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# BIVALVE SHELLFISH FILTRATION AND AQUACULTURE POLICY: TOWARDS A GREATER UNDERSTANDING OF MULTISPECIES ECOSYSTEM SERVICES AND A BLUEPRINT FOR IMPROVING DOMESTIC ESTUARINE PRODUCTION

By

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# ABSTRACT OF THE DISSERTATION

Bivalve shellfish filtration and aquaculture policy: Towards a greater understanding of multispecies ecosystem services and a blueprint for improving domestic estuarine production

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Molluscan bivalve shellfish are well-known for the suite of ecosystem services they provide within the nation's estuaries, with one of the most highlighted being their ability to filter the water column. Based on previous research, increasing bivalve species biodiversity should augment the provision of ecosystem services and theoretically, stabilize estuaries. A direct examination of potential interspecific population interactions amongst species within this functional group, however, has not yet been explored. This study examines that gap and forges a better link between theory and potential real-world limitations. This research used two common species within Delaware Bay, USA – *Crassostrea virginica* (Gmelin) and *Geukensia demissa* (Dillwyn) – to explore potential particle removal relationships when placed in monoculture and cohabitation. The objective was to answer the question of whether these two species exhibit interspecific competition for food resources. Using the highly controlled Clearance Method as well as a more variable flow through system, interspecific competition did not result between these two bivalve filter-feeding species. Particle removal amounts under the static and variable conditions were similar among single and mixed species populations. In the flow through systems, where filtration of a native particle community was examined, *C. virginica* exhibited

particle preferences based on size and possibly quality (the methods did not allow for examination of plankton speciation). Overall, the results provide evidence of complementary resource use and link previously modeled results of ecosystems with experimental data on coexisting bivalve species.

The second component of this research was an exploration of the management of bivalve shellfish, specifically focusing on the regulation of aquaculture within the United States. This section of the research analyzed the diverse policy systems implemented within four states to develop the thesis that U.S. shellfish aquaculture production is dependent upon industry supportive state-level regulations. Several key factors to successful industry development arose out of the four state analysis. The first factor in successful state industry growth is a single contact point between grower and the state permitting authorities (point-of-contact). Connected to the point-of-contact is the premise that vertical integration of permitting from federal permits through to local boards or review councils (vertical integration) occurs via that singular regulatory liaison. Furthermore, states with older regulatory structure (age) continually work to improve permitting efficiencies but do not have recent changes in regulation that cause short-term industry instability. Changes to regulation can cause uncertainty within the industry, limiting short-term growth while growers wait for regulatory measures to finalize. This is especially evident in states with numerous, recent changes. Finally, the primary factor to successful state shellfish aquaculture is political will or capital within the Executive Branch (political will). It is through cohesive agreement among all agencies within the Executive Branch, including the Governor's Office, that progress within states can be achieved with greatest efficiency.

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# DEDICATION

This work is dedicated to Anastasia and Catherine.

You know why.

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# Introduction

#### **1. Bivalve Ecosystem Services**

Bivalve filter feeders are known to provide ecosystem services (Dame 2012, Grabowski & Peterson 2007), with one of the most cited bivalve services being the removal of water column particles and management of system nutrients via filtration, sequestration, and remineralization (Cucci et al. 1989, Dame 2012, Grabowski & Peterson 2007, Hawkins et al. 1998, Humphries et al. 2016, Kellogg et al. 2013, Newell 2004, Newell et al. 2005, Prins et al. 1998, Porter et al. 2004, Rose et al. 2014). Much of the research on common, native species has focused on restoration efforts (harvestable population as well as filtration of excess nitrogen), with more recent research pointing to the need for multiple species to realize the full impact of service provision (Cerco and Noel 2007, Fulford et al. 2007, Fulford et al. 2010, Pomeroy et al. 2006). These modeling studies are not surprising since many bivalve species coexist or inhabit neighboring physical spaces within an estuary. Theoretically, these studies are evidence of the positive biodiversity-ecosystem health relationship (Figure I.1) that forms the basis of ecological literature on this topic (Cardinale et al. 2006, Dame 2012, Grant 1996, Loreau et al. 2001, Naeem et al. 1994, Rey Benayas et al. 2009, Worm et al. 2007). In scaling down from the ecosystem level to the potential interspecific relationships, however, few studies examine multispecies filtration relationships at the species level. As bivalve shellfish are increasingly used in restoration and aquaculture, this missing connection between the ecosystem literature and potential species-specific relationships has become a priority and is the overarching objective of this dissertation.



Figure I.1: Positive Biodiversity-Ecosystem Relationship. An increase in the biodiversity filterfeeding bivalves (primary consumers) results in greater service provision (and removal of primary productivity via filtration), which ultimately yields net benefits for the ecosystem, such as enhanced stability, maintenance of production changes, and improved function.

# 2. Multispecies Filtration

The increasing use of estuaries for marsh restoration as well as structural oyster aquaculture provided a real need to further examine coexisting bivalve species. The first two chapters in this dissertation are experimental studies on the ecology of interspecific interactions of common bivalve species. Broadly, this research examined interspecific relationships at the species-level to determine if ecosystem health could benefit from increasing biodiversity while individual species could experience a competitive disadvantage. For restoration and aquaculture, if the species of interest (being restored for filtration services or cultured for sale) is at the competitive disadvantage relative to the other members of the functional group, the management actions may yield inefficient or unsuccessful results. In other words, how do interspecific interactions relate to the effectiveness of policy and management decisions?

This present study examined two common, key species on the East Coast of North America, the eastern oyster Crassostrea virginica (Gmelin) and the ribbed mussel Geukensia demissa (Dillwyn). Both species have been studied extensively for their filtration capacity and efficiency within native environs (Crosby et al. 1990, Langdon and Newell 1990, Pales Espinosa et al. 2008, Riisgard 1988, Shumway et al. 1985, Wright et al. 1982, Wetz et al. 2002), but not together to understand their interactions. Chapters One and Two of this dissertation provide research to fill the gap between modeled results and lack of in-situ empirical measurements of interspecific relationships among functional group members. The use of a highly controlled system experiment (see Chapter One, Clearance Method) as well as flow through experiments with variable, native particle quantities and food species, (Chapter Two) allowed for examination of the specific research question: is there an identifiable interspecific feeding relationship between these two species? The research herein is the first step in connecting theory and real world species behaviors, and is the first known use of multiple species as one population within manipulated filtration studies. The results have implications for restoration efficiency such as competition potentially limiting a restored population's ability to provide services. The focus of this research was on potential limitations for a shellfish aquaculture farm. A competitive relationship between these two species, with G. demissa the dominant, could result in slower growth of on-farm C. virginica, or potentially stress stocks to point of increased mortality events. These studies could have real, near-term, and significant impacts for the industry.

# 3. Aquaculture & Policy

The third and final chapter in this work shifts focus a bit and analyzes policy regimes for shellfish aquaculture to determine avenues for individual state industry growth. At the advent of this research, the premise was to examine ecological interactions (Chapters One and Two) to gain information in support of management decisions. Chapter Three was originally conceptualized with the intent to apply the results of the previous chapters to regulatory actions within New

Jersey and East Coast states, essentially applying ways that ecosystem services from multiple species can be supported via regulatory measures at the state-level. After initial analysis, understanding successful state policy (regulatory and nonregulatory measures) became a greater priority. That prioritization included an analysis of the shellfish aquaculture regulatory regimes of the states of Maryland, New Jersey, Virginia, and Washington. These state-level case studies are used to develop the main chapter thesis that U.S. shellfish aquaculture production can only be increased via state-level actions that support industry development (or conversely, a decrease or stasis in national production will result from uniform state-level inhibitory policy measures). At the time the chapter was written, no shellfish harvest had occurred within U.S. territorial waters; all shellfish aquaculture was within State territorial waters (0-3 nautical miles, except the Gulf of Mexico bordering states). Farming in federal waters is still costly and cannot be viewed as a near-term reality for many of the typical, small businesses within the shellfish aquaculture industry, and is not considered within Chapter 3.

# 4. Format

Each of the chapters included in this dissertation are formatted for publication within a journal, noted after the chapter title. Since the third chapter is focused on policy, the format for reference citations is different from that of the first two chapters, but is correctly formatted for the journal identified at the top of the page. Additionally, there is purposeful redundancy within the text to ensure each chapter is a successful, independent publication.

# References

- Cardinale, B.J., D.S. Srivastava, J.E. Duffy, J.P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 443:989-992.
- Cerco, C. F.; M. R., Noel. 2007. Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? Estuaries Coasts. 30(2):331–343.

- Crosby, M.P., R.I.E. Newell, C.J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. Limnol. Oceanogr. 35(3):625-639.
- Cucci, T.L., S.E. Shumway, W.S. Brown, and C.R. Newell. 1989. Using Phytoplankton and Flow Cytometry to Analyze Grazing by Marine Organisms. Cytometry. 10:659-669.
- Dame, R.F. 2012. Ecology of marine bivalves: an ecosystem approach. Second edition. CRC Press, Boca Raton, 271 pp.
- Fulford, RS., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., Luckenbach. M., 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Mar. Ecol. Prog. Ser. 336:43-61.
- Fulford, RS., Breitburg, D.L., Luckenbach, M., Newell R.I.E., 2010. Evaluating ecosystem response to oyster restoration and nutrient load reductions with a multispecies bioenergetics model. Ecological Applications. 20(4):915-934.
- Grabowski, J.H., Peterson, C.H., 2007. Restoring oyster reefs to recover ecosystem services. Theoretical Ecology Series. 4:281-297.
- Grant, J. 1996. The relationship of bioenergetics and the environment to the field growth of cultured bivalves. Journal of Experimental Marine Biology and Ecology. 200:239-256.
- Hawkins, A.J.S., B.L. Bayne, S. Bougrier, M. Heral, J.I.P. Iglésias, E. Navarro, R.F.M. Smith, M.B. Urrutia. 1998. Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. Journal of Experimental Marine Biology and Ecology. 219:87-163.
- Humphries A.T., S.G., Ayvazian, J.C. Carey, B.T. Hancock, S. Grabbert, D. Cobb, C.J. Strobel and R.W. Fulweiler. 2016. Directly Measured Denitrification Reveals Oyster Aquaculture and Restored Oyster Reefs Remove Nitrogen at Comparable High Rates. Front. Mar. Sci. 3:74. doi: 10.3389/fmars.2016.0007
- Kellogg, M.L., J.C. Cornwell, M.S. Owens, K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. Mar Ecol Prog Ser. 480:1-19. doi: 10.3354/meps10331
- Langdon, C.J., R.I.E. Newell. 1990. Review: Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58:299-310.
- Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. Science. 294:804-808.
- Naeem, S., L.J. Thompson, S.P. Lawler, J.H. Lawton, and R.M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature. 368:734-737.
- Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: a review. Journal of Shellfish Research. 23:51-61.

- Newell, R.I.E., T.R. Fisher, R.R. Holyoke, and J.C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. Pages 93-120 *in* R. Dame and S. Olenin, editors. The comparative role of suspension feeders in ecosystems. Springer, Dordrecht, The Netherlands.
- Pales Espinosa, E., B. Allam, and S. Ford. 2008. Particle selection in the ribbed mussel *Geukensia demissa* and the Eastern oyster *Crassostrea virginica*: Effect of microalgae growth stage. Estuarine, Coastal and Shelf Science. 79:1-6.
- Pomeroy, L. R., D'Elia, C. F., Schaffner, L.S., 2006. Limits to Top-Down Control of Phytoplankton by Oysters in Chesapeake Bay. Mar. Ecol. Prog. Ser. 325:301-309.
- Porter, E.T., J.C. Cornwell, and L.P. Sanford. 2004. Effects of oyster *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. Mar. Ecol. Prog. Ser. 271:61-75.
- Prins, T.C., Smaal, A. C., Dame, R.F., 1998. A review of the feedbacks between bivalve grazing and ecosystem processes. Aquatic Ecology. 31:349-359.
- Rey Benayas, J.M., A.C. Newton, A. Diaz, and J.M. Bullock. 2009. Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. Science. 325:1121-1124.
- Riisgård, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of North American bivalves. Mar. Ecol. Prog. Ser. 45:217-223.
- Rose, J.M., S.B. Bricker, M.A. Tedesco, G.H. Wikfors. 2014. A Role for Shellfish Aquaculture in Coastal Nitrogen Management. Environ Sci Technol. 48:2519-2525. dx.doi.org/10.1021/es4041336
- Shumway, S.E., T.L. Cucci, R.C. Newell, C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Bio. Eco. 91:77-92.
- Wetz, M.S., A.J. Lewitus, E.T. Koepfler, K.C. Hayes. 2002. Impact of the Eastern oyster Crassostrea virginica on microbial community structure in a salt marsh estuary. Aquatic Microbial Ecology. 28:87-97.
- Worm, B. E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpem, J.B.C. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J.Stachowicz, and R. Watson. 2007. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science.314:787-790.
- Wright, R.T., R.B. Coffin, C.P. Ersing, and D. Pearson. 1982. Field and Laboratory Measurements of Bivalve Filtration of Natural Marine Bacterioplankton. Limnology and Oceanography. 27(1):91-98.

## Chapter 1

# Static condition particle removal from single and mixed species populations of *Crassostrea* virginica and *Geukensia demissa*

# ABSTRACT

Many of the landmark bivalve shellfish studies within ecology have been the result of a noted management need to restore a single key species with much of today's management protocols stemming from these single species studies. Those studies provide the foundational basis for today's research and are critical to the collective knowledge of ecosystems. In estuarine communities, however, recent ecosystem modeling studies have consistently found that single species restoration is not sufficient for filtration service provision. Specifically for this paper, researchers modeling filtration have found that particle removal and nutrient management are best provided by multiple species, but few experimental studies have explored this topic further. This study examines the filtration interaction of two potentially coexisting species within Delaware Bay - Crassostrea virginica (Gmelin) and Geukensia demissa (Dillwyn). Closed system experiments were used to determine particle clearance under highly controlled monoculture and multispecies arrangements. The results of this study showed that the species removed particles at similar levels whether in monoculture or cohabitating in a dual species population. Measured clearance rates, however, were much lower than other published rates, providing several lines of future inquiry with these species. Overall, the results support further examination of multispecies management actions to enhance ecosystem service provision.

