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FATTY ACID COMPOSITION OF ADULT AND LARVAL SUNRAY VENUS CLAMS *MACROCALLISTA NIMBOSA*: ENVIRONMENTAL AND GAMETOGENIC IMPACTS

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ABSTRACT The sunray venus clam has been advanced as an alternative species to hard clams for aquaculture in Florida. Although interest and market potential are high, hatchery operators continue to experience problems with consistent year-round spawns. Gametogenesis in other species has been linked to changes in fatty acid (FA) profiles, which are in turn affected by temperature and diet. Knowledge of specific FAs that are important for the development of high-quality gametes has implications in determining optimal conditions for maturation systems. This study compared the FA profile of sunray venus clams (wild, cultured) collected from two locations during the natural fall spawning cycle to (1) determine which FAs are associated with gametogenesis and (2) assess the impact of exogenous influences. The FA profile of developing clam larvae was likewise examined to offer additional insight into endogenous and exogenous influences. Males and wild females exhibited fall spawning peaks, with the majority in gametogenesis in the spring. Males had a second spawning peak in February, and cultured females spawned continuously throughout the 6-mo study period. The FA profile appeared to be less associated with gametogenesis than with sex or exogenous influences. Decreases in n-6 PUFAs (inverse relationship) and increases in n-3 PUFAs (positive relationship) were associated with gametogenesis in males and wild females, but not cultured females, perhaps because of the protracted spawning period. Differences were noted between the sexes, regardless of month or location, with females having a higher amount of total FAs, MUFAs (16:1 n-7), and SFAs (16:0 and 14:0), but lower n-3 PUFAs (22:6 n-3). There was little difference in the FA profile of clams collected at the two locations, although the wild population tended towards higher total FAs and n-3 PUFAs. Diet (chlorophyll a concentration and turbidity) appeared to have a stronger link with changes in FA profile and gametogenesis than temperature. Discernable differences were observed in the FA composition (n-3 and n-6 PUFAs) of larvae and 5-day postset that reflected the impact of both endogenous and exogenous (diet) influences. These results indicate that algal species with select FA profiles may increase maturation, particularly in females, leading to more consistent spawns.

KEY WORDS: sunray venus clam, *Macrocallista nimbosa*, reproductive pattern, fatty acid composition

INTRODUCTION

The sunray venus clam *Macrocallista nimbosa* (Lightfoot, 1786), is an attractive venerid clam, distributed from North Carolina to Florida and the Gulf of Mexico in shallow waters with sandy substrates (Abbott 1974). Although adult sunray venus clams can attain 10–12.5 cm in length and have an excellent meat flavor, a commercial fishery was not established until the 1960s, when a large population of clams was found near Alligator Harbor, FL (Akin & Humm 1960). From 1967 to 1972, two million pounds of clams were landed in Saint Joseph Bay with a value estimated at $0.25 million (Godcharles & Jaap 1973). Processing plants were established in the area where the clam meats were shucked and stripped for the fried clam market (Stokes et al. 1968). Because of the potential economic value of the sunray venus, fishery surveys were conducted in the early 1970s to locate new clam beds of possible commercial significance along Florida's northwest coast (Jolley 1972); however, insufficient natural stocks limited the development of the fishery (Ritchie 1977).

Limited information pertaining to the life history of sunray venus clams existed at that time (e.g., Futch 1967, Cake 1970). Growth experiments conducted with marked individuals in the wild indicated that these clams could attain a length of 7.5 cm (40 g) in 12 mo (Stokes et al. 1968). Haines (1975, 1976) performed the most comprehensive study for culture and demonstrated that sunray venus clams were found to be ripe year-round, but peak spawning occurred in the fall (Haines 1976).

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 & 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, 2014). 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 (e.g., fuel), growers recognized that species diversification could increase economic stability, and alternative bivalve species were explored. 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 existence of a latent market and the demonstrated rapid growth rate of the sunray venus clam, along with it being a native species, made it a logical choice as a candidate culture species.

To advance sunray venus clam culture, multiparental crosses were produced to ensure genetic variation and develop initial founder broodstock lines for the commercial hatchery sector to use in their genetic selection program (J. Scarpa, TX A&M University-Corpus Christi and L. Sturmer, University of Florida,

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unpublished data). Hatchery operators reported that males and females were often not ripe at the same time and did not undergo synchronous spawning when attempted outside the presumptive natural cycle. Haines (1975) attempts at induced spawning in the laboratory were only successful with adults collected in October and November in northwest Florida during the peak natural spawning season.

Thus far, only one study has been conducted that examined the reproductive cycle of the sunray venus clam. Although Haines (1976) measured the glycogen content of the sunray venus clam during gametogenesis, no other physiochemical changes were examined nor were environmental influences explored. The FA composition of gonads is altered in response to diet and physiochemical characteristics associated with location (De Moreno et al. 1980, Langdon & Waldock, 1981, Fernández-Reiriz et al. 1996, Pazos et al. 1996, Fernández-Reiriz et al. 1998, Soudant et al. 1999, Ojea et al. 2004, Dridi et al. 2007, Narváez et al. 2008). Total FAs and FA composition have been shown to follow a seasonal cycle related to the gametogenic cycle in bivalves (DeMoreno et al. 1980, Pazos et al. 1996, Soudant et al. 1999, Pazos et al. 2003, Ojea et al. 2004, Palacios et al. 2005, Dridi et al. 2007, Narváez et al. 2008), and polyunsaturated fatty acids (PUFAs) levels in particular have been shown to be associated with chlorophyll *a* in the environment (Ojea et al. 2004, Dridi et al. 2007, Narváez et al. 2008). The aims of this study were to (1) compare the FA profile of two populations of sunray venus clams (cultured and wild) during a 6-mo period that encompassed the fall spawning period; (2) compare the FA profile of cultured sunray venus larvae and postset; and (3) determine the relationship between FA profile, temperature, and diet. Knowledge gained may assist in optimizing conditioning parameters (temperature and diet) to achieve consistent year-round spawning of the sunray venus clam.

MATERIALS AND METHODS

Sunray Venus Clam Adults: Reproductive Pattern and Fatty Acid Profile

Study Area and Sampling

Samples of wild and cultured *Macrocallista nimbosa* populations were collected monthly from November 2014 through April 2015 (Table 1). Wild sunray venus clams ($n = 158$) were collected offshore of Seahorse Key in the Gulf of Mexico, whereas cultured cohorts ($n = 187$) were collected from populations held in submerged cages located at the University of Florida experimental lease within the Dog Island Aquaculture Use Zone near Cedar Key (Levy County) (Fig. 1). Cultured sunray venus clams originated from a February 2012 spawn using wild stocks collected from Seahorse Key and cultured clams originating in Alligator Harbor (Franklin County).

After collection, clams were shipped to Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU) overnight for subsequent processing. Sunray venus clams were weighed (gram) and measured (shell length, height, width; millimeter). Clams were opened, tissues removed, and soft tissues and empty shell weighed and recorded. A gonadal cross section was taken for histological processing and the remaining gonadal visceral mass collected and frozen for FA analysis.

Environmental Parameters

Temperature, salinity, turbidity, and dissolved oxygen measurements were continuously measured (30 min intervals) from November 2014 to April 2015 at a monitoring station located within the Dog Island Aquaculture Use Zone. The real time station consisted of a YSI 6600 multiparameter system. Water samples were collected at both Seahorse Key and Dog Island during monthly clam collections; chlorophyll *a* and pheophytin *a* concentrations were determined by ethanol extraction and spectrophotometric analysis (Sartory & Grobbelar 1984).

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 h 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 1 h each, followed by clearing with toluene and paraffin embedding (Howard et al. 2004).

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 2014 through April 2015.

