Building Hatchery Capacity Production of a Promising Shellfish Aquaculture Species: the Sunray Venus Clam *Macrocallista nimbosa*

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FINAL REPORT

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GOAL AND OBJECTIVES:

The goal of this project was to build hatchery capacity in Florida by providing the necessary infrastructure via a public-private partnership with researchers, Sea Grant specialists, and industry members to commercialize production of sunray venus clam *Macrocallista nimbosa* seed. We hypothesized that by defining maturation and conditioning protocols for spawning, assessing genetic diversity of broodstock and seed stocks, and refining performance in commercial land-based nurseries, reliable seed production will be accomplished, which has been achieved with hard clams. Specifically, we proposed to:

1) Determine optimal conditioning requirements and spawning techniques that result in consistent spawning success,
2) Determine optimal hatchery techniques that result in increased production and metamorphosis to post-set,
3) Develop additional multi-generational culture lines for commercial operations,
4) Develop methodologies to analyze the genetic diversity of wild sunray venus clam populations and current and future created culture lines, and
5) Evaluate the performance of nursery systems and practices on production of nursery seed.

Objective 1: Conditioning and Spawning

Objective 1a: Effect of feeding rates and temperatures

Three changes were made to the original proposal:

1. The original proposal specified that spawning attempts would take place after 4, 6 and 8 weeks of conditioning. Feedback from industry indicated that the initial 4 weeks was too short of a conditioning period and that 2 weeks between spawns was likewise too short of a period, spawning attempts were made 6, 9, and 12 weeks post-conditioning.

2. The original proposal specified that 4 maturation temperatures (18, 21, 24, 27^{o}C) and 3 maturation feed rates (2, 4, 6%) would be evaluated. Due to feedback from industry and maturation tank constraints the highest temperature (27^{o}C) was eliminated from the study. For comparison between hatcheries, and due to the need to balance the needs of running a commercial operation with that of research our industry partner, Bay Shellfish chose to evaluate one temperature (21^{o}C), that is typically used for hard clam maturation, and all 3 feed rates.

3. Bay Shellfish has been removed from the project (effective September 2020). We have been unable to obtain any information from experiments he stated previously that he had completed. The issue was due to financial accounting requirements by FAU, that unbeknownst to Bay Shellfish and the PI of this project were necessary for a Co-PI. An offer to instead convert Bay Shellfish status to a vendor was rejected by Bay Shellfish.

Materials and Methods:

Thirty adult clams were placed into one of nine 3-ft dia round broodstock tanks (150 gallons). Each tank contained 4 trays filled with sand to which 7-8 clams were placed. Tanks were fitted with temperature

controllers to maintain one of three temperatures (18, 21, 24°C). The daily feed ration was calculated based on % dry meat clam weight and clams were fed a combination of *Isochrysis galbana*, Tahitian strain and *Chaetocerous gracilis* delivered at one of three feed rates (2, 4, 6%) by drip feeding 24 hrs/day. Tanks were cleaned every 10 days. Spawning was attempted after 6, 9 or 12 weeks of conditioning. If spawning was unsuccessful during the first attempt (6 weeks), a second attempt was made at 9 weeks, followed by a 3rd attempt at 12 weeks, if necessary. As there were not enough tanks available to perform replicated trials (n=27 necessary, only 12 available), three separate trials were conducted over one year (April 2019 to April 2020).

Following fertilization counts, an equal number of fertilized eggs (≤ 3 million) from each treatment group were added to three separate larval tanks, and the percentage that developed to D stage was calculated. Larvae were fed 4 liters of T-iso (~ 6 million cells/ml) for the first few days, which was increased to 20 liters over time. Drain downs were conducted each day, and larvae transferred to a new larval tank. Larvae were set within the larval tanks (screen, diameter) and transferred to downwellers within 2 weeks. Post-set were fed ~ 90 L (0.1 million cells/ml).

Results HBOI:

Forty-six spawns were attempted within 4 time periods between 2019 and 2020. Of that 19 spawns (41%) were successful (i.e. males, females or both spawned), and 16 (35%) resulted in the production of fertilized eggs/D-stage larvae (Table 1).

For each spawning attempt, if no successful clam spawn was achieved on day one, clams were collected, loosely bagged and left dry in an air conditioned room overnight. The following day a second spawn was attempted. If no clams spawned on day two, they were returned to their respective broodstock conditioning tanks. Of those successful spawns 9 (47%) required a second attempt on day two (Table 1).

Trial 1 began in April and ended August 2019. Trial 2a began August 2019, but was aborted due to Hurricane Dorian (*see problems encountered*). Trial 2b began October 2019 and ended in January 2020. Trial 3 began in January and ended April 2020. Based on results obtained at Bay Shellfish, a small scale trial was conducted from June to August 2020, in which clams were fed at a 12% feed rate.

Spawning and fertilization rate

In the 1st trial, clams fed at a 6% feed rate spawned after 9 weeks of conditioning, those fed at 2% at 12 weeks, while clams fed at 4% failed to spawn following 12 weeks of conditioning (Table 1). Clams maintained at all 3 temperatures spawned, and all spawns occurred on the first attempted day of spawning. Fertilization was less successful at 18°C than at 21 or 24°C.

In the 2nd trial clams fed at a 2% feed rate spawned after 6 weeks of conditioning, those fed at 4% at 9 weeks, and clams fed at 6% at 12 weeks (Table 1). No clams spawned at 18°C in the 6% group, and few clams spawned at 21°C in the 2% group, and at 24°C in the 6% group. For both the 2% and 4% groups, regardless of temperature clams did not spawn on the first day of the attempt. Fewer eggs were fertilized overall regardless of temperature. Whether this was related to nutritional stress following Hurricane Dorian that occurred between the two trials (all groups received 2% feed) or that more conditioning time was needed between spawning trials for conditioning is unclear.

The only group that spawned during trial 3 were clams fed at a 6% feed rate, after 12 weeks of conditioning. Spawning occurred on the first attempted day and no differences were seen between temperatures. Fertilization was less successful at 18°C than at 21 or 24°C.

Feeding to satiation did not result in earlier spawns, and only those at 21°C spawned. It would appear, that after an initial SRV spawn, a minimum of 12 weeks of further conditioning is needed before a subsequent successful spawn, regardless of feed rate.

Table 1. Successful spawns obtained with SRV clams fed at 2, 4 or 6%, and maintained at 18, 21 or 24°C. An * indicates spawning occurred on day 2, of the attempted spawn.

Treatment	Weeks Cond	Cycles (n)	Temp range (°C)	Cycle phase	Spawned males (n) (%/total)	Spawned females (n) (% total)	Fertilized eggs (mill)
Trial 1 (Apr-Aug'19)							
6%, 18C (Jun)	9	1	21-28	Heat 1	7 (23%)	2 (6.7%)	3.4
6%, 21C	9	1	21-28	Heat 1	17 (57%)	7 (23%)	18.5
6%, 24C	9	1	21-28	Heat 1	11 (37%)	6 (20%)	10.4
2%, 18C (July)	12	3	21-30	Heat 1	13 (43%)	5 (17%)	4.3
2%, 21C	12	3	21-30	Heat 1	9 (30%)	4 (13%)	21.4
2%, 24C	12	3	21-30	Heat 1	8 (27%)	9 (30%)	21.9
Trial 2 (Oct'19-Jan'20)							
2%, 18C*(Nov)	6	3	21-31	Heat 1	21 (75%)	4 (14%)	8.9
2%, 21C*	6	3	21-31	Heat 1	2 (25%)	0	NA
2%, 24C*	6	3	21-31	Heat 1	6 (23%)	12 (46%)	10.5
4%, 18C*(Dec)	9	1	21-31	Heat 1	16 (59%)	7 (26%)	5.2
4%, 21C*	9	1	21-31	Heat 1	12 (52%)	4 (17%)	3.1
4%, 24C*	9	1	21-31	Heat 1	21 (78%)	4 (15%)	3.6
6%, 18C (Jan)	12	3	21-31	Heat 1	0	0	NA
6%, 21C	12	3	21-31	Heat 1	1 (3.5%)	3 (10.7%)	1.47
6%, 24C	12	3	21-31	Heat 1	1 (3.5%)	0	NA
Trial 3 (Feb-Apr'20)							
6%, 18C (Apr)	12	3	21-31	Heat 1	4 (16%)	5 (20%)	1.2
6%, 21C	12	3	21-31	Heat 1	2 (7.7%)	6 (23%)	2.3
6%, 24C	12	3	21-31	Heat 1	3 (11.5%)	5 (19%)	1.9
Trial 4 (June-Aug'20)							
12%, 18C*(Aug)	12	4	21-31	Heat 3	1 (3.3%)	0	0
12%, 21C*	12	4	21-31	Heat 3	7 (30.4%)	6 (26%)	3.6
12%, 24C*	12	4	21-31	Heat 3	3 (10.7%)	0	0

D stage Development

In the 1st trial, the percent of fertilized eggs that developed to D stage was highest in the 6% treatment (98.6%) regardless of maturation temperature. Only 42% of fertilized eggs in the 2% treatment developed to D stage; temperature differences were evident, with fewer clams maintained at 18°C developing to D stage (Table 2).

