EXPANDING AQUACULTURE OPPORTUNITIES IN THE NORTHEASTERN UNITED STATES THROUGH ECOLOGICALLY-SOUND DIVERSIFICATION

By

MICHAEL PETER ACQUAFREDDA

A dissertation submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Ecology and Evolution

Written under the direction of

Daphne Munroe

And approved by

__________________________

__________________________

__________________________

__________________________

New Brunswick, NJ

January 2021
ABSTRACT OF THE DISSERTATION

Expanding Aquaculture Opportunities in the Northeastern United States through Ecologically-Sound Diversification

By MICHAEL PETER ACQUAFREDDA

Dissertation Advisor:

Daphne Munroe

The goal of this dissertation was to investigate ways to expand bivalve aquaculture opportunities in the northeastern United States through ecologically-sound diversification (ESD). In an aquaculture context, ESD is defined as the practice of cultivating multiple species in a manner that minimizes negative impacts on the surrounding ecosystem while remaining adaptive and resilient to environmental change. Bivalve aquaculture is among the least environmentally damaging forms of food production and many of its impacts serve a net benefit to surrounding ecosystems, making it an ideal starting point for ESD. In the Northeast, production is dominated by the Eastern oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*), and high-efficiency yet risk-prone monoculture is the norm. In the first unit (Chapters 2 and 3), various aspects of Atlantic surfclam (*Spisula solidissima*) husbandry were evaluated to test whether this species can become an alternative species for shellfish growers. In Chapter 2, the effect of rearing temperature on the survival and growth of early juvenile surfclams was investigated. The results show that of the temperatures tested, 20°C was optimal for promoting survival and growth. In Chapter 3, a series of controlled experiments were conducted to evaluate the efficacy of various surfclam
nursery and grow-out techniques. In the nursery phase, juvenile surfclams were reared in various gear types. In the grow-out phase experiments, surfclam growth, survival, and condition were evaluated at three New Jersey farms. Results show that multiple rearing methods can effectively produce surfclams, but flow rate, food availability, and temperature are important factors that affect gear efficiency. In the grow-out experiments, survival varied across farms and across years; growth and condition were consistent across farms but varied seasonally. The second unit (Chapters 4 and 5) expands upon the results of the previous chapters, which demonstrated the vulnerability of surfclams to heat stress. Research presented in Unit 2 takes a proactive approach towards climate-informed aquaculture. In Chapter 4, the feasibility of breeding surfclams for greater heat tolerance is explored. The results indicated that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent heat stress for significantly longer than individuals that did not experience heat stress as juveniles. Additionally, selective breeding enhanced heat tolerance in first-generation surfclam progeny. To understand how selection via heat stress could alter the gene expression of a surfclam population, a transcriptomics study was conducted (Chapter 5). The results show that randomly selected (control) clams differentially expressed more genes in response to heat stress than the heat-selected clams, yet the latter group exhibited significantly stronger expression of heat shock proteins and other stress-response genes. In the third and final unit (Chapter 6), the efficacy of bivalve polyculture was evaluated using four cultured species: the Eastern oyster \((C.\ virginica)\), the Atlantic surfclam \((S.\ solidissima)\), the hard clam \((M.\ mercenaria)\), and the softshell clam \((Mya\ arenaria)\). Three particle depletion experiments were conducted to determine if more diverse
bivalve assemblages had greater clearance rates than those which were less diverse. Likewise, the effect of species richness on bivalve productivity (growth and survival) was evaluated. When supplied with natural seston, the four-species polyculture demonstrated a significantly greater tank-level clearance rate for particles <25 µm compared to most monocultures. However, nearly all productivity metrics were unaffected by species richness. Overall, this dissertation finds that the surfclam is a good candidate for species diversification in the Northeast, and the species fits well into the region’s established farming framework. Despite the current and future risks that the warming climate poses for the surfclam, this dissertation also finds that selective breeding may be a viable strategy for enhancing the survival of cultured surfclams. Finally, this dissertation provides some of the first evidence for niche complementarity among four cultured bivalves and demonstrates that in non-food limited systems, the focal species can be co-cultured without outcompeting one another. Taken together, this dissertation concludes that ecologically-sound diversification can be achieved spatially, temporally, and genetically in the northeastern United States.
Acknowledgements

I would first like to thank all of the funding sources that made my graduate research possible. I am grateful for the Rutgers University Department of Ecology, Evolution, and Natural Resources, the Graduate Program in Ecology and Evolution, and the Haskin Shellfish Research Laboratory for providing me with Graduate Assistantships. I am also grateful for the Rutgers University Division of Life Sciences and Department of Marine and Coastal Sciences for providing me with Teaching Assistantship opportunities.

Partial funding for this dissertation was provided by New Jersey Sea Grant with funds from the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under the NOAA grants #NA14OAR4170085, #NA18OAR4170087, #NA18OAR4170357, and the New Jersey Sea Grant Consortium. This dissertation was also partially supported by funds awarded by the Northeast Sustainable Agriculture Research and Education (SARE) Program of the United States Department of Agriculture (USDA), under SARE grant #GNE17-141-31064. The statements, findings, conclusions, and recommendations of this dissertation are solely mine, and do not necessarily reflect the views of New Jersey Sea Grant, the U. S. Department of Commerce, the Northeast SARE Program, or the USDA.

This dissertation was also supported by funds supplied by the Rutgers University New Jersey Aquaculture Innovation Center (AIC) Student Grant Program, the Rutgers University Ecology & Evolution Small Grant Program, and the Manasquan River Marlin and Tuna Club’s Captain George Burlew Scholarship. Notably, Sea-Bird Scientific generously supplied equipment that made much of this dissertation possible. Pilot research that was foundational to this dissertation was funded by Bumble Bee Seafoods.
Next, I must thank my advisor, Dr. Daphne Munroe, for her superb supervision and exceptional advisement throughout my graduate school experience. With her help and guidance, I was able to conduct, analyze, and publish my research in several journals and was given opportunities to present my work at more than a dozen conferences and fora. I would not have been able to complete this dissertation without her encouragement and confidence in my abilities. I feel proud and honored to have been given the chance to work alongside her, and I have become a better scientist because of her incomparable mentorship. I am incredibly appreciative of everything Daphne has done for me, and I look forward to working closely with her to answer future research questions.

I am thankful to my dissertation committee for their guidance, encouragement, and critical feedback, which helped improve the quality of this dissertation. I therefore would like to thank Dr. David Bushek, Dr. Judith Grassle, Dr. Ximing Guo, and Dr. Helen Gurney-Smith for their continued support.

I would also like to thank Dr. Michael De Luca and Lisa Ragone Calvo, who served as advisors, collaborators, and co-authors on some of the published work that came from this dissertation. In particular, I am grateful for Mike’s mentorship and his endless support for all the research I conducted at the NJ Aquaculture Innovation Center. Lisa has also been a spectacular mentor. Her guidance, encouragement, and Sweet Amalia’s oysters have kept me intellectually, emotionally, and physically nourished.

I received an incalculable amount of support and assistance from the faculty, staff, and interns of the Haskin Shellfish Research Laboratory and the NJ Aquaculture Innovation Center. This includes the use of laboratory space and supplies, help with organism collection and husbandry, and assistance with data collection. Specifically, I
would like to thank Iris Burt, Robert Cacace, Brenden Campbell, Nicole Deck, Jenn Gius, David Jones, Joshua Kiernan, Frederick Klie, Matthew Kozak, Horst Lehmann, Sean Maguire, Elise McKean, Nate Morris, Matthew Neuman, Jenny Paterno Shinn, Emily Scarpa McGurk, Sean Towers, Bea Vazquez, Angela White, Patty Woodruff, and Marila Xie for all of their help.

I would also like to thank Dr. Libby Jewett and Dr. Dwight Gledhill of the NOAA Ocean Acidification Program for providing me with excellent mentorship and support throughout my time with them as a John A. Knauss Marine Policy Fellow. Despite challenges created by the covid-19 pandemic, Libby and Dwight fostered a sense of belonging and gave me ample time for professional development.

This dissertation would not be possible without the tremendous contributions of the shellfish farmers who participated in the research. I learned so much from these individuals and hope that some of the conclusions of this dissertation can be useful to them. Specifically, I am so thankful for Bill Avery of Bill Avery’s Quality Bay Clams, Dale S. Parsons of Parsons Seafood, Marc Zitter of Northern Cape Sea Farms, and Matthew Gregg and Scott Lennox of Forty North Oyster Farms and the Barnegat Bay Oyster Collective. I am also grateful for ReClam the Bay, The Downeast Institute, and Sweet Amalia’s Oyster Farm for supplying resources, equipment, space, and organisms used in this dissertation.

I am fortunate to have been surrounded by an exceptional group of graduate students throughout my time at Rutgers University. I am honored to have been a part of the Ecology and Evolution Graduate Student Association; the members of the EcoGSA helped me grow as an individual and helped make me a better scientist. Specifically, I
would like to thank Janine Barr, Sarah Borsetti, Katrina Catalano, Dr. Joseph Caracappa, Samantha Gilbert Lynch, Emily Manuel, Dr. Jason Morson, Michael Whiteside, and Dan Zeng. Throughout my time at Rutgers University, these individuals gave me immense research support, but more importantly, they gave me endless intellectual and emotional support. I feel proud to call these individuals my colleagues and my friends.

Finally, I would not have achieved this goal without my wonderful family. To my Nana, Joan Ferraro, for being my biggest fan and always encouraging me to be curious about the world. To my Popa, Joseph Ferraro, for his inquisitive mind and engineering skills that shaped the polyculture mesocosm experiments. To my grandmother, Rose Acquafredda, for being my first surfclam taste tester. To my aunt and godmother, Luciann Acquafredda, for opening her home to me, which made my commute easier and my writing more productive. To my brother, Daniel Acquafredda, and my sister-in-law, Coleen Shanagher, for always showing me love, support, and interest in my work. To my parents, Joni and Dan Acquafredda, for their unconditional love, for pushing me to be my best, and for fostering my passion for science from a young age. And finally, to my partner, Emily Baldi, for her support, patience, and sacrifice. Emily has fostered my intellectual and emotional growth, and without her love and encouragement, this dissertation would not be possible.
Dedication

To my parents, Joni and Dan, and to my partner, Emily, for their never-ending support, encouragement, and love.
Table of Contents

ABSTRACT OF THE DISSERTATION.................................................................................. II
ACKNOWLEDGEMENTS................................................................................................... V
DEDICATION....................................................................................................................... IX
TABLE OF CONTENTS....................................................................................................... X
LIST OF TABLES .................................................................................................................. XIII
LIST OF FIGURES ............................................................................................................... XV

CHAPTER 1: ECOLOGICALLY-SOUND DIVERSIFICATION IN AQUACULTURE..................... 1
  1.0 Introduction................................................................................................................. 1
  1.1 Defining ecologically-sound diversification (ESD) ..................................................... 3
  1.2 Bivalve aquaculture: an ideal starting point for ESD ................................................ 6
  1.3 Risky efficiency: bivalve monoculture in the Northeast USA ..................................... 9
  1.4 Expanding aquaculture opportunities through ecologically-sound diversification .... 11
  1.5 Literature Cited......................................................................................................... 14

UNIT 1 – SUPPORTING AQUACULTURE DIVERSIFICATION BY EXPANDING
UNDERSTANDING OF SURFCLAM HUSBANDRY ....................................................... 19

CHAPTER 2: THE EFFECT OF REARING TEMPERATURE ON THE SURVIVAL AND GROWTH OF EARLY JUVENILE ATLANTIC SURFCLAMS (SPISULA SOLIDISSIMA) .......................................................... 20
  2.0 Abstract..................................................................................................................... 20
  2.1 Introduction................................................................................................................. 22
  2.2 Materials and methods ............................................................................................... 25
    2.2.1 Spawning and larval culture ............................................................................... 25
    2.2.2 Nursery rearing temperature experiment ......................................................... 25
    2.2.3 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater .......................................................... 30
    2.2.4 Statistical analyses .............................................................................................. 32
  2.3 Results ....................................................................................................................... 33
    2.3.1 Effects of rearing temperature on early juvenile surfclam survival .................... 33
    2.3.2 Effects of rearing temperature on early juvenile surfclam growth ....................... 36
    2.3.3 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater: survival .................................................. 39
    2.3.4 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater: growth .................................................................................. 39
  2.4 Discussion ................................................................................................................. 42
    2.4.1 Effects of temperature on juvenile surfclam survival and growth ....................... 42
    2.4.2 Factors that may modulate temperature effects on surfclams .............................. 44
    2.4.3 Concluding remarks ............................................................................................ 46
  2.5 Acknowledgements ................................................................................................... 48
  2.6 Literature Cited ....................................................................................................... 50

CHAPTER 3: EVALUATING VARIOUS NURSERY AND GROW-OUT CONDITIONS ON FARMED ATLANTIC SURFCLAMS (SPISULA SOLIDISSIMA) PERFORMANCE ................................................. 55
  3.0 Abstract..................................................................................................................... 55
  3.1 Introduction................................................................................................................. 57
  3.2 Materials and methods ............................................................................................... 60
    3.2.1 Spawning and larval culture of Atlantic surfclams .............................................. 60
    3.2.2 Early nursery gear type experimental design ..................................................... 61
    3.2.3 Late nursery gear type experimental design ....................................................... 66
    3.2.4 Data collection for nursery experiments ............................................................. 67
    3.2.5 Statistical analyses for nursery experiments ....................................................... 68
    3.2.6 Grow-out experimental design .......................................................................... 76
5.5 Acknowledgements .............................................................................................................. 246
5.6 Literature Cited .................................................................................................................... 248

UNIT 3 – EVALUATING THE EFFICACY OF BIVALVE POLYCULTURE .......................... 255

CHAPTER 6: EFFECT OF SPECIES DIVERSITY ON PARTICLE CLEARANCE AND
PRODUCTIVITY IN FARMED BIVALVES .............................................................................. 256

6.0 Abstract ................................................................................................................................. 256
6.1 Introduction ......................................................................................................................... 258
6.2 Materials and methods ....................................................................................................... 264
  6.2.1 General particle depletion experimental set-up .............................................................. 266
  6.2.2 Cultured algae depletion experiments ......................................................................... 271
  6.2.3 Natural seston depletion experiment ............................................................................ 272
  6.2.4 Productivity experimental set-up .................................................................................. 273
  6.2.5 Statistical analyses ....................................................................................................... 276
6.3 Results .................................................................................................................................. 277
  6.3.1 Clearance rates of cultured algae: Pavlova lutheri ....................................................... 277
  6.3.2 Clearance rates of cultured algae: P. lutheri and Nannochloropsis oculata ................ 280
  6.3.3 Clearance rates of seawater containing natural seston ................................................ 282
  6.3.4 Effect of species diversity on productivity ................................................................. 286
6.4 Discussion ............................................................................................................................ 291
  6.4.1 The effect of species diversity on particle clearance ..................................................... 291
  6.4.2 Variations in particle capture processes ....................................................................... 294
  6.4.3 Variations in diet requirement or preference ............................................................... 296
  6.4.4 The effect of species diversity on bivalve productivity ................................................. 298
  6.4.5 Implications for aquaculture ....................................................................................... 302
6.5 Acknowledgements ............................................................................................................. 304
6.6 Literature Cited .................................................................................................................... 306
6.7 Supplement ........................................................................................................................... 317

CHAPTER 7: CONCLUDING REMARKS ............................................................................. 321

7.0 Conclusion ............................................................................................................................ 321
7.1 Literature Cited .................................................................................................................... 329
List of Tables

Table 2-1. Growth rates of early juvenile surfclams reared at five different temperatures. Values are mean ± SE (N=3 replicate cohorts). Please see Section 2.2 for calculations of average daily and instantaneous growth rates. .................................................................37

Table 3-1. Percentage of clams reaching market sizes (shell length) over the duration of the grow-out experiment. AB = Absecon Bay, recruitment box only; UBB = Upper Barnegat Bay; LEH = Little Egg Harbor .................................................................105

Table 3-2. Survival, growth, and condition data for surfclams deployed in 2017. UBB = Upper Barnegat Bay; LEH = Little Egg Harbor .................................................................110

Table SP2-1. Shell dimensions from stocking density experiment. 95% confidence intervals (CI) were measured across replicates, unless only one replicate was available. In that case, it was measured across sampled clams (*). ........................................................................................................160

Table 5-1. Notable and highly significant differentially expressed genes (DEGs) induced by heat stress. HS29 = Heat-Selected clams at 29°C; HS16 = Heat-Selected clams at 16°C; RC29 = Random-Control clams at 29°C; RC16 = Random-Control clams at 16°C. Unless otherwise noted (*), the Transcript ID for each DEG listed in this table contains the prefix “Cluster-17909.”.........................................................220

Table 5-2. All differentially expressed genes (DEGs) between HS29 and RC29. HS29 = Heat-Selected clams at 29°C; RC29 = Random-Control clams at 29°C. The Transcript ID for all DEGs listed in this table contain the prefix “Cluster-17909.” ........................................................................................................228

Table 5-3. All differentially expressed genes (DEGs) between HS16 and RC16. HS16 = Heat-Selected clams at 16°C; RC16 = Random-Control clams at 16°C. Unless otherwise noted (*), the Transcript ID for each DEG listed in this table contains the prefix “Cluster-17909.” .................................................................231

Table 6-1. Species-specific constants. Species-specific constants that relate a bivalve’s dry tissue weight (W, g) to its clearance rate (CR, l h⁻¹) and are used in allometric equations that take the form, CR = aWᵇ. These values were selected or adapted from the listed sources, which experimentally derived allometric clearance equations in controlled laboratory-based studies. ..............................................................................268

Table 6-2. Stocking densities and the estimated initial tank-level clearance capacities used in the particle depletion experiments. In all assemblages, the tank-level clearance capacity was 22 l h⁻¹ .................................................................................................................................269

Table 6-3. Stocking densities and the estimated initial tank-level clearance capacities used in the productivity experiment .................................................................................................270

Table 6-4. Tank-level clearance rates of natural seston particles sized ≤6 µm. Values are mean ± standard error of the mean for three replicate mesocosms. Bivalve species abbreviations refer to Crassostrea virginica (Cv), Spisula solidissima (Ss), Mercenaria mercenaria (Mm), and Mya arenaria (Ma). Assemblages sharing a letter are statistically similar: ≤6 µm (p≤0.006, except Ss-Ma p=0.05 & Mm-Control p=0.01); 5-6 (p≤0.004, except Ss-Mm & Ss-Ma p<0.04); 4-5 (p≤0.002, except Ss-Mm & Ss-Ma p=0.04); 3-4 (p≤0.008, except Ss-Ma p<0.04); 2-3 (p≤0.007, except Ss-Mm p<0.02, n.b. Ss-Ma p=0.09); ≤2 (p≤0.004, except All-Cv p<0.02, n.b. Ss-Ma & Mm-Control p=0.07). ........................................................................................................284

Table 6-5. Output of productivity experiment linear regressions. Results of linear regressions of various productivity metrics on bivalve assemblage species richness. Bold represent statistically
Table 6-6. Species-specific productivity across bivalve assemblages. Values are mean ± standard error of the mean for three replicate mesocosms. Focal species within assemblages are bolded. Bivalve species abbreviations refer to *Crassostrea virginica* (Cv), *Spisula solidissima* (Ss), *Mercenaria mercenaria* (Mm), and *Mya arenaria* (Ma).

Table 6-S1. Morphometric data of bivalves used in the particle clearance experiments. These values represent mean ± standard deviation and were used to calculate tank-level clearance capacity of each mesocosm. Shell length represents the length of the anterior–posterior axis on the clams and the dorsal–ventral axis on the oysters. Shell height represents the dorsal–ventral axis on the clams and the anterior–posterior axis of the oysters. Shell width represents the dextral–sinistral axis of all bivalves. Whole wet weight is defined as the weight of the whole living organism with its shells and any external tissue patted dry. Dry tissue weight was determined after bivalve soft tissue was placed in a 68 °C drying oven for 48 h, cooled, and weighed.

Table 6-S2. Initial morphometric data of bivalves used in the productivity experiment. These values represent mean ± standard deviation and were used to calculate the initial tank-level clearance capacity of each mesocosm. Shell length represents the length of the anterior–posterior axis on the clams and the dorsal–ventral axis on the oysters. Shell height represents the dorsal–ventral axis on the clams and the anterior–posterior axis of the oysters. Shell width represents the dextral–sinistral axis of all bivalves. Whole wet weight is defined as the weight of the whole living organism with its shells and any external tissue patted dry. Dry tissue weight was determined after bivalve soft tissue was placed in a 68 °C drying oven for 48 h, cooled, and weighed.
List of Figures

Figure 2-1. Rearing temperature experimental design. For each temperature treatment (18°C, 20°C, 23°C, 24°C, 26°C), three independent closed recirculating aquaculture systems (RAS) were established. Each RAS contained three rearing silos, which were populated with clams from a single, independently-spawned cohort. When downwelling, each RAS held approximately 75 L and had a flowrate of 4.10 ± 0.28 L min⁻¹. When upwelling, each RAS held approximately 85 L and had a flow rate of 12.28 ± 0.84 L min⁻¹. Rearing silos were composed of PVC pipe and Nitex screen. The mesh size of the downwelling silos was 236 µm; the mesh sizes of upwelling silos were 500 µm and 750 µm. .........................................................................................................................28

Figure 2-2. The effect of temperature on early juvenile surfclam survival. Survival curves over the duration of the experiment. Points represent mean survival ± SE (N=3 replicate cohorts). Exponential decay functions were fit to the curves using nonlinear weighted least-squares estimation of the model parameters. Two-sample Kolmogorov-Smirnov tests were used to determine goodness-of-fit, where P-value >0.05 indicates a good fit (18°C, D=0.18, P=0.65; 20°C, D=0.18, P=0.65; 23°C, D=0.15, P=0.84; 24°C, D=0.09, P>0.99; 26°C, D=0.09, P>0.99). Models take the form %S=a*exp(-b*t), where %s is percent survival, and t is the time in days...34

Figure 2-3. Final (Day 32) survival percentage (A) and shell length (B). Bars nested under ‘Cohort Average’ represent the mean ± SE (N=3 replicate cohorts) and are equivalent to Day 32 values in Figures 2 and 4 for 3A and 3B, respectively. Bars nested under Cohorts A, B, and C represent the mean ± SE (3A, N=3 silos per cohort per treatment; 3B, N=60 clams per cohort per treatment). Neither final survival nor final shell length significantly differed across rearing temperatures (3A, Day 32 survival, ANOVA, F=0.78, P=0.56; 3B, Day 32 growth, ANOVA, F_{4,10}=1.9, P=0.19)....35

Figure 2-4. The effect of temperature on early juvenile surfclam growth. Growth curves over the duration of the experiment. Points represent mean shell length ± SE (N=3 replicate cohorts). Linear regressions were conducted to fit lines of best fit to each curve (18°C, r²=0.82, P<0.001; 20°C, r²=0.89, P<0.001; 23°C, r²=0.86, P<0.001; 24°C, r²=0.91, P<0.001; 26°C, r²=0.92, P<0.001). Models take the form SL=m*t+b, where SL is shell length in mm, and t is the time in days. .................................................................38

Figure 2-5. Survival and growth of juvenile surfclams reared with ambient, flow-through seawater. Circles represent daily mean temperature ± standard deviation of the seawater pumped from the Cape May Canal and used for rearing the surfclams. Triangles represent the median bootstrapped shell length ± 95% confidence intervals of the surfclam population. Squares represent the percentage of surviving surfclams. ............................................................................................................41

Figure 3-1. Bell-siphon raceway system diagram. (A) A bucket encloses a drainage pipe within a raceway. (B) Water flows into the raceway, filling it up. (C) When the water level rises to the level of the drainage pipe (i.e., the standpipe) within the bucket, a siphon is created. (D) The water drains from the system faster than it enters. (E) When the water level reaches the bottom of the bucket, the siphon breaks. (F) The system is automatically reset, and the raceway once again begins to fill (adapted from Gould, 2013). .................................................................................................................64

Figure 3-2. Temperature and salinity timeseries for the early nursery experiments. Points represent mean daily temperatures (°C, circles) and salinities (ppt, diamonds). Error bars represent standard deviation. Black points are for unfiltered seawater from the Cape May Canal and grey points indicate treated seawater (recirculated through a UV system and filtered to 1-µm). ..........................65

Figure 3-3. Early nursery experiment: shell length – shell height correlation. Points represent all shell length and shell height data collected during the early nursery experiment. Linear regression was conducted to generate a model that takes the form, SH = m*SL + b, where SH is shell height, m is slope, SL is shell length, and b is the y-intercept. Blue diamonds represent clams reared in the bell
siphon/bell siphon systems, purple squares represent clams reared in bell siphon/upweller systems, and red circles represent clams reared in downweller/upweller systems. ..............................................70

Figure 3-4. Late nursery experiment: shell length – shell height correlation. Points represent all shell length and shell height data collected during the late nursery experiment. Linear regression was conducted to generate a model takes the form, \( SH = m*SL + b \), where \( SH \) is shell height, \( m \) is slope, \( SL \) is shell length, and \( b \) is the Y-intercept. Green triangles represent clams reared in upwellers, orange squares represent clams reared in raceways with sand, and blue circles represent clams reared in raceways without sand. .................................................................71

Figure 3-5. Map of deployment sites used for the grow-out experiments. Points represent the locations of the four commercial shellfish farms where surfclams were deployed in during the 2016 and 2017 grow-out experiments. All sites are located in coastal bays of New Jersey, USA. Maps courtesy of NOAA National Centers for Environmental Information (NCEI). ..............................................72

Figure 3-6. Gear types used in the grow-out experiments. (A & B) 5-mm nylon mesh bags used at LEH (A) and UBB (B). (C & D) box bags in a bottom cage at LEH. The bags were composed of 5-mm HDPE mesh, and the cage was composed of vinyl-coated wire. (E) An anti-predator screen at an intertidal clam farm, similar to the piloted gear used in this study. Photo by Patrick and Barbara Woodbury. (F) Recruitment boxes at an intertidal clam farm, similar to the piloted gear used in this study. Photo by Dr. Brian Beal, Downeast Institute. ..............................................................73

Figure 3-7. Grow-out experiments: correlations of shell dimensions. Points represent all shell length, height, and width data collected during the grow-out experiments. Linear regressions were conducted to generate the models that take the form, \( y = m*x + b \), where \( m \) is slope \( b \) is the y-intercept, and \( x \) and \( y \) are the shell dimensions. 2016 deployment (left panel): red diamonds (LEH, October 2016), orange diamonds (LEH, December 2016), blue circles (UBB), purple squares (AB). 2017 deployment (right panel): red diamonds (LEH), blue circles (UBB), black squares (RC). .................................................................75

Figure 3-8. Early nursery experiment: survival and growth. Points represent mean percent survival (top panel) and shell length (bottom panel) of three replicate rearing silos for each treatment. Error bars represent 95% confidence intervals. Blue diamonds represent clams reared in the bell siphon/bell siphon systems (BSBS), purple squares represent clams reared in bell siphon/upweller systems (BSUW), and red circles represent clams reared in downweller/upweller systems (DWUW). Points are jittered for clarity. ..................................................................................................................85

Figure 3-9. Late nursery experiment: survival. Points represent mean percent survival of three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. Green triangles represent clams reared in upwellers (UW), orange squares represent clams reared in sand-filled raceways (SR), and blue circles represent clams reared in raceways with no sand (NS). .................................................................88

Figure 3-10. Late nursery experiment: growth. Points (top panel) and bars (bottom panel) represent mean shell length of three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. (Top) Growth (shell length) over the duration of the experiment: green triangles represent clams reared in upwellers (UW), orange squares represent clams reared in sand-filled raceways (SR), and blue circles represent clams reared in raceways with no sand (NS). (Bottom) Final shell length: bars sharing a letter are not significantly different from one another. .............................................................89

Figure 3-11. Late nursery experiment: sediment accumulation. Bars represent mean height of material that accumulated on the clams between cleanings, calculated across the three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. Letters above the bars indicate that all treatments were significantly different from one another (\( p < 0.001 \)) ........................................................................90
Figure 3-12. Sediment grain size of three surfclam grow-out sites as of fall 2016. Distribution of sediment grain size classes in bottom sediment at deployment farms UBB = Upper Barnegat Bay, LEH = Little Egg Harbor, AB = Absecon Bay. .................................................................92

Figure 3-13. Survival of clams deployed in fall 2016. Bars show mean percent survival of replicate mesh bags for a given grow-out site. Error bars represent 95% confidence intervals calculated across replicate bags. Error bars equal zero on initial deployment; other bars without error bars represent sampling events when only a single replicate bag was sampled. .................................................................95

Figure 3-14. Surfclam growth rates across farms, seasons, and deployments. Mean daily shell length growth rate for a given grow-out site and gear type. Error bars represent 95% confidence intervals calculated across replicate bags. Points without error bars represent sampling events when only a single replicate unit was examined. Points are jittered for clarity. .................................................................96

Figure 3-15. Shell length growth of clams deployed in fall 2016. Mean shell length of replicate mesh bags for a given grow-out site. Error bars represent 95% confidence intervals calculated across replicate bags. Bars without error bars represent sampling events when only a single replicate bag was examined. .................................................................97

Figure 3-16. Condition of clams deployed in fall 2016. Mean condition of 20 clams collected from each site and gear type on a given sampling event. Error bars represent 95% confidence intervals calculated across clams. .................................................................98

Figure 3-17. Sex ratio and gonad development of clams across farms, seasons, and deployments. UBB = Upper Barnegat Bay, LEH = Little Egg Harbor, AB = Absecon Bay, RC = Rose Cove. .................................................................99

Figure 3-18. Growth models for clams deployed in fall 2016. Shell length data collected on a given sampling event at: (Top left) Absecon Bay (AB), mesh bags only; (Top right) Upper Barnegat Bay (UBB); (Bottom left) Little Egg Harbor (LEH), October 2016 deployment; (Bottom right) Little Egg Harbor (LEH), December 2016 deployment. Black points represent the shell length data of dead clams measured throughout the duration of the experiment; each dead clam’s shell length has been plotted on its predicted death date, given by the best-fit linear or polynomial growth models. X = day of last sampling for Absecon Bay and Upper Barnegat Bay when no live clams were recovered. ........................................................................................................104

Figure 3-19. Environmental data at three grow-out sites. Temperature, salinity, and dissolve oxygen (DO) timeseries at Upper Barnegat Bay (top left), Little Egg Harbor, (middle left), and Absecon Bay (bottom left). Chlorophyll and turbidity timeseries at Upper Barnegat Bay (top right) and Little Egg Harbor (middle right). Left panels: mean daily temperature (˚C, black circles), salinity (purple diamonds), and DO (mL/L). Right panels: mean daily chlorophyll a (µg/L, green circles) and turbidity (NTU, brown diamonds). Error bars represent 95% confidence intervals for all panels. ........................................................................................................105

Figure 3-20. Environmental data at three grow-out sites. Temperature, salinity, and dissolve oxygen (DO) timeseries at Upper Barnegat Bay (top left), Little Egg Harbor, (middle left), and Absecon Bay (bottom left). Chlorophyll and turbidity timeseries at Upper Barnegat Bay (top right) and Little Egg Harbor (middle right). Left panels: mean daily temperature (˚C, black circles), salinity (purple diamonds), and DO (mL/L). Right panels: mean daily chlorophyll a (µg/L, green circles) and turbidity (NTU, brown diamonds). Error bars represent 95% confidence intervals for all panels. ........................................................................................................105

Figure SP1-1. Map of surfclam nursery locations. Points represent the locations of the land-based upwelling systems used to rear juvenile surfclams in this study. All sites are located near coastal bays of New Jersey, USA. Maps courtesy of NOAA National Centers for Environmental Information (NCEI). .................................................................115

Figure SP1-2. Surfclam growth at various NJ nursery locations. Mean final shell length after four months of rearing in upwellers (silos with 600-1000 µm mesh). Error bars represent standard error of all clams measured (N=50 per silo, one silo per site/maintenance level). High maintenance refers to a cleaning frequency of at least once per week for the duration of the study. ........................................................................................................117

Figure SP3-1. Surfclam shelf-life. Point represents the mean survival of all clams in a given treatment on day 8 for each of the four trials. The box and whisker plots represent the interquartile length of each treatment with the horizontal bar in each representing its mean. ........................................................................................................118
Figure SP4-1. Market potential survey for farm-raised surfclams. This survey form was developed and reviewed by Rutgers IRB and used in side-by-side taste tests of farmed hard clams (*Mercenaria mercenaria*, 227) and farmed Atlantic surfclams (*Spisula solidissima*, 421)............167

Figure 4-1. Schematic of experimental design. In Experiment I, selection pressure was applied when the clams were early juveniles, two to six weeks post-metamorphosis (Acquafredda et al., 2019). In Experiment II, selection pressure was applied when the clams were 21-month old adults. For the heat tolerance assessments, circles represent replicate buckets, which shared a common water bath (heat challenge) or shared a temperature-controlled room (control conditions). In the Experiment I heat tolerance assessment, each bucket initially contained six adult HS-16 (black circles) or NS-16 (grey circles) clams, ~2.5 years old. In the Experiment II heat tolerance assessment, each bucket initially contained 100 HSF1-19 (black circles) or NSF1-19 (grey circles) juvenile clams, ~5 months old. Abbreviations for Atlantic surfclam treatments are as follows: NS-16 = non-selected clams spawned in 2016; HS-16 = heat-selected clams, spawned in 2016, selected in 2016 as juveniles; NS-17 = non-selected clams, spawned in 2017; HS-17 = heat-selected clams, spawned in 2017, selected in 2018 as adults; HSF1-19 = F1 progeny of the HS-17 broodstock, spawned in 2019; NSF1-19 = F1 progeny of the NS-17 broodstock, spawned in 2019. ..................................................181

Figure 4-2. Survival of heat-selected and non-selected surfclams at control temperatures (A) and under severe heat stress (B). Points represent the survival of replicate buckets of heat-selected surfclams (HS-16, black squares, N = 4) and non-selected surfclams (NS-16, grey circles, N = 7). Each bucket initially contained six adult clams. (A) Control conditions: percent survival (%S) was 100% for both groups for the entirety of the experiment. (B) Heat challenge: lines of best fit were generated using generalized linear mixed models. Models take the form %S=(1/(1+exp(b0+b1*t)))*100, where %S is percent survival, t is the time in days, and b0 and b1 represent the model intercept and slope, respectively. .................................184

Figure 4-3. Growth (A, B) and survival (C) of heat-selected and non-selected surfclam progeny. NSF1-19 clams are F1 progeny spawned from non-selected surfclam broodstock (gray circles/bars). HSF1-19 clams are F1 progeny spawned from heat-selected surfclam broodstock (black squares/bars). Each progeny group was produced from a single spawning event. Points represent the mean shell length and error bars represent 95% confidence intervals. (A) Larval phase growth, N = 25 clams each day. (B) Nursery phase growth, N = 50 clams each day, except day 37 (N = 60) and day 142 (N = 300). (C) Bars show the survival of the two groups through development (N = 1 cohort per progeny group). ..................................................................................187

Figure 4-4. Survival of heat-selected and non-selected surfclam progeny at control temperatures (A) and under severe heat stress (B). NSF1-19 clams were spawned from non-selected surfclams (gray circles). HSF1-19 clams were spawned from heat-selected surfclams (black squares). Points represent the survival of replicate buckets (N = 3 per progeny group), with each bucket initially containing 100 juvenile clams. (A) Control conditions: linear regressions were used to determine lines of best fit for each curve. Models take the form %S=m*t+b, where %S is percent survival, t is the time in days, and b and m represent the model intercept and slope, respectively.  (B) Heat challenge: lines of best fit were generated using generalized linear mixed models. Models take the form %S=(1/(1+exp(b0+b1*t)))*100, where %S is percent survival, t is the time in days, and b0 and b1 represent the model intercept and slope, respectively..................................................189

Figure 5-1. FPKM cluster analysis of differentially expression genes. Clustered using the log10(FPKM+1) values. Red denotes genes with high expression levels, and blue denotes genes with low expression levels. ..................................................................................233

Figure 5-2. DEGs across group and temperature. (Top) Within-group/ across-temperature gene expression. The Venn diagram denotes the number of DEGs identified in each comparison, with the overlapping region showing the number of DEGs shared between the comparisons. (Bottom)
Figure 5-3. KEGG pathway enrichment for the within-group/across-temperature comparisons. Enrichment factor is the ratio of differentially expressed genes to the background frequency of genes associated with a given pathway. Plotted points convey the comparisons (point shape), the number of DEGs (point size), and the adjusted p value (point shade). ........................................237

Figure 6-0. Conceptual figure of particle clearance in bivalve polyculture. This conceptual figure shows how pre-capture niche complementarity may occur in four species of bivalves, each represented by the colored curves. All four species capture particles greater than 6 µm with 100% efficiency. However, as particle size decreases, interspecific variation in capture efficiency becomes apparent. More small particles (≤ 6 µm) would be captured in polyculture than any individual monoculture. ..........................................................263

Figure 6-1. Mesocosm assembly. Conceptual figure showing how a monoculture, biculture, triculture, and four-species polyculture would be assembled in these experiments. Oysters are represented in mesh bags in the tanks. The left and center panels depict the side and top views of the mesocosms, respectively. The right panel shows the bivalves used in this study, as well as the dimensions (centimeters) of each mesocosm. Black and gray lines outline the volume of the container and sediment layer, respectively. ..............................................................265

Figure 6-2. The effect of species richness on tank-level clearance of cultured microalgae. (A) Particle clearance rates of bivalve assemblage when solely supplied with P. lutheri; (B) Particle clearance rates of bivalve assemblage when supplied a mixture of P. lutheri (dark triangles) and N. oculata (light triangles). Points represent mean values and error bars represent SEM. For each curve, N=12 replicate monoculture mesocosms, N=18 biculture mesocosms, N=12 triculture mesocosms, and N=3 four-species polyculture mesocosms. ..........................................................278

Figure 6-3. Tank-level clearance rates of cultured microalgae. (A) Particle clearance rates of bivalve assemblage when solely supplied P. lutheri; (B) Particle clearance rates of bivalve assemblage when supplied a mixture of P. lutheri (dark) and N. oculata (light). Bars represent mean clearance rates of three replicate mesocosms and error bars represent standard error of the mean. Bivalve species abbreviations refer to Crassostrea virginica (Cv), Spisula solidissima (Ss), Mercenaria mercenaria (Mm), and Mya arenaria (Ma). ..........................................................279

Figure 6-4. Clearance of P. lutheri plotted against clearance of N. oculata for each bivalve assemblage. Points represent mean clearance rates of three replicate mesocosms and error bars represent standard error of the mean. The diagonal line denotes the one-to-one line of equal clearance. Bivalve species abbreviations refer to Crassostrea virginica (Cv), Spisula solidissima (Ss), Mercenaria mercenaria (Mm), and Mya arenaria (Ma). ..........................................................281

Figure 6-5. Tank-level clearance rates of natural seston particles. (A) Clearance rates for particles sized ≤6 µm; (B) Clearance rates for particles sized ≤25 µm; (C) Clearance rates for particles sized >25 µm. Bars represent mean clearance rate of three replicate mesocosms and error bars represent standard error of the mean. Bars sharing a letter are statistically similar (p ≤ 0.05). Bivalve species abbreviations refer to Crassostrea virginica (Cv), Spisula solidissima (Ss), Mercenaria mercenaria (Mm), and Mya arenaria (Ma). ..........................................................285

Figure 6-6. The effect of species richness on bivalve productivity. The effect of species richness on the average daily growth rates of bivalve (A) shell length, (B) shell height, (C) shell width, and (D) whole wet weight. Points represent mean values and error bars represent standard error of the mean. Squares represent C. virginica; Down-pointing triangles represent S. solidissima; Diamonds represent M. mercenaria; Up-pointing triangles represent M. arenaria. For each focal species, N=3 for monocultures, N=9 for bicultures, N=9 for tricultures, and N=3 for four species polyculture. ..........................................................290
Figure 6-S1. Temperature of the productivity experiment. All temperature (°C) observations collected are plotted. Data were collected at 10-minute intervals using Sea-Bird Scientific SBE 56 temperature loggers.

Figure 6-S2. Salinity of the productivity experiment. All salinity (ppt) observations collected are plotted. Most data were collected at 15-minute intervals using a YSI 6600 V2-4 multi-parameter water quality sonde. Data from a NOAA buoy (Station CMAN – 8536110) were used to fill a gap in the dataset from August 29 to September 21. These data were collected every six minutes. The buoy is located less than a mile from the NJ Aquaculture Innovation Center and data collected by the buoy is highly consistent with data collected from the laboratory’s flow-through seawater system.
Chapter 1: Ecologically-sound diversification in aquaculture

1.0 Introduction

Aquaculture, or the rearing of aquatic organisms, provides food and economic opportunity to millions of people worldwide. According to the Food and Agriculture Organization [FAO] of the United Nations (2020), aquaculture production in 2018 reached an all-time high of 114.5 million tons, an increase of 536% from 1990. By contrast, wild capture fisheries have largely plateaued over the last 30 years, with annual production ranging from 84 to 96 million tons.

As the demand for seafood continues to grow, the sea has the potential to produce a significantly larger fraction of sustainable food than it currently does; as much as 25% of all the protein necessary to feed 9.8 billion people in 2050 could be sustainably sourced from the sea (Costello et al., 2020). Increased sustainability in aquaculture will require policy reforms, shifting dietary demand, and advances in technology (Costello et al., 2020). Species diversification, a facet of any sustainable food system, will also play an important role because it builds resilience against environmental and economic stressors (Cochrane et al., 2009; Khoury et al., 2014; Altieri et al., 2015). Not only must the existing set of domesticated species and their culture systems become adapted to global environmental change, but the number of farmed species overall must also increase (De Silva et al., 2009; Boyd et al., 2020). Culturing more species provides additional adaption possibilities under various climate change scenarios, in addition to unexpected diseases and market issues (Duarte et al., 2007; Cochrane et al., 2009).

About 97% of all currently cultured aquatic species have been domesticated since the start of the 20th century, and the number continues to rise rapidly (Duarte et al.,
2007). To select a candidate species for aquaculture diversification, numerous biological and economic criteria are typically used (Webber & Riordan, 1976). The species should exhibit a rapid growth rate and high survival when reared in high-density culture systems. Instead of relying on collections from wild populations, the species should be easy to propagate in controlled conditions so there is a consistent supply of juvenile stock or seed available. Tolerating a broad range of environmental conditions is another advantage because it allows production to occur at numerous sites using a variety of gear types and through periods of stress. In short, the species should exhibit traits that make it conducive to domestication.

When considering species for food production, the species must also be appealing to the consumer, in taste, texture, and appearance (Webber & Riordan, 1976). Understanding these organoleptic qualities of any species is crucial for marketing and public acceptance. Additionally, whether at home, in restaurants, or at industrial-scale facilities, processing and cooking the species should not be complex. The final consideration for any aquaculture candidate species is whether it can be produced in sufficient quantities at low enough cost to make its farming profitable. Mann (1984) remarked that the factors for choosing a candidate aquaculture species have largely remained unchanged since the first documented selection criteria were developed by Fan Lee in his treatise describing common carp (*Cyprinus carpio*) pond aquaculture in China, circa 500 BCE. However, since aquaculture production has industrialized and exists in the modern context of global environment change, aquaculture operations must consider other factors beyond simply whether a species is “tasty, hardy, non-cannibalistic, inexpensive to culture, and fast growing” as Fan Lee stated 2500 years ago (Mann, 1984).
1.1 Defining ecologically-sound diversification (ESD)

I propose expanding the set of criteria used when considering aquaculture diversification to include the ecological impacts of rearing new culture candidates and the vulnerabilities of new and existing species to climate change and other global environmental shifts. In the context of aquaculture, I define ecologically-sound diversification (ESD) as the practice of cultivating multiple species in a manner that minimizes negative impacts on the surrounding ecosystem while remaining adaptive and resilient to environmental change. ESD must be ecologically sustainable, meaning it does not jeopardize the diversity, adaptability, resilience, and productivity of an ecosystem over time (Chapin et al., 2009).

ESD can be applied broadly, at a regional or national level, or within the context of a single farm. ESD can also be implemented spatially, temporally, and genetically. In the spatial sense, ESD can occur through aquatic polyculture, or multispecies aquaculture, which is the farming of two or more aquatic species that share space or resources. Integrated multi-trophic aquaculture (IMTA), a special case of aquatic polyculture, is the practice of cultivating organisms of different trophic levels in close proximity to one another; IMTA has received particular attention for its ability to reduce negative environmental impacts (Chopin et al., 2012). For instance, unfed crops like seaweed (e.g., Alaria esculenta) and mussels (e.g., Mytilus edulis) can mitigate the waste produced by a nearby fed species, like salmon (e.g., Salmo salar), by extracting the excess nutrients and particulates that the fed species produces (Chopin et al., 2012). In the temporal sense, ESD can occur through crop rotation, where the rearing of each species is alternated or varies seasonally to optimize each species’ growing conditions.
For example, some aquaculturists in Maine, USA, grow oysters (*Crassostrea virginica*) as a summer crop and sugar kelp (*Saccharina latissimi*) as a winter crop (Walsh, 2020). ESD also extends to genetic diversity, since it is key to keeping farmed species adaptive and resilient. Breeding programs have proven successful for many cultured species, although too little is known about the capacity of farmed organisms to cope with future environmental change (Reid et al., 2019b).

To fully achieve ESD, the production of a particular diversified farm must not exceed the carrying capacity of any constituent species, and nor should the collective production of a diversified farm exceed the ecological carrying capacity of the surrounding ecosystem (Dame & Prins, 1997; Byron et al., 2011). Moreover, farms that implement ESD should aim to be adaptive to changes in ecological carrying capacity that may result from climate change, invasive species introductions, biodiversity loss, or other environmental perturbations (Byron et al., 2011; Comeau et al., 2015; Guyondet et al., 2015). ESD is also generally not conducive with the introduction or use of non-native species, since they can have adverse ecological and genetic impacts on native fauna (De Silva et al., 2009). However, there are caveats to the use of non-native species for ESD, particular for biosecure land-based recirculating aquaculture operations that minimize the risk of non-native species escaping (Tal et al., 2009). For aquaculture that occurs in the natural environment, thoughtful consideration should be given to the facilitated introduction of species whose ranges are highly likely to shift to a new area due to climate change (Lunt et al., 2013; Bricknell et al., 2020).

ESD must also be economically viable if growers are to put this philosophy into practice. Ideally, the production of each species should be equal to or greater than
monoculture production, or the profit generated from the assemblage should be greater than that of a monoculture. If cultivating a diverse farm is less profitable than monoculture, then the lost profit must be less than the costs associated with the cumulative monoculture risk. Diversification is a form of insurance, so in this sense, the difference between lost profit and anticipated costs is the premium. Finally, economic sustainability also means that the environmental usage and resource extraction necessary to produce each species in the present must not compromise the production of the species in the future (World Commission on Environment and Development, 1987).
1.2 Bivalve aquaculture: an ideal starting point for ESD

Bivalve aquaculture is an ideal starting point for ESD, because it is widespread, economically important, and generally ecologically sustainable. At 17.3 million tons, bivalve aquaculture already composes the majority (56.3%) of all marine and coastal aquaculture production (FAO, 2020). Consequently, farming bivalve molluscs contributes nearly 34.6 billion USD or 13.1% of the global first-sale (farm-gate) value of cultivated aquatic species (FAO, 2020). In the United States, a mid-sized aquaculture producing country, more than 21,000 metric tons of farm-raised clam, oyster, and mussel meat were harvested in 2016, valued at over US $340 million (National Marine Fisheries Service [NMFS], 2018). Bivalves also contribute substantially to food security because large quantities can be produced at relatively low cost, thus generating low-cost and accessible food (Costello et al., 2020). Depending on future demand scenarios and policy decisions, bivalve mariculture may contribute as much as 43% of all sustainable seafood production by 2050 (Costello et al., 2020).

All forms of food production have some environmental impacts, but bivalve aquaculture is among the least environmentally damaging. Compared to nearly all other forms of animal protein production, bivalve aquaculture uses less energy and produces far fewer greenhouse gas emissions (Hilborn et al., 2018). Unlike the vast majority of finfish aquaculture, no feed inputs are required to farm bivalves (Costello et al., 2020); instead, farmed bivalves graze on naturally occurring food, primarily phytoplankton. As such, bivalve aquaculture is the only form of animal protein production that can mitigate eutrophication in estuarine and marine ecosystems, instead of contributing to it (Rose et al., 2014; Hilborn et al., 2018).
In this respect and others, bivalve aquaculture benefits the surrounding ecosystem. Bivalve farms suspended in the water column operate as breakwaters, attenuating wave action and protecting coastal habitats from storm surge (Zhu et al., 2020). Likewise, intertidal or nearshore shallow subtidal aquaculture operations can act as living shorelines, defending against coastal flooding and erosion through wave attenuation (Hossain et al., 2013). In addition to protecting habitats, bivalve farms actually become habitats. A bevy of research has shown that shellfish aquaculture gear, especially those used to rear oysters and mussels, can act as artificial reefs by increasing the structural complexity of an area and providing greater habitat value than unstructured areas (Dumbauld et al., 2009; Suplicy et al., 2018). The presence of oyster aquaculture gear recruits an equivalent or higher abundance and diversity of species compared to sandy, non-vegetated bottoms, eelgrass meadows, and cobble/boulder reefs (Dealteris et al., 2004; Dumbauld et al., 2009; Mercaldo-Allen et al 2020). Specifically, the gear provides nursery habitat and foraging opportunities for finfishes, substrate upon which sessile organisms can attach, and protection and provisioning for a plethora of invertebrates (Dealteris et al., 2004; Erbland & Ozbay, 2008; Ozbay et al., 2014; Mercaldo-Allen et al., 2020). Clam aquaculture, which generally uses lower-relief gear types, tends to have a negligible or positive affect on biodiversity (Brown & Thuesen, 2011; Luckenbach et al., 2016). Moreover, the particle clearing capacity of any bivalve species can locally reduce turbidity and cause a greater abundance of submerged aquatic vegetation (Shumway et al., 2003; Luckenbach et al., 2016). Despite these ecological enhancements, bivalve aquaculture can occasionally supply excessive organic content and silt to the sediments immediately around a farm (Nugues et al., 1996). This phenomenon can have negative
impacts, particularly for benthic fauna; however, this is largely dependent on farm management and the hydrodynamics of the site (Nugues et al., 1996).