	Wild sunray venus clam population					Cultured sunray venus clam population				
Month	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)	Number (*n*)	Width (mm)	Length (mm)	Height (mm)	Weight (g)
November	31	29.0 ± 3.8	104.3 ± 13.3	53.2 ± 6.9	104.9 ± 40.8	31	26.2 ± 0.75	73.3 ± 2.8	41.5 ± 1.7	52.2 ± 4.5
December	36	29.7 ± 3.0	104.9 ± 9.2	53.2 ± 4.3	105.1 ± 30.9	32	26.7 ± 1.1	74.5 ± 3.5	41.8 ± 2.2	53.7 ± 6.1
January	33	30.3 ± 3.4	106.8 ± 9.9	54.5 ± 5.3	111.5 ± 30.8	30	27.2 ± 1.9	74.9 ± 3.1	42.4 ± 1.5	53.8 ± 5.0
February	5	28.7 ± 2.5	100.5 ± 10.4	52.2 ± 5.6	97.2 ± 29.6	32	27.2 ± 1.2	75.5 ± 2.8	43.0 ± 1.4	57.4 ± 5.5
March	31	29.7 ± 3.2	106.6 ± 10.9	53.8 ± 5.4	108.3 ± 28.8	31	27.1 ± 1.0	74.7 ± 2.4	43.5 ± 1.9	55.7 ± 4.8
April	16	29.0 ± 3.7	104.3 ± 13.0	52.6 ± 6.4	104.5 ± 41.6	33	27.5 ± 0.8	75.7 ± 2.9	42.8 ± 1.6	56.2 ± 5.1

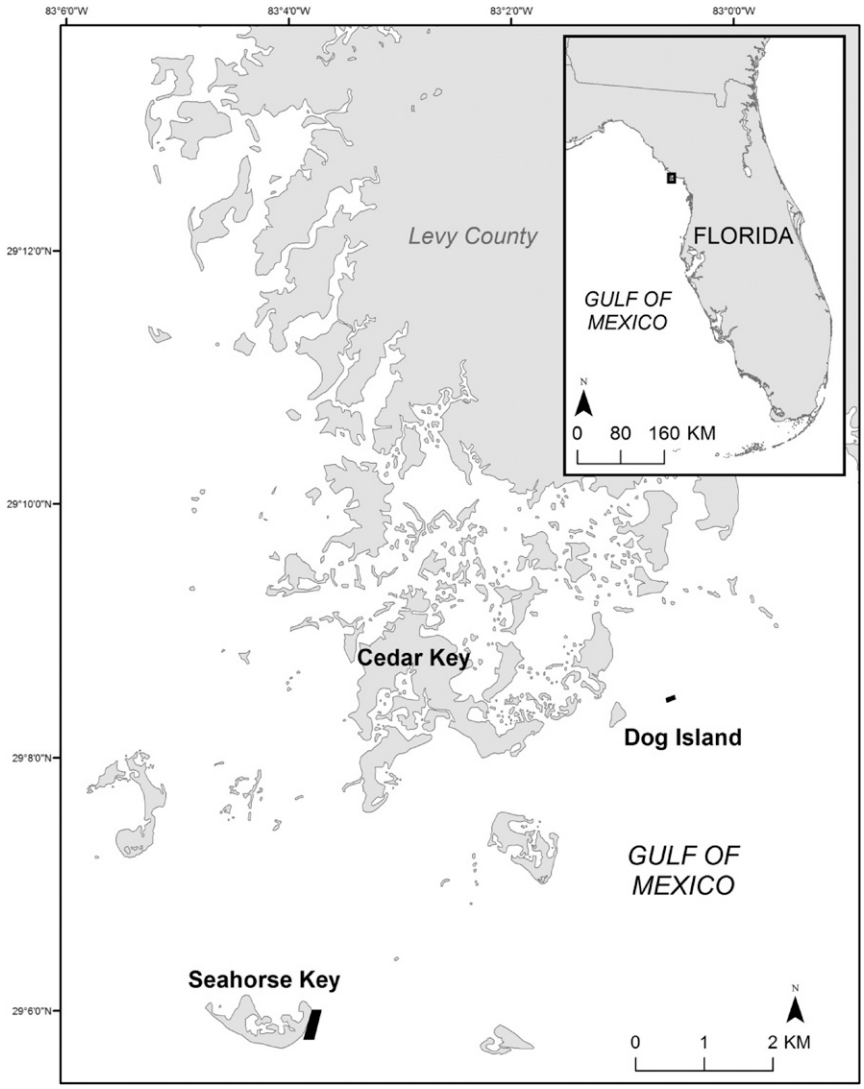

Figure 1. Map of locations in the Gulf of Mexico near Cedar Key, FL where sunray venus clams were collected. Cultured sunray venus clams were collected from the University of Florida experimental lease adjacent to Dog Island; wild sunray venus clams were collected from natural populations off of Seahorse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

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 prelabeled glass slides, and examined at 100–400× with a compound microscope.

After sex determination, sunray venus clams were categorized into one of six qualitative reproductive stages based on Hesselman et al. (1989), that is, resting/inactive (0), early development (3), late development (4), ripe (5), spawning/early post spawning (2), and late post spawning (1). The entire gonadal region of each individual was examined microscopically and the percentage of follicles in each reproductive stage in each field of view recorded. 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).

Fatty Acid and Statistical Analyses

After removal of a section of the gonad for histological evaluation, the remaining gonad was frozen for FA analysis. Once sex had been determined, gonadal samples were pooled into groups of five to eight clams and duplicate sample sets sent to University of Missouri's Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for crude fat (%) and FA profile analysis. An additional set of samples from November 2014 to January 2015 was sent to Microbial ID Inc. (Newark, DE) for FA determination. The FA profile in all instances was expressed as the percent of total fat.

A three-way analysis of variance (SAS 9.2, Cary, NC) using a nested linear model was conducted to determine if there were differences between male and female clams collected monthly from each location with respect to FA composition and gametogenic phase. Proportion data were arc-sine square root transformed before analysis, and data distribution was estimated before analysis. For biological measurements, two sample *t*-tests or Wilcoxon tests were used.

Sunray Venus Clam Larvae and Postset: Fatty Acid Profile

To determine the FA profile of larval and postset sunray venus clams, cultured clams were spawned on three occasions: February 19, March 18, and August 19, 2015. Larvae were reared through metamorphosis (set) following standard hard clam hatchery protocols. Larvae were fed *Isochrysis galbana,* Tahitian strain (Tiso). Algae were cultured following standard methods of algal production using f/2 media (Guillard & Ryther 1962). Before set (5–7 days), sunray venus clams were placed in downwellers and fed Tiso. Postset were fed a combination of Tiso and *Chaetoceros gracilis*.

Fatty Acid and Statistical Analyses

Larvae (seven samples) and postset (five samples) sunray venus clams were collected from each of the three spawns and the FA profile analyzed (Microbial ID, Inc.). An analysis of variance (SAS 9.2) was run to determine whether there were differences between the FA profiles of larvae and postset. Proportion data were arc-sine square root transformed before analysis, and data distribution was estimated before analysis.

RESULTS

Sunray Venus Clam Adults: Reproductive Pattern and Fatty Acid Profile

Size

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 regard to all measurements (Table 1). Shell length ($P \leq 0.0055$), height ($P \leq 0.0201$), and total weight ($P < 0.0394$) were significantly different between the two populations in all months sampled, whereas shell width was significantly different November to January and March ($P \leq 0.0003$). Shell length, height, and weight in both populations were highly correlated ($R = 0.94$–0.96). Width showed less correlation with other measurements ($R = 0.7589$–0.878); correlation was greatest for weight and least for length.

Environmental Parameters

Mean monthly water temperature and salinity for the Dog Island sample location are shown in Figure 2. Water temperatures exemplified a seasonal pattern, and monthly averages ranged from 13.8°C to 25.4°C. Salinity was relatively constant, and monthly averages ranged from 22.3‰ to 26.3‰. Lowest temperature and salinity were recorded in February.

Chlorophyll *a,* pheophytin *a* concentrations, and turbidity are shown in Figure 3. Chlorophyll *a* concentrations were low at both sites in December. Levels rose in January at Dog Island and remained high through April. Levels rose in February at Seahorse Key, peaked in March, and then dropped substantially in April. Turbidity at Dog Island was lowest in December and January and highest in March.

Histology

Sex Ratio

Of the 158 wild sunray venus clams analyzed histologically, 85 were male and 73 were female. The overall male:female sex ratio of 1.2:1 did not differ significantly from an expected 1:1 ratio ($P = 0.5573$); there were no significant differences between the months ($P > 0.05$) (Fig. 4).

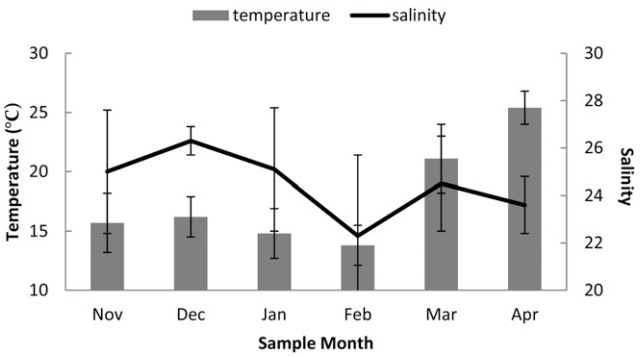

Figure 2. Mean monthly water temperature and salinity for the Dog Island collection site from November 2014 through April 2015. Averages consist of data taken at 30 minute intervals daily.

Of the 187 cultured sunray venus clams analyzed histologically, 85 were male and 102 were female. The overall male: female sex ratio of 0.8:1 did not differ significantly from an expected 1:1 ratio ($P = 0.1747$); the only month in which the ratio was significantly different between the two populations was in December ($P = 0.0134$) (Fig. 4).

