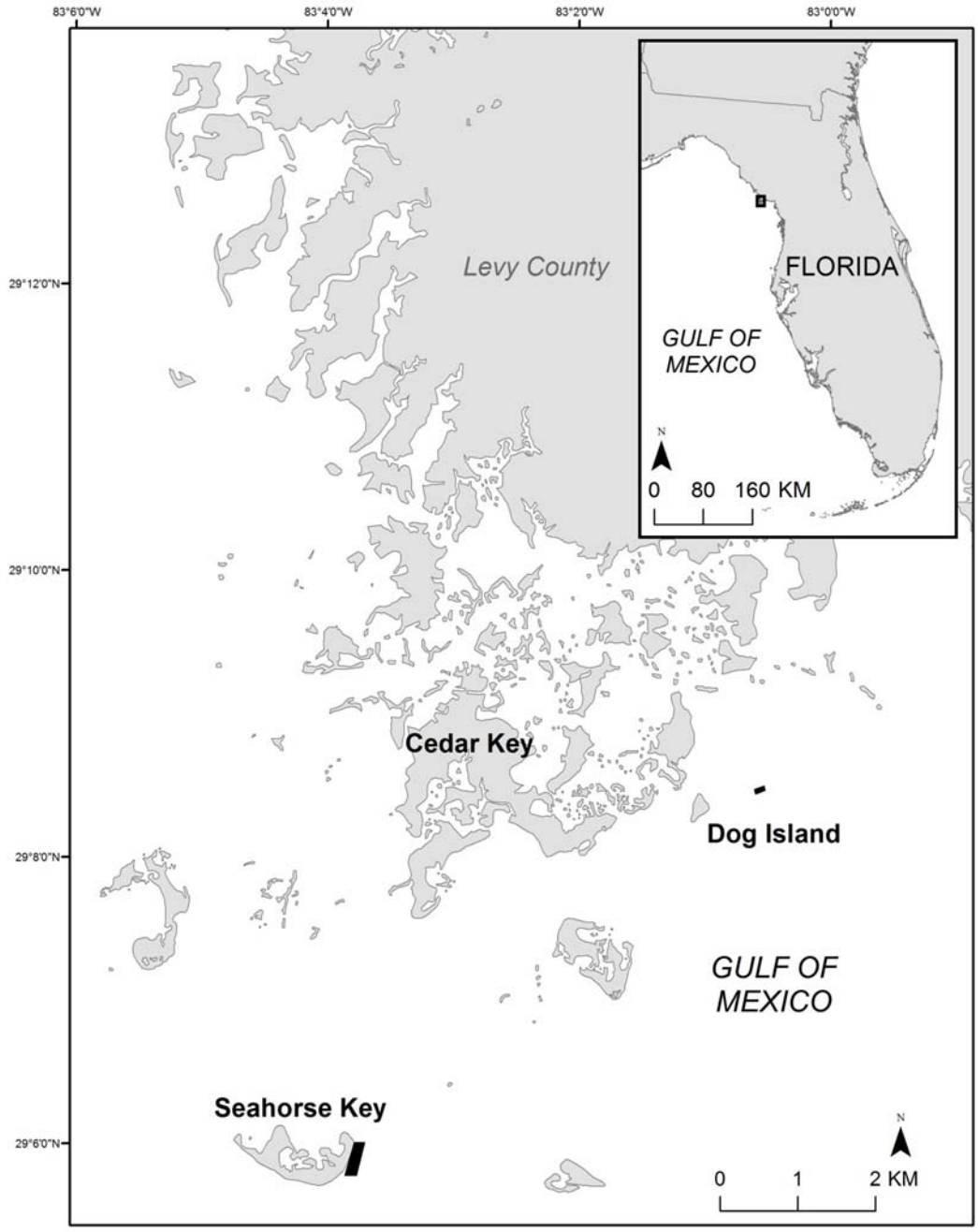

Figure 1. Map of locations in the Gulf of Mexico near Cedar Key, Florida where sunray venus clams were collected. Cultured sunray venus clams were collected from the University of Florida experimental lease adjacent to Dog Island; wild sunray venus clams were collected from natural populations off of Seahorse Key.