# 1. Introduction

Bivalve filter-feeders are considered ecosystem service providers and are well-studied for their role of removing seston from the surrounding water column and depositing particulate material

within the benthos (Cucci et al. 1989, Dame 2012, Grabowski & Peterson 2007, Hawkins et al. 1998, Newell 2004, Newell et al. 2005, Prins et al. 1998, Porter et al. 2004). The provision of benthic-pelagic coupling and top-down control on phytoplankton via filtration serves maintains estuarine health by regulating nutrient fluxes (Arfken et al. 2017, Caffrey et al. 2016, Humphries et al. 2016, Kellogg et al. 2013, Rose et al. 2014) and limits excess primary production (Dame 2012, Newell 2004). These characteristic services of bivalve shellfish have often been studied under the premise of single-species research or managing for restoration of a single species. As has been shown by several researchers working on restoring oysters in the Chesapeake Bay, filtration services measured by oysters alone are not providing the level of service expected; rather a compliment of species within the same functional group is required (Cerco and Noel 2007, Fulford et al. 2007, Fulford et al. 2010, Pomeroy et al. 2006). To explore the conclusion that multiple species are required for more efficient provision of filtration services, this study examined the filtration of two common east coast species – Crassotrea virginica (eastern oyster) and Geukensia demissa (ribbed mussel). The objective was to determine if these study species exhibit interspecific competition for food resources when placed together in a population (coexistence).

This study replicated what Riisgard (2001) refers to as the Clearance Method (first used by Fox et al. 1937 with *Mytilus californianus*; reviewed for clearance rate calculations in Coughlan 1969; additional references in Riisgard 2001). Under the Clearance Method, the study organisms are held within a controlled environment to remove variables outside of those introduced by the researcher (Riisgard 2001). For the two study species, control of the food species was deemed critical in exploring their potential feeding relationship based on their known particle size and type preferences (Riisgard 1988, Langdon and Newell 1990, Pales Espinosa et al. 2008, Shumway et al. 1985, Wright et al. 1982, Wetz et al. 2002). *Geukensia demissa* is known to utilize bacteria and detritus as a food resource (Langdon and Newell 1990, Wright et al. 1982)

with some studies showing assimilation efficiencies as high as 75-90% (Bushaw-Newton et al. 2008, Kreeger and Newell 2001). *Crassostrea virginica*, on the other hand, does not preferentially ingest microbial food resources available within estuarine communities (Crosby et al. 1990, Langdon and Newell 1990, Wetz et al 2002), with assimilation efficiencies around 50% for particles at  $2\mu$ m (*G. demissa* has an absorption efficiency around 70% for that size) (Riisgard 1988; see also Haven and Morales-Alamo 1973). By using known algal species (with a known size range), both bivalve species are expected to efficiently remove the algae cells, thereby establishing experimental treatments where competition is hypothesized to occur due to a multispecies population feeding on a limited food resource. Experimentation with greater particle variability is detailed in Chapter 2.

# 2. Methods

#### 2.1 Study Location

Closed system experiments were conducted at the Rutgers University Aquaculture Innovation Center (AIC) located on the Cape May Canal in North Cape May, Cape May County, New Jersey, USA. Filtered seawater was available under two levels of filtration- 50µm and larger removed from water, used in the closed system experiments; and 1µm and larger removed from seawater, used within sample analysis equipment.

# 2.2 Bivalve Populations

This research focused on two species native to Delaware Bay, NJ, USA- *C. virginica*, a common species within the state and regional aquaculture industries, and *G. demissa*, a species ubiquitous to coastal marshes. Under natural conditions, these two species are, for the most part, spatially separated within the Bay, with wild *C. virginica* beds near the main stem of the Bay (the location of New Jersey's managed oyster fishery) and *G. demissa* surrounding coastal marshes. There are some observations of *C. virginica* within intertidal and marsh areas of the State where they

cohabitate with *G. demissa*, but those have yet to produce sustained populations. The increasing desire for *C. virginica* aquaculture coupled with potential experimental aquaculture for *G. demissa*, has created human-induced relationships and unknown interactions. Additionally, due to endangered species concerns along the western shores of the Cape May peninsula, *C. virginica* aquaculture will foreseeably be directed to locations away from protected species but possibly towards other filter-feeders. Prior to commencing multispecies aquaculture or expansion of *C. virginica* culture within areas of *G. demissa*, this research will provide information on potential relationships that could occur between these two species, specifically with regards to their feeding behavior.

Three populations of bivalve shellfish were used for this research, including:

- 1. *Crassostrea virginica* (CV) one year of age, hatchery-reared, maintained under constant subtidal conditions, shell height 10mm to 50mm;
- 2. *Geukensia demissa*, subtidal- (GDS) unknown age, wild source, maintained under constant subtidal conditions, shell height 10mm to 60mm; and
- 3. *Geukensia demissa*, intertidal- (GDI) unknown age, wild source, maintained under intertidal conditions with two low (emersion 4 hours) and two high tides (immersion 8 hours) per 24-hour cycle, shell height 10mm to 60 mm.

Preliminary trials with constantly submerged *G. demissa* resulted in unexpected filtration rates. Two populations of *G. demissa* were deemed necessary based on those early results and the work of Galimany et al. (2013a), which showed that in as little as three days *G. demissa* can change filtration behavior based on tidal placement (intertidal versus subtidal) (see also Borrero 1987, Gillmor 1982 for growth studied and Kreeger et al. 1990 for long-term effects of tidal exposure). As best could be controlled, the *G. demissa* intertidal were held out of water several hours prior to the commencement of trials to ensure replication of their noted pulse feeding behavior (Charles and Newell 1997).

# 2.3 Biomass Estimates

Due to the nature of the experimental systems, it was desirable to limit the size of each bivalve to allow for the testing of populations as opposed to individuals. The smaller size of the individuals used in this research relative to sizes of individuals found within indigenous populations, led to the development of unique shell height to dry weight allometric equations. For each species, 100 animals were sacrificed with the resulting relationship for *C. virginica* represented in Figure 1.1 and *G. demissa* represented Figure 1.2. Both of these figures show that using population level equations would have yielded overestimates of biomass for the experimental subjects, therefore the relationships developed for the smaller test subjects were utilized.

Figure 1.1 and Figure 1.2 show both species exhibit differing relationships between the population level data and the experimental sized organisms. In Figure 1.1, the experimental *C. virginica* size range has a lower dry weight to shell height relationship as compared to the population level. The relationships exhibited, however, were similar. Figure 1.2, on the other hand, shows an overlap of the *G. demissa* relationships at the smaller shell heights. It is hypothesized that this overlap occurred due to those shell heights occurring within both the population level data and the experimental population data. For *C. virginica*, the data for the population level and the experimental organisms did not have a similar shell height co-occurrence.



Figure 1.1: The calculated shell height to dry weight relationship for the smaller, experimental *C. virginica* size group is represented by the dark grey squares; the allometric equation is signified by the dark grey line. Data extracted from annual sampling of the fishery oyster beds of Delaware Bay was used to develop a population level equation, which included all sizes from the yearly sampling efforts. The light grey boxes show the dry weight estimates obtained by entering measured shell heights for smaller, experimental subjects into the population-wide equation. The population level equation would have yielded an overestimate of biomass for all oysters used in this research.

#### 2.4 Tank Systems

The closed system consisted of filling six liters of 50µm filtered seawater (maintained at 28C) into experimental aquaria (2 gallon acrylic fish tanks, which equates to circa 7.75 liters), placement of the treatment bivalves within the appropriate tank, and subsequent feeding of all tanks with cultured algae. The following treatments were employed: 1) empty, but fed (control); 2) *C. virginica*; 3) *G. demissa* subtidal; 4) *G. demissa* intertidal; 5) *C. virginica* & *G. demissa* 

subtidal; and 6) *C. virginica* & *G. demissa* intertidal. Each tank, except for the control, contained three grams dry weight biomass of the identified bivalve species. When multiple species were placed into a tank, the biomass was estimated at one and one-half grams per species for a total tank biomass of three grams to ensure a consistent biomass for all treatment tanks.



Figure 1.2: The shell height to dry weight data for the measured, smaller *G. demissa* are represented with the darker grey boxes; the resulting allometric equation is signified by the dark grey line. The measured shell heights from that smaller group were then entered into the allometric equation obtained from previous unpublished studies using a set of *G. demissa* from a Delaware Bay tributary, which included a larger range in subject size. The light grey boxes represent estimated dry weight for the measured shell heights of the smaller experimental *G. demissa*, with the light grey line signifying the equation used to obtain these estimates. A divergence in estimated biomass between the two data series begins at around 30mm shell height, with the equation used to estimate larger *G. demissa* overestimating biomass from 30mm shell height and larger.

Between 200 and 300 mL of cultured *Isochrysis* sp. (4-6µm) was fed to each tank, with the same volume per trial date (the variation in volume occurred on different dates, not between tanks). An *Isochrysis* sp. was selected due to the continuous growth phase cultures available at the facility

(see Pales Espinosa et al. 2008 for additional consideration of culture phase with *G. demissa*) and is considered an industry standard for the culture of shellfish (Epifanio 1979, Pales Espinosa and Allam 2006, Pales Espinoa et al. 2008, Ponis et al. 2003). The control tank was fed similarly to all other tanks to determine particle settling rates under non-consumptive conditions. During each sampling event, the amount of algae fed into each of the six tanks yielded a change in water color discernable to the naked eye, and was therefore considered adequate to commence the trial.

The bivalves were allowed to acclimate for 20 minutes prior to feeding. Feeding and sampling occurred in the same order, from Tank 1 through Tank 6. The water was only agitated during feeding to ensure adequate mixing. Immediately after feeding, a five mL sample was extracted from each tank. Continuous five mL sampling from water above the bivalves (minimal agitation of the water) in five minute intervals occurred after the initial sample for a total of 40 minutes or 54 samples per experiment. It was determined in pre-trial experiments that samples beyond 40 minutes were not showing significant results, likely due to substantially reduced algal populations and resultant diminished filtration inherent to this method (Riisgard 2001). All samples were preserved with Lugols iodine solution and kept in the dark until analyzed. A total of 15 closed system sampling events occurred.

Samples were analyzed via a Beckman-Coulter Multisizer IV. Prior to feeding, a sample of the *Isochrysis* sp. was run through the particle analyzer to determine a peak particle frequency size range. Particle removal from each tank was then determined based on removal of particles within the peak size range. Variation in *Isochrysis* sp. density within each tank was expected, therefore, the removal levels for each tank were obtained by using the feeding sample (T0) for an individual tank as the baseline for that tank only; there were no between tank comparisons for removal counts. All samples after feeding (T5 through T40) were compared to the initial sample (T0), within the appropriate size range.

To account for inter-trial variability in algal culture densities, all particle counts for each tank during a specific trial date were normalized to the first sample (T0) for that tank. This allowed for the aggregation of particle count data for each tank throughout the 15 trials to then compare particle removal amounts between tanks. Mean clearance rates for each tank at every sampling time point are also provided and are estimated based on the Clearance Method equation provided in Coughlan (1969):

$$CR = (\ln(C_0) / \ln(C_t)) / t *V$$
 (1)

where Co and Ct = algal concentrations at time 0 and time t, t=time, and V = volume of water.

# 3. Results

The closed systems resulted in all treatment tanks removing particles through the first thirty to thirty-five minutes of sampling (Figure 1.3). Particle removal occurred immediately in all tanks except for *C. virginica* (Tank 2), which exhibited an increased particle count at the five-minute sampling point (T5). By the 10 minute sample (T10), all treatment tanks resulted reduced particle counts (removal), including *C. virginica*. None of the treatment tanks had significantly greater or lesser removal relative to the other treatment tanks. A plateau began to form for the tank containing *C. virginica* + *G. demissa* subtidal (tank 5) around 30 minutes (T30). By the 35 minute sampling interval, the plateau was obvious as all other treatment tanks except for *C. virginica* + *G. demissa* intertidal (tank 6) exhibit similar particle levels. The slowing of particle removal corresponds to circa 60% particle removal from the experimental systems. The control tank did not exhibit any signs of particle settling within the sampling timeframe.



Figure 1.3: Normalized peak particle counts. Results show particle removal in all treatment tanks, with near constant particle counts throughout the forty minutes in the control tank (dashed top line). The values here are normalized to the initial feeding (T0) sample and then averaged across the 14 sampling events.

The clearance rates calculated for each sampling interval (Figure 1.4) showed no clearance by the control tank, which corresponds with the relatively uniform particle counts over the 40 minutes of sampling from Figure 1.3. All treatment tanks showed similar clearance rates, both amongst the treatments as well as over the 40-minute experimental timeframe. The treatment tanks were between 0.1-0.2 liters per hour. Clearance rates for the five-minute interval were low for most of the treatment tanks, followed by a spike in the rate at the 10-minute sample. These clearance rates were not adjusted to a standard biomass measure (see Galimany et al. 2013b and Kreeger and Newell 2001), and reflect the full population, three grams dry weight.



Figure 1.4: Population (3g dry weight) clearance rates. Clearance rates at each five-minute sampling interval over the 40 minute experimental timeframe. The control tank (bottom dotted line) showed no clearance of particles, while all treatments showed clearance between 0.1-0.2 liters per hour.

# 4. Discussion

The clearance method experiments confirmed that the experimental bivalves were filtering when placed into a controlled environment after being manipulated. All treatment tanks removed particles in similar amounts, with coexisting bivalve species behaving in a similar manner to that of single species populations. Using standardized methods, this study found no discernable difference between the single species populations and the mixed species populations for particle removal and clearance rates. No changes in feeding for the mixed populations (or mixed populations relative to the single species populations) shows that no competition for food resources resulted in this study and therefore competition is not expected with these two species under restoration or aquaculture conditions.