By improving water quality, providing coastal defenses, and creating habitat for wild species, bivalve aquaculture can achieve conservation objectives, thus serving in the dual roles of sustainable food source and conservation measure (Froelich et al., 2017). For bivalve farming to be considered environmentally sustainable or even a form of conservation, proper farm management and a thorough understanding of the ecological system—inclusive of the aquaculture operation—is required. Furthermore, bivalve aquaculture faces numerous risks that may limit its long-term sustainability, in particular threats related to climate change (Reid et al., 2019a). ESD aims to mitigate these risks and bolster both the ecological and economic benefits of bivalve aquaculture.
1.3 Risky efficiency: bivalve monoculture in the Northeast USA

Throughout much of the world, bivalve aquaculture is most commonly conducted as monoculture. Monocultures can be considered artificial ecosystems dominated by relatively high densities of a single species. Monocultures prioritize efficiency over resilience and jeopardize food security because they are prone to numerous risks (Khoury et al., 2014; Altieri et al., 2015). By contrast, cultivating a diverse suite of species insulates farmers from any individual crop failure, whether it occurs from disease (Felton et al., 2016), predation (Russell, 1989), natural disasters, or fluctuating environmental conditions (Gaudin et al., 2015). The benefits go further when the species in the assemblage are each genetically diverse (Zhu et al., 2000; Reusch et al., 2005). Diversification can also sustain economic viability of farm operations, and even increase profitability, by allowing farmers to more easily navigate market forces as the price of each individual crop fluctuates (Chopin et al., 2012; Isaacs et al., 2016). Although most of the evidence of the risk-mitigating advantages of diversification originates from terrestrial agricultural research, these principles are likely to be applicable to aquaculture.

While aquaculture diversification is rapidly occurring in some places, such as China and Spain, other places lag behind (Cochrane et al., 2009). In the Northeast United States, farmed bivalve production is dominated by just two species, the Eastern oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*), and most farmers grow only one of these. This means that both the regional industry and individual farmers lack resiliency. The most recent illustration of the risks posed by monoculture occurred during 2020, when the SARS-CoV-2 (covid-19) pandemic forced restaurants to close. This caused substantial disruption throughout the region’s industry, since approximately
80% of the region’s farmed shellfish were consumed at restaurants (van Senten et al., 2020). Oyster farmers were at a greater disadvantage than clam farmers, since they relied more heavily on restaurant sales. For example, in Maryland, USA, it was estimated that 100% of water-column cultured oysters were sold to restaurants for the “half-shell market” (Parker et al., 2020).

Climate change poses an immense risk to aquaculture throughout the world as well as in the northeastern United States (Cochrane et al., 2009; Rosa et al., 2012; Bell et al., 2013; Chapman et al., 2020). Modeling efforts conducted for three coastal bays in Maine suggest that specific consequences of climate change (i.e., reduction in phytoplankton biomass, increase in predator biomass, or synergism of ocean acidification and warming) could lead to drastic declines in bivalve carrying capacity, which may decrease or eliminate the potential for bivalve aquaculture in those areas (Chapman et al., 2020). Cultivating species that can adapt to changes like warming and acidification will be necessary. Identifying alternative species with varying nutritional requirements or that utilize different growing seasons may offset challenges posed by a reduction in phytoplankton or environmental variability. Furthermore, breeding species for traits that make them more adaptive to future changes will bolster the resiliency of the region’s industry.
1.4 Expanding aquaculture opportunities through ecologically-sound diversification

A resilient industry has the ability to sustain an expansion. Thus, the goal of this dissertation is to investigate ways to expand aquaculture opportunities in the Northeast United States through ecologically-sound diversification.

Unit 1 (Chapters 2 and 3) of this dissertation describes a series of experiments related to Atlantic surfclam (*Spisula solidissima*) husbandry. The surfclam represents a top candidate for diversification in the Northeast because it is native, grows rapidly, and is somewhat recognizable in the marketplace. Historically, the surfclam was called by many different common names include the sea clam, beach clam, bar clam, hen clam, skimmer clam, and dipper clam (Yancey & Welch, 1968). Farmed surfclams have been marketed as butter clams (*personal observations*). As such, surfclam aquaculture is not entirely novel, and some culture techniques have previously been developed (Goldberg, 1989). The intent of Unit 1 was to create regionally-relevant husbandry information and evaluate whether this species can become an alternative species reared by the region’s shellfish growers. This unit supports both spatial and temporal ESD. In Chapter 2, the effect of rearing temperature on the survival and growth of early juvenile surfclams was investigated through a month-long study at a shellfish nursery where clams were exposed to different temperature treatments. In Chapter 3, a series of controlled experiments was conducted to evaluate the efficacy of various surfclam nursery and grow-out techniques. In the nursery phase experiments, juvenile surfclams were reared in various gear types (downwellers, bell siphon systems, upwellers, and shallow raceways with and without sand). In grow-out phase experiments, surfclam growth, survival, and condition were evaluated at multiple farm location across southern New Jersey.
In Unit 2, I expand upon the findings of the previous chapters, which demonstrate the degree to which surfclams are vulnerable to heat stress. High temperature conditions are particularly problematic on shallow coastal farms and will be exacerbated by rising ocean temperatures caused by climate change. Research presented in this unit takes a proactive approach towards climate-informed aquaculture and supports the genetic aspect of ESD. In Chapter 4, the feasibility of breeding surfclams for greater heat tolerance is explored. Specifically, the response of adult farmed surfclams to heat stress after juvenile exposure was assessed; additionally, the ability for heat tolerance to be passed to subsequent generations was tested. To further understand how a selective pressure could alter the gene expression profile of a surfclam population, a transcriptomics study was conducted and presented in Chapter 5. Here, gene expression profiles of heat-selected and non-selected surfclams were compared during an exposure to ambient or heat shock conditions.

In Unit 3, I discuss a study that sought to evaluate the efficacy of bivalve polyculture in the northeastern United States, with the intent of determining if it is a viable form of shellfish aquaculture diversification. This unit supports spatial ESD. To my knowledge, this is the first study that examined bivalve polyculture in the region using these four focal species: *C. virginica, M. mercenaria, S. solidissima*, and *Mya arenaria*. A series of mesocosm experiments was conducted using all 15 combinations of the focal species. Three particle depletion experiments were conducted to determine if more diverse bivalve assemblages had greater clearance rates than those that were less diverse. Likewise, the effect of species richness on bivalve productivity (growth and survival) was tested and evaluated.
Finally, I conclude my dissertation with a few remarks that summarize the results of these experiments and discuss the path towards greater ecologically-sound diversification.
1.5 Literature Cited


Gaudin, A.C., Tolhurst, T.N., Ker, A.P., Janovicek, K., Tortora, C., Martin, R.C., &


Unit 1 – Supporting aquaculture diversification by expanding understanding of surfclam husbandry

In Unit 1 (Chapters 2 and 3) of this dissertation, I discuss a series of experiments I conducted related to Atlantic surfclam (*Spisula solidissima*) husbandry. The intent of these studies was to create regionally-relevant husbandry information and evaluate whether this species can become a productive alternative species for shellfish growers. This unit supports both spatial and temporal ESD.

In Chapter 2, I ask the following question:

1. How does water temperature affect the growth and survival of early juvenile surfclams during the nursery phase of production?

In Chapter 3, I ask the following questions:

1. How do various nursery gear type (downwellers, bell siphon systems, upwellers, and shallow raceways with and without sand) affect the growth and survival of juvenile surfclams?
2. How do the growth, survival, and condition of surfclams vary across grow-out sites in New Jersey’s coastal bays?
Chapter 2: The effect of rearing temperature on the survival and growth of early juvenile Atlantic surfclams (*Spisula solidissima*)

With the exception of a few minor modifications, this chapter appears in its entirety in *Aquaculture Reports*. The citation for the published article is:


2.0 Abstract

The Atlantic surfclam (*Spisula solidissima*) is not currently cultivated on commercial scales, but it represents a potentially beneficial target species for crop diversification in the Northeast region of the United States. Surfclams are native to the region and rapid growth gives this species the potential to reach marketable sizes within 12 to 18 months.

Since heating and chilling seawater are major operating costs for bivalve seed production, a thorough understanding of how temperature influences juvenile surfclam survival and growth during the nursery phase – the period between metamorphosis and out-planting – must be determined before mainstream production can occur. I assessed early juvenile rearing temperature by conducting a month-long study where post-metamorphic surfclams (initial shell length ≈0.7 mm) were exposed to one of five temperature treatments (18.0, 20.2, 23.2, 24.4, and 26.3°C). Survival was approximately twice as high at 18°C compared to 26°C. Growth was maximized at intermediate temperatures between 20 and 24°C. Additionally, I monitored the survival and growth of juvenile surfclams for 20 weeks during the Northeast’s typical bivalve nursery operating season. Surfclams (initial shell length ≈1.5 mm) were reared at a commercial-scale using flow-
through upwelling systems, supplied with ambient seawater from the Cape May Canal. Surfclam seed survival after the 20-week study was 52%, with most mortality occurring after the temperature peaked around 27°C. The average growth rate over this study was 0.049 mm d⁻¹. These results suggest that juvenile surfclams should be reared in temperatures close to 20°C for the duration of the nursery phase. Colder temperatures increase survival but tend to slow growth. Prolonged exposure to warm temperatures can cause severe mortality, yet juvenile surfclams seem to have the ability to tolerate short-term exposures to unfavorably warm temperatures. Across the Northeast, the ambient seawater should be sufficient for surfclam seed production at commercial scales.
2.1 Introduction

Like other bivalves, the physiology and ecology of the Atlantic surfclam (*Spisula solidissima*) is influenced by the temperature of the surrounding environment (Newell & Branch, 1980). Temperature fundamentally affects enzyme reaction rates, protein folding, and membrane integrity (Wieser, 1973). In turn, temperature influences a bivalve’s ability to build an immune response (Yu et al., 2009), its motility (Savage, 1976), and essential metabolic processes such as respiration rate, filtration rate, and assimilation efficiency (Winter, 1969; Widdows & Bayne, 1971). For the Atlantic surfclam, temperature is a significant factor in the species’ range boundaries (Henderson, 1929; Golikov & Scarlato, 1973; Ropes, 1980), and plays an important role in reproduction (Ropes, 1968; Spruck et al., 1995), survival, and growth (Hurley & Walker, 1997; Jones et al., 1983).

Most studies that have examined temperature effects on surfclams have focused on larvae or adults rather than juveniles. Hurley & Walker (1997) found the optimal rearing temperature for larvae to be 20˚C, with larvae cultured at 15 or 25˚C experiencing little to no growth and ultimately succumbing to mortality. Even short-term exposure (<60 min) to temperatures above 25˚C led to significant mortality for all stages of larval surfclams (Wright, 1983). Studies of adult surfclams suggest that their optimal temperature range is from 16 to 22˚C, with prolonged exposure to 30˚C water thought to be lethal (Munroe et al., 2013; 2016). No previous study has explicitly studied the effect of temperature on juvenile surfclams.

Since heating and chilling seawater are major operating costs associated with bivalve seed production (Helm & Bourne, 2004), a thorough understanding of how
temperature influences juvenile surfclam survival and growth must be determined before mainstream production of this species occurs. The Atlantic surfclam is not currently cultivated on commercial scales, but it represents a potentially beneficial target species for crop diversification in the Northeast region of the United States. Surfclams are native to the Northeast (Merrill & Ropes, 1969), and rapid growth gives this species the potential to reach marketable sizes within 12-18 months (Goldberg, 1980; Krzynowek et al., 1980; Krzynowek & Wiggin, 1982; Davis et al., 1997). In the 1980s and 1990s, many of the hatchery techniques for surfclam aquaculture were developed (Clotteau & Dubé, 1993; Walker & Hurley, 1995; Hurley & Walker, 1996; 1997; Walker et al., 1998), along with some of the methods for the nursery and grow-out phases of production (Goldberg, 1980; 1989; Goldberg & Walker, 1990; Walker & Heffernan, 1990a; 1990b; 1990c; Walker, 2001). Despite early success with this species, research efforts into surfclam aquaculture waned during much of the early 2000s. Remaining gaps in the surfclam husbandry literature have left aquaculture practitioners without a complete understanding of how to best cultivate this species. In particular, the optimal nursery rearing temperature for juvenile surfclams (shell length 0.7 mm – 10.0 mm) to my knowledge had not previously been investigated.

I tested how nursery water temperature affects growth and survival of early juvenile surfclams. The treatment levels chosen for this study range from approximately 18 to 26°C and are biologically relevant because they encompass a subset of temperatures wild surfclams may encounter within their native range (Merrill & Ropes, 1969; Castelao et al., 2010). The treatment levels also have practical application because they are likely temperatures that a bivalve nursery facility in the Northeast region will experience during
the typical spring through fall operating season. Additionally, I present a case study of commercial-scale surfclam nursery culture using upwellers and ambient flow-through seawater from the Cape May Canal in Cape May, New Jersey. Surfclam survival and growth along with the ambient seawater temperature were tracked for 20 weeks until the majority of surfclam seed approached out-planting sizes.
2.2 Materials and methods

2.2.1 Spawning and larval culture

Three independent cohorts (Cohorts A, B and C) were spawned in late May 2016. Cohort A clams were the offspring of solely aquacultured individuals, while Cohort B and C clams had mixed parentage of aquacultured and wild surfclams. The aquacultured parents of all three cohorts were siblings. All parental clams developed ripe gonads prior to arriving at the laboratory. The clams were induced to spawn using thermal manipulation (Loosanoff & Davis, 1963; Jones et al., 1993).

Larvae were reared in treated seawater (recirculated through a UV system and filtered to 1 µm), which was maintained at a temperature and salinity of 20.3 ± 0.3°C and 31.1 ± 0.1, respectively. Larvae were reared in static tanks until they reached the pediveliger stage. At that time, pediveligers were moved to downwelling systems with rearing silos composed of fiberglass cylinders (diameter x height: 60.96 x 60.96 cm) and Nitex mesh screen (130, 180, and 200 µm). Post-metamorphic clams were reared in these downwelling conditions until the start of the experiment. Larvae were fed a mixed diet (1:1:1:2) of the following species of live algae Isochrysis galbana, Tisochrysis lutea, Pavlova lutheri, and Nannochloropsis oculata. The feeding ration was incrementally increased from $1.0 \times 10^4$ cells ml$^{-1}$ to $1.0 \times 10^5$ cells ml$^{-1}$. Larval stocking density was incrementally decreased from approximately 30 fertilized eggs ml$^{-1}$ to 1 larva ml$^{-1}$. Full water changes were conducted three times per week, and survival and growth were likewise monitored thrice weekly.

2.2.2 Nursery rearing temperature experiment
A controlled experiment was conducted to assess how early juvenile surfclam survival and growth respond to temperature. The experiment took place at the New Jersey Aquaculture Innovation Center, Rutgers University in North Cape May, NJ, USA, and was conducted from June through July 2016. The experiment was conducted in independent closed recirculating aquaculture systems (RAS) that could be set-up for downwelling or upwelling flow conditions (Figure 2-1). Rearing silos inside each RAS were composed of PVC cylinders (diameter x height: 15.24 x 20.32 cm) and Nitex mesh screen (235 µm when downwelling; 500 and 750 µm when upwelling).

When downwelling, each RAS held approximately 75 L of treated seawater (settled, sand filtered to 50 µm, and recirculated through a UV system) and had a flowrate of 4.10 ± 0.28 L min⁻¹. Each RAS was supplied a daily diet of 6.0*10⁴ cells mL⁻¹ of Isochrysis galbana and 1.6*10⁵ cells mL⁻¹ of Nannochloropsis oculata (≈ 2.0*10⁴ cells mL⁻¹ equivalent Isochrysis galbana (Helm & Bounre, 2004)). Full water changes were conducted weekly. When upwelling, each RAS contained approximately 85 L of unfiltered seawater from the Cape May Canal and had a flow rate of 12.28 ± 0.84 L min⁻¹. Partial water changes (25% for 500 µm silos and 50% for 750 µm silos) were conducted daily, and full changes were conducted weekly.

To maintain a near constant temperature across the replicates, the sump of each RAS was placed inside a fresh water reservoir that was heated (Marineland Visi-Therm® submersible 100W heater, item ML90443-00), chilled (Aqua Logic Cyclone chiller 1/3 HP, model CY-4), or left at the ambient room temperature (Figure 2-1). The five temperatures tested were (mean ± SE): 18.0 ± 1.2, 20.2 ± 0.6, 23.2 ± 0.6, 24.4 ± 0.8, 26.3 ± 1.0°C. Temperature was continuously logged at 15-minute intervals using iBCod.
submersible temperature loggers (Alpha Mach, model 22L). Data from the \textit{iBCod} loggers were validated with twice-daily temperature spot checks via a handheld YSI (Model 86). The \textit{iBCod} temperature logger failed for the 24°C treatment, so the data for this treatment came solely from the point measurements. Salinity data were collected twice-daily using a handheld YSI (Model 86). Daily salinity maintained across treatments was 32.1 ± 0.2. Each RAS contained a preconditioned biofilter, and ammonia levels never exceeded 0.25 ppm (LaMotte® Nitrogen Test Kits).
Figure 2-1. Rearing temperature experimental design. For each temperature treatment (18°C, 20°C, 23°C, 24°C, 26°C), three independent closed recirculating aquaculture systems (RAS) were established. Each RAS contained three rearing silos, which were populated with clams from a single, independently-spawned cohort. When downwelling, each RAS held approximately 75 L and had a flowrate of 4.10 ± 0.28 L min⁻¹. When upwelling, each RAS held approximately 85 L and had a flow rate of 12.28 ± 0.84 L min⁻¹. Rearing silos were composed of PVC pipe and Nitex screen. The mesh size of the downwelling silos was 236 µm; the mesh sizes of upwelling silos were 500 µm and 750 µm.
Clams used for this experiment were placed into the experimental temperature treatments at approximately two-weeks post-metamorphosis and had a mean shell length of 0.695 mm. The initial stocking density of each silo was $40.26 \pm 1.18$ clams cm$^{-2}$ (Liu et al., 2011). When the clams grew to a mean shell length of 0.965 mm, the clams were moved from downwelling to upwelling flow conditions and moved from 235 to 500 µm mesh screen rearing silos. When the clams grew to a mean shell length of 1.325 mm, the clams were moved into 750 µm mesh screen rearing silos, and the stocking density of each was decreased to $8.97 \pm 0.41$ clams cm$^{-2}$ (Liu et al., 2011).

Survival and growth were monitored for 32 days, with data collection occurring every 2-3 days. Survival was assessed by taking repeated volumetric abundance estimates: three replicate subsamples of known volumes of clams were counted, and the average count was multiplied by the total volume of clams in each rearing silo. Percent survival was calculated by dividing the final number of live clams by the initial number in each rearing silo. Percent survival data were corrected for any clams sacrificed during sampling and for the cull that took place when the clams were moved into 750 µm mesh screen rearing silos. Mean survival of a cohort within a given treatment was calculated as the average percent survival of the three rearing silos ($N=3$ rearing silos per cohort per treatment; Figure 2-1). Survival of a given treatment was calculated as the mean survival of the three cohorts ($N=3$ cohorts per treatment; Figure 2-1).

Growth was assessed by recording shell length (anteroposterior axis). Clams were randomly sampled, placed onto a Sedgewick-Rafter slide, and measured with an ocular micrometer on a VWR compound microscope (100X or 25X). Shell length of each cohort within a given treatment was calculated as the mean shell length from 60 clams (20 clams
from each of three rearing silos, N=60 clams per cohort per treatment; Figure 2-1). Mean shell length for a given treatment was calculated using mean shell lengths of the three cohorts (N=3 cohorts per treatment; Figure 2-1). Average daily and instantaneous growth rates were calculated as:

Equation 1. \[ \text{Gr}_{\text{ave}} = \frac{\text{SL}_{\text{time2}} - \text{SL}_{\text{time1}}}{\Delta \text{time}} \]

Equation 2. \[ \text{Gr}_{\text{inst}} = \log\left(\frac{\text{SL}_{\text{time2}}}{\text{SL}_{\text{time1}}}\right) / \Delta \text{time} \]

where SL are the median shell lengths for each cohort at time 1 and time 2, and \( \Delta \text{time} \) is the time interval in days between time 1 and time 2 (Munroe, 2016). Growth rates of the three cohorts were then averaged to generate an average daily growth rate and an instantaneous growth rate for each temperature treatment.

2.2.3 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater

The survival and growth of a mixed population of 1,752,740 surfclams from Cohorts B and C were monitored from mid-July to early December 2016. Monitoring began approximately one month after metamorphosis. These clams were reared in flow-through upwelling systems supplied with unfiltered ambient seawater from the Cape May Canal. From mid-July through October, temperature and salinity were continuously logged on site at 15-minute intervals using a YSI 6600 V2-4 Multi-Parameter Water Quality Sonde. From early to mid-November, temperature data were collected from the Cape May Canal NOAA buoy (Station CMAN4–853611), approximately 1-mile from the study site. From late November through December, temperature data were again continuously logged on site at 10-minute intervals using a SBE 56 Temperature Logger.
(Seabird-Scientific). All temperature and salinity data were validated with twice-daily temperature spot checks via a handheld YSI (Model 86). The mean salinity over the duration of the study was 28.3, however daily mean values ranged from 17.6 to 32.8.

During this study, clams were handled using standard commercial bivalve nursery practices (Jones et al., 1993; Helm & Bourne, 2004). On sampling events, they were sorted based on size using stainless steel wire sieves. Clams were reared in cylindrical fiberglass rearing silos (diameter x height: 45.72 x 45.72 cm), and incrementally moved into silos with larger mesh screen, from 400 to 600 to 750 to 1000 µm; clams were stocked on each mesh screen size at densities of 126.91 ± 50.32, 106.52 ± 11.36, 95.26 ± 8.74, 24.90 ± 1.27 clams cm⁻², respectively. The upwelling flow rate was maintained between 45 and 55 L min⁻¹. Survival was assessed with repeated volumetric abundance estimates. Growth was assessed by recording shell length. Clams less than 3.0 mm in shell length were measured as previously described. Clams larger than 3.0 mm were measured with a caliper (Mitutoyo Absolute™ Digimatic). An inconsistent number of clams were measured from each size class on each sampling event (N=40-500 clams); to produce an unbiased estimate of the population’s growth over time, a bootstrapped data set was generated from the available shell length data. For each sampling event, the median shell length of the population was calculated by resampling the available data with replacement 1000 times in proportion to the abundance of live clams in each size class. From the bootstrapped data set, 95% confidence intervals were generated. Average daily and instantaneous growth rates were calculated as previously described (Equations 1 and 2).
2.2.4 Statistical analyses

All data were analyzed using R (Version 0.99.484–© 2009-2015 RStudio, Inc.). Normality and homoscedasticity were confirmed using the Shapiro-Wilk Test and Levene's Test, respectively. All measures of dispersion reported in this paper are one standard error of the mean, unless otherwise noted.

Two-sample Kolmogorov-Smirnov tests were used with a Bonferroni adjustment to determine significant differences between the survival curves of surfclams reared at different temperatures. Exponential decay functions were fit to the curves using nonlinear weighted least-squares estimation of the model parameters. Two-sample Kolmogorov-Smirnov tests were also used to compare the estimated model parameters to the underlying data of each curve to determine goodness-of-fit. The final (Day 32) survival of surfclams was analyzed by one-way ANOVA; final percent survival data were square root-transformed prior to this analysis to meet the assumption of normality.

ANCOVA was used to determine significant differences between the growth of surfclams reared at different temperatures. A post-hoc Tukey’s HSD test was performed to determine significant differences between treatment pairs. Lines of best fit were generated using linear regression. The final (Day 32) growth of surfclams was analyzed by one-way ANOVA.
2.3 Results

2.3.1 Effects of rearing temperature on early juvenile surfclam survival

As rearing temperature increased, early juvenile surfclam survival decreased (Figure 2-2). The survival of surfclams reared in the coldest treatment was approximately twice as high as the those reared in the warmest (Figure 2-2, Figure 2-3A). Significant differences were observed between the coldest and two of the warmest survival curves (18 & 26°C: Kolmogorov–Smirnov test, D=0.42, P=0.05; 18 & 23°C: Kolmogorov–Smirnov test, D=0.45, P=0.02). Despite this, no significant differences in survival were observed at the end of the experiment (Day 32 survival: ANOVA, F=0.78, P=0.56; Figure 2-3A). Each cohort exhibited substantial variation in their response to the rearing temperatures; most notably, the survival of all Cohort B clams, regardless of rearing temperature, was greater than the survival of all Cohort C clams (Figure 2-3A). Survival of Cohort A clams was most similar to the three-cohort average (Figure 2-3A).
Figure 2-2. The effect of temperature on early juvenile surfclam survival. Survival curves over the duration of the experiment. Points represent mean survival ± SE (N=3 replicate cohorts). Exponential decay functions were fit to the curves using nonlinear weighted least-squares estimation of the model parameters. Two-sample Kolmogorov-Smirnov tests were used to determine goodness-of-fit, where $P$-value >0.05 indicates a good fit (18°C, $D=0.18$, $P=0.65$; 20°C, $D=0.18$, $P=0.65$; 23°C, $D=0.15$, $P=0.84$; 24°C, $D=0.09$, $P>0.99$; 26°C, $D=0.09$, $P>0.99$). Models take the form $%S=a*\exp(-b*t)$, where $%S$ is percent survival, and $t$ is the time in days.
Figure 2-3. Final (Day 32) survival percentage (A) and shell length (B). Bars nested under ‘Cohort Average’ represent the mean ± SE (N=3 replicate cohorts) and are equivalent to Day 32 values in Figures 2 and 4 for 3A and 3B, respectively. Bars nested under Cohorts A, B, and C represent the mean ± SE (3A, N=3 silos per cohort per treatment; 3B, N=60 clams per cohort per treatment). Neither final survival nor final shell length significantly differed across rearing temperatures (3A, Day 32 survival, ANOVA, $F=0.78$, $P=0.56$; 3B, Day 32 growth, ANOVA, $F_{4,10}=1.9$, $P=0.19$).
2.3.2 Effects of rearing temperature on early juvenile surf clam growth

Over the duration of the experiment, rearing temperature (ANCOVA, $F_{4,155}=2.9$, $P=0.02$), rearing day (ANCOVA, $F_{1,155}=1139.7$, $P<0.001$), and the interaction between rearing temperature and rearing day (ANCOVA, $F_{4,155}=3.5$, $P=0.01$) had significant effects on growth (Figure 2-4). A post-hoc Tukey’s HSD test showed a significant pairwise difference in growth between surfclams reared at 18 and 24˚C ($P=0.008$). Over the duration of the experiment, average growth rates ranged from 0.033 (26˚C) to 0.045 mm d$^{-1}$ (20˚C), and instantaneous growth rates ranged from 0.012 (18 and 26˚C) to 0.015 d$^{-1}$ (20, 23, and 24˚C) (Table 2-1).

The response to temperature appeared to change over the experimental period (Figure 2-4). For the first 14 days of the experiment, clams in the two warmest treatments outperformed those in the three colder treatments (Figure 2-4). Over the next week, clams across all treatments appeared to grow at similar rates. However, over the last 11 days, growth in the three intermediate temperatures began to diverge from those in the extremes (Figure 2-4).

Although surfclams reared in intermediate temperatures (20, 23, 24˚C) rather than extremes (18, 26˚C) achieved greater shell lengths, at the end of the experiment no significant difference was observed (ANOVA, $F_{4,10}=1.9$, $P=0.19$; Figure 2-3B). Again, each cohort exhibited substantial variation in their response to the rearing temperatures. For instance, among Cohort A clams, those reared in the warmest treatment achieved the smallest final shell length. Yet, these poor performing clams still grew to approximately the same size as the best performing individuals from Cohort B, which were reared at 20˚C (Figure 2-3B).
Table 2-1. Growth rates of early juvenile surfclams reared at five different temperatures. Values are mean ± SE (N=3 replicate cohorts). Please see Section 2.2 for calculations of average daily and instantaneous growth rates.

<table>
<thead>
<tr>
<th>Rearing temperature (°C ± SE)</th>
<th>Average daily growth rate (mm d⁻¹ ± SE)</th>
<th>Instantaneous growth rate (d⁻¹ ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.0 ± 1.2</td>
<td>0.034 ± 0.006</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>20.2 ± 0.6</td>
<td>0.045 ± 0.004</td>
<td>0.015 ± 0.001</td>
</tr>
<tr>
<td>23.2 ± 0.6</td>
<td>0.044 ± 0.005</td>
<td>0.015 ± 0.001</td>
</tr>
<tr>
<td>24.4 ± 0.8</td>
<td>0.043 ± 0.004</td>
<td>0.015 ± 0.001</td>
</tr>
<tr>
<td>26.3 ± 1.0</td>
<td>0.033 ± 0.001</td>
<td>0.012 ± 0.001</td>
</tr>
</tbody>
</table>
Figure 2-4. The effect of temperature on early juvenile surfclam growth. Growth curves over the duration of the experiment. Points represent mean shell length ± SE (N=3 replicate cohorts). Linear regressions were conducted to fit lines of best fit to each curve (18°C, $r^2=0.82$, $P<0.001$; 20°C, $r^2=0.89$, $P<0.001$; 23°C, $r^2=0.86$, $P<0.001$; 24°C, $r^2=0.91$, $P<0.001$; 26°C, $r^2=0.92$, $P<0.001$). Models take the form SL=m*t+b, where SL is shell length in mm, and t is the time in days.
2.3.3 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater: survival

Survival was high (>98%) between mid-July and early August, despite an upward trend in daily mean temperature from 22 to nearly 26°C (Figure 2-5). Between early August and early September, temperatures remained above 22°C, with the daily mean temperature reaching a maximum of 27.8°C (Figure 2-5). During this period, survival dropped from 98% to 60% (Figure 2-5). Following this warm period, very little mortality was observed. From early September to early December, survival decreased from 60% to 52% (Figure 2-5).

2.3.4 Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater: growth

Over the duration of the study, from mid-July to early December, the median shell length of the population grew from 1.5 to 8.3 mm (Figure 2-5), the average growth rate was 0.049 mm d\(^{-1}\), and the instantaneous growth rate was 0.012 d\(^{-1}\). From mid-July to mid-August, when daily mean temperatures steadily increased from 22 to the maximum of 27.8°C, the median shell length increased by 89% (Figure 2-5). This trend continued between mid-August and early September, as temperatures stayed above 22°C; over this interval, the median shell length increased by 114% (Figure 2-5). From early September to early October, temperatures remained between 22 and 24°C, but relatively little growth was observed; over this interval, the median shell length increased by 1% (Figure 2-5). From early October to early December, seawater temperatures stayed below 20°C, and
the median shell length increased by approximately 32% (Figure 2-5). The maximum shell length observed at the study’s conclusion was 16.97 mm (data not shown).
Figure 2-5. Survival and growth of juvenile surfclams reared with ambient, flow-through seawater. Circles represent daily mean temperature ± standard deviation of the seawater pumped from the Cape May Canal and used for rearing the surfclams. Triangles represent the median bootstrapped shell length ± 95% confidence intervals of the surfclam population. Squares represent the percentage of surviving surfclams.
2.4 Discussion

2.4.1 Effects of temperature on juvenile surfclam survival and growth

When measured over the entire experimental period, juvenile surfclam survival and growth were significantly affected by temperature. Less than half as many surfclams reared in the warmest temperature survived compared to those in the coldest temperature, while clams reared in intermediate temperatures (20, 23, and 24°C) reached greater shell lengths than those reared in the extreme temperatures (18 and 26°C). Substantial inter-cohort variation likely caused the lack of statistical significance on Day 32, yet the observed trends suggest that a longer exposure would have likely produced more pronounced results.

Long-term monitoring of juvenile surfclams raised with ambient, flow-through seawater reinforced the experimental results. Most of the mortality observed followed a six-week period of sustained temperatures above 22°C, and growth occurred in a wide range of temperatures, ranging from 12 to 26°C. The greatest average daily growth rates coincided with a severe mortality event, suggesting that the rapid increase in median shell length over this interval was caused by the removal of smaller (<5.0 mm), potentially less fit individuals from the population, not a substantial increase in growth from surviving surfclams. It does not appear that temperatures up to 26°C have an immediate lethal effect on juvenile surfclams. Instead, the lack of growth observed from early September to early October may indicate that after sustained exposure to temperatures above their maximum, juvenile surfclams require a recovery period before reestablishing shell growth. Alternatively, the composition, quality, and quantity of the available food during this period may have been suboptimal for growth and subsequently exacerbated the
necessary recovery time following the previous month’s temperature shock. Overall, it appears that temperature effects are modulated by other factors such as food availability, dissolved oxygen concentration, and by a clam’s energy reserves.

The results of this study align well with the existing literature, specifically the observed growth rates. At sites along the coast of Maine, juvenile aquacultured surfclams that experienced temperatures between 19-22°C grew from 3.0 to 8.9 mm from June through September, somewhat faster than the clams observed in this study (Davis et al., 1997). Growth rates observed in this study are most similar to those observed in studies of wild surfclams collected off the coast of New Jersey. Surfclams settling in July at inshore locations reached a maximum shell length of 16.0 mm by December and experienced growth rates between 0.0146 and 0.0635 mm d⁻¹ (Weissberger & Grassle, 2003). A similar study that assessed larval settlement and early juvenile growth rates found that post-metamorphic surfclams with shell lengths <0.360 mm grew between 0.010 and 0.020 mm d⁻¹, while growth rates of individuals >0.360 mm was closer to 0.025-0.050 mm d⁻¹ (Ma et al., 2006). Growth rates presented here for surfclams across all experimentally tested rearing temperatures fall in the range of 0.033-0.045 mm d⁻¹, with the July-December growth rate for surfclams reared in ambient water during the long-term monitoring at the upper end of that range (0.049 mm d⁻¹).

A number of studies have also examined how different wild surfclam populations from various regions throughout their geographic and depth range respond to temperature. Ambrose and colleagues (1980) found a positive correlation between growth and depth, a proxy for temperature, in surfclams from the Mid-Atlantic Bight and suggested that although inshore surfclams may have greater food availability, offshore
clams may experience more stable and preferred temperatures. Surfclams harvested from colder water offshore from the Delmarva peninsula had better body condition than warmer, inshore conspecifics (Kim & Powell, 2004). A comprehensive analysis of adult surfclams collected from areas ranging from southern New England to the Delmarva peninsula, from various depths in each region, found that the clams with the highest condition were found at intermediate depths where the optimum habitat was near 18°C; the worst habitats were those that experienced temperatures above 24°C (Marzec et al., 2010). Again, the data presented here largely correspond to trends observed in wild surfclam populations.

2.4.2 Factors that may modulate temperature effects on surfclams

Although these factors were not explicitly examined in this study, food quantity and quality, and intrinsic metabolic functions like filtration rate, assimilation efficiency, and respiration rate, modulate how a particular bivalve species responds to temperature changes. The ocean quahog (Arctica islandica) is a bivalve whose range overlaps with that of the surfclam (Merrill & Ropes, 1969) and tolerates temperatures between 0 and 16°C (Begum et al., 2009). The ocean quahog increased its filtration rate when warmed from 4 to 12 °C, but showed a negligible difference in its absorbance (assimilation) efficiency (Winter, 1969). However, when it was warmed from 12 to 20 °C, a moderate increase in filtration rate coincided with a dramatic increase in absorbance efficiency; this observed behavior suggests a possible mechanism for offsetting the metabolic losses incurred when this species is exposed to unfavorably warm temperatures (Winter, 1969). For bivalves generally, and surfclams specifically, food assimilation increases linearly
with shell length and temperature up to their thermal maximum (Powell et al., 1992; Munroe et al., 2016), but respiration increases exponentially with biomass (Møhlenberg & Kiørboe, 1981). Thus, in temperatures at or above their thermal maximum, surfclams will essentially starve to death as their respiration rate exceeds their assimilation rate. (Munroe et al., 2013; 2016).

No study has experimentally tested or modelled respiration and assimilation rates of juvenile surfclams below 20 mm, so it is unclear if certain oxygen concentrations or food abundances would be sufficient to offset the losses sustained by small surfclams grown in unfavorably warm temperatures. The data, which utilize natural conditions, suggest that temperatures above 26 °C for 2–4 weeks would not be able to sustain juvenile surfclams, at least at commercial scales. Yet, juvenile surfclams can cope with short-term exposures at or above 26 °C, on the order of days to weeks.

Genetic differences in a surfclam’s ability to filter water, respire, and assimilate food may be an important source of the variation observed in this study. The survival and growth of Cohort A clams, which were the offspring of solely aquacultured individuals, largely mirrored the average response of all three cohorts. The parents of Cohort A clams may have been inadvertently selected to fare well in nursery conditions. A similar instance of “cultivation vigor” has been observed in the Manila clam (Ruditapes philippinarum). At 35 °C, the wild-type Manila clam hit a tipping point, and its oxygen consumption rate consequently declined; however, no such tipping point was observed in two cultivated lines (zebra-striped and white-colored), suggesting that the lethal temperature for the wild-type was below that of the two cultivated lines (Nie et al., 2017). Cohort B and C clams, which were also used in the long-term monitoring, were generated
from mixed parentage of aquacultured and wild clams. Across the various experimental
temperature treatments, Cohort B clams exhibited the shortest final shell lengths, yet
demonstrated the greatest survival. On the other hand, Cohort C exhibited similar growth
to the average of all three cohorts yet suffered severe mortality regardless of rearing
temperature. Taken together, there appears to be some trade-off between growth and
survival with respect to surfclam nursery rearing temperature. Such a trade-off has been
observed in other bivalves (Fitzgerald-Dehoog et al., 2012). Future studies should
examine the degree to which warm water (>26 °C) tolerance is a heritable trait in the
Atlantic surfclam.

2.4.3 Concluding remarks

Recently, the regional aquaculture industry of the Northeast United States has
expressed interest in crop diversification (Bochter et al., 2014). Currently, the Eastern
oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*) are the
dominant species cultivated in the region, with a smaller fraction of farmers growing blue
mussels (*Mytilus edulis*) and scallops (*Placopecten magellanicus* and *Argopecten
irradians*) (United States Department of Agriculture [USDA], 2014). The Atlantic
surfclam (*Spisula solidissima*) is a potentially beneficial target for crop diversification in
this region because it is native, has an early rapid growth rate, and fits into the established
clam farming framework. Commercial-scale surfclam aquaculture production remains in
its infancy, despite the fact that some literature on surfclam husbandry does exist. The
results presented here contribute to that literature by providing information regarding the
temperature conditions necessary for successfully rearing juvenile surfclams (shell length
0.7 mm – 10.0 mm) during the nursery phase of production – the period between metamorphosis and out-planting.

Heating and chilling seawater are among the highest costs associated with operating commercial bivalve hatchery and nursery facilities, so a thorough understanding of optimal rearing temperatures is essential for hatchery managers, farmers, or other aquaculture practitioners (Helm & Bourne, 2004). The results suggest that after metamorphosis, surfclams should be raised in temperatures close to 20°C for the duration of their nursery period to maximize survival and to keep growth rates high. Importantly, juvenile surfclams can tolerate temperatures that deviate significantly from 20°C, yet some survival and growth may be compromised. Currently, the ambient seawater available at many locations across the Northeast United States should be sufficient for surfclam seed production at commercial scales.
2.5 Acknowledgements

Daphne Munroe, Lisa Ragone Calvo, and Michael De Luca served as advisors and collaborators of this study; all were listed as co-authors on the published article. The contributions of Michael Acquafredda include project conceptualization, investigation, acquisition of resources, project administration, data collection, data analyses, manuscript writing, manuscript revising and editing, and preparation of the final manuscript. The contributions of D. Munroe, L. Ragone Calvo, and M. De Luca include acquisition of resources, project supervision, validation, and manuscript revising and editing. D. Munroe was partially supported by the USDA National Institute of Food and Agriculture [Hatch project accession number 1002345] through the New Jersey Agricultural Experiment Station [Hatch project NJ32115].

This research was sponsored by New Jersey Sea Grant with funds from the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under NOAA grant #NA14OAR4170085 and the New Jersey Sea Grant Consortium. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of New Jersey Sea Grant or the U.S. Department of Commerce. Additionally, Sea-Bird Scientific generously supplied some of the temperature monitoring equipment through the 2016 Student Equipment Loan Program.

I am grateful to the staff and technicians of the New Jersey Aquaculture Innovation Center at Rutgers University, especially B. Campbell, D. Jones, J. Kiernan, F. Klie, M. Neumann, and S. Towers, for providing logistical and technical support. I would
also like to thank the anonymous reviewers for providing valuable feedback and helping to improve this article.

Finally, this work was supported by Rutgers University through the Haskin Shellfish Research Laboratory, the Department of Ecology, Evolution, and Natural Resources, and the Graduate Program in Ecology and Evolution.
2.6 Literature Cited


Widdows, J., & Bayne, B. L. (1971). Temperature acclimation of *Mytilus edulis* with


Chapter 3: Evaluating various nursery and grow-out conditions on farmed Atlantic surfclams (*Spisula solidissima*) performance

3.0 Abstract
Throughout much of the Northeast region of the United States, shellfish aquaculture is dominated by only two species: the hard clam (*Mercenaria mercenaria*) and the Eastern oyster (*Crassostrea virginica*). Despite this, local shellfish farmers are eager to diversify and have expressed interest in culturing new species. The Atlantic surfclam (*Spisula solidissima*) represents a potentially beneficial target species for species diversification because it is native and grows rapidly. However, remaining gaps in the surfclam husbandry literature have left aquaculture practitioners without a complete understanding of how to best cultivate this species on commercial scales. To fill this gap, a series of controlled experiments were conducted to evaluate the efficacy of various surfclam nursery and grow-out techniques. In the nursery phase experiments, early (0.4 – 2.7 mm) and late (1.1 – 18.0 mm) juvenile surfclams were reared in various gear types (downwellers, bell siphon systems, upwellers, and shallow raceways with and without sand). In the grow-out phase experiments, surfclam growth, survival, and condition were evaluated at multiple farm location across southern New Jersey. Sediment composition, temperature, salinity, dissolved oxygen, turbidity, and chlorophyll concentration were recorded throughout these experiments in order to provide additional information about how environmental conditions may modulate surfclam performance. Results from the nursery gear experiments indicate that multiple rearing methods can effectively produce surfclams, but flow rate, food availability, and temperature are important factors that can limit a gear’s efficiency. Sand-filled raceways were the exception, proving to be the least effective gear for rearing surfclam seed. In the grow-out experiments, survival varied
considerably across farms and across years. Surfclam mortality events were associated with anoxic conditions, warm temperatures, aerial exposure during a harsh winter, and predation. Generally, growth and condition were consistent across farms, but varied seasonally. Within 9 months, 67-98% of clams reached a market size (31.75 ≤ x < 44.45 mm). This study demonstrates that surfclam aquaculture is feasible in coastal New Jersey bays, and fits well into the Northeast region’s established shellfish farming framework.
3.1 Introduction
Throughout much of the Northeast region of the United States, shellfish aquaculture is
dominated by only two species: the hard clam (*Mercenaria mercenaria*) and the Eastern
oyster (*Crassostrea virginica*). As of April 2020, New Jersey had 54 operations permitted
for shellfish aquaculture (R. Schuster, Bureau of Marine Water Monitoring, *personal
communication*); of these, 17 oyster farms, 18 clam farms, and two farms culturing both
species reportedly sold more than 5.3 million oysters and 6.4 million clams in 2019.

Aquaculture is among the fastest-growing sectors of food production in the world (Food
and Agriculture Organization of the United Nations [FAO], 2020), and an increased
demand for shellfish has prompted the aquaculture industry in the Northeast to develop
an interest in culturing new species (Bochter et al., 2014). The Atlantic surfclam (*Spisula
solidissima*) represents a candidate for species diversification in the region because it is
native, grows rapidly, and is somewhat recognizable to the public. Yet before a species
can be aquacultured at a commercial scale, its husbandry techniques must be thoroughly
studied.

Surfclam aquaculture is not entirely novel. The earliest culture techniques were
developed in the 1950s, when the surfclam was used as a model organism by
developmental biologists interested in studying fertilization and parthenogenesis (Allen,
1953). Later, as the concept of rearing surfclams for food productions became
popularized (Goldberg, 1980; Krzynowek et al., 1980; Krzynowek & Wiggins, 1982),
various aspects of all three production phases–hatchery, nursery, and grow-out–were
investigated. This interest coincided with the late 1970s, when surfclam landings from the
wild fishery in state waters dramatically declined (Goldberg, 1989). However, once a
federal fisheries management plan was implemented in 1977, the first among fishery
management plans nationally, surfclam stocks in federal waters came available to the fishery allowing harvest to expand beyond state waters; by the 1990s, the federal surfclam fishery was well-developed and producing sustainable harvests (Mid Atlantic Fishery Management Council, 1998). Coincidentally, studies focused on surfclam aquaculture began to wane, leaving gaps in the husbandry literature.

The hatchery phase is perhaps the best studied phase of surfclam production (Wright, 1983; Clotteau & Dubé, 1993; Walker & Hurley, 1995; Hurley, 1996; Hurley & Walker, 1996; 1997). Since hatcheries maintain highly controlled conditions, many of the techniques established in these studies are applicable to current operations, no matter the location of the grower. Yet, nursery and grow-out phases have been studied to a lesser extent. Notably, most of the research focused on nursery and grow-out techniques occurred in only a few specific areas in Long Island Sound (Desbonnet, 1989; Goldberg, 1989) and coastal Georgia (Goldberg & Walker, 1990; Walker & Heffernan, 1990a; 1990b; 1990c; O’Beirn et al, 1997; Walker et al., 1997). Although many of the conclusions may be transferrable, several of the studies cited above investigated the southern or Raveneli’s surfclam (*Spisula solidissima similis*), a subspecies of the Atlantic surfclam (*S. s. solidissima*). Consequently, nursery and grow-out culture methods that may be suitable for commercial-scale production of the Atlantic surfclam remain understudied, especially those which could be feasible and affordable for growers in the Northeast. Today, surfclam aquaculture is limited to a few small-scale operations in New Jersey and Massachusetts (*author observations*).

Thus, the intent of this study was to identify suitable husbandry techniques and provide additional information about surfclam aquaculture to shellfish growers in New
Jersey and throughout the Northeast. Specifically, a series of controlled experiments were conducted to evaluate the efficacy of various surfclam nursery and grow-out techniques. These experiments were conducted at commercial-scale and occurred on operating nurseries and farms. In the nursery phase, early (0.4 – 2.7 mm) and late (1.1 – 18.0 mm) juvenile surfclams were reared in various gear types and their growth and survival were compared. The early nursery gear types tested in this study included downwelling systems, bell siphon systems, and upwelling systems. The late nursery gear types tested included upwelling systems and shallow raceway systems, filled with or without sand. In the grow-out phase experiments, surfclam growth, survival, and condition were evaluated at multiple farm location across southern New Jersey. Sediment composition, temperature, salinity, dissolved oxygen, turbidity, and chlorophyll concentration were recorded throughout these experiments to provide additional information about how environmental conditions may modulate surfclam performance.
3.2 Materials and methods

3.2.1 Spawning and larval culture of Atlantic surfclams

The larval spawning and juvenile rearing methods used to produce Atlantic surfclams (*Spisula solidissima*) for deployment in 2016 experiments are outlined in Acquafredda et al. (2019). The following methods were used to spawn the surfclams which were used in the nursery experiments as well as the clams used in the 2017 deployment of the grow-out experiments.

Spawning and larval culture took place at the New Jersey Aquaculture Innovation Center (AIC) at Rutgers University in North Cape May, NJ, USA. In March 2017, eleven female and seven male farm-raised surfclams, which had naturally developed ripe gonads prior to arriving at the laboratory, were induced to spawn using thermal manipulation (Jones et al., 1993; Loosanoff & Davis, 1963). Larvae were reared at a temperature of 20°C and a salinity of 30 ppt in treated seawater (TSW), which was created via recirculation through a UV system and filtered to 1-µm.

Before reaching the pediveliger stage, all larvae were reared in static tanks. Pediveligers and early post-metamorphic clams were reared in downwelling systems with rearing silos composed of fiberglass cylinders (diameter x height: 60.96 x 60.96 cm) and Nitex mesh screen (125 and 150-µm) until the start of the experiment when the clams reached a mean shell height (SH) of 372.4 µm.

During this larval and post-larval period, the clams were fed a mixed diet that contained no more than 50% (by biomass) of the each of the following species of live microalgae per day: *Isochrysis galbana*, *Tisochrysis lutea*, *Pavlova lutheri*, and *Nannochloropsis oculata*. The feeding ration was incrementally increased from 1.0*10^4 cells ml⁻¹ to 1.0*10^5 cells ml⁻¹. The stocking density was incrementally decreased from
approximately 30 fertilized eggs ml⁻¹ to 1 larva ml⁻¹. Full water changes were conducted two to three times per week, and survival and growth were likewise monitored twice to thrice weekly.

Sequencing analysis of the mitochondrial cytochrome oxidase subunit I (COI) gene, using the primers Spis-L and Spis-R described by Hare & Weinberg (2005) indicated that all clams used for spawning in this study were the northern subspecies of the Atlantic surfclam, *Spisula solidissima solidissima*.

### 3.2.2 Early nursery gear type experimental design

Three early nursery rearing methods were tested for their effect on juvenile surfclam survival and growth (0.4 – 2.7 mm). Each rearing method consisted of two phases, where the gear type was switched or retained half-way through the experiment. The treatments include: (1) a downwelling/upwelling system (DWUW), (2) a bell siphon/upwelling system (BSUW), and (3) a bell siphon/bell siphon system (BSBS). The six-week experiment occurred from 1 May to 12 June 2017 and took place at the AIC. Initially, the experiment was set-up with surfclams retained on 230-µm mesh (SH >340 µm) and stocked at a density of 185.02 clams cm⁻² (Jones et al., 1993). The initial mesh size for all rearing silos was 200-µm (Nitex). The stocking densities used in this experiment were based on those recommended for Manila clam (*Venerupis philippinarum*) culture (Jones et al., 1993).

For the first phase of the experiment (day 0-21), surfclams were placed into downwelling systems or bell-siphon systems. Three independent replicate downwelling systems were established, each composed of a cubic tank (60.96 x 60.96 x 75.57 cm)
filled with TSW, a rearing silo composed of a fiberglass cylinder (diameter x height: 30.48 x 45.72 cm) and Nitex screen, two airlifts, and a pre-conditioned biofilter. Each unit was supplied a daily diet of 6.0*10^4 cells mL^-1 of *I. galbana* and 1.6*10^5 cells mL^-1 of *N. oculata*, which collectively is equivalent to 8.0*10^4 cells mL^-1 of *I. galbana* biomass, according to Helm and Bourne (2004). Full water changes were conducted twice weekly. Nitrogenous waste (ammonia, nitrite, nitrate) levels were measured daily, but never exceeded 0.25 ppm (LaMotte® Nitrogen Test Kits).