Table 1. Summary of shell measurements (width, length, height) and total weight (mean ± SD) for sunray venus clams collected from wild and cultured populations during November 2015 through March 2016.

Month	Wild sunray venus clam population					Average of cultured sunray venus clam population				
	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)
December	30	32.1 ± 2.8	114.4 ± 9.8	57.6 ± 4.6	133.6 ± 33.9	47	28.8 ± 0.8	74.9 ± 5.6	42.5 ± 3.3	58.9 ± 8.4
January	23	31.7 ± 1.8	112.6 ± 7.5	56.5 ± 3.3	125.3 ± 21.2	48	29.3 ± 1.32	74.5 ± 6.4	43.9 ± 2.9	61.5 ± 8.7
February	23	32.9 ± 2.4	115.9 ± 9.9	58.1 ± 4.6	138.8 ± 31.4	48	29.5 ± 0.7	76.2 ± 2.9	43.5 ± 1.6	62.8 ± 3.9
March	32	32.1 ± 1.7	114.4 ± 6.7	57.1 ± 3.2	131.1 ± 20.7	48	29.6 ± 1.1	76.5 ± 2.9	43.5 ± 1.9	63.1 ± 5.7

Table 2. Summary of shell measurements (width, length, height) and total weight (mean ± SD) of cultured sunray venus clams populations August 2015 through July 2016.

Month	Cultured Anna Maria Island clam population					Cultured F2 clam population					Cultured Seahorse Key clam population				
	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)
Aug	16	28.1 ± 2.0	70.6 ± 5.5	40.0 ± 2.7	50.0 ± 7.5	16	27.9 ± 1.3	77.8 ± 3.6	44.6 ± 1.8	61.0 ± 6.8	16	27.4 ± 0.9	70.2 ± 3.2	40.3 ± 2.7	49.2 ± 5.9
Sep	16	28.2 ± 1.5	71.3 ± 3.6	40.5 ± 1.9	51.1 ± 6.6	16	28.3 ± 1.8	76.7 ± 3.8	43.5 ± 2.0	59.2 ± 8.1	16	27.5 ± 2.7	69.3 ± 8.5	38.7 ± 3.9	47.3 ± 12.9
Oct	16	29.5 ± 1.0	74.9 ± 2.7	42.3 ± 1.9	59.2 ± 5.2	16	27.9 ± 1.1	78.5 ± 4.0	44.7 ± 2.2	63.5 ± 7.5	16	27.8 ± 2.1	69.4 ± 6.8	39.1 ± 3.3	49.6 ± 10.6
Nov	16	29.5 ± 1.5	74.6 ± 4.6	42.3 ± 2.7	60.0 ± 8.5	16	28.9 ± 1.4	78.7 ± 3.2	45.1 ± 2.2	65.9 ± 8.1	16	28.9 ± 1.6	73.0 ± 3.5	41.9 ± 1.8	55.5 ± 6.1
Dec	16	29.6 ± 1.3	76.4 ± 3.1	43.4 ± 2.4	61.7 ± 6.1	16	28.8 ± 1.2	79.6 ± 3.5	45.3 ± 2.1	65.7 ± 6.1	15	28.0 ± 2.5	68.7 ± 7.9	38.9 ± 4.1	49.5 ± 12.9
Jan	16	30.7 ± 1.0	77.6 ± 4.0	43.9 ± 3.0	65.8 ± 8.4	16	29.0 ± 1.4	78.8 ± 3.1	46.8 ± 2.2	67.3 ± 7.7	16	28.1 ± 2.4	67.2 ± 9.3	41.0 ± 4.5	51.5 ± 14.2
Feb	16	29.4 ± 1.3	73.7 ± 4.9	41.9 ± 2.6	58.5 ± 7.1	16	28.9 ± 1.0	79.3 ± 5.1	45.0 ± 2.4	66.2 ± 7.2	16	30.2 ± 1.1	75.5 ± 2.1	43.6 ± 1.3	63.7 ± 4.2
Mar	16	30.9 ± 1.0	76.8 ± 3.2	43.7 ± 1.4	65.9 ± 5.9	16	29.2 ± 1.5	79.2 ± 3.5	45.3 ± 2.0	66.9 ± 8.8	16	28.8 ± 1.7	73.4 ± 5.7	41.5 ± 2.5	56.5 ± 8.0
Apr	16	30.6 ± 1.4	77.0 ± 3.0	44.0 ± 1.5	66.4 ± 6.4	16	29.4 ± 0.9	80.0 ± 3.3	45.6 ± 2.4	68.7 ± 6.2	16	29.4 ± 1.7	74.0 ± 4.5	41.6 ± 2.5	58.6 ± 6.7
May	16	30.6 ± 1.3	77.4 ± 3.1	45.7 ± 1.8	67.7 ± 6.4	16	28.2 ± 0.8	79.1 ± 3.6	46.8 ± 2.1	65.4 ± 6.1	16	29.8 ± 1.3	75.9 ± 2.5	44.8 ± 2.2	63.6 ± 6.4
June	16	31.0 ± 1.6	77.8 ± 3.3	44.3 ± 2.4	68.0 ± 8.5	16	29.7 ± 2.4	81.5 ± 4.9	47.0 ± 2.8	72.1 ± 13.1	16	29.3 ± 2.1	73.1 ± 6.6	41.8 ± 3.3	57.4 ± 10.8
July	16	30.3 ± 1.5	77.2 ± 2.8	45.8 ± 2.2	65.4 ± 6.2	16	29.1 ± 1.2	80.4 ± 3.7	46.1 ± 2.3	67.2 ± 7.3	16	29.2 ± 1.9	74.7 ± 6.6	43.2 ± 3.4	58.7 ± 9.4

