GULF SHELLFISH INSTITUTE
Research for Sustainable Aquaculture & Healthy Ecosystems

Report to Manatee County

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Submitted by:

Gulf Shellfish Institute, Inc.

1905 Intermodal Circle, Suite 330

Palmetto, FL 34221

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1.0 INTRODUCTION

1.1 Current Status of Aquaculture

World aquaculture production surpassed 111.9 million tons in 2017, worth nearly $250 billion; production of shellfish was 25.8 million tons worth $91 billion (FAO 2020). Aquaculture now accounts for more than half of the seafood consumed globally, and this contribution is expected to grow as world population continues to increase and harvest fisheries approach maximum sustainable yields.

The U.S. currently imports over 90% of its seafood. The resulting trade deficit reached $16.8 billion in 2018 (NOAA 2019). Imports of shellfish exceeded $79 million for clams, $87 million for oysters, $102 million for mussels and $243 million for scallops (NMFS 2018).

In spite of this level of seafood demand, the U.S. ranks only 17th in global aquaculture production (NMFS 2018). Total aquaculture production in 2017 was 626 million pounds, worth $1.47 billion, for both freshwater and marine species (NMFS 2018). Shellfish aquaculture totaled 46,361,103 kg, worth almost $325 million, with the Gulf states accounting for 54% of the volume and 27% of the value (NMFS 2018). Most shellfish aquaculture in the Gulf of Mexico occurred in Florida, where 132 farms produced over $16 million worth of clams and in Louisiana, where 28 farms accounted for over $29 million of oysters (USDA 2019).

The U.S. is a world leader when it comes to production of safe, sustainable seafood, as aquaculture production must adhere to local, state, and national regulations aimed at ensuring the safety of both the produced seafood and the environment (US Congress 1980, Gulf of Mexico Fishery Management Council and NOAA 2009, NOAA 2011). With responsible expansion of the aquaculture industry, the U.S. is in a prime position to provide safe, sustainable seafood for consumption to meet the growing demands of the human population. Increasing domestic aquaculture will help preserve working waterfront communities by creating jobs, increasing the supply of high quality, locally produced seafood, and help to reduce the large seafood trade deficit.

1.2 Environmental Benefits of Shellfish Aquaculture

Increasing shellfish aquaculture in the U.S. makes sense from both an economic and environmental perspectives. The feeding activity of marine bivalves, especially when in large assemblages such as oyster reefs, provide important ecological services. These animals are capable of filtering seston (suspended particulates) from large volumes of water as they feed.

Clearance rates vary seasonally, but for oysters, rates of 5 l h^{-1} g^{-1} dry tissue weight are typical (Newell, 1988). This results in the reduction of particle concentrations by 30%-45%; chlorophyll-a concentrations by as much as 90%; and nitrogen removal up to 25% of daily load (Carmichael et al. 2012). Some of cleared particulate matter is ingested and assimilated into tissue and shell growth; some is excreted (primarily as ammonium) and is available for primary production. The remainder ends up on the bottom as biodeposits (feces and pseudofeces). The process of transferring nutrients from the water column to the sediment, or benthic-pelagic coupling, benefits both the water column (pelagic) and bottom (benthic) communities in several ways:

1) Nutrients contained in biodeposits can be removed from the system by burial in sediments.

2) Nitrogen in biodeposits can be microbially denitrified to N_2 gas and permanently removed from the system. For oyster biodeposits, this process removes 17-24% of nitrogen from the sediments (Newell et al. 2002). Kellogg et al. (2013) found that seasonal denitrification rates at a restored oyster reef ranged from 0.3 to 1.6 mmol N_2-N m^{-2} h^{-1}.

3) Assimilation of nutrients into shell and tissue of bivalves (secondary production) effectively removes nitrogen and phosphorus from the water column (Rose et al. 2014). For oysters (*Crassostrea virginica*), the nitrogen content of dry tissue ranges from 7-9.7% and the nitrogen content of shells ranges from 0.08-0.24%; phosphorus contents are lower, ranging from 0.8-1.26% for dry tissue and 0.04-0.1% for shell (Newell 2004, Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2013, Reitsma et al. 2016). Data for quahogs, *Mercenaria mercenaria*, is limited, but nitrogen content of dry tissue and shell was reported to be 7.69% and 0.18%, respectively (Reitsma et al. 2016). Harvest of commercially important species thus results in a permanent removal of nutrients from the ecosystem. Bivalve aquaculture is now being embraced as a means of increasing nutrient bioassimilation and bioextraction in eutrophic estuaries (Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2014, Rose et al. 2014, Reitsma et al. 2016).

4) Filter feeding exerts “top-down” grazer control on phytoplankton, reducing water column turbidity and increasing the depth to which photosynthetically active radiation penetrates and seagrass can grow (Newell, 2004). Newell and Koch (2004) determined that even a modest density of oysters (25 g dry tissue weight m^{-2}) reduced suspended particulate matter in the water column by an order of magnitude, which in turn increased water clarity and the depth to which seagrasses were predicted to grow.

5) An additional ecological benefit provided by infaunal bivalves is the potential to increase seagrass productivity. Peterson and Heck (1999) demonstrated that biodeposition of nutrients by filter feeding bivalves (*Modiolus americanus*) increased pore water ammonium and phosphate concentrations. In a subsequent study, seagrass (*Thalassia testudinum*) leaf widths

and lengths were significantly greater in the presence of *M. americanus*, demonstrating that nutrients derived from filter feeding bivalves were taken up by seagrass and resulted in enhanced seagrass productivity (Peterson and Heck 2001a, 2001b).