In the second trial a higher percentage of fertilized eggs in the 2% treatment group (60%) achieved D stage compared to the 1st trial, with little difference seen between temperatures (Table 2). A higher percentage of fertilized eggs in the 2% group achieved D stage compared to the 4% group (43%). In contrast, temperature differences were seen in the 4% group, with fewer fertilized eggs developing to D stage at 18C.

In the 3rd trial, temperature impacted development to D stage, with less development occurring at lower temperatures.

In the 4th trial only those kept at 21°C spawned. Survival to D stage was high, but could not be compared to performance at other temperatures.

D stage to post-set

In the 1st trial, few D stage larvae in the 6% group developed to post-set. This was the first successful spawn and technician mistakes in larval rearing occurred. Improvement in larval rearing protocols were initiated by the 2nd spawn resulting in higher survival. Survival to post-set was slightly higher in the 18°C group, but this group also had a lower density. Survival to 1 mm was higher in the 21°C group.

In the 2nd trial, survival was higher at 21 and 24°C regardless of % fed. Post set survival was only compared for the 2% group and was higher at 18°C than 24°C. A water quality issue in the nursery affected post set clams from the 4% and 6% spawns, resulting in spawns being dumped prior to reaching 1 mm.

Survival from D stage to post-set was lower in the 3rd trial compared to previous trials, due to the necessity of maintaining post-set clams in larval tanks for too long due to nursery space issues. Highest survival was seen at 24°C and lowest at 18C. Survival of post-set to 1 mm followed a similar pattern.

Clams fed to satiation in trial 4 had comparable survival to post-set and to 1 mm as those fed at 6% in and maintained at 21°C.

Table 2. Development of fertilized SRV eggs to D-stage and post-set under various maturation conditions.

Treatment	Fertilized eggs tank (mill)	D-stage larvae (mill)	% Fert to D stage	Post-set (n)	% D stage to Post-set	% Post-set to 1 mm
Trial 1 (Apr-Aug)						
6%, 18 C	3.0	2.88	96%	40,000	1.3%	NA
6%, 21 C	3.0	3.4	100%	20,000	0.6%	NA
6%, 24 C	3.0	3.1	100%	890,000	30%	NA
2%, 18 C	3.0	0.77	26%	610,00	79%	17%
2%, 21 C	3.0	1.83	61%	1.2 mil	66%	29%
2%, 24 C	3.0	1.19	40%	820,00	69%	16%
Trial 2 (Oct-Jan)						
2%, 18 C	3.0	2.1	70%	1.4 mil	70%	56%
2%, 21 C	NA	NA	NA	NA	NA	NA
2%, 24 C	3.0	1.5	50%	1.2 mil	80%	36%
4%, 18 C	3.0	0.840	28%	570,000	68%	NA
4%, 21 C	3.0	1.3	43%	960,000	74%	NA
4%, 24 C	3.0	1.7	57%	1.5 mill	88%	NA
6%, 18 C	NA	NA	NA	NA	NA	NA
6%, 21 C	1.4	0.710	50%	500,000	70%	NA
6%, 24 C	NA	NA	NA	NA	NA	NA
Trial 3 (Feb-Apr)						
6%, 18 C	0.460	0.080	17%	20,000	25%	21%
6%, 21 C	0.460	0.116	25%	40,000	34%	46%
6%, 24 C	0.460	0.263	57%	148,000	56%	60%
Trial 4 (June-Aug)						
12%, 18 C	NA	NA	NA	NA	NA	NA
12%, 21 C	3.6	2.7	75%	1.5 mill	56%	42%
12%, 24 C	NA	NA	NA	NA	NA	NA

Twenty clams of each sieve size from the 2% group from trial 1 were measured. The average length and min/max values are given below (Table 3). Survival of the 2% post-set from trial 1 to 1 mm was 22% (577,787/2.6 million), with the 21°C treatment showing highest survival (Table 4). Clams had been in the hatchery for 3 months prior to sieving (July 7 2019 spawn, October 10 2019 sieve date)

Table 3. Average length (mm) of sieved clams from trial 1, 2% feed rate

Sieve Size (mm)	Avg Length (mm)	Range (mm)
<1.0	1.19 ± 0.18	0.7-1.35
1.0	1.67 ± 0.065	1.6-1.8
1.2	2.20 ± 0.141	2.0-2.4
1.6	3.03 ± 0.216	2.8-3.5
2.0	4.98 ± 0.349	4.3-5.4
4x3	6.96 ± 0.925	5.9-8.6

Table 4. Survival and sieve size of 2% post-set (1 mm) from trial 1.

	Initial Post set	Sieve count	Survival	< 1.0 mm	1-1.2 mm	1.2-1.6 mm	1.6-2.0 mm	2.0 mm	3x4 mesh
2%, 18C	610 K	105.6 K	17%	6,748	8,521	35,706	32,013	21,682	923
2%, 21C	1.2 mill	339.6 K	28%	57,358	51,125	113,299	71,865	43,364	2,586
2%, 24C	820 K	132.5 K	16%	9,559	9,748	44,976	34,299	31,058	2,955
Total	2.6 mill	577.7K	22%	73,665	69,394	193,981	138,177	96,104	6,464

Results obtained from Bay Shellfish:

Bay Shellfish was unable to achieve spawns at 6, 9 or 12 weeks at 21°C when feeding at a 4% rate. When the feed rate was doubled to 8% SRV's spawned, however, it is unclear how long they were conditioned at that rate before an attempt was made and no additional information was forthcoming, such as number of clams that spawned, number of males and females, fertilization rates or development to D stage. Based on results obtained from Bay Shellfish a 4th trial was conducted at FAU-HBOI to determine the effect of feeding at a higher rate.

Problems encountered:

We were in the midst of the 2nd replicate run when Hurricane Dorian struck. Although there was no damage to facilities, we were not allowed onto campus for a week, during which time tank temperatures were at ambient (~ 24°C) and clams were not fed. Algae cultures crashed and it took approximately 1 month before algae production was up and 2 months before we could re-start the experiments, as priority for micro-algae was given to on-site industry partners. The result was a 3 month delay in achieving this objective. During this time we also lost a third of the clams in the 2%, 21°C treatment group, necessitating a redistribution of the 2% clams for the 3rd trial (20/group). Smaller loses were seen in the 4% treatments (16% loss) and in the 6% treatments (5% loss).

Objective 1a Conclusions: Even without additional input from Bay Shellfish it would seem that higher feed rates (≥6%) are necessary for SRV maturation, and that both 21° and 24°C are preferable to 18°C for SRV broodstock conditioning.

Objective 1b: *Effect of diet on spawning and fertilization success*

Due to die-offs experienced during closures due to Hurricane Dorian (2019) and more recently Hurricane Iasiah (2020) not enough cultured broodstock (n=65) remained to conduct replicate studies. In November and December 2020 wild broodstock were collected from two locations (80, 30) that were used to achieve both this objective and objective 3. Clams were conditioned in January at 21°C and spawns were conducted in April 2021.

Although a diet consisting of either *Chaetoceros gracilis* alone or a 50:50 ratio of *C. gracilis* and T-iso resulted in earlier spawning, the T-iso spawn that occurred at 12 weeks resulted in an increased number of spawning clams and therefore a greater number of fertilized eggs (Table 5).

Table 5. Spawns obtained after conditioning broodstock with one of three dietary treatments. An * indicates spawning occurred on day 2 of the attempted spawn.

Treatment	Weeks Cond	Cycles (n)	Temp range (°C)	Cycle phase	Spawned males (n) (% total)	Spawned females (n) (% total)	Fertilized eggs (mill)
100% Cg*	9	1	21.0-31.0	Heat 1	1 (3%)	2 (5%)	920,000
50%/50% Cg/Ig	9	1	21.0-31.0	Heat 2	1 (3%)	2 (5%)	630,000
100% Ig	9	3	21.0-31.0	NA	0	0	0
100% Ig	12	1	21.0-31.0	Heat 1	9 (26%)	9 (26%)	12,051,000

Although not significant, a greater percentage of fertilized eggs (20-25%) achieved D-stage with *C. gracilis* or a 50:50 *C. gracilis*, T-iso mix (Table 6). Although not significant, the mixed diet resulted in slightly higher survival to post-set (5-15%). Both *C. gracilis* alone and the 50:50 mix resulted in higher survival from fertilized eggs to post-set (50.5, 55% vs. 40%) even though clams fed T-iso had been conditioned longer (Table 6).

Table 6. Development of fertilized SRV eggs to D-stage and post-set for groups fed one of three dietary treatments.

Treatment	Fertilized eggs tank	D-stage larvae	% Fert to D stage	Post-set (n)	% D stage to Post-set
100% Cg*	920,000	655,000	71.2	465,000	71.0
50%/50% Cg/Ig	630,000	422,000	67.0	350,000	82.9
100% Ig	2mil, 2mil, 2mil	1.0mil, 1.15mil, 1.1mil	50 58 55	680,000, 875,000, 825,000	68 87.5 83

Objective 1b Conclusions: It is typical hatchery practice to feed a mixed diet during conditioning. This experiment supports that this industry standard mix is the best diet of the 3 tested for broodstock conditioning and reiterates the conclusion reached in objective 1a that a minimum of 12 weeks conditioning is preferable. Due to time and space constraints we weren't able to evaluate 25:75 or 75:25

ratios or effects of other species (i.e. *Thalassiosira* sp. versus *C. gracilis*), however this limited study suggests the importance of diatoms over that of flagellates in conditioning.