Reproductive Stage

Peak spawning activity in wild females occurred in November (86%) and remained high through January, as indicated by a gonadal index (G.I.) of 1.75–2.75, after which gametogenesis increased substantially (Fig. 5). Peak spawning activity in cultured females occurred in January (75.6%) and was continuous throughout the 6-mo study period (Fig. 6). Peak spawning activity in males occurred in November (cultured), December (wild), and February (both populations), with highest levels of gametogenesis activity in March and April (Figs. 5 and 6). Significant differences were seen with regard to the interactions of sex and location ($P < 0.0001$), sex and time ($P < 0.0001$), and location and time ($P = 0.0067$), but not all three.

Fatty Acid Profile

The FA composition of the gonadal visceral mass of collected sunray venus clams, expressed as a percent of total

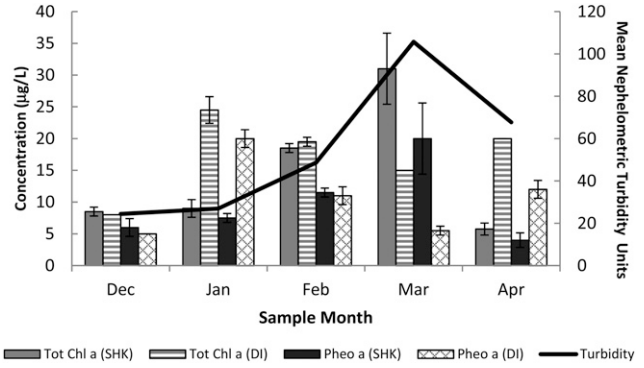

Figure 3. Mean chlorophyll *a* and pheophyton *a* concentrations ($n = 2$) for the sunray venus clam collection sites at Seahorse Key (SHK) and Dog Island (DI) and mean monthly turbidity Dog Island from December 2014 through April 2015.

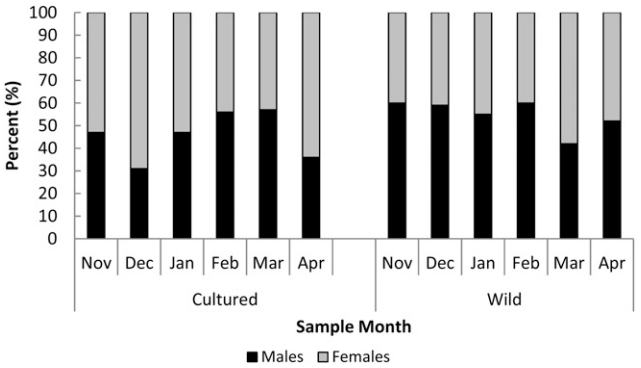

Figure 4. Ratio of male to female sunray venus clams for cultured and wild populations collected from November 2014 through April 2015. Approximately 30 sunray venus clams from both populations were examined each month except for February 2015 (wild $n = 5$).

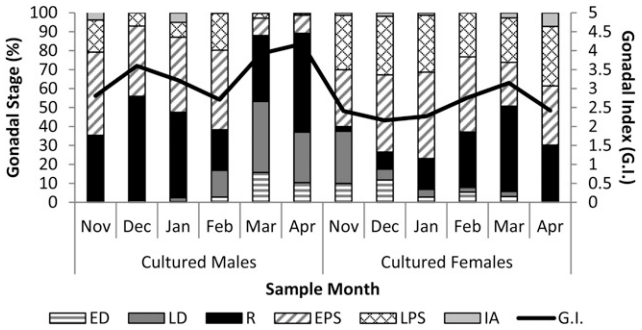

Figure 6. Percentage of sunray venus clams collected from cultured populations from November 2014 through April 2015 at each of the gonadal stages of development (ED, early development; LD, late development; R, ripe; EPS, early post spawning; LPS, late post spawning; IA, inactive). Monthly gonadal index (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).

lipids, is shown in Tables 2 and 3. In general, the predominant FA type was the saturated fatty acids (SFAs), followed by n-3 PUFAs, monounsaturated fatty acids (MUFAs), and n-6 PUFAs. The SFA profile was mainly represented by palmitic acid (16:0), the MUFA profile by palmitoleic acid (16:1 n-7), the PUFA n-6 profile by arachidonic acid (AA) (20:4 n-6) and the PUFA n-3 profile predominately by eicosapentanoic acid (EPA) (20:5 n-3), although similarly high levels of docosahexanoic acid (DHA) (22:6 n-3) were sometimes noted, particularly in males.

Significant interactions between sex, month, and population were only seen for MUFAs ($P = 0.037$). Significant interactions between sex and month were only seen between MUFAs ($P = 0.004$) and n3/n6 ratios ($P = 0.002$). No significant interactions were seen between month and population.

All four categories of FAs, including n-3/n-6 PUFAs, were significantly affected by month ($P < 0.027$). The SFA levels differed significantly between April (highest concentration) and February (lowest concentration) ($P = 0.0163$) because of decreased concentrations of 16:0 in cultured populations and 14:0 and 18:0 in wild females. The MUFA levels differed significantly between January (highest level) and February and April ($P < 0.0116$) primarily because of decreases in all MUFAs in males. Levels of PUFA n-6 were highest in November and December and significantly lower in January to March ($P < 0.0085$) because of decreases in 18:2 n-6 and 20:4 n-6. Levels of PUFA n-3 were highest in February and significantly lower in March and April ($P < 0.0073$) because of decreases in females. The ratio of n-3/n-6 was significantly higher in December to February and lower in November ($P < 0.0058$).

The concentration of SFAs, MUFAs, and PUFA n-3s were significantly affected by sex ($P < 0.0001$). Total SFAs were significantly lower in cultured males ($P < 0.0394$), primarily because of decreased 14:0. Total MUFAs were significantly lower in all males in December and April ($P < 0.0204$), cultured males in November and February ($P < 0.0500$), and wild males in March ($P = 0.0374$), primarily because of lower levels of 16:1 n-7. Total PUFA n-3s were influenced by sex ($P = 0.0002$), and lower in females, primarily because of lower levels of 22:6 n-3. PUFA n-3/n-6 ratios were likewise significantly influenced by sex ($P = 0.0011$).

Population influenced PUFA n-3s and n-3/n-6 ratios ($P < 0.018$); however, the only significant difference was seen in April ($P = 0.0379$), when levels in cultured populations were lower.

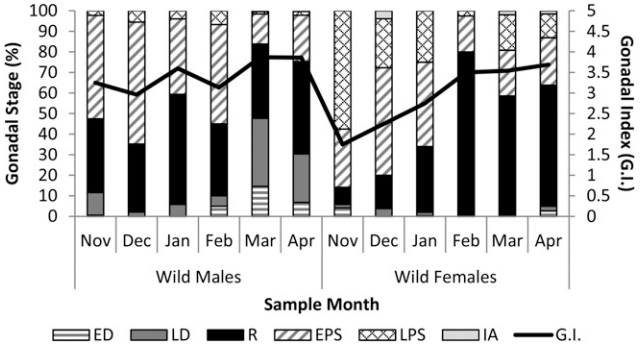

Figure 5. Percentage of sunray venus clams collected from natural populations (wild) from November 2014 through April 2015 at each of the gonadal stages of development (ED, early development; LD, late development; R, ripe; EPS, early post spawning; LPS, late post spawning; IA, inactive). Monthly gonadal 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).

Sunray Venus Clam Larvae and Postset: Fatty Acid Profile

Fatty Acid Profile

The FA profiles of D-stage larvae (day 1, day 5) and postset (day 6) sunray venus clams are shown in Table 4. When data across spawns were compared, significant differences were seen between larvae (day 1, day 5) and postset for total PUFA n-6s ($P = 0.041$) and the ratio of n3/n-6 PUFAs ($P = 0.049$) (Table 4). There were no significant differences in the FA profile of day-1 and day-5 larvae; however, significant differences were seen between day-1 larvae and postset with respect to PUFA n-3 levels ($P = 0.031$) and n3/n6 ratios ($P = 0.045$) and between day-5 larvae and postset with respect to PUFA n-6 levels ($P = 0.001$).

TABLE 2.

Fatty acid composition (mean ± SD), expressed as percent of the total fatty acids, of pooled (n = 5–7; 2–3 February) gonadal visceral masses of male and female sunray venus clams collected from natural populations during November 2014 through April 2015.