The concept of enhancing bivalve populations, especially oyster reefs, for their environmental benefits is being universally embraced (Higgins et al. 2011, Kellogg et al. 2014, Rose et al. 2014, Reitsma et al. 2016). The ability to quantify these ecological services further supports the ability of state and local agencies to justify the expense of restoration efforts. Grabowski et al (2012) estimated that the value of ecological services provided by oyster reefs is between $5,500 and $99,000 per hectare per year and that the reefs recover their restoration costs in 2 to 14 years.

As a result of these services, molluscan aquaculture has the lowest environmental impact of any food production method (Hilborn et al. 2018). While bivalves are growing on leased bottom, they are continually filtering and removing organic matter (phytoplankton and seston) and incorporating those nutrients into their biomass. When the animals are harvested those nutrients are permanently removed from the ecosystem. As mentioned above, biodeposits are incorporated into sediments, which leads to greater benthic productivity and denitrification.

1.3 Potential for Shellfish Aquaculture in Florida

Florida ranks eights in aquaculture production in the U.S., with total sales of $72 million in 2018 (USDA, 2019). The northern quahog, *Mercenaria mercenaria*, is the most important food item cultured in Florida, having a gross revenue impact of $40 million (Adams et al. 2014). Most of this production is in Cedar Key (Levy County), Florida (Colson & Sturmer 2000), but commercial leases are also located in Tampa Bay (Manatee County), Charlotte Harbor (Lee and Collier Counties in southwest Florida), and in the Indian River on the east coast. In addition, oyster aquaculture has recently begun in counties along the panhandle (https://www.fdacs.gov/Agriculture-Industry/Aquaculture/Aquaculture-Submerged-Land-Leasing).

Given the extensive coastline and variety of habitats and species, the potential for increasing shellfish aquaculture in Florida is tremendous. There are approximately 995,000 acres of approved or conditionally approved shellfish harvesting area, but only 0.15% (1550 acres) is currently being utilized for aquaculture (FDACS staff, pers. comm.). In addition to the northern quahog, several other species of marine bivalves have tremendous commercial potential. The Florida panhandle, with its brackish bays, is ideal habitat for oyster (*Crassostrea virginica*) aquaculture. The southwest coast of Florida, with sandy bottom and high salinity, is home to the indigenous southern quahog, *Mercenaria campechiensis*, and the sunray venus, *Macrocallista nimbosa*. The Big Bend region south to Tampa Bay, with its seagrass habitat, is the preferred habitat for the bay scallop, *Argopecten irradians*[1].

1.4 Role of Research

The factors that limit aquaculture in the U.S. are complex and multifaceted, but one of the factors has been the limited availability of funding for applied research. Applied research has been instrumental in the development of land-based agriculture for increasing yields and productivity; reducing losses to disease; increasing efficiency of farm operations; increasing food quality and safety; and, protecting the environment. The history of funding for agriculture and aquaculture research was reviewed by Love et al. (2015). They found that from 1990-2015, USDA funding for agriculture was $41 billion, while that for aquaculture (all sources) was only $1 billion. In spite of that disparity, funding for aquaculture since 2000 has had a 37-fold return on investment. Bosch et al. (2010) concluded that for oyster aquaculture, research aimed at increasing growth rates, reducing mortality, and reducing seed costs could significantly increase returns to oyster aquaculture. It thus follows that increasing the effort to obtain funding for applied research in support of expanding aquaculture in Florida will help us meet our goal of expanding shellfish production for both economic and environmental benefit.

2.0 OBJECTIVE

This project was a one-year planning grant (funded from the U.S. Department of Treasury (RESTORE POT 1) to Manatee County, subcontracted to the Gulf Shellfish Institute (GSI)) to identify the current obstacles to the expansion of shellfish aquaculture in Florida so that research needs could be identified and efforts to obtain funding could be prioritized. It was envisioned that subsequent funding (RESTORE POT 3) would be allocated to GSI to commence research directed at addressing the identified obstacles.

Our overall goal is to increase the number of marine bivalves in the coastal waters of Florida. This can be accomplished either through increased commercial production (for consumption) or increased restoration of native bivalve populations for their ecological services.

3.0 APPROACH

Our approach involved identifying research needs of both the commercial shellfish aquaculture industry and the shellfish restoration community. For the determining the needs of the commercial aquaculture industry, we met with farmers individually and at meetings of the

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[1] This is currently a recreationally harvested species, but there is interest in exploring the feasibility of commercial aquaculture.

three shellfish trade organizations in Florida: The Cedar Key Aquaculture Association, the Southwest Florida Shellfish Association, and the Florida Shellfish Aquaculture Association. In addition, we attended a regional meeting of oyster growers throughout the southeastern U.S. (Oyster South). We also attended national (National Shellfisheries Association) and international (World Aquaculture Association) meetings to get a sense of how universal some of these obstacles were and how they are being addressed in other regions of this country and other countries.

These national and international meetings were also informative with respect to shellfish restoration. To determine the research needs of the restoration community in Florida, GSI hosted a 3-day workshop entitled: “The Science and Application of Clam Restoration in Florida: Identify Research Needs to Quantify Ecological Services and Maximize Benefits”. It was held virtually through Zoom on February 9-11th, 2021 and attended by representatives of academia, industry, government regulatory/management, non-profit, education/extension/outreach and interested citizens.

4.0 SUMMARY OF RESEARCH NEEDS

The meetings GSI staff attended to solicit research needs of both commercial shellfish aquaculture and shellfish restoration are provided in Table I.