Objective 2: Hatchery Techniques

Objective 2a*: Effect of temperature and drain downs*

At 25°C development from fertilized egg to D stage averaged of 16% for tanks that were drained down each day compared to 14.8% survival for drain downs conducted every other day (P=0.49) (Table 7). Average survival from D-stage to post-set was slightly higher (55.6%) for tanks drained down on alternate days, but not significantly different (P=0.86) from daily drain-downs (43.9%).

At 28°C development from fertilized egg to D stage averaged 55% for tanks that were drained down every day compared to 57% for tanks drained down on alternate days (P=0.75) (Table 6). Average survival from D-stage to post-set was slightly higher (71.3%) for tanks drained down on alternate days, but not significantly different (P=0.23) from daily drain-downs (62.6%).

However, development to D stage from fertilized egg was significantly different dependent on temperature ($F_{1,8}$=111, P<0.0001), but not with respect to whether tanks were drained down every day or every other day ($F_{1,8}$=0.0004, P=0.98) and there was no interaction between the two ($F_{1,8}$=0.014, P=0.75). However, no significant differences were seen between survival from D-stage to post-set, with regards to either drain down time ($F_{1,8}$=0.033, P=0.895), temperature affect ($F_{1,8}$=4.2, P=0.073) or the interaction of the two ($F_{1,8}$=1.48, P=0.257).

Objective 2a Conclusions: Development of fertilized egg to D stage is not impacted if drain-downs do not take place every day at either 25°C or 28°C, however development is affected by decreased temperature. Survival of D stage to post-set is not affected by either drain down time or whether cultured at 25° or 28°C, although there was a slight trend towards higher survival at 28°C. Hatchery personnel could therefore cut labor cost by conducting drain downs on alternate days.

Table 7. The survival impact of conducting daily drain downs versus drain downs conducted on alternate days.

Drain Down Frequency	Group	Temperature	Fertilized eggs (n)	D-Stage (n)	Survival to D-stage (%)	Post-set (n)	Survival to Post-set from Egg (%)	Survival to Post-set from D-stage (%)
Everyday	#4	25' C	590,000	80,000	13.5	20,000	3.4	25
	#5	25' C	1,130,000	76,667	6.8	40,000	3.5	52.2
	#6	25' C	945,000	263,333	27.8	143,333	15.2	54.4
Alternate days	#1	25' C	590,000	72,500	12.3	31,667	5.4	43.6
	#2	25' C	1,130,000	143,333	12.7	61,667	5.5	43
	#3	25' C	945,000	185,000	19.6	148,333	15.7	80.2
Everyday	#4	28'C	2,000,000	1,070,000	54	680,000	34	63
	#5	28'C	2,000,000	1,150,000	58	875,000	44	76
	#6	28'C	2,000,000	1,100,000	55	825,000	41	75
Alternate days	#1	28'C	2,000,000	997,000	50	625,000	31	62
	#2	28'C	2,000,000	1,250,000	63	905,000	45	71
	#3	28'C	2,000,000	1,150,000	58	650,000	32	55

Objective 2b: Effect of diet **Note these experiments were the focus of the MS student thesis*

Experiment 1, Live micro-algae dietary treatments: Six treatment groups (n=3 replicates) were stocked with SRV post set (1079 ± 39 clams per replicate, initial average shell length of 0.91±0.24 mm). Growth and survival were assessed for six weeks. Treatment groups were:

1. *Isochrysis galbana* T-iso (50%) + *Pavlova lutheri* (50%)
2. *Chaetoceros gracilis* (50%) + *Thalassosoria wessflogii* (50%)
3. *I. galbana* T-iso (50%) + *C. gracilis* (50%)
4. *I. galbana* T-iso (25%) + *P. lutheri* (25%) + *C. gracilis* (25%) + *T.wessflogii* (25%)
5. *I. galbana* T-iso (33.3%) + *P. lutheri* (33.3%) + *C. gracilis* (33.3%)
6. *I. galbana* T-iso (33.3%) + *C. gracilis* (33.3%) + *T.wessflogii* (33.3%)

Daily growth rate was significantly higher ($F_{(5,354)} = 6.8$, $P < 0.05$) in T4, the tetra-algal diet (Ig+Pl+Cg+Tw) (80.4 µm ± 6.1 µm) than that of the bi-algal dietary treatments (T1, T2, T3) and T6 ($P < 0.05$) (Figure 1). Clams fed the bi-algal diet T2 diatom diet (Cg+Tw) had the lowest daily growth rate (57.4 µm ± 10 µm) as well as the most variation in size. Unexpecting, SRV clams fed T3, the hard clam industry standard diet (Ig+Cg) also exhibited slow growth; all 3 bi-algal diets resulted in lower growth than the tri-algal and tetra-algal diets. No differences were seen between the two tri-algal or the three bi-algal dietary treatments. Final size (shell length, mm) of SRV clams (n=60/treatment) showed a similar pattern, with SRV post-set in T4 attaining the largest size (3.5±1 mm) over a six-week period, and clams in T2 being the smallest (2.5±1.5 mm) and showing greatest variability in shell length (Figure 2).

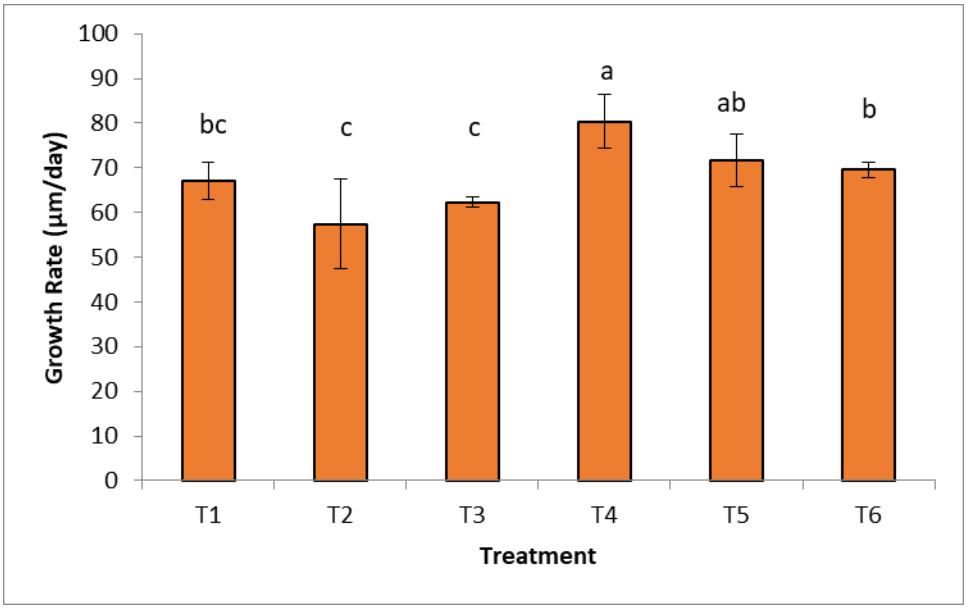

Figure 1: Daily growth rate (±SD) of SRV clam (*Macrocallista nimbosa*) post-set fed live micro-algal diets.

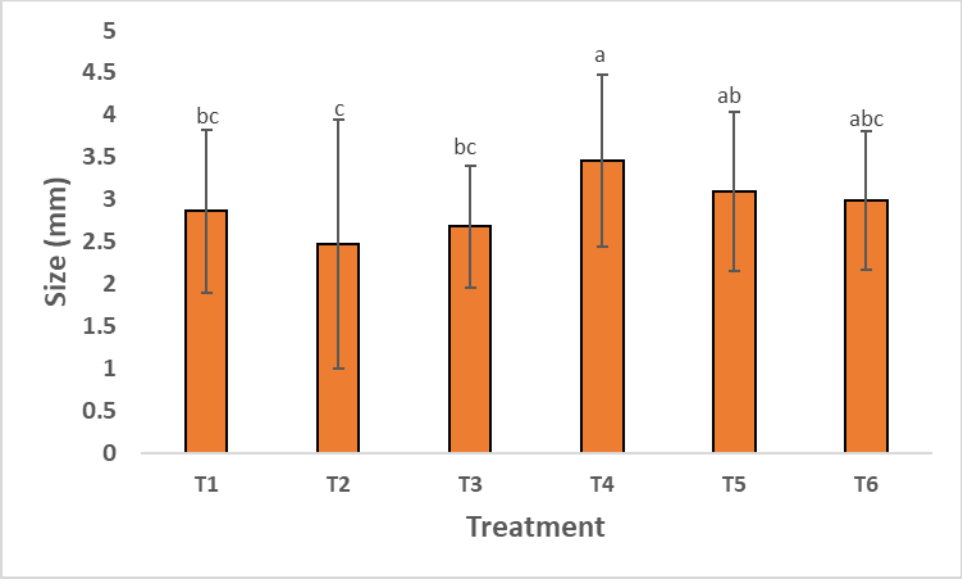

Figure 2: Final size (±SD) of SRV clam (*Macrocallista nimbosa*) post-set fed live micro-algal diets.