Fatty acid	November Male/Female (n = 3)	December Male/Female (n = 3)	January Male/Female (n = 3)	February Male/Female (n = 2)	March Male/Female (n = 2)	April Male/Female (n = 2)
14:0	2.9 ± 1.6/2.5 ± 1.2	1.9 ± 0.2/5.5 ± 2.5	3.4 ± 1.4/5.5 ± 1.7	1.3 ± 0.1/4.0 ± 0.1	1.7 ± 0.1/4.3 ± 1.1	1.8 ± 0.2/3.2 ± 0.2
15:0	0.8 ± 0.2/0.8 ± 0.2	0.7 ± 0.1/0.8 ± 0.0	0.7 ± 0.1/0.8 ± 0.0	0.6 ± 0.1/0.7 ± 0.0	0.5 ± 0.0/0.6 ± 0.1	0.6 ± 0.0/0.6 ± 0.0
16:0	20.1 ± 2.1/22.1 ± 1.3	20.1 ± 1.9/20.7 ± 3.8	19.2 ± 1.4/21.8 ± 1.0	19.3 ± 0.4/22.6 ± 1.1	18.8 ± 0.2/23.2 ± 0.4	19.1 ± 0.5/24.2 ± 1.3
17:0	2.8 ± 1.7/2.1 ± 0.3	2.2 ± 0.5/1.3 ± 0.3	1.9 ± 0.4/0.8 ± 0.6	1.5 ± 0.0/0.9 ± 0.0	1.7 ± 0.0/1.1 ± 0.1	1.8 ± 1.1/1.3 ± 0.1
18:0	5.7 ± 1.6/5.9 ± 1.3	6.5 ± 1.2/3.7 ± 1.6	6.0 ± 1.0/3.2 ± 1.4	6.0 ± 0.3/3.7 ± 0.5	6.2 ± 1.0/4.4 ± 0.4	8.2 ± 0.2/5.0 ± 0.7
Total SFA	31.5 ± 2.0/34.0 ± 1.5	31.4 ± 0.9/32.0 ± 3.2	31.2 ± 0.9/32.1 ± 0.2	28.6 ± 0.2/31.9 ± 1.7	28.9 ± 1.4/33.6 ± 1.0	31.5 ± 0.2/34.4 ± 1.9
16:1 (n-7)	7.7 ± 3.0/7.8 ± 2.7	6.0 ± 0.5/13.2 ± 2.7	8.8 ± 1.5/15.3 ± 3.6	4.9 ± 0.1/11.1 ± 0.5	5.6 ± 0.4/11.2 ± 0.4	4.2 ± 0.1/10.5 ± 0.3
18:1 (n-7)	4.0 ± 0.8/3.1 ± 0.6	3.3 ± 0.3/4.1 ± 0.4	3.1 ± 0.0/3.8 ± 0.2	2.0 ± 0.2/3.4 ± 0.2	2.7 ± 0.2/4.3 ± 0.1	2.6 ± 0.5/3.9 ± 0.7
18:1 (n-9)	2.5 ± 0.1/4.6 ± 1.6	2.0 ± 0.6/3.2 ± 1.1	1.8 ± 0.5/3.0 ± 0.3	1.4 ± 0.4/2.7 ± 0.1	1.4 ± 0.1/4.2 ± 0.6	1.7 ± 0.3/3.5 ± 0.2
20:1 (n-9)	1.5 ± 0.5/1.1 ± 0.9	1.7 ± 0.2/1.4 ± 0.7	1.4 ± 0.4/1.0 ± 0.5	1.5 ± 0.0/1.4 ± 0.1	1.6 ± 0.1/1.6 ± 0.3	1.6 ± 0.1/1.8 ± 0.2
Total MUFA	15.7 ± 3.8/16.6 ± 4.8	13.0 ± 1.1/21.9 ± 2.5	15.0 ± 1.6/23.2 ± 3.2	9.8 ± 0.3/18.6 ± 0.6	11.4 ± 0.1/21.3 ± 0.2	10.3 ± 0.1/19.6 ± 0.6
18:2 (n-6)	0.8 ± 0.3/0.7 ± 0.1	1.0 ± 0.6/0.7 ± 0.0	0.6 ± 0.0/0.7 ± 0.1	0.3 ± 0.1/0.4 ± 0.1	0.3 ± 0.1/0.5 ± 0.0	0.3 ± 0.1/0.4 ± 0.1
18:3 (n-6)	0.4 ± 0.1/0.4 ± 0.2	0.3 ± 0.1/0.4 ± 0.2	0.3 ± 0.0/0.3 ± 0.1	0.6 ± 0.0/0.9 ± 0.1	0.5 ± 0.0/0.7 ± 0.1	0.7 ± 0.1/1.0 ± 0.1
20:4 (n-6)	3.0 ± 0.5/2.9 ± 0.3	2.5 ± 0.1/1.8 ± 0.5	2.1 ± 0.4/1.5 ± 0.5	1.8 ± 0.6/2.2 ± 0.7	1.8 ± 0.7/1.6 ± 0.2	1.9 ± 0.5/1.7 ± 0.2
Total PUFA (n-6)	4.2 ± 0.5/4.0 ± 0.6	3.8 ± 0.8/2.9 ± 0.6	3.0 ± 0.4/2.5 ± 0.5	2.7 ± 0.6/3.5 ± 0.7	2.6 ± 0.8/2.9 ± 0.3	3.0 ± 0.6/3.1 ± 0.2
18:4 (n-3)	1.3 ± 0.3/1.1 ± 0.6	1.0 ± 0.0/1.8 ± 0.3	1.1 ± 0.1/1.8 ± 0.11	1.3 ± 0.2/2.3 ± 0.1	1.1 ± 0.1/1.8 ± 0.1	1.0 ± 0.1/2.2 ± 0.2
20:5 (n-3)	9.5 ± 1.2/6.4 ± 2.7	13.0 ± 1.6/14.6 ± 4.6	13.8 ± 1.5/15.2 ± 0.3	14.6 ± 0.3/15.7 ± 0.6	13.7 ± 0.4/10.7 ± 0.2	12.4 ± 0.2/10.8 ± 0.1
22:5 (n-3)	1.9 ± 0.6/1.7 ± 0.1	3.1 ± 0.1/1.4 ± 0.3	3.1 ± 0.5/1.4 ± 0.3	4.6 ± 0.6/1.5 ± 0.1	3.4 ± 0.1/1.2 ± 0.1	3.5 ± 0.1/1.4 ± 0.0
22:6 (n-3)	8.3 ± 3.7/6.6 ± 1.8	9.7 ± 1.8/5.2 ± 1.8	8.5 ± 2.1/4.8 ± 1.7	14.4 ± 1.3/7.4 ± 1.1	13.6 ± 0.2/6.2 ± 0.5	11.5 ± 0.7/6.4 ± 0.4
Total PUFA (n-3)	21.0 ± 4.8/15.8 ± 5.0	26.9 ± 3.3/23.0 ± 4.9	26.5 ± 3.9/23.4 ± 2.3	34.8 ± 1.9/26.9 ± 1.7	31.8 ± 0.6/19.9 ± 0.9	28.4 ± 0.7/20.9 ± 0.7
(n-3)/(n-6)	5.0 ± 0.8/3.8 ± 0.7	7.2 ± 0.7/7.9 ± 2.1	9.0 ± 2.1/9.6 ± 1.0	13.6 ± 4.0/7.7 ± 1.1	12.7 ± 4.2/6.9 ± 0.4	9.7 ± 1.7/6.6 ± 0.7
Total FAs	72.4 ± 2.8/70.4 ± 3.0	75.2 ± 1.5/79.8 ± 2.8	75.6 ± 3.4/81.2 ± 2.1	75.9 ± 1.5/80.6 ± 2.3	74.7 ± 2.9/77.7 ± 1.2	73.2 ± 0.5/78 ± 1.2

TABLE 3.

Fatty acid composition (mean ± SD), expressed as percent of the total fatty acids, of pooled (n = 5–7) gonadal visceral masses of male and female sunray venus clams collected from cultured populations during November 2014 through April 2015.