Table I.
Meetings attended by GSI staff and board members to determine the research needs of the commercial shellfish aquaculture industry and the shellfish restoration community.

Date	Focus	Name	Location	No. Attendees
Jan. 30, 2019	Aquaculture	Cedar Key Aquaculture Association	Cedar Key, FL	75
Feb. 21, 2019	Aquaculture Restoration	Ocean Acidification Workshop	Orange Beach, AL	44
Feb. 22-23, 2019	Aquaculture	Oyster South Symposium	Orange Beach, AL	235
Mar. 7-12, 2019	Aquaculture Restoration	World Aquaculture Society / National Shellfisheries Association	New Orleans, LA	80*
May 16, 2019	Aquaculture	Southwest Florida Shellfish Growers Association	Port Charlotte, FL	8
Aug. 22, 2019	Aquaculture	Florida Shellfish Aquaculture Association	Palmetto, FL	45
Oct. 9-10, 2019	Restoration	Oyster Mapping Workshop	St. Petersburg, FL	77
Jan. 22, 2020	Aquaculture	Florida Shellfish Aquaculture Association	Gainesville, FL	25
Jan. 22, 2020	Aquaculture	Cedar Key Aquaculture Association	Cedar Key, FL	50

Feb. 8, 2020	Restoration	Southwest Estuarine Restoration Team (SWERT)	Englewood, FL	22
Feb. 9-11, 2021	Restoration	GSI Workshop	Zoom	131

* Attendance at sessions where presentations were made by GSI staff. Overall attendance at the conference was 4,000.

4.1 Research Needs for Commercial Aquaculture

In assessing all the suggestions for research needs within the shellfish aquaculture industry, there were several basic themes that came to the forefront. These can be broken down into three categories: issues limiting seed production; issues limiting growout; and issues related to water quality.

4.1.1 Hatchery Seed Production

The entire industry relies on a consistent, reliable supply of seed (juvenile) organisms. Seed is produced by hatchery facilities that condition and spawn adult bivalves, rear the larvae and post-set stages, then grow the juveniles to about 5 mm in nurseries for sale to commercial growers. Growers plant the seed on bottom leases (in bags or under nets) until they are market size. There are currently 15 hatcheries within Florida, only a few of those supply the majority of seed to commercial growers (FDACS, 2020).

4.1.1.1 Unexplained mortalities

All hatchery facilities experience unexplained mortality of larvae or post-set animals from time to time[2]. However, the frequency and severity of these mortality events have been increasing in recent years.

Sometimes the cause is known (e.g., an equipment failure or human error), but at other times, the cause is not obvious and is simply attributed to “poor water quality” (also see Section 4.1.3). These events can occur at any time of year and can result in the loss of 100% of that cohort. In addition, mortalities can occur throughout the seed production process, including larval, post set, and nursery phases. All species are affected. To minimize financial losses, mortality events require hatcheries to increase their effort to produce the same amount of seed, stop seed production during part of the year, or some combination, none of which are economically desirable.

Until the cause of the mortality is determined, however, nothing can be done to manage it. Although “water quality” includes a wide variety of physical, chemical, and biological parameters, the aspect most likely to be involved is the bacterial community within the hatchery, also called its “microbiome”.

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[2] This has been is a problem affecting hatcheries throughout the world from the very beginning.

There are several reasons to suspect that changes in the microbiome throughout the year are affecting seed survival:

- The mortality patterns observed to date do not correlate to any obvious physical or chemical water quality parameter.
- Mortality is species-specific; some species are affected, while others are not.
- Histological examination of post-set clams during a mortality event revealed no obvious nutritional or pathological etiology (B. Barber, unpublished data).
- Preliminary analysis of the microbiome of post-set clams during the same mortality event (as above) indicated that about 30% of the microbial community consisted of *Vibrios*, including *V. aestuarianus*, a known pathogen of Pacific oysters (B. Barber, unpublished data).
- Species other than *Vibrios*, however, can cause diseases in bivalves and should be evaluated (Travers et al. 2015). For example, the etiological agent of Juvenile Oyster Disease (JOD) was determined to be a previously undescribed bacterium in the *Roseobacter* clade (Boettcher et al. 1999, 2000, 2005).

Research Priority

Intensively sample and identify (using next generation sequencing) the microbiome in a large, commercial bivalve hatchery over an entire year, sampling water, larvae and post-set animals, to correlate the composition of the microbiological community in the system to larval and seed survival. This effort could lead to determination of specific pathogens as well as possible probiotic bacteria, both of which can lead to improved management options for seed producers.

4.1.1.2 Triploid oyster seed

Another aspect of seed production that is paramount to the growth of oyster aquaculture in Florida is the availability of triploid oyster seed. Much research over the years has demonstrated that triploid (having three sets of chromosomes) oysters grow faster than diploid (having the normal two sets of chromosomes) oysters, due primarily to the fact that triploids are essentially sterile so can devote maximum energy to growth rather than reproduction (Allen & Downing 1986, Barber & Mann 1991). This also avoids the seasonal variation in oyster meat quality due to the natural reproductive cycle (oysters tend to be thin and water during the summer months after spawning). The potential advantage of producing triploid shellfish has been evaluated for commercially important oyster species worldwide (Nell 2002).

Triploid oysters can be produced by several methods, but the most common and effective way is by using cytochalasin B (CB) to block either meiosis I or II (Allen & Bushek 1992, Barber et al. 1992). These are commonly called “chemical triploids”. This method, however, does not produce 100% triploids. A more recent approach to producing triploid oysters uses CB to block polar body extrusion in the few ova that are produced by some triploid females. This results in tetraploid (four sets of chromosomes) oysters. When tetraploids (4n) are crossed with diploids (2n), 100% triploids (3n) are produced (Guo et al. 1996; Wang et al. 2005). These are termed "natural triploids".