Survival was also affected by dietary treatment, however in contrast to growth, survival was significantly higher ($F(5, 12) = 9.2$, $P = 0.0008$) in T3 (Ig+Cg) (Figure 3). SRV clams fed the hard clam industry standard bi-algal diet had significantly higher survival (88.1% ± 6.9%) compared to all other dietary treatments (Figure 2). The lowest survival was seen in the T2 bi-algal diatom diet (Cg+Tw) and theT5 tri-algal diet (Ig+Pl+Cg), 65.3% ± 4.1%, and 61.9% ± 6.6% respectively. These two treatment groups showed significantly lower survival than all other groups ($P < 0.05$).

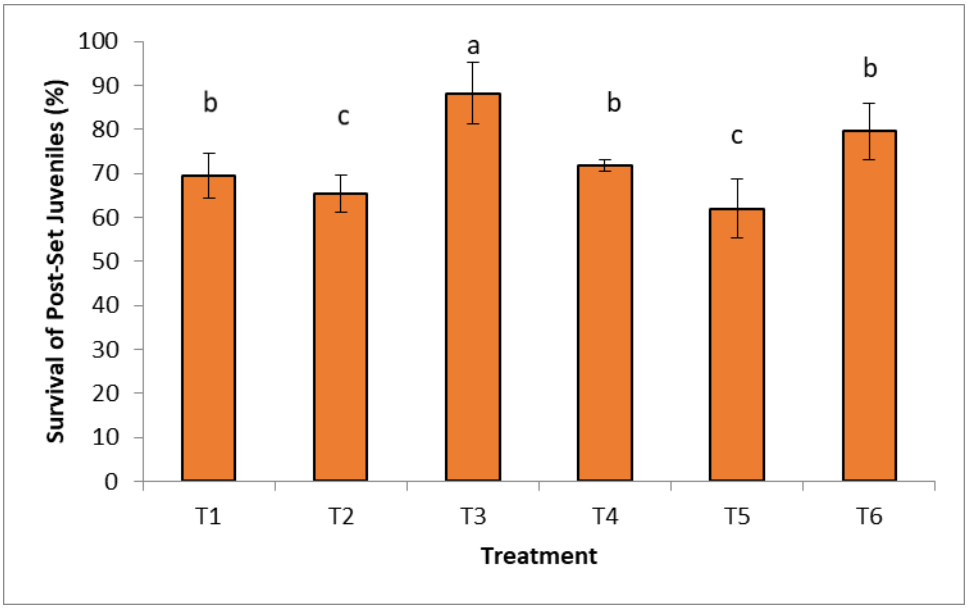

Figure 3: Survival of post-set sunray venus clam (*Macrocallista nimbosa*) fed live micro-algal diets.

Objective 2b, experiment 1 conclusions: The best diet for growth of SRV clam is not necessarily the diet that results in optimal survival. Although SRV clams fed the bi-algal industry standard diet had the highest survival diets mixed diets with multiple species (3-4 flagellates and diatoms) were larger. The

diet that consistently performed poorly (both growth and survival) was the diatom only diet. Therefore, a diet consisting of equal proportions of diatoms and flagellates results in increased production, and that a mixed species diet of three or more algal species may be beneficial.

Experiment 2, Live micro-algae dietary treatments versus concentrate diets Six treatment groups (n=3 replicates) were stocked with SRV post set (1079 ± 43 clams per replicate, initial average shell length of 0.89 mm). Growth and survival were assessed for six weeks. Treatment groups consisted of either 100% live microalgae, a 50% partial or a 100% complete replacement diet (Reed Mariculture). Treatment groups were:

1. *I. galbana* T-iso (live) (100%)
2. *I. galbana* T-iso (live) (50%) + *Chaetoceros gracilis* (live) (50%)
3. *I. galbana* T-iso (live) (50%) + Isochrysis 1800 concentrate (50%)
4. *I. galbana* T-iso (live) (25%) + *C. gracilis* (live) (25%) + Shellfish Diet 1800 concentrate (50%)
5. Isochrysis 1800 concentrate (100%)
6. Shellfish Diet 1800 concentrate (100%)

SRV clams, fed T2, the bi-algal Ig+Cg live microalgal diet were significantly larger (3.7 ± 0.7 mm) (Figure 4) than all other treatments ($P < 0.05$). Post-set clams fed diets that incorporated concentrates as either a partial or complete replacement for live microalgae resulted in lower growth. The smallest clams were seen in treatments fed only concentrate diets, with poorest growth (1.4±.0.6 mm) in T6 (100% Shellfish Diet 1800™).

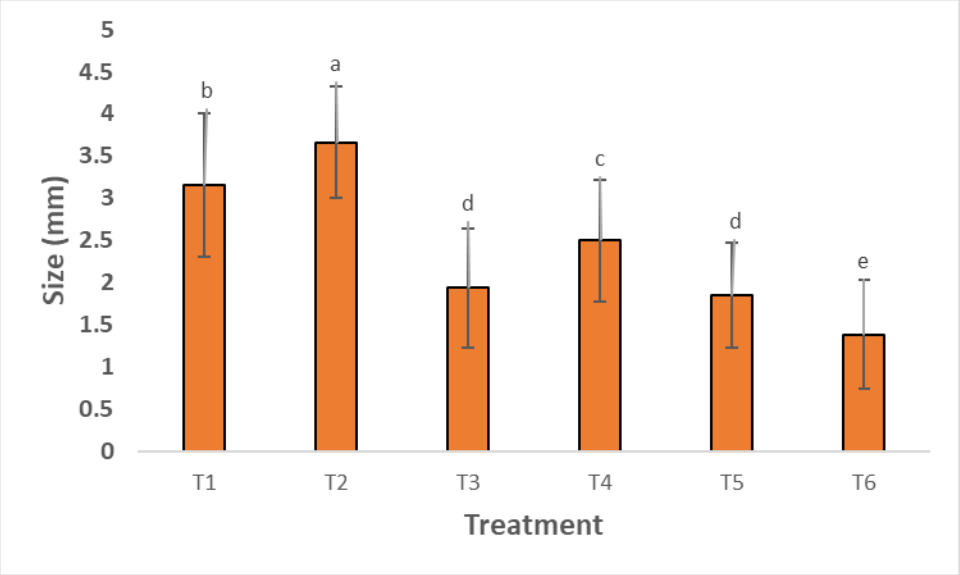

Figure 4: Final size (±SD) of sunray venus clam (*Macrocallista nimbosa*) fed live or concentrate diets.

Highest survival (78%±0.8; 76%±2) was seen in the two live diets T1 (Ig) and T2 (Ig+Cg) (Figure 5). Survival was significantly lower (44±2.5, 30±1.8) in treatments fed a 100% algal concentrate diet (T5, T6). Although clams fed the two live diets did not differ significantly from each other those fed the partial and complete replacement diets did. While the mixed partial replacement diet (T4) incorporating Shellfish 1800 had better survival than the single algal species partial replacement diet (T3) incorporating Isochrysis 1800, the opposite effect was seen with complete replacement diets with higher survival noted with Isochrysis 1800 (T5).

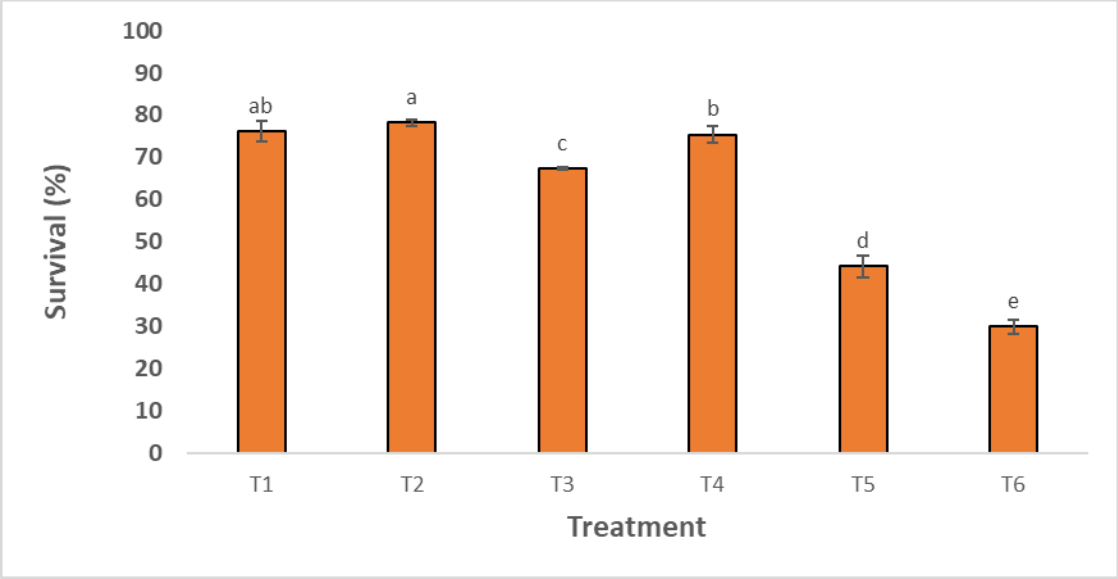

Figure 5: Survival (±SD) of sunray venus clam (*Macrocallista nimbosa*) fed live or concentrate diets.