A bell siphon system is a rearing method that supplies bivalves with a continuous flow of seawater, which ebbs and flows between a set maximum and minimum height (Figure 3-1). In this study, three independent replicate bell siphon systems were established. Each system consisted of a raceway (length x width: 7.1 x 0.58 m) and a rearing silo composed of fiberglass cylinders (diameter x height: 60.96 x 60.96 cm) and Nitex screen. Each system was continuously supplied with unfiltered seawater from the Cape May Canal at a flow rate of ≈56.6 L min^-1. The effluent flow of each system was controlled by a bell siphon, a modified 5-gallon bucket resting atop the raceway’s standpipe, which cycled the water in the raceway between a maximum and minimum height of 33 cm and 5 cm, respectively. A complete cycle of took approximately 27 minutes, with 24 min to fill and 3 min to drain. Raceways were cleared of settled sediment twice weekly.

On day 21, 95% of surfclams were retained on 500-µm mesh (SH >710 µm). At that time, the stocking density was reduced to 102.8 clams cm^-2 (Jones et al., 1993), and the rearing silo mesh size was increased to 400-µm. All rearing silos were made of fiberglass cylinders (diameter x height: 30.48 x 45.72 cm). All surfclams in downwelling
systems were placed into independent flow-through upwelling systems (DWUW). Similarly, one half of the surfclams in the bell siphon system were placed into independent flow-through upwelling systems (BSUW), while the other half remained in the bell siphon systems (BSBS). All upwellers were supplied with unfiltered water from the Cape May Canal at a flow rate of ≈50 L min⁻¹. Raceways were cleared of settled sediment twice weekly. With the exception of rearing silo mesh size and stocking density, no changes were made to the three independent bell siphons systems used in this half of the experiment.

For clams experiencing TSW conditions (i.e., downwellers), temperature data were continuously recorded at 10-minute intervals using SBE 56 (Seabird Scientific) data loggers; salinity data were collected twice-daily using a handheld YSI (Model 86) (Figure 3-2). For clams that experienced unfiltered seawater (i.e., bell siphons and upwellers), temperature and salinity data were continuously recorded at 15-minute intervals using a YSI 6600 V2-4 multi-parameter water quality sonde (Figure 3-2).
Figure 3-1. Bell-siphon raceway system diagram. (A) A bucket encloses a drainage pipe within a raceway. (B) Water flows into the raceway, filling it up. (C) When the water level rises to the level of the drainage pipe (i.e., the standpipe) within the bucket, a siphon is created. (D) The water drains from the system faster than it enters. (E) When the water level reaches the bottom of the bucket, the siphon breaks. (F) The system is automatically reset, and the raceway once again begins to fill (adapted from Gould, 2013).
Figure 3-2. Temperature and salinity timeseries for the early nursery experiments. Points represent mean daily temperatures (°C, circles) and salinities (ppt, diamonds). Error bars represent standard deviation. Black points are for unfiltered seawater from the Cape May Canal and grey points indicate treated seawater (recirculated through a UV system and filtered to 1-µm).
3.2.3 Late nursery gear type experimental design

Three late nursery gear types were tested for their effect on surfclam survival and growth (1.1-18.0 mm). The gear types included: (1) upwellers (UW), (2) shallow raceways partially filled with sand (SR), and (3) shallow raceways without sand (NS). The experiment lasted 48 days, from 21 June to 8 August 2017 at the AIC. Three independent replicate systems were established for each rearing method. All were flow-through systems supplied with unfiltered seawater from the Cape May Canal. The rearing silos for the upweller treatment were composed of fiberglass cylinders (diameter x height: 45.72 x 45.72 cm) and Nitex mesh (600-µm mesh from day 0 to 14, 1000-µm mesh from day 14 to 28, and 1500-µm mesh from day 28 to day 48). Both raceway treatments consisted of three 85 L raceways (length x width x depth: 102.11 x 30.48 x 27.31 cm). The sand-filled raceway treatment had a 5 cm layer of medium-grain sand (Udden–Wentworth scale). Flow rates differed across the treatments. The mean (± standard deviation) flowrates for the SR, NS, and UW treatments were 8.1±1.4 L min⁻¹, 8.1±1.7 L min⁻¹, and 50.1±2.0 L min⁻¹, respectively. Twice weekly, all treatments were cleared of any sediments and waste products that accumulated on top of the clams. The UW and NS treatments were sprayed with TSW, while a siphon was used to extract the accumulated material above the sand of the SR treatment. Temperature and salinity were continuously recorded at 15-minute intervals using a YSI 6600 V2-4 multi-parameter water quality sonde (Figure 3-2).

Stocking densities used in the late nursery gear type experiment were based on those recommended for Manila clam (*Venerupis philippinarum*) culture (Jones et al., 1993). Initially, the stocking density in each treatment was 60.9 clams cm⁻² and contained
clams that could be retained on 750-µm mesh (SH >1.1 mm). On day 14, stocking
density in each treatment was reduced to 15.2 clams cm\(^{-2}\) and only restocked with clams
retained on 1.5-mm mesh (SH >2.2 mm). On day 28, the stocking density was again
reduced to 3.8 clams cm\(^{-2}\) and only restocked with clams retained on 4-mm mesh (SH
>5.7 mm).

3.2.4 Data collection for nursery experiments

Survival and growth data were collected weekly or biweekly. Survival was
assessed as described by Acquafredda and colleagues (2019). Percent survival data were
corrected for any clams sacrificed during sampling and after culls that took place when
stocking densities were reduced. Mean survival for a given treatment was calculated as
the mean percent survival of the three replicate units.

Growth was assessed by recording shell length (anterior-posterior axis) and shell
height (dorsal-ventral axis) as described by Acquafredda and colleagues (2019). Since
shell length and shell height correlated extremely well (Figure 3-3, Figure 3-4, \(R^2\) >0.99
for both), only shell length data will be discussed in detail. On each sampling event, fifty
clams were randomly sampled per replicate rearing unit, and the mean shell length was
calculated for each unit. On each sampling day, the mean shell length for each treatment
was calculated as the mean value of its three replicate units (n=3).

On day 2, 5, 9, 28, and 40 of the late nursery experiment, sediment accumulation
was measured. In each rearing unit, the height of accumulated sediments was measured
using a ruler. Additionally, 50 mL samples of the accumulated sediments were collected.
The samples were analyzed for sediment grain size by drying the samples in a 68°C oven
for 48 hours and passing them through a series of sieves in a sediment shaker (Dunham et al., 2013). The Udden–Wentworth scale was used to categorize the size classes. Loss-on-ignition analysis was conducted by sequentially burning the samples in pre-weighed crucibles at 550˚C and 750˚C for 4 hours each, to determine the organic and inorganic carbon content of the samples, respectively. Between being burned and weighed, the samples were stored in a desiccator overnight.

3.2.5 Statistical analyses for nursery experiments

All data were analyzed with R (Version 4.0.2 © 2020-06-22 The R Foundation) using RStudio (Version 1.3.1056 © 2009–2020 RStudio, Inc.). Normality and homoscedasticity were confirmed using the Shapiro-Wilk Test and Levene's Test, respectively. All measures of dispersion reported for the early and late nursery experiments are 95% confidence intervals calculated across replicate treatments (rearing silos), unless otherwise noted. Average daily growth rates were calculated using the method outlined by Acquafredda and Munroe (2020). All percent survival data were arcsine-transformed prior to this analysis to meet the assumption of normality.

For the early nursery experiment, growth data from the first half of the experiment (day 0-21) were squared prior to analysis to meet the assumption of normality. Independent two-sample t-tests were used to compare the survival and growth of surfclams during the first half of the early nursery experiment. During the latter half of the early nursery experiment (day 21-42), the BSBS and BSUW rearing methods were not independent. Therefore, paired (dependent) t-tests were used to compare the survival and growth of surfclams in the BSBS and BSUW rearing methods. Independent two-
Sample t-tests were used to compare the survival and growth of surfclams in the DWUW rearing method to both the BSBS and the BSUW methods. To compensate for the multiple comparisons during the latter half of the early nursery experiment, a Bonferroni correction was applied to the resulting p-values. ANCOVA was used to determine the effect of gear type on surfclam growth rate for each half of the experiment.

During the late nursery experiment, ANOVA was used to compare final survival and growth of surfclams reared in different treatments, as well as to compare average sediment accumulation by treatment, between cleanings. A post-hoc Tukey’s HSD test was used to determine significance between treatment pairs. Additionally, ANCOVA was used to determine the effect of rearing method and flow rate on surfclam growth rate over the duration of the experiment.
Figure 3-3. Early nursery experiment: shell length – shell height correlation. Points represent all shell length and shell height data collected during the early nursery experiment. Linear regression was conducted to generate a model that takes the form, $SH = m \times SL + b$, where $SH$ is shell height, $m$ is slope, $SL$ is shell length, and $b$ is the y-intercept. Blue diamonds represent clams reared in the bell siphon/bell siphon systems, purple squares represent clams reared in bell siphon/upweller systems, and red circles represent clams reared in downweller/upweller systems.
Figure 3-4. Late nursery experiment: shell length – shell height correlation. Points represent all shell length and shell height data collected during the late nursery experiment. Linear regression was conducted to generate a model takes the form, $SH = m \times SL + b$, where $SH$ is shell height, $m$ is slope, $SL$ is shell length, and $b$ is the Y-intercept. Green triangles represent clams reared in upwellers, orange squares represent clams reared in raceways with sand, and blue circles represent clams reared in raceways without sand.
Figure 3-5. Map of deployment sites used for the grow-out experiments. Points represent the locations of the four commercial shellfish farms where surfclams were deployed in during the 2016 and 2017 grow-out experiments. All sites are located in coastal bays of New Jersey, USA. Maps courtesy of NOAA National Centers for Environmental Information (NCEI).
Figure 3-6. Gear types used in the grow-out experiments. (A & B) 5-mm nylon mesh bags used at LEH (A) and UBB (B). (C & D) Box bags in a bottom cage at LEH. The bags were composed of 5-mm HDPE mesh, and the cage was composed of vinyl-coated wire. (E) An anti-predator screen at an intertidal clam farm, similar to the piloted gear used in this study. Photo by Patrick and Barbara Woodbury. (F) Recruitment boxes at an intertidal clam farm, similar to the piloted gear used in this study. Photo by Dr. Brian Beal, Downeast Institute.
Figure 3-7. Grow-out experiments: correlations of shell dimensions. Points represent all shell length, height, and width data collected during the grow-out experiments. Linear regressions were conducted to generate the models that take the form, $y = mx + b$, where $m$ is slope, $b$ is the $y$-intercept, and $x$ and $y$ are the shell dimensions. 2016 deployment (left panel): red diamonds (LEH, October 2016), orange diamonds (LEH, December 2016), blue circles (UBB), purple squares (AB). 2017 deployment (right panel): red diamonds (LEH), blue circles (UBB), black squares (RC).
3.2.6 Grow-out experimental design

Surfclam grow-out experiments were conducted over two years from October 2016 to August 2018. In the fall of 2016, surfclams were deployed at three commercial shellfish farms situated in coastal New Jersey bays (Figure 3-5). All of the farms were located in high salinity estuarine areas within six kilometers of inlets to the Atlantic Ocean. All farms were subtidal and had an approximate mean low water height of 60 cm. These farms were located in Absecon Bay (AB), Little Egg Harbor (LEH), and the Upper Barnegat Bay (UBB) (Figure 3.1). Most clams deployed at these sites in 2016 were monitored through the summer of 2017, although some were monitored through the end of the study. In the fall of 2017, a second cohort of clams were deployed at three farms. These included LEH and UBB, but the AB site was discontinued. Instead, a new farm located in Rose Cove (RC) was selected. The RC site was situated approximately 5.3 km north-northwest of the LEH site.

In the first round of grow-out experiments (2016 deployment), the surfclams were deployed in replicate 5-mm nylon mesh bags (1.22 x 1.22 m) and anchored to the bottom with bent rebar corner pins (Figure 3-6). These bags allow the surfclams to burrow and become partially covered by the surrounding sediments. Five mesh bags were deployed at UBB and AB. Six replicate bags were deployed at LEH. At AB, the bags were covered with an anti-predator screen (6.35-mm mesh), at the behest of the farmer. All clams deployed in the mesh bags at UBB and AB, and half of the clams deployed at LEH, had an initial shell length of 13.35 ± 0.27 mm (N=200 clams). The other half of LEH clams were deployed approximately six weeks later than all other clams and had a slightly larger initial shell length (15.49 ± 0.81 mm, N=50 clams) at outplanting. The initial
stocking density of each mesh bag was 5000 surfclams (3400 clams m\(^2\); 34% of each bag’s was area covered when the clams were arranged as a monolayer). During the summer 2017 sampling event, some of these clams were transferred into 9-mm mesh bags, which were identical in every other way than mesh opening to the 5-mm bags.

Two other gear types were piloted (N=1 per type) at the Absecon Bay site during the 2016 deployment, at the whim of the farmer. The first consisted of placing clams in a demarcated area of the farm and covering them with an anti-predator (i.e., predator exclusion) screen. Anti-predator screens are a typical method for rearing hard clams (Mercenaria mercenaria) in the region (Luckenbach et al., 2016; Figure 3-6). This screen was composed of high-density polyethylene (HDPE) mesh (6.35-mm) and was outlined with lead rods to ensure the screen would stay in place. The second consisted of placing clams inside a wooden box, colloquially known as a recruitment box. This structure was akin to an enlarged version of what has been used for recruiting and rearing softshell clams (Mya arenaria) in Maine (B. Beal, Downeast Institute, personal communication; Figure 3-6). The box was framed with lumber (10.16 cm x 1.83 m x 0.61 m) and its bottom was made of vinyl coated wire (Aquamesh®, Riverdale Mills) and landscaping fabric. The box was filled with sediment from the surrounding area and the clams were placed inside. Then, the box was covered with an anti-predator screen, as described above. The clams deployed in these piloted gear types were approximately half the size as those deployed in the replicated mesh bag experiment, having a mean shell length of 6.21 ± 0.31 mm (N=50 clams).

During the second round of grow-out experiments, which began in October 2017, two replicate 5-mm mesh bags were deployed at UBB and LEH. This gear type was not
deployed at Rose Cove. A new gear type was also tested during this grow-out season to assess whether surfclams can be grown to market sizes without providing any substrate. This gear type consisted of placing surfclam seed in 5-mm HDPE mesh box bags (50.8 x 91.44 cm) and deposited those bags into a vinyl-coated wire cage that was moored with a helical shed anchor (Figure 3-6). This system is commonly used for rearing oysters in New Jersey (author observations). Each cage had four compartments (length x width x height: 91.44 x 60.96 x 10.16 cm each) that were situated in a stacked (2 x 2) array. The mesh size of each cage was 1.27-cm. Each cage had short legs (height: 15.24 cm) that raised the compartments above the sediment. One bottom cage was placed at each farm, and each cage contained four replicate box bags of clams (one per compartment). Across gear types, the initial stocking density was kept at the 2016 level; the nylon mesh bags were each filled with 5000 surfclams, and the box bags were each filled with 1562 surfclams. The initial shell length of clams deployed in 2017 was 15.62 ± 0.33 mm (N=200).

To characterize the farms, environmental data were collected at AB, LEH, and UBB. In fall 2016, three sediment cores were collected from each site. Samples from each site were pooled and sediment grain size was analyzed with the same methods used for the late nursery experiment. At the three aforementioned sites, water temperature, salinity, and dissolved oxygen (DO) data were recorded with SBE 37-SM MicroCAT (Sea-Bird Scientific) data loggers. These CTD sondes continuously recorded temperature, salinity, and DO at 10-minute intervals. Fluorometers (ECO FLNTU, WET Labs) were also deployed at LEH and UBB for portions of the grow-out experiments. The fluorometers measured ambient chlorophyll (λ_{excitation}=470 nm; λ_{emission}=695 nm) and
turbidity (700 nm) and collected ten one-second samples every 15-minutes. At each site, the data loggers were placed inside a box bag (1.27-cm mesh) and attached to mooring constructed out of cinderblocks and a wire shelf. Therefore, the loggers sat approximately 25 cm above the sediment. These loggers were placed within 0.5 m of the clams at LEH and UBB and within 10 m of the clams at AB. No environmental data were recorded at RC.

The clams were sampled approximately every four months. Between sampling events, the bags were not monitored or manipulated in any way. On each sampling event, surfclam growth, survival, and condition data were collected. On some occasions, data could only be collected from one or a few replicates due to time, tide, and weather constraints. To assess growth, the shell length (anterior-posterior axis), height (dorsal-ventral axis), and width (dextral-sinistral axis) of 25 surfclams per replicate were measured using calipers (Mitutoyo Absolute™ Digimatic). However, on other occasions the number of clams measured ranged from 10 to 69 per replicate. Additionally, shell dimensions of empty shells from dead clams were also measured (N=15–25 per replicate). Since both shell height and width correlated extremely well with shell length (Figure 3-7, $R^2>0.94$ for each), only shell length data will be discussed in detail. Survival was estimated by conducting volumetric abundance estimates of live and dead clams. When the number of surviving clams in a given bag was estimated to be below 250, the clams were counted individually.

On most sampling events, a subset of 20 clams was returned to the laboratory for further analysis. However, on certain occasions the number of clams measured ranged from 5 to 28 per replicate. Gonad development was assessed, first by macroscopic
inspection. If the gonad was present, a capillary tube was inserted into it to collect a sample. Then, the sample was plated on a slide and inspected under a VWR compound light microscope at 100X magnification. The stages of gonad development were categorized as “immature/inactive”, “developing”, “ripe”, and “spawned out”. These categories are based on those described by Ropes (1968). However, since histological examination was not conducted, some of the stages were merged. Specifically, the “early active” and “late active” stages were categorized as “developing”; the “partially spawned” and “spent” stages were categorized as “spawned out”. Sex was determined by the presence of eggs, sperm, or their respective progenitor cells. However, since histological examination was not conducted, sex could not be determined for some individuals in the “developing” stage. Biomass data (whole wet weight, wet tissue weight, dry tissue weight, and dry shell weight) were also collected from the subset of clams returned to the laboratory. The condition of each clam was determined by a condition index (CI), which was calculated using the following formula:

\[ CI = \frac{d}{s} \times 100 \]

where \( d \) is the dry tissue weight of a clam in grams and \( s \) is the dry shell weight of a clam in grams. A higher condition index score indicates a healthier (i.e., meatier) clam. Since there is no standardized market size for surfclams, the size ranges for the pastaneck (31.75 ≤ \( x \) < 44.45 mm), littleneck (44.45 ≤ \( x \) < 57.15 mm), and middleneck sizes (57.15 ≤ \( x \) < 69.85 mm) were chosen by the authors after consultation with the partnering farmers of this study and other colleagues studying surfclam aquaculture (J. Reitsma, Woods Hole Sea Grant, personal communication).
3.2.7 Statistical analyses for grow-out experiments

All data were analyzed with R (Version 4.0.2 © 2020-06-22 The R Foundation) using RStudio (Version 1.3.1056 © 2009–2020 RStudio, Inc.). All measures of dispersion presented for the grow-out experiments represent the 95% confidence intervals calculated across replicate bags of clams. When only one replicate was available, 95% confidence interval were calculated across the clams within the single sample and the sample size was provided in-text.

Due to limited replication, sampling inconsistencies, and the lack of independence, robust statistical analysis for comparing growth and survival across farms was limited to the data collected during the summer of 2017. Where statistical tests were conducted on growth data, normality and homoscedasticity were confirmed using the Shapiro-Wilk Test and Levene's Test, respectively. Average daily growth rates were calculated as stated above. Percent survival data were arcsine-transformed prior to statistical analysis to meet the assumption of normality. Paired (dependent) t-tests were used to compare the growth and survival of surfclams deployed in October and December 2016. Independent two-sample t-tests were used to compare the growth and survival of surfclams between farms. To compensate for the multiple comparisons, a Bonferroni correction was applied to the resulting p-values. For all clams deployed in mesh bags during the 2016 deployment, growth models were fit to all shell length data collected from live clams. To generate the model for clams reared at AB, a linear regression was conducted. To generate the models for clams reared at UBB and LEH, polynomial regressions were conducted using Excel (Version 16.42, Microsoft®). The resulting
models were used to predict the death dates for each dead clam measured over the course of the experiment.
3.3 Results

3.3.1 The effect of early nursery gear type on surfclam survival and growth

Survclam survival monotonically declined for the duration of the experiment, regardless of rearing method. After the first two weeks, 93.2 ± 10.6% of surfclams reared in downwellers (DW) survived compared to 79.3 ± 20.2% in bell siphon systems (BS). Due to variation across replicates, no significant difference was detected between the two rearing methods (Figure 3-8; Two-sample t-test, p > 0.2). By week three, mortality of surfclams reared in DW accelerated, and was indistinguishable from survival of clams reared in BS (Figure 3-8; DW: 73.5 ± 9.0%; BS: 68.8 ± 9.9%; Two-sample t-test, p > 0.5). During the second phase, when clams were moved into upwellers or continued in bell siphon systems, survival continued to decrease, albeit at a slower rate. At the end of the 42-day experiment, surfclam survival across rearing methods was statistically similar and averaged 66.3 ± 8.5% (Figure 3-8; Multiple t-tests with a Bonferroni correction, p >0.8).

For the first two weeks of the experiment, surfclams reared in the DW grew faster than those in BS (Figure 3-8; DW: 37.6 ± 0.0005 µm day⁻¹; BS: 30.7 ± 0.003 µm day⁻¹), and both gear type and experimental day had a significant effect on growth rate (Figure 3-8; ANCOVA (gear type) F(1, 20) = 18.57 , p <0.001; (experimental day) F(1,20) = 250.08, p < 0.001). However, by day 21 the surfclams in BS had a mean shell length that was nearly equivalent to that of the clams reared in DW (Figure 3-8; DW: 1.22 ± 0.03 mm; BS: 1.08 ± 0.16 mm; Two-sample t-test, p=0.14). Variation in shell length was markedly lower for clams reared in DW.
After being transferred from downwellers to upwellers (DWUW), clam growth in this group appeared to pause for approximately one week. Throughout the latter half of this experiment, average daily growth rate of clams moved from downwellers to upwellers (Figure 3-8; DWUW: 52.7 ± 0.001 µm day⁻¹) remained slower than that of clams moved from bell siphons to upwellers (Figure 3-8; BSUW: 75.4 ± 0.002 µm day⁻¹) or clams that remained in bell siphon systems (Figure 3-8; BSBS: 65.5 ± 0.002 µm day⁻¹). Despite the growth rate differences, gear type did not have a significant effect on growth rate (Figure 3-8; ANCOVA (gear type) F(2, 30) = 2.875, p = 0.07; (experimental day) F(1, 30) = 311.79, p < 0.001). Final (day 42) surf clam shell length was also statistically similar across rearing methods and averaged 2.48 ± 0.15 mm (Figure 3-8; Multiple t-tests with a Bonferroni correction, p >0.35).
**Figure 3-8. Early nursery experiment: survival and growth.** Points represent mean percent survival (top panel) and shell length (bottom panel) of three replicate rearing silos for each treatment. Error bars represent 95% confidence intervals. Blue diamonds represent clams reared in the bell siphon/bell siphon systems (BSBS), purple squares represent clams reared in bell siphon/upweller systems (BSUW), and red circles represent clams reared in downweller/upweller systems (DWUW). Points are jittered for clarity.
3.3.2 The effect of late nursery gear type on surfclam survival and growth

After two weeks of rearing in the sand-filled raceway (SR), surfclam survival was 57.8 ± 1.5%, while survival of clams in the other two rearing methods was above 80% (Figure 3-9). Surfclam survival in SR rapidly declined, falling below 20% by the end of the experiment. Clams reared in shallow raceways with no sand (NS) fared well until day 14, after which survival rapidly declined. Clams in NS continued to experience mortality similar to clams reared in SR. Conversely, 72.0 ± 4.6% of surfclams reared in upwellers (UW) had survived by day 28, and after this point, little mortality was observed. At the end of the 48-day experiment, survival of surfclams reared in UW was significantly greater than clams reared in either raceway treatment (Figure 3-9; Two-way ANOVA (gear type) F(2,3) = 21.981, p = 0.016; (flow rate) F(1,3) = 1.303, p = 0.34; Tukey’s HSD: UW-NS p = 0.03, UW-SR p = 0.02). A post-hoc Tukey’s HSD test showed that there was no significant difference in the final survival of surfclams reared in raceways with or without sand (p = 0.68).

For the first 28 days, no difference in shell length growth were observed for surfclams among the three rearing methods (Figure 3-10). Surfclam growth rate during the first two weeks was negligible in all treatments and averaged 0.0047 ± 0.0068 mm day⁻¹. During the second two weeks, clams exhibited similar growth rates, which ranged from 0.14 to 0.16 mm day⁻¹. However, between day 28 and the end of the experiment, gear type, flow rate, and experimental day all had a significant effect on growth rate, and there was a significant interaction between rearing method and flow rate (Figure 3-10; ANCOVA (gear type) F(2,11) = 11.20, p = 0.002; (flow rate) F(1,11) = 14.76, p = 0.003; (experimental day) F(1,11) = 122.48, p < 0.001; (gear type *flow rate), F(2,11) = 5.36, p
Average daily growth rate of clams reared in UW was \(0.38 \pm 0.012\ \text{mm day}^{-1}\), more than twice as fast as what clams experienced in either SR \((0.16 \pm 0.057\ \text{mm day}^{-1})\) or NS \((0.18 \pm 0.050\ \text{mm day}^{-1})\). At the end of the experiment, surfclams reared in UW had significantly greater mean shell length compared to clams reared in NS or SR (Figure 3-10; Two-way ANOVA (gear type) \(F(2,3) = 82.497, p = 0.002\); (flow rate) \(F(1,3) = 2.557, p = 0.21\); Tukey’s HSD: UW-NS \(p = 0.007\); UW-SR \(p = 0.002\)). A post-hoc Tukey’s HSD test showed that while not significant, length of surfclams grown in NS trended to be greater than that observed for surfclams grown in SR \((p=0.06)\).

Between the twice weekly cleanings, considerable amounts of sediment accumulated on top of the clams across all treatments, forming a layer of grey mud. The accumulated material was mainly composed of fine particulate matter \((\sim 75\% \leq 500\ \mu\text{m})\) that fell out of suspension from the flow-through raw seawater in addition to accumulated pseudofeces. Loss-on-ignition analysis showed that \(16.4 \pm 2.9\%\) of the material was composed of organic carbon and \(10.8 \pm 3.4\%\) was composed of inorganic carbon. There was no difference in the composition of the material that accumulated on the clams across treatments (ANOVA \(p>0.17\)). However, the quantity of accumulated matter differed significantly across each gear type (Figure 3-11; ANOVA \(F(2,6) = 3002, p < 0.001\); Tukey’s HSD: \(p < 0.001\) for all pairwise comparisons). On average, 4.4-times as much material accumulated in SR between cleanings compared to UW. Similarly, 2.7-times as much material accumulated in NS compared to UW.
Figure 3-9. Late nursery experiment: survival. Points represent mean percent survival of three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. Green triangles represent clams reared in upwellers (UW), orange squares represent clams reared in sand-filled raceways (SR), and blue circles represent clams reared in raceways with no sand (NS).
Figure 3-10. Late nursery experiment: growth. Points (top panel) and bars (bottom panel) represent mean shell length of three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. (Top) Growth (shell length) over the duration of the experiment: green triangles represent clams reared in upwellers (UW), orange squares represent clams reared in sand-filled raceways (SR), and blue circles represent clams reared in raceways with no sand (NS). (Bottom) Final shell length: bars sharing a letter are not significantly different from one another.
Figure 3-11. Late nursery experiment: sediment accumulation. Bars represent mean height of material that accumulated on the clams between cleanings, calculated across the three replicate rearing units for each treatment. Error bars represent 95% confidence intervals. Letters above the bars indicate that all treatments were significantly different from one another (p < 0.001)
3.3.3 Evaluating surfclam performance across multiple NJ shellfish farms

3.3.3.1 Fall 2016

The first round of surfclam grow-out experiments was initiated during a two-week period in the fall of 2016. Clams were first deployed at Little Egg Harbor (LEH) on 25 October, at Absecon Bay (AB) on 31 October, and at Upper Barnegat Bay (UBB) on 8 November. However, three of the six total replicate bags at LEH were deployed on 6 December.

As of fall 2016, sediment at UBB was the most homogenous (Figure 3-12). The substrate here was chiefly composed of fine sand (78%), with medium sand (13%) and very fine sand (7%) composing the next largest size class. Notably, this site exhibited very little silt (< 1%) or larger particles sized between coarse sand and pebbles (< 2%). LEH had the most balanced mix of sediments (Figure 3-12). Here, small sediments such as silt (15%), very fine sand (33%), and fine sand (29%) made up the majority of particles. Larger particles sized between coarse sand and pebbles together made up less than one quarter of the substrate. The sediment at AB exhibited the smallest grain sizes, with a majority of particles characterized as either very fine sand (53%), fine sand (21%), or silt (11%) (Figure 3-12). Larger particles sized between coarse sand and pebbles together made up approximately 15% of the substrate.
Figure 3-12. Sediment grain size of three surf clam grow-out sites as of fall 2016. Distribution of sediment grain size classes in bottom sediment at deployment farms UBB = Upper Barnegat Bay, LEH = Little Egg Harbor, AB = Absecon Bay.
3.3.3.2 Spring 2017

The first sampling event occurred during the following spring. Sampling occurred on 20 March at UBB, on 5 April at LEH, and on 13 April at AB. Only one replicate bag per farm was assessed during this sampling event. Survival was high, ranging from 84 to 95% across the three sites (Figure 3-13). Average daily growth rates varied more widely (Figure 3-14). Of clams reared in mesh bags, those at the LEH site exhibited the fastest growth rate at 0.073 mm day\(^{-1}\), while those at the UBB site exhibited the slowest growth rate at 0.050 mm day\(^{-1}\). Clams reared in mesh bags at AB experienced an intermediate growth rate of 0.065 mm day\(^{-1}\), while clams in the piloted gear types had the greatest growth rates overall during this period. Clams in the recruitment box and under the anti-predator screen, grew at 0.11 and 0.083 mm day\(^{-1}\), respectively. The mean shell length of the sampled clams ranged from 19.26 ± 0.96 mm at UBB to 25.06 ± 0.65 mm at LEH (N= 50 clams per farm) (Figure 3-15).

Clam condition also varied across sites (Figure 3-16). Clams had high condition at UBB and LEH (UBB: 33.4 ± 4.48, N=20; LEH: 32.2 ± 2.36, N=24 clams). However, clams at AB, regardless of which gear type, had a condition that was approximately one-third lower (AB bag: 21.7 ± 4.3; AB recruitment box: 18.6 ± 2.8; AB screen: 25.2 ± 4.0; N=20 clams per gear type).

Two-thirds of the clams sampled at LEH were ripe males, one-quarter were ripe females, and the remaining portion had no gonad present (Figure 3-17). At AB, clams in the mesh bags were further along in maturation than those in the piloted gear types (Figure 3-17). Of clams in mesh bags, 55% were developing as males, 20% were developing as females, and 25% had no gonad present. By contrast, only 25% of clams sampled from the recruitment box had initiated gametogenesis and all were developing as
males. Of the clams reared under the anti-predator screen, 25% were developing as males and 20% were developing as females. Gonad maturation and sex were not assessed for the clams sampled from UBB.

Numerous other organisms were observed in, on, and around the bags of the cultured surfclams. Atlantic mud crabs (Panopeus herbstii) and Say’s mud crabs (Dyspanopeus sayi) were observed at all three sites. At UBB, the commensal species living alongside the surfclams included decorator worms (Diopatra spp), swimming clams (Solemya velum), and isopods (Cirolana spp). At LEH, the commensal species included false angel wings (Petricolaria pholadiformis), stout razor clams (Tagelus plebius), and marsh grass shrimp (Palaemon vulgaris). At AB, ribbon worms (Cerebratulus lacteus) and peanut worms (Sipunculids) were found living among the clams. Few biofouling organisms were observed during this sampling event. However, at UBB and AB, macroalgae like Gracilaria spp and sugar kelp (Saccharina latissima) were observed growing on the bags. The bags at LEH hosted these macroalgae in addition to Ulva spp. Several species were also found living within a few meters of the clams. At LEH, this included several gastropods, such as knobbed whelk (Busycon carica), mud snails (Tritia obsoleta), and slippershells (Crepidula fornicata). At AB, an Atlantic purple sea urchin (Arbacia punctulate) was found foraging on top of the mesh bags.
Figure 3-13. Survival of clams deployed in fall 2016. Bars show mean percent survival of replicate mesh bags for a given grow-out site. Error bars represent 95% confidence intervals calculated across replicate bags. Error bars equal zero on initial deployment; other bars without error bars represent sampling events when only a single replicate bag was sampled.
Figure 3-14. Surfclam growth rates across farms, seasons, and deployments. Mean daily shell length growth rate for a given grow-out site and gear type. Error bars represent 95% confidence intervals calculated across replicate bags. Points without error bars represent sampling events when only a single replicate unit was examined. Points are jittered for clarity.
Figure 3-15. Shell length growth of clams deployed in fall 2016. Mean shell length of replicate mesh bags for a given grow-out site. Error bars represent 95% confidence intervals calculated across replicate bags. Bars without error bars represent sampling events when only a single replicate bag was examined.
Figure 3-16. Condition of clams deployed in fall 2016. Mean condition of 20 clams collected from each site and gear type on a given sampling event. Error bars represent 95% confidence intervals calculated across clams.
Figure 3-17. Sex ratio and gonad development of clams across farms, seasons, and deployments. UBB = Upper Barnegat Bay, LEH = Litte Egg Harbor, AB = Absecon Bay, RC = Rose Cove.
3.3.3.3 Summer 2017

The next sampling event occurred in the summer, on 29 June for LEH, 10 July for AB, and 20 July for UBB. All replicate bags were assessed at each farm. Survival was highly variable across sites. Clams deployed at LEH exhibited significantly higher survival compared to those at UBB and AB (Figure 3-13; Multiple t-tests with a Bonferroni correction, p<0.001). Clams deployed at LEH in October showed lower, yet not significantly different, survival compared to clams deployed in December (Oct: 77.6 ± 12.5%; Dec: 91.1 ± 13.6%; Paired t-test with a Bonferroni correction, p=0.86). There was particularly high mortality observed at AB and UBB (Figure 3-13).

The AB site showed evidence of smothering; mesh bags were filled with highly anoxic mud and empty but articulated clam shells (i.e., boxes). No live clams were recovered at the AB site in any of the five replicate bags. However, a linear growth model was fit to the shell length data collected from the live clams during the previous sampling events, and this model was used to predict the death dates for each dead clam measured on this and all previous sampling events, where data were available (Figure 3-18; Linear Regression, \( R^2 = 0.85 \)). Death assemblage data indicate that clams may have been dying at a relatively constant rate from 90 days post deployment. Although survival could not be estimated for the clams at AB in the piloted gear types, there was no evidence of smothering or anoxic conditions in the recruitment box or under the anti-predator screen.

The UBB site showed evidence of predation mortality. Specifically, torn bags and broken clam shells suggested predation by blue crabs (Callinectes sapidus). Blue crabs were also observed hovering over the bags and pulling at the mesh. Additionally, several large moon snails (Euspira heros) were found in the bags and some shells showed the
hallmark holes of gastropod predation. Here, clam survival over the duration of the study was $5.12 \pm 5.8\%$ across the five replicate bags (Figure 3-13). There was no statistical difference in survival between UBB and AB (Independent $t$-test with a Bonferroni correction, $p=0.75$).

Between the spring and summer sampling events of 2017, average daily growth rate at UBB (0.11 mm day$^{-1}$) exceeded growth observed at LEH (0.082 mm day$^{-1}$) (Figure 3-14). Since no live clams were recovered from the mesh bags at AB, growth rate could not be determined for this group over this interval. However, much like the clams reared at the UBB site, clams reared in the piloted gear types at AB experienced faster growth between the spring and summer compared to the fall and spring. Clams reared in the recruitment box grew at 0.12 mm day$^{-1}$ and clams reared under the anti-predator screen grew at 0.11 mm day$^{-1}$ (Figure 3-14).

From fall 2016 to summer 2017, clams reared at UBB exhibited a slightly faster ($0.086 \pm 0.0070$ mm day$^{-1}$), although not significantly different, growth rate than what was observed at LEH (Figure 3-14; Multiple $t$-tests with a Bonferroni correction, $p>0.11$). Likewise, no significant difference in growth rate was observed for clams deployed in October ($0.071 \pm 0.0087$ mm day$^{-1}$) compared to December ($0.074 \pm 0.0075$ mm day$^{-1}$) at LEH (Paired $t$-test with a Bonferroni correction, $p=0.67$). Although survival was low at the UBB site, over 80% of the surviving clams reached pastaneck market size (Table 3-1) and the mean shell length across all replicate bags was 35.85 ± 1.99 mm. By contrast, 43% of LEH October deployment clams and 31% of LEH December deployment clams reached pastaneck market size by the summer (Table 3-1), and the mean shell lengths across replicates were 30.45 ± 2.35 mm and 30.70 ± 0.25 mm,
respectively (Figure 3-15). At AB, approximately two-thirds of clams reared in recruitment box reached pastaneck size, while none of those reared in the other treatments grew to that size (Table 3-1). As such, mean shell length for clams in the recruitment box was 34.41 ± 1.42 mm (N= 55 clams), while it was only 29.06 ± 0.86 mm (N= 55 clams) for clams reared under the anti-predator screen (Figure 3-15).

Mean condition of clams across sites decreased dramatically from spring to summer 2017, ranging from 9.1 at UBB (N=5 replicate bags, N=5-12 clams per bag) to 10.6 ± 0.8 (N=20 clams) at LEH (Figure 3-16). The gonad condition of all inspected clams across the three sites indicated that all individuals had recently spawned out (Figure 3-17).

The experiment concluded at AB after summer 2017 sampling. At UBB, all surviving clams (N=1188), other than those harvested to conduct the condition analysis, were pooled and redeployed in a single 9-mm nylon mesh bag. At LEH, each of the six replicate bags of clams was split evenly; the 12 new bags had an average stocking density of 2025 ± 154 clams bag⁻¹. Three of the six bags containing October deployment clams had 9-mm mesh, and two of the six bags containing December deployment clams had 9-mm mesh. All remaining bags had 5-mm mesh.

In addition to the predators observed at UBB, many other organisms were observed near the clams during this sampling event. For instance, all bags contained numerous gravid Atlantic mud crabs (P. herbstii), and all bags were biofouled with both Ulva spp and Gracilaria spp. At AB, mud snails (Tritia obsoleta) and decorator worms (Diopatra spp) were found living commensally with the surfclams, and at LEH, blood arks (Anadara ovalis) were found growing alongside the clams.
Figure 3-18. Growth models for clams deployed in fall 2016. Shell length data collected on a given sampling event at: (Top left) Absecon Bay (AB), mesh bags only; (Top right) Upper Barnegat Bay (UBB); (Bottom left) Little Egg Harbor (LEH), October 2016 deployment; (Bottom right) Little Egg Harbor (LEH), December 2016 deployment. Black points represent the shell length data of dead clams measured throughout the duration of the experiment; each dead clam’s shell length has been plotted on its predicted death date, given by the best-fit linear or polynomial growth models. X = day of last sampling for Absecon Bay and Upper Barnegat Bay when no live clams were recovered.
Table 3-1. Percentage of clams reaching market sizes (shell length) over the duration of the grow-out experiment.
AB = Absecon Bay, recruitment box only; UBB = Upper Barnegat Bay; LEH = Little Egg Harbor.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Initial deployment date</th>
<th>Sampling date</th>
<th>Pastaneck $31.75 \leq x &lt; 44.45$ mm (1.25 ≤ $x$ &lt; 1.75 in)</th>
<th>Littleneck $44.45 \leq x &lt; 57.15$ mm (1.75 ≤ $x$ &lt; 2.25 in)</th>
<th>Middleneck $57.15 \leq x &lt; 69.85$ mm (2.25 ≤ $x$ &lt; 2.75 in)</th>
<th>N clams measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEH</td>
<td>10/25/16</td>
<td>6/29/17</td>
<td>42.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>100</td>
</tr>
<tr>
<td>LEH</td>
<td>10/25/16</td>
<td>10/2/17</td>
<td>73.8%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>145</td>
</tr>
<tr>
<td>LEH</td>
<td>10/25/16</td>
<td>4/12/2018</td>
<td>88.7%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>53</td>
</tr>
<tr>
<td>LEH</td>
<td>10/25/16</td>
<td>8/10/2018</td>
<td>20.0%</td>
<td>80.0%</td>
<td>0.00%</td>
<td>25</td>
</tr>
<tr>
<td>LEH</td>
<td>10/25/2016 &amp; 12/6/16</td>
<td>7/17/2018</td>
<td>68.0%</td>
<td>32.0%</td>
<td>0.0%</td>
<td>25</td>
</tr>
<tr>
<td>LEH</td>
<td>12/6/16</td>
<td>6/29/17</td>
<td>31.4%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>102</td>
</tr>
<tr>
<td>LEH</td>
<td>12/6/16</td>
<td>10/2/17</td>
<td>65.3%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>75</td>
</tr>
<tr>
<td>LEH</td>
<td>12/6/16</td>
<td>8/10/2018</td>
<td>33.3%</td>
<td>64.4%</td>
<td>2.2%</td>
<td>45</td>
</tr>
<tr>
<td>LEH</td>
<td>10/11/17</td>
<td>4/12/2018</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>90</td>
</tr>
<tr>
<td>LEH</td>
<td>10/11/17</td>
<td>7/17/2018</td>
<td>98.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>50</td>
</tr>
<tr>
<td>UBB</td>
<td>11/8/16</td>
<td>7/20/17</td>
<td>84.4%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>32</td>
</tr>
<tr>
<td>UBB</td>
<td>10/2/17</td>
<td>7/2/2018</td>
<td>68.18%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>22</td>
</tr>
<tr>
<td>AB</td>
<td>10/31/16</td>
<td>7/10/17</td>
<td>67.3%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>55</td>
</tr>
</tbody>
</table>
3.3.3.4 Fall 2017

The second round of surfclam grow-out experiments was initiated for the 2017-2018 growing season and began during a two-week period in early October. Clams were deployed at UBB on 2 October and clams were deployed at LEH on 11 October. Clams were deployed at a new site, Rose Cove (RC), on 13 October.

After the 2017 seed was outplanted at the UBB and LEH sites, all replicate bags from the 2016 deployment were assessed. No live clams were recovered at the UBB site (Figure 3-13); therefore, the first round of grow-out experiments concluded at UBB. A polynomial growth model was fit to the shell length data collected from the live clams during the previous sampling events, and this model was used to predict the death dates for each dead clam measured on this and all previous sampling events, where data were available (Figure 3-18; Polynomial Regression, $R^2=0.88$). It supports the survival data collected over the course of the experiment, showing that most mortality occurred between the first and second sampling events.

At LEH, surfclam survival declined (Figure 3-13), growth slowed (Figure 3-14), and condition fell to $7.9 \pm 0.17$ (Figure 3-16; Oct 2016 deployment: N=2 replicate bags, N=20 clams per bag). Gonads of all sampled individuals were in the inactive phase (Figure 3-17). Clams moved into 9-mm mesh bags survived less well than those retained in 5-mm bags. For the October 2016 deployment, survival was $24.3 \pm 15.7\%$ for clams exclusively grown in 5-mm bags compared to $3.9 \pm 3.1\%$ for clams moved into 9-mm mesh bags after spending 8 months in 5-mm bags. This trend was also true for clams initially deployed in December 2016. Here, surfclam survival of was $37.9 \pm 8.9\%$ for clams exclusively grown in 5-mm bags, whereas none of the clams moved into 9-mm mesh bags survived.
Between summer and fall 2017, growth rate slowed to $0.032 \pm 0.0074$ mm day$^{-1}$ and $0.019 \pm 0.011$ mm day$^{-1}$ for LEH clams deployed in October and December 2016, respectively (Figure 3-14). Despite these reduced growth rates, clams reached mean shell lengths of $33.79 \pm 1.50$ mm and $32.83 \pm 1.18$ mm, respectively (Figure 3-15).

Additionally, 74% of October deployment clams and 65% of December deployment clams reached pastaneck market size by this time (Table 3-1). After this sampling event, three bags from the October 2016 deployment (two 5-mm and one 9-mm bag) and three bags from the December 2016 deployment (all 5-mm) remained on the farm.

### 3.3.3.5 Spring 2018

The farms were next sampled over a one-month period in early spring. Clams were sampled on 16 March at UBB, on 18 March at RC, and on 12 April at LEH. At all three farms, clams deployed in the box bag/cage system suffered severe mortality (Table 3-2). Shells of dead clams were articulated and did not show any signs of predation. This mortality event was likely due to harsh winter weather that caused the cages to become exposed during extreme low tides. At both LEH and UBB, no clams in any of the box bags survived (Table 3-2). At RC, three of the four bags suffered 100% mortality, but survival of the single spared box bag was 79.3% (Table 3-2). Mean condition of these clams was high at $20.1 \pm 2.5$ (N=19 clams), and of these, only a single clam was undergoing the early stages of gametogenesis (Table 3-2; Figure 3-17). Clams at RC that did survive exhibited a slow growth rate (Figure 3-14; 0.012 mm day$^{-1}$) and reached a mean shell length of $16.9 \pm 0.55$ mm (N=69 clams; Table 3-2). An examination of the dead clam shells from the other three bags suggested they also had not grown very much
since deployment. Due to loss of replication at RC as well as the complete loss of this treatment at the other two farms, sampling at RC was discontinued.

Due to foul weather, mesh bags could not be assessed at UBB during this sampling period. At LEH, survival of clams in mesh bags was highly variable and lower than what was observed during the same period during the first round of deployments (Table 3-2). Although the two bags were placed immediately adjacent to one another, the survival of one replicate was 61.6%, while the other was 25.3%. By contrast, the growth and condition of these clams was consistent across these two replicates. These surfclams exhibited a growth rate of $0.049 \pm 0.0044$ mm day$^{-1}$ (Figure 3-14) and had a mean shell length was $25.72 \pm 0.83$ mm (Table 3-2). These surfclams also exhibited high condition (31.9 ± 3.1), although very few of the sampled clams had yet to initiate gametogenesis (Table 3-2; Figure 3-17).

Clams from the 2016 deployment were also examined during this sampling event, specifically, one 5-mm bag from the October 2016 deployment and one 5-mm bag from the December 2016 deployment. Cumulative survival for the clams continued to fall (Oct 2016: 8.0%; Dec 2016: 15.1%; Figure 3-13). At this time, growth and condition data were not collected for clams deployed in December 2016. However, between fall 2017 and spring 2018, the growth rate of clams deployed in October 2016 fell to 0.022 mm day$^{-1}$ (Figure 3-14). This growth rate was noticeably lower than what was observed over the same interval a year prior (Figure 3-14). Despite this, surfclam condition increased to $24.3 \pm 1.1$ (N=28), the highest it had been since the previous spring (Figure 3-16). Mean shell length of these clams was $36.69 \pm 0.95$ mm (N=53), and over 88% had reached the pastaneck market size (Figure 3-15; Table 3-1). All clams had gonad present; 61% were
ripe females, 29% were ripe males, and 10% were in the developing phase. (Figure 3-17).

After this sampling event, the clams from these two bags were pooled into a single 5-mm bag.

During this sampling event, few species were found near the clams at any of the farms. Oyster spat was observed on the bags at RC, and a few other organisms including, Say’s mud crabs (*D. sayi*) and polychaete worms, were found among the mesh bags at LEH.
Table 3-2. Survival, growth, and condition data for surfclams deployed in 2017.
UBB = Upper Barnegat Bay; LEH = Little Egg Harbor; RC = Rose Cove

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sampling date</th>
<th>Gear type</th>
<th>Survival ± 95% CI (%)</th>
<th>Mean shell length ± 95% CI (mm)</th>
<th>Condition ± 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEH</td>
<td>10/11/2017</td>
<td>Box bags in bottom cage</td>
<td>100</td>
<td>16.74 ± 0.69</td>
<td></td>
</tr>
<tr>
<td>LEH</td>
<td>10/11/2017</td>
<td>5-mm mesh bags</td>
<td>100</td>
<td>16.74 ± 0.69</td>
<td></td>
</tr>
<tr>
<td>LEH</td>
<td>4/12/2018</td>
<td>Box bags in bottom cage</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>LEH</td>
<td>4/12/2018</td>
<td>5-mm mesh bags</td>
<td>43.45 ± 35.57</td>
<td>25.72 ± 0.83</td>
<td>31.9 ± 3.1</td>
</tr>
<tr>
<td>LEH</td>
<td>7/17/2018</td>
<td>5-mm mesh bags</td>
<td>34.95 ± 36.16</td>
<td>37.23 ± 0.53</td>
<td>11.1 ± 0.3</td>
</tr>
<tr>
<td>UBB</td>
<td>10/2/17</td>
<td>Box bags in bottom cage</td>
<td>100</td>
<td>15.63 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>UBB</td>
<td>10/2/17</td>
<td>5-mm mesh bags</td>
<td>100</td>
<td>15.63 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>UBB</td>
<td>3/16/18</td>
<td>Box bags in bottom cage</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>UBB</td>
<td>7/2/18</td>
<td>5-mm mesh bags</td>
<td>0.2</td>
<td>32.65 ± 0.33</td>
<td>10.7 ± 0.2</td>
</tr>
<tr>
<td>RC</td>
<td>10/13/17</td>
<td>Box bags in bottom cage</td>
<td>100</td>
<td>15.06 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td>3/18/18</td>
<td>Box bags in bottom cage</td>
<td>19.83 ± 3.11</td>
<td>16.9 ± 0.55</td>
<td>20.1 ± 2.5</td>
</tr>
</tbody>
</table>
3.3.3.6 Summer 2018

All grow-out experiments concluded after the summer 2018 sampling event. The clams deployed in 2017 were resampled on 2 July at UBB and on 17 July at LEH. Similar to what was observed during the first round of deployments, final surf clam survival was again much higher at LEH compared to UBB (Figure 3-13; Table 3-2). Evidence of dramatic surf clam predation was again found at UBB; only 11 live clams were found in each of the replicate bags (Table 3-2). Both bags present at LEH lost approximately the same number of clams (8% of initial stocking density), unlike the highly disparate survival observed during the previous sampling event (Table 3-2). Final survival of the two surf clam bags deployed in 2017 was 53.4% and 16.5%, respectively.