Using this approach, triploid oyster seed are now becoming the standard within the U.S. for oyster aquaculture. Like other forms of agriculture, aquaculture benefits from utilizing strains of organisms that are bred for maximizing growth and survival under local conditions. Because oyster aquaculture is so new to the Gulf of Mexico, there has been little time (and few resources) to produce a line of tetraploid oysters (to produce triploid seed) specifically adapted to environmental conditions in Florida for use by Florida growers.

Research Priority

> ***Develop a line of Florida-specific tetraploid oysters that can be crossed with selectively bred diploid oysters (see section 4.1.2.1). The techniques for accomplishing this are already known; it is merely a matter of finding a source of funding to support the work[3]. Having an available supply of triploid oyster seed, adapted to Florida waters, will dramatically increase production and profitability of oyster farmers throughout the Gulf region.***

4.1.1.3 Conditioning oyster broodstock

As previously mentioned, the ultimate goal of shellfish hatcheries is to have the ability to produce seed of any species of bivalve throughout the year. This requires that the environmental conditions (primarily temperature and food supply) that initiate gametogenesis and produce healthy gametes must be known so they can be recreated in the hatchery. This is a process termed "conditioning", in which adult bivalves are provided the conditions necessary to undergo gametogenesis, regardless of the time of year. These parameters are known for quahogs, *Mercenaria spp*.,) but not eastern oysters, *C. virginica*. At the present time, there is no standard approach for conditioning oysters (Bill Walton, Alabama Sea Grant; John Supan, LSU, pers. comm.). The few hatcheries currently producing oyster seed merely wait until oysters become ripe naturally (usually in the spring). Occasionally oysters will naturally re-condition and spawn a second time in the autumn, but this is not consistent.

Similarly, research on the Pacific oyster, *Crassostrea gigas*, has shown that autumn conditioning using typical temperature and dietary approaches does not work. However, if oysters are first subjected to an "accelerated" temperature and photoperiod regime, in which winter conditions (8.5 °C and 8 hours of light per day) are provided in October, oysters can then be conditioned for 7 weeks (at 19 °C and 16 hours of light per day) to produce mature gametes (Chávez-Villalba et al. 2002, Fabioux et al. 2005). In addition, it was shown that once ripe, oysters can be held for prolonged periods at lower temperatures to prevent spawning.

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[3] This work has already begun at the University of Florida, under the direction of Dr. H. Yang.

Research Priority

> ***Determine the parameters required to condition C. virginica "out of season" in Florida. This should be addressed by determining whether the internal clock regulating gametogenesis in C. virginica can be changed using an approach similar to that shown to be effective for C. gigas. Increasing the availability of oyster seed throughout the year will lead to greater production to meet current market demands.***

4.1.1.4 Conditioning sunray venus clams

The sunray venus, *Macrocallista nimbosa*, ranges from North Carolina to Florida and Texas in the Gulf of Mexico (Abbott 1974). Within that range, it is found in sandy, coastal habitats. It has been considered a potential commercial species in Florida for over 50 years (Akin and Humm 1959).

In spite of considerable recent interest within the aquaculture industry, commercial production of the sunray venus has been limited by an inability to consistently and predictably produce juvenile (seed) clams. This species does not respond to the conditioning protocols currently practiced for the northern quahog, *M. mercenaria*. A comprehensive understanding of the reproductive cycle of this species and the environmental factors that control gametogenesis will be required for hatcheries to produce a consistent seed supply. Normally, this is some combination of temperature and food supply (Barber, 2017).

Research Priority

> ***Determine the environmental factors (e.g., temperature and food supply) that regulate gametogenesis in the sunray venus, M. nimbosa, so that hatcheries can produce seed on a consistent basis. This will help expand and diversify the industry throughout Florida.***

4.1.1.5. Algal production

Marine bivalves are filter feeders. Their primary source of nutrition in nature provided by single-celled plants, called phytoplankton or microalgae. In hatcheries, production of bivalve seed depends on the ability to also produce adequate quantities of microalgae. Algal production can demand up to half of the operating costs of a hatchery. Obviously, the success of seed production depends on maximizing the production (volume) and quality of microalgae.

Much research has been done to determine the biochemical composition of many species of microalgae and how a single species or combination of species affects growth rates of bivalves (Davis and Guillard 1958, Walne 1970). It has been found that a diet consisting of multiple species is superior to unialgal diets (Epifanio 1979). Considerable research has also been done correlating specific biochemical constituents with growth performance (Wikfors et al. 1984, 1992, 1996). Some preliminary research has been conducted on formulating artificial diets for bivalves (Langdon and Siegfried 1984). Compared to finfish aquaculture, however, relatively little is known regarding bivalve nutrition. Much more research into the nutritional requirements of bivalves and the manipulation and production of large quantities of nutritious, well balanced diets is needed.

Research Priority

> ***Undertake research that defines the nutritional requirements for commercially important bivalve species; genetically improving the biochemical composition of microalgae; and increasing the efficiency of production systems for microalgae. Progress in bivalve nutrition will lower the cost of seed production and improve the health of both broodstock and their offspring.***

4.1.2 Growout

Bivalve seed purchased from a commercial hatchery is planted on shellfish leases (obtained from the Florida Division of Aquaculture) until large enough to meet the intended market, in a process called “growout”. Generally, protection from predators is the biggest concern. Growout techniques depend on the species being grown. Clams are either placed into mesh bags or under nets, or some combination. Oysters are placed into mesh cages that can be placed on the bottom or suspended on the surface. Growth rate and survival to market size are dependent on environmental conditions and the genetics of the seed purchased. The ultimate goal for growers is to have the greatest growth rate and lowest mortality rate possible. Both of these factors can be improved with selective breeding.