# 4.1 Particle Counts

A plateau formed in all treatment tank at 35 minutes (T35) after feeding (Figure 1.3). A reduction in removal efficiency, as was seen here, was expected due to the method limitations (Riisgard 2001). As particles decrease within a closed system, filtration and clearance rates begin to mirror that decrease with reduced particle removal, according to species dependent characteristics (Riisgard 1988). In this study, the plateau (Figure 1.3) was around 60% particle removal, whereas in Riisgard's work (1988), the study ceased around 40-50% particle reduction for larger particles. The timeframe within which Riisgard (1988) reached the threshold for cessation of the experiment and the 40-50% mark is unknown, however, no trials lasted longer than 60 minutes. A longer sampling timeframe could have occurred for the study herein, using similar conditions, if additional algae were fed to the tanks throughout the sampling timeframe. Clausen and Riisgard (1996) and Galimany et al. (2013a) employed similar methods that allowed for sampling over hours (Clausen and Riisgard 1996) and weeks (Galimany et al. 2013a). By maintaining levels of the algae within the system, cyclic feeding related to food resources availability can be attained (see Clausen and Riisgard, 1996 p.42, Figure 5).

# 4.2 Clearance Rates

Clearance rates from this study were well below what has been reported by others for both species (Kreeger and Newell 2001, Newell et al. 2005, Riisgard 1988, Wright et al. 1982). In a study of the species used here, Riisgard (1988) showed clearance rates of 2.0-3.0 liters per hour for *C. virginica* and a lower rate of just above 2.0 liters per hour for *G. demissa* for a population with three grams dry weight biomass. Newell et al. (2005) explored clearance relative to seasonal changes and reported no clearance in Chesapeake Bay oysters in the winter months of January and February, followed by a constant increase to the maximum of just below 10 liters per hour per gram (dry weight) in July and August. In September and October, the clearance rate was

almost 7.5 liters per hour per gram and 2.34 liters per hour per gram, respectively (Newell et al. 2005).

The *G. demissa* clearance rates were similar to the other treatment tanks but were well below reported rates for this species. Wright et al. 1982 examined *G. demissa* removal of bacteria and algae and found clearance rates ranging from 0.46 liters per hour (bacteria) to 1.1 liters per hour (algae). Other studies of *G. demissa* have similarly reported higher clearance rates for both subtidal and intertidal *G. demissa* (Galimany et al. 2013a, Galimany et al. 2013b) than was found in this study. Examining the portfolio of food options for the intertidal mussel, Kreeger and Newell (2001) reported lower rates than other literature for *G. demissa* intertidal within their 4-5  $\mu$ m food size categories, but they were reflective of a standardized mussel size of 0.27grams dry weight. Compared with three grams dry weight, there is clearly a scale discrepancy with the results provided herein.

Although not mentioned in all the above studies, it is common to acclimate the study organisms to the food resource to ensure an accurate examination of filtration and clearance. For instance, Rissgard (1988) did not mention an acclimation timeframe for any of the six study species he examined, but Pales Espinosa et al. (2008) note that they acclimated their study species for one week prior to use. Interestingly, Galimany et al. (2013a) never acclimated their study organisms and used them within the study system immediately after epibiont removal and extraction from holding location (Galimany et al. 2013a). It is unclear if the lower clearance rates reported in this chapter were a result of using study species that were simply not ready for the warmer water and specific food resource used of this research. Since the supply of study organisms was limited and the study outlined in this chapter occurred concurrent with that of Chapter 2, organisms from all three populations were used interchangeably. It is possible that handling could have caused the lower clearance rates reported here.

# 4.3 Geukensia demissa Populations

No differences between the two G. demissa populations were found in this study. Several other researchers have also concluded that no difference in feeding occurs with this species due to tidal condition (Bayne et al. 1998, Griffiths 1980, Kreeger et al. 1990, Widdows and Shick 1985). The conclusion is that assimilation of specific particles as well as digestive physiology may differ due to tidal niche for this species (Kreeger et al. 1990).

Galimany et al (2013a), is one of the few studies where intertidal *G. demissa* showed greater particle removal than similarly sourced *G. demissa* that had been held in subtidal conditions for two months. In that study, a great deal of emphasis is placed on assimilation of particles and the food resource. For more on the long-term implications of assimilation differences in this species see Kreeger et al. (1990). Regarding the food resource, it is important to consider that in the 2013 study, Galimany et al. (2013) used *Tetraselmis chui* (PLY 429) and *Rhodomonas* sp. (RHODO strain). Those species are similarly used in shellfish culture and research but could produce different results from *Isochrysis* sp. under experimental conditions. Unfortunately, that does not entirely explain the difference in clearance rates for those in this Chapter to previously reported literature since Riisgard (1988) also used an *Isochrysis* sp (*Isochrysis galbana*) as well as a larger, cultured algae, *Cryptomonas* sp. and recorded higher clearance rates than those found here.

# 5. Conclusion

The closed system experiments resulted in no interspecific interactions influencing filtration behavior when *C. virginica* and *G. demissa* were placed into the same system. Under monoculture and co-habitation, these species reflected similar algal removal amounts and clearance rates. Additional study, under controlled conditions is required to determine why the clearance rates reported here are lower than those previously reported in literature for these two

species, and to determine if the food resource or handling may have influenced how the two G.

demissa populations filtered within this study.

# References

- Arfken, A. B. Song, J.S. Bowman, M. Piehler. 2017. Denitrification potential of the eastern oyster microbiome using a 16S rRNA gene based metabolic inference approach. PLoS ONE 12(9): e0185071. <u>https://doi.org/10.1371/journal.pone.018507</u>
- Bayne, B.L., J.S. Hawkins, E. Navarro. 1988. Feeding and Digestion in Suspension-Feeding Bivalve Molluscs: The Relevance of Physiological Compensations. Amer. Zool. 28:147-159.
- Borrero, F.J. 1987. Tidal height and gametogenesis: Reproductive variation among populations of *Geukensia demissa*. The Biological Bulletin. 173(1).
- Bushaw-Newton, K.L., D.A. Kreeger, S. Doaty, D.J. Velinsky. 2008. Utilization of *Spartina-* and *Phragmites*-Derived Dissolved Organic Matter by Bacteria and Ribbed Mussels (*Geukensia demissa*) from Delaware Bay Salt Marshes. Estuaries and Coasts. 31:694-703.
- Caffrey, J.M., J.T. Hollibaugh, B. Mortazavi. 2016. Living oysters and their shells as sites of nitrification and denitrification. Marine Pollution Bulletin. 112:86-90. https://doi.org/10.1016/j.marpolbul.2016.08.038
- Cerco, C. F.; M. R., Noel. 2007. Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? Estuaries Coasts. 30(2):331–343.
- Charles, F., R.I.E. Newell. 1997. Digestive physiology of the ribbed mussel *Geukensia demissa* (Dillwyn) held at different tidal heights. Journal of Experimental Marine Biology and Ecology. 209:201-213.
- Clausen, I., and H.U. Riisgård. 1996. Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to nutritional needs. Mar. Ecol. Prog. Ser. 141:37-45.
- Coughlan, J. 1969. The estimation of filtration rate from the clearance of suspensions. Marine Biology 2: 356-358.
- Crosby, M.P., R.I.E. Newell, C.J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. Limnol. Oceanogr. 35(3):625-639.
- Cucci, T.L., S.E. Shumway, W.S. Brown, and C.R. Newell. 1989. Using Phytoplankton and Flow Cytometry to Analyze Grazing by Marine Organisms. Cytometry. 10:659-669.
- Dame, R.F. 2012. Ecology of marine bivalves: an ecosystem approach. Second edition. CRC Press, Boca Raton, 271 pp.

- Epifanio, C.E. 1979. Growth in Bivalve Molluscs: Nutritional Effects of Two or More Species of Algae in Diets Fed to the American Oyster *Crassostrea virginica* (Gmelin) and the Hard Clam *Mercenaria mercenaria* (L.). Aquaculture. 18:1-12.
- Fulford, RS., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., Luckenbach. M., 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Mar. Ecol. Prog. Ser. 336:43-61.
- Fulford, RS., Breitburg, D.L., Luckenbach, M., Newell R.I.E., 2010. Evaluating ecosystem response to oyster restoration and nutrient load reductions with a multispecies bioenergetics model. Ecological Applications. 20(4):915-934.
- Fox, D.L., H.U. Sverdrup, J.P. Cunningham. 1937. The rate of water propulsion by the California mussel. Biol Bull 72:417-438.
- (Galimany et al. 2013 a) Galimany, E., J. Alix, M.S. Dixon, G.H. Wikfors. 2013. Short communication: Adaptability of the feeding behavior of intertidal ribbed mussels (*Geukensia demissa*) to constant submersion. Aquaculture International. 21(5):1009-1015.
- (Galimany et al. 2013 b) Galimany, E., J.M. Rose, M.S. Dixon, G.H. Wikfors. 2013. Quantifying Feeding Behavior of Ribbed Mussels (*Geukensia demissa*) in Two Urban Sites (Long Island Sound, USA) with Different Seston Characteristics. Estuaries and Coasts. 36:1265-1273.
- Gillmor, R.B. 1982. Assessment of intertidal growth and capacity adaptations in suspension-feeding bivalves. Mar. Bio. 68(3):277-286.
- Grabowski, J.H., Peterson, C.H., 2007. Restoring oyster reefs to recover ecosystem services. Theoretical Ecology Series. 4:281-297.
- Griffiths, R.J. 1980. Filtration, Respiration and Assimilation in the Black Mussel Choromytilus meridionalis. Mar. Ecol. Prog. Ser. 3:63-70.
- Haven, D.S. and R. Morales-Alamo. 1970. Filtration of particles from suspension by the American oyster, *Crassostrea virginica*. Biol. Bull. 139:248-264.
- Hawkins, A.J.S., B.L. Bayne, S. Bougrier, M. Heral, J.I.P. Iglésias, E. Navarro, R.F.M. Smith, M.B. Urrutia. 1998. Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. Journal of Experimental Marine Biology and Ecology. 219:87-163.
- Humphries A.T., S.G., Ayvazian, J.C. Carey, B.T. Hancock, S. Grabbert, D. Cobb, C.J. Strobel and R.W. Fulweiler. 2016. Directly Measured Denitrification Reveals Oyster Aquaculture and Restored Oyster Reefs Remove Nitrogen at Comparable High Rates. Front. Mar. Sci. 3:74. doi: 10.3389/fmars.2016.0007
- Kellogg, M.L., J.C. Cornwell, M.S. Owens, K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. Mar Ecol Prog Ser. 480:1-19. doi: 10.3354/meps10331

- Kreeger, D.A., and R.I.E. Newell. 2001. Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. J. Exp. Mar. Bio. Eco. 260:71-91.
- Kreeger, D.A., R.I.E. Newell, C.J. Langdon. 1990. Effect of tidal exposure on utilization of dietary lignocellulose by the ribbed mussel *Geukensia demissa* (Dillwyn) (Mollusca: Bivalvia). J. Exp. Mar. Bio. Eco. 144(2-3):85-100.
- Langdon, C.J., R.I.E. Newell. 1990. Review: Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58:299-310.
- Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: a review. Journal of Shellfish Research. 23:51-61.
- Newell, R.I.E., T.R. Fisher, R.R. Holyoke, and J.C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. Pages 93-120 in R. Dame and S. Olenin, editors. The comparative role of suspension feeders in ecosystems. Springer, Dordrecht, The Netherlands.
- Pales Espinosa, E. and B. Allam. 2006. Comparative Growth and Survival of Juvenile Hard Clams, *Mercenaria mercenaria*, Fed Commercially Available Diets. Zoo Biology. 25:513-525.
- Pales Espinosa, E., B. Allam, and S. Ford. 2008. Particle selection in the ribbed mussel *Geukensia demissa* and the Eastern oyster *Crassostrea virginica*: Effect of microalgae growth stage. Estuarine, Coastal and Shelf Science. 79:1-6.
- Pomeroy, L. R., D'Elia, C. F., Schaffner, L.S., 2006. Limits to Top-Down Control of Phytoplankton by Oysters in Chesapeake Bay. Mar. Ecol. Prog. Ser. 325:301-309.
- Ponis, E., R. Robert, G. Parisi. 2003. Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture. 221:491-505.
- Porter, E.T., J.C. Cornwell, and L.P. Sanford. 2004. Effects of oyster *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. Mar. Ecol. Prog. Ser. 271:61-75.
- Prins, T.C., Smaal, A. C., Dame, R.F., 1998. A review of the feedbacks between bivalve grazing and ecosystem processes. Aquatic Ecology. 31:349-359.
- Riisgård, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of North American bivalves. Mar. Ecol. Prog. Ser. 45:217-223.
- Riisgård, H.U. 2001. On measurement of filtration rates in bivalves—the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser. 211:275-291.
- Rose, J.M., S.B. Bricker, M.A. Tedesco, G.H. Wikfors. 2014. A Role for Shellfish Aquaculture in Coastal Nitrogen Management. Environ Sci Technol. 48:2519-2525. dx.doi.org/10.1021/es4041336

- Shumway, S.E., T.L. Cucci, R.C. Newell, C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Bio. Eco. 91:77-92.
- Wetz, M.S., A.J. Lewitus, E.T. Koepfler, K.C. Hayes. 2002. Impact of the Eastern oyster Crassostrea virginica on microbial community structure in a salt marsh estuary. Aquatic Microbial Ecology. 28:87-97.
- Widdows J, J.M. Shick. 1985. Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. Mar Biol 85:217–232.
- Wright, R.T., R.B. Coffin, C.P. Ersing, and D. Pearson. 1982. Field and Laboratory Measurements of Bivalve Filtration of Natural Marine Bacterioplankton. Limnology and Oceanography. 27(1):91-98.