Objective 2b, Experiment 2 Conclusions: SRV clams performed better with live microalgal diets than with partial or complete concentrate replacement diets. Yet there is some benefit in using concentrates at least as a supplement to live algae, as survival over a six week period was adequate (5-12% lower than the corresponding live diet). This suggests that algal concentrates can be used as a supplement to live algae in situations in which live micro-algae production is not adequate. Although SRV clams fed a complete replacement diet did not perform well over a six-week period, consideration of their use during short-term situations in which live micro-algae production is halted, i.e. storms, power outages, may be warranted. Unfortunately, shorter term growth and survival was not assessed. Use of these algal concentrate diets, originally designed for oyster production, is not recommended as a replacement for live microalgae for SRV clams. In addition to decreased production, clumping, tank fouling and accumulations of concentrates on the tank bottom and problems with keeping them suspended in the water column were noted, all of which may have affected the ability of the clams to successfully ingest the concentrates.

Objective 3: Develop additional multi-generational culture lines

The original objective involved the creation of 4 new lines (AHxAH, SHKxAMI, SHKxF2, AMIxF2 to supplement the previously 2006 to 2008 created F2(AHxSHK), SHKxSHK and AMIxAMI stocks:. Due to multiple hurricanes that have impacted the panhandle over the past few years Alligator Harbor (AH) wild stocks have disappeared from previous locations. The few that could be found during this study were used for objective 4, reducing the creation of 4 new additional lines to 3: SHKxAMI, SHKxF2, AMIxF2.

Prior to the start of this study it was realized that all previously held lines, were quite old, well past breeding age, and few individuals remained and that the younger F2 stocks produced in 2015-2016 were actually from F2xAMI crosses. We therefore elected to create two new culture lines from wild stock: SHKxSHK and AMIxAMI, for a total of five new genetic lines. We were successful in creating four of those lines (Table 8).

Wild stocks of SRV's from Sea Horse Key (SHK), located near Cedar Key and wild stocks from Anna Maria Island (AMI) near Tampa Bay, were collected in November 2020, conditioned for three months and spawned 1/12/2021 to create three new/replacement lines were created: SHKxSHK, AMIxAMI and SHKxAMI. In conjunction with the broodstock dietary experiments (Obj 1b) we attempted to create the two cultured x wild stock lines but were only successful in producing culturedxSHK. The cultured stock used in all of these experiments (F2(=AHxSHK)xAMI) originated from 2015-2016 HBOI-SLP and FL-ARC funded studies. By the time genetic crosses were attempted these clams were five years old, had spawned numerous times, and only about ½ of the original population remained from the 2018 starting population resulting in a "spawned out" population. In January only 1 male produced sperm and in April only 2 cultured females produced eggs.

Table 8. Summary of newly developed SRV lines.

Cross	# M and F	Date Produced	# Eggs	# D stage	# Post set (1 Wk)	# Post Set (8 Wk)	Recipients
SHKxSHK	7 M, 4 F	1/13/2021	3,000,000	2,300,000		189,735	3
							(H,S,W)
AMIxAMI	4 M, 6 F	1/13/2021	3,700,000	2,500,000		298,990	3
							(H,S,M)
SHKxAMI	11 M, 10 F	1/13/2021	1,900,000	1,000,000		42,624	2, HBOI
							(H,S,HBOI)
SHKxAMI	1 M SHK, 2 F AMI	4/24/2021	920,000	655,000	465,000		HBOI
CulturedxSHK	1 M SHK, 2 F cult	4/25/2021	630,000	422,000	350,000		HBOI

The resulting 1 mm post set from the January spawn were distributed to three participating (objective 5) partners in March/April 2021 and HBOI (Table 8). The participants agreed to keep these genetic lines separate from previous distributed seed so that they can be evaluated genetically. The four created lines will be maintained at both FAU-HBOI and UF for use as broodstock for future projects, for future genetic analysis and as known lines of genetic stock for distribution to individual hatcheries.

**Addendum: April 2021 spawns were maintained at HBOI through 2021 and later divided between HBOI and one participating nursery. Four additional hatcheries/nurseries (2 East coast, 2 West coast) received SRV clams from the SHKxAMI and CULxSHK spawns, that had been maintained at Cedar Key, in April 2022 to use as broodstock.*

Objective 4: Analysis of Genetic Diversity

Mitochondrial DNA analysis, used to determine female lineage, was used to compare genetic diversity between wild SRV from different locations (Figure 5 map) and cultured clams. Ninety-four clams (44 wild, 60 cultured) were analyzed for genetic diversity. Wild collected clams included 24 from SHK, 15 from AMI, 2 from AH, 3 from North Carolina. Cultured stock included 18 from a 2019 spawn of the parental F2xAMI (=line F2A 2015/2016 spawn), 15 from F2AxSHK, 13 from AMIxSHK and 14 from AMIxSHK (Table 9). In addition a haplotype from Genbank collected from Ten thousand Islands was added for comparison.

Problems encountered: Only four clams were collected from Alligator Harbor during two trips in 2020 in areas where they had previously been plentiful due to hurricane impacts. Only three clams were found in North Carolina (Dave Cerino, Carteret Co. Comm. College), collected from two locations in 2020 in which they had previously been found historically.

Sixteen haplotypes were identified (Table 9). The most genetic diversity was seen within the SHK population. Of the 16 haplotypes 10 were found in the wild SHK clams. In contrast the 15 wild AMI clams had very little genetic diversity with only 2 distinct haplotypes. Two haplotypes were represented by the 2 AH clams, one shared with the dominant SHK haplotype and the other unique.

Progeny of the parental culture line (F2xAMI) line also showed low diversity with only two haplotypes, 1 unique (H06) and 1 (H01) shared with wild SHK, AH and NC clams. The original F2 line was developed from an SHKxAH cross. That parentage is reflected in 77% of the progeny and indicates that the females were likely from that line, while males were likely from the AMI line, as their genetic contribution would not be reflected in mtDNA analysis. The remaining 22% of those clams have a unique signature which could be reflective of any of the 3 locations. Three of the five lines created during this project have been analyzed an SHKxculture (c1), and 2 AMIxSHK (c2, c3) crosses. Both the C1 and C2 crosses share an identical AMI haplotype, while the C3 cross shares both AMI and SHK haplotypes as would be expected and is the most genetically diverse of the 3 lines. No analysis was done of the AMIxAMI or SHKxSHK crosses. It would be interesting to see, especially for the SHKxSHK cross whether genetic diversity was retained or loss.

Of most interest were the NC haplotypes. Two of the clams were collected from one location and the third from another. The single collected clam had a distinct haplotype, expected for an “outlier” group. The other two clams, collected from a different location, had the most common haplotype H01, shared with SHK, and AH wild clams and the F2A culture line. This was unexpected. One possible explanation is that NC has been receiving SRV clams from FL hatcheries for their SRV program for a few years and it is possible that these 2 collected clams could have somehow “escaped” from grow-out trials there. However, this is pure speculation at this point.

Figure 5. Location of natural populations of SRV clams collected from Florida's west coast.

Table of Haplotypes of mtDNA COI shared among SRV											
	Wild Stock							Cultured Lines			
Haplotype	SHK	AMI	AH	NC	10K	Wild Total		F2A	C1	C2	C3	Total
H01	11		1	2		14		14			12	26
H02				1		1						
H03		11				11			15	13	1	29
H04		4				4					1	1
H05	4					4						
H06								4				4
H07	2					2						
H08	1					1						
H09	1					1						
H10	1					1						
H11	1					1						
H12	1					1						
H13	1					1						
H14	1					1						
H15			1			1						
H16					1	1						
N	24	15	2	3	1	45		18	15	13	14	60

Wild Stocks	Culture Lines
SHK - Sea Horse Key, Cedar Key FL	F2A -F2 x AMI
AMI- Anna Maria Island Broodstock	C1- SHK x culture Apr2021
NC - North Carolina	C2- AMI x SHK Apr2021
AH- StTeresa Beach/Alligator Harbor, FL	C3- AMI x SHK Jan2021
10k- Ten thousand Islands area (Genbank)	

Table 6. SRV wild and cultured clams showing 16 diverse haplotypes.

Objective 4 Conclusions: With such a small sample size from some locations it is hard to draw any conclusions. What can be stated at this time is that the SHK wild stock is the most genetically diverse of all the wild stock. Sea Horse Key is located mid-way between Alligator Harbor and Anna Maria Island (Figure 5) but only shares one haplotype with either location (AH, HO1). That AH and AMI clams share no haplotypes thus far may reflect their geographically distance from each other. That one of the F2xAMI haplotypes is shared with AH and SHK is not surprising as the F2 parental stock was created from a cross of the two. That this cross does not share a haplotype with AMI clams is somewhat surprising but as mentioned above it is unknown where the unique HO6 haplotype originated from. Of the three cultured crosses created for this project, two (C1, C2) exhibit no genetic diversity and are like AMI wild stock, while the third (C3) is more diverse representing both SHK and AMI haplotypes. With such a small sample size no conclusions can be drawn with regards to North Carolina and Florida SRV populations. That there are no known FL east coast SRV locations makes conclusions even more difficult.

