Scope of Work

Hatchery/Nursery Facility Selection

Florida hard clam aquaculture takes place primarily in three geographic regions: the Big Bend along the Gulf of Mexico coast (Dixie and Levy Counties), southwest Florida (Tampa Bay to Ten Thousand Islands), and the Indian River Lagoon on the east central coast. The project team worked with 10 certified clam seed producers located across the state, who operate hatchery and nursery facilities. Selection of commercial operations was based on willingness to participate, commitment to assist in sampling protocols, location (to capture geographic diversity), source water (well versus surface, Atlantic versus Gulf), and scale of operation (to capture operational diversity). Project personnel met individually with industry partners at their operations to develop a sampling schedule tailored to their location, business plan, production schedule, and observations of problematic periods. Each operation was assigned a code to maintain anonymity.

Facility Monitoring and Assessment

Water Quality Indicators – Four types of water quality samples were scheduled for production periods, typically fall through late spring/early summer, but were modified based on the operation's production schedule.

Continuous logging **–** Three shellfish operations, one in each geographic region, were equipped with palm-sized Onset HOBO™ pH and Temperature Data Loggers, Dissolved Oxygen Data Loggers, and Conductivity/Salinity Data Loggers. Project personnel programmed the Loggers to collect data every 30 min and were deployed at participating facilities during production seasons. Project personnel performed maintenance and downloaded data via optic base stations regularly.

Weekly measurements **–** All participating operations were provided with kits of water quality equipment to remain on the premises. Kits included 1) refractometers, 2) temperature, dissolved oxygen, and pH meters, 3) alkalinity test kits, and 4) data sheets to record measurements. Project personnel in each geographic area worked with participating hatcheries and nurseries in obtaining measurements of these basic water quality indicators.

Biweekly sampling **–** Hatchery source water (well water versus surface water) was sampled every two weeks at all participating operations by project personnel located in each geographic area. Sampling per year was limited to nine months when facilities were operational from fall through late spring/early summer. Water samples were transported or shipped to FAU-HBOI and analyzed for ammonia, nitrite, nitrate, and calcium using a Hach DR1900 spectrophotometer and indicator reagent kits. Additional water samples were archived for potential future analysis of other parameters.

Annual sampling **–** Hatchery and/or nursery source water was sampled once per year in all participating operations by project personnel located in each geographic area. Sampling was timed to coincide with production schedules and periods of high rainfall, runoff, and aerial pest control spraying. Water samples were sent to Midwest Laboratory, Omaha, NE, for a full suite of analyses for ions (e.g., sodium, chloride, phosphorus, calcium, potassium, magnesium, sulfate, boron, iron, nitrate nitrogen, copper, manganese, carbonate, zinc, bicarbonate, total dissolved solids), harmful metals and regulated inorganic chemicals (e.g., antimony, arsenic, fluoride, selenium, barium, lead, beryllium, mercury, sulfate, cadmium, nickel, thallium, chromium, nitrite nitrogen), water volatile

organics (80 VOCs), and 20 organophosphorus pesticides and herbicides in common lawn and agricultural use.

Local meteorological conditions were documented in each geographic region, including regional rainfall and river gage heights by accessing NOAA meteorological stations and databases maintained by the DACS Shellfish Environmental Assessment Section field stations. Further, FAU-HBOI maintains a series of land/ocean biogeochemical observatory (LOBO) units and weather sensors to provide real-time, high-accuracy and high-resolution water quality/weather data in the Indian River Lagoon.