# Chapter 2

# Mixed species populations of *Crassostrea virginica* and *Geukensia demissa* exhibit signs of complimentary resource use

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# ABSTRACT

Many states along the U.S. East Coast are determining the best methods for increasing sustainable molluscan bivalve shellfish aquaculture production within territorial waters. The ecosystem services and economic stability provided by the industry are enhanced by the ingenuity of growers to use new space for farms, implement new techniques, and in some cases develop multispecies systems as a way to diversify production lines. Theoretically, the increased biodiversity of the functional group when additional bivalve filter feeding species are added to the system should provide stability to the provision of ecosystem services, which in turn increases system resilience. A direct examination of potential population interactions, however, has not yet been explored. The potential for a negative relationship among two species when placed in cohabitation or proximity such that there is competition for food resources could lower farm output even as system-wide stability increases. To examine this potential interaction further, a study of the feeding relationship between two common species within Delaware Bay, New Jersey- the cultured Crassostrea virginica and the native, marsh-fringing Geukensia demissa- was conducted. This study examined particle clearance under monoculture and multispecies arrangements while the organisms were allowed to feed on natural plankton communities within a flow through system. Crassostrea virginica removal rates were reduced for the smallest particle category. Sampling coincided with potential changes in plankton community which may have led to changes in feeding behavior due to different food resources. Overall, this study provides experimental evidence to support the complementary resource use theory that biodiversity enhances ecosystem functioning.
## 1. Introduction

Throughout many areas of the US East Coast, molluscan bivalve shellfish aquaculture is becoming more prominent within the coastal zone (USDA, NASS 2013). As the industry expands, siting farms within new areas has the potential to reveal new or previously unknown interactions among the culture species and indigenous populations. Increasing biodiversity through the culture of native species, theoretically, should be positively linked with enhanced ecosystem function, productivity, and stability (Cardinale et al. 2006, Dame 2012, Grant 1996, Loreau et al. 2001, Naeem et al. 1994, Rey Benayas et al. 2009, Worm et al. 2007). Augmenting populations of bivalve filter feeders should further the provision of ecosystem services such as particle filtration which yields greater benthic-pelagic coupling and regulation of nutrient fluxes (Grabowski & Peterson 2007, Humphries et al. 2016, Kellogg et al 2013, Prins et al. 1998, Porter et al. 2004). Bivalve mollusc grazing also provides a top-down control on phytoplankton production (Dame 2012, Newell 2004, Newell et al. 2005, Prins et al. 1998), which leads to increased light availability (Newell 2004, Porter et al. 2004) and microphytobenthos and submerged aquatic vegetation (Porter et al. 2004).

Applied research on the provision of ecosystem services from restored oyster reefs supports the positive biodiversity-service provision theory. Researchers have concluded that single species restoration cannot achieve the nutrient reduction goals set forth by resource managers and that a mix of filtration providers is required (Cerco and Noel 2007, Fulford et al. 2007, Fulford et al. 2010, Grizzle et al. 2006; Ulanowicz & Tuttle 1992). At the community level, marine filter-feeding has been shown to be most efficiently provided by a complex assemblage of organisms rather than a single keystone species (Pomeroy et al. 2006). Increasing the diversity of a focal trophic group, such as marine bivalves (primary consumers), results in greater resource depletion by that group (primary producers; phytoplankton) (Cardinale et al. 2006). The added benefit of increased biodiversity is likely due to resource partitioning amongst species, with the trophic

group collectively capturing a greater amount and more diverse assemblage of planktonic species (Fulford et al. 2010).

In previous studies supporting the biodiversity-ecosystem benefit theory, much of the work has focused on modeling data from single species and environmental parameters. There is little direct experimental evidence linking the ecosystem-scale models with aquaculture production (e.g., Lefebvre et al. 2009 using isotopes to determine particle assimilation at a multispecies culture site in France), and most often, this research focused on the end goal of mapping carrying capacity for systems near threshold maxima (see Byron et al. 2011, Smaal et al. 2013). To reduce the data gap between theory and on-farm aquaculture production, as well as to provide information for culture systems prior to reaching capacity, this paper examined whether a farmed species may be negatively influenced by the presence of a neighboring filter feeding bivalve. Particle removal resulting from filtration by the cultured species, *Crassostrea virginica* (eastern oyster), relative to a potentially interacting species, Geukensia demissa (ribbed mussel) was studied. Monoculture and multispecies conditions were employed to answer the research question of whether there is a competitive interspecific relationship for food resources between the two study bivalve species. Based on the previous studies finding that diversity within filter-feeders is net benefit for ecosystems, it was hypothesized competition would be avoid via resource partitioning (Fulford et al. 2010) between the two species.

## 2. Methods

## 2.1 Study Location

Dual bivalve species filtration experiments were conducted at the Rutgers University Aquaculture Innovation Center (AIC) located on the Cape May Canal in North Cape May, Cape May County, New Jersey, USA. Raw seawater from the Cape May Canal was continuously pumped throughout the facility allowing for experimentation under controlled conditions but with a water mass containing a typical particle community for lower Delaware Bay. Tidal fluctuations can result in minor variations in the salinity and temperature of the incoming water; however, during sampling the average salinity was 30. There were high sediment loads within the raw seawater entering the facility, and it was not possible to remove all of the fine sediment prior to the water entering the experimental flow through units.

## 2.2 Bivalve Populations

This research focused on two species native to Delaware Bay, NJ, USA- *C. virginica*, a common species within the state and regional aquaculture industries, and *G. demissa*, a species ubiquitous to coastal marshes. Three populations of bivalve shellfish were used for this research, including:

- 1. *Crassostrea virginica-* (CV) one year of age, hatchery-reared, maintained under constant subtidal conditions, shell height 10mm to 50mm;
- 2. *Geukensia demissa*, subtidal- (GDS) unknown age, wild source, maintained under constant subtidal conditions, shell height 10mm to 60mm; and
- Geukensia demissa, intertidal- (GDI) unknown age, wild source, maintained under intertidal conditions with two low (emersion 4 hours) and two high tides (immersion 8 hours) per 24-hour cycle, shell height 10mm to 60 mm.

For more information on the need for three populations and the size ranges used, see Chapter 1.

## 2.3 Flow Through System

The flow through experiments consisted of the two bivalve species placed within monoculture and multispecies arrangements. The system included a series of three foot long gutters connected via one-half inch PVC piping (Figure 2.1). The main inflow to the system ("P"), originating from the AIC facility raw seawater system, was split into two distinct water flows ("I" and "II"). One path for the water entered into a series with two gutters (I-A-B), the other path of water to a separate series, also containing two gutters (II-C-D). This allowed for simultaneous testing of two series during each sample date. Each gutter in this design represented a distinct population, and the series allowed for the comparison of initial population filtration versus secondary population filtration.



Figure 2.1: Flow through system schematic. Gutter labeling shown here is used again in the results section of this paper. Once waters enters the I-A-B side of the system it did not flow into the other side of the system. The same was true for the II-C-D side of the system, allowing for two series testing within one event. Arrows in this diagram indicate water flow.

The treatment populations, outlined according to series, are included in Table 2.1. For each series, the species listed under "Population #1" was placed into the first treatment gutter in the series, the species listed under "Population #2" was placed into the second treatment gutter in the series.

A total gutter biomass of 10 grams dry weight was used. For the gutter treatments with a mix of species present, 5 grams dry weight biomass per species was used, maintaining a total gutter biomass of 10 grams.

Series	Population #1 (First gutter in series)	Population #2 (Second gutter in series)
1	C. virginica	G. demissa subtidal
2	C. virginica	G. demissa intertidal
3	C. virginica	C. virginica
4	G. demissa subtidal	C. virginica
5	G. demissa intertidal	C. virginica
6	G. demissa subtidal	G. demissa intertidal
7	G. demissa intertidal	G. demissa subtidal
8	MIX: C. virginica + G. demissa subtidal	EMPTY
9	MIX: C. virginica + G. demissa intertidal	EMPTY

Table 2.1: Treatment populations for the flow through experiments. Each species is listed according to their respective placement within each two gutter series.

At the beginning of each experiment, the gutters were filled with the appropriate bivalves, the water was turned on to the system, and the flow rates monitored to ensure water was flowing through the entire system and all experimental individuals submerged. Flow rates were not continuously monitored or altered throughout the three hour trial unless an issue in the main pipe necessitated such actions. The bivalves were allowed to acclimate for one hour in this system due to noted "closing up" during early sampling (predominantly the oysters, but both species seemed to close when a sampler's shadow darkened the gutter).

A series of reverse samples were taken at the gutter outflows and the main inflow at hours one, two, and three. These are categorized as reverse samples because the lowest outflow, or the last outflow within a series, was sampled first. The remainder of the series was then sampled, continuing in reverse order. Three replicates were taken from each sampling point, with a complete set of the seven outflow ports sampled once prior to initiating replicate two, followed by replicate three. This sampling order was intentional to ensure minimal disruption to flow rates within the gutters which could have increased turbidity and particle counts in the samples. The hour timeframe for sampling was intended to capture any changes that may occur due to tidal fluctuations and possible changes in particle taxa over the ebb or flood tide. The timing also allowed the experimental organisms to reacclimatize to any changes within the gutter such as changes in flow rates, short-term increased turbidity, and movement of the gutter.



Figure 2.2: Image of experimental system during operation. Samples were extracted in the reverse of the water flow. For the I-A-B series, sampling occurred B-A-I. After both sides were sampled simultaneously, the main inflow "P" was sampled.

Water samples from the flow through system were analyzed on a Beckman-Coulter Multisizer IV Particle Analyzer. Unless there were particles visible to the naked eye, the samples were not filtered prior to particle analysis. For those samples with visibly larger material, the sample was filtered through 50 micron nitex mesh prior to analysis. The flow through samples were analyzed immediately after capture to minimize the potential loss of particles, which could skew the size frequencies. The particle communities within the raw seawater were expected to be variable with high sediment loads. The total number of particles present within a sample as well as the number of particles greater than three microns were recorded. Via simple subtraction, those particles less than three microns were also recorded. The three micron threshold was selected as a good demarcation in the data for analyzing bacterioplankton removal signals (for *C. virginica* limitations see Crosby et al. 1990, Langdon and Newell 1990, Wetz et al. 2002; for *G. demissa* preferential removal see Kreeger et al. 1990, Langdon & Newell 1990, Wright et al. 1982).

The data corresponding to a set sampling regime- events with same gutter series present, typically four dates within one calendar week- were analyzed via two-way ANOVA to examine the effects of gutter (proxy for treatment) as well as time (tidal fluctuations). A Tukey post-hoc test was performed on the significantly different data to identify uniquely performing gutters within each sampling event. Statistical analyses were run for three particle size categories: all particles within a sample, particles greater than three microns in size, and particles less than three microns in size. All statistical analyses were run on R Studio platform.

#### 3. Results

The flow through system results reported here are organized according to the sampling regime (see Table 2.2 for order), with no inter-regime comparisons of raw data due to variability in the initial particle quantities. Basic water parameter ranges for temperature and salinity during the week of each sampling series are provided in Table 2.2. For all sampling regimes, when "time" or "time and gutter interaction" were statistically significant, a Tukey analysis was conducted to examine the results of "time" further. None of those produced a recognizable pattern (tide, date, sampling hour), and therefore all time-related results are excluded here even when the interaction terms are significant. All results below are for all three particle size categories.

- ····· - ····························					
Series	Dates (2014)	Temperature	Salinity		
		Range (C)	Range (psu)		
GDI-CV;GDS-CV	Sept. 10,11, 15, 16, 17	22.9-22.1	31.9-29.1		
CV-GDI;CV-GDS	Sept. 22, 23, 24, 25	20.0-21.5	31.2-31.7		
GDI-GDS; CV-CV	Sept. 30, Oct. 1, 2, 3	20.8-20.7	30.3-27.8		
GDS-GDI; Empty-CV	Oct. 6, 8, 9	19.5-18.6	30.7-30.6		
GDS+CV; GDI+CV	Oct. 13, 14, 15, 16	21.3-18.2	30.7-27.1		

Table 2.2: Sampling Series, dates, and associated water parameters.

In the first sampling regime GDI-CV; GDS-CV (Figure 2.3), analyses for all three particle categories resulted in significant interactions between time and gutter (ANOVA interaction, p <0.001). In all three particle size categories, the first treatment population had particles counts significantly different from the three inflow ports, but there was no significant difference between the first bivalve populations located on separate sides of the system. Similarly, the two second treatment populations had particle counts significantly different from that particle counts significantly different from that particle counts significantly different from that of the first treatment populations, but there was no significant difference between the secondary bivalve populations on separate sides of the system. These results do not show any signs the experimental setup (tandem gutter pairs) influenced the results since the two CV populations on separate sides of the system yielded particle counts that are statistically similar. No effect of subtidal versus intertidal replication for the two *G. demissa* populations could be discerned from these results either.



Figure 2.3: Results of Sampling Regime 1: GDI-CV; GDS-CV. P= main inflow that was split into the two series, one including I (inflow) to gutters labelled A (GDI) and B (CV); the other side contained II (inflow) flowing to gutters labelled C (GDS) and D (CV). This figure provides the results for all particles within a sample (far left), all particles greater than three microns in size (center), and those smaller than three microns (far right). The Tukey notation at the top of bars is only representative of an analysis for data within the respective particle size category and does not translate across the boxes. All data represent significant interaction effects between time and gutter at p < 0.001.

In the second sampling regime, CV-GDI; CV-GDS (Figure 2.4), the interaction of time and gutter was significant at p < 0.001 for all three particle size categories. All particle size categories resulted in significantly different particle counts for all treatment gutters. Based on each gutter yielding a statistically different particle count, there is no evidence of a species effect since *C*. *virginica* was the first population on both sides of the system. The results could be revealing experimental design flaws, however, there were no noted issued during sampling and the results of the other sampling regimes prove otherwise.



Figure 2.4: Results of Sampling Regime 2: CV-GDI; CV-GDS. P= main inflow that was split into the two series, one including I (inflow) to gutters labelled A (CV) and B (GDI); the other side contained II (inflow) flowing to gutters labelled C (CV) and D (GDS). This figure provides the results for all particles within a sample (far left), all particles greater than three microns in size (center), and those smaller than three microns (far right). The Tukey notation at the top of bars is only representative of an analysis for data within the respective particle size category and does not translate across the boxes. All data represent significant interaction effects between time and gutter at p < 0.001.



Figure 2.5: Results of Sampling Regime 3: GDI-GDS; CV-CV. P= main inflow that was split into the two series, one including I (inflow) to gutters labelled A (GDI) and B (GDS); the other side contained II (inflow) flowing to gutters labelled C (CV) and D (CV). This figure provides the results for all particles within a sample (far left), all particles greater than three microns in size (center), and those smaller than three microns (far right). The Tukey notation at the top of bars is only representative of an analysis for data within the respective particle size category and does not translate across the boxes. Only the data for particles greater than three microns (center box) resulted in a positive interaction between time and gutter (p < 0.001). The data represented here include the interaction ANOVA and Tukey significance only for the center box, the left and right do not include analysis for interaction.