Future considerations: It would be of interest to carry on additional analysis in the future, outside of the scope of this project, especially of the two wild crosses (SHKxSHK; AMIxAMI) produced, additional NC

collections and additional batches of F2A progeny, as a number of spawns were produced with that line during the course of this project.

Objective 5: Evaluate performance of nursery systems

The focus of this objective was to compare two systems (upwellers and raceways) in two seasons (spring, fall) to evaluate system and seasonal influences on post-set nursery clams (1mm-6mm). Although nurseries have been willing to participate it has proven difficult to find nurseries that have raceway systems as currently the preferred commercial nursery system used for rearing hard clams are upwellers. Therefore, the majority of the data obtained has been with upweller systems. In addition, a few nurseries that had been willing to participate in trials when this study was first proposed in 2017 either chose not to or were unable to participate (business closed) by the time SRV clams produced in this study became available. As a result only five nurseries participated in achieving this objective. Although not ideal from a replicate standpoint, valuable data can still be gleaned concerning system, coastal and seasonal differences and this data can be compared to results obtained in previous FLSG projects that evaluated SRV nursery performance.

Clams were distributed to five participating nurseries in fall 2019 (two east coast and three west coast). In spring 2020 clams were distributed to three participating nurseries (two east coast and one west coast). Low salinity at two of the west coast locations hampered participation. In the fall of 2020, the three west coast locations participated again. All nurseries except one (WC2) were field based nurseries. Data evaluated included salinity, temperature, growth (sieve sizes) and estimated survival.

SRV nursery clam production is detailed in Table 7. There are two raceway/downweller comparisons in the fall of 2019, none in spring 2020, and one in fall 2020. Only two facilities (east coast, west coast) had raceway systems available. One nursery (east coast) directly compared upweller and raceway systems, as well as downwellers. Two nurseries (west coast) located in close proximity used either upweller or raceway systems, which could be compared.

As different nursery operators, not only received different sized seed, but also kept them in their respective nurseries for various lengths of times, particularly in the fall of 2019, growth statistics were based on mm/wk. Unfortunately as survival was not assessed until clams were removed from the nursery and placed in grow-out bags, this likely resulted in skewing of survival results, particularly in fall of 2019, where the trial period ranged from 4-24 weeks. The trial periods in the spring of 2020 and the fall of 2020 were closer (10-12 and 8-12 weeks, respectively).

Systems

There was no statistically significant difference ($P>0.05$) in growth or survival between raceways and upwellers in fall 1 (2019) when data was compared for all facilities on both coasts or between paired facilities (EC1, WC1, WC2). However, if each paired facility on each coast is compared there was significantly higher survival in upwellers ($P=0.0001$), than raceways at the east coast facility, but no difference in growth. On the west coast there was significantly higher survival ($P>0.0001$) and growth ($P=0.044$) in raceways in fall 1 (2019), while in fall 2 (2020), growth was significantly higher in upwellers ($P=0.017$) but survival was significantly higher ($P>0.0001$) in raceways.

Season

Only three facilities could be compared with regards to season (2 east coast, 1 west coast), as two of the west coast facilities were unable to accept clams in the spring due to salinity issues. Therefore the only seasonal comparisons that could be made were with upweller systems. There were no seasonal differences with regards to either growth or survival (P>0.05) between the three systems. If each facility is compared separately with regards to season, EC1 and WC1 showed higher growth in fall (P>0.005), while EC2 showed higher growth in spring (P=0.006); however, no differences were seen in survival.

Table 7. Growth and survival of SRV clams in both fall and spring in various nursery systems.

Nursery	System	Sieve size	# Clams (initial)	Trial Period	# Clams (final)	Survival (%)	Initial Size (mm)	Final size (mm)	mm/wk
Fall 2019									
East Coast 1	Upweller	>1.2	64,660	8 weeks	48,000	74.0	2.6 ± 0.46	6.0 ± 1.4	0.425
East Coast 1	Race Way	>1.2	64,660	8 weeks	24,000	37.0	2.6 ± 0.46	6.0 ± 1.4	0.425
East Coast 1	Downweller	>1.2	64,660	4 weeks	30,000	46.0	2.6 ± 0.46	3.6 ± 2.0	0.25
East Coast 2	Upweller	>1.6	138,177	16 weeks	20664	14.9	4.0 ± 1.03	6.2	0.137
West Coast 1	Upweller	>2.0	108,113	24 weeks	65,000	60.1	5.2 ± 1.2	10.4 ± 1.3	0.217
West Coast 2	RW tray	3x4mesh	6,465	6 weeks	6,000	92.8	7.6 ± 1.3	9.6 ± 1.8	0.33
West Coast 3	Upweller	>1.2	250,000	8 weeks	172,000	68.8	2.2 ± 0.14	6.1	0.487
Spring 2020									
East Coast 1	Upweller	>1.0, >1.6	186,300/85,560	12 weeks	180,000	66.2	1.7 ± 0.10/3.0 ± 0.22	5.1 ± 2.2	0.229
East Coast 2	Upweller	>1.0, >1.2	93,150/128,400	12 weeks	24333	10.9	1.7 ± 0.10/2.2 ± 0.14	6.7	0.39
West Coast 3	Upweller	>1.0, >1.2	176,030/128,000	10 weeks	207,000	68	1.7 ± 0.10/2.2 ± 0.14	6.2	0.425
Fall 2020									
West Coast 1	Upweller	>1.0	113,400	12 weeks	61,408	67.2	1.2	4.86+0.98	0.305
West Coast 2	RW tray	>1.0	56,700	12 weeks	54,264	94.3	1.2	3.58+0.72	0.198
West Coast 3	Upweller	>1.0	390,000	8 weeks	336,700	86	2.2	6.4	0.525

Abiotic factors

Temperature and salinity are known to affect both abundance and type of phytoplankton. In addition, SRV's prefer high salinities, and experience both lower growth and survival at salinities <25 ppt. Temperature and salinity were highest at WC3 in both seasons and years (Table 8). The two east coast sites experienced similar temperatures and salinities in both seasons; therefore, abiotic factors alone can not explain the differences seen in terms of performance, particularly survival, at the two facilities. No particular mortality event was associated with low survival at EC2; poor survival appeared to be consistent week to week. The two west coast sites located in close proximity, had similar temperatures and salinities in both years, and were lower than at other sites; however, they varied in terms of performance. One was a land-based and one a field-based nursery (FLUPSY), which may somewhat explain differences seen. Higher survival seen at WC2 may also be partially explained in that it was the only nursery that was not part of a commercial operation.

Objective 5 Conclusions: SRVs perform well in upwellers typically used in hard clam production. As only two nurseries used raceways it is difficult to compare performance, especially survival, which varied significantly, however, growth was similar in both systems. The facility that saw higher survival with raceway systems has experience growing clams in those systems while the other facility does not routinely use them which may account for differences seen.

Season has little impact on nursery performance of SRVs in terms of survival, but did appear to impact growth, with better overall growth seen in fall. Geographic location (east versus west coast) appears to have little impact on nursery performance; however, best overall performance in upwellers was seen at the most southerly west coast location, which has both higher salinity and temperature.

Table 8. Temperature and salinity at Florida nurseries during fall and spring.

Nursery	Temperature (F)	Min/Max	Salinity (ppt)	Min/Max
Fall 2019 (Oct/Nov to Dec/Jan)				
East Coast 1	71.7 ± 5.5	52.2 - 86.3	28.7 ± 3.3	23 - 35
East Coast 2	73.0 ± 5.5	62.4 - 84.6	27.2 ± 1.9	23 - 30
West Coast 1	67.7 ± 7.7	43.6 - 86.5	25.0 ± 2.0	20 - 28
West Coast 2	67.3 ± 5.8	55.4 - 86.6	25.0 ± 2.0	18 - 28
West Coast 3	73.5 ± 4.8	57.2 - 83.9	32.4 ± 1.2	30 - 35
Spring 2020 (Feb to Apr)				
East Coast 1	74.9 ± 4.9	57.4 - 84.3	29.9 ± 2.3	24 - 34
East Coast 2	75.1 ± 4.8	63.8 - 84.4	26.8 ± 2.1	24 - 31
West Coast 3	78.3 ± 5.4	59.2 - 88.3	32.8 ± 0.9	31 - 35
Fall 2020 (Aug/Sept to Oct/Nov)				
West Coast 1	78.1 ± 6.6	56.2 - 94.1	23 ± 3	17 - 27
West Coast 2	79.9 ± 5.0	66.9 - 89.8	23 ± 3	16 - 28
West Coast 3	84.4 ± 2.5	79 - 89	27 ± 3.5	20 - 31

Research Project Accomplishments

Optimal Conditioning Requirements for Sunray Venus Clams

Relevance

The primary issue which hatcheries face is the ability to spawn SRV clams year-round. Previous FLSG funded research showed that SRV clams could be spawned similar to hard clams and successful spawns were achieved 46% of the time with larvae produced 37.5% of the time. However, the intent of that research was to provide proof of concept and did not address optimization. Histological data collected from wild SRV clams showed the importance of temperature and diet in gamete production.