Over the duration of the study, these LEH clams exhibited a growth rate of 0.073 ± 0.0019 mm day⁻¹ (Figure 3-14) and had a mean shell length of 37.2 ± 0.5 mm (Table 3-2). Moreover, between April and July 2018, the growth rate of these clams was 0.12 ± 0.014 mm, the highest growth rate documented for clams at LEH over the two-year study (Figure 3-14). The few surviving clams at UBB had a similar growth rate over the study duration (0.062 ± 0.0012 mm day⁻¹; Figure 3-14) and achieved a similar mean shell length (32.65 ± 0.33 mm; Table 3-2) to those at LEH. Although 68.18% of UBB clams and 98% of LEH clams were pastaneck market size (Table 3-1), clams from both sites were in similarly poor condition (LEH: 11.1 ± 0.3; UBB: 10.7 ± 0.2; Figure 3-16).

Clams initially deployed at LEH in 2016 were sampled twice during the summer, on 17 July and approximately one month later, on 10 August. In July, the 5-mm bag that contained the set of pooled clams was assessed. Survival over this interval (April – July 2018) was 56.9% and condition fell to 10.8 ± 0.7 (N=25 clams) (Figure 3-13; Figure 3-16). However, average daily growth rate of these clams rose to 0.076 mm day⁻¹ (Figure 3-
112

14), with a mean shell length of 43.94 ± 1.18 mm (Figure 3-15). Additionally, 32% of clams reached the littleneck market size and 68% were at the pastaneck size (Table 3-1).

August 2018 represents the conclusion of grow-out experiments for clams at LEH. The remaining four bags of surfclams were sampled. Final survival of clams deployed in October 2016 varied immensely depending on bag mesh size (5-mm: 12.4%; 9-mm: 0.8%) (Figure 3-13). Survival of clams deployed in December 2016 also varied considerably between the two remaining bags, despite both replicates being reared continuously in 5-mm bags (8.6 ± 6.9%; Figure 3-13). Surfclam condition was similar across deployments (Oct 2016: 9.2 ± 0.7, N=1 replicate, N = 25 clams; Dec 2016: 9.5 ± 0.3, N=2 replicates, N= 25 clams per replicate; Figure 3-16) and similar to clams examined a month earlier.

Over the entire study duration, growth rate of surfclams deployed in October 2016 and December 2016 was nearly identical (Oct 2016: 0.052 mm day⁻¹; Dec 2016: 0.052 ± 0.0029 mm day⁻¹; Figure 3-14). Surfclams deployed in October 2016 had a final mean shell length of 47.16 ± 1.34 mm (N=1 replicate bag; N=25 clams), while those deployed in December 2016 had a final mean shell length of 47.39 ± 1.79 mm (Figure 3-15). Moreover, between 20% and 33.3% of all clams that reached pastaneck size, between 64.4% and 80% were littleneck size, and no more than 2.2% were middleneck size (Table 3-1).

Polynomial growth models were fit to the shell length data collected from the live clams measured during this and previous sampling events. This was conducted for both of the 2016 LEH deployments. These models were then used to predict the death dates for each dead clam measured on this and all previous sampling events, where data were
available (Figure 3-18; Polynomial Regression, Oct 16: $R^2=0.87$; Dec 2016: $R^2=0.88$). These plots support the survival data, showing that most mortality occurred during the summer months.

Similar to the previous sampling events, several species continued to be associated with the surfclams. At LEH, the was heavy macroalgae fouling (*Ulva* spp, *Enteromorpha* spp, *Gracilaria* spp), which formed thick sheet over the mesh bags. These fouling organisms were observed at UBB but in much smaller abundances. Several small blue crabs (*C. sapidus*) were seen swimming around both sites. Likewise, numerous mud crabs (*D. sayi* and *P. herbstii*) and polychaete worms were found inside and around the mesh bags. Interestingly, an oyster toadfish (*Opsanus tau*) was found foraging among the surfclams; this fish was also brooding a clutch ≈300 eggs that were deposited on the cinderblock mooring for the data loggers.
Figure 3-19. Environmental data at three grow-out sites. Temperature, salinity, and dissolve oxygen (DO) timeseries at Upper Barnegat Bay (top left), Little Egg Harbor, (middle left), and Absecon Bay (bottom left). Chlorophyll and turbidity timeseries at Upper Barnegat Bay (top right) and Little Egg Harbor (middle right). Left panels: mean daily temperature (°C, black circles), salinity (purple diamonds), and DO (mL/L). Right panels: mean daily chlorophyll a (µg/L, green circles) and turbidity (NTU, brown diamonds). Error bars represent 95% confidence intervals for all panels.
3.3.3.7 UBB environmental data

The CTD and fluorometer were both deployed with the initial cohort of clams on 8 November 2016. Temperature, salinity and dissolved oxygen data were collected continuously throughout the two-year study period. Temperature followed predictable seasonal trends, within annual maxima observed in July and annual minima observed in December or January (Figure 3-19). Temperatures declined from 12.64 ± 0.21°C on 8 November to a minimum of 0.35 ± 0.52°C on 16 December. During this time, 38 of the 100 days from 13 December 2016 to 22 March 2017 had mean daily temperatures ≤ 4.0°C. The temperature fell below 1°C on 9 January 2017. Water temperature briefly rose between 7.53 ± 0.24 and 9.06 ± 0.24°C from 23 February and 3 March, before declining again to 1.29 ± 0.43°C on 16 March. From this local minimum, water temperature steadily increased until it reached the maximum mean daily temperature for both the year and for the clams of the first deployment: 26.50 ± 0.41°C on 22 July 2017. The only other day in 2017 with a mean daily temperature above 26°C was 23 July. Moreover, 95 of the 122 days from 12 June to 11 October 2017 had mean daily temperatures ≥ 21°C. After reaching this peak, water temperature declined for the remainder of the summer and fall. Winter water temperatures were colder than the previous year, with 55 of the 100 days from 13 December 2017 to 22 March 2018 having mean daily temperatures ≤ 4.0°C. The temperature fell below 1°C for over two weeks, between 31 December 2017 and 15 January 2018. Interestingly, another local maximum occurred around the third week in February 2018. Temperature then briefly fell back down to 5°C before steadily rising for the remainder of the study. Maximum mean daily temperature observed during 2018 was
23.41 ± 0.66˚C, and 14 of the 30 days from 2 June to 2 July had mean daily temperatures ≥ 21˚C.

Generally, during the fall and winter of 2016-2017, the salinity at UBB ranged from 26 to 31 (Figure 3-19). However, a number of low salinity events were recorded over these seasons. At least five separate events caused the salinity to fall below 20. Each event corresponded to a temperature minimum. The maximum mean daily salinity recorded in 2017 was 31.5 ± 0.15, and after this peak, salinity declined, and remained between 22 to 27 until the middle of July. It is unclear whether this decrease in salinity represents a natural phenomenon or an instrument malfunction. Data were extracted from the logger and the device was redeployed after the summer 2017 sampling event. After that sampling event, salinity generally remained between 25 and 30 for the duration of 2017. However, from 17-19 December 2017, another low salinity event occurred, where salinity fell sharply and stayed between 11 and 12 for three days. Another less drastic low salinity event was documented between 1-3 January, where salinity fell to 20.9 ± 1.3. Soon after this local minimum, maximum mean daily salinity for 2018 was recorded: 30.8 ± 0.1 on 15 January 2018. Salinity remained between 27 and 30 for several weeks, but then began to decline for the remainder of the study period. On the final day of sampling on 2 July, mean daily salinity was recorded at 23.0 ± 0.2. Again, it is unclear whether the spring decrease in salinity represents a natural phenomenon or an instrument malfunction.

Throughout the two-year experiment, the environment around the clams was well oxygenated (Figure 3-19). Mean daily dissolved oxygen remained extremely consistent; over the two years, mean daily standard deviation was less than 0.24 mL L⁻¹. Clams in
the first deployment experienced conditions which ranged from a high of 9.13 ± 0.23 mL L⁻¹ on 16 December 2016 to a low of 4.75 ± 0.032 mL L⁻¹ on 22 July 2017. Clams in the second deployment experienced conditions which ranged from a high of 8.57 ± 0.0055 mL L⁻¹ on 9 January 2018 to a low of 5.25 ± 0.057 mL L⁻¹ on 1 July 2018.

At UBB, chlorophyll and turbidity data were collected over three intervals over the two-year study period: from 8 November 2016 to 5 February 2017, from 2 October 2017 to 1 January 2018, and from 16 March to 14 June 2018 (Figure 3-19). High concentrations of chlorophyll were observed during all three occasions. During fall 2016 and winter 2017, chlorophyll rose dramatically after the initial deployment and reached a maximum mean daily value of 47.8 ± 1.2 µg L⁻¹ on 21 December 2016. Although there were several large fluctuations, chlorophyll was generally above 25 µg L⁻¹ for this entire period, and only began to decline during the last week of sampling. During this period, the turbidity data distribution, although much smaller in magnitude, roughly followed the shape as the chlorophyll data distribution. Two high turbidity events occurred during which turbidity increased above 20 NTU; these occurred between 25-28 November 2016 and 23-27 January 2017. During fall 2017 and winter 2018, the chlorophyll data followed a bimodal distribution, with maxima of 51.5 ± 0.01 µg L⁻¹ and 51.4 ± 0.1 µg L⁻¹ occurring on 2 November 2017 and 3 December 2017, respectively. The chlorophyll stayed above 45 µg L⁻¹ for over three weeks between 13 October and 8 November 2017. During the second peak, between 20 November and 11 December 2017, the chlorophyll remained above 50 µg L⁻¹. After this, chlorophyll declined for the remainder of this sampling period. Turbidity similarly exhibited a bimodal distribution with peaks aligned to those observed for chlorophyll. However, maximum mean daily turbidity observed
during this interval was 26.3 ± 0.3 NTU. During the spring of 2018, chlorophyll again followed a bimodal distribution, with maxima occurring on 23 April 2018, and towards the end of the sampling interval on 14 June 2018. During the first peak, the chlorophyll remained above 36 µg L⁻¹ for one month. Again, turbidity data followed a similar bimodal distribution but exhibited a smaller magnitude.

3.3.3.8 LEH environmental data

The CTD and fluorometer were both initially deployed on 6 December, with the second 2016 surfclam deployment. Temperature, salinity and dissolved oxygen data were collected continuously through 17 July 2018. Overall, temperature at LEH followed predictable seasonal trends, matching those observed at UBB (Figure 3-19). At LEH, annual maxima were observed in July and annual minima were observed in December or January. Throughout the winter of 2017, 48 of the 100 days from 9 December 2016 to 18 March 2017 had mean daily temperatures ≤4.0°C. On four occasions, local minima occurred with mean daily temperatures dropping below 1°C. These events, which occurred on similar dates to minima observed at UBB, occurred 16 December 2016, 7-10 January 2017, 5 March, and 15-16 March. As with UBB, there was a brief warming between 23 February and 2 March and another between March 7-10, which saw mean daily temperatures briefly rise between 7 and 9°C. After mid-March, the water temperature steadily increased until it reached the maximum mean daily temperature of both the year: 26.85 ± 0.21°C on 18 July 2017. Mean daily temperature stayed above 26°C from 17 to 22 July. Additionally, 103 of the 146 days from 19 May to 11 October 2017 had mean daily temperatures ≥ 21°C. After reaching this peak, the water temperature declined for the remainder of the summer and fall. Throughout the winter of
2018, 49 of the 100 days from 13 December 2017 to 22 March 2018 had mean daily temperatures ≤ 4.0°C. As with UBB, temperatures at LEH fell below 1°C for a prolonged period (27 December 2017 - 20 January 2018), and temperatures briefly rose around the third week of February 2018. The temperature then briefly fell back below 5°C before steadily rising for the remainder of the study. For 35 of the 54 days from 25 May to 17 July 2018, the mean daily temperature was greater than 21°C. The mean daily temperature rose above 26°C every day between 2 and 5 July and culminated with the warmest recorded mean daily temperature of the year (28.60 ± 0.27°C).

Generally, during the fall and winter of 2016-2017, the salinity at LEH fluctuated between from 25 to 30 (Figure 3-19). Salinity at LEH also followed many of the same trends observed at UBB, including low salinity events, where salinity fell below 21. The lowest salinity observed was 13.3 ± 0.051 on 14 December 2016. Like UBB, each event corresponded to a dip in temperature. Salinity declined throughout much of the spring, reaching a low of 22.0 ± 0.011 on 2 June 2017. Notably, the salinity began to rise two weeks before the summer 2017 sampling event when the data were extracted from the logger and the device was redeployed. After that sampling event, the salinity generally stayed between 24 and 27 for the remainder of 2017. Much like conditions observed at UBB, salinity at LEH fluctuated during the winter of 2017-2018, with several low salinity events and a general decline in salinity after mid-January. After the spring 2018 sampling event, salinity briefly rose above 30, but then declined again for the remainder of the study. Again, it is unclear whether these gradual declines in salinity represents a natural phenomenon or instrument drift.
Based on the DO data collected, clams at LEH also did not experience oxygen limitation at any point throughout the two-year study (Figure 3-19). The mean daily dissolved oxygen remained extremely consistent, and generally varied no more than 0.19 mL L\(^{-1}\) per day. During the first round of grow-out experiments, clams experienced conditions which ranged from a high of 9.11 ± 0.021 mL L\(^{-1}\) on 29 December 2016 to a low of 4.80 ± 0.017 mL L\(^{-1}\) on 18 July 2017. During the second round, clams experienced conditions ranging from 9.49 ± 0.0080 mL L\(^{-1}\) on 8 January 2018, to 4.69 ± 0.021 mL L\(^{-1}\) on 5 July 2018.

At LEH, chlorophyll and turbidity were measured over three intervals spanning the two-year study period: from 6 December 2016 to 27 February 2017, from 29 June 2017 to 31 August 2017, and from 12 April to 11 July 2018 (Figure 3-19). In contrast to what was observed at UBB, chlorophyll concentrations at LEH rarely rose above 8 µg L\(^{-1}\). During the first sampling interval, mean daily chlorophyll concentration generally ranged from 3 to 8 µg L\(^{-1}\), although some short-lived peaks were observed with concentrations as high as 26.7 ± 2.8 µg L\(^{-1}\). Turbidity fluctuated widely throughout the first sampling interval, with values ranging from 5 to 25 NTU. During the second sampling interval, the mean daily chlorophyll concentrations were lower. Only a single, short-lived peak was observed at 15.7 ± 1.4 µg L\(^{-1}\). Turbidity was also low during this interval. Three peaks with turbidity values above 20 NTU were observed; each peak lasted a single day. The highest chlorophyll concentrations at LEH were documented during the third sampling event, which took place during the spring and summer of 2018. Here, chlorophyll concentration followed a bimodal distribution, with maxima occurring on 2 May 2018 (40.9 ± 2.6 µg L\(^{-1}\)) and on 21 June 2018 (51.5 ± 0.0029 µg L\(^{-1}\)). With
concentrations above 30 µg L⁻¹, the first peak lasted approximately four days and the second lasting for 17 days. However, beyond these peaks, chlorophyll concentrations stayed between 3 and 8 µg L⁻¹. Turbidity once again fluctuated throughout the sampling interval, with values ranging from 5 to 25 NTU. Notably, a prolonged peak in turbidity with values above 21 NTU.

3.3.3.9 AB environmental data

Conductivity, temperature, salinity and DO were continuously recorded at AB beginning on 12 December 2016. Overall, temperature, salinity, and DO at AB exhibited very limited diel variability (Figure 3-19). During the winter of 2016-2017, mean daily water temperatures ranged from 2 to 5°C. Throughout the winter, 30 of the 100 days from 13 December 2016 to 22 March 2017 had mean daily temperatures ≤ 4.0°C. Much like conditions observed at UBB and LEH, water temperature briefly fell below 1°C between 8-10 January, and briefly rose above 10°C between 24-26 February. After 15 March, the temperature began to steadily increase and reached a maximum mean daily temperature of 24.70 ± 0.13 on 10 July 2017. Additionally, 25 of the 53 days from 19 May to 10 July had mean daily temperatures ≥21°C.

Initially, mean daily salinity ranged from 29.1 to 31.5, but suddenly dropped on 23 January 2017 and remained between 22.1-25.2 until the next sampling event (Figure 3-19). After the data were extracted from the logger and redeployed, mean daily salinity rose above 30.5 for a few days before declining once again. Between the middle of April and the final sampling event, mean daily salinity at AB ranged from 24.1 to 27.2. These gradual declines in salinity were similarly observed at UBB and LEH, but it remains
unclear whether this represents a natural phenomenon across the region or a malfunction with all three sondes.

Similar to conditions observed at the other two sites, DO at AB suggests that the water remained well oxygenated throughout the duration of the study (Figure 3-19). Mean daily dissolved oxygen at AB ranged from $8.55 \pm 0.024 \text{ mL L}^{-1}$ on 9 January 2017 to $4.95 \pm 0.017 \text{ mL L}^{-1}$ in on 10 July 2017.
3.4 Discussion

3.4.1 The effect of early nursery gear type on surfclam survival and growth

Multiple rearing methods tested in the early nursery phase proved relatively equally effective at producing surfclams. The optimal method may vary by the needs and conditions of a given nursery. This result supports earlier research which found that various iterations of commonly used bivalve culture methods can effectively rear surfclams, if proper levels of food availability and stocking density are met (Goldberg, 1980; Liu et al., 2011).

Bivalves generally experience high mortality during larval and early post-larval development (Mann, 1984). In this experiment, survival was highly variable among treatment replicates, suggesting early post-metamorphic surfclams will succumb to some level of mortality, regardless of rearing method. Immediately after metamorphosis, surfclams gills are underdeveloped and can take up to two weeks before becoming fully functional. During this period, surfclams may rely on pedal feeding, where the foot is used to collect food (J. Grassle, Rutgers University, personal communication). Therefore, one hypothesis that may explain the observed post-metamorphic mortality is that surfclams that do not have access to adequate food resources during the peri-metamorphosis period will experience latent mortality. It should also be noted that many clams in both treatments became covered in biofouling organisms, specifically the stalked ciliates, *Vorticella spp.* and *Zoothamnium spp.* These biofouling organisms have been associated with nursery failures in previous studies (Goldberg, 1980). Additionally, clams reared in raw water flow-through conditions have more opportunities for contaminant
exposure, which underscores the risk associated with exposing early juvenile bivalves to unfiltered seawater too early in development.

During the first two weeks of the experiment, surfclams reared in downwellers grew faster than those in bell siphon systems. Temperature likely drove the observed differences, although food availability, flow rate, and interspecific interactions may have also contributed. With a mean temperature of 18.7°C, temperature in the downwelling system was closer to optimal surfclam conditions (Acquafredda et al., 2019). Clams in the bell siphon experienced a mean temperature of 15.7°C over the same 21-day period. Although it had a slower flow rate than the bell siphon system, the feeding ration in the downwelling system should have provided food at sufficient concentrations to sustain growth and survival (Walker et al., 1998; Acquafredda et al., 2019). Unfortunately, no measurements of food availability were collected in the bell siphon treatment. Despite this, food limitation was unlikely to occur due to high flow rates and relatively low stocking density in each experimental unit.

Interestingly, when clams reared in downwellers were moved to upwellers, their growth rate became more similar to those reared entirely in bell siphon systems. Growth effectively stopped for the first week after clams were transferred. It is possible that these clams were not yet acclimatized to the unfiltered water conditions and temporally ceased growth. These clams subsequently grew slower, than those reared entirely in flow-through conditions. Goldberg (1989) showed that juvenile surfclam growth was inversely related to stocking density when reared in upwellers receiving ambient seawater at a fixed flow rate, but if flow rate varied, growth was also influenced by phytoplankton availability. In the present study, clams received seawater from the same source and were
stocked at identical densities. Although flow rates were not consistently measured in the early nursery experiment, they did vary between bell siphon and upwelling treatments. Thus, the slightly, albeit not significantly, different growth rates may have been caused by food availability. Taken together, results of this experiment reinforce the concept that environmental parameters like temperature and food availability are important modulators of surfclam growth.

Ultimately, recirculating downwelling systems serve to provide highly controlled conditions for clams in the nursery phase. In turn, clams can be provided constant and optimal environmental conditions and shielded from predators, biofouling organisms, and pathogens they may become exposed to in flow-through conditions. However, if costs associated with heating and cooling seawater are prohibitive, or algal production capacity is limited, adequate early juvenile surfclam growth and survival can be achieved using the bell siphon method. Transferring clams from either method to an upwelling system is recommended due to increased flow, which can provide more diverse and more abundant food for growing clams. Additionally, aquaculturists should carefully acclimatize surfclams when transferring them from controlled to flow-through condition to sustain growth.

3.4.2 The effect of late nursery gear type on surfclam survival and growth

Upwelling systems tended to support greater surfclam survival and growth compared to raceways systems, with or without the addition of sand. Although clams received seawater from the same source and were stocked at identical densities, flow rate in the upwelling system was more than six-times faster than the raceway treatments.
Interestingly, flow rate only had a significant influence on growth, not survival. This may suggest that flow rates of the raceway systems did provide enough food for somatic maintenance but were inadequate for providing the additional nutrition necessary for sustaining growth.

Phytoplankton availability via flow rate has previously been shown to be an important control on juvenile surfclam growth (Goldberg, 1989). A previous study showed that surfclams can be successfully reared in both flow-through upwellers supplied with unfiltered seawater and recirculating upwellers supplied with filtered seawater and cultured algae (Goldberg, 1980; O’Beirn et al., 1997). However, recirculating upwelling systems supplied with cultured algae tend to become inefficient after surfclams reach 5 mm in shell length. This is because stocking densities must be kept low and algae production must be kept high to achieve the growth necessary for commercial surfclam production (Goldberg, 1980). This was the case when O’Beirn and colleagues (1997) grew surfclams in recirculating upwellers and fed them with cultured algae, since it took 14 weeks for those clams to grow from 6.4 to 15.3 mm.

In the present study, all clams were reared in flow-through conditions, yet the flow rates varied significantly. Clams reared in upwellers grew from 1.1 to 18 mm in 48 days, and doubled their growth rate after reaching 10 mm. By contrast, clams in raceway systems grew at effectively the same rate between 4.25 and 6.50 mm as they did between 10 and 15 mm. If flow rate was indeed a proxy for food availability in this study, then these results align well with previous research.

Although most species of clams, including surfclams, are infaunal organisms, land-based nursery systems tend not to include sediments because sediments make it
difficult to keep cultures clean, healthy, and pest-free. The unidirectional flow of water through a shallow raceway system is unlike the tidal flow experienced by clams in the wild. Depending on certain factors, like flow rate and tank dimensions, a raceway system may hinder transport of waste products away from the clams. Sediment that falls out of suspension and accumulates on the clams, further limiting water flow. Collectively, these raceway system inefficiencies may reduce food and oxygen delivery to the clams.

However, Goldberg (1980) did utilize sand in raceways as a grow-out system and successfully grew clams from 18 mm to 50 mm in 6 months. However, in that study, the flow rate was substantially higher (50 L min\(^{-1}\) vs. 8.1 L min\(^{-1}\)) and stocking density was much lower than even the lowest stocking density used in this experiment (100-500 clams m\(^{-2}\) vs. 38,500 clams m\(^{-2}\)).

I observed the clams burrowing up through the mud to maintain a constant distance from the sediment/water interface. On many occasions, clams in the sand-filled raceway treatment became entirely surrounded by mud, not sand. Although neither dissolved oxygen (DO) nor pH were measured in this study, I hypothesize that the poor growth and high mortality in the raceway treatments may have been caused by mud accumulation that became hypoxic and/or hypercapnic. In fine sediments rich in organic matter, there is strong coupling between hypoxic and acidified conditions via microbial respiration (Gobler & Baumann, 2016). In two-month old hard clams (*Mercenaria mercenaria*), low DO reduced survival, low pH reduced growth, and the combination resulted in additive reductions of both metrics (Gobler et al., 2014). Interestingly, the same study showed that while growth of four-month old hard clams was not impacted by either stressor alone, growth was synergistically reduced when the two stressors were
Surfclams are sensitive to low-oxygen conditions; in fact, mass die-offs of wild surfclams have been documented during hypoxic events off the coast of New Jersey (Garlo et al., 1979). More recent research also suggests surfclams are vulnerable to acidified conditions (Pousse et al., 2020). No study has examined how surfclams respond to the combined impact of these stressors, highlighting a need for further research.

Nursery systems that utilize substrate typically do so because some infaunal bivalves rely on pressure exerted by the surrounding substrate to provide the necessary environment for proper shell formation. For instance, basket cockles (*Clinocardium nuttallii*) occasionally exhibited shell deformities when they were not reared in substrates (Epelbaum et al., 2011; Dunham et al., 2013). Substrate also protects infaunal bivalves from biofouling organisms (Marshall & Dunham, 2013). Yet there are additional, more practical problems with using sand in raceway systems that make it an unattractive option for shellfish growers. Nursery systems involving sediments can more easily harbor pathogens, predators, and other contaminants. Although not quantified, the sand-filled raceway system undoubtedly proved to be the most laborious and time-consuming to maintain in this study. While the raceways without sand stayed significantly cleaner than those with sand, they still accumulated considerably more sediment than the upwellers. Upwelling systems required the least maintenance, accumulated the least sediment, and promoted the greatest surfclam growth and survival. In the present study, no deformed surfclams were observed and no biofouling organisms were observed beyond the stalked ciliates, which disappeared after the surfclams reached approximately 2 mm in shell length.
Taken together, these results suggest that rearing in substrate provides no advantage to juvenile surfclams, with respect to survival or growth. Furthermore, substrate is unnecessary for proper juvenile shell formation to at least a shell length of 18 mm. Shallow raceways without sand were not an effective nursery culture method, although they may prove to be suitable if used with greater flow rates and less turbid seawater. Since upwellers were the most efficient late nursery gear type tested in this study, and they are already among the most common systems used by Northeast growers, they will most likely be promoted if surfclam aquaculture becomes more widely adopted.

3.4.3 Evaluating surfclam performance across several NJ farms

3.4.3.1 Survival

During the grow-out experiments, surfclam survival was highly variable across farms and was influenced by two main factors: poor environmental conditions and predation. At best, the 9-month (fall 2016 – summer 2017) survival of farmed surfclams was 78-91% and the 20-month (fall 2016 – summer 2018) survival was 9-12%. At worst, 100% mortality was observed. Despite this, it is possible that survival could be greatly improved with minor adjustments to the tested culture techniques and by utilizing other information gained from this study.

The poor environmental conditions that led to surfclam mortalities fall into three categories: smothering and low DO, warming during summer months, and aerial exposure during freezing temperatures. With 85% of sediment grains less than 125 µm, the sand at AB was the finest among the farms. It is likely that the combination of fine sediments and anti-predator screen laid on top of the mesh bag ultimately led to the
observed anoxic conditions and complete surfclam mortality. Based on the size of dead shells (death assemblage), hypoxic conditions may have started as early as 90 days post deployment and caused mortality slowly over time. This is supported by previous research that showed that at 10°C, it took 8-10 weeks before 50% of adult surfclams died in response to DO levels of 1 mL L$^{-1}$ (Thurberg & Goodlet, 1979). Low DO may have also contributed to the relatively poor condition of AB clams, which were more than 30% lower than clams at the other two farms in the spring of 2017. However, low DO conditions stand in contrast to the information collected by the data logger, which recorded well-oxygenated water throughout the duration of the study. Most likely, the microhabitat around the clams in the sediment differed from what the data logger was recording above the bottom. Not only was the data logger 10 m away from the clams, but it also was raised 25 cm above the sediment. Interestingly, the clams in the piloted gear types at AB (the recruitment box and under the anti-predator screen) did not experience anoxic conditions but did show low condition. This suggests that fine sediments alone did not cause the severe mortality. Instead, the double containment (mesh bags plus an anti-predator screen) most likely exacerbated the natural propensity of fine sediments to go anoxic by limiting water flow, trapping waste produced by the clams, and collecting particles that fell from the water column. Moreover, low DO conditions would have worsened as water temperature increased, particularly between May and July 2017 when temperatures rose above 21°C.

Although more subtle, heat-induced mortality was also observed during the grow-out experiments. This is perhaps best exemplified by the mortality experienced by clams at LEH. For clams deployed in October 2016, mean survival of clams retained in 5-mm
bags was reduced from greater than 75% in June 2017 to lower than 25% in October 2017. Likewise, for clams deployed in December 2016, mean survival over this period was reduced from more than 90% to less than 40%. Although smaller individuals (SL < 50 mm) have a wider thermal tolerance than large-bodied individuals (SL > 120 mm), prolonged exposure to temperatures above 21°C can range from tolerable to lethal depending on the condition of the clam and other factors like food availability and DO (Weinberg, 2005; Munroe et al. 2013; Acquafredda et al., 2019; Acquafredda et al., 2020). Temperatures between 26 and 28°C are almost certainly lethal for surfclams if sustained for several days (Acquafredda et al., 2019; Acquafredda et al., 2020). At all sites, temperatures regularly rose above 21°C for most of the late spring, summer, and early fall. Yet the mean daily temperature of a farm rarely rose above 26°C; the longest duration occurred at LEH for six days during July 2017. The chlorophyll concentration observed over the same period was low, staying well below 5 µg L^{-1}. Together, warm temperatures and low food availability could explain the aforementioned mortalities at LEH.

Substantial mortality was also observed during the 2017 deployment, particularly among clams deployed in box bags in bottom cages. Dead shells remained articulated and there were no signs of predation. Together with the environmental data collected at UBB and LEH, it is possible that the substantial mortality was caused by severe winter weather. In late December 2017 and early January 2018, coastal areas of southern New Jersey experienced large snowfall events and extremely low air temperatures (Office of the New Jersey State Climatologist [ONJSC], 2020). There were also blow-out tide events that may have exposed the cages to freezing air. It remains unclear whether the
observed clam mortality was a result of the low-salinity conditions caused by the influx of snow and meltwater, or if the clams were exposed to freezing air. Based on observations at other, non-participating shellfish farms in the region, the cages may even have become encapsulated in ice. This may also be the reason why one of the two mesh bags deployed in 2017 at LEH suffered substantially worse mortality, since it was oriented closer to the bottom cage and in a slightly shallower area than the bag next to it. While this represents an unfortunate loss for our ability to gauge the effectiveness of this gear system, preliminary research suggests that this method is capable of productive surfclam culture. Nevertheless, this incident does illustrate an important aspect of surfclam aquaculture: subtidal surfclam aquaculture is less risky in areas that are not prone to low salinity events, large tidal fluctuations, and/or freezing conditions.

Surfclam aquaculture appears to be associated with a diversity of macrofauna, similar to what has been observed at other clam and oyster farms (Luckenbach et al., 2016; Mercaldo-Allen et al., 2020). Although many of the taxa observed were commensal, some were quite clearly predators. Predator management is a key facet of all bivalve aquaculture, and the vulnerability of surfclams to predation was well documented in this study. This is best highlighted by the data collected at UBB. Between 95 and 99% of clams suffered mortality between the fall and the following summer of both deployments, and much of the mortality can be attributed to blue crab (*Callinectes sapidus*) and moon snail (*Euspira heros*) predation. Both species are well known predators of the surfclam (Weissberger, 1999; Weissberger & Grassle, 2003). Notably, this level of predation was not apparent at the other sites, and predation by other species was not observed. However, mud crabs (*Panopeus herbstii* and *Dyspanopeus sayi*) were
abundant throughout the two-year study, but their interspecific interactions with surfclam remains unclear. Further research should be devoted to understanding whether these species are in fact surfclam predators.

Unlike the bags at LEH and AB, which were placed among extensive aquacultured hard clam beds, the bags at UBB were placed in a sandy, unstructured area several meters away from bottom cage oyster culture. Burrowing bivalves experience increased predation pressure at high densities, but when individuals > 20 mm are reared together, hard clams experience lower predation pressure compared to surfclams (Desbonnet, 1989). This is because hard clams enter a partial predator refuge at 20 mm (Malinowski, 1985), whereas surfclams do not gain this protection until reaching 35 mm (Desbonnet, 1989). The difference in refuge size is related to differences in shell thickness, convexity of shell, and shell gape; surfclams have thinner, less convex shells that are easier for predators to break (Elner & Hughers, 1978; Boulding, 1984). At UBB, predators may have benefited from increased foraging efficiency. Although each replicate bag was stocked at the same density among the farms, the bags at LEH and AB, may have been shielded by the abundant hard clams around them, while small surfclams at UBB were targeted by predators, skewing the size frequency of the survivors larger, to approximately 36 mm.

Although the 5-mm nylon bags were prone to being torn by crabs, the 9-mm bags allowed predators to freely enter and feed on the clams. Similar results were seen in Georgia, where 3-mm mesh bags produced greater surfclam survival than bags with larger mesh (Walker, 2001). Therefore, mesh bags with small mesh size (3-5-mm) composed of a more durable material should be tested as a way to mitigate crab and snail
predation. Partially buried cages should be evaluated in other parts of the Northeast, since previous research in Long Island Sound and coastal Georgia has shown surfclams reared in cages outperformed those reared in mesh bags (Goldberg, 1989; Walker, 2001). A hybrid concept is being piloted in Massachusetts, where surfclams are grown-out in partially buried HDPE mesh bags strung together with a longline (J. Reitsma, Woods Hole Sea Grant, personal communication).

3.4.3.2 Condition and gonad maturation

Condition is an important metric for shellfish growers because it provides insight into the health of a farm and the intensity of its production (Filgueira et al., 2013). Clams with low condition are more prone to predation and succumbing to other forms of mortality. When clams are in poor condition, they are also less marketable, especially if the clams are sold by the pound instead of by the piece. In this study, the seasonality of surfclam condition and gonad maturation followed predictable trends, where low temperatures and high food availability promoted higher condition in spring and the opposite led to low condition during summer and fall. Decrease in condition observed during summer appeared to be related to surfclam spawning, which occurred between the spring and summer sampling events. Since temperatures remained above 21°C well into October and chlorophyll data suggest food quantities were low, it is unsurprising that surfclam condition during fall was equivalent to what was observed during summer.

Previous studies have found that surfclams on farms and in the wild have higher condition and develop gonads during the spring and fall, and condition tends to decrease immediately after spawning (Ropes, 1968; Spruck, 1995). Diatom algal blooms have
been proposed as a critical component of successful gametogenesis (Goldberg, 1980; 1989). Wild surfclams off the coast of New Jersey usually spawn during the early summer and occasionally during the fall (Ropes, 1968; Chintala & Grassle, 1995). None of the clams sampled in the fall had gonad present, indicating that farmed surfclams in coastal New Jersey bays are unlikely to spawn during the fall, possibly because environmental conditions are not conducive for maintaining gonad through the summer or developing new gonads during a second round of gametogenesis.

Maturation and time of first spawning vary across surfclam populations. Belding (1910) remarked that one-year-old surfclams are capable of spawning if they attain a shell length of 39 mm, but more successful spawning occurred during a surfclam’s second year, upon reaching 67 mm. In Prince Edward Island, Canada, surfclams may not spawn until they reach four years of age and a shell length of 80-95 mm (Sephton, 1987). However, surfclams as young as three months post-settlement (SL = 7-10 mm) were documented with ripe and spent gonads off the coast of New Jersey and were shown to produce viable larvae after being spawned under laboratory conditions (Chintala & Grassle, 1995).

In the present study, gametogenesis was observed in clams as young as 11-months old with shell lengths of 25 mm. Although many individuals were still immature in the spring of 2017, the sex ratios at both LEH and AB skewed male. Wild populations of surfclams generally have even sex ratios (Ropes, 1968; Sephton, 1987). However, male-skewed sex ratios are not uncommon in juvenile bivalves and have previously been described in yearling farmed surfclams (Spruck, 1995). Clams in the mesh bags also matured more quickly than those in the piloted gear types. In the spring of 2017, between
75-90% of clams in the mesh bags across all farms were developing gonad compared to only 25-45% of clams in the recruitment box or the anti-predator screen. Although the clams were all the same age, the clams in the piloted gear types were approximately half the size of those in the mesh bags at initial deployment. Their smaller size was a consequence of higher stocking densities in the nursery. By the spring sampling event, these clams had grown to the same size as those deployed in mesh bags. This demonstrates that the clams in the piloted gear types exhibited compensatory growth, directing more energy to shell and somatic tissue biomass at the expense of gonad development. During spring 2018, two-year-old clams had initiated gametogenesis earlier than the yearling clams, with 90% of the former being ripe by April. This discrepancy in the onset of gametogenesis among different clam cohorts requires further investigation, primarily because of its implications for producing surfclam broodstock.

By controlling the temperature of the holding tank, practitioners can maintain ripe broodstock or promote the maturation of clams that have begun the early stages of gametogenesis (Goldberg, 1980; 1989; Walker & Hurley, 1995). However, there is currently no published protocol for conditioning immature surfclams or surfclams that are in the inactive phase of gametogenesis. There is also very little information about the nutritional requirements necessary for adequate surfclam ripening. Currently, growers must procure ripe broodstock from individuals conditioned on the farm. In order for surfclam aquaculture to become more widely adopted, broodstock conditioning protocols must be developed.
3.4.3.3 Growth

Despite the highly variable survival, surfclam growth remained relatively consistent across farms throughout the two-year study. Wild surfclams prefer medium to coarse sand habitats that contain less than 5% silt (Yancey & Welch, 1968; Franz, 1976), and surfclam abundance correlates with increasing sediment grain size up to coarse sand (Olsen, 1970). They also prefer clear water with suspended solid concentrations below 5 mg L$^{-1}$ (Robinson et al., 1984). However, studies of farmed surfclams demonstrate that they tolerate environments that deviate from the optimal conditions they experience on the continental shelf. Goldberg & Walker (1990) found no effect of sediment type on growth when clams were reared in mud ($\leq 64 \, \mu$m), sand (64 - 2 µm), or an even mix of the two. Yet a follow-up study did find that clams grew significantly worse in mud than sand (Walker & Heffernan, 1990a). In this study, growth rates were relatively consistent across farms despite the farms having considerably different sediment compositions. Therefore, sediment grain size likely did not have a substantial influence on surfclam growth. Similarly, turbidity likely did not substantially affect surfclam growth, since all recorded turbidity values were far below levels considered detrimental to surfclams (Robinson et al., 1984).

Surfclam growth did align with seasonal trends in temperatures and food availability. Growth rates were consistently highest (0.06-0.12 mm day$^{-1}$) between spring and summer, corresponding to a rapid rise in temperatures from 5 to 20°C. Peaks in chlorophyll concentration were also documented at UBB and LEH during this period. Conversely, growth rates were low (0.02-0.03 mm day$^{-1}$) between summer and fall, when temperatures peaked and slowly fell. Chlorophyll data during this period was only collected at LEH, and chlorophyll concentration was generally less than 5 µg L$^{-1}$.
Interestingly, growth rates between fall and spring were inconsistent across years. Between fall 2016 and spring 2017, growth rates were very high (0.05-0.11 mm day\(^{-1}\)). However, between fall 2017 and spring 2018, growth rates were among the lowest recorded (0.01-0.05 mm day\(^{-1}\)).

I hypothesize that the discrepancy between the fall-spring growth rates was related to the varying environmental conditions each year. Although more cold-tolerant than other commercially reared bivalves, surfclams become inactive when water temperature falls below 4°C (Saila & Pratt, 1973). Temperatures fell below this threshold for long periods each winter. AB experienced the mildest conditions with only 30 of 100 days falling below 4°C. At UBB, it was considerably colder during the winter of 2017-2018, with two and half more weeks below 4°C compared to the previous winter. However, cold temperatures alone do not seem to explain the discrepancy in growth among years. Poor growth was observed at LEH, and at the nearby site, RC, during fall 2017-spring 2018, despite LEH experiencing approximately the same number of days below 4°C both winters. During fall 2016-spring 2017, surfclams performed better at all sites, despite several low salinity/low temperature events at LEH and UBB. Each of these events was short-lived, lasting at most a few days. These events may have been caused by the data loggers becoming partially encapsulated in ice, in which case they would be recording fresher water than the surrounding environment. Alternatively, partial aerial exposures could have caused these anomalies. In either case, the clams would not have experienced the harsh conditions, since the data loggers were elevated 25 cm above the bags. If the clams did encounter stressful condition during these events, the impact was minimal, since high survival and growth were observed at all farms in spring 2017.
By contrast, there was a considerable period of time during the winter of 2017-2018 when temperatures were below 1°C (27 December to 20 January). As stated above, this period corresponded to harsh winter conditions that would have caused the clams in the box bag/bottom cage gear system to suffer heavy mortalities. These clams almost certainly became exposed for an extended period and may have even been encapsulated in ice. The fluorometer deployed at UBB during this period also recorded this event; a large peak in chlorophyll concentration fell as the logger became exposed in late December 2017. During this month-long period, clams that ultimately survived may have cycled between short periods of aerial exposures and short periods in shallow water. Therefore, the clams may not have been able on capitalize on this highly productive time, causing them to be less well provisioned throughout the remainder of the winter and early spring. Other studies have found that when surfclams were grown-out at various tidal levels, growth and survival decreased as exposure time increased; like all bivalves, surfclams are incapable of feeding and are at risk of freezing or desiccating when they are not submerged (Walker & Heffernan, 1990a; 1990b; 1990c).

Beyond temperature, food availability, and depth, salinity may have also influenced surfclam growth. Although the lower salinity threshold for adult surfclams is 12.5, surfclams prefer more oceanic salinities (Castagna & Chanley, 1973). Beyond the aforementioned low salinity events, salinity at the farms rarely fell below 20. However, salinity also rarely rose above 30. Consequently, sub-optimal salinity may have contributed to slower surfclam growth relative to what has been reported in other studies (Goldberg, 1980; 1989; Walker, 2001).
Stocking density is also an important factor that influences surfclam growth, both in the wild and on the farm (Weinberg, 1998; Goldberg, 1989; Walker, 2001). Wild surfclams exhibit density-dependent growth, an indicator of intraspecific competition for resources, throughout a significant portion of their range (Weinberg, 1998). Previous studies that have examined cultured surfclam stocking density found that surfclams can reach 50 mm within a single growing season (Goldberg, 1980; 1989; Goldberg & Walker, 1990; Walker & Heffernan, 1990a; Walker, 2001). In the present study, surfclams were initially deployed at 3400 clams m$^{-2}$, which ranges from 6.8 to 34-times higher than densities used by Goldberg (1980) and Walker (2001). When Goldberg (1989) stocked at 2000 clams m$^{-2}$, clams grew from 15.7 to 32.0 mm between June and November; this corresponds more closely to the results of this study. Prior research also showed that clams exhibited greater growth in bottom cages compared to mesh bag, and mesh size had no effect on growth (Walker, 2001). Collectively, high stocking densities and the use of mesh bags may explain why growth observed in the present study was slower than previous research. Notably, when stocking density was reduced, whether intentionally after the first summer sampling event or from mortality, growth did not increase. This suggests that the aforementioned environmental conditions may have placed an upper bound on growth.

One important consideration when comparing the present study to previous research is that stocking densities used in many earlier studies are not efficient for commercial-scale production by modern aquaculturists. While it took 21 months for more than 65% of surfclams to reach the littleneck size ($44.45 \leq x \leq 57.15$ mm), between 67-98% of clams reached pastaneck size ($31.75 \leq x < 44.45$ mm) within 9 months. The
pastaneck size is a relatively new size class of farmed clams. As its name implies, pastanecks are marketed as a product intended to be cooked with pasta or other accompanying ingredients, rather than for the half-shell market to be consumed raw. Rearing larger quantities of smaller surfclams has advantages for farmers. It reduces the implicit risk of growing any crop by shortening the amount of time it remains on the farm. The largest risk that cannot be mitigated with adjustments to husbandry practices is vulnerability of surfclams to warm water temperatures, which depresses growth and causes mortality. Future research should go beyond studying optimal stocking densities for surfclam growth, and instead consider optimal densities for commercial-scale surfclam production. Additionally, more research should be devoted to developing heat-tolerant surfclam stocks that could cope with the summer conditions on many Northeast shellfish farms.

3.4.4 Concluding summary

In this study, various methods of surfclam rearing during the nursery and grow-out phase of production were tested and evaluated. In accordance with earlier bivalve nursery work, results of this study indicate that multiple rearing methods can effectively produce surfclam seed (Goldberg, 1980; Liu et al., 2011). Although the optimal method may depend on the needs and capacity of a given nursery, some central tenets are important for aquaculturists to consider. Higher flow conditions (~50 L min\(^{-1}\) vs. ~8 L min\(^{-1}\)) lead to greater growth and supported survival. Rearing early post-set clams in highly controlled conditions, like downwellers, can provide constant, optimal environmental conditions and shield clams from the predators, biofouling organisms, and
pathogens. However, these conditions can be cost prohibitive in some cases. As such, adequate early juvenile surfclam growth and survival can also be achieved using the bell siphon method. At some point during the nursery phase, clams should be transferred from static or recirculating systems to flow-through conditions, which generally provide clams with a greater abundance and diversity of food. Aquaculturists should carefully acclimatize surfclams during this transition. In this study, upwellers were the superior late nursery rearing type. There was no advantage to rearing surfclam seed in sediment during the nursery phase (shell length: 0.28-18.0 mm).

Grow-out survival varied considerably across farms and across years. Surfclams mortality events were associated with anoxic conditions, warm temperatures, aerial exposure during a harsh winter, and predation from blue crabs (C. sapidus) and moon snails (E. heros). Generally, growth, condition, and gonad development were consistent across farms, but varied seasonally. Within 9 months, 67-98% of clams reached the pastaneck market size (31.75 ≤ x < 44.45 mm). Surfclam aquaculture also appear to be associated with a diversity of macrofauna, similar to what has been observed at other clam and oyster farms (Luckenbach et al., 2016; Mercaldo-Allen et al., 2020). Optimal gear type for grow-out phase production remains uncertain; however, 5-mm mesh bags were clearly superior to 9-mm mesh bags, but mesh bags were generally insufficient at deterring predators.

The best time of year to harvest surfclams reared at New Jersey shellfish farms would be during spring before water temperature increases to an unfavorable level and before clams spawn to ensure that the clams are at their best condition. Farmers should ensure clams are safely subtidal for the duration of winter to reduce the likelihood of
mortality. Moreover, results from this study suggest that the cold-tolerant surfclam can exploit winter and spring algal blooms during the fall-spring season to sustain growth. This stands in contrast to other commonly cultivated bivalves in the Northeast, like the Eastern oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*), which are largely dormant during this period. If growers were to culture surfclams in addition to one or both of these other species, they may be able to better exploit available farm resources to sustain harvests throughout a longer portion of the year. However, until heat-tolerant surfclams can be successfully bred and mass produced, farmers should consider moving clams to deeper, cooler water during the warmest weeks of summer to avoid excessive mortality.

This study demonstrates that surfclam aquaculture is feasible in coastal New Jersey bays, and fits well into the Northeast region’s established shellfish farming framework. Surfclam aquaculture is already expanding, particularly in Massachusetts. In 2020, a directory of East Coast shellfish growers listed 5 hatcheries/nurseries (two in Maine, two in Massachusetts, and one in New Jersey), from which surfclam seed could be ordered (Zemeckis, 2020).

Further research should be devoted to investigating the optimal grow-out gear, as well as optimal stocking density for commercial-scale production. For instance, clams reared under anti-predator screens and inside the recruitment box at AB exhibited rapid growth. While not experimentally robust, these farmer innovations provided information upon which future experiments can be designed. Furthermore, the box bag/bottom cage treatment should be re-tested, since its failure in this study was largely related to a stochastic event. Finally, research should be devoted toward examining whether surfclam
aquaculture is suitable for the near-shore coastal environment, which provides more ideal conditions (higher salinity, lower temperatures) compared to coastal bays used in most studies conducted to date.
3.5 Acknowledgements

This work would not be possible without the tremendous contributions from the following partner farmers: Bill Avery of Bill Avery’s Quality Bay Clams, Dale S. Parsons of Parsons Seafood, Marc Zitter of Northern Cape Sea Farms, and Matthew Gregg and Scott Lennox of the Barnegat Bay Oyster Collective. I am also grateful to the personnel of the Haskin Shellfish Research Laboratory and the New Jersey Aquaculture Innovation Center who provided assistance with this research, particularly S. Borsetti, D. Bushek, J. Caracappa, B. Campbell, J. Gius, X. Guo, D. Jones, J. Kiernan, F. Klie, N. Morris, M. Neuman, J. Paterno Shinn, S. Towers, M. Whiteside, P. Woodruff, and M. Xie.

Daphne Munroe, Lisa Ragone Calvo, and Michael De Luca served as advisors and collaborators of this study. The contributions of Michael Acquafredda include project conceptualization, investigation, project administration, data collection, and data analyses. The contributions of D. Munroe, L. Ragone Calvo, and M. De Luca include project conceptualization, investigation, acquisition of resources, and project supervision.

This research was sponsored by New Jersey Sea Grant with funds from the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under NOAA grant #NA14OAR4170085 and the New Jersey Sea Grant Consortium. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of New Jersey Sea Grant or the U.S. Department of Commerce. Additionally, Sea-Bird Scientific generously supplied some of the temperature monitoring equipment through the 2016 Student Equipment Loan Program.
Finally, this work was supported by Rutgers University through the Haskin Shellfish Research Laboratory, the Department of Ecology, Evolution, and Natural Resources, and the Graduate Program in Ecology and Evolution.
3.6 Literature Cited


Desbonnet, A. (1989). The effect of size, density, and growth strategy on survival of


Goldberg, R. (1980). Biological and technological studies on the aquaculture of yearling


Liu, W., Pearce, C. M. Alabi, A. O., Beerens A., & Gurney-Smith, H. (2011). Effects of


Walker, R. L., & Heffernan, P. B. (1990c). The effects of cage mesh size, tidal level
placement on the growth and survival of clams *Mercenaria mercenaria* (L.) and *Spisula solidissima* (Dillwyn 1817) in coastal waters of Georgia. *Northeast Gulf Science, 11*, 29-38.


Supplemental Surfclam Projects

A number of supplemental projects related to surfclam husbandry were conducted. These include studies that assessed both rearing conditions (i.e., nursery location) and post-harvest aspects of surfclam aquaculture (i.e., shelf-life and marketing).

Supplemental Project 1 – The effect of nursery location on juvenile surfclam growth

The growth of juvenile surfclams reared in flow-through upwellers supplied with ambient, unfiltered seawater was compared across four locations: (1) the NJ Aquaculture Innovation Center at Rutgers University in North Cape May, NJ (AIC), (2) Barnegat Light, Long Beach Island, NJ (LBI), (3) Waretown, NJ, and (4) Atlantic City, NJ (Figure SP1-1). Initial shell length was 1.8 mm, and growth was monitored three times, in June, July, and October 2017. However, this experiment was not well-controlled, so it may be inappropriate to draw conclusions beyond those that are anecdotal. Conditions that varied without controls in this experiment include: upwelling rearing silos with different mesh sizes, upwelling rearing silos with different diameters (and therefore stocking densities), irregular sampling, and inconsistent maintenance (removing biofouling, clam excrement, and sediment from silos), across locations. Moreover, there was no replication at any of the locations.