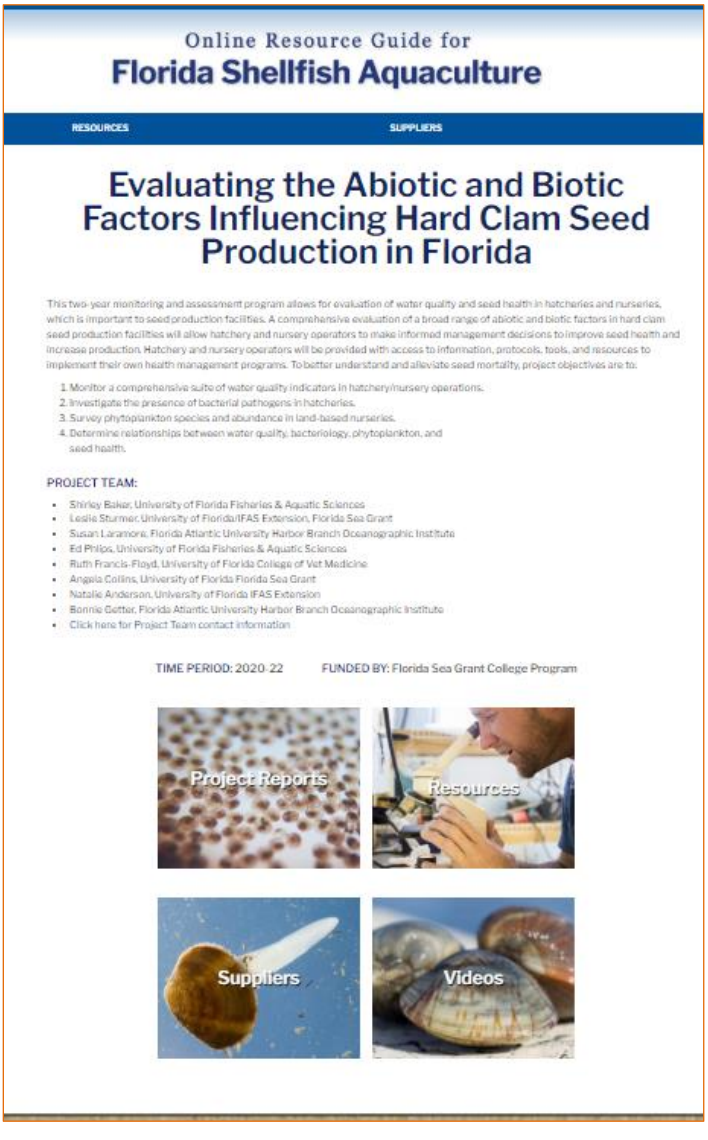
Carbonate Chemistry – Using water quality parameters measured weekly (pH and alkalinity), calcite and aragonite carbonate saturation states were estimated using the CO2SYS program developed for CO_2 system calculations. Calcium carbonate can readily dissolve if the saturation state is less than one. On the other hand, calcium carbonate does not readily dissolve if the saturation state is greater than one. Clams are calcifying organisms and live in conditions where the calcium carbonate saturation state is greater than one and thrive in conditions where it is much greater than one (VMRC 2013). Ocean acidification can contribute to lower calcium carbonate state values. However, inshore coastal waters influenced by rivers and rainfall can also periodically have lower saturation values due to freshwater dilution. This occurrence may be experienced by some hatcheries in Florida particularly during and after heavy rainfall and flood events.

Bacterial Pathogens – Potential problem areas that chronically harbor microbes (bacterial “hot spots”) in hatcheries (e.g., incoming water, larval tank water, maturation tank water, post-set tank water, algae cultures) were sampled during the production season (beginning, middle, end) with additional samples taken if mortality events occurred. Water samples were collected in sterile containers and chilled or placed in a refrigerator prior to same day or next day processing (from east coast hatcheries) or shipped with gel packs overnight (from west coast hatcheries) and processed upon receipt at FAU-HBOI. Samples collected from each hatchery were plated in triplicate on TCBS (*Vibrio* selective) agar plates. Total *Vibrios* were enumerated, and the proportion of non-pathogenic and pathogenic *Vibrios* assessed. Potentially pathogenic bacteria were identified using biochemical tests or sequencing. Agar plates were wrapped in parafilm, placed in a resealable plastic bag, and are stored in a refrigerator at Harbor Branch for short term future analysis of non-identified colonies.