Response

To answer what triggers maturation (production of ripe, viable gametes) and spawning, temperature (18, 21, 24C), diet (Tiso 100%, Cg 100%, Tiso:Cg 50%/50%), feeding rate (2%,4%,6%), and conditioning time (6, 9, 12 weeks) were evaluated. Detailed data was collected on the number of male and female broodstock that spawned, the temperature and spawning cycle at which eggs and sperm were produced, the number of eggs fertilized, survival to D-stage larvae and survival to post-set.

Results

Although successful spawns were achieved with as little as 6 weeks of conditioning, at all 3 feed rates and at all 3 temperatures, optimal conditioning time was 12 weeks, optimal temperature was 21-24C and optimal feed rate was 6%. Successful spawns were achieved in trials conducted in various seasons including, summer (June, July, August), winter (November, December, January) and spring (April). Spawning always occurred during the heat phase of the spawning cycle, and in all but one instance in the first heat/cold cycle. The number of fertilized eggs produced in each spawn ranged from 1.2 to 21.9 million. The percent of fertilized eggs that developed into D stage larvae ranged from 28-100%. The percent of D stage larvae that developed into post set ranged from 1% to 80%.

Conditioning: Feeding Rates and Temperature

Relevance

The primary issue which hatcheries face is the ability to spawn SRV clams year-round. Previous FLSG funded research showed that SRV clams could be spawned similar to hard clams and successful spawns were achieved 46% of the time with larvae produced 37.5% of the time. However, the intent of that research was to provide proof of concept and did not address optimization. Histological data collected from wild SRV clams showed the importance of season (temperature) in gamete production. SRV clams are a subtropical species therefore a higher temperature than used for hard clam maturation may be optimal. In addition, hatchery operators that have worked with SRV clams have noted that they appear to require more algae than hard clams.

Response

To determine optimal conditioning requirements in terms of feed rate and temperature for maturation conditioning, spawning and production of ripe, viable gametes three temperatures (18, 21, 24°C), and three feed rates (2%,4%,6%) were evaluated. Three replicate treatments with 30 clams each maintained in 1 diameter (150-gallon tanks). Clams were conditioned for 6, 9, or 12 weeks. Detailed data was collected on the number of male and female broodstock that spawned, the temperature and spawning cycle at which eggs and sperm were produced, the number of eggs fertilized, survival to D-stage larvae and survival to post-set.

Results

Although successful spawns were achieved with as little as 6 weeks of conditioning, at all 3 feed rates and at all 3 temperatures, optimal conditioning time was 12 weeks, optimal temperature was 21-24°C and optimal feed rate was >6%. Successful spawns were achieved in 41% of trials conducted in various seasons including 9 in summer (June, July, August), 8 in winter (November, December, January) and 3 in spring (April), which showed that SRV clams can be spawned year-round. Spawning always occurred during the heat phase of the spawning cycle, and in all but one instance in the first heat/cold cycle. In 19% of trials spawns did not occur on the first attempted day but were successful on the 2nd day. The number of fertilized eggs produced in each spawn ranged from 1.2 to 21.9 million. The percent of fertilized eggs that developed into D stage larvae ranged from 28-100% and was lowest at 18°C regardless of feed rate. The percent of D stage larvae that developed into post set ranged from 1% to 80%, with human error accounting for development that was <25%.

Conditioning, Effects of Diet

Relevance

The primary issue which hatcheries face is the ability to spawn SRV clams on demand. Hatcheries typically feed a combination of diatoms (i.e., *Chaetoceros gracilis*) and flagellates (i.e., *Isochrysis galbana*) during maturation but the amounts vary and are based on algae condition and therefore availability rather than predetermined ratios. Evaluation of temperature and feed rate conducted earlier in this study suggested a feed rate >6% and a temperature of 21-24°C resulted in increased fertilization, and development to D stage. During those trials a mixture of the species were fed, as is typical practice, based on algae condition and availability. Based on literature a mix of algae are better than mono-species diets due to complementary nutritional profiles. Diatoms are typically higher in Omega 3 fatty acids, and flagellates are typically higher in Omega 6 fatty acids.

Response

To determine whether conditioning SRV broodstock with an equivalent ratio of a diatom and flagellate results in better production of spawns two mono-species diets consisting of a diatom (*Chaetoceros gracilis*, Cg) or a flagellate (*Isochrysis galbana*, T-ISO) were compared to a bi-algal 50:50 ratio diet of

Cg:T-ISO. SRV broodstock were maintained at a temperature of 21°C, with an 8% feed rate, in 1-meter (150 gallon) tanks. Replicate dietary treatments (n=3, 30 clams each) were conditioned for 9 weeks prior to spawning attempts. If spawning did not occur at 9 weeks on day 2, a second spawn was attempted at 12 weeks of conditioning. Detailed data was collected on the number of male and female broodstock that spawned, the temperature and spawning cycle at which eggs and sperm were produced, the number of eggs fertilized, survival to D-stage larvae and survival to post-set.

Results

A higher percentage of fertilized eggs (20-25%) achieved D-stage with *C. gracilis* or a 50:50 *C. gracilis*, T-ISO mix, but differences were not significant. Both *C. gracilis* and the 50:50 mix resulted in higher survival to post-set (50.5, 55% vs. 40%) even though clams fed T-ISO were conditioned longer. The mixed diet resulted in slightly higher, but not significant differences, in survival to post-set (5-15%). Although a mixed diet appears to be optimal for conditioning SRV broodstock compared to a mono-species, a diet that is Cg compared to T-ISO. Like the temperature, feed rate experiments longer conditioning time seems to result in an increased number of spawning clams.

Hatchery Practices, Drain Down Frequency and Temperature

Relevance

Typical hard clam hatchery practices involve conducting daily drain downs of larval tanks followed by transfer to a tank containing new water to maintain water quality. Whether the practice of daily drain drowns results in increased larval production is unclear. Decreasing drain down frequency from every day to every other day would result in decreased labor that could translate into an economic benefit. However, there may be a seasonal component at play, in that when temperature is higher, drain downs may need to be conducted more frequently than when temperatures are lower.

Response

To evaluate the effect of changing drain down frequency during larval production equal amounts of fertilized eggs were added to larval tanks and development to D-stage and post set evaluated. Tanks (n=3 replicates) were maintained at one of two temperatures (25°C, 28°C) and either drained down daily or on alternate days. T-ISO was initially added to tanks, with *Chaetoceros gracilis* introduced gradually until post-set was achieved.

Results

There was no significant difference in survival from fertilized egg to D stage larvae or from D stage larvae to post-set whether drain downs were conducted every day or on alternative days. Although drain down frequency did not affect development to D-stage, temperature did, with more development (75% increase) occurring at 28°C compared to 25°C. On the other hand, larval tank temperature did not affect survival of D-stage larvae to post-set. Therefore, larval tanks may be drained down every other day, cutting down on labor requirements in the hatchery.

Hatchery Practices, Microalgal Diets

Relevance

Typical hard clam hatchery practices during larval culture involves feeding a mono-species flagellate diet, such as *Isochrysis* spp., or a mono-species flagellate diet followed by gradual addition of a diatom, such as *Chaetocerus* spp., resulting in a mixed bi-algal diatom/flagellate diet prior to or at post-set. However, there are other flagellate (i.e. *Pavlova lutheri*) and diatom species (i.e. *Thalassiosira sp.*), that

are also used in aquaculture. Whether the typical hard clam diet of T-ISO/Cg is optimal for SRV post-set production has not been explored.

Response

To determine whether the "typical" hard clam post-set diet was optimal for SRV clam post-set multiple bi-, tri-, and quad-algal species diets consisting of various combinations of flagellates (*Pavlova lutheri*, *Isochrysis galbana*) and/or diatoms (*Chaetoceros gracilis, Thalassosoria wessflogii*) were compared. The six dietary treatments included 2 flagellates, 2 diatoms, the "standard diet", all 4 species or both diatoms and T-ISO or both flagellates and *C. gracilis*. Each treatment consisted of ~1000 one-week post set clams (0.89 mm shell length). Survival and growth were evaluated over a six-week period.

Results

Growth of SRV post-set was significantly higher in treatments receiving an equal proportion of all four live microalgal species, but survival was significantly higher in treatments receiving the typical commercial combination of *I. galbana* and *C. gracilis*. Lowest growth (30% lower) and survival (25% lower) was seen with the bi-algal diatom diet. Therefore, post-set SRV clams may be fed the same diet as hard clam post-set.