Fatty acid	November Male/Female (n = 3)	December Male/Female (n = 3)	January Male/Female (n = 3)	February Male/Female (n = 2)	March Male/Female (n = 2)	April Male/Female (n = 2)
14:0	1.1 ± 0.0/3.2 ± 0.4	1.4 ± 0.9/3.9 ± 0.7	3.1 ± 1.4/3.3 ± 1.4	0.8 ± 0.2/3.1 ± 0.2	1.1 ± 0.0/2.8 ± 0.7	0.3 ± 0.1/3.5 ± 0.4
15:0	0.8 ± 0.2/1.1 ± 0.1	0.8 ± 0.2/1.0 ± 0.1	1.0 ± 0.2/1.0 ± 0.1	0.4 ± 0.0/0.6 ± 0.0	0.6 ± 0.0/0.6 ± 0.0	0.5 ± 0.0/0.8 ± 0.1
16:0	19.6 ± 1.0/24.6 ± 1.5	19.6 ± 1.1/22.8 ± 2.7	21.0 ± 2.7/21.4 ± 0.3	16.5 ± 0.9/20.1 ± 1.3	19.1 ± 1.1/23.8 ± 1.4	18.5 ± 0.4/23.5 ± 0.6
17:0	2.7 ± 0.6/2.4 ± 0.6	2.8 ± 0.4/1.7 ± 0.33	2.1 ± 0.3/2.2 ± 0.6	2.1 ± 0.1/1.4 ± 0.1	2.2 ± 0.3/1.3 ± 0.1	2.7 ± 0.2/2.2 ± 0.3
18:0	7.2 ± 1.3/4.9 ± 1.0	7.1 ± 1.3/3.8 ± 1.2	4.6 ± 1.9/4.3 ± 1.4	7.4 ± 0.5/4.6 ± 0.3	7.6 ± 0.4/5.1 ± 0.2	6.9 ± 0.0/5.6 ± 0.2
Total SFA	31.4 ± 0.2/36.2 ± 1.9	31.8 ± 1.2/33.3 ± 2.8	31.8 ± 2.9/32.2 ± 1.2	27.2 ± 1.4/29.8 ± 0.7	30.6 ± 0.5/33.7 ± 2.2	29.0 ± 0.6/35.6 ± 1.5
16:1 (n-7)	4.6 ± 0.6/10.0 ± 1.6	4.7 ± 1.1/11.4 ± 1.8	9.9 ± 4.3/10.7 ± 3.7	3.2 ± 0.1/9.9 ± 0.8	6.4 ± 0.2/11.8 ± 0.3	2.7 ± 0.6/11.3 ± 0.8
18:1 (n-7)	2.8 ± 0.1/3.9 ± 0.1	2.8 ± 0.3/3.9 ± 0.0	3.3 ± 0.3/3.3 ± 0.3	2.0 ± 0.2/3.6 ± 0.2	3.0 ± 0.4/3.8 ± 0.3	1.6 ± 0.2/3.6 ± 0.3
18:1 (n-9)	3.1 ± 0.9/4.6 ± 0.9	2.4 ± 0.5/3.9 ± 1.2	2.9 ± 1.6/3.0 ± 0.3	1.6 ± 0.2/3.3 ± 0.3	1.7 ± 0.3/4.5 ± 0.2	2.5 ± 0.2/4.6 ± 0.4
20:1 (n-9)	1.3 ± 1.2/1.1 ± 1.0	1.7 ± 0.6/1.3 ± 0.5	1.4 ± 0.7/1.3 ± 0.5	1.3 ± 0.1/1.5 ± 0.1	1.4 ± 0.1/1.8 ± 0.1	1.3 ± 0.1/1.7 ± 0.2
Total MUFA	11.8 ± 0.7/19.6 ± 2.2	11.6 ± 1.1/20.5 ± 2.5	17.5 ± 5.6/18.4 ± 3.8	8.0 ± 0.2/18.3 ± 0.7	12.5 ± 0.8/21.9 ± 0.2	8.1 ± 0.3/21.2 ± 0.9
18:2 (n-6)	0.6 ± 0.1/0.6 ± 0.2	0.5 ± 0.1/0.7 ± 0.1	0.6 ± 0.2/0.7 ± 0.1	0.2 ± 0.0/0.3 ± 0.0	0.4 ± 0.0/0.3 ± 0.0	0.3 ± 0.0/0.3 ± 0.0
18:3 (n-6)	0.3 ± 0.1/0.3 ± 0.1	0.2 ± 0.0/0.3 ± 0.1	0.3 ± 0.1/0.3 ± 0.1	0.3 ± 0.1/0.6 ± 0.1	0.4 ± 0.1/0.4 ± 0.0	0.2 ± 0.0/0.6 ± 0.0
20:4 (n-6)	3.4 ± 0.0/2.2 ± 0.4	2.9 ± 0.2/2.1 ± 0.6	2.2 ± 0.6/2.1 ± 0.6	1.8 ± 0.9/2.0 ± 0.4	1.8 ± 0.8/2.3 ± 0.5	2.5 ± 1.1/2.4 ± 0.6
Total PUFA (n-6)	4.2 ± 0.0/3.1 ± 0.3	3.6 ± 0.2/3.2 ± 0.6	3.2 ± 0.5/3.2 ± 0.6	2.3 ± 1.1/3.0 ± 0.4	2.6 ± 0.7/3.0 ± 0.4	3.0 ± 1.1/3.3 ± 0.6
18:4 (n-3)	0.9 ± 0.1/1.3 ± 0.2	0.9 ± 0.1/1.8 ± 0.4	1.4 ± 0.3/1.8 ± 0.4	0.8 ± 0.1/1.9 ± 0.1	1.0 ± 0.1/1.5 ± 0.1	1.1 ± 0.3/1.6 ± 0.6
20:5 (n-3)	9.4 ± 1.6/7.0 ± 1.1	9.8 ± 1.3/10.8 ± 3.3	12.0 ± 1.8/11.8 ± 0.3	12.5 ± 0.3/11.0 ± 0.3	8.7 ± 0.4/7.4 ± 0.4	6.6 ± 0.7/5.2 ± 0.6
22:5 (n-3)	2.6 ± 0.2/1.3 ± 0.2	3.1 ± 0.5/1.4 ± 0.2	2.3 ± 0.7/2.1 ± 0.6	3.9 ± 0.1/1.7 ± 0.2	2.6 ± 0.2/1.4 ± 0.2	2.8 ± 0.1/1.3 ± 0.0
22:6 (n-3)	9.0 ± 2.1/4.6 ± 1.6	9.4 ± 2.1/5.5 ± 1.7	7.6 ± 1.9/7.2 ± 1.9	12.7 ± 0.6/7.5 ± 0.3	8.3 ± 0.3/5.3 ± 0.4	9.8 ± 0.3/4.5 ± 0.4
Total PUFA (n-3)	22.0 ± 3.9/14.1 ± 3.1	23.2 ± 3.7/19.5 ± 4.8	23.3 ± 3.2/23.0 ± 2.0	29.9 ± 0.6/22.1 ± 0.9	20.7 ± 0.9/15.7 ± 1.2	20.3 ± 0.6/12.7 ± 1.0
(n-3)/(n-6)	5.2 ± 0.9/4.5 ± 0.6	6.5 ± 1.0/6.1 ± 1.1	7.4 ± 0.7/7.3 ± 0.7	14.4 ± 6.3/7.5 ± 0.6	8.8 ± 2.6/5.2 ± 0.3	7.3 ± 2.9/3.9 ± 0.4
Total FAs	69.5 ± 2.9/73.2 ± 1.8	70.2 ± 5.0/76.5 ± 6.2	76.2 ± 3.6/76.7 ± 5.0	67.5 ± 2.1/73.2 ± 1.3	66.3 ± 1.3/74.3 ± 2.4	67.1 ± 1.6/72.8 ± 2.1

TABLE 4.

Fatty acid composition (mean ± SD), expressed as percent of the total fatty acids, for straight hinge veliger larvae (D-stage) and postset sunray venus clams produced from successful spawns conducted from February through August 2015.

Fatty acid	February 2015 Postset ($n = 2$)	March 2015 24-h D-stage ($n = 2$)	March 2015 5 day D-stage ($n = 3$)	March 2015 Postset ($n = 2$)	August 2015 24-h D-stage ($n = 2$)	August 2015 Postset ($n = 1$)
14:0	7.9 ± 0.1	4.3 ± 0.1	11.7 ± 2.2	9.9 ± 1.2	6.6 ± 0.9	8.6
15:0	0.3 ± 0.0	0.7 ± 0.0	0.3 ± 0.2	0.6 ± 0.0	0.5 ± 0.0	0.6
16:0	14.6 ± 0.1	28.8 ± 0.6	17.5 ± 3.9	19.1 ± 0.8	25.7 ± 5.5	18.6
17:0	0.8 ± 1.2	0.9 ± 1.3	0.5 ± 0.8	0.9 ± 1.1	0.0 ± 0.0	1.3
18:0	6.9 ± 0.3	4.9 ± 1.8	5.0 ± 2.8	6.2 ± 1.6	2.8 ± 0.6	5.3
Total SFA	30.7 ± 0.7	39.6 ± 3.7	34.9 ± 8.7	36.7 ± 4.9	35.7 ± 5.2	34.5
16:1 (n-7)	4.8 ± 0.1	13.6 ± 0.2	5.0 ± 0.5	13.4 ± 11.7	18.6 ± 2.4	21.2
18:1 (n-7)	4.8 ± 0.0	4.6 ± 0.1	1.7 ± 2.9	4.4 ± 0.5	2.3 ± 0.6	4.9
18:1 (n-9)	17.5 ± 0.8	6.0 ± 0.7	16.0 ± 5.8	13.4 ± 7.6	7.1 ± 0.9	7.7
20:1 (n-9)	3.9 ± 0.0	1.7 ± 0.5	2.2 ± 1.1	2.4 ± 1.2	0.0 ± 0.0	1.7
Total MUFA	31.0 ± 0.3	25.9 ± 1.1	25.0 ± 8.3	33.6 ± 3.4	28.1 ± 0.8	35.5
18:2 (n-6)	6.7 ± 0.1	0.8 ± 0.2	5.2 ± 1.2	6.1 ± 0.8	2.5 ± 0.1	4.8
18:3 (n-6)	0.4 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.4	0.8 ± 0.1	0.5
20:4 (n-6)	0.6 ± 0.1	1.3 ± 0.3	0.5 ± 0.4	1.0 ± 1.4	4.4 ± 0.1	2.0
Total PUFA (n-6)	7.6 ± 0.2	2.2 ± 0.2	5.9 ± 1.7	7.4 ± 1.0	7.6 ± 0.0	7.4
18:4 (n-3)	4.5 ± 0.2	1.2 ± 0.1	6.7 ± 2.3	3.1 ± 1.1	1.8 ± 0.6	2.0
20:5 (n-3)	0.5 ± 0.2	7.4 ± 1.6	0.7 ± 0.7	1.0 ± 1.4	6.5 ± 1.5	2.0
22:5 (n-3)	0.0 ± 0.0	0.8 ± 1.1	0.3 ± 0.3	0.1 ± 0.1	0.5 ± 0.1	0.1
22:6 (n-3)	5.8 ± 0.3	4.3 ± 1.3	6.2 ± 5.4	4.0 ± 1.5	4.6 ± 0.1	2.7
Total PUFA (n-3)	10.8 ± 0.7	13.8 ± 4.1	13.9 ± 7.7	8.1 ± 1.1	13.4 ± 1.9	6.9
(n-3)/(n-6)	1.4 ± 0.0	6.2 ± 1.2	2.3 ± 1.4	1.1 ± 0.3	1.8 ± 0.3	0.9
Total FAs	89.1 ± 0.5	81.5 ± 3.6	79.7 ± 6.2	77.8 ± 2.4	84.8 ± 3.7	84.3