Selective breeding has been used throughout history to improve the performance numerous agricultural crops. The premise is simple. Within a cohort of individuals, some will exhibit a particular (desirable) trait to a greater extent than others. For example, some will grow faster or some will survive exposure to disease, even though most will succumb. If the fastest growing individuals or the survivors of disease are then bred to produce the next generation, their offspring will inherit (presumably) the genes responsible for those characteristics.

4.1.2.1 Genetic selection of oysters

Selective breeding has been effective for both improving growth rate and disease resistance in oysters, *C. virginica* (Ford & Haskin 1987, Barber et al. 1998, Ragone et al. 2003). Improved lines of oysters could be grown as diploids or crossed with Florida-specific tetraploids (see section 4.1.1.2) to produce superior triploids. In all cases, continuation of this approach over multiple generations resulted in increased gains. So once started, this approach needs to be maintained.

Although diseases have yet to have a major impact on oyster aquaculture in Florida, the parasite *Perkinsus marinus,* which has caused major disease mortality in both Delaware Bay and Chesapeake Bay, is present and can present problems in the future.

Research Priority

> ***Initiate a selective breeding program for Florida oysters with the goal of producing lines of oysters having faster growth and greater disease resistance at locations throughout the state. By themselves these oysters will be superior to wild oysters. Using these selected broodstock for crossing with tetraploids to produce triploid oysters should further enhance growth and improve meat quality and marketability (see section 4.1.1.2). Either approach will result in economic benefits to the industry and is a logical starting point given the current depleted status of the oyster fishery and existing market demand.***

4.1.2.2 Genetic selection of clams

A recent concern of clam (*M. mercenaria*) growers in Florida is an increase in mortalities on leases during the summer. The correlation of mortality with high summer water temperature could be explained by the fact that *M. mercenaria* is not native to the west coast of Florida is thus beyond its southern distributional limit. Either of these factors would make this species more sensitive to even a small increases in water temperature near its upper lethal limit. This problem is likely to continue or worsen, given that global temperature is projected to increase by 2 to 9.7°F (1.1 to 5.4°C) by 2100 (https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature-projections). Also see Section 4.1.3.

We are aware of no studies directly examining the upper thermal tolerance limit of *M. mercenaria*. Several studies, however, examined aspects of the physiology of *M. mercenaria* as affected by temperature. Ansell (1968) reported that growth of *M. mercenaria* was maximal at temperatures between 20 and 24 °C, decreased above 24 °C and ceased at 31 °C. Pumping rate of *M. mercenaria* declined sharply above 26 °C (Hamwi & Haskin 1969). Talmage & Gobler (2011) found that survival of larvae and growth of juvenile *M. mercenaria* were both significantly lower at 28 °C than at 24 °C. Together, these studies indicate that *M. mercenaria* performs best at temperatures between 20 and 25 °C, but becomes physiologically stressed as temperature exceeds 26 °C.

For reference, water temperatures on the west coast of Florida often exceed 30 °C during the summer months. Barber (unpublished) recorded a maximum water temperature in lower Tampa Bay of 33.3 °C and found that temperature exceeded 30 °C from June 24 to July 30, 2017. Summer water temperature in Pine Island Sound exceeded 33 °C in September 2017; the ten year average water temperature in September is between 26.7 and 32.2 °C (http://chnep.wateratlas.usf.edu/contour/). Cedar Key water temperature averages 30.2 °C in August and can exceed 31 °C (https://www.seatemperature.org/north-america/united-states/cedar-key.htm). It is possible that temperatures may be even higher at shallow lease sites, especially during low tides in the summer. Thus it is not surprising that *M. mercenaria* is stressed and mortalities occur under these conditions.

One approach to address this problem would be to attempt to increase heat tolerance in clams using a genetic selection approach, as described in Section 4.1.2. Adult clams could be exposed to increasing water temperature until mortality begins to occur. Any surviving clams would be presumed to have a greater tolerance to high temperature and could be used as broodstock to be conditioned and spawned to produce a cohort of more heat tolerant clams.

Research Priority

> ***Initiate a selective breeding program for clams, <u>M. mercenaria</u>, to increase tolerance to high water temperature. Heat tolerant strains of clams would increase survival during the summer months when water temperature is greatest and thus increase profitability.***

<u>*4.1.2.3 Use of an alternate species*</u>

Another approach to alleviate summer, temperature-related mortality would be to use a more heat tolerant clam species. Considerable evidence supports the contention that the southern quahog, *M. campechiensis,* which is endemic to the west coast of Florida, is more tolerant of higher temperature than the northern quahog, *M. mercenaria*.

M. campechiensis has been found to grow faster than *M. mercenaria*, especially at higher temperatures (Menzel 1963, 1989). Growth of *M. mercenaria* was minimal in late summer and fall and greatest in the winter and spring while growth of *M. campechiensis* was more consistent throughout the year (Menzel 1964, Arnold et al. 1991, 1998, Jones et al. 1990). In addition, growth of *M. mercenaria* was found to be negligible at temperatures above 26 °C while growth of *M. campechiensis* continued at temperature up to 35 °C in northwest Florida (Menzel 1963). Thus the preponderance of published evidence suggests that *M. campechiensis* has a greater growth rate (and therefore tolerance of high water temperature) than *M. mercenaria* at temperatures typical of summer conditions in Florida.