Table 3. Monthly averages ± standard deviations (min-max Values) at the Dog Island Lease Area, Cedar Key Water Quality Monitoring Station.

	Temperature (°F)	Salinity (ppt)	Dissolved Oxygen (mg/l)	Turbidity (NTU)
Aug-15	86.06 ± 2.59 (79.4 - 93.7)	22.72 ± 1.81 (17.21 - 27.04)	6.47 ± 1.37 (2.39 - 11.81)	37.7 ± 28.6 (6.2 - 259.1)
Sep-15	82.34 ± 2.33 (77.5 - 89)	20.89 ± 2.23 (15.55 - 27.4)	6.59 ± 0.98 (3.82 - 10.74)	22.55 ± 19.65 (4 - 133.8)
Oct-15	75.98 ± 3.13 (65.91 - 84.4)	25.42 ± 1.03 (21.91 - 27.31)	7.19 ± 0.78 (5.47 - 11)	123.51 ± 246.99 (2.5 - 1252)
Nov-15	72.91 ± 6.42 (55.53 - 84.4)	25.75 ± 1.33 (21.85 - 28.48)	7.05 ± 1.08 (4.46 - 10.2)	63.04 ± 97.98 (5.8 - 1230)
Dec-15	67.78 ± 4.08 (53.87 - 76.1)	24.77 ± 1.56 (21.76 - 28.96)	7.58 ± 0.85 (5.55 - 10.17)	73.08 ± 272.75 (2.2 - 2248)
Jan-16	56.59 ± 5.3 (41.88 - 75.3)	23.89 ± 2.61 (18.07 - 27.99)	8.83 ± 0.96 (5.4 - 11.52)	35.96 ± 120.4 (2 - 2234)
Feb-16	58.79 ± 4.41 (48.06 - 68.46)	23.56 ± 1.42 (18.19 - 26.36)	9.43 ± 1.11 (6.18 - 12.87)	37.37 ± 42.49 (1.8 - 384.6)
Mar-16	69.03 ± 4 (59.3 - 76)	24.23 ± 1.21 (20.78 - 27.04)	7.58 ± 0.77 (5.63 - 10.04)	41.23 ± 40.55 (-3 - 365.8)
Apr-16	73.89 ± 3.87 (63.32 - 84.8)	24.48 ± 2.37 (19.17 - 29.12)	7 ± 0.98 (4.64 - 9.7)	113.83 ± 187.01 (9.1 - 1397)
May-16	81.58 ± 5.46 (67.96 - 92.3)	23.17 ± 2.91 (18.08 - 29.93)	6.18 ± 1.4 (2.91 - 11.49)	46.35 ± 44.02 (4.6 - 528.6)
Jun-16	84.97 ± 2.11 (80 - 91.4)	24 ± 2.75 (18.54 - 28.68)	5.81 ± 1.32 (2.03 - 9.84)	51.97 ± 37.45 (6.2 - 242.7)