DISCUSSION

Sunray Venus Clam Adults: Reproductive Pattern and Fatty Acid Profile

The FA profile and reproductive cycle of two populations (wild and cultured) of sunray venus clams were compared for a 6-mo period that encompassed the presumptive spawning season described by Haines (1976). The FA profile varied with respect to month and sex, and to a lesser extent with population. Differences in the reproductive cycle of the two populations were noted between females, but not males. Concentrations of PUFAs changed during the reproductive cycle.

Size

Sunray venus clams collected from natural assemblages at Seahorse Key were larger than the population of cultured clams held at the Dog Island lease in Cedar Key; however, only the age of the cultured population (~3 y at study initiation) is known. Clams collected offshore from sandy bottoms at Seahorse Key were found at a depth of 7–12 cm. Stokes et al. (1968) suggested that subadults move from inshore to offshore areas, and Jolley (1972) reported that clam size increased with increased depth. The average shell length of clams from Seahorse Key was slightly larger (104.5 mm) than that reported by Akin and Humm (1960) from Alligator Harbor populations (95.6 mm), but smaller than that reported by others (125–149 mm) (Stokes et al. 1968, Jolley 1972, Haines 1976). Jolley suggested that clams collected by Akin & Humm (1960) were medium sized rather than mature individuals; however, clams with smaller shell length from both populations in this study were found in ripe and spawning stages.

Histology

Sex Ratio

Although monthly variations were observed, overall male: female ratios did not differ significantly from the expected 50:50 ratio and are in line with ratios reported previously (Haines 1976). Scarpa et al. (2009) reported a slightly skewed male to female sex ratio of 1.4:1 based on a total of 66 wild sunray venus clams collected over a 6-mo period, but this was likely a result of the small number of clams collected monthly. No evidence of hermaphroditism was observed in any of the aforementioned studies and Haines (1975) suggested that this species may be dioecious; however, data provided evidence for protandry; clams less than 60 mm in length were either male or undifferentiated.

Reproductive Stage

Haines (1975, 1976) provides the only other study detailing the reproductive cycle of *M. nimbosa*. That 1-y study indicated that clams are in early stages of gametogenesis during the first 5 mo of the year, with spawning beginning in late summer and peaking between October and December. Scarpa et al. (2009) reported limited reproductive data for sunray venus clams collected from Alligator Harbor from July to November 2006, with most clams in early and ripe stages in July and peak spawning occurring in October. The sample size was small and results of males and females were combined. In this study, peak spawning occurred later and was more protracted. In contrast to Haines (1975, 1976), spawning activity did not decline abruptly in January. Spawning activity of males was highest in November, December, and February, declining in March. Spawning activity was high in females from November through

January, declining abruptly in wild females in February but not cultured females, which spawned continuously from November through April. Although the sample size could offer a partial explanation for this discrepancy, results were similar in male populations, and previously reported results were based on smaller sample sets. Haines (1975) evaluated 7–20 clams and Scarpa et al. (2009) 6–10 clams compared with 30 clams examined monthly in this study. Although Scarpa et al. (2009) did not distinguish between males and females, the raw data clearly shows reproductive stage differences between sexes. In October, most males and all females were spawning; however, by December, all males were undergoing gametogenesis, whereas most females continued to spawn (Scarpa & Laramore, unpublished data). Cultured sunray venus clams ($n = 15$–60) examined histologically from October to December 2008 and in April 2009 showed that the majority of males were spawning from October to December, and in gametogenesis by April (Laramore unpublished data). During the same period, spawning activity of cultured females was low with the majority of females undergoing gametogenesis (Laramore, unpublished data). Collectively, these results indicate that male sunray venus clams could be considered fall to winter spawners, as Haines (1975, 1976) had indicated previously, but females exhibit disparate spawning patterns, which may be a consequence of environmental or dietary variability. These studies provide evidence that lends support to claims of Florida clam seed producers of nonsynchronous spawning in male and female sunray venus clams and hence their labeling of these clams as "dribble spawners".

Spawning activity in southerly latitudes is less dependent on temperature than in northern latitudes and therefore may be more protracted. Hesselman et al. (1989) reported continual spawning of hard clams in Florida, with peak spawning occurring in fall and spring. The results of the present study are in agreement with this observation in that although fall spawning peaks were noted, sunray venus clams were in multiple reproductive stages simultaneously, with males continuously spawning from November to February and females exhibiting a more protracted spawning period.

Fatty Acid Profile

Lipids and FAs are important in the reproductive process (Pazos et al. 1996) and accumulate in maturing oocytes, serving as an energy source during larval development (Gallager & Mann 1986, Soudant et al. 1996, Hendriks et al. 2003). Polyunsaturated fatty acids (PUFAs), especially EPA (20:5 n-3) and DHA (22:6 n-3), are major membrane components and important for membrane function (Hendriks et al. 2003, Palacios et al. 2005). The gonadal visceral mass was specifically targeted in this study to discern tissue specific changes in FAs that might occur during gametogenesis.

A few studies have examined biochemical changes in bivalves. The FA composition of bivalves has been explored in the mussels, *Mytilus platensis, Mytilus galloprovincialis,* and *Perna perna,* (DeMoreno et al. 1980, Freites et al. 2002, Narváez et al. 2008), the clams, *Ruditapes decussatus*, and *Ensis siliqua* (Ojea et al. 2004, Baptista et al. 2014), the oyster, *Crassostrea gigas*, (Pazos et al. 1996, Soudant et al. 1999, Dridi et al. 2007), and the scallops *Pecten maximus*, *Argopecten pupuratus*, and *Nodipecten nodosus* (Pazos et al. 1997, Caers et al. 1999, Palacios et al. 2005). With the exception of Haines (1976) study in which seasonal changes in glycogen content were examined, biochemical changes during gametogenesis have not been explored in the sunray venus clam.

In general, the highest SFA values in sunray venus clams were associated with 16:0 (palmitic acid), highest MUFA values with 16:1 n-7 (palmitoleic acid), highest n-3 PUFA values with EPA (20:5 n-3), and highest n-6 PUFA values with 20:4 n-6 (AA). Similar findings have been reported for mussels (DeMoreno et al. 1980, Narváez et al. 2008) and clams (Ojea et al. 2004, Baptista et al. 2014). SFAs were the dominant FA type in sunray venus clams, followed by n-3 PUFAs, which is in agreement with reported findings in other clam and mussel species (DeMoreno et al. 1980, Ojea et al. 2004, Narváez et al. 2008). In contrast, n-3 PUFAs were the dominant FA type, followed by SFAs, in oysters and scallops (Pazos et al. 1996, Caers et al. 1999, Palacios et al. 2005, Dridi et al. 2007).