Nevertheless, the data show noteworthy trends. At all locations, surfclams that received more maintenance grew to greater final shell lengths than those that were not tended as consistently (Figure SP1-2). Surfclams reared in high-salinity, high-flow back bay habitats near Atlantic City and LBI grew to greater final shell lengths than clams grown at the AIC or in Waretown (Figure SP1-2).
Figure SP1-1. Map of surfclam nursery locations. Points represent the locations of the land-based upwelling systems used to rear juvenile surfclams in this study. All sites are located near coastal bays of New Jersey, USA. Maps courtesy of NOAA National Centers for Environmental Information (NCEI).
Figure SP1-2. Surfclam growth at various NJ nursery locations. Mean final shell length after four months of rearing in upwellers (silos with 600-1000 µm mesh). Error bars represent standard error of all clams measured (N=50 per silo, one silo per site/maintenance level). High maintenance refers to a cleaning frequency of at least once per week for the duration of the study.
Supplemental Project 2 – The effect of early grow-out stocking density on surfclam growth

A pilot study investigating the effect of stocking density on surfclam growth and survival during the initial months of grow-out was conducted. Surfclam seed was outplanted in cylindrical cages (diameter x height: 7.62 x 36.20 cm = 1.651 L) tethered to partially buried cinderblocks at two locations: the Cape May Canal at the NJ Aquaculture Innovation Center (AIC) and the Rutgers University Cape Shore Laboratory (CS). Three replicates of each of the following four stocking densities were deployed at each site: 9300 clams (500 mL, 30% of the container’s total volume), 4650 clams (250 mL, 15%), 2325 clams (125 mL, 7.5%), 1116 clams (60 mL, 3.6%). The clams used in this study were from the same population generated for the 2017 deployment grow-out experiments described in Chapter 3. Prior to deployment, surfclams had initial shell lengths (± 95% CI) of 7.52 ± 0.25 mm (N=100; Table SP2-1), a size slightly smaller than what would normally be deployed on shellfish farms. Clams were randomly assigned to location and stocking density treatment. Units deployed at the AIC were subtidal, with the exception that one replicate of each density became partially exposed during extreme low tides. All units deployed at CS were intertidal and were exposed for at least two hours twice daily. The experiment took place from 4 August 2018 to November 18, 2018. Initial and final survival and growth data were collected as stated in Chapter 3, Section 3.2.6 Grow-out experimental design. Data were analyzed using Microsoft Excel® and with R (Version 4.0.2 © 2020-06-22 The R Foundation) using RStudio (Version 1.3.1056 © 2009–2020 RStudio, Inc.).
At CS, all replicates of all stocking densities experienced 100% mortality. Shell sizes from dead clams indicate that they died soon after initial deployment (Table SP2-1), suggesting that in intertidal areas, surfclams cannot be reared outside of sediments.

At AIC, some replicates became unmoored; and some cages deteriorated, releasing the clams inside. Therefore, only 1-2 replicates of each stocking density treatment were available for final data collection. In the lowest stocking density, surfclams grew approximately 13 mm over 15 weeks, achieving a mean shell length of more than 20 mm. By contrast, clams in the highest stocking density grew less than 3 mm over the 15-week study. While robust statistical analysis is precluded due to loss of replicates, these pilot data suggest that as stocking density increases, growth decreases (Table SP2-1). This agrees with previous research related to farmed surfclam stocking density.
Table SP2-1. **Shell dimensions from stocking density experiment.** 95% confidence intervals (CI) were measured across replicates, unless only one replicate was available. In that case, it was measured across sampled clams (*).

<table>
<thead>
<tr>
<th>week</th>
<th>Location</th>
<th>Stocking density</th>
<th>Mean shell length (± 95% CI) mm</th>
<th>Mean shell height (± 95% CI) mm</th>
<th>live/dead</th>
<th>replicates</th>
<th>N clams per replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Initial population</td>
<td></td>
<td>7.52 ± 0.25*</td>
<td>5.51 ± 0.17*</td>
<td>live</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>AIC</td>
<td>3.60%</td>
<td>20.30 ± 0.41</td>
<td>15.81 ± 0.56</td>
<td>live</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>AIC</td>
<td>7.50%</td>
<td>18.56 ± 1.81*</td>
<td>13.37 ± 1.13*</td>
<td>live</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>AIC</td>
<td>15%</td>
<td>12.78 ± 1.43</td>
<td>9.22 ± 0.85</td>
<td>live</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>AIC</td>
<td>30%</td>
<td>9.20 ± 0.49*</td>
<td>7.35 ± 0.37*</td>
<td>live</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>CS</td>
<td>3.60%</td>
<td>7.71 ± 0.59</td>
<td>5.89 ± 0.36</td>
<td>dead</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>CS</td>
<td>7.50%</td>
<td>8.48 ± 0.64</td>
<td>6.36 ± 0.39</td>
<td>dead</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>CS</td>
<td>15%</td>
<td>8.29 ± 0.61</td>
<td>6.18 ± 0.43</td>
<td>dead</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>CS</td>
<td>30%</td>
<td>7.84 ± 0.26</td>
<td>5.95 ± 0.18</td>
<td>dead</td>
<td>3</td>
<td>25</td>
</tr>
</tbody>
</table>
Supplemental Project 3 – Shelf-life of farm-raised surfclams

This project entailed comparing various methods of storing harvested farm-raised surfclams. Market size surfclams, which were originally produced during a 2014 pilot study or during the 2016 surf clam production discussed in Chapter 2, were collected from a partnering shellfish farm on four sampling (harvest) dates. The dates sampled and mean shell length of the clams were April 2016: 46.4 mm; October 2016: 52.1 mm; March 2017: 54.8 mm; and June 2017: 42.4 mm. These dates were intentionally chosen to cover a range of harvest conditions under which live clams may be collected and subsequently put into cold storage. All ambient conditions during the harvest were recorded (i.e., air temperature, water temperature, weather conditions, and time of transport). Clams were washed, as per industry practice, and transported to the Rutgers University Haskin Shellfish Research Laboratory in a cooler on ice. A total of 20-30 clams were collected for each shelf-life treatment group on each harvest date.

The various shelf-life methods tested in this study included: surfclams in a mesh bag nestled within ice, surfclams wrapped in a moist towel, surfclams in a mesh bag (dry), surfclams loose on top of ice, and surfclams loose on a tray (dry). Each harvested clam was randomly assigned to one of the treatments. All treatments were kept in refrigerated conditions (6°C and 93% humidity). Surf clam shelf-life was also compared to the shelf-life of similarly sized hard clams (M. mercenaria), which were harvested at the same farm and at the same time as the surfclams. Since hard clams are the industry and consumer “standard” for the northeastern United States, hard clam shelf-life served as a useful comparison.
Each day for at least 12 days, the number of dead clams and the number of
gaping, yet still live, clams were monitored. Any dead clams were noted and
immediately removed from the experiment. At the end of each trial, all remaining live
clam were measured. Results for each of the four trials were relatively consistent and
suggest that surfclams have a shorter shelf-life than hard clams. Additionally, surfclams
have a tendency to gape, which might confuse consumers who are not familiar with this
product. Gaping in hard clams indicates that the clams have spoiled, whereas gaping in
surfclams is normal; a gaping surfclam is still fresh as long as it remains closed when
pressure is applied to its shells.

However, when maintained in a mesh bag covered with a damp towel, surfclam
survival after eight days was comparable to that of hard clams (Figure SP3-1). There was
no correlation between clam size and shelf-life performance. During the final trial, clams
were often inadvertently left sitting in meltwater. This resulted in very high mortality for
the mesh bag nestled within ice treatment, suggesting that ice must be drained as it melts
to prevent freshwater mortality.

Overall, the results suggest that surfclams wrapped in a moist towel have the
longest shelf-life and can stay fresh in this way for at least 8 days. This treatment is likely
the best because the damp towel helps keep the clams moist without the threat of
submerging them to freshwater. Moreover, the towel helps keep the clams shut, which
minimizes gaping and prevents water loss from the clams’ tissues.
Figure SP3-1. Surfclam shelf-life. Point represents the mean survival of all clams in a given treatment on day 8 for each of the four trials. The box and whisker plots represent the interquartile length of each treatment with the horizontal bar in each representing its mean.
Supplemental Project 4 – Evaluating the market potential of farm-raised surfclams

Meetings were held with experts from the Rutgers University Food Innovation Center to discuss best approaches to assess market potential and sensory evaluations of the Atlantic surfclam (*Spisula solidissima*). A survey form was developed and reviewed by Rutgers Institutional Review Board (IRB) (Figure SP4-1). The first survey evaluation was held on March 20, 2017 at the monthly meeting of the American Federation of Chefs (ACF), Southern Jersey Chapter. A presentation about shellfish aquaculture and surfclams was also presented by Dr. Daphne Munroe about the ecology and farming of the species. Approximately 40 chefs attended and participated in the survey. Each participant received the survey form and two different species of clams, which were both simply steamed in the same manner. One of the clams was a surfclam (designated 421 on the survey form) and the other was a hard clam (*Mercenaria mercenaria*, designated 227). Since the hard clam is the industry and consumer “standard” for the northeastern United States, it served as a useful comparison.

Out of 25 attendees of the ACF meeting that tasted each sample, 20 surveys were completed (or at least attempted). The averages for the assessment of 421 (Surfclams) on a five-point Likert scale (1-5) were: 3.90 for appearance, 4.00 for texture and 4.06 for flavor. The averages for the assessment of 227 (Hard Clams) were: 4.55 for appearance, 4.32 for texture and 4.33 for flavor.

Out of the 20 surveys completed, 9 participants claimed they currently buy/serve local fresh seafood, while 6 participants claimed they do not – the remaining 5 did not complete this question. Types of seafood that these current buyers/servers of local fresh seafood included clams, mussels, oysters, soft shell crabs, shellfish, little tops, cherry
stones, fish/shrimp, flounder, crabs, scallops and lobster. The averages of how important fresh seafood attributes are to each participant were 2.20 for grown locally, 2.60 for price, 2.87 for sustainability and 1.87 for quality.

Out of 17 participants that responded to “would you be willing to pay more for this product,” 5 replied no and 12 replied yes. From those participants, six responded that they would be willing to pay up to 10% more, 3 claimed up to 25% more and none of the participants claimed that they would be willing to pay up to 50% more. Of the 16 participants that responded to their involvement in the foodservice sector, nine were chefs, two distributors, zero were owners and five identified as others (others included three guests and two culinary students). Additional comments included: “larger clams were fishy/gritty,” “very enjoyable to palate,” “really enjoyed both clams,” “(421) definitely caught my attention,” “thought the farm raised (421) were plumper/sweeter,” and “sample 421 tasted a little sweeter and 227 tasted a little salty.”

Overall, there was a positive response for both clams that were sampled. The Likert scores for sample 227 (Hard Clam) were all slightly higher compared to sample 421(Surf Clam). This shows that a majority of the participants preferred the hard clam to the surf clam. The surfclam has a very different texture and flavor to the commonly available hard clam and will likely need some exposure before it is thought to be of comparable quality.
Local Farm Raised Clams Survey

Please circle the number that best describes your opinion.

**Appearance:** Thinking only about the appearance of the clams, please circle the statement that best reflects your opinion:

<table>
<thead>
<tr>
<th>Sample 421</th>
<th>Sample 227</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly like</td>
<td>Strongly like</td>
</tr>
<tr>
<td>Like</td>
<td>Like</td>
</tr>
<tr>
<td>Neither like or dislike</td>
<td>Neither like or dislike</td>
</tr>
<tr>
<td>Dislike</td>
<td>Dislike</td>
</tr>
<tr>
<td>Strongly dislike</td>
<td>Strongly dislike</td>
</tr>
</tbody>
</table>

**Texture:** Thinking only about the texture of the clams, please circle the statement that best reflects your opinion:

<table>
<thead>
<tr>
<th>Sample 421</th>
<th>Sample 227</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly like</td>
<td>Strongly like</td>
</tr>
<tr>
<td>Like</td>
<td>Like</td>
</tr>
<tr>
<td>Neither like or dislike</td>
<td>Neither like or dislike</td>
</tr>
<tr>
<td>Dislike</td>
<td>Dislike</td>
</tr>
<tr>
<td>Strongly dislike</td>
<td>Strongly dislike</td>
</tr>
</tbody>
</table>

**Flavor:** Thinking only about the flavor of the clams, please circle the statement that best reflects your opinion:

<table>
<thead>
<tr>
<th>Sample 421</th>
<th>Sample 227</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly like</td>
<td>Strongly like</td>
</tr>
<tr>
<td>Like</td>
<td>Like</td>
</tr>
<tr>
<td>Neither like or dislike</td>
<td>Neither like or dislike</td>
</tr>
<tr>
<td>Dislike</td>
<td>Dislike</td>
</tr>
<tr>
<td>Strongly dislike</td>
<td>Strongly dislike</td>
</tr>
</tbody>
</table>

Do you currently buy and serve local fresh seafood?

☐ Yes  ☐ No

**If yes,** what type? ____________________________________________

Please rank order each of the following attributes for fresh seafood, 1 to 4, with 1 being most important and 4 being the least important. Do not use a number more than once.

- [ ] Grown locally
- [ ] Price
- [ ] Sustainably grown
- [ ] Quality
Sample 421 is a locally grown farm raised surf clam. Would you be willing to pay more for this product than you currently do for hard clams?

☐ Yes  ☐ No

If yes, please circle how much:

☐ 10%  ☐ 25%  ☐ 50%

Are you a: ☐ Chef  ☐ Distributor  ☐ Restaurant owner  ☐ Other ______________________

Please share any additional comments or thoughts on the products you just tasted.

________________________________________________________________________

Thank you!!

Figure SP4-1. Market potential survey for farm-raised surfclams. This survey form was developed and reviewed by Rutgers IRB and used in side-by-side taste tests of farmed hard clams (Mercenaria mercenaria, 227) and farmed Atlantic surfclams (Spisula solidissima, 421)
Unit 2 – Building climate change resilience by breeding surfclams for greater heat tolerance

In Unit 2 (Chapters 4 and 5) of this dissertation, I expand upon the findings of the previous chapters, which demonstrate the degree to which farmed Atlantic surfclams (*Spisula solidissima*) are vulnerable to heat stress. High temperature conditions are particularly problematic on shallow coastal farms and will be exacerbated by rising ocean temperatures caused by climate change. Research presented in this unit takes a proactive approach towards climate-informed aquaculture and supports the genetic aspect of ESD.

In Chapter 4, I ask the following questions:

1. How do surfclams that survive a lethal heat stress as juveniles respond to a second lethal heat stress as adults?
2. Can surfclams be selectively bred for greater heat tolerance?

In Chapter 5, I ask the following questions:

1. How does the surfclam transcriptome differ between ambient and heat stress conditions?
2. How does a selective pressure (i.e., an acute lethal heat challenge) alter the gene expression profile of a surfclam population?
Chapter 4: Exploring the feasibility of selectively breeding farmed Atlantic surfclams (*Spisula solidissima*) for greater heat tolerance

With the exception of a few minor modifications, this chapter appears in its entirety in *North American Journal of Aquaculture*. The citation for the published article is:

[https://doi.org/10.1002/naaq.10168](https://doi.org/10.1002/naaq.10168)

4.0 Abstract

Bivalve aquaculture is an important and rapidly expanding sector in global food production, yet climate change presents numerous challenges to its continued expansion. The Atlantic surfclam (*Spisula solidissima*) is emerging as an attractive alternate species by aquaculturists across the northeastern United States, since it is native, grows rapidly, and complements the region’s established farming framework. However, the species is vulnerable to prolonged high temperatures conditions, an issue that will be exacerbated by rising ocean temperatures and particularly problematic on shallow coastal farms. In this study, I evaluated the response of adult farmed surfclams to heat stress after juvenile exposure and the ability for heat tolerance to be passed to subsequent generations. I found that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent heat stress for significantly longer than individuals not exposed to lethal temperatures as juveniles. I also found that selective breeding enhanced heat tolerance in first-generation surfclam progeny. Moreover, growth of the heat-selected progeny was not significantly different from that of control clams. Although more research on this topic is necessary, this work suggests selective breeding may be a viable strategy for enhancing survival of cultivated bivalves vulnerable to heat stress.
4.1 Introduction

The world’s oceans have absorbed more than 90% of the heat trapped by anthropogenic greenhouse gas emissions (Intergovernmental Panel on Climate Change [IPCC], 2014), and recent research suggests that ocean warming exceeds previous estimates. For instance, Resplandy and colleagues (2018) measured the outgassing of O\textsubscript{2} and CO\textsubscript{2} from the world’s oceans, or the amount of gas the oceans release as they warm, and found that every year since 1991, humans put 60% more energy into the oceans than previously thought. Local warming of the continental shelf along the northeastern United States has been documented for decades (Scavia et al., 2002). Recent estimates of the Northeast’s sea surface temperatures suggest that this region is warming two- to three-times faster than the global average (Saba et al., 2016; Pershing et al., 2015). A plethora of marine species have already begun shifting their distributions in response to the temperature change (Sunday et al., 2012; Kleisner et al., 2017; Free et al., 2019; Morson et al., 2019). Among them is the Atlantic surfclam (*Spisula solidissima*), whose northward range shift is well documented (Munroe et al., 2013; 2016; Powell et al., 2016; Hennen et al., 2018; Hofmann et al., 2018).

As a temperate species, surfclams are known to be vulnerable to high temperature conditions (Goldberg & Walker, 1990; Weinberg, 2005; Hornstein et al., 2018). This issue is expected to be exacerbated as the region’s waters continue to warm (Narváez et al., 2015; Munroe et al., 2016), and one that may already be problematic on shallow coastal farms. Historically, the habitat for wild populations of surfclams off the coast of New Jersey extended from shallow beaches along barrier islands and the mouths of estuaries to a depth of 60 m on the continental shelf (Jacobson & Weinberg, 2006).
Recently, the range of wild surfclams off the coast of New Jersey has shifted towards deeper, cooler water (Weinberg et al., 2002; Weinberg et al., 2005; Weinberg, 2005; Timbs et al., 2019).

Temperature greatly influences surfclam growth and performance, and their ability to cope with warm water temperatures is in part related to their size (Cerrato & Keith 1992). Large-bodied individuals caught in the surfclam fishery (shell length >120 mm) cannot survive prolonged exposure to temperatures greater than 21°C (Munroe et al., 2013; Weinberg, 2005). However, juveniles and smaller individuals seem to have a wider thermal tolerance, succumbing to mortality when temperatures above 26°C are sustained for days or weeks (Acquafredda et al., 2019). During an experimental evaluation of nursery rearing temperatures of early post-metamorphic juvenile surfclams (shell length ~0.7–3.0 mm), individuals under heat stress (~26°C) survived less than half as well as those reared under cooler (≤ 20°C) conditions (Acquafredda et al., 2019). Notably, surfclams produced from different parent stock responded differently to temperature during these trials, suggesting that thermal tolerance may be a heritable trait (Acquafredda et al., 2019).

The surfclam has strong potential to benefit farmers who are eager to build diversity and resiliency into their farm plans (Acquafredda & Munroe, 2020). However, I anticipate this species’ vulnerability to heat stress will be one of the greatest limitations to further surfclam aquaculture development across the Northeast. Selective breeding for greater heat tolerance might be a viable strategy for enhancing survival of cultivated surfclams. Selective breeding programs have been the foundation of viable Eastern oyster production along the east coast of the United States (Haskin & Ford, 1979). As such,
similar programs that produce heat-tolerant surfclam stocks and lead to improved and consistent annual yields would provide stability to farmers and facilitate industrial scale production.

Here, I present observations on the response of adult farmed surfclams to heat stress after juvenile exposure and explore the feasibility of selectively breeding surfclams for greater heat tolerance. In Experiment 1, I examined whether surfclams that survived a month-long heat stress as early juveniles would be more resilient than control clams when re-exposed to similarly stressful conditions as adults. In Experiment 2, I sought to determine whether surfclams could be selectively bred for greater heat tolerance. To address this, surfclam broodstock that survived a lethal heat shock were bred, and the growth, survival, and heat tolerance of their first-generation progeny were compared to the progeny of control broodstock.
4.2 Materials and methods

4.2.1 Assessing heat tolerance of adult farmed surfclams after juvenile exposure to heat stress.

The Atlantic surfclams (*Spisula solidissima*) used in Experiment I were generated during a previous study (Acquafredda et al., 2019). A brief explanation is provided here. In June and July 2016, a controlled temperature tolerance experiment was conducted at the New Jersey Aquaculture Innovation Center (AIC) in North Cape May, NJ. In this study, juvenile surfclams (initial shell length ~0.7 mm) from three replicate cohorts were assigned to temperature treatments two weeks after metamorphosis. One treatment consisted of a continuous month-long exposure of ~26˚C, which caused a selection event where approximately 79% of clams died. While clams in this study most likely succumbed to heat-induced mortality, it is plausible that a bacterial infection, borne of the high temperature conditions, contributed to or exacerbated the mortality attributed to heat stress alone. However, no latent mortality was observed after the heat-exposure concluded and once the clams were returned to control conditions (≤ 20˚C). The survivors of this treatment were pooled, and the group was designated heat-selected 2016 (HS-16). Another treatment in that study consisted of control conditions (≤ 20˚C) where clams did not experience stress. This group was designated non-selected 2016 (NS-16). Prior to this experiment, both groups were exposed to the same larval conditions in the hatchery (see Acquafredda et al., 2019). Likewise, both groups also experienced the same environmental conditions after the study. For the three months immediately following the selection event, the groups were reared in flow-through upwelling conditions at the AIC (Acquafredda et al., 2019). When the mean shell length reached approximately 13 mm,
the NS-16 and HS-16 clams were outplanted at a shallow subtidal farm in southern Barnegat Bay, NJ.

In September and October 2018, a fully-crossed controlled experiment (Experiment 1) was conducted using the NS-16 and HS-16 clams to determine whether prior exposure to heat stress conferred protection during a subsequent exposure to high-temperature conditions (Figure 4-1). The experiment took place at the Haskin Shellfish Research Laboratory in Port Norris, NJ, and occurred when the clams were approximately 2.5 years old. The NS-16 and HS-16 clams had a mean shell length of 45.00 mm (SD, 4.46) and 48.67 mm (SD, 2.78), respectively. For 12 days, surfclams were exposed to control temperatures between 9 and 11°C (mean ± SD, 10.2 ± 0.6°C) or a lethal heat challenge at temperatures between 28 and 30°C (mean ± SD, 29.4 ± 0.7°C). The experiment occurred after a one-week acclimation period where the water temperature was slowly adjusted from 16°C, the conditions of the field from which the clams were retrieved. The control conditions were adjusted below field conditions due to equipment and space constraints at the laboratory. The heat challenge temperatures used in this and subsequent experiments presented here were chosen based on preliminary studies and previous work (Acquafredda et al., 2019). Due to the number of available clams harvested, four and seven replicate buckets were used for HS-16 and NS-16, respectively, for each temperature treatment. Each replicate consisted of six clams placed in a bucket containing 15-L of treated seawater (TSW), which had been 1-µm filtered and UV-sterilized.

Replicate buckets in the heat challenge treatment shared a common water bath, which was heated with multiple aquarium heaters (300–400W Aqueon) that were
controlled by a single-stage digital temperature controller (Aqua Logic, Inc.). Buckets in the control treatment were maintained in a temperature-controlled room set to the target temperature. Continuous water temperature data were logged with SBE 56 (Seabird Scientific) devices using a 600 second sampling frequency. Point temperature, salinity, and nitrogen waste (NH₄⁺, NO₂⁻, NO₃⁻) data were collected daily with an analog thermometer, refractometer, and API® test kits, respectively. Across all experimental units, the mean salinity was 30.4 ppt (SD, 1.1). Water changes occurred daily or when ammonia, nitrite, or nitrate levels exceeded 0.5 ppm. Buckets were continuously aerated, contained a preconditioned biofilter, and were fed 3% dry weight daily of Shellfish Diet 1800 (Reed Mariculture, Instant Algae), as per manufacturer's instructions. Clam survival was monitored daily, and dead clams were immediately removed from buckets. Survival was monitored until all individuals in the heat challenge suffered mortality.

4.2.2 Selecting heat-selected and non-selected surfclam broodstock groups

Experiment II began with generating a heat-tolerant surfclam broodstock group. In December 2018, 21-month old farmed-raised surfclams were collected from a farm in Barnegat Bay and transferred to the AIC. The surfclams used in Experiment II were from a separate cohort, distinct from those used in Experiment I (Figure 4-1). These surfclams had a mean wet tissue weight of 2.5 g (SD, 0.7), a mean dry tissue weight of 0.3 g (SD, 0.08), and a mean shell length of 36.55 mm (SD, 2.46). A random sample of 500 clams was selected as the control broodstock, designated here as the non-selected group (NS-17). The NS-17 group was placed in 900-L of 50-µm filtered and UV-sterilized seawater, which was maintained at a mean temperature of 11.6°C (SD, 0.6) (Figure 4-1). The tank
was set up as a recirculating system, which was continuously aerated and contained a preconditioned biofilter. The clams were fed a ration of 3% dry weight per day of Shellfish Diet 1800 (Reed Mariculture, Instant Algae).

The remaining clams were separated into two tanks, each containing 555 individuals. These clams were maintained in identical conditions to the control tank, except the temperature was incrementally increased by 2°C per day until the clams were exposed to an acute heat shock. The acute heat shock consisted of a continuous exposure to temperatures between 27.5 and 30°C (mean ± SD, 28.3 ± 2.1°C) for approximately five days (Figure 4-1). Throughout the broodstock selection processes, clam survival was monitored daily; dead clams were immediately removed from the tanks. At the end of the heat shock, 53.8% (SD, 6.2) of clams suffered mortality. Immediately following the heat shock, the temperature was decreased to 20°C and subsequently lowered over several days to match the conditions of the control tank. However, latent mortality continued to occur for approximately one month after the clams were returned to favorable conditions. This occurred even though the feeding ration for these clams was increased to 6% dry weight per day following the heat shock. Due to uneven latent mortality, the final selection differential, or the overall percentage of clam suffering mortality from the selection event, was 74.8% (SD, 13.9). The surviving clams were pooled and designated the heat-selected (HS-17) broodstock group. No NS-17 clams were lost during that period.

Throughout the broodstock selection process, continuous temperature data were logged with SBE 56 (Seabird Scientific) devices using a 600 second sampling frequency. Point temperature, salinity, and nitrogen waste (NH$_4^+$, NO$_2^-$, NO$_3^-$) data were also
collected daily with an analog thermometer, refractometer, and API® test kits, respectively. Across all tanks, the mean salinity was 28.8 ppt (SD, 1.1). Water changes occurred daily or when ammonia levels exceeded 0.5 ppm. Nitrite and nitrate levels did not exceed 0 ppm. Approximately six weeks after the selection event, both broodstock groups were moved back into the Barnegat Bay, so they could become naturally conditioned to spawn.

4.2.3 Spawning broodstock and rearing progeny

Experiment II continued in May 2019, when ripe surfclam broodstock were transferred from the Barnegat Bay conditioning site to the AIC (Figure 4-1). Spawning was induced using thermal manipulation (Loosanoff & Davis, 1963; Jones et al., 1993). Two males and two females from each broodstock group contributed to their respective progeny groups. NSF1-19 refers to the F1 progeny of the non-selected broodstock group (NS-17). HSF1-19 refers to the F1 progeny of the heat-selected broodstock group (HS-17). The small number of parents used to produce each progeny group was not intentional, but rather an unfortunate consequence of the limited number of broodstock that had ripe gametes at the time of spawning.

The larvae and juvenile clams from both progeny groups were reared identically using established culture methods (Jones et al., 1993; Acquafredda et al., 2019). Larvae were reared in static 200-L tanks containing TSW. The larval stocking density was reduced from 14 to 2 larvae/mL by metamorphosis (Jones et al., 1993). At metamorphosis, larvae were set in downwelling silos, which were composed of fiberglass cylinders (diameter x height, 60.96 x 60.96-cm) and mesh screen (Nitex) and reared in
TSW. Incrementally, clams were moved into new rearing silos with larger mesh screen, from 125 to 150 to 180 to 200-µm. The initial stocking density for post-metamorphic juveniles was 185 clams/cm². While the larval and juvenile clams were reared in TSW, the mean temperature and mean salinity were maintained at 20.9°C (SD, 0.7) and 30.2 ppt (SD, 0.4), respectively. Larvae and juveniles were fed a mixed diet of *Tisochrysis lutea* and *Pavlova pinguis*. The feeding ration was incrementally increased from $1.0 \times 10^4$ cells/mL on day 1 to $8.5 \times 10^4$ cells/mL on day 21.

By day 23, the rearing silos were moved to flow-through raceways (length x width, 7.1 x 0.58-m) supplied with unfiltered (raw) seawater from the Cape May Canal. The flow was controlled with a bell siphon, which produced continuous ebb and flow conditions that cycled the height of the raceway between 33 cm and 5 cm approximately every 27 minutes. On day 37, the rearing silos were moved to upwelling conditions, which experienced flow rates between 45 and 55 L/min. Incrementally, clams were moved into new rearing silos (diameter x height, 45.72 x 45.72-cm) with larger mesh screen, from 400 to 600 to 750 to 1000-µm. Concurrently, the stocking density was reduced in accordance with established culture methods, and by the end of the nursery phase, the clams were stocked at 0.38 clams/cm² (Jones et al., 1993). During the period when these clams were reared in raw seawater, they experienced mean daily temperatures that ranged from 18.1 to 25.4°C (mean ± SD, 22.6 ± 1.6°C). Likewise, the clams experienced mean daily salinities that ranged from 24.2 to 30.3 (mean ± SD, 28.6 ± 1.3).

Survival of the surfclam progeny was assessed with repeated volumetric abundance estimates (Acquafredda et al., 2019). Growth of the surfclam progeny was determined by recording shell length (anteroposterior axis) in proportion to the
abundance of each determined size class. Larvae and juvenile clams less than 3.0 mm in shell length were measured by placing individuals onto a Sedgewick-Rafter slide and measuring each with an ocular micrometer on a VWR compound microscope (100X or 25X). Clams larger than 3.0 mm were measured with digital calipers (Mitutoyo Absolute™ Digimatic).

**4.2.4 Assessing heat tolerance of heat-selected and non-selected surfclam progeny**

Experiment II continued in September 2019 at the Haskin Shellfish Research Laboratory to determine whether selectively bred surfclam progeny had greater heat tolerance than non-selected control progeny (Figure 4-1). This controlled heat tolerance assessment was conducted in the same manner outlined above for Experiment I, except for the following modifications. The temperature of the heat challenge was maintained between 27 and 30˚C (mean ± SD, 28.9 ± 1.2˚C), while the control conditions were kept between 9 and 15˚C (mean ± SD, 11.2 ± 2.4˚C). Before the exposure, the clams underwent an acclimation period wherein the water temperature was slowly adjusted over several days from 23.6˚C, the raw seawater temperature the clams had previously been experiencing. Across all experimental units, the mean salinity was 32.3 ppt (SD, 0.9). Water changes occurred daily or when ammonia levels exceeded 0.5 ppm. Nitrite and nitrate levels did not exceed 0 ppm. Three replicate buckets were established for each progeny group, and each replicate bucket initially contained 100 clams. This approximated the biomass of the six adult clams used per replicate in Experiment I. The clams used in this experiment had a mean shell length of 9.65 mm (SD, 0.72).
4.2.5 Statistical analyses

All data were analyzed with R (Version 4.0.2 © 2020-06-22 The R Foundation) using RStudio (Version 1.3.1056 © 2009–2020 RStudio, Inc.). Normality and homoscedasticity of all growth and survival data were confirmed using the Shapiro-Wilk Test and Levene's Test, respectively. Measures of dispersion presented in this paper are reported as ± standard deviations (SD) or 95% confidence intervals (CI), wherever noted. To determine the significance of the survival of clams during the heat challenge experiments, generalized linear mixed models fit by maximum likelihood were fit to the data. Experimental day and group were fixed effects, while replicate bucket was a random effect. Due to the linearity of the data, ANCOVA was used to determine the significance of the survival of clams experiencing the control conditions during these experiments. Similarly, ANCOVA was used to determine whether breeding group had a significant effect on the growth rates of larval and juvenile surfclam progeny. Average daily growth rates were determined using the formula described by Acquafredda & Munroe (2020):

\[ X_{GR} = \frac{X_t - X_{t0}}{\Delta t} \]

Here, \( X \) represents the mean value of a particular growth variable (shell length), collected on the first and last day of the study, and \( \Delta t \) represents the number of days of the study. A Student’s t-test was used to compare the final size of surfclam progeny at the end of their nursery phase (day 142).
Figure 4-1. Schematic of experimental design. In Experiment I, selection pressure was applied when the clams were early juveniles, two to six weeks post-metamorphosis (Acquafredda et al., 2019). In Experiment II, selection pressure was applied when the clams were 21-month old adults. For the heat tolerance assessments, circles represent replicate buckets, which shared a common water bath (heat challenge) or shared a temperature-controlled room (control conditions). In the Experiment I heat tolerance assessment, each bucket initially contained six adult HS-16 (black circles) or NS-16 (grey circles) clams, ~2.5 years old. In the Experiment II heat tolerance assessment, each bucket initially contained 100 HSF1-19 (black circles) or NSF1-19 (grey circles) juvenile clams, ~5 months old. Abbreviations for Atlantic surfclam treatments are as follows: NS-16 = non-selected clams spawned in 2016; HS-16 = heat-selected clams, spawned in 2016, selected in 2016 as juveniles; NS-17 = non-selected clams, spawned in 2017; HS-17 = heat-selected clams, spawned in 2017, selected in 2018 as adults; HSF1-19 = F1 progeny of the HS-17 broodstock, spawned in 2019; NSF1-19 = F1 progeny of the NS-17 broodstock, spawned in 2019.
4.3 Results

4.3.1 Experiment I – Response of Adult Farmed Surfclams to Heat Stress after Juvenile Exposure

No clams from either group, HS-16 or NS-16, died when held at the control conditions (Figure 4-2A). When exposed to the heat challenge (28–30°C), both experimental day and group were significant predictors of surfclam survival ($P < 0.001$). Mortality of NS-16 clams was first observed on day 4, while mortality of HS-16 clams was not observed until day 5 (Figure 4-2B). The largest difference in survival between the groups was observed on day 7, where mean survival was 36% (CI, 18) for NS-16 and 71% (CI, 16) for HS-16. All NS-16 clams died by day 9, while complete mortality of HS-16 clams was not observed until day 12 (Figure 4-2B).

In response to the heat challenge, clams from both groups displayed similar phenotypes and behaviors that suggested they were succumbing to heat stress. As early as 24-hours into the heat challenge, clams were observed gaping. Clams were also seen extending their feet into the water column for prolonged periods of time, but they did not attempt to walk or explore their immediate surroundings by feeling the bottom of the experimental containers. Similarly, the clams responded more slowly to touch stimuli than clams in the control conditions; when probed, a gaping clam did not immediately close its valves or retract its foot or siphon. After five days into the heat challenge, the shell margins of the clams had become very soft, almost pliable. The shell margins of dead clams easily cracked under moderate finger pressure. The soft tissues of live clams took on a moribund appearance, becoming thin, watery, and translucent. Dead clams
were often found disintegrated, with their feet becoming detached from their watery, degraded viscera.
Figure 4-2. Survival of heat-selected and non-selected surfclams at control temperatures (A) and under severe heat stress (B). Points represent the survival of replicate buckets of heat-selected surfclams (HS-16, black squares, N = 4) and non-selected surfclams (NS-16, gray circles, N = 7). Each bucket initially contained six adult clams. (A) Control conditions: percent survival (%S) was 100% for both groups for the entirety of the experiment. (B) Heat challenge: lines of best fit were generated using generalized linear mixed models. Models take the form %S = (1/(1+exp(b0 + b1*t)))*100, where %S is percent survival, t is the time in days, and b0 and b1 represent the model intercept and slope, respectively.
4.3.2 Experiment II – Selective Breeding for Greater Heat Tolerance: Rearing heat-selected and non-selected surfclam progeny

Overall, no notable differences in larval or juvenile growth were observed between the HSF1-19 and NSF1-19 surfclam progeny groups (Figure 4-3A, B). Progeny group had no effect on the growth rate of larval clams (Figure 4-3A, ANCOVA (Progeny Group), $F(1,5) = 0.50, P = 0.51$). The average daily larval growth rates observed for HSF1-19 and NSF1-19 clams were 12.7 and 11.4 µm/d, respectively. All larvae metamorphosed between day 16 and 23.

Larvae spawned in early May 2019 had grown to a size ready for deployment on clam farms by September. At that time (142 days post-fertilization), HSF1-19 and NSF1-19 clams had a mean shell length of 14.14 mm (CI, 0.16) and 14.02 mm (CI, 0.17), respectively. There was no significant difference in size (Figure 4-3B; t-test, $t = -0.9, P = 0.35$). Similarly, progeny group had no effect on the growth rate of juvenile clams (Figure 4-3B; ANCOVA (Progeny Group), $F(1,23) = 0.27, P = 0.61$). The average daily juvenile growth rates observed for HSF1-19 and NSF1-19 clams were 0.115 and 0.114 mm/d, respectively.

Survival varied between the two progeny groups over the larval and nursery rearing phases; however, since only one cohort of each progeny group was produced, statistical inference is inhibited (Figure 4-3C). During the larval phase (day 0–16), the survival of the NSF1-19 group was more than double that of the HSF1-19 group (Figure 4-3C). During the juvenile phase (day 23–142), the survival of the HSF1-19 group was 26% greater that the NSF1-19 group (Figure 4-3C). Measured over the entire study
period (day 0–142), the survival of the NSF1-19 group was 2.68-times greater than that of the HSF1-19 group (Figure 4-3C).
Figure 4-3. Growth (A, B) and survival (C) of heat-selected and non-selected surfclam progeny. NSF1-19 clams are F1 progeny spawned from non-selected surfclam broodstock (gray circles/bars). HSF1-19 clams are F1 progeny spawned from heat-selected surfclam broodstock (black squares/bars). Each progeny group was produced from a single spawning event. Points represent the mean shell length and error bars represent 95% confidence intervals. (A) Larval phase growth, $N = 25$ clams each day. (B) Nursery phase growth, $N = 50$ clams each day, except day 37 ($N = 60$) and day 142 ($N = 300$). (C) Bars show the survival of the two groups through development ($N = 1$ cohort per progeny group).
4.3.3 Experiment II – Selective Breeding for Greater Heat Tolerance: Heat tolerance of heat-selected and non-selected surf clam progeny

When exposed to control conditions between 9 and 15°C, surf clam mortality was negligible for both progeny groups; each progeny group only lost a single clam (Figure 4-4A). In these conditions, there was no significant difference in survival between the NSF1-19 and HSF1-19 progeny groups (ANCOVA (Progeny Group), $F(1,50) = 0.12, P = 0.73$). When exposed to the heat challenge (28–30°C), again both experimental day and group were significant predictors of surf clam survival ($P < 0.001$). NSF1-19 clams began to die as early as day 2. HSF1-19 clams did not begin to die until day 4 (Figure 4-4B). The largest difference in survival between the progeny groups was observed on day 6. On this day, mean survival was 48.0% (CI, 13.7) for NSF1-19 and 78.7% (CI, 12.4) for HSF1-19. However, on day 7, the mean survival of NSF1-19 and HSF1-19 clams was much more similar at 2.7% (CI, 3.3) and 8.3% (CI, 10.7), respectively. All clams from NSF1-19 died by day 8, but clams from the HSF1-19 persisted slightly longer, until day 9 (Figure 4-4B).

Clams in the heat challenge displayed the same heat-stress associated phenotypes and behaviors as those explained in Experiment I.
Figure 4-4. Survival of heat-selected and non-selected surfclam progeny at control temperatures (A) and under severe heat stress (B). NSF1-19 clams were spawned from non-selected surfclams (gray circles). HSF1-19 clams were spawned from heat-selected surfclams (black squares). Points represent the survival of replicate buckets ($N = 3$ per progeny group), with each bucket initially containing 100 juvenile clams. (A) Control conditions: linear regressions were used to determine lines of best fit for each curve. Models take the form $%S = mt + b$, where $%S$ is percent survival, $t$ is the time in days, and $b$ and $m$ represent the model intercept and slope, respectively. (B) Heat challenge: lines of best fit were generated using generalized linear mixed models. Models take the form $%S = (1/(1 + \exp(b_0 + b_1 * t))) \times 100$, where $%S$ is percent survival, $t$ is the time in days, and $b_0$ and $b_1$ represent the model intercept and slope, respectively.
4.4 Discussion

This study represents a small yet promising first step towards developing heat-tolerant Atlantic surfclams (*Spisula solidissima*), which in turn may facilitate more resilient aquaculture production of this species in a warming climate. I found that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent thermal stress for significantly longer than individuals that did not experience an earlier exposure. Moreover, I found that through selective breeding, heat tolerance was improved in first-generation surfclam progeny. Together, the results from these experiments suggest that heat-induced selection can identify clams genetically predisposed to withstanding high temperature conditions.

Although the manner of selection differed between Experiment I and II, the selection differentials achieved in both experiments (HS-16 = 79%; HS-17 = 75%) were similar, and both had the desired effect of identifying surfclams predisposed to withstand heat stress. The HS-16 surfclams were generated with a chronic stress (26°C for several weeks), while the HS-17 surfclams were selected using an acute heat shock (27–30°C for several days). The age of the clams when the selection pressure was administered also varied; HS-16 clams were selected as early juveniles, while HS-17 clams were selected as adults. No latent mortality was observed among HS-16 juveniles after the chronic stress. In contrast, the acute stress used to generate the HS-17 group caused substantial carry-over mortality, despite the adults being quickly returned to favorable temperatures and provided with high food availability. This observation aligns with findings from earlier work which indicate that a surfclam’s capacity to cope with heat stress is dependent on
multiple factors, such as age, size, duration of the heat stress, and the intensity of the heat stress (Narváez et al., 2015; Acquafredda et al., 2019).

No differences in larval or juvenile growth rate were observed between the heat-selected and non-selected progeny groups. Yet across different developmental stages, survival did vary between the progeny groups. Larvae of the control parents (NSF1-19) exhibited substantially greater survival than larvae from heat-selected parents (HSF1-19). I suspect that the difference in larval survival observed in this study may be related to the lipid reserves these progeny groups received from their parents. Bivalves provision their eggs with polyunsaturated fatty acids and other lipids they ingest from their diet, and the quantity and quality of lipid reserves available to embryos can influence subsequent larval growth and survival (Utting & Millican, 1997). I hypothesize that while the heat-selected broodstock (HS-17) were capable of developing gonad over the five-month period between the selection event and spawning, they may not have adequately rebuilt their lipid stores and thus, insufficiently provisioned their eggs. However, if the difference in larval survival was indeed a maternal effect, it was largely mitigated after metamorphosis. The survival of the two progeny groups was similar when measured over just the juvenile or nursery phase of development; in fact, juvenile survival was slightly higher in the offspring of heat-selected parents (HSF1-19). An alternative explanation for the observed variation in survival could be due to random chance since only one cohort of each progeny group was produced in this study.

After a single generation of selection, heat-selected surfclam progeny had significantly greater survival during continuous exposure to lethal temperatures compared to control progeny. It should be noted that the extreme lethal temperature exposure used
in this experiment (~29°C continuous exposure) is more severe than what would currently be observed at typical subtidal farm sites in New Jersey (described in Chapter 3); however, temperatures can occasionally reach that high on intertidal surfclam farms in Massachusetts (J. Reitsma, Woods Hole Sea Grant, personal communication). Although the progeny of heat-selected parents (HSF1-19) were more tolerant to heat stress, with the greatest difference in survival occurring after six days of exposure, after eight days, the survival of both progeny groups was comparable. I predict that this heat-tolerant phenotype could be enhanced through additional rounds of selection, further differentiating the heat-selected line from non-selected controls. While this study did not address whether the growth rates of the heat-selected and non-selected progeny groups differed under elevated temperature conditions, the findings of Experiment II suggest that selective breeding for greater heat tolerance in farmed surfclams is possible without compromising growth rate in ambient conditions. However, it must be noted that this study is constrained by the fact that each progeny group was produced from merely four parents in a single spawning event. Undoubtedly, the limited genetic diversity and lack of spawning replication are notable shortcomings of this study. Critically, future studies must use a greater diversity of broodstock and spawning replication when conducting surfclam breeding experiments.

Selective breeding is a critical tool for adapting food systems to the changing climate. Climate change will exacerbate food insecurity, particularly in regions already facing instability, limited food access, and undernutrition (Wheeler & von Braun 2013). For decades, bivalves have been selectively bred to enhance traits relevant to improving aquaculture production (Newkirk, 1980; Guo, 2004; Abdelrahman et al., 2017; Mizuta &
Much attention has been paid to improving fundamental performance measures such as survival and growth for numerous commercially-important species (Manzi et al., 1991; Utting et al., 1996; Zheng et al., 2006; Deng et al., 2009; Li et al., 2011; Dove & Connor, 2012; Vu-Van Sang et al., 2019). Additionally, genetic improvements have been made to enhance species’ resistance or tolerance to diseases (Haskin & Ford 1979; Guo et al., 2003; Ragone Calvo et al., 2003; Proestou et al., 2016). However, only recently have bivalve breeding efforts begun to explicitly focus on traits that confer protection against climate change-induced stressors, such as acidification (Fitzer et al., 2019) and thermal stress (Lang et al., 2009; Nie et al., 2017; Tan et al., 2020). For instance, a Manila clam selective breeding program was initiated in China in part to improve stocks that had been suffering devastating summer mortalities (Yan et al., 2005; Zhang & Yan, 2010). Since bivalve farming produces fewer greenhouse gas emissions than nearly all other forms of animal protein production (Hilborn et al., 2018), the resilience and growth of bivalve farming is crucial for increasing the sustainability of food systems.

The present study suggests that farmed surfclams may have the capacity to cope with some degree of warming. However, this species remains at risk from the continued rise in ocean temperatures, particularly on shallow coastal farms that currently contain suitable surfclam habitat but occasionally reach temperatures at or near the species’ lethal limit (Narváez et al., 2015; Timbs et al., 2019). Another plausible route for improving the heat tolerance of surfclams farmed in the northeastern United States is to cross it with its southern subspecies, *S. solidissima similis*. Superior survival and growth due to hybrid vigor, or heterosis, have been observed across several cultured bivalve species (Zhang et
al., 2007; Yan et al., 2008; 2009; Wang et al., 2010; Mlouka et al., 2020). The range of the southern surfclam extends from the Gulf of Mexico to Cape Hatteras, with patchy populations found as far north as Long Island Sound (Hare et al., 2010), yet neither wild nor laboratory-produced hybrids are documented. Therefore, investigations into whether crossing the northern and southern subspecies will produce a superior clam with a greater propensity for enduring heat stress should be carefully explored.

Finally, more research should be devoted to understanding the genetic underpinnings of the surfclam’s response to heat stress. To that end, candidate alleles could be identified and could facilitate marker-assisted selection. Furthermore, other studies have documented enhanced heat tolerance in bivalves following sublethal exposure, with the sustained expression (days to weeks) of heat shock proteins mediating induced thermal tolerance (Shamseldin et al., 1997; Clegg et al., 1998; Sung et al., 2011). Studying factors that may modulate thermal tolerance, like heat shock protein expression or epigenetic modifications, will complement the on-going efforts that are using genetic techniques to generate heat-tolerant surfclams. Collectively, these alternate approaches may expedite the process of breeding Atlantic surfclams for greater heat tolerance. Ultimately, more research into selectively breeding surfclams is warranted and necessary in order to ensure improved and consistent annual yields of farmed Atlantic surfclams in a warming climate.
4.5 Acknowledgements

Daphne Munroe and Ximing Guo served as advisors and collaborators of this study; both were listed as co-authors on the published article. The contributions of Michael Acquafredda include project conceptualization, investigation, acquisition of resources, project administration, data collection, data analyses, manuscript writing, manuscript revising and editing, and preparation of the final manuscript. The contributions of X. Guo and D. Munroe include acquisition of resources, project supervision, validation, and manuscript revising and editing. D. Munroe was partially supported by the USDA National Institute of Food and Agriculture (NIFA) Hatch project accession number 1020831 through the New Jersey Agricultural Experiment Station, Hatch project NJ32140. X. Guo is also supported by USDA NIFA Hatch project NJ30401.

This research was sponsored by New Jersey Sea Grant with funds from the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under NOAA grant #NA18OAR4170357 and the New Jersey Sea Grant Consortium. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of New Jersey Sea Grant or the U. S. Department of Commerce. The NJSGC publication number is NJSG-20-957. This publication was also funded in part through a graduate student grant awarded by the Northeast Sustainable Agriculture Research and Education (SARE) Program of the United States Department of Agriculture (USDA), under SARE grant #GNE17-141-31064. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the Northeast SARE Program or the
USDA. Additionally, Sea-Bird Scientific generously supplied the water quality monitoring equipment, which M. Acquafredda was awarded through the 2016 Student Equipment Loan Program.

I am grateful to the personnel of the Haskin Shellfish Research Laboratory and the New Jersey Aquaculture Innovation Center who provided assistance with this research, particularly S. Borsetti, R. Cacace, J. Caracappa, N. Deck, M. De Luca, D. Jones, J. Kiernan, E. McKean, M. Neuman, L. Ragone Calvo, S. Towers, P. Woodruff, and M. Xie. I would also like to thank the anonymous reviewers for providing valuable feedback and helping to improve this article.

Finally, this work was supported by Rutgers University through the Haskin Shellfish Research Laboratory, the Department of Ecology, Evolution, and Natural Resources, and the Graduate Program in Ecology and Evolution.
4.6 Literature Cited


Results from the 2004 Cooperative Survey of Atlantic Surfclams, pp. 1-41. NEFSC Ref. Doc. 05-01.