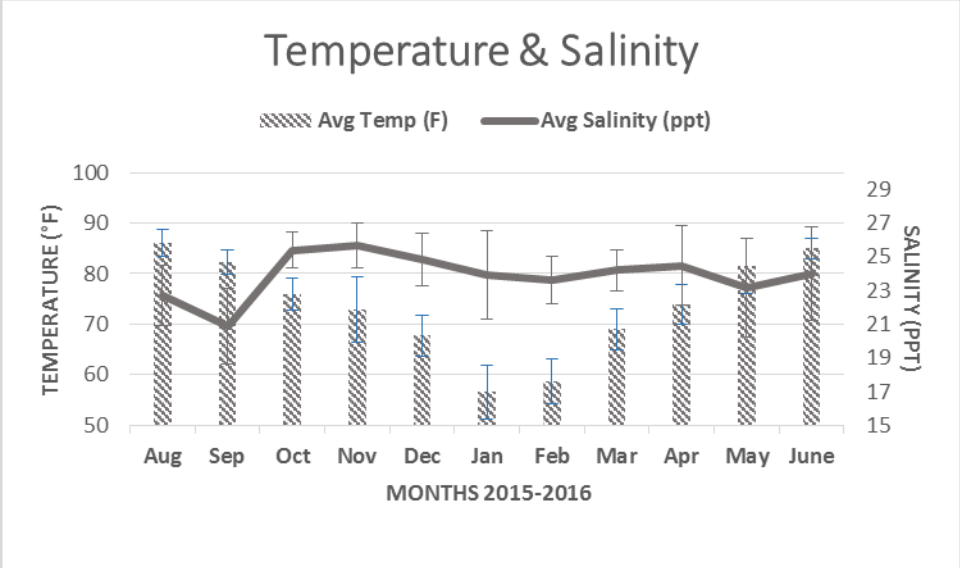

Figure 2. Temperature and salinity monthly averages (± standard deviations) at the Dog Island Lease Area, Cedar Key Water Quality Monitoring Station.