Seed Health – Seed were sampled from hatcheries and nurseries during the production season (beginning, middle, end) with additional samples preserved in the event of mortalities or other unusual observations. Live seed were collected in sterile sample bags and shipped to FAU-HBOI for processing. The number of seed samples from each hatchery/nursery was dependent on size of operation. Briefly, seed were decalcified in CalEx and placed in Davidson’s fixative for 48 hours before transfer into 70% ethanol. Preserved samples were processed (tissue dehydration in alcohol followed by rehydration in toluene), paraffin-embedded, sectioned at 6 mM, and stained with hematoxylin and eosin. Histopathological examinations documented tissue condition. Any degeneration or inflammatory reactions in the digestive gland, mantle, or gills were noted and condition ranked on a 1-4 scale. Tissue blocks and slides are archived at FAU and can be maintained indefinitely. In addition, hatchery/nursery operators were asked to provide weekly observations of seed health indicators. In collaboration with operators, a checklist of seed health indicators was developed, which included gut content, production of feces, presence of fouling, deformities, behavior, and evidence of recent growth or mortality.

Phytoplankton Quality and Quantity – All nursery operations were provided with kits of phytoplankton sampling supplies to remain on the premises. Kits included 1) Lugol's solution and amber glass bottles for general phytoplankton sample preservation, 2) buffered glutaraldehyde solution and scintillation vials for optimal preservation of picoplankton, and 3) data sheets. Project personnel in each geographic region assisted in sampling and preserving nursery source water weekly during production. Water samples were collected at the same time (mid-day) and location (primary water entry point) within the nursery each week, noting local meteorological and hydrological conditions that may influence phytoplankton in the source water (i.e., tide level, recent rainfall, cloud cover, air temperature, and wind direction). Although collected weekly, only one to two sets of samples were processed each month, with additional samples processed if mortality events occurred or if incidents of harmful or nuisance phytoplankton species were suspected. Samples were processed at the UF Algal Ecology Lab in Gainesville; preserved samples were analyzed microscopically for phytoplankton abundance and species composition, noting any harmful algal bloom species. Phytoplankton carbon values were estimated by applying conversion factors to biovolume estimates. Preserved samples are archived at UF.

Training and Educational Materials – After initial meetings with hatchery/nursery operators, a sampling protocol was developed for each facility. Project personnel routinely worked with operators in each geographic region – training staff on how to properly use test kits, collect and preserve water and seed samples, and record data. This information was used in developing summaries and extension-type factsheets. In the second year, a series of video modules (3-5 minutes) was created that addressed the proper use of water quality testing equipment, including how to calibrate equipment, and protocols for collecting and preserving samples for phytoplankton and bacteriological analyses. In addition, a video was developed describing how to use plating methods for bacterial monitoring. Further, a set of standard protocols for monitoring and assessing water quality, bacterial loads, phytoplankton, and seed health was developed. These protocols include "how to" instructions and "shopping lists" of equipment and supplies, as well as connection with people and laboratories available to help beyond the project. Educational materials generated through this project were used in developing a problem-solving guide to assist seed producers in understanding and alleviating seed mortality at their facilities. These resources can be found at the website: https://shellfish.ifas.ufl.edu/clam-seed-project-2020-22/.



Sampling Schedule
Florida Clam Hatcheries and Nurseries
2021-2022

Parameters	Hatcheries	Nurseries	Sample analysis
Water quality			
Temperature (T), Salinity (S), Dissolved Oxygen (DO), pH.	Continuous+	Continuous+	UF, FAU-HBOI
T, S, DO, pH, alkalinity	Weekly*	Weekly*	UF, FAU-HBOI
Ammonia, nitrite, nitrate, calcium	Biweekly*	-	FAU-HBOI
Full analysis	Annually**	Annually**	Midwest Laboratory
Herbicides/Insecticides	-	Annually*****	Water Ag Labs
Bacteriology	Three times***	-	FAU-HBOI
Seed health			
Histology	Three times***	Three times***	FAU-HBOI
Health feedback	Weekly*	Weekly*	-
Phytoplankton	-	Monthly****	UF

+During production at one facility per geographic location

*During production at all participating facilities

**One sample from each operation, unless hatchery and nursery water sources differ. Analyses includes ions, heavy metals, pesticides, etc.

***During production cycle, up to nine months, additional samples if problems detected

****Weekly samples collected during production, 1-2 samples per month processed (up to 10 processed samples per facility per year), additional samples processed if problems observed

*****Herbicides – Summer 2021/Insecticides – Summer 2022