Chapter 5: Transcriptomic response of Atlantic surfclams (*Spisula solidissima*) to heat stress

5.0 Abstract

There is clear evidence that the oceans are warming due to anthropogenic climate change, and the northeastern United States contains some of the fastest warming areas. This warming is projected to continue with serious biological and social ramifications for fisheries and aquaculture. One species particularly vulnerable to warming is the Atlantic surfclam (*Spisula solidissima*). The surfclam is a critically important species, linking marine food webs and supporting a productive, lucrative, and sustainable fishery. The surfclam is also emerging as an attractive candidate for aquaculture diversification, but the warming of shallow coastal farms threatens the expansion of surfclam aquaculture. Little is known about the adaptive potential of surfclams to cope with ocean warming. In this study, the surfclam transcriptome under heat stress was examined. Two groups of surfclams were subjected to heat stress to assess how artificial selection may alter gene expression. One group of clams had been selected for greater heat tolerance (HS) and the other was composed of random control clams (RC). After a six-hour exposure to 16 or 29°C, gill transcriptome expression profiles of the four temperature/group combinations were determined by RNA-sequencing and compared. When surfclams experienced heat stress, they exhibited upregulation of heat shock proteins (HSPs), inhibitors of apoptosis (IAPs), and other stress-response related genes. RC clams differentially expressed 1.7-times more genes than HS clams, yet HS clams had a stronger response of key stress response genes, including HSPs, IAPs, and genes involved with mitigating oxidative stress. The findings also indicate that the HS clams may have been primed to withstand
subsequent heat stress after undergoing the initial selection event. This work provides insights into how surfclams adapt to heat stress and should inform future breeding programs that attempt to breed surfclam for greater heat tolerance, and ultimately bring greater resiliency to shellfish farms.
5.1 Introduction

There is clear evidence that the oceans are warming due to anthropogenic climate change (Intergovernmental Panel on Climate Change [IPCC], 2014; Ishii et al., 2017; Resplandy et al., 2018; Chan et al., 2019; Zanna et al., 2019). The northeastern coast of the United States contains some of the fastest warming coastal areas in the world and has experienced episodic marine heat waves (Pershing et al., 2015; Saba et al., 2016; Northeast Fisheries Science Center [NEFSC], 2020). Since 2000, this region has also undergone a regime shift, experiencing a significant increase in the number of warm core rings, which could bring warmer, saltier water from the Gulf Stream to the continental shelf (Gangopadhyay et al., 2019). Ocean warming is projected to cause serious biological and social implications for fisheries and aquaculture, and the northeast this region has already experienced numerous socioecological changes driven or exacerbated by this phenomenon. This includes the collapse of the Gulf of Maine cod (Gadus morhua) fishery (Pershing et al., 2015), shifting spatial distributions of numerous finfish species (Nye et al., 2009; Kleisner et al., 2017; Free et al., 2019; Morson et al., 2019), and heat-related mortalities in blue mussel (Mytilus edulis) aquaculture (Mallet et al., 1990; LeBlanc et al., 2005).

One species particularly vulnerable to ocean warming is the Atlantic surfclam (Spisula solidissima), because high temperature reduces its growth and increases mortality (Goldberg & Walker, 1990; Weinberg 2005; Acquafredda et al., 2019; Acquafredda et al., 2020). The surfclam is one of the largest non-symbiotic suspension feeding bivalves, and it plays an ecologically important role linking primary productivity to higher trophic-level consumers in the nearshore ecosystem (Munroe et al., 2013). The
surfclam is a principal prey item for many species, including the horseshoe crab (*Limulus polyphemus*; Bottom & Haskin, 1984), the moon snail (*Euspira heros*), the lady crab (*Ovalipes ocellatus*), the rock crab (*Cancer irroratus*; Mackenzie et al., 1985), haddock (*Melanogrammus aeglefinus*) and cod (Clark, 1954). In the United States, the surfclam also supports a productive, lucrative, and sustainable fishery. As of 2017, the fleet landed 40.2 million pounds of meats valued at 32.7 million USD, and the fishery was neither overfished nor was overfishing occurring (NEFSC, 2017; National Marine Fisheries Service [NMFS], 2018). However, across its range, the surfclam has shifted away from southern and shallow locations towards more northern and deeper areas (Munroe et al; 2013; 2016; Powell et al., 2016; Hennen et al., 2018; Hofmann et al., 2018; Timbs et al., 2019). Additionally, the surfclam is emerging as an attractive species for aquaculture in the northeastern United States, since it is native, grows rapidly, and complements the growing seasons of other regionally farmed bivalves (Acquafredda et al., 2019; Acquafredda & Munroe, 2020). However, the warming of shallow coastal farms threatens the expansion of surfclam aquaculture.

The adaptive potential of the surfclam to cope with heat stress is an active area of research. Juvenile surfclams that survived prolonged heat stress had greater survival upon re-exposure to those conditions as adults compared to clams that had never experienced severe heat stress (Acquafredda et al., 2020). Moreover, first generation progeny of heat-selected surfclams survived significantly longer during a lethal heat challenge compared to control progeny spawned from non-selected individuals (Acquafredda et al., 2020).

Together, these findings suggest that heat tolerance is a heritable trait in surfclams, and
selective breeding may produce surfclams with greater heat tolerance for farmers seeking to cultivate this species.

In marine bivalves, heat stress can cause oxidative damage (Verlecar et al., 2007), immune system impairment (Chen et al., 2007), and negatively impact feeding processes and energy budgets (Ezgeta-Balić et al., 2011). In surfclams specifically, heat stress decreases clearance rate, respiration rate, and assimilation rate, thereby reducing scope for growth and negatively impacting survival (Hornstein et al., 2018; Acquafredda et al., 2019). Although the phenotypic response of surfclams to heat stress has been documented, little is known about the molecular underpinnings of the surfclam’s response to heat stress.

While only a few species have been studied, some patterns have emerged in the gene expression profiles of bivalves under heat stress. During exposure to unfavorably high temperatures, the Pacific oyster (*Crassostrea gigas*) upregulates genes associated with stress response (e.g., heat shock proteins), lipid biosynthesis, and immune response, while also suppressing growth and downregulating genes that encode for lipid catabolism and mobilization (Meistertzheim et al., 2007; Lang et al., 2009). Manila clams (*Ruditapes philippinarum*) are also known to increase expression of stress and immune response genes when subjected to heat stress (Menike et al., 2014; Ding et al., 2018). Multiple studies also suggest that genes that mitigate the effects of reactive oxygen species (ROS) are upregulated during thermal stress (Meistertzheim et al., 2007; Truebano et al., 2010; Menike et al., 2014).

In this study, I examined the surfclam transcriptome under heat stress and assessed how selection could alter the gene expression profile of surfclams. Two groups
of clams were used, one which had been selected for greater heat tolerance via an acute heat stress four months prior to the study, and a group of randomly selected control clams that never experienced severe heat stress. After a six-hour exposure to 16 or 29°C, gill transcriptome expression profiles of the four temperature x group combinations were determined and compared. Specifically, genes and pathways that were differentially expressed by these surfclam groups during heat stress were identified and analyzed.
5.2 Materials and methods

5.2.1 Experimental design

A cohort of 21-month-old Atlantic surfclams (*Spisula solidissima*) originally spawned in 2017 and farm-raised in Barnegat Bay, NJ were exposed to control conditions (9-11°C) or a lethal heat challenge (27.5-30°C) for a selective breeding study (Acquafredda et al., 2020). Over the five-day challenge, mortality reached ~55%. However, latent mortality occurred for an additional month, leading to a final mortality of 75% (Acquafredda et al., 2020). In that study, the heat-selected clams were designated HS-17; in the present study, this group will simply be referred to as HS. Likewise, the non-selected clams (randomly chosen control individuals) used in that study were designated NS-17, but in the present study, this group will be referred to as RC.

Four months following the challenge, a controlled experiment was conducted to determine whether surfclams that survived a lethal heat stress change their gene expression patterns upon re-exposure to similarly stressful thermal conditions. The experiment was conducted at the New Jersey Aquaculture Innovation Center (AIC) at Rutgers University in North Cape May, NJ. Eighteen individuals were randomly selected from each of the two aforementioned groups, HS and RC. Nine from each group were placed into one of the two treatments, a six-hour heat shock (29.0 ± 0.1°C) and designated either RC29 or HS29, or six-hours in favorable control conditions (16.0 ± 0.5°C) and designated RC16 or HS16. No acclimation period was afforded to the individuals that were placed in the heat shock conditions. Instead, clams were moved out of 15.8°C water and immediately placed into the experimental conditions. Within each treatment, the nine clams from each group were divided into three replicate buckets, each
containing three clams and 15 L of treated (1 µm filtered, UV-sterilized) seawater. Buckets in the heat shock treatment shared a common water bath, which was heated with multiple aquarium heaters (300-400W Aqueon) controlled by dual-stage digital temperature controllers (Inkbird ITC-308). Buckets in the control treatment also shared a common water bath, and its temperature was maintained with an immersion chiller (Aqua Logic, Cyclone 1/4 HP CY-3) and a single-stage digital temperature controller (Aqua Logic, Inc.). A YSI model 86 was used to collect temperature and salinity data, the latter of which ranged from 30.5-31.3 across all experimental units. All buckets were continuously aerated and dissolved oxygen concentration data were collected with a YSI model 55. Dissolved oxygen ranged from 5.90-6.65 mg L\(^{-1}\) in the heat-shock treatment and from 8.48-9.67 mg L\(^{-1}\) in the control treatment. The mean shell length for the HS and RC clams were statistically similar at 35.47 ± 1.34 and 35.25 ± 1.94 mm, respectively (two-sample t-test, \(p = 0.70\)).

After the six-hour experiment, 12 pooled samples (2 clam groups x 2 temperature treatments x 3 replicate buckets) were collected; each was composed of gill tissue from the three clams from each replicate bucket. Gill tissue was chosen for sampling because it has been shown to respond to thermal stress in other marine bivalves (Meistertzheim et al., 2007; Lang et al., 2009; Ding et al., 2018). The tissue from each pool was stored in 1.5 mL Eppendorf tube filled with RNAlater. The RNAlater in each tube was replaced 24 hours after sampling. The samples were then sent to Novogene (CA, USA) for RNA extraction, library construction, and sequencing. After the RNA was extracted from gill tissues and before libraries were constructed, extensive quality control measures were conducted. RNA degradation and contamination were assessed using agarose gel (1%)
electrophoresis. Preliminary RNA quantification and purity were assessed with a Nanodrop spectrophotometer (Thermo Fisher, USA). The RNA quality was assessed by determining the RNA integrity number for each sample by using an Agilent Bioanalyzer 2100 (Agilent Technologies, USA). After the quality control procedures, the NEBNext® Ultra™ RNA Library Prep Kit (New England Biolabs, Inc., USA) was used to prepare the samples for sequencing. The mRNA was enriched from total RNA using oligo(dT) beads, and the mRNA was then fragmented randomly in fragmentation buffer. First-strand cDNA synthesis was carried out with random hexamers and reverse transcriptase. A custom second-strand synthesis buffer (Illumina) was added with dNTPs, RNase H and Escherichia coli polymerase I to generate the second strand by nick-translation. The final cDNA libraries were ready following purification with AMPure XP beads, terminal repair, A-tailing, ligation of sequencing adapters, size selection, and PCR enrichment. Library concentration was first quantified using a Qubit 2.0 fluorometer (Life Technologies, USA). Libraries were then diluted to 1 ng μL⁻¹ before checking insert size on an Agilent Bioanalyzer 2100 (Agilent Technologies, USA), and then quantified to greater accuracy using quantitative PCR (Q-PCR) (library activity >2 nM). High-quality cDNA libraries were then sequenced by an Illumina HiSeq platform. Clean reads were obtained from the raw reads by removing low quality sequences, sequences with adaptor contamination, and sequences with uncertain nucleotides constituting more than 10 percent of the read (N > 10%).
5.2.2 Transcriptome analyses

Trinity (Grabherr et al., 2011) was used to assemble a reference transcriptome since the surfclam genome has not been sequenced. Corset (Davidson & Oshlack, 2014), which clusters contigs based on shared reads and separates contigs when different expression patterns between samples are observed, was used for the hierarchical clustering. Longest transcripts of each cluster were designated as unigenes. Seven databases including, NCBI non-redundant protein sequences, NCBI nucleotide sequences, NCBI euKaryotic Orthologous Groups (KOG), Protein family (Pfam), Swiss-Prot, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genome (KEGG) were used to annotate the resulting transcripts and identify genes (or homologs of genes) based on sequence homology. RSEM (Li et al., 2011) was used to convert read counts to fragments per kilobase of transcript sequence per millions base pairs sequenced (FPKM) and was used to determine expression levels. Subsequently, a hierarchical clustering analysis was used to identify differences and patterns of gene expression across the group/temperature comparisons. The clustering analysis was applied to the union of, or common set, of differentially expressed genes (DEGs) found across all pairwise group/temperature comparisons (see below). A complete list of all DEGs with associated information from all seven databases is hosted at the Rutgers University Haskin Shellfish Research Laboratory (Port Norris, NJ), and portions of the dataset can be provided upon request. Finally, KEGG pathway analysis was conducted to identify significantly enriched metabolic or signal transduction pathways; this analysis compared the frequency of DEGs to the background frequency of genes associated with a given pathway.
5.2.3 Statistical analyses

All measures of dispersion reported in this paper are standard deviation, unless otherwise noted. DESeq was the software package used for the differential gene expression analysis (Anders & Huber, 2010). The resulting p values from the DESeq analysis were corrected using the Benjamini and Hochberg's approach for controlling the false discovery rate (FDR). Genes with an adjusted p value < 0.05 were designated as DEGs. A built-in R package (Version 1.1.383–© 2009–2017 RStudio, Inc.) called pheatmap was used to conduct the clustering analysis of DEGs and to generate the hierarchical heat map. In the heat map, DEGs were clustered based on the centered and normalized log_{10}(FKPM+1) values. For the KEGG pathway analysis, significance was assessed using Fisher’s exact test (hypergeometric test) with the Benjamini and Hochberg's FDR correction. Pathways with an adjusted p value < 0.05 were designated as significantly enriched.
5.3 Results

5.3.1 Transcriptome reconstruction

A total of 555,039,028 raw reads were obtained from the 12 pooled samples containing tissue from 36 clams. After quality control, 541,307,436 clean reads, or 97.53% of all raw reads, were acquired. The mean GC content of sequencing reads across all samples was 37.84 ± 0.38%. The raw sequence data from this study was submitted to NCBI Sequence Read Archive (SAR) under BioProject ID PRJNA596792. The data can be accessed at https://www.ncbi.nlm.nih.gov/sra using the accession numbers SAMN13638038–SAMN13638049. The mean percentage of total reads mapped to the reference transcriptome assembly was 72.60 ± 1.32%, and 43.05% were annotated in at least one of seven functional gene databases.

A total of 185,825 transcripts were identified after de novo assembly of the sample transcriptomes, and of those, 185,675 were classified as unigenes. The mean length of the transcripts was 1142 nucleotides, while the median was 692. The minimum and maximum lengths were 201 and 29,827 nucleotides, respectively. This descriptive information was identical for the identified unigenes, with the exception that the mean length of the unigenes was 1143 nucleotides. Overall, 6086 genes were used in the cluster analysis, which depicts differential gene expression across group and temperature. Noteworthy and highly significant DEGs with their Swiss-Prot IDs and descriptions were assembled in Table 5-1, Table 5-2, and Table 5-3.
5.3.2 Differentially expressed genes (DEGs) induced by heat stress

The within-group/across-temperature (RC29vs.RC16 and HS29vs.HS16) comparisons were examined to determine how naïve surfclams (RC) respond to heat stress and how it may differ from the way clams that survived a prior lethal heat stress (HS) responded to a repeated exposure. A six-hour heat shock of 29˚C induced a significant response in both the RC and HS groups, compared to their respective controls held at 16˚C. The cluster analysis revealed that the RC clams differentially expressed significantly more genes than the HS clams did (Figure 5-1). Heat stress induced more nearly 2000 more DEGs in RC29 compared to HS29. In total, 4908 DEGs were detected in RC29 relative to clams at 16˚C, 79% of which were upregulated under heat stress (Figure 5-2). In contrast, 2916 DEGs were detected in HS29 relative to clams at 16˚C and 86% were upregulated under heat stress (Figure 5-2). Collectively, 1786 DEGs that were shared between the two groups, and 1638 DEGs were upregulated at 29˚C, regardless of group (Figure 5-2). The most notable expression patterns were related to genes in the heat shock protein family (HSPs), inhibitors of apoptosis (IAPs), immune-response genes, and oxidative stress-response genes (Table 5-1).

HS29 and RC29 both exhibited robust differential expression of approximately 60 genes in the HSP family, including hsp40 (dnaja), hsp70, hsp90, and hsp110 (Table 5-1). Activators and other proteins that interact with HSPs were also identified as DEGs. Compared to their 16˚C counterparts, HS29 exhibited a 75-fold increase in HSP expression compared to a 22-fold increase exhibited by RC29.

Expression of genes in the IAP family followed a similar pattern (Table 5-1). HS29 and RC29 expressed approximately 33 and 47 IAPs, respectively. Although RC29
expressed a greater number of IAPs, the expression level of these genes was collectively lower than what was observed in HS29. Whereas HS29 had a 50-fold increase in IAP expression relative to HS16, RC29 had a 19-fold increase relative to RC16. At 29°C, both groups of clams upregulated between 15 and 20 DEGs that promote apoptosis, such as *caspase-3*. However, for both RC29 and HS29, the upregulation of pro-apoptosis genes (3 to 5-fold increase compared to clams at 16°C) was far lower than the upregulation of IAPs.

Numerous immune-related genes, such as toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible (RIG) I-like receptors (RLRs), C-type lectins and galectins, were also differentially expressed under heat stress (Table 5-1). In HS29, several TLRs were identified as DEGs, including *tlr13, tlr14, toll2*, and every gene was upregulated. Although more TLRs were differentially expressed in RC29 relative to RC16, several were downregulated. Consequently, HS29 had a more than 20-fold increase in TLR expression compared to HS16, while the expression of TLRs in RC29 was less than twice as high as RC16. Only one NLR, *nlrq4*, was differentially expressed by RC29, and it was downregulated relative to RC16. HS29 also downregulated this gene relative to HS16, but HS29 also upregulated two other NLRs. Therefore, HS29 had an overall increase in NLR expression while RC29 exhibited a decrease. No RLRs were differentially expressed in the HS29vs.HS16 comparison, yet three DEGs in the RC29vs.RC16 comparison had the C-terminal domain of RIG-I. Relative to RC16, RC29 upregulated two and down regulated one of these DEGs, leading RC to exhibit a general decrease in RLR expression at 29°C. More C-type lectins and galectins were differentially expressed in the RC29vs.RC16 comparison than
the HS29vs.HS16 comparison. For RC clams, expression decreased at 29°C, with RC29 having approximately one-third of the levels of RC16. By contrast, there was a greater than 2-fold increase in overall expression of C-type lectins and galectins in HS29 relative to HS16.

Compared to clams at 16°C, both HS and RC clams at 29°C demonstrated significant upregulation of genes that confer protection from oxidative stress, including genes such as superoxide dismutase 1 (sod1), glutaredoxin (grx), and glutathione s-transferase 1 (gst1) (Table 5-1). Overall, HS29 differentially expressed approximately 18 genes, leading to a 12-fold increase in the expression oxidative stress response genes. A similar number of DEGs were identified in the RC29vs.RC16 comparison, but RC29 only exhibited a 6-fold increase in the expression of ROS-quenching genes.

5.3.3 Differentially expressed genes (DEGs) across group/within temperature

The across-group/within-temperature comparisons (HS29vs.RC29 and HS16vs.RC16) were also examined to determine if HS and RC clams expressed distinct genes under the same conditions. Far fewer DEGs were observed in these comparisons. At 29°C, 51 DEGs were identified between HS and RC clams; of these, 35 were upregulated in RC and 16 were upregulated in HS clams (Table 5-2; Figure 5-2). The most notable difference between HS29 and RC29 relates to the expression of a heat shock protein. As mentioned previously, both HS29 and RC29 exhibited robust expression of many heat shock proteins. However, one gene (heat shock protein 70 B2, ID: 21841) had significantly higher expression (2.4-fold) in HS29 than RC29.
Of the 51 DEGs, three lipid metabolism genes were not expressed in HS29, but were expressed in RC29. These includes \textit{acetyl-CoA carboxylase}, \textit{sterol regulatory element-binding protein cleavage-activating protein (SCAP)}, which is a regulatory protein involved with cholesterol biosynthesis, and \textit{phospholipase (ABHD3)}, which is involved with phospholipid remodeling. However, HS29 did show significantly higher expression of one lipid metabolism gene, \textit{Sn1-specific diacylglycerol lipase beta}.

Although the expression of IAPs was robust in both RC29 and HS29, both expressed at least one DEG associated with apoptosis induction. Compared with RC29, HS29 had significantly higher expression of \textit{mitochondrial apoptosis-inducing factor 1}, but lower expression of \textit{MAP kinase-activating death domain protein}. Other notables DEGs associated with immunity. Unlike RC clams at 29˚C, HS clams did not express a \textit{probable polyketide synthase (Pks16)}, which encodes an enzyme that facilitates the production of antimicrobial agents; however, HS clams did express a leucine-rich repeat-containing G-protein coupled receptor, which is likely a unique toll-like receptor (ID: 116707).

At 16˚C, only 31 genes were differentially expressed between HS and RC clams; of these, 22 were downregulated and nine were upregulated in HS16 (Table 5-3; Figure 5-2). HS clams downregulated genes associated with one-carbon metabolism \textit{(cytoplasmic C-1-tetrahydrofolate synthase)} and innate immunity \textit{(techylectin-5A)}, while upregulating genes associated with chromatin decondensation and ribosome assembly \textit{(nucleolin)}, reactive oxygen species production \textit{(NADPH oxidase 5)}, and protein glycosylation \textit{(beta-1,4-galactosyltransferase 1)}. Both groups expressed genes associated with apoptosis (RC16: \textit{caspase 3}; HS16: \textit{netrin receptor UNC5D}) and cell signaling.
(RC16: regulator of G-protein signaling 22; HS16: parathyroid hormone/parathyroid hormone-related peptide receptor), in addition to expressing genes that contain leucine-rich repeats (RC16: lrcc71; HS16: lrcc74a).
Table 5-1. Notable and highly significant differentially expressed genes (DEGs) induced by heat stress. HS29 = Heat-Selected clams at 29°C; HS16 = Heat-Selected clams at 16°C; RC29 = Random-Control clams at 29°C; RC16 = Random-Control clams at 16°C. Unless otherwise noted (*), the Transcript ID for each DEG listed in this table contains the prefix “Cluster-17909.”

<table>
<thead>
<tr>
<th>ID</th>
<th>Read count HS29</th>
<th>Read count HS16</th>
<th>Fold Change (log2 [HS29/HS16])</th>
<th>Adjusted p value HS29 vs. HS16</th>
<th>Read count RC29</th>
<th>Read count RC16</th>
<th>Fold Change (log2 [RC29/RC16])</th>
<th>Adjusted p value RC29 vs. RC16</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>73258</td>
<td>709</td>
<td>74</td>
<td>3.27</td>
<td>5.73E-04</td>
<td>797</td>
<td>68</td>
<td>3.54</td>
<td>9.14E-06</td>
<td>Q64433</td>
<td>10 kDa heat shock protein, mitochondria, Mus musculus</td>
</tr>
<tr>
<td>64477</td>
<td>14808</td>
<td>49</td>
<td>8.25</td>
<td>5.29E-21</td>
<td>9337</td>
<td>45</td>
<td>7.70</td>
<td>4.78E-05</td>
<td>Q94738</td>
<td>97 kDa heat shock protein, Strongylocentrotus franciscanus</td>
</tr>
<tr>
<td>107949</td>
<td>12903</td>
<td>6</td>
<td>11.13</td>
<td>3.70E-08</td>
<td>3563</td>
<td>6</td>
<td>9.15</td>
<td>5.22E-28</td>
<td>P0CB32</td>
<td>Heat shock 70 kDa protein 1-like, Bos taurus</td>
</tr>
<tr>
<td>39118</td>
<td>226</td>
<td>18</td>
<td>3.69</td>
<td>4.49E-04</td>
<td>Inf</td>
<td>0</td>
<td>Inf</td>
<td>4.22E-08</td>
<td>O43301</td>
<td>Heat shock 70 kDa protein 12A, Homo sapiens</td>
</tr>
<tr>
<td>32880</td>
<td>334</td>
<td>0</td>
<td>Inf</td>
<td>1.78E-06</td>
<td>207</td>
<td>0</td>
<td>Inf</td>
<td>4.22E-08</td>
<td>Q06248</td>
<td>Heat shock 70 kDa protein IV, Paracentrotus lividus</td>
</tr>
<tr>
<td>72056</td>
<td>22043</td>
<td>0</td>
<td>Inf</td>
<td>1.88E-20</td>
<td>12425</td>
<td>1</td>
<td>13.34</td>
<td>1.93E-06</td>
<td>P08106</td>
<td>Heat shock 70 kDa protein, Gallus gallus</td>
</tr>
<tr>
<td>71067</td>
<td>42312</td>
<td>199</td>
<td>7.73</td>
<td>5.41E-17</td>
<td>38474</td>
<td>213</td>
<td>7.50</td>
<td>3.94E-10</td>
<td>Q92598</td>
<td>Heat shock protein 105 kDa, Homo sapiens</td>
</tr>
<tr>
<td>113554</td>
<td>11343</td>
<td>16</td>
<td>9.50</td>
<td>6.12E-19</td>
<td>7062</td>
<td>52</td>
<td>7.10</td>
<td>1.46E-10</td>
<td>P02518</td>
<td>Heat shock protein 27, Drosophila melanogaster</td>
</tr>
<tr>
<td>21519</td>
<td>26739</td>
<td>0</td>
<td>15.74</td>
<td>3.93E-39</td>
<td>11694</td>
<td>0</td>
<td>15.08</td>
<td>1.91E-04</td>
<td>P41825</td>
<td>Heat shock protein 70 A1, Anopheles albimanus</td>
</tr>
<tr>
<td>21841</td>
<td>73617</td>
<td>2</td>
<td>15.03</td>
<td>1.25E-33</td>
<td>10850</td>
<td>1</td>
<td>13.57</td>
<td>6.73E-09</td>
<td>P41827</td>
<td>Heat shock protein 70 B2, Anopheles albimanus</td>
</tr>
</tbody>
</table>
Table 5-1 Continued

<table>
<thead>
<tr>
<th>ID</th>
<th>Read count HS29</th>
<th>Read count HS16</th>
<th>Fold Change (log2 [HS29/HS16])</th>
<th>Adjusted p value HS29vs.HS16</th>
<th>Read count RC29</th>
<th>Read count RC16</th>
<th>Fold Change (log2 [RC29/RC16])</th>
<th>Adjusted p value RC29vs.RC16</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>123079</td>
<td>154928</td>
<td>2</td>
<td>16.10</td>
<td>3.28E-30</td>
<td>41390</td>
<td>1</td>
<td>15.07</td>
<td>6.01E-31</td>
<td>P41827</td>
<td>Heat shock protein 70 B2, <em>Anopheles albimanus</em></td>
</tr>
<tr>
<td>73231</td>
<td>375787</td>
<td>13</td>
<td>14.82</td>
<td>1.09E-21</td>
<td>118870</td>
<td>14</td>
<td>13.06</td>
<td>1.06E-12</td>
<td>P41827</td>
<td>Heat shock protein 70 B2, <em>Anopheles albimanus</em></td>
</tr>
<tr>
<td>79243</td>
<td>195584</td>
<td>10219</td>
<td>4.26</td>
<td>2.96E-04</td>
<td>175553</td>
<td>9155</td>
<td>4.26</td>
<td>7.58E-06</td>
<td>O02192</td>
<td>Heat shock protein 83, <em>Drosophila auraria</em></td>
</tr>
<tr>
<td>83430</td>
<td>148425</td>
<td>7046</td>
<td>4.40</td>
<td>6.58E-05</td>
<td>133078</td>
<td>6912</td>
<td>4.27</td>
<td>2.32E-06</td>
<td>P34058</td>
<td>Heat shock protein HSP 90-beta, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>87575</td>
<td>1480</td>
<td>121</td>
<td>3.62</td>
<td>2.18E-05</td>
<td>1002</td>
<td>130</td>
<td>2.95</td>
<td>1.13E-02</td>
<td>P02513</td>
<td>Heat shock protein Hsp-16.48/Hsp-16.49, <em>Caenorhabditis elegans</em></td>
</tr>
<tr>
<td>40108</td>
<td>1577</td>
<td>123</td>
<td>3.68</td>
<td>1.31E-05</td>
<td>1643</td>
<td>155</td>
<td>3.40</td>
<td>3.15E-06</td>
<td>Q5E954</td>
<td>DnaJ homolog subfamily A member 1, <em>Bos taurus</em></td>
</tr>
<tr>
<td>45742</td>
<td>129362</td>
<td>274</td>
<td>8.89</td>
<td>1.67E-15</td>
<td>58985</td>
<td>335</td>
<td>7.46</td>
<td>5.64E-04</td>
<td>Q5BIP8</td>
<td>DnaJ homolog subfamily B member 5, <em>Bos taurus</em></td>
</tr>
<tr>
<td>114424</td>
<td>1101</td>
<td>120</td>
<td>3.20</td>
<td>3.55E-04</td>
<td>1787</td>
<td>133</td>
<td>3.75</td>
<td>4.03E-07</td>
<td>Q99615</td>
<td>DnaJ homolog subfamily C member 7, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>57044</td>
<td>120427</td>
<td>90</td>
<td>10.39</td>
<td>1.84E-19</td>
<td>93260</td>
<td>93</td>
<td>9.96</td>
<td>1.48E-22</td>
<td>P82147</td>
<td>Protein lethal(2)essential for life, <em>Drosophila melanogaster</em></td>
</tr>
<tr>
<td>88757</td>
<td>13697</td>
<td>732</td>
<td>4.23</td>
<td>3.31E-06</td>
<td>15866</td>
<td>634</td>
<td>4.65</td>
<td>8.83E-11</td>
<td>Q0VCX2</td>
<td>78 kDa glucose-regulated protein, <em>Bos taurus</em></td>
</tr>
<tr>
<td>107948</td>
<td>2121</td>
<td>1</td>
<td>11.72</td>
<td>3.06E-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P34933</td>
<td>Heat shock-related 70 kDa protein 2, <em>Bos taurus</em></td>
</tr>
<tr>
<td>34330</td>
<td>2008</td>
<td>0</td>
<td>Inf</td>
<td>6.72E-07</td>
<td>1568</td>
<td>0</td>
<td>12.18</td>
<td>3.28E-03</td>
<td>P63017</td>
<td>Heat shock cognate 71 kDa protein, <em>Mus musculus</em></td>
</tr>
<tr>
<td>ID</td>
<td>Read count HS29</td>
<td>Read count HS16</td>
<td>Fold Change (log2 [HS29/HS16])</td>
<td>Adjusted p value HS29vs.HS16</td>
<td>Read count RC29</td>
<td>Read count RC16</td>
<td>Fold Change (log2 [RC29/RC16])</td>
<td>Adjusted p value RC29vs.RC16</td>
<td>Swissprot ID</td>
<td>Swissprot description</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>-------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>79227</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P47773</td>
<td>Heat shock cognate 71 kDa protein, <em>Ictalurus punctatus</em></td>
</tr>
<tr>
<td>83667</td>
<td>18</td>
<td>0</td>
<td>Inf</td>
<td>2.23E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q5ZLF0</td>
<td>Hsc70-interacting protein, <em>Gallus gallus</em></td>
</tr>
<tr>
<td>10553</td>
<td>52</td>
<td>1</td>
<td>5.19</td>
<td>1.47E-03</td>
<td>100</td>
<td>8</td>
<td>3.69</td>
<td>5.08E-03</td>
<td>P38532</td>
<td>Heat shock factor protein 1, <em>Mus musculus</em></td>
</tr>
<tr>
<td>123681</td>
<td>26</td>
<td>0</td>
<td>6.90</td>
<td>1.01E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8BK64</td>
<td>Activator of 90 kDa heat shock protein ATPase homolog 1, <em>Mus musculus</em></td>
</tr>
<tr>
<td>44006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6987</td>
<td>96</td>
<td>6.18</td>
<td>3.03E-17</td>
<td>Q95433</td>
<td>Activator of 90 kDa heat shock protein ATPase homolog 1, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>123126</td>
<td>28000</td>
<td>49</td>
<td>9.16</td>
<td>2.46E-22</td>
<td>16800</td>
<td>68</td>
<td>7.95</td>
<td>1.59E-24</td>
<td>Q99P31</td>
<td>Hsp70-binding protein 1, <em>Mus musculus</em></td>
</tr>
<tr>
<td>82358</td>
<td>747</td>
<td>151</td>
<td>2.31</td>
<td>4.51E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P41436</td>
<td>Apoptosis inhibitor IAP, <em>Cydia pomonella</em> granulosis virus (isolate Mexico/1963)</td>
</tr>
<tr>
<td>124109</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>416</td>
<td>44</td>
<td>3.24</td>
<td>2.00E-04</td>
<td>Q13490</td>
<td>Baculoviral IAP repeat-containing protein 2, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>33852</td>
<td>52</td>
<td>1</td>
<td>5.19</td>
<td>2.01E-03</td>
<td>67</td>
<td>8</td>
<td>3.12</td>
<td>4.05E-02</td>
<td>Q13075</td>
<td>Baculoviral IAP repeat-containing protein 1, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>61784</td>
<td>15474</td>
<td>49</td>
<td>8.30</td>
<td>2.23E-18</td>
<td>9649</td>
<td>53</td>
<td>7.52</td>
<td>4.25E-23</td>
<td>Q62210</td>
<td>Baculoviral IAP repeat-containing protein 2, <em>Mus musculus</em></td>
</tr>
<tr>
<td>ID</td>
<td>Read count HS29</td>
<td>Read count HS16</td>
<td>Fold Change (log₂ [HS29/HS16])</td>
<td>Adjusted p value HS29 vs. HS16</td>
<td>Read count RC29</td>
<td>Read count RC16</td>
<td>Fold Change (log₂ [RC29/RC16])</td>
<td>Adjusted p value RC29 vs. RC16</td>
<td>Swissprot ID</td>
<td>Swissprot description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>--------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>85410</td>
<td>30402</td>
<td>228</td>
<td>7.06</td>
<td>2.78E-15</td>
<td>18167</td>
<td>278</td>
<td>6.03</td>
<td>2.75E-16</td>
<td>A9JTP3</td>
<td>Baculoviral IAP repeat-containing protein 7, <em>Xenopus tropicalis</em></td>
</tr>
<tr>
<td>85910</td>
<td>73110</td>
<td>200</td>
<td>8.51</td>
<td>4.26E-17</td>
<td>41050</td>
<td>279</td>
<td>7.20</td>
<td>1.23E-18</td>
<td>Q8JHV9</td>
<td>Baculoviral IAP repeat-containing protein 7-A, <em>Xenopus laevis</em></td>
</tr>
<tr>
<td>96167</td>
<td>845</td>
<td>111</td>
<td>2.92</td>
<td>9.30E-03</td>
<td>1120</td>
<td>139</td>
<td>3.02</td>
<td>1.34E-04</td>
<td>Q24306</td>
<td>Death-associated inhibitor of apoptosis 1, <em>Drosophila melanogaster</em></td>
</tr>
<tr>
<td>82696</td>
<td>9741</td>
<td>111</td>
<td>6.46</td>
<td>3.61E-15</td>
<td>6556</td>
<td>157</td>
<td>5.38</td>
<td>6.30E-10</td>
<td>Q24307</td>
<td>Death-associated inhibitor of apoptosis 2, <em>Drosophila melanogaster</em></td>
</tr>
<tr>
<td>107780</td>
<td>123</td>
<td>2</td>
<td>6.14</td>
<td>7.42E-04</td>
<td>88</td>
<td>5</td>
<td>4.18</td>
<td>1.49E-03</td>
<td>Q60989</td>
<td>E3 ubiquitin-protein ligase XIAP, <em>Mus musculus</em></td>
</tr>
<tr>
<td>124618</td>
<td>119</td>
<td>0</td>
<td>8.72</td>
<td>4.58E-09</td>
<td>174</td>
<td>0</td>
<td>Inf</td>
<td>1.72E-05</td>
<td>Q90660</td>
<td>Inhibitor of apoptosis protein, <em>Gallus gallus</em></td>
</tr>
<tr>
<td>128226</td>
<td>437</td>
<td>0</td>
<td>Inf</td>
<td>3.71E-07</td>
<td>284</td>
<td>0</td>
<td>Inf</td>
<td>8.49E-03</td>
<td>Q6R7D0</td>
<td>Putative apoptosis inhibitor ORF99, Ostreid herpesvirus 1 (isolate France)</td>
</tr>
<tr>
<td>81101</td>
<td>6646</td>
<td>1185</td>
<td>2.49</td>
<td>1.46E-02</td>
<td>7564</td>
<td>1291</td>
<td>2.55</td>
<td>5.84E-04</td>
<td>P53563</td>
<td>Bcl-2-like protein 1, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>64488</td>
<td>1293</td>
<td>283</td>
<td>2.19</td>
<td>4.12E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q13625</td>
<td>Apoptosis-stimulating of p53 protein 2, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>62315</td>
<td>323</td>
<td>2</td>
<td>7.35</td>
<td>2.95E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q16611</td>
<td>Bcl-2 homologous antagonist/killer, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>ID</td>
<td>Read count HS29</td>
<td>Read count HS16</td>
<td>Fold Change (log2 [HS29/HS16])</td>
<td>Adjusted p value HS29vs.HS16</td>
<td>Read count RC29</td>
<td>Read count RC16</td>
<td>Fold Change (log2 [RC29/RC16])</td>
<td>Adjusted p value RC29vs.RC16</td>
<td>Swissprot ID</td>
<td>Swissprot description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>--------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>62314</td>
<td></td>
<td></td>
<td>687</td>
<td>77</td>
<td>3.15</td>
<td></td>
<td>1.85E-04</td>
<td></td>
<td>Q16611</td>
<td>Bcl-2 homologous antagonist/killer, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>62312</td>
<td></td>
<td></td>
<td>208</td>
<td>1</td>
<td>7.68</td>
<td></td>
<td>2.57E-02</td>
<td></td>
<td>Q16611</td>
<td>Bcl-2 homologous antagonist/killer, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>45629</td>
<td>41</td>
<td>0</td>
<td>Inf</td>
<td>2.48E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8MJC3</td>
<td>Caspase-3, <em>Oryctolagus cuniculus</em></td>
</tr>
<tr>
<td>91728</td>
<td></td>
<td></td>
<td>1000</td>
<td>209</td>
<td>2.26</td>
<td></td>
<td>8.85E-03</td>
<td></td>
<td>Q08DY9</td>
<td>Caspase-3, <em>Bos taurus</em></td>
</tr>
<tr>
<td>40442</td>
<td>124</td>
<td>0</td>
<td>Inf</td>
<td>5.87E-10</td>
<td>58</td>
<td>2</td>
<td>4.83</td>
<td>7.59E-04</td>
<td>F7D3V9</td>
<td>Leucine-rich repeat-containing G-protein coupled receptor 5, <em>Xenopus tropicalis</em></td>
</tr>
<tr>
<td>87580</td>
<td>14</td>
<td>0</td>
<td>Inf</td>
<td>3.75E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A2RUW1</td>
<td>Toll-interacting protein, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>62923</td>
<td>211</td>
<td>16</td>
<td>3.72</td>
<td>9.29E-04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q6R5N8</td>
<td>Toll-like receptor 13, <em>Mus musculus</em></td>
</tr>
<tr>
<td>76880</td>
<td>32</td>
<td>198</td>
<td>-2.63</td>
<td>-2.63</td>
<td>17</td>
<td>216</td>
<td>-3.63</td>
<td>2.91E-04</td>
<td>F6R2G2</td>
<td>NLR family CARD domain-containing protein 4, <em>Xenopus tropicalis</em></td>
</tr>
<tr>
<td>75226</td>
<td></td>
<td></td>
<td>76</td>
<td>8</td>
<td>3.25</td>
<td></td>
<td>3.14E-02</td>
<td></td>
<td>Q8TC21</td>
<td>Zinc finger protein 596, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>101224</td>
<td></td>
<td></td>
<td>160</td>
<td>655</td>
<td>-2.03</td>
<td></td>
<td>2.79E-02</td>
<td></td>
<td>Q9N2M8</td>
<td>Headcase protein, <em>Drosophila melanogaster</em></td>
</tr>
<tr>
<td>74838</td>
<td>898</td>
<td>6</td>
<td>7.18</td>
<td>8.38E-15</td>
<td>868</td>
<td>0</td>
<td>Inf</td>
<td>8.32E-07</td>
<td>A3FM55</td>
<td>C-type lectin 1, <em>Hydrophis hardwickii</em></td>
</tr>
<tr>
<td>ID</td>
<td>Read count HS29</td>
<td>Read count HS16</td>
<td>Fold Change (log2 [HS29/HS16])</td>
<td>Adjusted p value HS29 vs HS16</td>
<td>Read count RC29</td>
<td>Read count RC16</td>
<td>Fold Change (log2 [RC29/RC16])</td>
<td>Adjusted p value RC29 vs RC16</td>
<td>Swissprot ID</td>
<td>Swissprot description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>------------------------------</td>
<td>--------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>119089</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>115</td>
<td>0</td>
<td>Inf</td>
<td>5.61E-08</td>
<td>A3FM55</td>
<td>C-type lectin 1, <em>Hydrophis hardwickii</em></td>
</tr>
<tr>
<td>99637</td>
<td>10</td>
<td>98</td>
<td>-3.31</td>
<td>3.29E-02</td>
<td>18</td>
<td>166</td>
<td>-3.21</td>
<td>2.58E-03</td>
<td>Q6ZS10</td>
<td>C-type lectin domain family 17, member A, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>46153</td>
<td>95</td>
<td>11</td>
<td>3.15</td>
<td>4.21E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q66S03</td>
<td>Galactose-specific lectin nattectin, <em>Thalassophryne nattereri</em></td>
</tr>
<tr>
<td>82703</td>
<td>56</td>
<td>333</td>
<td>-2.57</td>
<td>2.54E-02</td>
<td>90</td>
<td>383</td>
<td>-2.08</td>
<td>4.53E-02</td>
<td>P47845</td>
<td>Galectin-3, <em>Oryctolagus cuniculus</em></td>
</tr>
<tr>
<td>*Cluster 1827.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P46413</td>
<td>Superoxide dismutase [Cu-Zn], <em>Macaca fascicularis</em></td>
</tr>
<tr>
<td>57252</td>
<td>96</td>
<td>0</td>
<td>Inf</td>
<td>2.18E-08</td>
<td>219</td>
<td>0</td>
<td>Inf</td>
<td>4.48E-04</td>
<td>P46436</td>
<td>Glutathione S-transferase 1, <em>Ascaris suum</em></td>
</tr>
<tr>
<td>21716</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>546</td>
<td>27</td>
<td>4.32</td>
<td>1.42E-02</td>
<td>P46413</td>
<td>Glutathione synthetase, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>21714</td>
<td>368</td>
<td>1</td>
<td>8.82</td>
<td>9.21E-07</td>
<td>347</td>
<td>1</td>
<td>8.04</td>
<td>7.14E-04</td>
<td>P46413</td>
<td>Glutathione synthetase, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>42537</td>
<td>0</td>
<td>24</td>
<td>-Inf</td>
<td>2.00E-02</td>
<td>0</td>
<td>26</td>
<td>-Inf</td>
<td>4.56E-03</td>
<td>Q54B10</td>
<td>NADPH oxidoreductase A, <em>Dictyostelium discoideum</em></td>
</tr>
<tr>
<td>13852</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>208</td>
<td>6</td>
<td>5.13</td>
<td>1.94E-07</td>
<td>Q1ENI8</td>
<td>Peroxidasin homolog, <em>Caenorhabditis elegans</em></td>
</tr>
<tr>
<td>114824</td>
<td>5685</td>
<td>49</td>
<td>6.85</td>
<td>3.48E-04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q3UQ28</td>
<td>Peroxidasin homolog, <em>Mus musculus</em></td>
</tr>
<tr>
<td>43138</td>
<td>1487</td>
<td>259</td>
<td>2.52</td>
<td>1.00E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8CDN6</td>
<td>Thioredoxin-like protein 1, <em>Mus musculus</em></td>
</tr>
</tbody>
</table>
Table 5-1 Continued

<table>
<thead>
<tr>
<th>ID</th>
<th>Read count HS29</th>
<th>Read count HS16</th>
<th>Fold Change (log2 [HS29/HS16])</th>
<th>Adjusted p value HS29vs.HS16</th>
<th>Read count RC29</th>
<th>Read count RC16</th>
<th>Fold Change (log2 [RC29/RC16])</th>
<th>Adjusted p value RC29vs.RC16</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>113966</td>
<td>115</td>
<td>3</td>
<td>5.27</td>
<td>4.87E-05</td>
<td>188</td>
<td>4</td>
<td>5.57</td>
<td>3.08E-07</td>
<td>Q7ZU99</td>
<td>Transitional endoplasmic reticulum ATPase, <em>Danio rerio</em></td>
</tr>
<tr>
<td>79018</td>
<td>1579</td>
<td>197</td>
<td>3.00</td>
<td>7.28E-04</td>
<td>2287</td>
<td>217</td>
<td>3.40</td>
<td>3.87E-06</td>
<td>P27824</td>
<td>Calnexin, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>113304</td>
<td>16</td>
<td>0</td>
<td>Inf</td>
<td>2.59E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A8QMS7</td>
<td>Myeloid differentiation primary response protein MyD88, <em>Takifugu rubripes</em></td>
</tr>
<tr>
<td>124151</td>
<td>61</td>
<td>0</td>
<td>Inf</td>
<td>2.93E-06</td>
<td>72</td>
<td>2</td>
<td>5.43</td>
<td>1.66E-04</td>
<td>Q9UHD2</td>
<td>Serine/threonine-protein kinase TBK1, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>120157</td>
<td>830</td>
<td>67</td>
<td>3.63</td>
<td>5.46E-05</td>
<td>1127</td>
<td>59</td>
<td>4.26</td>
<td>4.30E-08</td>
<td>P47809</td>
<td>Dual specificity mitogen-activated protein kinase 4, <em>Mus musculus</em></td>
</tr>
<tr>
<td>16418</td>
<td>69</td>
<td>0</td>
<td>7.28</td>
<td>1.86E-05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9R0T8</td>
<td>Inhibitor of nuclear factor kappa-B kinase subunit epsilon, <em>Mus musculus</em></td>
</tr>
<tr>
<td>15961</td>
<td>86</td>
<td>1</td>
<td>6.02</td>
<td>2.37E-05</td>
<td>59</td>
<td>1</td>
<td>5.90</td>
<td>2.03E-04</td>
<td>P16157</td>
<td>Ankyrin-1, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>69500</td>
<td>12802</td>
<td>410</td>
<td>4.97</td>
<td>9.07E-03</td>
<td>9721</td>
<td>403</td>
<td>4.59</td>
<td>3.43E-11</td>
<td>Q03017</td>
<td>NF-kappa-B inhibitor cactus, <em>Drosophila melanogaster</em></td>
</tr>
<tr>
<td>76792</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11824</td>
<td>3467</td>
<td>1.77</td>
<td>3.84E-02</td>
<td>Q04861</td>
<td>Nuclear factor NF-kappa-B p105 subunit, <em>Gallus gallus</em></td>
</tr>
</tbody>
</table>
Table 5-1 Continued

<table>
<thead>
<tr>
<th>ID</th>
<th>Read count HS29</th>
<th>Read count HS16</th>
<th>Fold Change (log2 [HS29/HS16])</th>
<th>Adjusted p value HS29vs.HS16</th>
<th>Read count RC29</th>
<th>Read count RC16</th>
<th>Fold Change (log2 [RC29/RC16])</th>
<th>Adjusted p value RC29vs.RC16</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>80223</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8NFZ5</td>
<td>TNFAIP3-interacting protein 2, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>80224</td>
<td>11325</td>
<td>2285</td>
<td>2.31</td>
<td>2.40E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8NFZ5</td>
<td>TNFAIP3-interacting protein 2, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>115179</td>
<td>47</td>
<td>2</td>
<td>4.41</td>
<td>1.71E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O08727</td>
<td>Tumor necrosis factor receptor superfamily member 11B, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>17060</td>
<td></td>
<td></td>
<td>4.41</td>
<td>4.41E-05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O08712</td>
<td>Tumor necrosis factor receptor superfamily member 11B, <em>Mus musculus</em></td>
</tr>
<tr>
<td>46751</td>
<td></td>
<td></td>
<td>Inf</td>
<td>4.04E-04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q61382</td>
<td>TNF receptor-associated factor 4, <em>Mus musculus</em></td>
</tr>
<tr>
<td>95615</td>
<td></td>
<td></td>
<td>Inf</td>
<td>2.88E-07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q5BIS9</td>
<td>5'-AMP-activated protein kinase subunit beta-1, <em>Bos taurus</em></td>
</tr>
<tr>
<td>56519</td>
<td></td>
<td></td>
<td>Inf</td>
<td>1.62E-02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q91309</td>
<td>6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, <em>Lithobates catesbeiana</em></td>
</tr>
</tbody>
</table>
Table 5-2. All differentially expressed genes (DEGs) between HS29 and RC29.
HS29 = Heat-Selected clams at 29°C; RC29 = Random-Control clams at 29°C. The Transcript ID for all DEGs listed in this table contain the prefix “Cluster-17909.”.