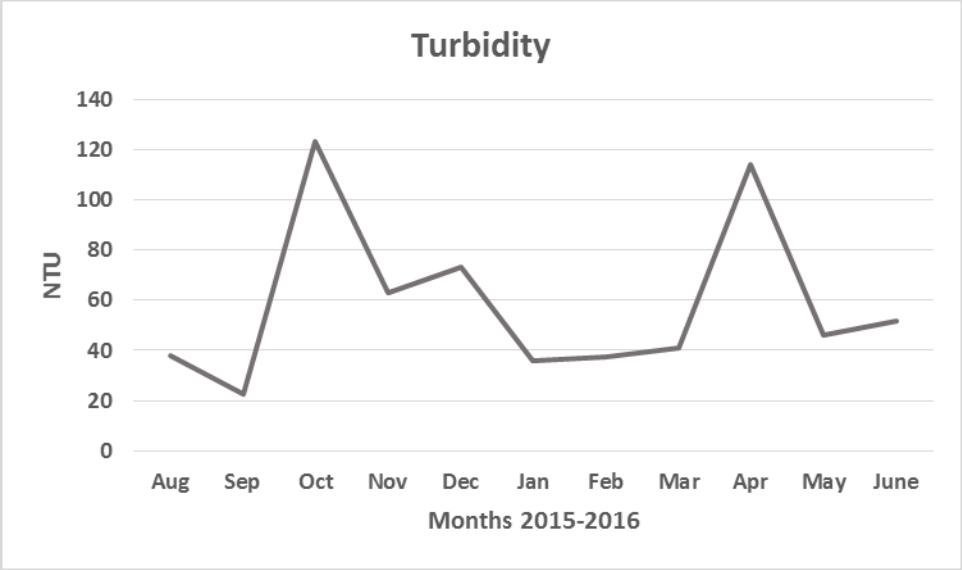

Figure 3. Monthly turbidity averages (± standard deviations) at the Dog Island Lease Area, Cedar Key Water Quality Monitoring Station.

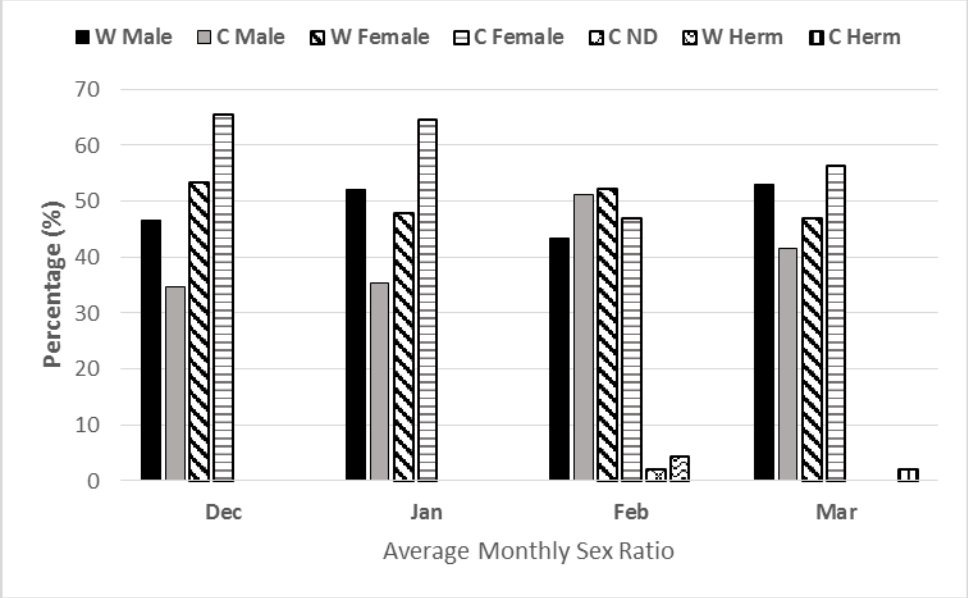

Figure 4. Sex ratios of wild (n= 23-32) and cultured (n=47-48) adult broodstock.