In the sampling regime GDI-GDS; CV-CV (Figure 2.5), the time-gutter interaction is only significant (p < 0.001) for particles greater than three microns, with no significant interaction of variables in the other particle size categories. The greater than three micron data show significantly different particle counts for all gutters, similar to the second sampling regime. In the other two size categories, where the data was only reviewed for gutter (time-gutter interaction was not significant), the second *C. virginica* population (gutter D) exhibited particle removal similar to both of the initial bivalve populations (*G. demissa* intertidal and *C virginica*) as well as the other secondary population (*G. demissa* subtidal). Only the *G. demissa* half of the sampling

regime resulted in significant removal by population two after the water mass interacted with population one. This held true for all particle sizes.



Figure 2.6: Results of Sampling Regime 4: GDS-GDI; Empty-CV. P= main inflow that was split into the two series, one including I (inflow) to gutters labelled A (GDS) and B (GDI); the other side contained II (inflow) flowing to gutters labelled C (Empty) and D (CV). This figure provides the results for all particles within a sample (far left), all particles greater than three microns in size (center), and those smaller than three microns (far right). The Tukey notation at the top of bars is only representative of an analysis for data within the respective particle size category and does not translate across the boxes. All data represent significant interaction effects between time and gutter at p < 0.001.

In the fourth sampling regime GDS-GDS; Empty-CV (Figure 2.6), analyses for all three particle categories resulted in significant interaction between time and gutter at p <0.001. This sampling regime was useful in substantiating results from previous regimes where the experimental system was deemed to not be a contributing factor in the results. For this regime, the empty gutter particle counts were not statistically different from that of the inflow ports, but were significantly different from the treatment populations. Under the data analyses for all particles within a sample and only those less than three microns, the three treatment gutters, representing an isolated CV

population, as well as interacting GD populations, resulted in significantly different particle counts from one another (and the inflow ports).

Conversely, data analysis for particles greater than three microns resulted in no significant difference between the first treatment populations on each side of the experimental system (Figure 2.6). Both of those initial populations exhibited particle counts that were significantly different from the second treatment population of GDS. Collectively, these results are potentially revealing a particle size influence on the filtration potential for the experimental bivalves. When the particle size category includes only those particles which are known to be removed by CV, CV removes a similar amount to GD. When the particle size category includes particles smaller than what is efficient for CV, the results here show that CV filters significantly fewer particles relative to GD.

In the final sampling regime examining a mix of GDS + CV; and a mix of GDI + CV (Figure 2.7) the interaction of time and gutter is significant for the "all particles" size category (p < 0.01) and the less than three microns (p < 0.05). This sampling regime resulted in the mixed species populations filtering a significant amount of particles relative to the inflow ports, but not significantly different from each other. The results here, therefore do not show any subtidal-intertidal influence on the filtration interactions of these two species.



Figure 2.7: Results of Sampling Regime 5: GDS+CV and GDI+CV. P= main inflow that was split into the two series, one including I (inflow) to gutters labelled A (GDS+CV) and B (Empty, no data); the other side contained II (inflow) flowing to gutters labelled C (GDI+CV) and D (Empty, no data). This figure provides the results for all particles within a sample (far left; significant interaction of time and gutter at p < 0.01), all particles greater than three microns in size (center; no significant interaction of time and gutter at p < 0.05). The Tukey notation at the top of bars is only representative of an analysis for data within the respective particle size category and does not translate across the boxes.

## 4. Discussion

#### 4.1 Reduced filtration- Crassostrea virginica

No interspecific competition resulted from the flow through study, supporting the hypothesis that the two study species would not compete for food. The most prominent result from the study was the reduced particle removal from the *C. virginica* populations, evident in three out of the five series. Based on the three particle size categories used for the analyses, particle size influenced this result with decreased filtering efficiency for particles less than three microns. The threshold of three microns was intentional to account for bacterial removal and is just below the size range of algae commonly used to support the culture of shellfish (Epifanio 1979, Pales Espinosa and Allam 2006, Pales Espinoa et al. 2008, Ponis et al. 2003). The reduced removal of smaller particles by *C. virginica* corresponds with known efficiency decreases for particles this size (Riisgard 1988, *C. virginica* 50% efficiency at  $2\mu$ m; see also Haven and Morales-Alamo 1970, 53% at 3-4 µm), however, the results were unexpected. At the onset of the trials, it was presumed that ample food within the preferred size range would be present for both bivalve species since the lower Delaware Bay typically experiences a bloom of nannoplankton in late summer (Pennock and Sharp 1986). Under the late summer conditions, a greater percentage of the total particulate matter would have been within the preferred *C. virginica* size range. The first sampling regime may have occurred at the tail end of the late summer bloom conditions, reflected in the uniform filtration results. The sampling data, however, do not provide evidence of a bloom or significant reduction in particles signifying decreasing plankton levels (Figures 2.3-2.7 show a cyclic change in particle counts between regimes, likely a result of tide).

Seasonality in plankton species may explain the different particle removal efficiencies exhibited by the *C. virginica*. According to Watling et al. (1979), the Delaware Bay is a system dominated by small flagellates from summer through early fall. The sampling for this study commenced in September, at the end of this time range. From October through May, the Bay experiences a shift in plankton composition to become diatom-dominated (Watling et al. 1979), with the spike in diatoms overlapping with the last four sampling regimes in this study. This shift in resources, which some researchers have shown can impact *C. virginica* removal efficiencies (Epifanio et al. 1981, Wetz et al. 2002), could have resulted in *C. virginica* exhibiting lower particle removal. Although both bivalve species are known for particle sorting and preferential selection (Pales Espinosa et al. 2008, Langdon and Newell 1990, see review of bivalve sorting in Ward and Shumway 2004), *C. virginica* has greater limitations for both size (Newell and Jordan 1983,

Riisgard 1988, Ward et al. 1998) and food quality (Crosby et al. 1990, Langdon and Newell 1990, Mafra Jr. et al. 2009, Newell and Jordan 1983, Shumway et al. 1985). *G. demissa* is not as selective due to its intertidal, marsh-fringing niche (Charles and Newell 1997, Jordan and Valiela 1982, Kemp et al. 1990, Kreeger and Newell 2001, Kreeger et al. 1990, Lent 1967). Studies of *C. virginica* have also shown a decrease in particle assimilation that correspond with the timing of the plankton community shift (Haven and Morales-Alamo 1966). Changes to food resources coupled with reduced temperature could have cause the depressed filtration by *C. virginica* (Loosanof 1958, Newell and Langdon 1996, Shumway 1996). It is unclear as to why a concurrent change in filtration would not also occur with *G. demissa* as temperatures decrease. Additionally, water temperatures over the entire sampling timeframe were never below 18C, which is above the temperature threshold for filtration reductions within this species (Loosanoff 1958). Collectively, these analyses led to the conclusion that statistically lower particle removal from *C. virginica* was a result of food quality changes.

#### 4.2 Geukensia demissa Populations

The influence of tidal holding on *G. demissa* was absent from the results, with both populations exhibiting similar feeding behavior. Additionally, in the final sampling regime (Figure 2.7), the mix of *G. demissa* with *C. virginica* did not show any impacts from the different subpopulation of *G. demissa*. These findings are counter to previous literature that shows a change in filtration with this species can occur within several days of holding under subtidal conditions (Galimany et al. 2013; see also Borrero 1987, Gillmor 1982). It is possible that the holding of the study organisms for a few weeks prior to experimentation missed any signal of altered filtration. In previous studies, the organisms were only held for days prior to the commencement of first testing. Additional study of this behavior is required.

## 4.3 Biodiversity and Ecosystem Health

No interspecific competition for food resources resulted from this study. Competition would have been evident by similar particle counts for population one and population two in a series such that the first population negatively influenced filtration of population two. Potential interspecific competition would have been noted in sampling regimes one and two, where the populations in a series included different study species. Interspecific species effects cannot explain the *C. virginica* removal rates since they occur without the presence of *G. demissa* (Figure 2.6) or in a series of two *C. virginica* populations (Figure 2.5).

This study provides experimental evidence in support of the theory that complementary resource use (Tillman 1997) by species within a functional group can stabilize ecosystem function via their redundancy within the system (Blondel 2003). In this study, the two bivalve filter feeding species filtered particles within the larger size range with similar effectiveness. A slight decrease by *C. virginica* was due to changes in plankton composition that could be explored further with additional sampling over numerous seasons. The different degrees of particle removal within certain sizes and potential quality by one of the study species created a dynamic of resource partitioning (Fulford et al. 2010), whereby the "functional repertoire" (Duarte 2000) of the system is enhanced by the presence of both species.

#### References

Blondel, J. 2003. Guilds or functional groups: does it matter? OIKOS. 100:223-231.

- Borrero, F.J. 1987. Tidal height and gametogenesis: Reproductive variation among populations of *Geukensia demissa*. The Biological Buletin. 173(1).
- Byron, C., D. Bengtson, B. Costa-Pierce, J. Calanni. 2011 Intergrating science into managemt: Ecological carrying capacity of bivalve shellfish aquaculture. Marine Policy. 35:363-370.

- Cardinale, B.J., D.S. Srivastava, J.E. Duffy, J.P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 443:989-992.
- Charles, F., and R.I.E. Newell. 1997. Digestive physiology of the ribbed mussel *Geukensia demissa* (Dillwyn) held at different tidal heights. Journal of Experimental Marine Biology and Ecology. 209:201-213.
- Cerco, C. F.; M. R., Noel. 2007. Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? Estuaries Coasts. 30(2):331–343.
- Crosby, M.P., R.I.E. Newell, C.J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. Limnol. Oceanogr. 35(3):625-639.
- Dame, R.F. 2012. Ecology of marine bivalves: an ecosystem approach. Second edition. CRC Press, Boca Raton, 271 pp.
- Duarte, C.M. 2000. Marine biodiversity and ecosystem services: an elusive link. J. Exp. Mar. Bio. Eco. 250:117-131.
- Epifanio, C.E. 1979. Growth in Bivalve Molluscs: Nutritional Effects of Two or More Species of Algae in Diets Fed to the American Oyster *Crassostrea virginica* (Gmelin) and the Hard Clam *Mercenaria mercenaria* (L.). Aquaculture. 18:1-12.
- Epifanio, C.E., C.C. Valenti, C.L. Turk. 1981. A Comparison of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* as Foods for the Oyster, *Crassostrea virginica*. Aquaculture. 23:347-353.
- Fulford, RS., D.L. Breitburg, R.I.E. Newell, W.M. Kemp, M. Luckenbach. 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Mar. Ecol. Prog. Ser. 336:43-61.
- Fulford, RS., D.L. Breitburg, M. Luckenbach, R.I.E. Newell. 2010. Evaluating ecosystem response to oyster restoration and nutrient load reductions with a multispecies bioenergetics model. Ecological Applications. 20(4):915-934.
- Galimany, E., J. Alix, M.S. Dixon, G.H. Wikfors. 2013. Short communication: Adaptability of the feeding behavior of intertidal ribbed mussels (*Geukensia demissa*) to constant submersion. Aquaculture International. 21(5):1009-1015.
- Gillmor, R.B. 1982. Assessment of intertidal growth and capacity adaptations in suspension-feeding bivalves. Mar. Bio. 68(3):277-286.
- Grabowski, J.H., Peterson, C.H., 2007. Restoring oyster reefs to recover ecosystem services. Theoretical Ecology Series. 4:281-297.
- Grant, J. 1996. The relationship of bioenergetics and the environment to the field growth of cultured bivalves. Journal of Experimental Marine Biology and Ecology. 200:239-256.

- Grizzle, R.E., Greene, J.K., Luckenbach, M.W., Coen L.D., 2006. A new in situ method for measuring seston uptake by suspension-feeding bivalve molluscs. Journal of Shellfish Research. 25(2):643-649.
- Haven, D.S. and R. Morales-Alamo. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol. And Oceanogr.. 11(4):487-498.
- Haven, D.S. and R. Morales-Alamo. 1970. Filtration of particles from suspension by the American oyster, *Crassostrea virginica*. Biol. Bull. 139:248-264.
- Humphries A.T., S.G., Ayvazian, J.C. Carey, B.T. Hancock, S. Grabbert, D. Cobb, C.J. Strobel and R.W. Fulweiler. 2016. Directly Measured Denitrification Reveals Oyster Aquaculture and Restored Oyster Reefs Remove Nitrogen at Comparable High Rates. Front. Mar. Sci. 3:74. doi: 10.3389/fmars.2016.0007
- Jordan, T.E. and I. Valiela. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. Limnol. Oceanogr. 27(1):75-90.
- Kellogg, M.L., J.C. Cornwell, M.S. Owens, K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. Mar Ecol Prog Ser. 480:1-19. doi: 10.3354/meps10331
- Kemp, P.F., S.Y. Newell, C. Krambeck. 1990. Effects of filter feeding by the ribbed mussel *Geukensia demissa* on the water-column microbiota of a *Spartina alterniflora* saltmarsh. Mar. Ecol. Prog. Ser. 59:119-131.
- Kreeger, D.A., and R.I.E. Newell. 1996. Ingestion and assimilation of carbon from cellulolytic bacteria and heterotrophic flagellates by the mussels *Geukensia demissa* and *Mytilus edulis* (Bivalvia, Mollusca). Aquatic Microbial Ecology. 11:205-214.
- Kreeger, D.A., and R.I.E. Newell. 2001. Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. J. Exp. Mar. Bio. Eco. 260:71-91.
- Kreeger, D.A., R.I.E. Newell, C.J. Langdon. 1990. Effect of tidal exposure on utilization of dietary lignocellulose by the ribbed mussel *Geukensia demissa* (Dillwyn) (Mollusca: Bivalvia). J. Exp. Mar. Bio. Eco. 144(2-3):85-100.
- Langdon, C.J. and R.I.E. Newell. 1990. Review: Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58:299-310.
- Lefebvre, S., J.C. Marín Leal, S. Dubois, F. Orvain, J-L. Blin, M-P., Bataillé, A. Ourry, R. Galois. 2009. Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in two shellfish culture dominated ecosystems. Estuarine, Coastal and Shelf Science. 82:415-425.
- Lent, C.M. 1967. Effect of Habitat on Growth Indices in the Ribbed Mussel, *Modiolus (Arcuatua) demissus*. Chesapeake Science. 8(4):221-227.

- Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. Science. 294:804-808.
- Loosanof, V.L. 1958. Some Aspects of Behavior of Oysters at Different Temperatures. Biol. Bull. 114:57-70.
- Mafra Jr., L.L., V.M. Bricelj, J.E. Ward. 2009. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. II. Selective rejection. Aquatic Biology. 6:213-226.
- Naeem, S., L.J. Thompson, S.P. Lawler, J.H. Lawton, and R.M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature. 368:734-737.
- Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: a review. Journal of Shellfish Research. 23:51-61.
- Newell, R.I.E., and S.J. Jordan. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginca*. Mar. Ecol. Prog. Ser. 13:47-53.
- Newell, R.I.E. and C.J. Langdon. 1996. Mechanisms and Physiology of Larval and Adult Feeding. Pages 185-229 in V.S. Kennedy, R.I.E. Newell, and A.F. Eble eds. The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, MD.
- Newell, R.I.E., T.R. Fisher, R.R. Holyoke, and J.C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. Pages 93-120 in R. Dame and S. Olenin, editors. The comparative role of suspension feeders in ecosystems. Springer, Dordrecht, The Netherlands.
- Pales Espinosa, E. and B. Allam. 2006. Comparative Growth and Survival of Juvenile Hard Clams, *Mercenaria mercenaria*, Fed Commercially Available Diets. Zoo Biology. 25:513-525.
- Pales Espinosa, E., B. Allam, and S. Ford. 2008. Particle selection in the ribbed mussel *Geukensia demissa* and the Eastern oyster *Crassostrea virginica*: Effect of microalgae growth stage. Estuarine, Coastal and Shelf Science. 79:1-6.
- Pennock, J.R., and J.H, Sharp. 1986. Phytoplankton production in the Delaware Estuary: temporal and spatial variability. Mar. Ecol. Prog. Ser. 34:143-155.
- Pomeroy, L. R., D'Elia, C. F., Schaffner, L.S., 2006. Limits to Top-Down Control of Phytoplankton by Oysters in Chesapeake Bay. Mar. Ecol. Prog. Ser. 325:301-309.
- Ponis, E., R. Robert, G. Parisi. 2003. Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture. 221:491-505.
- Porter, E.T., J.C. Cornwell, and L.P. Sanford. 2004. Effects of oyster *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. Mar. Ecol. Prog. Ser. 271:61-75.

- Prins, T.C., Smaal, A. C., Dame, R.F., 1998. A review of the feedbacks between bivalve grazing and ecosystem processes. Aquatic Ecology. 31:349-359.
- Rey Benayas, J.M., A.C. Newton, A. Diaz, and J.M. Bullock. 2009. Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. Science. 325:1121-1124.
- Riisgård, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of North American bivalves. Mar. Ecol. Prog. Ser. 45:217-223.
- Shumway, S.E. 1996. Natural Environmental Factors. Pages 467-513 in V.S. Kennedy, R.I.E. Newell, and A.F. Eble eds. The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, MD.
- Shumway, S.E., T.L. Cucci, R.C. Newell, C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Bio. Eco. 91:77-92.
- Smaal, A.C., T. Schellekens, M.R. van Stralen, J.C. Kromkamp. 2013. Decrease of the carrying capacity of the Oosterschelde estuary (SW Delta, NL) for bivalve filter feeders due to overgrazing? Aquaculture. 404-405:28-34.
- Tillman, D. 1997. Biodiversity and Ecosystem Functioning. Pages 93-112 *in* G. Daily ed. Nature's Services: Societal Dependence on Natural Ecosystems. Island Press, Washington, DC.
- Ulanowicz, R.E., Tuttle, J.H., 1992. The Trophic Consequences of Oyster Stock Rehabilitation in Chesapeake Bay. Estuaries. 15(3): 298-306.
- (USDA, NASS) United States Department of Agriculture, National Agricultural Statistics Service, Census of Aquaculture (2013), Volume 3, Special Studies, Part 2 (issued September 2014) 1-98. <u>https://www.agcensus.usda.gov/Publications/2012/Online\_Resources/Aquaculture/</u> (accessed 11/2/2016).
- Ward, J.E., and S.E. Shumway. 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Bio. Eco. 300:83-130.
- Ward, J.E., J.S. Levinton, S.E. Shumway, T. Cucci. 1998. Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Marine Biology. 131:283-292.
- Watling, L., D. Bottom, A. Pembroke, D. Maurer. 1979. Seasonal Variations in Delaware Bay Phytoplankton Community Structure. Marine Biology. 52:207-215.
- Wetz, M.S., A.J. Lewitus, E.T. Koepfler, K.C. Hayes. 2002. Impact of the Eastern oyster Crassostrea virginica on microbial community structure in a salt marsh estuary. Aquatic Microbial Ecology. 28:87-97.
- Wright, R.T., R.B. Coffin, C.P. Ersing, and D. Pearson. 1982. Field and Laboratory Measurements of Bivalve Filtration of Natural Marine Bacterioplankton. Limnology and Oceanography. 27(1):91-98.

Worm, B. E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpem, J.B.C. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J.Stachowicz, and R. Watson. 2007. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science.314:787-790.

#### **Chapter 3**

# State-Level Bivalve Shellfish Aquaculture Regulations Directing U.S. National Industry Development: Evidence from Four Coastal States

[Formatted for submission to Marine Policy Journal]

#### Abstract

Aquaculture production within the United States has been a topic of study for years as researchers and government officials look to improve the balance of domestic production relative to seafood imports. Often missing from this discussion are the tangible ways that state governmental policy actions serve to support or inhibit the industry, specifically with regard to molluscan bivalve shellfish aquaculture. This research serves to connect the current dialog at the national level with the state level aquaculture industry by exploring a diverse set of policy systems implemented within four states. Several key factors to successful industry development arose out of the four state analysis, including: a single contact point between grower and the state permitting authorities (*point-of-contact*); the political will of the entire executive branch- often through Governor mandate- to focus on easing restrictions to the industry (*political will*); vertical integration of permitting from federal permits through to local boards or review councils (vertical *integration*); as well as the age of the industry or related regulatory system, not including fisheries (or fisheries management) that may be present within state waters (age). From this analysis, more established states with a single liaison point-of-contact were associated with the most robust molluscan bivalve shellfish aquaculture industries. States with newer aquaculture industries or more recent changes in aquaculture-related policy, often have only a mix of these items and therefore are still lagging behind the top producers. The spectrum of industry development at the state level is thus reflected in the nation's production numbers, which are improving but far slower than other nations.

## 1. Introduction

The often-promoted roles of filtration and habitat creation that molluscan bivalve shellfish provide within estuarine communities [1-14] has recently taken on new focus as shellfish aquaculture becomes increasingly prominent within US estuaries. The negative impacts and externalities historically associated with the culturing of finfish species (use of antibiotics, threat of nonnative species escape, high nutrient loads through excess feeding) [15-16] are typically not associated with modern molluscan bivalve shellfish farming. Many researchers point to the culture of filter-feeding shellfish as a means of improving overall estuarine health through the use of native species (enhancing populations), the provision of filtration services (improved local water quality), and the stabilization of local economies [7, 17-20]. Even with abundant evidence for net positive socioecological benefits from molluscan bivalve shellfish aquaculture, there appears to be only moderate development of the industry within the US, and large variations in state-by-state industry growth rates (or depreciations).

At present, all molluscan bivalve shellfish aquaculture occurs within state territorial waters; therefore, state regulations are the primary mediating factor for industry development and facilitation of associated socioecological benefits. Managers regulating the industry may not consider, or be required to include, the beneficial services of the cultured products (filtration, habitat creation) when developing policy measures. It is through industry-supportive policies, however, that states are essentially fostering ecosystem services by developing a robust and sustainable molluscan bivalve shellfish aquaculture industry.

The critical nature of regulation at the state-level is absent from much of the dialog surrounding industry development from both the ecological scientific community and the regulatory researchers. This paper explores the theory that molluscan bivalve shellfish aquaculture within the US has been stymied at the national level due to obstacles imposed by some coastal state regulations. Farm and sales data from the United Stated Department of Agriculture (USDA) serves as a starting point to ascertain which states have industry supportive policies and which may regulate the industry via more restrictive policies or policies not updated for industry needs. Using the states of Maryland, New Jersey, Virginia, and Washington as informative case studies, this review identified: 1) policy regimes or actions that serve to support or inhibit the industry and 2) areas where improvements could be made to stimulate growth. These states were selected for their relative sales levels as leaders in the nation (Virginia and Washington) as well as more nascent industries that have shown recent changes towards supporting future growth (Maryland and New Jersey).

## 2. State Sales Trends

Shellfish aquaculture farm numbers and sales within the United States has seen a substantial increase over the past decade [21]. Although a majority (as high as 90%) of the seafood consumed within the US is imported [22], consumers are beginning to demand more domestic products [23]. The renaissance of oyster bars coupled with ever increasing demand for high quality local products has resulted in an ever increasing number of shellfish consumed within the US having domestic origin [24]. According to the USDA, National Agriculture Statistics Service (NASS) Census of Aquaculture, sales of domestic shellfish has followed consumption trends with a clear increase between survey years of 2005 and 2013. Molluscan bivalve shellfish sales, as reported by survey respondents, have risen within the U.S. from 2005 to 2013 nearly 35% [21] (Table 3.1).

Trends at the state level, however, are mixed, with states such as Virginia and Washington at the forefront of the shellfish aquaculture movement, and the slowly improving states of Maryland and New Jersey still near bottom of the sales list (see Table 3.1). According to the Census for Aquaculture, Virginia and Washington, respectively, represent 13% and 45% of the US sales for

aquacultured molluscan species in 2013, an increase of 45% in the market share for Washington relative to 2005 through a 94% increase in sales over that timeframe. Washington, which went from producing a third of the total US market value in 2005 to representing just under half in 2013 (Table 3.1), has consistently held the notoriety as the top molluscan bivalve producing state in the nation. The increase in market shares within the USDA data is likely due to several factors including diminishing number of farms and farm sales in other states as well as increased political and financial support within Washington state government for growth of the already premier industry [25]. Virginia's 18% change in sales between 2005 and 2013 was enough for that state to retain prominence on the U.S. East Coast, however, their market share dropped by almost 7%. It is unclear why Virginia was unable to replicate the national market jump experienced by Washington growers, but could be due to the species produced and their respective values. USDA data provided in Table 3.1 include sales for all mollusk species with no differentiation for those which may carry a higher or lower price. Other states such as Maryland (0.53% total U.S. sales in 2013) have more recently begun supporting development of their shellfish aquaculture industries [21, 26], which is reflected in their national standings for aquacultured molluscan shellfish sales. New Jersey is poised between the two extremes, reflecting elements of an historic aquaculture industry based on traditional (fishery-related) practices coupled with a more recent surge of newer, innovative shellfish farming methods. Although holding a much smaller share of the national market value (3% in 2013), New Jersey's shellfish aquaculture industry has witnessed a relative explosion in reported sales (percent change in sales over 200% between the reporting years of 2005 and 2013) from among a fewer number of farms. Of those states where sales are reported for both survey years, only Maryland reported a greater increase over the eight years. New Jersey and Maryland are similar in that both have experienced a more recent shift from solely focusing state-level resources on the management of fisheries to the coupled development of a continued but smaller fishery and the regulation of the structural aquaculture sector.

Table 3.1: Number of farms reporting sales of aquacultured molluscan species and the aggregate sales for those farms in the 2005 and 2013 USDA, NASS Census of Aquaculture. The four states reviewed in this research are provided at the top of the table, with the remainder of the reporting states following in alphabetical order.

	2005		2013				
	Farms	Sales	Sales†- 2013	Percentage	Farms	Sales	Percentage
		(\$1,000)	Dollars	of Total		(\$1,000)	of Total
			(\$1,000)	(Sales)			(Sales)
New Jersey	67	2,820	3,405	1%	50	10,303	3%
Virginia	53	29,028	35,052	14%	80	41,522	13%
Washington	174	63,710	76,933	31%	125	149,320	45%
Maryland	6	196	236	0.10%	10	1,738	0.53%
California	21	20,064	24,228	10%	27	16,992	5%
Connecticut	27	D			25	28,297	9%
Florida	154	10,694	12,913	5%	132	19,641	6%
Louisiana	135	28,499	34,414	14%	39	13,355	4%
New York	13	D*			15	5,658	2%
North	56	761	919	0.37%	22	337	0.10%
Carolina							
Oregon	21	11,584	13,988	6%	17	10,555	3%
Rhode Island	11	D			21	5,734	2%
South	35	2,505	3,025	1%	9	2,008	0.61%
Carolina							
Massachusetts	138	6,157	7,435	3%	132	D	
Maine	32	2,861	3,455	1%	22	D	
US, TOTAL	980	203,183	245,355	100%	756	328,567	100%

\*D= data not reported; information could be used to identify data from individual respondents. †, Inflation accounted for using the U.S. Department of Labor, Bureau of Labor Statistics Calculator https://www.bls.gov/data/inflation\_calculator.htm

## 3. Molluscan Bivalve Shellfish Aquaculture Policy

## 3.1 Public Policy

Broadly, policy includes measures developed at any level of government to serve their constituents. "Simply defined, public policy refers to a government action or inaction designed to serve a politically defined purpose. Policy should be seen as an output of government..." [27, p.3]. This may encompass measures to promote beneficial goods within society as well as restrictions to limit negative activities.