Fatty Acids and Reproductive Stage

Several studies have shown a relationship between the bivalve reproductive cycle and FA composition (DeMoreno et al. 1980, Pazos et al. 1996, Soudant et al. 1999, Ojea et al. 2004, Palacios et al. 2005, Dridi et al. 2007, Narváez et al. 2008, Baptista et al. 2014). In this study, no significant differences were observed in SFA or MUFA values in association with gametogenesis; however, no clear relationships have been established for these two FA categories in other studies. Researchers have reported increases in both (Narváez et al. 2008), decreases in both (DeMoreno et al. 1980), decreases in SFAs but not MUFAs (Pazos et al. 1996), decreases in SFAs, and increases in MUFAs (Ojea et al. 2004) in conjunction with gametogenesis. In this study, decreases were observed in PUFA n-6 levels during gametogenesis in males and wild females associated with lower 18:2 n-6 and 20:4 n-6 values. Similar decreases in 20:4 n-6 have been reported in oysters and mussels during gametogenesis (Dridi et al. 2007, Narváez et al. 2008). Concurrent with decreased PUFA n-6 levels, increases were observed in PUFA n-3 levels, primarily associated with increased levels of 20:5 n-3 (females) and 22:6 n-3 (males). Increased PUFA n-3 values have been associated with oocyte maturation in oysters (Pazos et al. 1996, Soudant et al. 1999, Dridi et al. 2007), clams (Ojea et al. 2004, Baptista et al. 2014), and scallops (Palacios et al. 2005). The specific n-3 PUFA associated with these increases, however, varies by species and study: 22:6 n-3 (Pazos et al. 1996, Soudant et al. 1999, Ojea et al. 2004, Dridi et al. 2007, Baptista et al. 2014), 20:5 n-3 (Pazos et al. 1996, Soudant et al. 1999, Ojea et al. 2004, Palacios et al. 2005, Baptista et al. 2014), and 22:5 n-3 (Ojea et al. 2004, Baptista et al. 2014).

Fatty Acids and Environmental Parameters

The relationship between temperature, diet, and gametogenic cycle may be intertwined as gonadal development increases when food is readily available, and occurrence of phytoplankton blooms are often temperature dependent, and therefore, determining which factor is responsible for changes in FA composition can be difficult. For instance, Narváez et al. (2008) reported links with all three, but concluded that temperature had the strongest impact on mussels. Other researchers concluded that changes in FA profiles of oysters and clams during gametogenesis were influenced more by diet than temperature (Pazos et al. 1996, Ojea et al. 2004).

Variations in SFAs and MUFAs in the present study could not be correlated with temperature. Although researchers have reported temperature-associated changes with SFAs (Pazos et al. 1996, Pazos et al. 2003, Narváez et al. 2008, Fernández-Reiriz et al. 2015), and MUFAs (Narváez et al. 2008, Fernández-Reiriz et al. 2015), some reported a positive correlation (Pazos et al. 1996, Pazos et al. 2003, Fernández-Reiriz et al. 2015), whereas others reported a negative relationship (Narváez et al. 2008).

Inverse relationships between temperature and total PUFAs (Piretti et al. 1988, Pazos et al. 1996, Pazos et al. 2003, Ojea et al. 2004, Dridi et al. 2007, Narváez et al. 2008, Fernández-Reiriz et al. 2015) have been reported. Narváez et al. (2008) determined that relationships differ dependent on specific PUFAs: some showed an inverse relationship (C20:5 n-3), some a positive relationship (C20:4 n-6), and others no relationship (C18:2n-6, C18:3n-3, C18:4n-3, and C22:6n-3). Although significant time-dependent differences were observed with n-6 PUFAs, n-3 PUFAs, and n3/n6 ratios during the present study, no temperature-related association was apparent.

Phytoplankton is the major food source of bivalves, as well as the major source of FAs (Sargent 1976). Studies have shown that the composition of FAs in bivalves reflects the type of phytoplankton consumed (De Moreno et al. 1980, Langdon & Waldock 1981, Fernández-Reiriz et al. 1996, Pazos et al. 1996, Fernández-Reiriz et al. 1998, Soudant et al. 1999, Ojea et al. 2004, Dridi et al. 2007, Narváez et al. 2008). Researchers have reported increased PUFAs in relation to phytoplankton abundance and increases in chlorophyll *a* (Ojea et al. 2004, Dridi et al. 2007). Ojea et al. (2004) determined that this increase was due to increased levels of 22:5 n-3. Narváez et al. (2008) reported a positive relationship with chlorophyll *a* concentration and certain SFAs (14:0) and MUFAs (16:1 n-7, 18:1 n-7) and a negative relationship with certain PUFAs (18:4 n-3, 20:4 n-6). During the course of the present study, an inverse relationship with n-6 PUFAs (20:4 n-6, 18:2 n-6) and a positive relationship with turbidity, chlorophyll *a*, and n-3 PUFAs was found. This was more pronounced at the inshore Dog Island site, where most n-3 PUFAs increased with increased chlorophyll *a*, whereas at the offshore Seahorse Key site only 22:6 n-3 increased, a potential reflection of variation in phytoplankton community composition between sites. Narváez et al. (2008) pointed out that chlorophyll *a* levels are not necessarily representative of the phytoplankton community and that significant shifts in the community occur over time consisting of organisms composed of different lipids. Fatty acid profiles may serve as biomarkers of food sources allowing one to determine whether phytoplankton consumed was predominately diatoms, dinoflagellates, or ciliates (Pazos et al. 1996). In addition, detritus, bacteria, and zooplankton are also part of the bivalve diet (Ezgeta-Balic et al. 2012). As a consequence, FA profiles of various dietary sources of bivalves may overlap (Pazos et al. 1996, Ezgeta-Balic et al. 2012), making interpretation difficult, although ratios of various FA types may aid in interpretation (Ezgeta-Balic et al. 2012). Reliance on comparison of chlorophyll *a* concentration alone to account for variation in FA profiles is not ideal, and determination of the phytoplankton community would have improved interpretation of the results in this study. Still, as an increase in n-3 PUFAs was noted during gametogenesis, algal species, such as *Isochrysis* and *Pavlova* sp., with high n-3 PUFA concentrations should be included in diets fed to sunray venus clams during broodstock conditioning.

Fatty Acids and Population/Location

Pazos et al. (1997) observed that populations of *Pecten maximus* from different areas showed discrepancies in the seasonal pattern of gonadal development and timing of spawning; however, the cause was not indicated and was thought to be as likely a result of location as of differing genetics. A genetic assessment of the two populations (wild, cultured) used in this study has not been conducted; however, as the cultured clam population is only two generations removed from natural stocks, genetics is likely to be less of a factor than location. Although the wild population generally contained higher total FAs, differences in the FA profile between the two sunray venus clam populations were only noted with regard to n-3 PUFAs and n3/n6 ratios, which were significantly higher in the wild population in the spring. Rather than genetic differences, this variation is likely due to location and phytoplankton community composition between the two sites. Other environmental parameters (temperature and salinity) were assumed to be similar at both sites because of the proximity of location.

Fatty Acids and Sex

Biochemical differences are expected between male and females because of the increased energy required for increased egg quality (Delgado et al. 2004). It is therefore assumed that FA profile will differ between sexes during the reproductive cycle.

Delgado et al. (2004) reported that female *Ruditapes decussatus* accumulated large amounts of free FAs compared with males. Differences were noted in the FA profile of sunray venus males and females, regardless of month, location, or reproductive stage. Males showed significantly reduced amounts of total SFAs and MUFAs compared with females. Female gonads contained higher levels of palmitic acid (16:0), myristic acid (14:0), palmitoleic acid (16:1 n-7), oleic acid (18:1 n-7), and vaccenic acid (18:1 n-9).

Other researchers report high levels of 14:0 in female bivalves (Kluytmans et al. 1985, Caers et al. 1999, Birkely et al. 2003, Martínez-Pita et al. 2012a, Fernández-Reiriz et al. 2015). High levels of 16:0 have also been reported in *Mytilus galloprovincialis* and *Mya truncata* females (Kluytmans et al. 1985, Birkely et al. 2003, Martínez-Pita et al. 2012b, Fernández-Reiriz 2015), whereas higher levels of 16:0 were reported in *Argopecten purpuratus* and *Donax trunculus* males (Caers et al. 1999, Martínez-Pita et al. 2012a). Regardless of location, sunray venus males had lower overall FAs than females. Although males had lower total SFAs they had higher levels of 18:0. All of the aforementioned studies are in agreement in noting higher levels of 18:0 in males. Results of this study agree with previous research that showed higher levels of 16:1 n-7 (Kluytmans et al. 1985, Caers et al. 1999, Birkely et al. 2003, Martínez-Pita et al. 2012a,b, Fernández-Reirez et al. 2015), 18:1 n-9 (Caers et al. 1999), and 18:1 n-7 (Caers et al. 1999, Birkely et al. 2003, Martínez-Pita et al. 2012a) in females than in males.

No significant difference in n-6 PUFAs was found between male and female sunray venus clams, which is similar to findings in other reported studies. Differences were, however, noted between the sexes with regard to n-3 PUFAs. In contrast to the high SFA and MUFA values in sunray venus females, n-3 PUFAs, and n-3/n-6 ratios, were lower because of low levels of

DHA (22:6 n-3) and DPA (22:5 n-3). This is in agreement with reports of higher DHA levels in males (Caers et al. 1999, 2003, Birkely et al. 2003, Martínez-Pita et al. 2012a, b, Fernández-Reiriz et al. 2015), which Caers et al. (2003) postulated was necessary for the formation of spermatocyte membranes. In contrast, in scallops, higher n-3 PUFAs were reported in females because of high levels of 18:3 n-3, 18:4 n-3, and 20:5 n-3 (Caers et al. 1999, Freites et al. 2010). These results suggest that the FA profile of males and females of various bivalve species may have a physiological basis. From an aquaculture perspective, particular attention should be paid toward using algal species in conditioning systems that meet these necessary FA requirements, and research focused on improving gamete quality through dietary influence should include an analysis of the FA profile of fed algae.