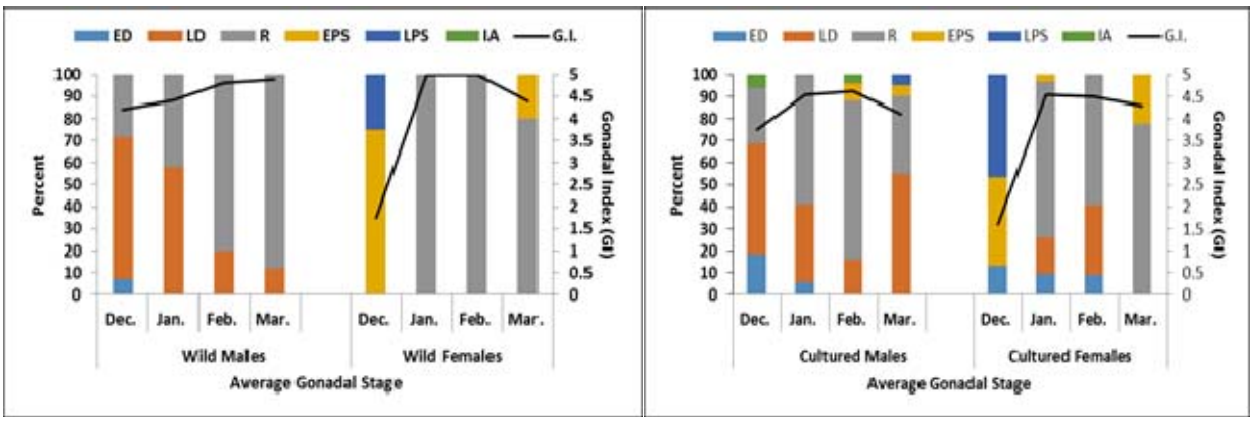

Figure 5. Percentage of natural (wild) and cultured sunray venus clams collected December 2015 to April 2016 at each of the gonadal stages of development. Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

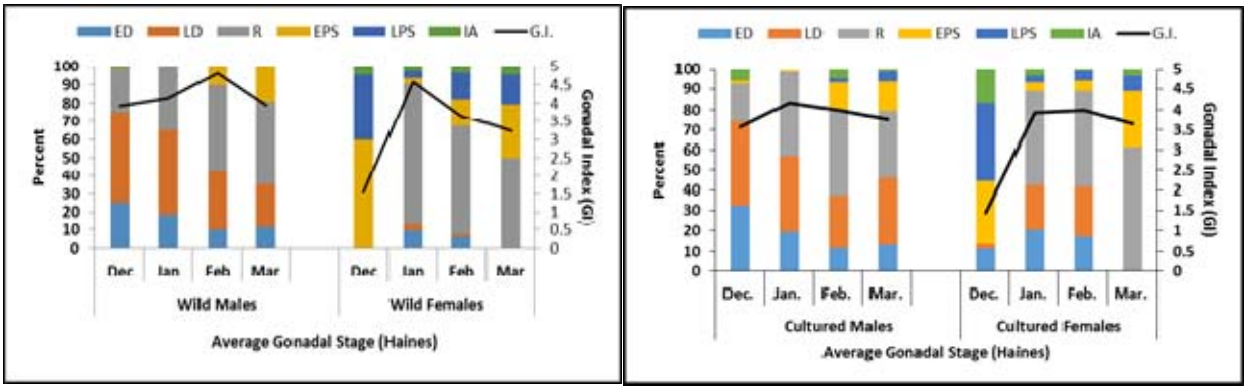

Figure 6. Percentage of natural (wild) and cultured sunray venus clams collected December 2015 to April 2016 at each of the gonadal stages using the methodology of Haines (1976). Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

Figure 7. Sex ratios of cultured (n=47-48) adult sunray venus clams. A=Anna Maria Island, F=F2 and S=Seahorse Key.