The primary policy instrument used to effect behavioral change is the implementation of rules or regulations (used synonymously herein with acknowledgement these words may carry different meaning according to agency, level of government, or activity regulated) [28]. Other nonregulatory measures can result in substantive change without the development of rules; however, for these processes to be successful, all parties must agree to the integrity of the process and legitimize the results [28]. Those types of public policy are used far less within government due to the lack of control over individual actors. Rules are typically more rigid than voluntary initiatives, striking a balance between precision (of what is regulated and under what conditions) and flexibility (to allow innovation and future control) [28]. They are a means to inform a large group of regulated society via indirect dialog (every individual is not directly informed of new rules and rule changes) and at a minimum must be perceived by the regulated community as legitimate [28]. While this method for controlling societal behavior is highly effective in many situations, newly developed rules must be coordinated with current regulations to ensure the regulated community is not overburdened. In instances where regulations become too complex, additional non-regulatory actions that work to streamline processes or enhance communication may become necessary.

## 3.2 Aquaculture Policy

In 2011, using the National Shellfish Initiative framework developed by the National Oceanic and Atmospheric Administration (NOAA) [29], Washington State implemented the Washington Shellfish Initiative [25, 30]. The Initiative has received full political support from the Governor's office, including a Shellfish Policy Advisor (housed in the Governor's office), as well as support from those in the executive branch regulating the industry, and from academia, and tribal and local governments. Goals of the statewide non-regulatory policy directive include, among other items, the streamlining of regulatory processes, funding research on native shellfish restoration, and increasing available shellfish growing area through the enhancement of water quality [30]. To

achieve greater efficiency with regulatory processes, the Shellfish Interagency Permitting (SIP) Team was developed, consisting of representatives from local, state, tribal, and federal entities. Of significance for this review, the team's primary goal was "permitting coordination [...] to make effective and efficient use of agency resources to facilitate timely and predictable delivery of quality decisions on shellfish aquaculture permit applications while protecting public health and the environment" [31, p.1]. Further, the main results of Phase I include the rejection that a "one-stop-shop" style permitting process would apply in Washington (declined to consolidate permitting or permit applications within one authorizing entity), but that a lead State Agency and Aquaculture Coordinator are a must [31]. To continue the work of facilitating dialog among the multitude of regulatory agencies, it was clear to the team leaders that a single point-of-contact within the State must be identified and financially supported for the initiative's efforts to continue to produce tangible results into the future [31]. In the Phase I report, the SIP team recommended that the Aquaculture Coordinator be housed at the Washington State Department of Agriculture, serving the role of facilitating dialog between applicants and all authorizing entities, which may include County and Local government as well as multiple state agencies. The placement of the Aquaculture Coordinator within the Washington State Department of Agriculture was deemed a good fit for the Department's statutory role as industry advocate and entity engaged in improving regulatory efficiency [31]. Additionally, to further consolidate permitting and potential interactions between applicants and the State, the SIP team recommended that a lead State Agency be agreed upon by all State regulatory entities; ideally, the agency within which the Aquaculture Coordinator is housed [31]. The crux of this recommendation is that the Aquaculture Coordinator must be provided sufficient authority to oversee permitting [31].

Should Washington State proceed with implementing the Phase I recommendations, and provide administrative and financial support for an Aquaculture Coordinator at a lead state agency, it may be helpful to review the hierarchy of regulatory agencies within the Commonwealth of Virginia,

where the coordinating role is already present. Currently the top east coast producer of molluscan bivalve shellfish (and top producer of hard clams in the nation), Virginia was able to be at the forefront of molluscan bivalve shellfish aquaculture due, in large part, to the early decision by regulators and industry to support leasing, vertical integration of services, and oyster farming as a compliment to their native fishery [32-33]. Building off early success with leasing, the state enhanced their permitting efficiency through their "one-stop-shop" permitting implemented via the Joint Permit Application (JPA) [34]. The JPA serves as the application for permits required at the State (Virginia Marine Resources Commission and Virginia Department of Environmental Quality), Federal (US Army Corps of Engineers), and Local (Local Wetlands Review Boards) levels. There is no mention of the need for an Aquaculture Coordinator with the role of advocate and permit efficiency review; however, the process of submitting a single application to the VMRC is a regulatory mirror to the recommendations from the non-regulatory Washington Shellfish Initiative. The critical item noted in Washington- that the lead State Agency and Aquaculture Coordinator be recognized for their authority to serve as liaison and advocate [31] is not necessary in Virginia as the lead agency is also a permitting agency. Applicants are required to have a relationship with the lead agency within Virginia. Fostering the relationship between the Aquaculture Coordinator in Washington State and the industry is paramount for that role to be successful.

Maryland and New Jersey are comparable states in that many coastal communities were founded on traditional oyster fishery roots [35]. The culturing of shellfish (predominantly oysters) is a more nascent industry within Maryland, resulting in a series of recent shifts in state level authorizations. The first, and perhaps most significant change occurred in 2011 when the Maryland legislature moved the authority to regulate molluscan bivalve shellfish aquaculture from the Maryland Department of Agriculture to the Department of Natural Resources [26]. That move condensed regulatory authority within a single state agency (Maryland Department of the Environment provides water classification for shellfish growing waters but has no regulatory role). Secondarily, several regulatory changes occurred to provide for the current joint application process which is a consolidated process (close to the Virginia JPA), but it can take almost a year to complete due to the stepwise review of the application [36]. The high demand for leases within Maryland provides incentive for the State to continue to review current processes and streamline additional items. Finally, even though the shift in regulatory authority from Maryland Department of Agriculture to the Department of Natural Resources moved the focus of aquaculture out of agriculture, it is still supported as a rural agricultural industry within the State. Through the quazipublic financing entity (independent corporation body that was formed and funded by General Assembly), Maryland Agricultural & Resource-Based Industry Development Corporation (MARBIDCO), aquaculturists can apply for low-interest loans to assist with start-up expenses [37]. Based on the collection of recent changes implemented at the state level, it is possible the 2013 USDA data did not fully capture growth of the industry in the past several years.

New Jersey is slowly increasing market share of the molluscan bivalve shellfish aquaculture industry, but not at a rate that has led to a change in the State's relative position nationally. This stagnation therefore leads to the question of why there is not a more significant change in New Jersey's relative position, nationally, as a producer of aquacultured molluscan species between the two reporting years of 2005 and 2013. Molluscan bivalve shellfish aquaculture within the State of New Jersey is comprised primarily of hard clams (*Mercenaria mercenaria*) and oysters (*Crassostrea virginica*), all of which is managed by nine state and federal agencies (see Table 3.2 for listing of agencies and primary roles with respect to molluscan bivalve shellfish aquaculture). For molluscan bivalve shellfish aquaculture located within Delaware Bay (and some Atlantic Coastal bays), there is additional oversight from the US Fish and Wildlife Service due to the 2015 listing of the Red Knot *rufa* subspecies (*Calidris canutus rufa*, Linnaeus) as federally threatened [38]. The consultation process for USFWS authority (through the US Army Corps of Engineer

(USACE) Nationwide or Individual permits) is similar to that of National Marine Fisheries Service (NMFS) for marine mammals or endangered fish species [39]; however, the USFWS authority is noted here because it is a substantial factor within the current regulatory matrix for growers in the affected area.

Table 3.2: State and federal agencies with direct oversight of New Jersey's bivalve shellfish aquaculture industry.

Agency	Agency Role
NJ Department of	Connect aquaculture to agriculture; ownership of crops
Agriculture (NJDA)	
NJ Department of	Commercial and recreational shellfish licensure;
Environmental	leasing of shellfish growing locations;
Protection(NJDEP), Bureau	management of natural shellfish resources
of Shellfisheries (BSF)	
NJDEP, Bureau of Marine	Shellfish Growing Water Classification;
Water Monitoring (BMWM)	Commercial Shellfish Aquaculture Permit;
	Hatchery and Nursery Permit
NJDEP, Division of Land	Coastal Zone and Waterfront Development Permits
Use Regulation (DLUR)	
NJDEP, Bureau of Tidelands	Manage use of State lands that are currently or were historically
Management (BTM)	flowed by the tides; staff for Tidelands Resource Council
NJDEP, Marine Law	Enforce permits and regulations
<b>Enforcement (MLE)</b>	
NJ Department of Health	Human health oversight; regulation of shellfish handling and
(NJDOH)	transport; wholesale transactions
<b>US Army Corps of Engineers</b>	Regulate activity and structure within navigable waters of the
(USACE)	nation
US Food and Drug	Federal coordinating agency for the Interstate Shellfish
Administration (FDA)	Sanitation Conference and associated National Shellfish
	Sanitation Program
US Fish and Wildlife Service	Management of actions within areas of federally listed species
(USFWS)	

Of the state departments listed in Table 3.2, the New Jersey Department of Environmental Protection (NJDEP) and the New Jersey Department of Health (NJDOH) have authority over all state issued permits related to shellfish aquaculture. Those two Departments also partner for administration of the State's *Vibrio* plan [40]. The regulatory responsibility of NJDEP is further distributed among five Bureaus or Divisions, each with a unique responsibility in managing the

industry. The New Jersey Department of Agriculture (NJDA) is responsible for promoting industry development, providing marketing assistance, and serving as industry advocate [41].

Table 3.3: Regulatory authorizations and associated agencies for all commercial shellfish aquaculture within the State of New Jersey (2016). New Jersey Authorizing Code (NJAC) are the regulations through which activities are managed by state agencies, the New Jersey Statutory Authority (NJSA), or statute (law), authorizing the development of the respective regulation is included in the far right column.

	Authorization	Agency	NJAC	NJSA
1	Commercial Shellfish License	NJDEP, BSF	None	50
2	Commercial Shellfish Lease	NJDEP, BSF Shellfish Council	7:25-24 (Atlantic ONLY)	50
3	Nationwide 48 (or Individual) Permit	USACE	N/A	N/A
4	Coastal/Waterfront Development Permit	NJDEP, DLUR	7:7	13:19 (12:3; 12:5-3)
5	Tidelands License	BTM/TRC	None	12:3
6	Aquatic Farmer License	NJDA	2:89	4:27
7	Commercial Shellfish Permit	NJDEP, BMWM	7:12-9	2C:64 4:27 13:1D-9
8	Hatchery/Nursery Permit*	NJDEP, BMWM	_	23:2B-1 50:1-5 et seq. 58:24
9	Certified Dealer <sup>†</sup>	NJDOH	8:13	24:15
10	Aquatic Organism Import*	NJDEP, BSF	None	50; 4:27
11	Vibrio Plan <sup>#</sup>	NJDEP/NJDOH	7:12-8.6 (NJDEP) 8:13-1.7(NJDOH)	See 7&8 above See 9 above

\*If necessary

<sup>†</sup>Optional; first point-of-sale for all molluscan bivalve shellfish must be to a certified dealer, however each grower is not required to become certified.

# Seasonal; Oysters Only

The authorizations and regulatory oversight can be further dissected by examining the specific permits required to culture shellfish within NJ waters, as is provided in Table 3.3. This table is arranged according to the 2016 permitting process within the state, with each authorization required in a mostly stepwise manner. Not all authorizations may be required for every application; all of the listed approvals are required to place structure within the water column or on the seafloor.

The authority for the NJDOH at N.J.S.A. 24:15-1 et seq. is purposefully disregarded in this review due New Jersey's regulations reflecting national standards that are implemented within all molluscan bivalve producing and harvesting states. The Interstate Shellfish Sanitation Conference (ISSC) - a network of all shellfish producing states, industry representatives, and the FDA-dictates conditions on the harvest, handling, and transport of shellfish through the National Shellfish Sanitation Program (NSSP), commonly referred to as the Model Ordinance (Section II of NSSP) [42]. Each molluscan shellfish producing state must comply with the minimum conditions dictated within the NSSP in order to transport and sell outside of state borders [42]. New Jersey has incorporated the appropriate sections of the Model Ordinance within regulations and differences between the New Jersey program and other states are considered minor relative to items of concurrence.

At the aggregate level the legislative intent within New Jersey is balanced to achieve industry development coupled with consideration of multi-user resources (coastal waters) and the protection of human health. It appears that at the laws within New Jersey are calling for efforts similar to those noted above for Washington. The Aquaculture Coordinator role recommended for Washington State, serving essentially as the liaison between permitting entities and applicant, is provided for within New Jersey according to N.J.S.A. 4:27, with late 2016 amendments to N.J.S.A. 4:27-10 reinforcing the permit coordination role of the NJDA (same Department in Washington where permit coordination is recommended [31]). It is still too soon to know how the executive branch Departments in New Jersey will implement the new requirements; however, it is important to note that permit coordination is being required via statutory change. In Washington, the charge for more efficient permit processes are the result of a non-regulatory initiative supported by the Governor's office. In the latter case, there is clear political support for the voluntary initiative, but there is no legal impetus and so if this recommendation is not implemented, the State can continue under present conditions. Since significant resources have

been allocated towards the Washington State Shellfish Initiative, it is unlikely that the recommendation will not be given full consideration, even if the Coordinator position is placed within another agency (which is a tertiary recommendation but not desired according to the SIP Team [31]).

Virginia and Maryland each have a process that is similar to the one outlined in the New Jersey legislative amendments to N.J.S.A. 4:27 (consolidated permit application), however in both of those states the primary point-of-contact agency has regulatory oversight over the industry. This seems to be an important difference between the efforts of New Jersey and its regional neighbors. The recently amended New Jersey legislation had originally required a coordinated permit process upon first passage in 1997, but that process had not been implemented. The NJDA does not have regulatory authority, providing a license that serves to show proof of ownership, but does not authorize the activity or use of State lands. In Virginia and Maryland, the point-ofcontact or lead state agency has a forced, direct relationship with applicants due to the permitting authority of the lead agency. No one can conduct molluscan bivalve shellfish aquaculture within the waters of either of those states without going through the lead agency for one or more authorizations. This nuance in permitting authority of lead agencies appears to be a crucial difference between these three states. Likely reflecting on this potential for their own failure, the Washington SIP team in their recommendation for the Department of Agriculture to be the lead state agency, stipulated that permit coordination authority must be recognized by all parties in order for the recommendation to be successful. Perhaps within New Jersey a similar recommendation or affirmation will be required as a companion to the recently amended statute.