A second species of bivalve that is native to southwest Florida is the sunray venus, *Macrocallista nimbosa*. It is known to inhabit open waters with high salinity and high summer temperatures (Barber 2017). As discussed in Section 4.1.1.4, however, production of this species is currently limited by an ability to consistently condition broodstock.

Research Priority

> ***Compare growth and survival of alternate species such as the southern quahog, <u>M. campechiensis</u> and the sunray venus, <u>Macrocallista nimbosa</u>, to the northern quahog, <u>M. mercenaria</u>, especially during the summer months. Commercial production of these species will depend on market demand. <u>M. campechiensis</u> is***

a good candidate for the frozen market, and M. nimbosa, in limited trials, is a popular half-shell product.

4.1.3 Water quality

4.1.3.1 Climate change

Climate change associated with the accumulation of greenhouse gases in the atmosphere is affecting fisheries and fisheries-based economies around the globe (Hallowed et al. 2013).

The two main aspects of climate change affecting production of bivalves are ocean acidification (OA) and increasing temperature (previously discussed in Section 4.1.2.2). Ocean acidification is a direct consequence of rising atmospheric levels of carbon dioxide and has resulted in an average decrease of 0.1 pH units in ocean surface water (Feely et al. 2009). Decreasing pH has implications for calcium carbonate producing organisms such as marine bivalves. Projections suggest that conditions in wide areas of open ocean, particularly at high latitudes, could favor the dissolution of biogenic carbonate minerals by the end of the century (Feely et al. 2009, Steinacher et al. 2009).

For bivalve aquaculture, the impacts of OA were first seen in oyster hatcheries in the Pacific Northwest. At a hatchery in Oregon during the summer of 2009, aragonite saturation state ranged from 0.8 to 3.2 and pH fluctuated between 7.6 and 8.2; both larval production and growth (120 to 150 mm) of the oyster *Crassostrea gigas* were significantly negatively correlated with the aragonite saturation state of waters in which larval oysters were spawned and reared for the first 48 h of life (Barton et al. 2012). As a result, shellfish hatcheries in the Pacific Northwest now routinely buffer their water.

Robbins and Lisle (2017) examined pH trends in Florida estuaries between 1980 and 2008 and found consistent decreases in 8 out of the 10 estuaries, with an average rate of decrease on the Gulf of Mexico side estuaries of 7.3×10^{-4} pH units year^{-1}. These rates are 2–3 times lower than decreases associated with ocean acidification in the Pacific Northwest. As a result, Florida hatcheries are not now experiencing problems associated with pH. This is something that warrants continued monitoring.

As reported in Section 4.1.2.2, maximum summer temperatures may be causing mortality of northern quahogs, *M. mercenaria*. This trend is likely to continue, as sea surface temperature is projected to increase 0.05 to 0.5 ^{o}C decade^{-1} through 2099 (Alexander et al. 2018). The aquaculture industry will have to adapt, either by developing a heat tolerant strain of *M. mercenaria* or incorporating more heat-tolerant native species, such as *M. campechiensis* or *M. nimbosa,* into the industry.

4.1.3.2 Harmful algal blooms (HABs)

Harmful algal blooms (HABs) have negative economic, environmental and human health impacts worldwide (Shumway 1990, Landsberg 2002). Associated costs are estimated to exceed $82 million annually (Hoagland and Scatasta, 2006).

In the Gulf of Mexico, the most prevalent HAB is the neurotoxic dinoflagellate, *Karenia brevis*. The most recent bloom lasted over a year, from October 2017 until early 2019, and extended from the Florida panhandle to southwest Florida and even made its way to the east coast (https://oceanservice.noaa.gov/hazards/hab/florida-2018.html#:~:text=An%20unusually%20persistent%20harmful%20algal,the%20east%20coast%20of%20Florida.)

The neurotoxin produced by *K. brevis* affects shellfish in two primary ways. First, it reduces the growth rate and survival of both clam and oyster larvae (Rolton et al. 2014). Adult bivalves are not directly affected by the toxin, but they accumulate the toxin in their tissues, making them unsafe for human consumption (Echevarria et al. 2012). An economic analysis revealed that the extended red tide event of 2015-16 resulted in a sales loss of cultured clams worth $1.33 million and an overall negative economic impact of $3.25 million to the Florida economy (http://shellfish.ifas.ufl.edu/news/%20Red-Tide-Causes-Economic-%20Losses-SW-Florida-Industry/ http://shellfish.ifas.ufl.edu/news/%20Red-Tide-Causes-Economic-%20Losses-SW-Florida-Industry/)

Shellfish harvesting areas are closed when cell counts reach 5,000 liter^{-1} and harvesting cannot resume until toxin levels in tissues are below 20 mouse units (FDACS 2019). Uncertainties regarding the relationships between cell counts, uptake rates and tissue toxin levels have resulted in a conservative regulatory environment. Research is needed that will allow the state (FDACS and FWC) to more effectively regulate shellfish harvesting in Florida with respect to HAB.

Research Priorities

1. Determine clearance rates of K. brevis by quahogs and oysters and establish relationships between clearance and toxin levels in tissue (differentiate among adductor muscle, gonad, digestive gland, etc).

2. Once shellfish become toxic, investigate means of increasing the rate of depuration of toxins so shellfish are safe for human consumption sooner than via in situ depuration.