Hatchery Practices, Concentrate Diets

Relevance

Bivalve production relies almost exclusively on the production of live microalgae throughout all life stages. The cost of live microalgal production is high due to that production is labor intensive. In addition to potential problems with contamination (bacteria, ciliates) that can impact microalgal production, storm events and resulting power outages and other issues can also reduce or halt production. Processed microalgal concentrates have been developed that have been advocated as both a replacement for or a backup for live algae in emergency situations. Whether these products can serve either purpose for SRV clams during any life stages, including post-set would be invaluable to hatchery operators.

Response

To evaluate the utility of using algal concentrates as either a partial or complete replacement for live microalgae for post-set SRV clams, week old clams were fed one of six diets. Two algal concentrates were tested: Shellfish diet 1800, and Isochrysis 1800. All treatment groups (n=3 replicates) were stocked with SRV post set (1079 clams, shell length 0.89 mm). Treatments consisted of 100% live microalgae (T-ISO or T-ISO/*C. gracilis*), 50% partial (T-ISO live, Isochrysis 1800 or T-ISO/*C. gracilis*, Shellfish diet 1800) or a 100% complete algal concentrate replacement diet. Growth and survival were assessed at six weeks.

Results

SRV clams fed the live bi-algal diet of T-ISO and *C. gracilis*, were significantly larger (27-60%) than clams fed any other diets. Both live microalgae diets had higher growth than either partial or complete replacements. Lowest growth was seen with Shellfish Diet 1800. High survival was seen with both T-ISO and *C. gracilis* or the partial replacement *I. galbana, C. gracilis*, Shellfish Diet 1800. Survival was significantly lower in treatments fed a 100% algal concentrate diet (47-62%). SRV post-set clams perform best with a live microalgal diet. Although live algae can be replaced up to 50% with algal concentrates tested, the use of algal concentrates as a total replacement diet is not recommended for SRV post-set clams.

Creation of New Sunray Venus Culture Lines

Relevance

Maintaining genetic diversity within cultured stocks is important to prevent inbreeding depression that may ultimately affect production performance. There are three geographical locations on Florida's west coast that have historically had large natural populations of SRV clams, Alligator Harbor (AH), Sea Horse Key (SHK) and Anna Maria Island (AMI). Previous funded SRV projects created three culture lines from these populations in 2012 and one crossed line (SHK/AH=F1). These lines were distributed to interested hatcheries at that time and maintained at the UF experimental lease, although it is doubtful that hatcheries that currently produce SRV clams know the origin of their stock. By the time this project was proposed only three lines remained as the AH line had been lost. In 2015-2016 another cultured cross was created (F2xAMI). At the start of this project few progeny from the remaining three lines remained. Only the line from 2015 had sufficient stock to be used as parental stock for this study.

Response

To ensure genetic diversity in current and future culture lines new and replacement crosses were made by collecting wild clams from previously known locations and creating crosses of those lines and crosses with the current cultured stock. Wild clams from two of the three original geographic areas were collected (N=30 to 45 clams) to create replacement lines for Sea Horse Key and Anna Maria Island. Few wild SRV clams could be found in Alligator Harbor (N=4) due to the impact of multiple hurricanes, which was not sufficient for creating this cross. In addition to pure geographic lines, crosses of these two wild populations lines as well as crosses of the current cultured line (F2xAMI) with wild clams were made to create five new lines. For each spawn between 30 and 50 clams, that had been conditioned for 12 weeks at 21C, at a 8% feed rate (50:50 T-ISO:*C. gracilis*) were spawned by thermal induction.

Results

Five lines were created between January and April 2021: SHKxSHK, AMIxAMI, SHKxAMI, SHKx(F2xAMI) and AMIx(F2xAMI). The 1 mm post-set from the January spawn were distributed to three participating partners in April. Participants have agreed to keep these lines separate from previous distributed cultured seed (F2xAMI). April spawns remain at HBOI. All lines will also be maintained at FAU-HBOI and UF for future use, and distribution to individual hatcheries.

Genetic Diversity Assessment

Relevance

Aquaculture programs rely on genetic information for selecting broodstock, increasing production, maintaining sustainability of production, reducing inbreeding depression, and safeguarding against disease. There is a strong need for SRV clam genetic data to support its future development as a cultured species. To date, no genetic analysis of wild population structure for this species has been conducted. The only published genetic information suitable for genetic analysis is a single individual *M. nimbosa* from the Ten Thousand Islands Area of South Florida (Mikkelsen et al. 2006). The genetic diversity of the current cultured line (F2=SHK/AHxAMI) used in this study will be compared to sunray venus clams collected for natural Florida populations used to create additional lines. North Carolina SRV populations will serve as the outlier group.

Response

The use of mitochondrial DNA (mtDNA) markers is an established method for the evaluation of genetic status and diversity in populations. Population genetic variability of *M. nimbosa* was examined using the highly variable mitochondrial cytochrome oxidase I (COI) gene that commonly resolves phylogeographic

structure in marine invertebrates including bivalves. Adductor muscle tissue was dissected from 30-50 individual clams per line/population for genetic comparison via sequencing, following DNA extraction and PCR amplification of a portion of the COI gene. The questions that will be answered include: 1) What is the genetic diversity of wild stocks of SRV clams based on mtDNA COI gene fragment, and 2) Is the genetic diversity of current cultured lines of SRV clams lower than in wild stocks.

Results

Sixteen haplotypes were identified among 63 analyzed clams. The most genetic diversity was seen within the wild SHK population (N=23), which had 10 haplotypes. In contrast wild AMI clams (N=15) had very little genetic diversity with only 2 distinct haplotypes, neither shared with other wild populations. The AH clams (N=2) had 2 haplotypes, one shared with an SHK haplotype and the other with the F2xAMI parental stock as well as an SHK haplotype. The cultured parental (F2xAMI) line (N=18) also showed low diversity with only two haplotypes, 1 unique and 1 shared with an SHK, an NC and an AH haplotype. The clams collected in NC (N=3) had 2 haplotypes, 1 distinct and one shared with the cultured parental line.

<u>Performance of Nursery Systems for Sunray Venus Clams</u>

Relevance

Two systems (wellers, raceways) are used by clam nurseries to rear hard clams from 1-2 mm to 8-10 mm. Although nurseries prefer the use of upwellers for hard clam culture this may not be the optimal system for SRV clams. Previous FLSG funded research showed that use of raceways resulted in higher survival and growth, but this has not been tested at a commercial facility. Seasonal differences with respect to growth and survival are likewise reported by nurseries, therefore seasonal trials were conducted at the same commercial facilities in fall and spring.

Response

Clams were distributed to five participating nurseries in the fall of 2019 (2 east coast, 3 west coast), to 3 participating nurseries in the spring of 2020 (2 east coast, 1 west coast) and to 3 participating nurseries in the fall of 2020. One facility on the east coast compared both systems, and two facilities on the west coast compared both systems. The remaining facilities had upweller systems only.

Results

SRVs performed well in upwellers typically used in hard clam production. As only two nurseries used raceways it is difficult to compare performance, especially survival, which varied significantly, however, growth was similar in both systems. The facility that saw higher survival with raceway systems has experience growing clams in those systems while the other facility does not routinely use them which may account for differences seen.

Season has little impact on nursery performance of SRVs in terms of survival, but did appear to impact growth, with better overall growth seen in fall. Geographic location (east versus west coast) appears to have little impact on nursery performance; however, best overall performance in upwellers was seen at the most southerly west coast location, which has both higher salinity and temperature.

Project Students Supported

Name: Edward Perri
Institution: FAU
Department: Aquaculture
Major Professor: Susan Laramore
Status: Masters
Graduation: July 2021
Thesis Title: Effect of microalgae (species, processing) on Sunray Venus Clam (*Macrocallista nimbosa*) and Hard Clam (*Mercenaria mercenaria*) Production and Fatty Acid Content

New Extramural Funding Based on the Project

Title Project: Enhancing Marine aquaculture in the tropical US: Methods for sustainable commercial co-cultivation of shellfish and seaweed in Florida
Funding Amount: $300K total
Project Completion Date: 8/31/2023
Project Sponsor: NOAA S&K

Outreach and Activities

Through presentations at conferences, published abstracts, and planned videos we will have informed industry, the public and academia concerning this project.

- Two abstracts were submitted, and one poster and one oral presentation given at a national shellfish and national aquaculture conference
- Two videos are planned for 2022 – SRV Maturation & SRV larval rearing

Presentations

1. Perri, E. (presenter), Hassan, M.M., Laramore, S., and Baptiste, R. 2021. Effect of microalgae (species) on Sunray venus clam (*Macrocallista nimbosa*) and hard clam (*Mercenaria mercenaria*) post-set production. National Shellfisheries Association 113 Annual meeting, (virtual), March 22 – March 25.
2. Laramore, S. (presenter), Perri, E., Hassan, M.M., Sturmer, L., Wills, P., and Baldwin, J. 2022. Effects of microalgal concentrates on the production of post-set sunray venus *Macrocallista nimbosa* and hard clams *Mercenaria mercenaria*, Aquaculture 2022, San Diego, CA February 28-March 4.

Publications

Perri, E., Sturmer, L., Wills, P.S. Baldwin, J. and Laramore S. 2022. Effect of Microalgal Diets on Sunray Venus Clam (*Macrocallista nimbosa*) Production and Fatty Acid Profile. J Shellfish Res (In review).