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Read count (HS29)</th>
<th>Read count (RC29)</th>
<th>Fold Change (log2 [HS29/RC29])</th>
<th>Adjusted p value</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>83849</td>
<td>92</td>
<td>0</td>
<td>Inf</td>
<td>2.23E-06</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>113308</td>
<td>87</td>
<td>0</td>
<td>Inf</td>
<td>3.18E-06</td>
<td>Q13449</td>
<td>Limbic system-associated membrane protein, Homo sapiens</td>
</tr>
<tr>
<td>79448</td>
<td>1093</td>
<td>64</td>
<td>4.10</td>
<td>5.73E-06</td>
<td>Q96SJ8</td>
<td>Tetraspanin-18, Homo sapiens</td>
</tr>
<tr>
<td>84879</td>
<td>77</td>
<td>0</td>
<td>Inf</td>
<td>3.95E-05</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>113190</td>
<td>57</td>
<td>0</td>
<td>Inf</td>
<td>1.75E-04</td>
<td>Q9JM53</td>
<td>Apoptosis-inducing factor 1, mitochondrial, Rattus norvegicus</td>
</tr>
<tr>
<td>94775</td>
<td>57</td>
<td>0</td>
<td>Inf</td>
<td>2.10E-04</td>
<td>O08874</td>
<td>Serine/threonine-protein kinase N2, Rattus norvegicus</td>
</tr>
<tr>
<td>116707</td>
<td>42</td>
<td>0</td>
<td>Inf</td>
<td>2.17E-03</td>
<td>F7D3V9</td>
<td>Leucine-rich repeat-containing G-protein coupled receptor 5, Xenopus tropicalis</td>
</tr>
<tr>
<td>77384</td>
<td>122</td>
<td>0</td>
<td>Inf</td>
<td>2.17E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>29187</td>
<td>35</td>
<td>0</td>
<td>Inf</td>
<td>1.07E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>112715</td>
<td>347</td>
<td>0</td>
<td>Inf</td>
<td>2.46E-02</td>
<td>Q8NCG7</td>
<td>Sn1-specific diacylglycerol lipase beta, Homo sapiens</td>
</tr>
<tr>
<td>102707</td>
<td>30</td>
<td>0</td>
<td>Inf</td>
<td>2.47E-02</td>
<td>Q8BGC0</td>
<td>HIV Tat-specific factor 1 homolog, Mus musculus</td>
</tr>
<tr>
<td>75342</td>
<td>28</td>
<td>0</td>
<td>Inf</td>
<td>2.80E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>88101</td>
<td>449</td>
<td>0</td>
<td>Inf</td>
<td>3.43E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>103534</td>
<td>39</td>
<td>0</td>
<td>Inf</td>
<td>3.51E-02</td>
<td>P23906</td>
<td>Interferon regulatory factor 2, Mus musculus</td>
</tr>
<tr>
<td>21841</td>
<td>60287</td>
<td>11411</td>
<td>2.40</td>
<td>3.90E-02</td>
<td>P41827</td>
<td>Heat shock protein 70 B2, Anopheles albimanus</td>
</tr>
<tr>
<td>86089</td>
<td>269</td>
<td>32</td>
<td>3.08</td>
<td>4.15E-02</td>
<td>P69309</td>
<td>Polyubiquitin, Avena fatua</td>
</tr>
</tbody>
</table>
Table 5-2 Continued

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Read count (HS29)</th>
<th>Read count (RC29)</th>
<th>Fold Change (log2 [HS29/RC29])</th>
<th>Adjusted p value</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>118366</td>
<td>0</td>
<td>164</td>
<td>-Inf</td>
<td>1.01E-08</td>
<td>Q869W9</td>
<td>Probable polyketide synthase 16, <em>Dictyostelium discoideum</em></td>
</tr>
<tr>
<td>84331</td>
<td>6</td>
<td>455</td>
<td>-6.21</td>
<td>1.35E-07</td>
<td>P18320</td>
<td>Profilin, <em>Heliocidaris crassispina</em></td>
</tr>
<tr>
<td>89926</td>
<td>5</td>
<td>247</td>
<td>-5.65</td>
<td>5.30E-06</td>
<td>P35605</td>
<td>Coatomer subunit beta, <em>Bos taurus</em></td>
</tr>
<tr>
<td>88863</td>
<td>0</td>
<td>99</td>
<td>-Inf</td>
<td>1.56E-05</td>
<td>A0JPI9</td>
<td>Leucine-rich repeat-containing protein 74A, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>77097</td>
<td>0</td>
<td>77</td>
<td>-Inf</td>
<td>3.10E-05</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>67247</td>
<td>0</td>
<td>72</td>
<td>-Inf</td>
<td>6.69E-05</td>
<td>P11029</td>
<td>Acetyl-CoA carboxylase, <em>Gallus gallus</em></td>
</tr>
<tr>
<td>98530</td>
<td>0</td>
<td>64</td>
<td>-Inf</td>
<td>1.81E-04</td>
<td>Q86T12</td>
<td>Dipeptidyl peptidase 9, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>97944</td>
<td>0</td>
<td>64</td>
<td>-Inf</td>
<td>1.88E-04</td>
<td>Q8IVF4</td>
<td>Dynein heavy chain 10, axonemal, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>74100</td>
<td>2</td>
<td>634</td>
<td>-8.17</td>
<td>1.11E-03</td>
<td>Q86UP9</td>
<td>Lipoma HMGIC fusion partner-like 3 protein, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>58004</td>
<td>0</td>
<td>59</td>
<td>-Inf</td>
<td>1.51E-03</td>
<td>P40222</td>
<td>Alpha-taxilin, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>22731</td>
<td>0</td>
<td>48</td>
<td>-Inf</td>
<td>2.17E-03</td>
<td>O96006</td>
<td>Zinc finger BED domain-containing protein 1, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>32477</td>
<td>0</td>
<td>44</td>
<td>-Inf</td>
<td>5.07E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>19261</td>
<td>0</td>
<td>58</td>
<td>-Inf</td>
<td>5.22E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>10854</td>
<td>0</td>
<td>42</td>
<td>-Inf</td>
<td>6.14E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>76238</td>
<td>860</td>
<td>6153</td>
<td>-2.84</td>
<td>6.14E-03</td>
<td>P41731</td>
<td>CD63 antigen, <em>Mus musculus</em></td>
</tr>
<tr>
<td>107353</td>
<td>0</td>
<td>42</td>
<td>-Inf</td>
<td>6.30E-03</td>
<td>Q3V3Q7</td>
<td>Phosphofurin acidic cluster sorting protein 2, <em>Mus musculus</em></td>
</tr>
<tr>
<td>102919</td>
<td>15</td>
<td>215</td>
<td>-3.87</td>
<td>1.44E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>129015</td>
<td>0</td>
<td>37</td>
<td>-Inf</td>
<td>1.66E-02</td>
<td>Q53HC0</td>
<td>Coiled-coil domain-containing protein 92, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>117326</td>
<td>0</td>
<td>64</td>
<td>-Inf</td>
<td>2.00E-02</td>
<td>A4HD2</td>
<td>Helicase ARIP4, <em>Xenopus tropicalis</em></td>
</tr>
<tr>
<td>13374</td>
<td>0</td>
<td>35</td>
<td>-Inf</td>
<td>2.00E-02</td>
<td>O13124</td>
<td>Vitamin D3 receptor, <em>Xenopus laevis</em></td>
</tr>
</tbody>
</table>
Table 5-2 Continued

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Read count (HS29)</th>
<th>Read count (RC29)</th>
<th>Fold Change (log2 [HS29/RC2])</th>
<th>Adjusted p value</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>124511</td>
<td>11</td>
<td>302</td>
<td>-4.74</td>
<td>2.46E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>111156</td>
<td>0</td>
<td>36</td>
<td>-Inf</td>
<td>2.52E-02</td>
<td>A0JPH4</td>
<td>Sterol regulatory element-binding protein cleavage-activating protein, <em>Xenopus laevis</em></td>
</tr>
<tr>
<td>74309</td>
<td>0</td>
<td>34</td>
<td>-Inf</td>
<td>2.52E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>118676</td>
<td>0</td>
<td>33</td>
<td>-Inf</td>
<td>2.80E-02</td>
<td>Q0VC00</td>
<td>Phospholipase ABHD3, <em>Bos taurus</em></td>
</tr>
<tr>
<td>18656</td>
<td>5</td>
<td>100</td>
<td>-4.44</td>
<td>2.80E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>39781</td>
<td>0</td>
<td>33</td>
<td>-Inf</td>
<td>2.80E-02</td>
<td>Q5S007</td>
<td>Leucine-rich repeat serine/threonine-protein kinase 2, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>42338</td>
<td>0</td>
<td>33</td>
<td>-Inf</td>
<td>2.80E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>96023</td>
<td>0</td>
<td>32</td>
<td>-Inf</td>
<td>3.50E-02</td>
<td>Q8WXG6</td>
<td>MAP kinase-activating death domain protein, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>120405</td>
<td>0</td>
<td>32</td>
<td>-Inf</td>
<td>3.51E-02</td>
<td>P15884</td>
<td>Transcription factor 4, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>123853</td>
<td>0</td>
<td>32</td>
<td>-Inf</td>
<td>3.55E-02</td>
<td>O08727</td>
<td>Tumor necrosis factor receptor superfamily member 11B, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>110619</td>
<td>0</td>
<td>31</td>
<td>-Inf</td>
<td>3.92E-02</td>
<td>O55043</td>
<td>Rho guanine nucleotide exchange factor 7, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>48703</td>
<td>0</td>
<td>31</td>
<td>-Inf</td>
<td>3.92E-02</td>
<td>O14981</td>
<td>TATA-binding protein-associated factor 172, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>18464</td>
<td>0</td>
<td>30</td>
<td>-Inf</td>
<td>4.45E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>21582</td>
<td>0</td>
<td>30</td>
<td>-Inf</td>
<td>4.59E-02</td>
<td>Q8AVC3</td>
<td>Glucose-6-phosphate exchanger SLC37A2, <em>Xenopus laevis</em></td>
</tr>
<tr>
<td>129906</td>
<td>0</td>
<td>30</td>
<td>-Inf</td>
<td>4.75E-02</td>
<td>Q0IHV1</td>
<td>Inverted formin-2, <em>Xenopus tropicalis</em></td>
</tr>
</tbody>
</table>
Table 5-3. All differentially expressed genes (DEGs) between HS16 and RC16.
HS16 = Heat-Selected clams at 16˚C; RC16 = Random-Control clams at 16˚C. Unless otherwise noted (*), the Transcript ID for each DEG listed in this table contains the prefix “Cluster-17909.”

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Read count (HS16)</th>
<th>Read count (RC16)</th>
<th>Fold Change (log2[HS16/RC16])</th>
<th>Adjusted p value</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>105853</td>
<td>63</td>
<td>0</td>
<td>Inf</td>
<td>4.25E-05</td>
<td>Q6UXZ4</td>
<td>Netrin receptor UNC5D, Homo sapiens</td>
</tr>
<tr>
<td>121604</td>
<td>53</td>
<td>0</td>
<td>Inf</td>
<td>2.23E-04</td>
<td>A0JPI9</td>
<td>Leucine-rich repeat-containing protein 74A, Rattus norvegicus</td>
</tr>
<tr>
<td>105537</td>
<td>160</td>
<td>9</td>
<td>4.19</td>
<td>3.69E-04</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>*Cluster-35747.4</td>
<td>43</td>
<td>0</td>
<td>Inf</td>
<td>1.97E-03</td>
<td>P25107</td>
<td>Parathyroid hormone/parathyroid hormone-related peptide receptor, Didelphis virginiana</td>
</tr>
<tr>
<td>81525</td>
<td>168</td>
<td>12</td>
<td>3.78</td>
<td>2.14E-03</td>
<td>P20397</td>
<td>Nucleolin, Xenopus laevis</td>
</tr>
<tr>
<td>29683</td>
<td>45</td>
<td>0</td>
<td>7.39</td>
<td>3.36E-03</td>
<td>Q96PH1</td>
<td>NADPH oxidase 5, Homo sapiens</td>
</tr>
<tr>
<td>107654</td>
<td>33</td>
<td>0</td>
<td>Inf</td>
<td>1.29E-02</td>
<td>P15291</td>
<td>Beta-1,4-galactosyltransferase 1, Homo sapiens</td>
</tr>
<tr>
<td>104679</td>
<td>32</td>
<td>0</td>
<td>Inf</td>
<td>1.29E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>78521</td>
<td>412</td>
<td>0</td>
<td>Inf</td>
<td>3.08E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>48476</td>
<td>0</td>
<td>262</td>
<td>-Inf</td>
<td>1.21E-07</td>
<td>G3UYX5</td>
<td>Regulator of G-protein signaling 22, Mus musculus</td>
</tr>
<tr>
<td>90948</td>
<td>14</td>
<td>323</td>
<td>-4.55</td>
<td>1.50E-07</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>45629</td>
<td>0</td>
<td>62</td>
<td>-Inf</td>
<td>4.3E-05</td>
<td>Q8MJC3</td>
<td>Caspase-3, Oryctolagus cuniculus</td>
</tr>
<tr>
<td>98727</td>
<td>2</td>
<td>85</td>
<td>-5.19</td>
<td>2.99E-04</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>99251</td>
<td>0</td>
<td>76</td>
<td>-Inf</td>
<td>3.0E-04</td>
<td>P11586</td>
<td>C-1-tetrahydrofolate synthase, cytoplasmic, Homo sapiens</td>
</tr>
<tr>
<td>45197</td>
<td>0</td>
<td>51</td>
<td>-Inf</td>
<td>3.0E-04</td>
<td>Q9U8W8</td>
<td>Techylectin-5A, Tachylepus tridentatus</td>
</tr>
<tr>
<td>129479</td>
<td>0</td>
<td>47</td>
<td>-Inf</td>
<td>4.1E-04</td>
<td>A6NDX5</td>
<td>Putative zinc finger protein 840, Homo sapiens</td>
</tr>
</tbody>
</table>
Table 5-3 Continued

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Read count (HS16)</th>
<th>Read count (RC16)</th>
<th>Fold Change (log2[HS16/RC16])</th>
<th>Adjusted p value</th>
<th>Swissprot ID</th>
<th>Swissprot description</th>
</tr>
</thead>
<tbody>
<tr>
<td>102001</td>
<td>0</td>
<td>44</td>
<td>-Inf</td>
<td>1.0E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>79147</td>
<td>0</td>
<td>48</td>
<td>-Inf</td>
<td>1.9E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>108984</td>
<td>8</td>
<td>121</td>
<td>-3.88</td>
<td>2.14E-03</td>
<td>Q9D6P8</td>
<td>Calmodulin-like protein 3, <em>Mus musculus</em></td>
</tr>
<tr>
<td>46678</td>
<td>0</td>
<td>40</td>
<td>-Inf</td>
<td>2.1E-03</td>
<td>Q6ZV73</td>
<td>FYVE, RhoGEF and PH domain-containing protein 6, <em>Homo sapiens</em></td>
</tr>
<tr>
<td>41923</td>
<td>2</td>
<td>69</td>
<td>-5.01</td>
<td>3.36E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>101484</td>
<td>0</td>
<td>36</td>
<td>-Inf</td>
<td>4.5E-03</td>
<td>E1B7L7</td>
<td>Ubinuclein-2, <em>Bos taurus</em></td>
</tr>
<tr>
<td>101617</td>
<td>0</td>
<td>35</td>
<td>-Inf</td>
<td>5.0E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>83532</td>
<td>0</td>
<td>62</td>
<td>-Inf</td>
<td>7.0E-03</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>60824</td>
<td>0</td>
<td>34</td>
<td>-Inf</td>
<td>1.0E-02</td>
<td>P24524</td>
<td>Glycine receptor subunit alpha-3, <em>Rattus norvegicus</em></td>
</tr>
<tr>
<td>120180</td>
<td>0</td>
<td>39</td>
<td>-7.13</td>
<td>1.12E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>103258</td>
<td>67</td>
<td>412</td>
<td>-2.62</td>
<td>1.29E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>83296</td>
<td>1</td>
<td>41</td>
<td>-5.75</td>
<td>1.85E-02</td>
<td></td>
<td>uncharacterized protein</td>
</tr>
<tr>
<td>90135</td>
<td>3</td>
<td>64</td>
<td>-4.68</td>
<td>2.26E-02</td>
<td>Q9D3W5</td>
<td>Leucine-rich repeat-containing protein 71, <em>Mus musculus</em></td>
</tr>
<tr>
<td>121414</td>
<td>0</td>
<td>29</td>
<td>-Inf</td>
<td>2.9E-02</td>
<td>Q9Z1N9</td>
<td>Protein unc-13 homolog B, <em>Mus musculus</em></td>
</tr>
<tr>
<td>108364</td>
<td>3</td>
<td>68</td>
<td>-4.34</td>
<td>3.08E-02</td>
<td>Q8BG94</td>
<td>COMM domain-containing protein 7, <em>Mus musculus</em></td>
</tr>
</tbody>
</table>
Figure 5-1. FPKM cluster analysis of differentially expression genes. Clustered using the log_{10}(FPKM+1) values. Red denotes genes with high expression levels, and blue denotes genes with low expression levels.
Figure 5-2. **DEGs across group and temperature.** (Top) Within-group/across-temperature gene expression. The Venn diagram denotes the number of DEGs identified in each comparison, with the overlapping region showing the number of DEGs shared between the comparisons. (Bottom) Across-group/within-temperature gene expression. Circles denote the number of DEGs identified in each comparison.
5.3.4 Notable KEGG pathway enrichment

Numerous KEGG pathways were significantly enriched in DEGs induced by heat stress. Overall, 20 significantly enriched pathways were identified in the RC group, 19 were identified in the HS group, and 15 pathways were shared across the two groups (Figure 5-3). The most significant pathway enriched by HS29 and RC29, relative to their respective controls, was the protein processing in endoplasmic reticulum pathway. This pathway predominantly features members of the HSP family, including HSP110, HSP70, HSP90, and DNAJA1 (HSP40), as well as other molecular chaperones that assist in protein folding, sorting, transport, and degradation. Several other enriched pathways also contained HSPs and other chaperones (e.g., calnexin and CDC48). Another pathway that was significantly enriched by both groups at 29°C was the antigen processing and presentation pathway; this pathway contains proteins involved with mounting an immune response (e.g., MyD88) in addition to serine/threonine kinases, which are involved with regulating cell growth and proliferation (e.g., TBK1 and MAP2K4). Some pathways were significantly inhibited by both groups at 29°C. These include the apoptosis pathway and the tumor necrosis factor (TNF) signaling pathway, which were associated with high expression levels of proteins that inhibit programmed cell death (e.g., BCL2, XIAP, BIRC2).

The Toll-like receptor pathway, which plays a key role in the innate immune system, was only significantly activated by HS29 relative to HS16. However, this pathway and the three others identified as uniquely significant to the HS group all trended in that direction for the RC group (0.06 < p < 0.12, Figure 5-3). Conversely, the five pathways unique to the RC group were not enriched to a substantial degree by HS clams.
One enriched pathway unique to the RC group was the AMPK signaling pathway, which is involved with cellular energy homeostasis. This pathway inhibits protein and fatty acid synthesis while activating fatty acid and glucose oxidation to increase supplies of ATP that may otherwise be depleted during periods of stress. Notably, this pathway was significantly downregulated in RC29 relative to RC16. The NF-kappa B signaling pathway was another pathway that was only significantly enriched by the RC group. RC29 exhibited significant differential expression of both \(NFkB1\), a transcription factor that promotes gene expression in response to inflammation and cytokines, and its inhibitor, \(NFkB1\), suggesting a complex or conflicted response.

In contrast to the within-group/across-temperature comparisons, few KEGG pathways were significantly enriched in the across-group/within-temperature comparisons. Three KEGG pathways were significantly enriched in RC29 relative to HS29, and all were related to lipid homeostasis (\(p < 0.05\)). These pathways include fatty acid biosynthesis, aflatoxin biosynthesis, and fatty acid metabolism and were associated with \(acetyl-CoA \text{ carboxylase (ACACA)}\) and the \(probable \text{ polyketide synthase 16 (Pks16)}\), which RC29 upregulated. No KEGG pathways were significantly enriched by the two groups at 16°C.
Figure 5-3. KEGG pathway enrichment for the within-group/across-temperature comparisons. Enrichment factor is the ratio of differentially expressed genes to the background frequency of genes associated with a given pathway. Plotted points convey the comparisons (point shape), the number of DEGs (point size), and the adjusted p value (point shade).
5.4 Discussion

In this study, the transcriptomes of two populations of surfclams were compared, a control group that was naïve to thermal stress (RC) and a group that was heat-selected for greater heat tolerance (HS) (Acquafredda et al., 2020). The gene expression patterns of these two groups were compared across two temperatures, a stressful condition (29°C for 6 hours) and a favorable or control condition (16°C for 6 hours). Each group/temperature comparison provides insight into the ways in which surfclams may cope with heat stress. The RC29vs.RC16 comparison is most representative of the response that wild type surfclams would have during an acute heat stress event. By contrast, the HS29vs.HS16 comparison illustrates the response of a heat-tolerant population that survived a previous exposure of sustained and lethal heat stress. The HS29vs.RC29 comparison is useful because it demonstrates which genes may be most beneficial for surviving heat stress and may facilitate marker-assisted selection. Finally, the HS16vs.RC16 comparison may highlight genes that are constitutively expressed during favorable conditions and predispose the HS clams to higher survival at the onset of heat stress, which would also facilitate marker-assisted selection.

In terms of the total number of genes expressed, RC clams mounted a broader transcriptomic response to 29°C. RC clams differentially expressed about 1.7-times more genes than HS clams did at this temperature, with approximately 2.8-times more unique DEGs. The expression pattern of RC29 may be interpreted as a broad over-reaction to heat stress, while the response of HS29 is more tempered or focused likely as an adaptive mechanism. This is supported by the fact that while HS29 differentially expressed fewer genes overall, HS29 showed higher expression levels of key stress response genes.
compared to RC29. Moreover, previous research has shown that bivalves can generate a maladaptive and overactive transcriptomic response when encountering novel stressors. For instance, Ostreid herpesvirus 1 µVar infection causes an explosive transcriptomic reaction in the Pacific oyster (Crassostrea gigas), which has been implicated as a contributing cause to the severe mortality that oysters suffer from this virus (He et al., 2015).

One possible mechanism that explains the response exhibited by HS clams at 29°C is based on the genetic predisposition of this group. As survivors from a previous heat shock, the HS individuals may be genetically determined to produce a tempered or more focused response whenever they were exposed to heat stress, including during the initial heat challenge selection event (Acquafredda et al., 2020). An alternative explanation is based on epigenetics. After the initial heat shock event, epigenetic modifications may have been established in the HS clams, which ensured that gene expression would be moderated or more focused during any subsequent heat stress. Under this hypothesis, the “epigenetic memory” effectively primed the organisms to cope with subsequent stress. Under both hypotheses, the need for HS clams to expend energy on gene expression upon re-exposure to heat stress would be reduced relative to the control clams, further supporting HS clam survival.

Comparisons of both groups across temperatures reveal that heat shock proteins (HSPs) were strongly upregulated at 29°C. Production of molecular chaperones, like HSPs, during periods of stress is a coping mechanism well conserved among taxa (Sung et al., 2011; Li et al., 2019). HSPs assist with the folding of denatured proteins and newly synthesized proteins during periods of stress (Pockley, 2003). In most organisms,
constitutive expression of HSPs normally comprise 5-10% of proteins in cells, but this percentage increases dramatically under stress (Pockey, 2003). Both RC29 and HS29 clams differentially expressed approximately 60 HSPs, yet it is unclear whether all these DEGs identified in this study represent independent genes as the transcriptome assembly was highly fragmented. Previous research has shown that bivalve genomes are highly polymorphic and contain highly expanded sets of stress response genes such as HSPs (Zhang, Fang, et al., 2012; Guo et al., 2015). Therefore, it is likely that the surfclam genome also contains an abundant array of distinct HSPs. Although a similar number of DEGs were expressed by both groups, the expression of HSPs was thrice as high in HS clams as RC clams. One gene in particular, which most closely resembles hsp70B2, was expressed to a significantly greater degree in HS clams than RC clams (p = 0.039). Together, these results imply that robust expression of HSPs is foundational to enhanced heat tolerance in surfclams.

Both groups expressed genes that facilitate apoptosis at 16°C, which likely reflects the fact that apoptosis is a process that occurs as a part of an organism’s normal growth and maintenance. Yet programmed cell death is also a consequence of temperature stress (Menike et al., 2014). While both groups expressed genes that promote apoptosis at 29°C, the induction of apoptosis was likely largely mitigated because both HS and RC clams significantly upregulated between 30 and 50 inhibitors of apoptosis (IAPs). Significant upregulation of an expanded set of IAPs is a key feature of stress response in bivalve molluscs (Zhang, Fang, et al., 2012; Guo et al., 2015). While the RC group expressed more IAPs, the HS group exhibited a stronger response; the HS group exhibiting a 50-fold increase in IAP expression, while the RC group had only a 19-fold
increase. These results suggest that robust expression of IAPs is of paramount importance to surfclam heat tolerance.

Both groups also highly expressed genes that mitigate the effects of reactive oxygen species (ROS), which often co-occur with apoptosis during periods of stress. In all cells, small amounts of ROS occur as byproduct from normal oxidative metabolism (Simon et al., 2000). However, periods of stress can lead to immense increases in ROS production, destroying numerous cellular components and triggering apoptosis (Simon et al., 2000). In other bivalves, genes that help quench ROS are among those most highly upregulated during thermal stress (Meistertzheim et al., 2007; Truebano et al., 2010; Menike et al., 2014). Although a similar number of DEGs were expressed by RC29 and HS29, the latter demonstrated stronger expression of oxidative stress response genes. These results indicate that the ability to respond to oxidative stress is another important component of surfclam heat tolerance.

Interestingly, HS and RC clams had notably different expression patterns of immune-related genes in response to heat stress. Compared with humans, the fruit fly Drosophila melanogaster, and other model organisms, bivalves have an expanded set of innate immune receptor genes, such as toll-like receptors (TLRs), retinoic acid-inducible (RIG) I-like receptors (RLRs), and C-type lectins (Zhang et al., 2015). These expanded gene families have supported structural and functional diversification that has caused many immune-related genes to adopt new roles, particularly with respect to abiotic stress response (Guo et al., 2015; Guo & Ford, 2016). More than half of all immune-related receptors and adaptors identified in the Pacific oyster are associated with abiotic stress response (Zhang et al., 2015). Specifically, members of the TLR family, which are
generally respond to pathogenic microbes and stimulate inflammatory signaling cascades, have been shown to be expressed during temperature stress (Kawasaki & Kawai, 2014; Zhang et al., 2015; Huang & Ren, 2019). Likewise, RLRs, MyD88, and TNF-related genes were also upregulated during temperature stress (Zhang et al., 2015). These genes may be upregulated to defend against infections that often co-occur with heat stress, or they may have developed other stress-mitigating functions (Guo et al., 2015). In this study, HS29 exhibited a general upregulation of TLRs, NLRs, carbohydrate-binding proteins (i.e., C-type lectins and galectins), and other immune-response related genes and pathways. By contrast, RC29 had a more mixed response. RC29 did uniquely express a probable polyketide synthase, which indicates this group may have been producing antimicrobial molecules (Sabatini et al., 2018). However, RC29 generally downregulated or had relatively low expression levels of members of the most prominent immune-response gene families. For instance, TLRs were highly upregulated by HS clams under stress (20-fold increase compared to clams at 16°C); at least one DEG was both significantly upregulated and unique to this group. By contrast, many TLRs were downregulated by RC29, and the overall expression pattern of TLRs was less than twice as high as RC16. Additionally, while C-type lectins and galectins were generally upregulated in HS29, RC29 showed the opposite trend. Together, these results suggest that regulation of certain homologs of immune-related genes may be key components of enhanced surf clam heat tolerance, and these genes may be good candidates for selection by breeding programs. However, additional research is required to determine the precise roles that these genes play in mitigating heat stress.
Another notable difference in gene expression between HS and RC clams at 29°C was related to lipid metabolism. During heat stress, RC clams upregulated genes that code for fatty acid synthesis and repair as well as genes that help remodel membranes, including ACACA and SCAP. Additionally, RC clams under heat stress also downregulated a KEGG pathway that inhibits fatty acid synthesis (i.e., AMPK signaling pathway) and upregulated KEGG pathways such as fatty acid biosynthesis and metabolism. HS clams did express other lipid metabolism genes at 29°C, so the observed distinctions may reflect subtle differences in how HS and RC clams respond to heat stress. The membranes of polar or cold-adapted animals tend to have more unsaturated fatty acids comprising the phospholipids of their cell membranes, compared to animals in temperate or tropical climates which have more saturated acids, like cholesterol, contributing to their cell membranes (Palmerini et al., 2009). When some bivalve species suffer heat stress, their lipid content becomes reduced, either because their lipids are metabolized as an energy source (Anacleto et al., 2014), or they undergo the process of lipid peroxidation, where lipids are degraded by ROS (Abele & Puntarulo, 2004). Surfclams are also known to exhibit a homeoviscous adaptation response to seasonal changes in temperature, adjusting membrane fluidity by altering the fraction of saturated and polyunsaturated fatty acids that comprise the phospholipids of their cell and mitochondrial membranes (Munro & Blier, 2015). Moreover, membrane remodeling is one hypothesis put forth to explain how multiple oyster species have induced thermal tolerance after being pretreated with a short-term heat shock (Shamseldin et al., 1997; Zhang, Hou, et al., 2012; Periera et al., 2020). Since the observed differences in this study reflect relatively minor distinctions between the groups, more research is necessary to
determine whether membrane remodeling plays any role in enhancing surfclam heat tolerance.

Ocean warming presents numerous challenges for marine species. For largely sessile organisms like bivalves, persistence depends on the capacity of populations to adapt or evolve to these rapid environmental changes (Bitter et al., 2019). The present study elucidates the gene expression profiles of a selectively bred surfclam population under heat stress, while also comparing those patterns to clams that are more representative of individuals found in the wild. Based on the findings of this study, when surfclams undergo acute heat stress, they respond by robustly expressing HSPs, IAPs, and genes mitigating ROS production. Although HS clams expressed fewer genes overall during heat stress, HS clams had a more focused response, exhibiting stronger expression of key stress-response gene compared to RC clams. This work should inform future breeding programs that attempt to breed surfclam for greater heat tolerance via marker-assisted selection, yet more work is required to fully understand the surfclam’s adaptive capacity to thermal stress. Proteomics research should be conducted to elucidate which of the identified heat-induced transcripts are ultimately translated and put into action by surfclam cells. Additionally, more research should be devoted to understanding whether there are trade-offs associated with enhanced heat-tolerance and understanding how chronic exposure influences surfclam gene and protein expression. In C. gigas, many of the genes that were upregulated during an acute heat challenge were not significantly expressed during chronic exposure, implying that oysters respond to short-term and long-term thermal stresses using different mechanisms (Clark et al., 2013). Since surfclams are more likely to experience long term stress both on farms and in the wild, the
surfclam’s response to prolonged thermal stress should be examined in future studies. Finally, future research efforts should aim to elucidate the standing genetic variation among surfclam individuals and populations to determine whether wild surfclams throughout their geographic range have the innate adaptive capacity to persist in a warming ocean.
5.5 Acknowledgements

Daphne Munroe and Ximing Guo served as advisors and collaborators of this study. The contributions of Michael Acquafredda include project conceptualization, investigation, acquisition of resources, project administration, data collection, and data analyses. The contributions of X. Guo and D. Munroe include project conceptualization, experimental design, acquisition of resources, project supervision, and data interpretation.

This research was sponsored by New Jersey Sea Grant with funds from the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under NOAA grants #NA18OAR4170087 and #NA18OAR4170357, and the New Jersey Sea Grant Consortium. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of New Jersey Sea Grant or the U. S. Department of Commerce. This publication was also funded in part through a graduate student grant awarded by the Northeast Sustainable Agriculture Research and Education (SARE) Program of the United States Department of Agriculture (USDA), under SARE grant #GNE17-141-31064. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the Northeast SARE Program or the USDA. Additionally, Sea-Bird Scientific generously supplied the water quality monitoring equipment, which M. Acquafredda was awarded through the 2016 Student Equipment Loan Program.

I am grateful to the personnel of the Haskin Shellfish Research Laboratory and the NJ Aquaculture Innovation Center who provided assistance with this research,

Finally, this work was supported by Rutgers University through the Haskin Shellfish Research Laboratory, the Department of Ecology, Evolution, and Natural Resources, and the Graduate Program in Ecology and Evolution.
5.6 Literature Cited


He, Y., Jouaux, A., Ford, S. E., Lelong, C., Sourdaine, P., Mathieu, M., & Guo, X.


Northeast Fisheries Science Center, National Oceanic and Atmospheric Administration


Saba, V. S., Griffies, S. M., Anderson, W. G., Winton, M., Alexander, M. A., Delworth,


Unit 3 – Evaluating the efficacy of bivalve polyculture

In Unit 3 (Chapters 6) of this dissertation, I discuss a study that sought to evaluate the efficacy of bivalve polyculture in the northeastern United States. The intent of this study was to determine if bivalve polyculture can be a viable form of shellfish aquaculture diversification, thereby supporting the spatial aspect of ESD. To my knowledge, this is the first study that examined bivalve polyculture in the region using these four focal species: *C. virginica, M. mercenaria, S. solidissima,* and *Mya arenaria.*

In Chapter 6, I ask the following questions:

1. Do more diverse bivalve assemblages have greater clearance rates than those which were less diverse?
2. Are more diverse bivalve assemblages more productive (i.e., exhibit greater biomass growth and have higher survival) than those which are less diverse?
Chapter 6: Effect of species diversity on particle clearance and productivity in farmed bivalves

With the exception of a few minor modifications, this chapter appears in its entirety in Marine Ecology Progress Series. The citation for the published article is:


6.0 Abstract

Most bivalve farms are designed as monocultures, yet diverse communities often outperform monocultures on various metrics of ecosystem functioning, including particle clearance and productivity. This study tested the feasibility of bivalve polyculture by examining particle clearance, growth, and survival of four species that are economically important to the northeastern United States. Three particle depletion experiments were conducted to determine if more diverse bivalve assemblages had greater clearance rates than those which were less diverse. Different assemblages of Crassostrea virginica, Spisula solidissima, Mercenaria mercenaria, and Mya arenaria were supplied with a single cultured algal species, a mix of two cultured algal species, or natural seston. To determine how species richness affects bivalve productivity, growth and survival were monitored in a flow-through mesocosm experiment, which simulated farm conditions. In the cultured algae experiments, more diverse assemblages did not exhibit significantly greater clearance rates than those that were less diverse. Instead, the clearance rates of each species were additive across assemblages. Surprisingly, most assemblages did not display a significant preference for the larger microalgae species, Pavlova lutheri (4.0 – 6.0 µm), over Nannochloropsis oculata (1.90 – 3.75 µm). Most notably, when supplied with natural seston, the four-species polyculture demonstrated a significantly greater
tank-level clearance rate for particles <25 µm compared to most monocultures. However, nearly all productivity metrics were not significantly affected by species richness. This work suggests that some degree of complementarity exists among these bivalves, and that in non-food limited systems, these bivalves could be co-cultured without outcompeting one another.
6.1 Introduction

Farming bivalve molluscs is of high economic importance, as it contributes nearly 11.8% (USD 19 billion) to the global first-sale value of cultivated aquatic animals (Food and Agriculture Organization of the United Nations [FAO], 2016). In the United States alone, more than 21,000 metric tons of farm-raised clam, oyster, and mussel meat were harvested in 2016, valued at over 340 million USD (National Marine Fisheries Service [NMFS], 2018). Typically, bivalve farms are designed as monocultures, which can be considered artificial ecosystems dominated by relatively high densities of a single species. While monocultures increase efficiency, they can also be vulnerable to a variety of risks (Altieri et al., 2015).

Cultivating a diverse suite of species insulates farmers from any individual crop failure, whether it occurs from disease (Felton et al., 2016), predation (Russell, 1989), or fluctuating environmental conditions (Gaudin et al., 2015). In this way, polyculture can sustain the economic viability of farm operations, and can even increase profitability by allowing farmers to more easily navigate market forces if the price of each individual crop fluctuates (Chopin et al., 2012; Isaacs et al., 2016). Although most of the evidence outlining the deficiencies of monoculture and the risk-mitigating advantages of polyculture originate from terrestrial agricultural research, these principles are possibly applicable to aquaculture.

Aquatic polyculture, or multispecies aquaculture, is the farming of two or more aquatic species that share space or resources. Multi-trophic aquaculture, where organisms of different trophic levels are cultivated in close proximity to one another, has received attention for its ability to reduce the environmental impacts of the fed species.
Incorporating unfed species like seaweed and mussels, which extract excess nutrients and particulates from the system, help mitigate the waste produced by a salmon farm (Chopin et al., 2012). Multispecies aquaculture can also occur on a single trophic level and still confer various advantages compared to single-species culture. For example, different bivalve species bioaccumulate toxins at different rates and store those toxins in varying organs throughout their bodies (Bricelj & Shumway, 1998). Having an alternate crop when one crop becomes too contaminated for sale would benefit a bivalve farmer. Likewise, the growth rates of different species vary by season (Stecher et al., 1996), meaning that different crops can be grown from seed to harvest on different schedules. When farmers grow multiple bivalve species that reach market size during different seasons, they ensure a sustained harvest throughout the year.

Exploitative interspecific competition for food resources among bivalves is a mechanism by which the productivity of a diversified farm may be constrained. However, diverse communities often outperform monocultures for various metrics of ecosystem functioning, including resistance to invasion and community-level resilience (Steiner et al., 2005; Steiner et al., 2006), ecosystem stability (Tilman et al., 2006), particle clearance (Whalen & Stachowicz, 2017; Valentine & Butler IV, 2019), and productivity, i.e., biomass growth (Tilman et al., 2001; Spooner & Vaughn, 2009; Liang et al., 2016; Duffy et al., 2016). There is evidence of positive relationships between diversity and ecosystem functioning in suspension feeders generally, and in bivalves specifically. For example, more species-rich communities of suspension-feeding bryozoans and tunicates demonstrated greater phytoplankton clearance rates, regardless of flow conditions.
Similarly, Spooner and Vaughn (2009) observed that freshwater mussel biomass increased with species richness across 21 communities.

One of the main mechanisms hypothesized to explain the positive relationship often observed between diversity and ecosystem functioning is niche complementarity (Fox, 2005; Tilman et al., 2006; Fargione et al., 2007). Species that perform similar ecosystem functions are often assigned to a collective functional group or guild. Since evolution shapes populations towards more efficient resource use (Duffy et al., 2016), the functional niches of a given species within a guild do not always neatly overlap, resulting in niche complementarity (Rosenfeld, 2002). Species within the bivalve suspension-feeding guild compete for suspended particles, but variations in particle selection adaptations that partition the available food resources among taxa would minimize interspecific competition (Lesser et al., 1992; Riera et al., 2002; Novais et al., 2016).

Nearly complete niche overlap likely occurs for the filtration of particles greater than 6 µm, since most bivalves can retain particles larger than this with 100% efficiency (Riisgård, 1988; Rosa et al., 2018). For smaller particles, less than 6 µm, niche complementary may occur among bivalves because interspecific variation in the morphology of feeding structures causes differences in interspecific capture efficiencies (Riisgård, 1988). Likewise, physicochemical interactions between seston and the mucus that lubricates bivalves’ feeding apparatus can facilitate differential particle capture (Ward et al., 1994; Beninger et al., 1997; Pales Espinosa & Allam, 2013; 2018). If each species specializes in or prefers filtering a specific set of particles, then more particles overall would be filtered in polycultures than monocultures (Figure 6.0). However, if
species are capable of filtering all particles with equal efficiency, then they would be directly competing for this suspended material.

To date, little work has investigated the viability of bivalve polyculture. Four bivalve species native to the northeastern United States were selected for this study, including the Eastern oyster (*Crassostrea virginica*), the Atlantic surfclam (*Spisula solidissima*), the hard clam (*Mercenaria mercenaria*), and the softshell clam (*Mya arenaria*). These species are of high economic importance to the region and their familiarity to farmers make them the most likely candidates for diversified bivalve farms. *C. virginica* and *M. mercenaria* are the two most commonly cultured bivalves in the northeastern United States, and their culture techniques are well-studied. Clam gardening and other small-scale aquaculture of *M. arenaria* have historically taken place in locations such as Massachusetts and Maine, but further research into the culture techniques for *M. arenaria* are in active development due to the high demand of this species and the declining wild fishery (Beal et al., 2016; Hagan & Wilkerson, 2018). Commercial-scale *S. solidissima* aquaculture remains in its infancy, but it has the potential to become an attractive alternate crop for farmers interested in diversifying their farms due to its rapid growth and culture techniques compatible with existing infrastructure (Acquafredda et al., 2019).

In this study, three particle depletion experiments were conducted to determine if more diverse bivalve assemblages had greater particle clearance capacity than those which were less diverse. In a series of experiments, different assemblages were supplied with a single cultured algal species, a mix of cultured algal species that differed significantly in size, or unfiltered seawater containing a diverse assemblage of natural
seston. Additionally, I investigated whether bivalves grown in diverse assemblages were more productive than those grown in less diverse groups. To assess how species richness affected bivalve productivity, I conducted a fully replicated, fully combinatorial, long-term, flow-through mesocosm experiment that closely matched farm conditions and tracked bivalve growth and survival through time.
Figure 6-0. **Conceptual figure of particle clearance in bivalve polyculture.** This conceptual figure shows how pre-capture niche complementarity may occur in four species of bivalves, each represented by the colored curves. All four species capture particles greater than 6 µm with 100% efficiency. However, as particle size decreases, interspecific variation in capture efficiency becomes apparent. More small particles (≤ 6 µm) would be captured in polyculture than any individual monoculture.
6.2 Materials and methods

All experiments were conducted at the Rutgers University New Jersey Aquaculture Innovation Center (AIC) in North Cape May, NJ. The bivalve seed (6–18 months old) used in the experiments represent cultured strains and sizes that are commonly used by growers during the initial outplanting of new seed on their farms. The oysters (*Crassostrea virginica*) and surfclams (*Spisula solidissima*) were produced at the AIC, the hard clams (*Mercenaria mercenaria*) were procured from a hatchery in Atlantic City, NJ, and the softshell clams (*Mya arenaria*) were obtained from a hatchery in Beals, Maine.

In all experiments, mesocosms were constructed out of 62-liter plastic tanks, each filled with approximately 8.5 cm of cleaned sand and 41 l of seawater (Figure 6-1). Corrugated plastic partitions were buried in the sediment and used to segregate the species (Figure 6-1). The bivalves were placed into the mesocosms prior to the start of the experiments, so that the clams had adequate time to bury. Oysters were split between two or four mesh bags and hung above the sediment. In the particle depletion experiments, an airstone was added to each mesocosm approximately 4 cm below the surface to gently aerate the tank and to keep the water well mixed.
**Figure 6-1. Mesocosm assembly.** Conceptual figure showing how a monoculture, biculture, triculture, and four-species polyculture would be assembled in these experiments. Oysters are represented in mesh bags in the tanks. The left and center panels depict the side and top views of the mesocosms, respectively. The right panel shows the bivalves used in this study, as well as the dimensions (centimeters) of each mesocosm. Black and gray lines outline the volume of the container and sediment layer, respectively.
6.2.1 General particle depletion experimental set-up

Clearance rate is defined as the volume of water cleared of suspended particles per unit of time, and only equals filtration rate when 100% of suspended particles are efficiently retained (Møhlenberg & Riisgård, 1979; Riisgard, 2001). Previous research on bivalve filtration has determined allometric equations that relate a bivalve’s size to its clearance rate. Here, I estimated the expected tank-level clearance capacity of a given mesocosm as the summed clearance rates of all individuals predicted by allometric equations, using the following formula:

\[
\text{Expected tank-level clearance rate} = \sum_{i=1}^{j} a_i \times W_i^b \times N_i
\]  

(1)

Here, \(a\) and \(b\) are species-specific constants derived from published allometric equations (Table 6-1), \(W\) is the mean dry tissue weight of a species at the onset of an experiment, \(N\) is the number of individuals of each species in the assemblage, and \(j\) is the number of species in the assemblage. To determine \(W\) for each species, the soft tissue of five individuals were placed in a 68°C drying oven for 48 hours, cooled, and weighed.

The actual particle clearance rate for a given assemblage was measured using the clearance method, which consists of taking water samples over a fixed time interval and measuring the particle concentrations that monotonically decline through time (Riisgård, 2001). The observed clearance rate for a given mesocosm was calculated using the following equation (Coughlan, 1969, Riisgard, 2001):

\[
\text{Observed tank-level clearance rate} = \frac{V}{t} \times \ln \left( \frac{C_0}{C_t} \right)
\]  

(2)
Here, $V$ is the known volume of the mesocosm, $t$ is the time in hours, and $C_0$ and $C_t$ represent the initial ($t = 0$) and final ($t = 5$) particle concentrations of the mesocosm, respectively. The temperature and salinity of the mesocosms were maintained between 19.0 – 20.5°C and 31.7 – 32.4 ppt, respectively. Each mesocosm was stocked such that the expected community clearance capacity was approximately 22 l hour$^{-1}$ (Table 6-2). The focal bivalves used in the particle clearance experiments had mean shell lengths and dry tissue weights that ranged from 18-24 mm and from 0.009-0.073 g, respectively (Table 6-S1) Before each experiment, the animals were starved for 48 hours. Experiments were performed between September and October 2017.
Table 6-1. **Species-specific constants.** Species-specific constants that relate a bivalve’s dry tissue weight (W, g) to its clearance rate (CR, l h\(^{-1}\)) and are used in allometric equations that take the form, CR = aW\(^b\). These values were selected or adapted from the listed sources, which experimentally derived allometric clearance equations in controlled laboratory-based studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>a</th>
<th>b</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crassostrea virginica</em> (Cv)</td>
<td>6.79</td>
<td>0.73</td>
<td>Riisgård (1988)</td>
</tr>
<tr>
<td><em>Spisula solidissima</em> (Ss)</td>
<td>4.015</td>
<td>0.76</td>
<td>Adapted from Riisgård (1988) and Riisgård (2001)</td>
</tr>
<tr>
<td><em>Mercenaria mercenaria</em> (Mm)</td>
<td>2.45</td>
<td>0.79</td>
<td>Adapted from Riisgård (1988) and Coughlan &amp; Ansell (1964)</td>
</tr>
<tr>
<td><em>Mya arenaria</em> (Ma)</td>
<td>4.79</td>
<td>0.71</td>
<td>Riisgård &amp; Seerup (2003)</td>
</tr>
</tbody>
</table>
Table 6-2. Stocking densities and the estimated initial tank-level clearance capacities used in the particle depletion experiments. In all assemblages, the tank-level clearance capacity was 22 l h\(^{-1}\).

<table>
<thead>
<tr>
<th>Assemblage</th>
<th>C. virginica (Cv)</th>
<th>S. solidissima (Ss)</th>
<th>M. mercenaria (Mm)</th>
<th>M. arenaria (Ma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cv</td>
<td>104</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ss</td>
<td></td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mm</td>
<td></td>
<td></td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Ma</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Cv + Ss</td>
<td>52</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cv + Mm</td>
<td>52</td>
<td></td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Cv + Ma</td>
<td>52</td>
<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Ss + Mm</td>
<td>20</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ss + Ma</td>
<td>20</td>
<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Mm + Ma</td>
<td></td>
<td></td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td>Cv + Ss + Mm</td>
<td>35</td>
<td>13</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Cv + Ss + Ma</td>
<td>35</td>
<td>13</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Cv + Mm + Ma</td>
<td>35</td>
<td></td>
<td>36</td>
<td>19</td>
</tr>
<tr>
<td>Ss + Mm + Ma</td>
<td></td>
<td>13</td>
<td>36</td>
<td>19</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>26</td>
<td>10</td>
<td>27</td>
<td>14</td>
</tr>
</tbody>
</table>
Table 6-3. Stocking densities and the estimated initial tank-level clearance capacities used in the productivity experiment.

<table>
<thead>
<tr>
<th>Assemblage</th>
<th>C. virginica (Cv)</th>
<th>S. solidissima (Ss)</th>
<th>M. mercenaria (Mm)</th>
<th>M. arenaria (Ma)</th>
<th>Tank-level clearance capacity (1 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cv</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>Ss</td>
<td></td>
<td>96</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Mm</td>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Ma</td>
<td></td>
<td></td>
<td></td>
<td>116</td>
<td>61</td>
</tr>
<tr>
<td>Cv + Ss</td>
<td>30</td>
<td>48</td>
<td></td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>Cv + Mm</td>
<td>30</td>
<td></td>
<td>20</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Cv + Ma</td>
<td>30</td>
<td></td>
<td></td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>Ss + Mm</td>
<td></td>
<td>48</td>
<td>20</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Ss + Ma</td>
<td></td>
<td>48</td>
<td></td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>Mm + Ma</td>
<td></td>
<td></td>
<td>20</td>
<td>58</td>
<td>36</td>
</tr>
<tr>
<td>Cv + Ss + Mm</td>
<td>20</td>
<td>32</td>
<td>13</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Cv + Ss + Ma</td>
<td>20</td>
<td>32</td>
<td></td>
<td>39</td>
<td>62</td>
</tr>
<tr>
<td>Cv + Mm + Ma</td>
<td>20</td>
<td></td>
<td>13</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Ss + Mm + Ma</td>
<td></td>
<td>32</td>
<td>13</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>15</td>
<td>24</td>
<td>10</td>
<td>29</td>
<td>49</td>
</tr>
</tbody>
</table>
6.2.2 Cultured algae depletion experiments

In order to compare clearance rates among bivalve assemblages that differ in species richness when such assemblages have limited food choice availability, mesocosms containing various combinations of bivalves were supplied with a single algal species, *Pavlova lutheri* (Strain: Mono), or with two species that differed significantly in size, *P. lutheri* and *Nannochloropsis oculata*. All combinations of the four species were compared (Table 6-2, 6-S1). The single-species and the two-species algal depletion experiments were each replicated three times; each mesocosm contained the same individuals on each occasion. Control mesocosms containing no animals were used to estimate the rate at which the algal cells fell out of the water column. The experiment was conducted in a windowless laboratory under ambient artificial light.

For the single-species algal depletion experiment, live *P. lutheri* were added to each mesocosm at a concentration of $2.6 \pm 0.4 \times 10^4$ cells ml$^{-1}$. For the two-species algal depletion experiment, live *P. lutheri* and *N. oculata* were added to each mesocosm at a concentration of $1.3 \pm 0.6 \times 10^4$ cells ml$^{-1}$ and $1.23 \pm 0.26 \times 10^5$ cells ml$^{-1}$, respectively. Although the concentrations of each algal species differed, the two species were similarly abundant in terms of the overall biomass (Helm & Bourne, 2004). The organic dry weight of algal cells used for both depletion experiments ranged from 0.56-0.61 µg ml$^{-1}$. A beaker was used to collect 50 ml water samples from each mesocosm immediately after the algae were added and once again after five hours.

The particle concentration of each sample was analyzed with a Beckman Coulter Counter, a particle analyzer, which can detect individual particles between 1 and 50 µm with high confidence. Each sample was passed through a Melitta$^\text{TM}$ coffee filter to
remove impurities that might damage the Coulter Counter. Ten ml of each sample were placed into a cuvette and diluted with 10 ml of 0.1 µm filtered seawater, then gently inverted to thoroughly mix their contents. To reduce sampling bias, all samples were run in triplicate, then averaged across the three runs. The concentration of *P. lutheri* present in each sample was calculated from the number of particles detected between the sizes of 4.00 and 6.00 µm. The concentration of *N. oculata* present in each sample was calculated from the number of particles detected between the sizes of 1.90 and 3.75 µm.

6.2.3 Natural seston depletion experiment

To compare clearance rates among bivalve assemblages that differ in species richness when supplied with natural seston, mesocosms containing various combinations of bivalves were supplied with unfiltered seawater from the Cape May Canal. For this experiment, only the four-species polyculture and the four monocultures were compared (Table 6-2). Three replicate mesocosms were established for each combination. Three control mesocosms containing no animals were also used to estimate the rate at which particles fell out of the water column. The mesocosms had an initial concentration of 3.1 ± 0.2 * 10^5 particles ml⁻¹.