Both of these aspects will help reduce the time that shellfish harvesting is prohibited and reduce the resulting economic impact. The regulatory agencies should also adopt methods that increase the accuracy and reduce the turn around time for both cell counts (DNA assay or HABscope) and toxin levels in tissue (LC-MS). These techniques are currently available and their accuracy and precision have been demonstrated.

4.2 Research Needs for Shellfish Restoration

The use of bivalve restoration to supplement declining populations of wild species and to enhance estuary resiliency is a recent environmental practice. Bivalve shellfish (e.g., clams, oysters, mussels) filter the water when feeding and as such, remove seston from the water. Consequently, bivalves are credited with ecosystem services such as improvement of water quality and clarity, removal nutrients, and reduction of phytoplankton from the water column (Newell et al. 2002, Kellogg et al. 2013). In Florida, restoration of hard clam populations (*Mercenaria spp*.) to improve estuarine conditions is gaining momentum, however not enough information exists on the benefits that restored clams can provide for the environment. The Gulf Shellfish Institute recognizes the importance of rigorous research to fill in the knowledge gaps associated to the practice and thus developed a workshop to take a first step at identifying data gaps and prioritizing research needs. Filling in these knowledge gaps will increase the support of hard clams as candidates for estuarine ecosystem restoration and positively influence the Florida shellfish aquaculture industry as hard clams form a major component of the industry.

The workshop consisted on three half-days from 9:00am until 12:00pm. There was a total of 131 participants registered for the workshop representing the academic, industry, government regulatory/management, non-profit, education/extension/outreach and interested citizen sector.

The first day of the workshop was focused on understanding the history of clam restoration in Florida through a presentation given by Dr. William Arnold. Subsequently, short presentations were given by the different groups conducting clam restoration projects in Florida. Sarasota Bay Watch is leading efforts on multiyear restoration efforts of southern hard clams (*Mercenaria campechienis*) in Sarasota Bay. Todd Osborne team from Florida Atlantic University, Harbor Branch Oceanographic Institute is focusing on northern hard clam (*Mercenaria mercenaria*) restoration in the Indian River Lagoon. The Sanibel Captiva Conservation Foundation including Melynda Brown, Eric Milbrandt, and Leah Reindenbach are focusing on clam restoration efforts in the Charlotte Harbor estuary.

During the second day of the workshop, three presentations were given by experts. Dr. Bruce Barber presented on hard clam filtration rates as an ecological service. Dr. Patrick Baker presented on carbon sequestration by hard clams. Dr. Ashley Smyth presented on nutrient removal by hard clams. The presentations were followed by a question-and-answer session with the participants. A group discussion session followed where participants were split into groups and asked to identify the areas where further research is needed.

During the third day of the workshop, two presentations were given by experts. Dr. Bradley Peterson discussed seagrass enhancement by hard clams. Dr. Matthew Parker presented on potentially obtaining nutrient credits through clam restoration efforts. Similar to day 2, the presentations were followed by a question-and-answer session with the participants and a breakout group discussion.

Throughout the workshop there were several basic themes that came to the forefront. These can be broken down into the limited available information on the ecological services conducted by hard clams including: water filtration, nutrient removal and flux, carbon sequestration, seagrass enhancement, and nutrient credits.

4.2.1 Water filtration

Filter feeding by clams can result in further ecological services such as a reduction in particulate matter, which in turn decreases turbidity, increases light penetration and benthic plant productivity. Filtration by clams also produces benthic-pelagic coupling which influences the amount of nutrient deposition (feces and pseudofeces) in sediments, the burial in sediments, and increases denitrification and seagrass production. Filtration rates of hard clams have been examined by a handful of studies and using static methods or a flow-through approach (Hibbert 1977, Riisgård 1988, Beals 2004, Echevarria et al. 2012). These studies have examined filtration rates by hard clams ranging from less than 60 minutes to four hours of experiments. Temperatures range from 12-30 °C and filtration rates have been calculated using particulate organic carbon removal, coulter counter, and flow cytometer. Many questions still remain related to hard clams and filtration rates.

Research Priorities

1. Determine how filtration rates of hard clams is affected by environmental variables such as temperature (10-32 °C), salinity (20-35 psu), food availability, and water flow.

2. Investigate how do all these factors simultaneously affect filtration rates and the amount of time spent filter feeding.

3. Examine clam behavior in the estuaries to understand how much water they filter daily. Clams have inactive and active times during the day and it is unclear how much time they spend filter feeding.

4.2.2 Nutrient removal and flux

Transformation rates of nutrients and nutrient pools can depend on environmental context, bivalve physiology and density, and on the microbial community present in the sediments. Nitrogen in the water fuels algae production, which is consumed by clams and repackaged into biodeposits. Consequently, nitrogen is removed from the system by biodeposits getting buried in the sediments. Once in the sediments, biodeposits through microbial processes can be denitrified into nitrogen gas (N_2), which further removes nitrogen from the system (Reitsma et al. 2016). Hard clams can also remove nitrogen by assimilating it into biomass through growth (Rose et al. 2014). Most of the available information on bivalve and nutrient removal and flux originates from studies focused on oysters. Very limited information exists on nitrogen cycling and hard clams.