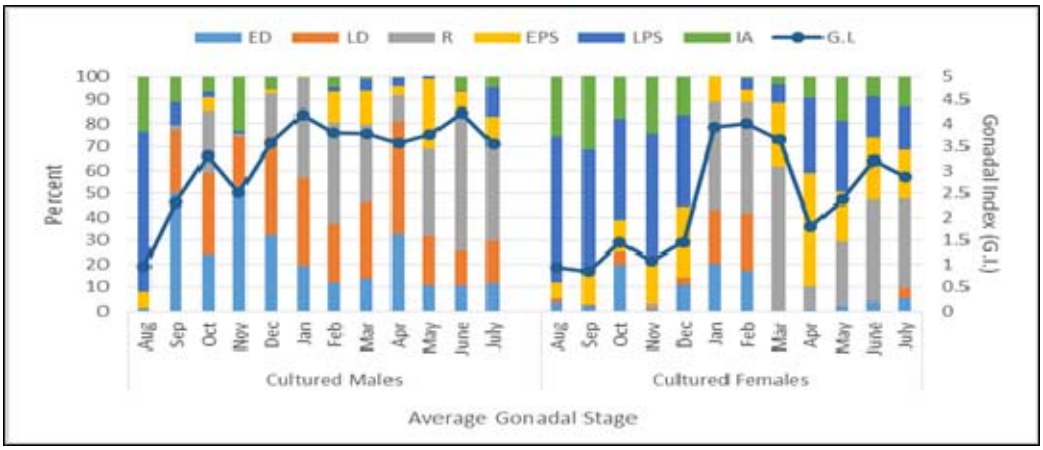

Figure 8. Percentage of cultured sunray venus clams from all lines collected August 2015 to July 2016 at each of the gonadal stages. Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

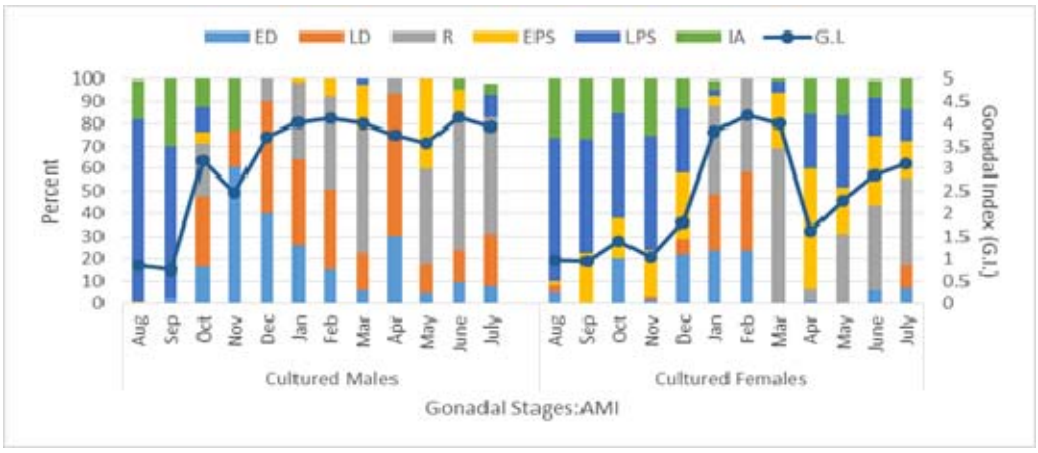

Figure 9. Percentage of cultured sunray venus clams from the Anna Maria Island line collected August 2015 to July 2016 at each of the gonadal stages. Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