From this four state review, it is clear that state governments are recognizing the need to continually adjust regulations related to molluscan bivalve shellfish aquaculture, with non-regulatory (Washington) and regulatory (Maryland and Virginia) items developed to improve

permitting efficiency. Although not explored fully above, a critical component to the success of exemplar states (in terms of sales) is the inclusion of all levels of government- most importantly the federal level authorizations through the US Army Corps of Engineers (USACE). Inclusion of all authorizing entities, not just those at the state level, ensures greater consistency in permit decision-making and can develop more transparent communication amongst all parties (reviewing the same information and speaking to the same application material).

#### 4. State-Level Policy Impacts

The review of regulatory highlights from four coastal states in the previous section has yielded a collection of regulatory factors that individually and collectively guide aquaculture industry development. These factors include: 1) a single point-of-contact between an applicant and the regulatory arena; 2) the political will within the executive branch to foster the industry, also considered "political capital" to provide favorable conditions for the industry; 3) vertical integration of the regulatory process such that permitting at all levels (federal through to local) is considered within a consolidated permit process; and 4) the age of the industry or management system. This last item was not expressly mentioned in the above discussion, but it was noted for the states of Maryland and New Jersey with regards to the shift in permitting authority within Maryland as well as recent changes to permitting and amended legislation within New Jersey. For the purposes of this review, those states (New Jersey and Maryland) are categorized as having younger programs relative to Washington and Virginia. A closer look at the four identified factors and their presence within each of the four review states (Table 3.4), reveal a few conclusions regarding state-level policy efficiencies.

First, states at the forefront of shellfish aquaculture sales have apparent (Washington) and assumed (Virginia) political capital within the industry, which is then manifest in industry development and progressive policy-making. In Washington State there is transparent support for
the industry from the Governor through to local and tribal governments; the political will to advance the industry is well defined for that state. Since Virginia has a robust industry and streamlined permitting process, it is assumed that the industry has abundant political capital which ensures continued development of industry-supportive policies within the State and maintenance of their top-tier status nationally. In addition, all states either possess or are working towards a single point-of-contact for permitting, with some vertical integration of processes following suite. For New Jersey, there are no local review boards like those present within Washington and Virginia, therefore the consolidation of all permits would only require state and federal permit coordination. Vertical integration is critical to any state's success but will yield the greatest impacts to states with multiply layers of industry review.

Table 3.4: Regulatory factors resulting from the analysis of molluscan bivalve shellfish aquaculture policy regimes in four coastal states.

Regulatory Factors				
	Point-of-Contact	Political Will/Capital	Vertical Integration of Regulatory Processes	Age of Industry/ Management
NJ	Numerous; see amended legislation	None apparent	No	Young, traditional aquaculture; fishery management
WA	Recommended single POC but not yet implemented	Yes; Governor's Office & Initiative	Recommended but not yet implemented	Long-standing aquaculture industry
VA	Single POC for JPA	Political capital at state and local levels	Yes	Long-standing aquaculture industry
MD	Single POC	None apparent	Yes	Young, oyster fishery focused with recent transition to aquaculture

Finally, the age of the industry or of the current management system is a clear indication of industry success. The two younger states of Maryland and New Jersey are well behind the sales levels of Washington State and Virginia. It is apparent from this analysis that age of industry is not the sole factor of industry success, however, age of management system is definitely leading to some of the state-by-state differentiation found within the USDA data (Table 3.1). Further changes to management systems within individual states will likely result in near-term slower

growth of the industry as it stabilizes to reconcile to new permitting or regulatory measures. On the plus side, if those measures include items to foster growth they should also lead to significant gains in future years.

## 5. Conclusion

This paper identified several state-level obstacles to the growth of the molluscan bivalve shellfish aquaculture industry. None of the identified hurdles are unique to an individual state and are present in varying degrees within the four case study states. The greatest means of improving a state's status, and in turn the nation's sales and production, is via concerted state-level executive branch support for the industry. The top bivalve producing states have long recognized that the industry as a socioeconomic engine for their state with prominent top-down support regardless of political changes. These states have also worked to streamline permitting throughout the entire hierarchy of governance (including federal and local approvals), and provide applicants with a single point-of-contact to serve as liaison between the industry and the regulatory arena. Wellestablished, top-producing states have an advantage with current sales numbers since they have been conducting and advancing aquaculture for decades; however, the intrinsic ability of the industry to innovate should allow younger states to recognize that small but purposeful regulatory and political changes could lead to substantial advances for the industry and state economy. Collectively, the actions at the state-level can shift the U.S. standing for bivalve shellfish sales, creating societal benefits and providing a reliable, safe supply of domestic goods. Recognition of the importance of efficient state-level policy (regulatory and non-regulatory) is required for a complete discussion on national domestic shellfish production now and in the future.

## References

- J.E. Cloern. Does the Benthos Control Phytoplankton Biomass in South San Francisco Bay? Mar. Ecol. Prog. Ser., 9 (1982), pp.191-202.
- [2] P.J. Cranford, P.M. Strain, M. Dowd, B.T. Hargrave, J. Grant, and M-C. Archambault. Influence of mussel aquaculture on nitrogen dynamics in a nutrient enriched coastal embayment. Mar. Ecol. Prog. Ser. 347 (2007), pp.61-78.
- [3] R.F. Dame Ecology of marine bivalves: an ecosystem approach. Second edition. CRC Press, Boca Raton, (2012), 271 pp.
- [4] R.S. Fulford, D.L. Breitburg, R.I.E. Newell, W.M. Kemp, and M. Luckenbach. Effects of oyster population resotoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Mar. Ecol. Prog. Ser. 336 (2007), pp.43-61.
- [5] R.S. Fulford, D.L. Breitburg, M. Luckenbach, and R.I.E. Newell. Evaluating ecosystem response to oyster restoration and nutrient load reductions with a multispecies bioenergetics model. Ecological Applications. 20(4) (2010), pp.915-934.
- [6] J.H. Grabowski, C.H. Peterson, Restoring oyster reefs to recover ecosystem services. Theoretical Ecology Series. 4 (2007) 281-297.
- [7] A.T. Humphries, S.G. Ayvazian, J.C. Carey, et al. Directly Measured Denitrification Reveals Oyster Aquaculture and Restored Oyster Reefs Remove Nitrogen at Comparable High Rates. Frontiers in Marine Science. 3 (2016). doi: 10.3389/fmars.2016.00074.
- [8] R.I.E. Newell. Ecological Changes in Chesapeake Bay: Are They the Result of Overharvesting the American Oysters, Crassostrea virginica? (1988), Pages 536-546 in M.P. Lynch and E.C. Krome, editors. Understanding the Estuary: Advances in Chesapeake Bay Research. Chesapeake Research Consortium Publication 129.
- [9] R.I.E. Newell. Ecosystem influences of natural and cultivated populations of suspensionfeeding bivalve mollusks: a review. Journal of Shellfish Research. 23 (2004), pp.51-61.
- [10] R.I.E. Newell, T.R. Fisher, R.R. Holyoke, and J.C. Cornwell. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. (2005), Pages 93-120 in R. Dame and S. Olenin, editors. The comparative role of suspension feeders in ecosystems. Springer, Dordrecht, The Netherlands.
- [11] C.B. Officer, T.J. Smayda, and R. Mann. Benthic Filter Feeding: A Natural Eutrophication Control. Mar. Ecol. Prog. Ser. 9 (1982), pp.203-210.
- [12] C.H. Peterson, J.H. Grabowski, S.P. Powers. Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. Mar.Ecol. Prog. Ser Vol. 264 (2003) 249-264.
- [13] T.C. Prins, A. C. Smaal, and R.F. Dame. A review of the feedbacks between bivalve grazing and ecosystem processes. Aquatic Ecology. 31 (1998), pp.349-359.
- [14] R.E. Ulanowicz and J.H. Tuttle. The Trophic Consequences of Oyster Stock Rehabilitation in Chesapeake Bay. Estuaries. 15(3) (1992), pp.298-306.

- [15] P.L. deFur, D.N. Rader, Aquaculture in Estuaries: Feast or Famine? Estuaries, 18 (1A) (1995) 2-9.
- [16] R.L. Naylor, R.J. Goldburg, J.H. Primavera, et al. Effect of aquaculture on world fish supplies. Nature. 405 (2000), pp. 1017-1024.
- [17] B.R. Dumbauld, J.L. Ruesink, S.S. Rumrill. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture. 290 (2009), pp.196-223. doi:10.1016/j.aquaculture.2009.02.033.
- [18] G. Krause, C. Brugere, A. Diedrich, et al. A revolution without people? Closing the peoplepolicy gap in aquaculture development. Aquaculture. 447 (2015), pp. 44-55.
- [19] M.A. Rice, M.A. Environmental Impacts of Shellfish Aquaculture: Filter Feeding to Control Eutrophication. pp. 77-86. In: M. Tlusty, D. Bengtson, H.O. Halvorson, S.Oktay, J. Pearce & R. Rheualt, (eds.). Marine Aquaculture and the Marine Environment: A Meeting for the Stakeholders in the Northeast. Held Jan. 11-13, 2001 at the Univ. of Massachusetts Boston. Cape Cod Press, Falmouth MA.
- [20] S.E. Shumway, C. Davis, R. Downey, R. Karney and others Shellfish aquaculture in praise of sustainable economies and environments. World Aquaculture. 34 (2003) 8-10.
- [21] United States Department of Agriculture, National Agricultural Statistics Service, Census of Aquaculture (2013), Volume 3, Special Studies, Part 2 (issued September 2014) 1-98. https://www.agcensus.usda.gov/Publications/2012/Online\_Resources/Aquaculture/ (accessed 11/2/2016).
- [22] National Marine Fisheries Service, Office of Science and Technology, Fisheries of the United States, 2015: Current Fishery Statistics No.2015, (2016) 1-151.
- [23] National Restaurant Association, Demand for Local Food on the Rise http://www.restaurant.org/News-Research/News/Demand-for-local-foods-is-on-the-rise (accessed 06/16/2017)
- [24] National Restaurant Association. "What's Hot for 2017" Survey http://www.restaurant.org/News-Research/News/Whats-Hot-Top-10-food-trends-in-2017 (accessed 6/16/2017)
- [25] Washington State Shellfish Initiative, Governor Jay Inslee webpage http://www.governor.wa.gov/issues/issues/energy-environment/shellfish (accessed 12/31/2016)
- [26] Maryland Department of Natural Resources, Shellfish Aquaculture, Getting Started. http://dnr2.maryland.gov/fisheries/Pages/aquaculture/getting-started.aspx (accessed 06/16/2017)
- [27] S.Z. Theodoulou, The Contemporary Language of Public Policy: Starting to Understand. pp. 1-11. In: S.Z. Theodoulou, M.A. Cahn (eds.) Public Policy: The Essential Readings. Second Edition. Pearson, 2013.

- [28] D. Stone, Policy Paradox: The Art of Political Decision Making. Third Edition. W.W. Norton & Company, Inc. 2012.
- [29] National Oceanic and Atmospheric Administration (NOAA), National Shellfish Initiative http://www.nmfs.noaa.gov/aquaculture/policy/shellfish\_initiative\_homepage.html (accessed 12/31/2016)
- [30] Washington State Shellfish Initiative, December 09, 2011 White Paper http://www.governor.wa.gov/sites/default/files/documents/WSI\_WhitePaper2001.pdf (accessed 01/06/2017).
- [31] Shellfish Interagency Permitting Team Phase I Report, May 05, 2016. Prepared by P.J. Lund, L.K. Hoberecht. http://www.ecy.wa.gov/programs/sea/aquaculture/sip.html (accessed 01/06/2017)
- [32] T.J. Murray, M.J. Oesterling. Virginia Shellfish Aquaculture Situation and Outlook Report Results of Virginia Shellfish Aquaculture Crop Reporting Survey 2004-2006, VIMS Marine Resource Report No. 2006-5 (2006). http://web.vims.edu/adv/aqua/MRR2006\_5.pdf (accessed 01/06/2017)
- [33] K. Hudson, Virginia Shellfish Aquaculture Situation and Outlook Report: Results of the 2016 Virginia Shellfish Aquaculture Crop Reporting Survey, VIMS Marine Resource Report No. 2017-7 (2017). http://www.vims.edu/research/units/centerspartners/map/aquaculture/docs\_aqua/2017\_shellf ish\_report.pdf (accessed 06/01/2017)
- [34] Virginia Tidewater Joint Permit Application, Revised May 2017 http://www.deq.virginia.gov/Portals/0/DEQ/Water/WetlandsStreams/fillable%20Tidewater %20JPA%20May%202017.pdf?ver=2017-05-23-162846-223 (accessed 06/03/2017)
- [35] D. Webster, Maryland Oyster Culture: A Brief History. 2007. https://extension.umd.edu/aquaculture/oysters/aquaculture-policy (accessed 06/16/2017)
- [36] See Commercial Shellfish Aquaculture Lease Application, Maryland Department of Natural Resources, Shellfish Aquaculture, Getting Started http://dnr2.maryland.gov/fisheries/Pages/aquaculture/getting-started.aspx (accessed 06/16/2017)
- [37] Maryland Agricultural & Resource-Based Industry Development Corporation, Maryland Shellfish Aquaculture Loan Program. http://www.marbidco.org/loans/msal.html (accessed 11/2/2016)
- [38] United States Fish and Wildlife Service, New Jersey Field Office. Biological Opinion on the Effects of Existing and Expanded Structural Aquaculture of Native Bivalves in Delaware Bay, Middle and Lower Townships, Cape May County, New Jersey on the Federally Listed Red Knot (Calidris canutus rufa). April 2016.
- [39] United States Fish & Wildlife Service and National Marine Fisheries Service, Endangered Species Consultation Handbook: Procedures for Conducting Consultation and Conference Activities under Section 7 of the Endangered Species Act. (1998).

 $https://www.fws.gov/endangered/esa-library/pdf/esa\_section7\_handbook.pdf (accessed 12/03/2015)$ 

- [40] NJ Admin Code § 7:12-8.6 (2016)
- [41] NJ Rev Stat § 4:27-6 (2017)
- [42] United States Food & Drug Administration, National Shellfish Sanitation Program, NSSP Guide for the Control of Molluscan Shellfish: 2015 Revision (Updated February 2017) https://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.ht m (accessed 06/16/2017)