Sunray Venus Clam Larvae and PostSet: Fatty Acid Analysis

Larval Fatty Acid Profile

As evidenced by a number of studies, the nutritional composition of diets affects survival and growth of larval bivalves (Napolitano et al. 1988, Brown et al. 1997, Labarta et al. 1999, Rico-Villa et al. 2006, Rivero-Rodriquez et al. 2007, Aranda-Burgos et al. 2014, Freites et al. 2016). Early larval stages rely on embryonic energy reserves, and therefore, feed intake is typically low (Labarta et al. 1999, Rico-Villa et al. 2006, Ben Kheder et al. 2010, Aranda-Burgos et al. 2014, daCosta et al. 2015).

Although larval sunray venus FA profiles tended toward lower MUFAs, no significant differences were found in total SFA or MUFA concentrations when larval (1 and 5 day) and postset FA profiles of three separate sunray venus clam spawns were compared. In contrast, changes in PUFA values were seen between larval and postset clams. Day-old larvae had significantly higher levels of n-3 PUFAs than postset, and 5-day larvae had significantly higher n-6 PUFAs than postset. Notable differences were evident when individual FAs within the four broad FA categories were compared, especially between day-old larvae and postset. The March spawn, in which three larval ages (1, 5 days, and postset) were compared, provides insight into the transition between reliance on embryonic reserves and dietary impacts, with 5-day larvae exhibiting a FA profile that was more similar to postset than to 1-day larvae.

Labarta et al. (1999) reported an increase in total FAs, SFAs, MUFAs, and PUFAs of *Ostrea edulis* larvae during the larval period, followed by a decrease in 10-day postset. Aranda-Burgos et al. (2014) reported higher SFAs and MUFAs, and lower PUFAs in 2-day-old *Ruditapes decussatus* larvae, regardless of diet, compared with 22-day-old larvae. Rico-Villa et al. (2006) reported higher SFA levels at early stages of *Crassostrea gigas* larvae (egg, D-stage) and higher MUFAs at later stages (pediveliger, spat).

To determine the influence of endogenous and exogenous in larvae, various ages need to be examined. Although 1-day-old *Ostrea edulis* larvae and 10-day-old spat had similar SFA and MUFA levels, levels increased during larval development, then decreased after metamorphosis to initial levels (Labarta et al. 1999). Total SFAs were similar for both larval and postset sunray venus clams, but age dependent patterns were noted. Although palmitic acid (16:0) was the dominant SFA, 1-day-old larvae had the highest concentrations, whereas postset had higher levels of 14:0 and 18:0. Five-day larvae represented a transitional stage with concentrations of 14:0 and 16:0 levels like that of postset and 18:0 concentrations like that of 1-day larvae. Similar age-related patterns have been reported for *Crassostrea gigas* and *Ruditapes decussatus* for 16:0 and 14:0, but not for 18:0 (Rico-Villa et al. 2006, Aranda-Burgos et al. 2014).

Although larval sunray venus clams tended toward lower MUFA levels than postset, the difference was not significant; however, MUFA profiles and amounts varied widely in postset from the three spawns. Aranda-Burgos et al. (2014) reported higher MUFA levels in younger *Ruditapes decussatus* larvae because of high 18:1 n-9 levels. Rico-Villa et al. (2006) reported higher MUFA levels in pediveligers and spat related to high 16:1 n-7 levels. In this study, similar or lower 18:1 n-9 and 16:1 n-7 levels were seen in larvae compared with postset.

The essential FAs, EPA (20:5 n-3) and DHA (22:6 n-3), are necessary for larval development, and concentrations impact growth and survival. One-day sunray venus larvae had higher levels of EPA than 5-day larvae and postset, and DHA levels increased from 1-day to 5-day larvae, followed by a decrease in postset. Labarta et al. (1999) reported a similar increase in DHA during oyster larval development, followed by a decrease in postset, but saw no changes in EPA levels, and postulated an energy related role for DHA and a structural role for EPA. In contrast, Aranda-Burgos et al. (2014) reported higher n-3 PUFAs in 22-day-old compared with 2-day-old *Ruditapes decussatus* because of increased EPA levels; however, the magnitude of increase was diet dependent. Rico-Villa et al. (2006) reported lower 22:5 n-3 levels in pediveligers and spat, whereas other PUFA n-3 levels were diet dependent.

Omega-6 FAs, especially DPA (22:5 n-6) and AA (20:4 n-6), are known to enhance larval growth and survival, and AA has been shown to be involved in stress response and pathogen resistance (Pernet et al. 2005, Aranda-Burgos et al. 2014). Aranda-Burgos et al. (2014) reported similar PUFA n-6 values for *Ruditapes decussatus* larvae and postset, and except for 18:2 n-6, which was higher in 2-day larvae, changes in specific n-6 PUFAs were diet, rather than age related. Rico-Villa et al. (2006) reported highest values of AA in early stages of *Crassostrea gigas*. Labarta et al. (1999) saw no age-associated changes in AA levels in *Ostrea edulis* larvae; however, 18:2 n-6 increased during development followed by a decrease in 10-day postset. Levels of AA were higher than other n-6 PUFAs in 1-day sunray venus larvae but decreased in 5-day larvae and postset, concurrent with a rise in 18:2 n-6.

Labarta et al. (1999) suggested that bivalve species have various FA requirements, reflected by species specific FA profiles and cited research suggesting clams required high amounts of DHA whereas oysters required more EPA. To further complicate matters, the aforementioned studies compared larvae of different ages from single spawns, whereas comparisons in this study include results of three distinct spawns.

The impact of endogenous and exogenous influences on the FA profile of developing sunray venus clams may be determined by comparing the FA profile of females collected and used to spawn to that of larvae and postset obtained from those spawns. The FA profile of 1-day-old larvae was more similar to broodstock females than it was to 5-day larvae. The FA profile of both

5-day larvae and postset from the March spawn likely represents a combination of decreasing endogenous and increasing exogenous influences, including differences between the diets of larvae and postset.

Larval sunray venus clams were fed *Isochrysis galbana* (T-iso clone), whereas postset received a mixture of *I. galbana* and *Chaetoceros gracilis*. Both algal species differ in their FA profile. Although the FA profiles of the algae fed in this study were not evaluated, various literature has shown that the two FA profile of these algal species vary; both species have similar SFA concentrations, whereas *C. gracilis* has higher levels of MUFA, and *I. galbana* has higher amounts of PUFAs, in particular n-3 PUFAs (Volkman et al. 1989, Napolitano et al. 1990, Ohse et al. 2014). The higher PUFAs seen in larvae may reflect that they are predominately fed *I. galbana,* whereas the increased MUFA concentration in postset may reflect that they are fed both algal species.

In summary, the FA profile and reproductive cycle of two sunray venus clam populations were compared over a six month period that encompassed the presumptive fall spawning season. Although previous research suggested that these clams are fall spawners, high levels of spawning were seen in males from November through February. The reproductive activity of females varied based on location with one population exhibiting protracted spawning and the other showing a pattern similar to that of males at both locations. This may suggest dietary influences at the two sites. Seasonal (monthly), sex, and population (location) differences were observed with respect to FA profile. Sex had a pronounced effect on FA profile, monthly variations in PUFA levels were associated with gametogenesis, whereas the influence of population was most likely due to location and variation in phytoplankton community. Diet had a stronger influence on FA profile than temperature, although phytoplankton blooms occurred in response to changes in temperature.

The FA profiles of larval and postset clams showed evidence of endogenous influence at early stages and dietary influence at later stages. When choosing algal species for broodstock conditioning, the FA profile of the broad categories (SFAs, MUFAs, PUFAs) as well as the individual FAs within each category need to be considered. This study suggests that the production of high quality SRV gametes is dependent on the FA profile of algal species fed during conditioning and that algal species rich in n-3 PUFAs and SFAs, such as *Isochrysis* and *Pavlova* sp. may be more important than those rich in n-6 PUFAs and MUFAs, such as *Chaetoceros* sp. It has been suggested that the use of multiple algal species, simulating the variety of phytoplankton in the natural environment, may result in increased gamete and larval production. The two most commonly used algal combination in commercial bivalve operations consist of *Isochrysis galbana* and *Chaetoceros gracilis*. Although these species differ in FA profile, as noted previously, the profiles complement each other. Additional research should focus on whether algal species other than combinations used in hard clam production would improve gamete production in sunray venus clams

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