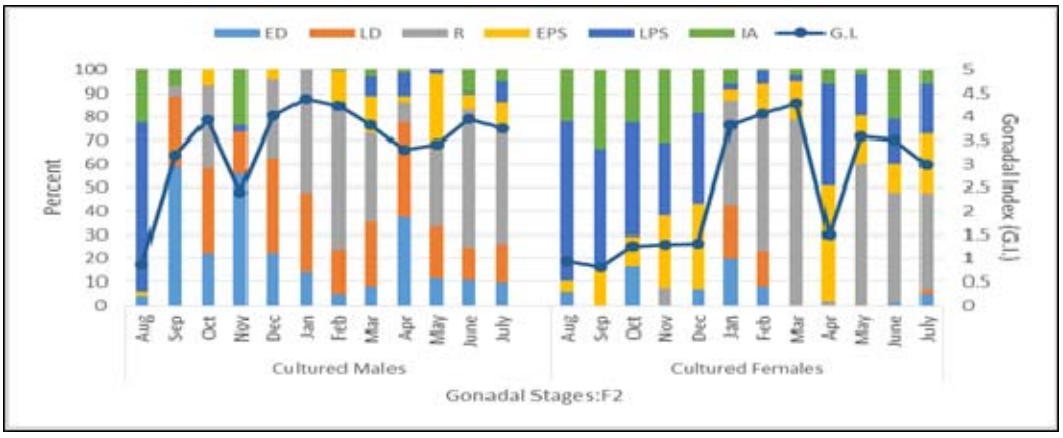

Figure 10. Percentage of cultured sunray venus clams from the F2 line collected August 2015 to July 2016 at each of the gonadal stages. Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

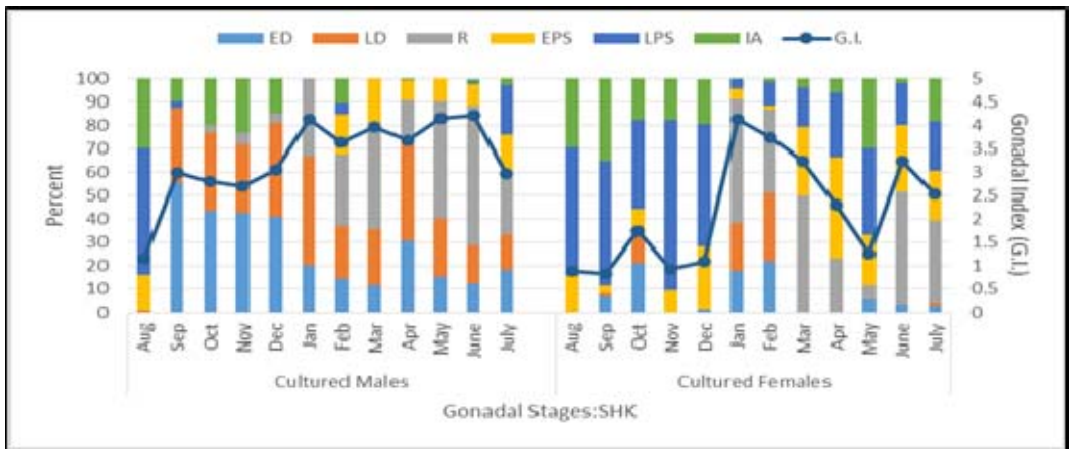

Figure 11. Percentage of cultured sunray venus clams from the Seahorse Key line collected August 2015 to July 2016 at each of the gonadal stages. Monthly gonadal index (G.I.) values were determined by averaging the number of clams in each category (ED=3, LD=4, R=5, EPS=2, LPS=1, IA=0).

APPENDIX 1:

WORKSHOP

A typical workshop was not conducted for this project. A workshop was initially planned for late summer or early fall of 2016 as part of an annual clam workshop typically held at Cedar Key and last held at FAU-HBOI in March of 2015. Due to hurricane Hermine in Cedar Key in September and illness and medical emergencies from August until the present time (Sturmer) the planned clam workshop has been put on hold until sometime in 2017.

However the results of this study, along with the results of the SLP funded study were presented to the general public on November 2, 2016 as part of the Ocean Science Lecture Series. Approximately 80 people attended the lecture, including IRSC aquaculture students, east coast clam farmers, FWC personnel, FAU-HBOI scientists and staff (including aquaculture staff). The presentation was one of three 15 minute presentations given that night, with 10 minutes for questions following each presentation. Five people, including 2 clam farmers, asked questions. A reception with snacks (cookies, water, soda, tea) followed the hour long lecture series in which the three speakers (including Laramore) mingled with interested attendees and answered questions on a one on one basis. During the reception two more inquiries were made concerning this research topic, one from a clam farmer and one from the FAU foundation.

APPENDIX 2:

PHOTODOCUMENTATION/Pictorial Guide

SRV Male and Female Development (Separate PDF file)