Research Priorities

1. ***Determine how nutrient cycling of nitrogen varies between hard clam species, location, environmental variables, and growing conditions in aquaculture settings.***

2. ***Investigate how hard clam bioturbation and oxygen availability influence denitrification.***

3. ***Assess how hard clam filtration rates affect sediment nitrogen cycling.***

4. ***Investigate how hard clam sensitivity to low quality seston influences sediment nitrogen cycling.***

5. ***Determine to what extent clams remove (denitrify) or retain (Dissimilatory Nitrate Reduction to Ammonia) nitrogen.***

4.2.3 Carbon sequestration

Hard clams can store carbon in their shells, but to increase their storage capacity the industry and restoration practices must expand throughout Florida. Carbon storage has not been assessed in clam restoration projects and the little information available is sourced from studies in clam aquaculture leases. In addition, there are several sources of error in calcium carbonate estimates, which need to be investigated for accurate calculation of carbon storage.

Research Priorities

1. ***Investigate how farming can affect naturally-occurring shell mineralization in hard clams.***

2. ***Clam shells may form and get lost from samples due to small size or other factors prior to harvesting; thus, calculations may underestimate carbon storage. Investigating how carbon storage varies with clam size and what percentage of clams escape the gear will help fill in this unknown.***

3. ***Investigate how different estuarine conditions (e.g., depth, temperature, salinity, dissolved oxygen) may affect carbon storage and the fraction of carbon dioxide (CO_2) that dissolves back into the seawater.***

4. Investigate how the process of carbon storage vary temporally (i.e., interannually and intra-annually).

5. Some metabolic carbon dioxide (CO_2) is used for calcification instead of being released into the atmosphere, but how does this fraction vary with hard clam age?

6. Assess what fraction of biological remineralization uses carbonate (CO_3^{2-}) instead of bicarbonate (HCO_3^-), and how it affects carbon storage.

4.2.4 Seagrass enhancement

There are several reasons why including infaunal bivalves such as, hard clams as a coastal management strategy might positively enhance seagrass habitats. Filter feeding by bivalves reduces turbidity and allows light to penetrate the benthos reaching primary producers, such as seagrasses, leading to enhanced productivity. Filtration by bivalves also results in the transfer of material and nutrients to the sediments as pseudo-feces and feces (Riisgård 1988, Carroll et al. 2008, Peterson and Heck 1999, 2001a, 2001b). Bivalves may therefore further support seagrass by creating nutrient rich biodeposits on the sediment. For example, recent research by the Gulf Shellfish Institute demonstrated that sediments containing infaunal clams at commercial densities had a total organic matter content that was 37% greater than control areas after nine months and 126% greater content after 18 months (Barber 2018). Additionally, bioturbation by infaunal bivalves can move the nutrient rich material to the rhizosphere and possibly stimulate seagrass growth. Consequently, hard clams likely have a positive effect on seagrass. Unfortunately, little information is available on the effects that hard clams may have on seagrasses.

Research Priorities

1. Determine how the addition of nutrients by clams into sediments (feces and pseudofeces) affect the reproductive output, leaf, and rhizome production of the different Florida seagrasses.

2. Determine the density of clams necessary to improve water quality and clarity and result in seagrass enhancement.

3. Examine the time scale expected to see results.

4. Investigate how upstream vs. downstream location and estuarine residence time affect clam impacts on seagrass.

4.2.5 Nutrient credits by hard clams

- There are no current programs in Florida that can provide nutrient credits for harvesting, purchasing, or restoring hard clam populations. In other US states (e.g., Maryland), the oyster nutrient program has first established a 'Best Management Practice' (BMP). This process involves:
    - Petition the EPA for permission to form a panel to look at the topic in hand
    - Submit a potential of panel members to EPA for approval
    - Schedule regular panel meetings
    - Research information to find out what is known and not known on the topic
    - Obtain group consensus on recommendations by the panel
    - Write a report on the panel findings
    - Present the report publicly to the corresponding agency
    - Address public comments
    - Re-submit the report with addressed comments
    - The corresponding agency approves or disapproves the recommendations. If this occurs then the Best Management Practice is created for items in the report.
    - Municipalities then, determine any potential compensation system.
- The panel will be in charge of:
    - Identifying and defining hard clam practices for BMP consideration
    - Developing a pollutant removal crediting decision framework for hard clam BMPs
    - Proposing nitrogen and phosphorus removal effectiveness estimates for hard clam practices that have sufficient science support to justify recommendations
- Panel members can include researchers, non-profit, academics, extension members

4.2.6 General conclusions from the workshop

- As we suspected, there are significant unknows on the ecological services performed by clams, which need to be addressed in order to further support or validate their use for restoration.

- There is preliminary research that can provide us with insights of what to expect for the different ecological services performed by clams. Hard clams likely impact sediment composition, water quality, and other trophic groups, such as clam predators and bivalve species. However, there is a tremendous need for research to fill in the details such as direction of relationships (positive/negative), magnitude of impacts, and quantification of the processes so we can ultimately understand how clams work as part of the entire ecosystem.
- While clams certainly have the potential to help our estuaries, they must not be singled out as the only solution for all the water quality issues of our estuaries. Clam restoration can serve as one of the many tools that need to be used as part of a larger management water quality strategy to ensure the health of our coastal environments.
- It is necessary to understand our estuarine systems thoroughly and identify the reasons why our estuaries are degraded. We must assess if the estuaries are healthy enough to sustain populations of clams over time. Otherwise, considerable effort and funds will be spent on restoring clams that might not survive under current conditions.
- One important initial aspect when considering a hard clam restoration project, is to identify the reason why clams are being restored (e.g., improve water quality, enhance seagrass, restore the native clam populations). This would allow the determination of the best approach to be used.
- Addressing the knowledge gaps and research priorities identified in this workshop will allow us to make accurate estimates of ecological services and/or suggest farm management practices that might maximize potential ecological benefits. This will ultimately contribute to Florida economics by possibly contributing an additional market for the shellfish aquaculture industry.

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