On the day of the experiment, the seawater in each mesocosm was replaced with unfiltered seawater from the Cape May Canal. Care was taken during the water changes to not disturb the sediment nor the buried bivalves. A beaker was used to collect 50 ml water samples from each mesocosm immediately after the tanks were filled and once again after five hours. Each sample was immediately preserved with 1 ml of 2.5%
Lugol’s Iodine Solution and wrapped in aluminum foil to reduce photodegradation of the preservative.

The particle concentration of each sample was analyzed with FlowCAM, an imaging particle analyzer, which can detect individual particles between 2 and 300 µm with high confidence. Each sample was passed over stacked 212 and 38 µm sieves (diagonals = 299 µm and 54 µm, respectively), to divide the samples into large and small size classes. All particles retained on the 212 µm screen (>299 µm) were discarded. Before being analyzed on FlowCAM, all samples were gently inverted to thoroughly mix their contents. To reduce sampling bias, all samples were run in triplicate, then averaged across the three runs. Particles retained on the 38 µm screen were washed into 20 ml of treated seawater. A 0.95 ml subsample of this size fraction was analyzed on a FC300-FV FlowCell with a 4X objective lens. Because these samples were concentrated prior to analysis on FlowCAM, the particle concentration data collected were multiplied by 0.4 in order to reflect the concentration of the original samples. Approximately 0.04 ml of the sample fraction passing through the 38 µm screen was analyzed on a FC80-7FV FlowCell with a 10X objective. All particle images were sorted into size classes using their Estimated Spherical Diameter (ESD).

6.2.4 Productivity experimental set-up

A fully replicated, fully combinatorial, long-term, flow-through mesocosm experiment was conducted to address the hypothesis that more diverse assemblages of bivalves will have greater productivity than those which are less diverse. Here, I define productivity as an increase in the rate at which biomass is assimilated into the individuals
of the population. Productivity was calculated as the increase in average daily growth rate of the shell dimensions and average daily growth rate of an individual’s whole wet weight. Whole wet weight is defined as the weight of the whole living organism with its shells and tissue patted dry; this metric captures changes in both the shell and tissue weights and is reflective of measures used by some aquaculturists to quantify the productivity of their farms (United States Department of Agriculture [USDA], 2014).

The experiment ran for 93 days from 11 July 2017 to 12 October 2017, and each of three replicates consisted of fifteen mesocosms, containing one of each of the potential combinations of *C. virginica*, *S. solidissima*, *M. mercenaria*, and *M. arenaria*. Each tank was supplied with unfiltered water from the Cape May Canal at a flow rate of 3.5 ± 0.16 l min⁻¹ into each mesocosm. A settling tank was used to reduce heavy sediment loads in incoming water. Inflow into each mesocosm was positioned approximately 8 cm below the surface and water supply was evenly distributed across the entire mesocosm. Water exited each mesocosm via displaced overflow. All mesocosms and associated plumbing were cleared of fouling organisms daily, to ensure that only the focal species contributed to the clearance capacity of each mesocosm.

Bivalves were placed into the mesocosms as previously stated prior to the start of the experiments; all clams were given adequate time to bury themselves and oysters placed in bags hanging above the sediment (Figure 6-1). Temperature was continuously recorded every 600 seconds over the course of the experiment with Seabird Scientific SBE 56 data loggers (Figure 6-S1). Salinity was continuously logged at 15-minute intervals using a YSI 6600 V2-4 multi-parameter water quality sonde; data from a nearby NOAA buoy (Station CMAN – 8536110) were used to fill a gap in the dataset (Figure 6-
All mesocosms were initially stocked at a tank-level clearance capacity of 62 l hr\(^{-1}\), with the exception of those containing *M. mercenaria*, which ranged from 10 l hr\(^{-1}\) in the *M. mercenaria* monocultures to 49 l hr\(^{-1}\) in the four-species polycultures (Table 6-3). The focal bivalves used in the productivity experiment had initial mean shell lengths and dry tissue weights that ranged from 17-28 mm and from 0.045-0.088 g, respectively (Table 6-S2).

Once monthly (day 0, 34, 65, 93), ten individuals of each species from each species assemblage from each replicate were randomly sampled and the following data were collected: shell length, shell height, shell width, and whole wet weight. Shell length represents the length of the anterior-posterior axis on the clams and the dorsal-ventral axis on the oysters. Shell height represents the dorsal-ventral axis on the clams and the anterior-posterior axis of the oysters. Shell width represents the dextral-sinistral axis of all bivalves. Shell dimension data were collected with calipers (Mitutoyo Absolute™ Digimatic). Wet weight was measured with a digital scale (Ohaus®). All live animals were returned to the same mesocosm after each data collection event. Average daily growth rate was calculated using the following equation (Munroe, 2016):

\[
X_{GR} = \frac{X_t - X_{t_0}}{\Delta t}
\]

Here, \(X\) represents the average value of particular growth variable (shell length, shell height, shell width, whole wet weight), collected on the first and last day of the experiment, and \(\Delta t\) represents the number of days of the experiment (=93). Survival was determined by collecting and counting all live and dead individuals within each mesocosm.
6.2.5 Statistical analyses

All data were analyzed with R (Version 3.4.2 © 2017-09-28 The R Foundation) using RStudio (Version 1.1383 © 2009-2017 RStudio, Inc.). Normality and homoscedasticity were confirmed using the Shapiro-Wilk tests and Levene's tests, respectively. Percent survival data were arcsine-transformed prior to analysis to meet the assumption of normality. All measures of dispersion reported in this paper are one standard error of the mean, unless otherwise noted. One-way ANOVAs were used to determine significant differences among bivalve assemblage in tank-level clearance rates, and post-hoc Tukey’s HSD tests were performed to determine significant differences between assemblage pairs. Chi-squared tests were employed to determine whether clearance rates for focal bivalve species within the given multispecies assemblage were additive. Finally, linear regressions were used to determine whether species richness had a significant effect on the clearance rates of assemblages and the productivity of given focal species across assemblages.
6.3 Results

6.3.1 Clearance rates of cultured algae: Pavlova lutheri

The species richness of an assemblage had no effect on its clearance rate of *Pavlova lutheri* (Linear Regression, $r^2 < 0.01$ $p = 0.40$, Figure 6-2A). On average, bivalve assemblages cleared the tanks of *P. lutheri* at a rate of $22.0 \pm 3.88 \text{l hr}^{-1}$ (Figure 6-2A, 6-3A). When supplied *P. lutheri*, the clearance rates of each focal species within any given multispecies assemblage were additive (Chi-squared: $0.004 \leq x \leq 0.260$, df = 1, $0.61 \leq p \leq 0.95$, Figure 6-3A). For example, the tank-level clearance rate for *P. lutheri* in oyster and surfclam monocultures was $15.5 \pm 2.77$ and $21.6 \pm 5.32 \text{l hr}^{-1}$, respectively. The tank-level clearance rate for the oyster/surfclam biculture, which contained half as many individuals as each monoculture, was $18.1 \pm 5.71 \text{l hr}^{-1}$. 
Figure 6-2. The effect of species richness on tank-level clearance of cultured microalgae. (A) Particle clearance rates of bivalve assemblage when solely supplied with *P. lutheri*; (B) Particle clearance rates of bivalve assemblage when supplied a mixture of *P. lutheri* (dark squares) and *N. oculata* (light triangles). Points represent mean values and error bars represent SEM. For each curve, N=12 replicate monoculture mesocosms, N=18 biculture mesocosms, N=12 triculture mesocosms, and N=3 four-species polyculture mesocosms.
Figure 6-3. Tank-level clearance rates of cultured microalgae. (A) Particle clearance rates of bivalve assemblage when solely supplied *P. lutheri*; (B) Particle clearance rates of bivalve assemblage when supplied a mixture of *P. lutheri* (dark) and *N. oculata* (light). Bars represent mean clearance rates of three replicate mesocosms and error bars represent standard error of the mean. Bivalve species abbreviations refer to *Crassostrea virginica* (*Cv*), *Spisula solidissima* (*Ss*), *Mercenaria mercenaria* (*Mm*), and *Mya arenaria* (*Ma*).
6.3.2 Clearance rates of cultured algae: *P. lutheri* and *Nannochloropsis oculata*

When supplied a two-species mixture of cultured algae, all assemblages demonstrated greater clearance rates for the larger microalga, *P. lutheri*, compared to the smaller *Nannochloropsis oculata* (Figure 6-4); however, none exhibited a significant preference (Paired t-test: $1.1 \leq t \leq 4.2$, df = 2, $0.05 < p < 0.42$). The species richness of an assemblage had no effect on the clearance rate of either microalga (*P. lutheri* - Linear Regression, $r^2 < 0.01$ $p = 0.62$, Figure 6-2B; *N. oculata* - Linear Regression, $r^2 < 0.01$ $p = 0.58$, Figure 6-2B). On average, when the bivalve assemblages were provided the algal mixture, they cleared the tanks of *P. lutheri* and *N. oculata* at rates of $15.1 \pm 3.56$ and $10.6 \pm 2.22$, respectively. When averaged across all assemblages, tank-level clearance rate for all particles was $25.7 \pm 5.50$ l hr$^{-1}$ (Figure 6-2B, 6-3B).

When supplied with the mixture of *P. lutheri* and *N. oculata*, the clearance rates of each focal bivalve species within any given multispecies assemblage were additive (*P. lutheri* Chi-squared: $3 \times 10^{-5} \leq x \leq 0.298$, df = 1, $0.59 \leq p \leq 0.99$; *N. oculata* Chi-squared: $6 \times 10^{-5} \leq x \leq 1.184$, df = 1, $0.28 \leq p \leq 0.99$, Figure 6-3B). This additive effect of bivalve clearance rates is again best demonstrated by the oyster/surfclam biculture. Oyster and surfclam monocultures cleared *P. lutheri* from the mixture at rates of $15.0 \pm 5.34$ and $15.5 \pm 7.20$ l hr$^{-1}$, while clearing *N. oculata* from the mixture at rates of $6.87 \pm 2.28$ and $13.0 \pm 4.42$ l hr$^{-1}$, respectively. The oyster/surfclam biculture, which again contained half as many individuals as each monoculture, depleted *P. lutheri* and *N. oculata* from the mixture at rates of $15.2 \pm 7.77$ and $9.88 \pm 3.89$ l hr$^{-1}$, respectively.
Figure 6-4. Clearance of *P. lutheri* plotted against clearance of *N. oculata* for each bivalve assemblage. Points represent mean clearance rates of three replicate mesocosms and error bars represent standard error of the mean. The diagonal line denotes the one-to-one line of equal clearance. Bivalve species abbreviations refer to *Crassostrea virginica* (*Cv*), *Spisula solidissima* (*Ss*), *Mercenaria mercenaria* (*Mm*), and *Mya arenaria* (*Ma*).
6.3.3 Clearance rates of seawater containing natural seston

Small particles, less than or equal to 25 µm, comprised 99.1% of all particles across samples; larger particles ranging from 25 to 299 µm made up the remaining 0.9%. Among the small particles, 82.5% were less than or equal to 6 µm. The tank-level clearance rate for particles equal to or less than 6 µm varied significantly among bivalve assemblages (ANOVA, F(5,12) = 30.77, p < 0.001, Figure 6-5A). Most notably, the four-species polyculture exhibited significantly greater clearance rates than the oyster, hard clam and softshell clam monocultures (Tukey’s HSD, p ≤ 0.006, Figure 6-5A). The polyculture tank-level clearance rate was 61%, greater than the oyster monoculture, 141% greater than the hard clam monoculture, and 91% greater than the softshell clam monoculture. Although the polyculture exhibited a mean clearance rate 26% greater than that of the surfclam monoculture, no significant difference was observed between the pair (Tukey’s HSD, p = 0.19, Figure 6-5A). There was also no significant difference in tank-level clearance rate among the oyster, hard clam, and softshell clam monocultures (Tukey’s HSD, p = 0.19, Figure 6-5A). The surfclam monoculture outperformed the other clam monocultures (Tukey’s HSD, p ≤ 0.05, Figure 6-5A), but not the oyster monoculture (Tukey’s HSD, p = 0.35, Figure 6-5A).

Tank-level clearance rates were also calculated for subsets of particles smaller than 6 µm (Table 6-4). Tank-level clearance rate declined with particle size, but in general, the same statistical trends emerged as when these size classes were grouped (Table 6-4). However, two exceptions were documented. For particles less than or equal to 3 µm, the softshell clam monoculture performed statistically similar to the surfclam monoculture (Tukey’s HSD, 0.06 < p ≤ 0.09, Table 6-4). For the smallest subset of
particles, hard clam tank-level clearance was not different from the control mesocosms (Tukey’s HSD, p = 0.07, Table 6-4).

The same trends for particles less than or equal to 6 µm were similarly observed for tank-level clearance rates of all particles up to 25 µm in size (ANOVA, F(5,12) = 35.85, p < 0.001, Figure 6-5B). However, a different trend emerged for particles greater than 25 µm. Only the surfclam and hard clam monocultures significantly reduced more particles than the no-animal controls (ANOVA, F(5,12) = 5.041, p ≤ 0.01, Figure 6-5C); no significant differences in tank-level clearance rates were observed across bivalve assemblages (Tukey’s HSD, p > 0.31, Figure 6-5C).
Table 6-4. Tank-level clearance rates of natural seston particles sized ≤6 µm. Values are mean ± standard error of the mean for three replicate mesocosms. Bivalve species abbreviations refer to *Crassostrea virginica* (Cv), *Spisula solidissima* (Ss), *Mercenaria mercenaria* (Mm), and *Mya arenaria* (Ma). Assemblages sharing a letter are statistically similar: ≤6 µm (p≤0.006, except Ss-Ma p=0.05 & Mm-Control p=0.01);
5-6 (p≤0.004, except Ss-Mm & Ss-Ma p<0.04);
4-5 (p≤0.002, except Ss-Mm & Ss-Ma p<0.04);
3-4 (p≤0.008, except Ss-Ma p<0.04);
2-3 (p≤0.007, except Ss-Mm & Ss-Ma p<0.04);
≤2 (p≤0.004, except All-Cv p<0.02, n.b. Ss-Ma p=0.09).

<table>
<thead>
<tr>
<th>Assemblage</th>
<th>Clearance rate of particles ≤2 µm (l h⁻¹)</th>
<th>Clearance rate of particles 2-3 µm (l h⁻¹)</th>
<th>Clearance rate of particles 3-4 µm (l h⁻¹)</th>
<th>Clearance rate of particles 4-5 µm (l h⁻¹)</th>
<th>Clearance rate of particles 5-6 µm (l h⁻¹)</th>
<th>Clearance rate of particles ≤6 µm (l h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>20.6 ± 0.49 a</td>
<td>24.3 ± 0.76 a</td>
<td>25.4 ± 0.61 a</td>
<td>24.7 ± 0.35 a</td>
<td>25.6 ± 0.39 a</td>
<td>22.0 ± 0.50 a</td>
</tr>
<tr>
<td>Cv</td>
<td>13.1 ± 1.45 bc</td>
<td>14.5 ± 1.65 bc</td>
<td>15.1 ± 1.65 bc</td>
<td>15.2 ± 1.52 bc</td>
<td>16.4 ± 1.34 bc</td>
<td>13.7 ± 1.48 bc</td>
</tr>
<tr>
<td>Ss</td>
<td>16.4 ± 0.58 ab</td>
<td>19.1 ± 1.09 ab</td>
<td>20.3 ± 0.93 ab</td>
<td>20.3 ± 1.12 ab</td>
<td>20.6 ± 1.73 ab</td>
<td>17.4 ± 0.72 ab</td>
</tr>
<tr>
<td>Mm</td>
<td>7.8 ± 1.44 dc</td>
<td>10.3 ± 2.48 c</td>
<td>12.0 ± 2.06 c</td>
<td>12.6 ± 2.00 c</td>
<td>14.0 ± 1.85 c</td>
<td>9.11 ± 1.67 c</td>
</tr>
<tr>
<td>Ma</td>
<td>10.7 ± 1.18 bc</td>
<td>12.5 ± 0.93 bc</td>
<td>13.7 ± 0.58 c</td>
<td>13.6 ± 0.77 c</td>
<td>13.7 ± 0.93 c</td>
<td>11.5 ± 1.02 c</td>
</tr>
<tr>
<td>Control</td>
<td>2.09 ± 1.85 d</td>
<td>-0.27 ± 1.67 d</td>
<td>1.25 ± 1.41 d</td>
<td>1.29 ± 1.41 d</td>
<td>1.00 ± 1.33 d</td>
<td>1.54 ± 1.70 d</td>
</tr>
</tbody>
</table>
Figure 6-5. Tank-level clearance rates of natural seston particles. (A) Clearance rates for particles sized ≤6 μm; (B) Clearance rates for particles sized ≤25 μm; (C) Clearance rates for particles sized >25 μm. Bars represent mean clearance rate of three replicate mesocosms and error bars represent standard error of the mean. Bars sharing a letter are statistically similar (p ≤ 0.05). Bivalve species abbreviations refer to *Crassostrea virginica* (Cv), *Spisula solidissima* (Ss), *Mercenaria mercenaria* (Mm), and *Mya arenaria* (Ma).
6.3.4 Effect of species diversity on productivity

Generally, the composition of the bivalve assemblages had no effect on individual bivalve growth. Average daily growth rates for shell length (Figure 6-6A), shell height (Figure 6-6B), shell width (Figure 6-6C), and whole wet weight (Figure 6-6D) remained constant for each focal species, regardless of the species richness of an assemblage (Linear Regression, $r^2 < 0.07$, $p > 0.11$, Table 6-5). The only exception was a slight, yet significant negative effect observed for surfclam shell length (Linear Regression, $r^2 = 0.17$, $p < 0.03$, Figure 6-6A, Table 6-5).

Across assemblages, oysters exhibited the greatest average daily growth rate for each of the parameters measured. On average, oyster shell lengths, heights, and widths grew at $0.24 \pm 0.0061$, $0.17 \pm 0.0051$, and $0.060 \pm 0.0019$ mm day$^{-1}$, respectively; oyster wet weights increased by $0.14 \pm 0.0046$ g day$^{-1}$ (Table 6-6). The three clam species exhibited remarkably similar growth rates across all measured parameters (Table 6-6). For instance, the average daily shell length growth rate for surfclams, hard clams, and softshell clams was $0.15 \pm 0.0036$, $0.16 \pm 0.0030$, and $0.18 \pm 0.0023$ mm day$^{-1}$, respectively (Table 6-6).

Over the 93-day experiment, survival of the focal bivalves was high and varied little among replicates. The species richness of an assemblage had no effect on the survival of the focal bivalves (Linear Regression, $r^2 < 0.08$, $p > 0.09$, Table 6-5). Hard clam survival was highest at $98 \pm 0.69\%$, and oyster survival was similarly high at $96 \pm 0.94\%$ (Table 6-6). Both the softshell clam and surfclam survival were lower, with softshell clam survival at $89 \pm 1.4\%$ and surfclam survival lowest at $87 \pm 3.7\%$ (Table 6-6). A notable outlier was surfclam survival in the oyster/surfclam/hard clam assemblages;
at 54 ± 22%, this combination represents the lowest and most variable survival for any species in any combination. This stands in contrast to the mean survival of hard clams, oysters, and softshell clams, which never fell below 92, 90, 81%, respectively, for any assemblage (Table 6-6).
Table 6-5. **Output of productivity experiment linear regressions.** Results of linear regressions of various productivity metrics on bivalve assemblage species richness. Bold represent statistically significant values. Bivalve species abbreviations refer to *Crassostrea virginica* (Cv), *Spisula solidissima* (Ss), *Mercenaria mercenaria* (Mm), and *Mya arenaria* (Ma).

<table>
<thead>
<tr>
<th>Species</th>
<th>Productivity metric</th>
<th>p value</th>
<th>adjusted $r^2$</th>
<th>m</th>
<th>b</th>
<th>$F_{1,22}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cv</td>
<td>shell length</td>
<td>0.776</td>
<td>-0.042</td>
<td>0.0021</td>
<td>0.23</td>
<td>0.083</td>
</tr>
<tr>
<td>Ss</td>
<td>shell length</td>
<td><strong>0.025</strong></td>
<td><strong>0.17</strong></td>
<td><strong>-0.0090</strong></td>
<td><strong>0.17</strong></td>
<td><strong>5.77</strong></td>
</tr>
<tr>
<td>Mm</td>
<td>shell length</td>
<td>0.546</td>
<td>-0.028</td>
<td>-0.0021</td>
<td>0.16</td>
<td>0.38</td>
</tr>
<tr>
<td>Ma</td>
<td>shell length</td>
<td>0.301</td>
<td>0.0052</td>
<td>0.0028</td>
<td>0.17</td>
<td>1.12</td>
</tr>
<tr>
<td>Cv</td>
<td>shell height</td>
<td>0.138</td>
<td>0.056</td>
<td>0.0089</td>
<td>0.15</td>
<td>2.37</td>
</tr>
<tr>
<td>Ss</td>
<td>shell height</td>
<td>0.074</td>
<td>0.10</td>
<td>-0.0047</td>
<td>0.12</td>
<td>3.52</td>
</tr>
<tr>
<td>Mm</td>
<td>shell height</td>
<td>0.640</td>
<td>-0.035</td>
<td>-0.0014</td>
<td>0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>Ma</td>
<td>shell height</td>
<td>0.993</td>
<td>-0.045</td>
<td>0.000015</td>
<td>0.10</td>
<td>0.000081</td>
</tr>
<tr>
<td>Cv</td>
<td>shell width</td>
<td>0.135</td>
<td>0.058</td>
<td>0.0072</td>
<td>0.051</td>
<td>0.80</td>
</tr>
<tr>
<td>Ss</td>
<td>shell width</td>
<td>0.139</td>
<td>0.056</td>
<td>-0.0027</td>
<td>0.070</td>
<td>2.36</td>
</tr>
<tr>
<td>Mm</td>
<td>shell width</td>
<td>0.325</td>
<td>0.00063</td>
<td>-0.0015</td>
<td>0.076</td>
<td>1.02</td>
</tr>
<tr>
<td>Ma</td>
<td>shell width</td>
<td>0.841</td>
<td>-0.044</td>
<td>-0.00024</td>
<td>0.058</td>
<td>0.041</td>
</tr>
<tr>
<td>Cv</td>
<td>whole wet weight</td>
<td>0.227</td>
<td>0.023</td>
<td>0.0065</td>
<td>0.12</td>
<td>1.54</td>
</tr>
<tr>
<td>Ss</td>
<td>whole wet weight</td>
<td>0.211</td>
<td>0.028</td>
<td>-0.0032</td>
<td>0.081</td>
<td>1.66</td>
</tr>
<tr>
<td>Mm</td>
<td>whole wet weight</td>
<td>0.494</td>
<td>-0.023</td>
<td>-0.0020</td>
<td>0.10</td>
<td>0.48</td>
</tr>
<tr>
<td>Ma</td>
<td>whole wet weight</td>
<td>0.117</td>
<td>0.067</td>
<td>0.0015</td>
<td>0.035</td>
<td>2.67</td>
</tr>
<tr>
<td>Cv</td>
<td>survival</td>
<td>0.132</td>
<td>0.059</td>
<td>0.069</td>
<td>1.18</td>
<td>2.45</td>
</tr>
<tr>
<td>Ss</td>
<td>survival</td>
<td>0.228</td>
<td>0.023</td>
<td>-0.093</td>
<td>1.39</td>
<td>1.54</td>
</tr>
<tr>
<td>Mm</td>
<td>survival</td>
<td>0.096</td>
<td>0.081</td>
<td>0.068</td>
<td>1.28</td>
<td>3.02</td>
</tr>
<tr>
<td>Ma</td>
<td>survival</td>
<td>0.612</td>
<td>-0.033</td>
<td>-0.020</td>
<td>1.17</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Table 6-6. Species-specific productivity across bivalve assemblages. Values are mean ± standard error of the mean for three replicate mesocosms. Focal species within assemblages are bolded. Bivalve species abbreviations refer to *Crassostrea virginica* (Cv), *Spisula solidissima* (Ss), *Mercenaria mercenaria* (Mm), and *Mya arenaria* (Ma).

<table>
<thead>
<tr>
<th>Assemblage</th>
<th>Average daily growth rates</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shell length (mm d⁻¹)</td>
<td>Shell height (mm d⁻¹)</td>
</tr>
<tr>
<td>Cv</td>
<td>0.25 ± 0.019</td>
<td>0.16 ± 0.011</td>
</tr>
<tr>
<td>Cv + Ss</td>
<td>0.27 ± 0.015</td>
<td>0.18 ± 0.010</td>
</tr>
<tr>
<td>Cv + Mm</td>
<td>0.21 ± 0.019</td>
<td>0.16 ± 0.013</td>
</tr>
<tr>
<td>Cv + Ma</td>
<td>0.21 ± 0.015</td>
<td>0.15 ± 0.014</td>
</tr>
<tr>
<td>Cv + Ss + Mm</td>
<td>0.24 ± 0.0081</td>
<td>0.18 ± 0.019</td>
</tr>
<tr>
<td>Cv + Ss + Ma</td>
<td>0.23 ± 0.016</td>
<td>0.16 ± 0.018</td>
</tr>
<tr>
<td>Cv + Mm + Ma</td>
<td>0.23 ± 0.013</td>
<td>0.18 ± 0.011</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>0.26 ± 0.0052</td>
<td>0.19 ± 0.018</td>
</tr>
<tr>
<td>Ss</td>
<td>0.16 ± 0.0038</td>
<td>0.11 ± 0.0018</td>
</tr>
<tr>
<td>Cv + Ss</td>
<td>0.15 ± 0.0071</td>
<td>0.11 ± 0.0046</td>
</tr>
<tr>
<td>Ss + Mm</td>
<td>0.17 ± 0.0096</td>
<td>0.13 ± 0.0066</td>
</tr>
<tr>
<td>Ss + Ma</td>
<td>0.15 ± 0.015</td>
<td>0.11 ± 0.0075</td>
</tr>
<tr>
<td>Cv + Ss + Mm</td>
<td>0.14 ± 0.0098</td>
<td>0.11 ± 0.0078</td>
</tr>
<tr>
<td>Cv + Ss + Ma</td>
<td>0.14 ± 0.012</td>
<td>0.11 ± 0.0073</td>
</tr>
<tr>
<td>Ss + Mm + Ma</td>
<td>0.15 ± 0.0035</td>
<td>0.11 ± 0.0031</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>0.13 ± 0.010</td>
<td>0.10 ± 0.0058</td>
</tr>
<tr>
<td>Mm</td>
<td>0.16 ± 0.0026</td>
<td>0.13 ± 0.0017</td>
</tr>
<tr>
<td>Cv + Mm</td>
<td>0.15 ± 0.012</td>
<td>0.12 ± 0.0069</td>
</tr>
<tr>
<td>Ss + Mm</td>
<td>0.17 ± 0.0047</td>
<td>0.14 ± 0.0035</td>
</tr>
<tr>
<td>Mm + Ma</td>
<td>0.16 ± 0.0065</td>
<td>0.13 ± 0.0082</td>
</tr>
<tr>
<td>Cv + Ss + Mm</td>
<td>0.14 ± 0.0097</td>
<td>0.12 ± 0.0078</td>
</tr>
<tr>
<td>Cv + Mm + Ma</td>
<td>0.16 ± 0.0092</td>
<td>0.13 ± 0.0051</td>
</tr>
<tr>
<td>Ss + Mm + Ma</td>
<td>0.17 ± 0.0091</td>
<td>0.14 ± 0.0096</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>0.15 ± 0.0086</td>
<td>0.13 ± 0.0099</td>
</tr>
<tr>
<td>Ma</td>
<td>0.18 ± 0.0093</td>
<td>0.10 ± 0.0043</td>
</tr>
<tr>
<td>Cv + Ma</td>
<td>0.16 ± 0.0083</td>
<td>0.09 ± 0.0046</td>
</tr>
<tr>
<td>Ss + Ma</td>
<td>0.17 ± 0.0043</td>
<td>0.10 ± 0.0037</td>
</tr>
<tr>
<td>Mm + Ma</td>
<td>0.18 ± 0.0027</td>
<td>0.10 ± 0.0027</td>
</tr>
<tr>
<td>Cv + Ss + Ma</td>
<td>0.17 ± 0.0016</td>
<td>0.09 ± 0.0016</td>
</tr>
<tr>
<td>Cv + Mm + Ma</td>
<td>0.18 ± 0.0027</td>
<td>0.10 ± 0.0028</td>
</tr>
<tr>
<td>Ss + Mm + Ma</td>
<td>0.19 ± 0.0039</td>
<td>0.10 ± 0.0042</td>
</tr>
<tr>
<td>Cv + Ss + Mm + Ma</td>
<td>0.18 ± 0.0076</td>
<td>0.10 ± 0.0066</td>
</tr>
</tbody>
</table>
Figure 6-6. The effect of species richness on bivalve productivity. The effect of species richness on the average daily growth rates of bivalve (A) shell length, (B) shell height, (C) shell width, and (D) whole wet weight. Points represent mean values and error bars represent standard error of the mean. Squares represent *C. virginica*; Down-pointing triangles represent *S. solidissima*; Diamonds represent *M. mercenaria*; Up-pointing triangles represent *M. arenaria*. For each focal species, N=3 for monocultures, N=9 for bicultures, N=9 for tricultures, and N=3 for four species polyculture.
6.4 Discussion

Niche complementarity predicts that diverse bivalve assemblages would filter more particles and exhibit greater productivity if the species in the assemblage utilize different resources (Schoener, 1974; Chesson, 2000). Although the results failed to detect this trend when assemblages were supplied cultured algae, I did detect enhanced particle clearance rates when the same experiments were conducted using natural seston. The focal bivalves of this study exhibit flexible diets, consuming picoplankton, mesozooplankton, and detritus as supplements to their main nutritional source, phytoplankton (Matthiessen, 1960; Kreeger et al., 1988; Prins & Escaravage, 2005; Kach & Ward, 2008). Unlike the cultured algae experiments, where bivalve mixtures were competing for only one or two algal species, the diversity of food available in the natural seston experiment provided the conditions necessary to observe resource partitioning among *Crassostrea virginica*, *Spisula solidissima*, *Mercenaria mercenaria*, and *Mya arenaria*. However, results from the productivity experiment show that growth was not enhanced in diverse assemblages, suggesting that clearance rate and productivity are decoupled. Nevertheless, I conclude that bivalve farms will benefit from growing a diverse set of crops, because in nearly every case, bivalve co-culture did not compromise growth for any component species in a multispecies group.

6.4.1 The effect of species diversity on particle clearance

I anticipated that if niche complementarity occurred among the focal bivalves, more diverse assemblages would show higher particle clearance rates. However, the results only demonstrated this increase in clearance with diversity when natural seston
was supplied. In the cultured algae experiments, clearance rates of each focal bivalve species within any given multispecies assemblage were additive. This finding suggests that the allometric equations used in this study adequately related a bivalve’s size to its clearance rate, despite some differences in the methodology used by the listed sources from which the equations were derived. Additive clearance rates of the focal species were observed when the assemblages were supplied solely with *P. lutheri* as well as when they were supplied a mixture of *P. lutheri* and *N. oculata*. When provided both microalgae, all communities had slightly greater clearance rates of *P. lutheri*; however, none displayed a significant preference. This result was unexpected given the capture efficiency of all four focal species is known to decrease with size for particles less than 6 µm (Riisgård, 1988). With a diameter between 4 and 6 µm, *P. lutheri* cells should be cleared with near complete efficiency. Conversely, *N. oculata* cells are typically 8x smaller in volume than *P. lutheri* and should be captured considerably less effectively (Riisgård, 1988). The near equivalence in clearance rates for these differently sized microalgae presents an intriguing opportunity for further study.

In the natural seston depletion experiments, tank-level clearance was equivalent across all assemblages for particles greater than 25 µm. This result reinforces the concept that all four focal species can extract particles between 25 and 299 µm with near complete efficiency. Beyond large phytoplankton such as diatoms and dinoflagellates, particles in this size class can include detritus and zooplankton. Some suspension-feeding bivalves utilize these other nutritional sources as a supplement to phytoplankton (Langdon & Newell, 1990; Davenport et al., 2000; Prins & Escaravelle, 2005). However, even if large particles are ingested, they may not necessarily be digested (Shumway et al.,
Since this study did not examine the extent to which particles were ingested, it is unclear whether the focal species rejected these larger particles as pseudofaeces or consumed them, and if so, whether they were actually assimilated.

Phytoplankton and other organic particles less than 25 µm are most often associated with preferential bivalve food (Hawkins et al., 1996; Ward et al., 1997; Bacon et al., 1998; Levinton et al., 2002). In the natural seston depletion experiments, the four-species polyculture had significantly greater tank-level clearance compared to all monocultures, with the exception of the surfclam monoculture. Interestingly, this trend was also true for particles less than 6 µm, the sizes at which bivalves filter at lower efficiency (Riisgård, 1988). The augmentation in particle clearance observed in the four-species polyculture compared to most monocultures suggests that some degree of niche complementarity exists among the focal species. Many studies that have examined niche complementarity have highlighted cases where species with narrow niches support increased diversity and occur in stable coexistence (Pyke, 1982; Grant & Grant, 2006; Fox & Bellwood, 2013). However, other studies have found that even species with broad, overlapping niches can promote diversity and coexistence through plasticity in their resource use (Behmer & Joern, 2008; Ashton et al., 2010). Species within the suspension-feeding guild have considerable overlap in the size of particles they can acquire. Yet, some suspension feeders do partition available food resources to minimize interspecific competition (Peterson, 1982; Lesser et al., 1992; Kang et al., 2009; Lacoste et al., 2016; Novais et al., 2016). I propose that in this study, the bivalve polyculture removed more suspended seston than monocultures due to variations in particle capture processes and differences in dietary requirements or preferences exhibited by the four focal species.
6.4.2 Variations in particle capture processes

Variations in gill structure and function as well as differences in mucous composition are mechanisms by which bivalves may control the efficiency of particle capture. Bivalve gill (ctenidium) morphology and physiology is diverse (Rosa et al., 2018). In this study, the three clam species have homorhabdic eulamellibranch gills, while the oyster has heterorhabdic pseudolamellibranch gills (Arakawa, 1971). Of these two types, eulamellibranch gills are more complex, unified structures that allow more control to be exerted over the flow of water passing by the gills and increases the opportunity for particle capture (Ward et al., 1994; Beninger et al., 1997). Such differences in gill structure and function could be responsible for differences in the observed particle capture efficiency.

Cilia are responsible for pumping water past the gills and collecting particles (Dame, 1996). Lateral cilia draw water through the pallial cavity and over the gills (Dame, 1996). The currents produced are regulated by species-specific traits and behaviors, including valve gape (Frank et al., 2007), the inhalant siphon aperture size (Jørgenson & Riisgård, 1988; Riisgård et al., 2003), and branchical musculature (Ward et al., 1994; Medler & Silverman, 2001; Gainey et al., 2003). Moreover, interspecific variation in ciliary beating and flow patterns are mediated by environmental conditions; for instance, the presence of brown tide alga (*Aureococcus anophagefferen*) reduced ciliary beating in *C. virginica* and *M. mercenaria* but not *M. arenaria* (Gainey & Shumway, 1991; Robbins et al., 2010). Laterofrontal cilia, or cirri, entrain particles through a combination of mucociliary and hydrodynamic mechanisms (Ward et al., 1994;
The size, structure, and spacing of laterofrontal cilia effect the capture efficiency of particles below 6 µm (Wright et al., 1982). Riisgård (1988) found that *S. solidissima, M. mercenaria, and C. virginica* all possess laterofrontal cirri and can capture particles as small as 2 µm with at least 50% efficiency, while *A. irradians* does not and captures those particles with only 15% efficiency. In this study, *C. virginica, S. solidissima,* and *M. arenaria* were highly efficient at capturing particles in the smallest size class. Monocultures of those three species cleared particles less than or equal to 2 µm approximately 80% as well as they cleared particles between 5 and 6 µm. By contrast, *M. mercenaria* cleared the smallest particles only 56% as efficiently.

The efficiency of bivalve suspension-feeding is largely dependent on the mucus that coats the pallial organs (Dufour & Beninger, 2001). Lectins, carbohydrate-binding proteins that are present in mucous, promote preferential particle selection by strongly binding to carbohydrate residues that cover the surfaces of microalgae (Pales Espinosa et al., 2010a; 2010b; 2016a; 2016b). Notably, a lectin that binds to *Nannochloropsis sp.* but not to *P. lutheri,* has been identified in *C. virginica* (Pales Espinosa & Allam, 2018). This finding may help explain why in this study, the bivalve assemblages cleared *P. lutheri* and *N. oculata* at similar rates despite the difference in particle size. Mucus may also help bivalves discriminate between particles through interactions with other physiochemical properties of seston, such as wettability and surface charge (Solow & Gallager, 1990; Rosa et al., 2017). Generally, microalgae with hydrophobic surfaces and those with a midrange of surface charges are preferentially selected over those with more hydrophilic surfaces and those with a neutral charge (Rosa et al., 2013; 2017; 2018).
However, different bivalves do not always respond to the same particle surface properties (Rosa et al., 2017). The relationships between bivalve particle selection and the physiochemical properties of seston require further study.

6.4.3 Variations in diet requirement or preference

Many studies have demonstrated that seston characteristics have a significant effect on bivalve clearance rates. Behavioral responses to seston vary across taxa; total seston concentration, the organic content of seston, and its nutritional profile can each influence bivalve feeding patterns (Hawkins et al., 1996; Navarro et al., 2000; Hewitt & Pilditch, 2004; Yahel et al., 2009; Galimany et al., 2013). Generally, clearance declines as the seston concentration increases, although the clearance curves for some species have an initial peak over low to moderate seston concentrations before declining (Hawkins et al., 1999; Velasco & Navarro, 2005). Species that inhabit more turbid environments tend to exhibit greater plasticity in their particle processing mechanisms. In response to seston concentration, some species such as *M. mercenaria* regulate clearance rate, while others like *Mytilus edulis* regulate pseudofaeces production (Foster-Smith, 1975; Bricelj & Malouf, 1984). The ribbed mussel (*Geukensia demissa*) employs multiple strategies; to compensate for the diluted nutritional quality of the seston they encountered in a highly turbid and urbanized estuary, ribbed mussels enhanced their pseudofeces production and selection efficiency, thus enriching the particles they ingested with more organic content (Galimany et al., 2013).

Yet beyond the mechanisms that bivalves evolved to cope with the quantity of particle in their environment, variations in particle selection mechanisms likely also arose
in response to species-specific dietary requirements. Differential capture of similarly sized, yet more nutritious, particles allows species to increase their feeding efficiency (Cucci et al., 1985; Yahel et al., 2009). Another adaptive strategy, particularly for largely or entirely sessile organisms living in dynamic environments, is to conserve mechanisms that alter a species’ diet when food availability changes due to competition or environmental factors. Phytoplankton are the principal source of nutrition for all four focal species, yet they also display flexibility in their particle capture selectivity and diet. While their capture efficiencies for picoplankton are low, *C. virginica* and *M. mercenaria* will consume them when other food is scarce (Kreeger et al., 1988; Langdon & Newell, 1990; Kach & Ward, 2008). *M. arenaria* does not capture bacterioplankton less than 1 µm effectively (Wright et al., 1982), but it will consume slightly larger detritus (Matthiessen, 1960). *S. solidissima* are voracious consumers, and population models suggest wild surfclam stocks are consuming other food beyond that which can be measured as water column primary productivity (Munroe et al., 2013). Farmed surfclams require the fall algal bloom to recover from the stress they encounter during the summer. Since the natural seston depletion experiment occurred in October, I hypothesize that surfclams increased their feeding efficiency during this time. Consequently, the surfclam monoculture clearance rate was lower than, but statistically equivalent to the four-species polyculture.

In this study, niche complementarity was observed despite the prevailing view that most suspension-feeding bivalves tend to be opportunistic generalists (Cranford et al., 2011). Plasticity and seasonality in bivalve feeding patterns may accommodate the niches space of multiple members of a community. To elucidate this, more research
needs to be conducted on the plasticity and seasonality of particle capture, particle processing, and the dietary requirements and preferences of bivalves outside laboratory contexts.

6.4.4 The effect of species diversity on bivalve productivity

Unlike the particle depletion experiments, where the stocking densities of all experimental mesocosms had equivalent clearance capacities, the clearance capacities of the bivalve assemblages in the productivity experiment varied more widely. The allometric equations used in this study were also derived from controlled laboratory-based studies that were conducted under a limited range of temperatures and seston characteristics, unlike the fluctuating conditions the bivalves of this study experienced. While these represent limitations of the study, some comparisons can still be made. If food were limiting and complementarity for food resources existed, individuals in species-rich assemblages should have exhibited greater productivity than conspecific grown in monoculture. This phenomenon has been demonstrated in a long-term grassland experiment where diverse communities produced greater biomass than monocultures when grown in fields where nutrients were limited (Tilman et al., 2001; 2006). Alternatively, if food were limiting and strong competition for food resources had existed, the species best equipped to acquire and assimilate the resources would grow while the others would languish. Though experimental and observational data suggest that competitive exclusion in bivalves due to interspecific consumptive competition is rare, weaker competitors may exhibit reduced growth rates (Peterson, 1992). For example, localized competitive preemption by mussels (M. edulis) caused cockles
(Cerastoderma edule) to grow to a smaller shell length, have reduced body weight, and experience lower recruitment (Kamermans, 1993; Donadi et al., 2013).

If food were not limiting, neither complementarity nor competition among species would be evident; if all individuals in a community are supplied with enough food, the species identity of the community should not influence its collective productivity (Levinton, 1972; Lesser et al., 1992; Peterson, 1992). Instead, other forces should regulate each species’ growth and in turn the productivity of the community. This was demonstrated by Peterson and Andre (1980), who examined the growth of the deep-dwelling infaunal species Sanguinolaria nuttali. When grown with two other species that occupy the same position in the sediment, S. nuttali growth was reduced by 80%.

However, when S. nuttalli was grown with the shallow-dwelling Prothaca staminea, no impact on growth was noticeable, indicating that competition for space, not food was the limiting resource. Similarly, Kamermans and colleagues (1992) found that interspecific competition between C. edule and Macoma balthica was minimal since food was highly available to both species, especially because M. balthica can switch from suspension- to deposit-feeding when seston becomes scarce.

In the present study, in almost every case, the species composition of bivalve assemblages had no effect on the rates of growth for any of the shell dimensions or the whole wet weight for any of the four focal bivalves. This occurred despite the discrepancies in the assemblages’ clearance capacities. It is likely that the mesocosms never experienced food limitation because full water replacement occurred hourly and food densities at this location are relatively high, thus the observed lack of response in productivity. However, increased species richness did have a slight negative effect on the
average daily shell length growth rate for surfclams. This result suggests surfclams succumbed to some degree of interspecific competition despite the lack of effect on other productivity metrics. Competition against surfclams may have been exacerbated by abiotic factors, particularly temperature and seston concentration.

Atlantic surfclams are vulnerable to warm temperature stress and respond by reducing their clearance rate, ingestion rate, assimilation efficiency, and slowing or ceasing their growth (Narváez et al., 2015; Hornstein et al., 2018; Acquafredda et al., 2019). The present study occurred during the warmest part of the year (July to October), when temperatures exceeded this species thermal maximum (Munroe et al., 2013; 2016; Acquafredda et al., 2019), but not that of the other three species (Loosanoff, 1958; Kennedy & Mihursky, 1971; Grizzle et al., 2001). Since its ability to acquire and utilize food becomes compromised during heat stress, the surfclam’s competitive ability may have been depressed during portions of this study. Hard clams, softshell clams, and oysters may also have had a competitive advantage over the surfclams because those taxa are better adapted to process the high sediment loads they experienced during the productivity experiment (Bricelj & Malouf, 1984; Grizzle & Morin, 1989; Bacon et al., 1998; Suedel et al., 2015). Unlike the other species, which are commonly exposed to high-turbidity conditions in estuaries, the surfclam’s preferred habitat is low-turbidity areas of the continental shelf (Merrill & Ropes, 1969; Snelgrove et al., 1998). Elevated seston concentrations create sub-optimal conditions for surfclams, which result in reduced clearance rates, increased pseudofaeces production, and decreased ingestion of phytoplankton (Robinson et al., 1984). This study was limited to a few months during the summer and fall, but future experiments should be conducted over a full year and
repeated in the field. A longer study would expose the focal species to a wider range of the abiotic factors that influence clearance rate and growth, including temperature (Jørgensen et al., 1990; Kittner & Riisgård, 2005), salinity (Navarro & Gonzalez, 1998), and changes in seston characteristics (Hawkins et al., 1999; Navarro et al., 2000; Hewitt & Pilditch, 2004; Velasco & Navarro, 2005).

Future studies of bivalve polyculture should also measure the net absorption efficiencies and other post-ingestion processes of the focal species, because productivity is constrained by how well the availability and quality of resources match the particular dietary requirements of a given species at a particular time. Bivalves can meet their energy and nutritional demands by regulating feeding and digestive processes, which can sometimes mitigate the effects of a limited or low-quality food supply. For instance, by increasing gut residence time, starved blue mussels (M. edulis) can achieve absorption efficiencies equivalent to well-fed conspecifics (Hawkins & Bayne, 1984). Similarly, ribbed mussels (G. demissa) increased their gut residence time when supplied with high concentrations of seston that contained relatively low nutritional content (Galimany et al., 2013). Interspecific competition, particularly with non-native species, can overwhelm these adaptive post-ingestion processes, thereby constraining growth (Galimany et al., 2017). Yet, seasonal differences in the ways that physiological and competitive processes interact among suspension feeders can also alter these dynamics (Riera et al., 2002).

Although the focal species of this study were native to the same region, they do not all exhibit high growth rates during the same seasons. The oysters, hard clams, and softshell clams grow best in the spring, summer, and fall, while farmed surfclams
perform best during the cooler seasons of fall, winter, and spring (Stecher et al., 1996; described in Chapter 3). If these species modulate their niches temporally, then the competitive interactions identified in this study may not hold true throughout all periods of the year.

6.4.5 Implications for aquaculture

Although more work around bivalve polyculture is necessary, the results of this study suggest that *C. virginica*, *S. solidissima*, *M. mercenaria*, and *M. arenaria* could be co-cultured in the northeast United States without outcompeting one another. While bivalve monoculture is still largely the norm for this region, some farmers are experimenting with raising multiple species in close proximity to one another (Kramer, 2019; *personal observations*). Aquaculture used for restoration of filtration services in estuarine and marine environments have mainly utilized monocultures of *C. virginica* (Kreeger et al., 2018). Results from this study suggest restoration efforts may benefit from deploying a diverse set of native taxa, which may remove more total suspended particles than any single species. Bivalve productivity was not enhanced in multispecies groups, but in almost every case, increased species richness did not have negative consequences for each species’ growth. Importantly, the other bet-hedging benefits of crop diversification still hold true.

Crop diversification gives aquaculture practitioners protection from any individual crop failure linked to natural phenomena. However, some efficiency may be lost when monocultures are abandoned. Growers must also consider the cost of alternate gear types, available space, product processing, and permitting when considering whether diversification is a feasible option for their farms. Until more research is conducted,
growers should also be aware that the strength and sign of these species interactions may be modulated by environmental conditions. The degree of niche partitioning or interspecific competition among these bivalves may vary temporally, spatially, or across other environmental dimensions, and this has implications for the farmed species stable coexistence.
6.5 Acknowledgements

Daphne Munroe served as advisors and collaborator of this study; she was listed as a co-author on the published article. The contributions of Michael Acquafredda include project conceptualization, investigation, acquisition of resources, project administration, data collection, data analyses, manuscript writing, manuscript revising and editing, and preparation of the final manuscript. The contributions of D. Munroe include project supervision, validation, and manuscript revising and editing. D. Munroe was partially supported by the USDA National Institute of Food and Agriculture Hatch project accession number 1020831 through the New Jersey Agricultural Experiment Station, Hatch project NJ32140.

This work was supported by a Student Award administered by the New Jersey Aquaculture Innovation Center of Rutgers University. Additionally, Sea-Bird Scientific generously supplied the water quality monitoring equipment, which M. Acquafredda was awarded through the 2016 Student Equipment Loan Program.

I am grateful to the following people for providing logistical and technical support: Sarah Borsetti, Joseph Caracappa, Nate Morris, Iris Burt, Lisa Calvo, Samantha Gilbert Lynch, Jenn Gius, Jenny Paterno Shinn, Michael Whiteside, Patty Woodruff, Michael De Luca, David Jones, Josh Kiernan, Matthew Neuman, Sean Towers, Dan Acquafredda, and Joseph Ferraro. I am also grateful to the entities which supplied the bivalves used in these experiments: The Downeast Institute, Sweet Amalia’s Oyster Farm, and Bill Avery’s Quality Bay Clam Company. I would also like to thank the anonymous reviewers for providing valuable feedback and helping to improve this article.
Finally, this work was supported by Rutgers University through the Haskin Shellfish Research Laboratory, the Department of Ecology, Evolution, and Natural Resources, and the Graduate Program in Ecology and Evolution.
6.6 Literature Cited


Gaudin, A.C., Tolhurst, T.N., Ker, A.P., Janovicek, K., Tortora, C., Martin, R.C., &


diverse cropping systems under a new agricultural policy environment in Rwanda. Food Security, 8, 491–506.


Munroe, D. M., Narvaez, D. A., Hennen, D., Jacobson, L., Mann, R., Hofmann, E. E.,


Pales Espinosa, E., Hassan, D., Ward, J. E., Shumway, S. E., & Allam, B. (2010b) Role
of epicellular molecules in the selection of particles by the blue mussel *Mytilus edulis*. Biological Bulleting, 219, 50-60.


Figure 6-S1. Temperature of the productivity experiment. All temperature (°C) observations collected are plotted. Data were collected at 10-minute intervals using Sea-Bird Scientific SBE 56 temperature loggers.
Figure 6-S2. Salinity of the productivity experiment. All salinity (ppt) observations collected are plotted. Most data were collected at 15-minute intervals using a YSI 6600 V2-4 multi-parameter water quality sonde. Data from a NOAA buoy (Station CMAN – 8536110) were used to fill a gap in the dataset from August 29 to September 21. These data were collected every six minutes. The buoy is located less than a mile from the NJ Aquaculture Innovation Center and data collected by the buoy is highly consistent with data collected from the laboratory’s flow-through seawater system.
Table 6-S1. Morphometric data of bivalves used in the particle clearance experiments. These values represent mean ± standard deviation and were used to calculate tank-level clearance capacity of each mesocosm. Shell length represents the length of the anterior–posterior axis on the clams and the dorsal–ventral axis on the oysters. Shell height represents the dorsal–ventral axis on the clams and the anterior–posterior axis of the oysters. Shell width represents the dextral–sinistral axis of all bivalves. Whole wet weight is defined as the weight of the whole living organism with its shells and any external tissue patted dry. Dry tissue weight was determined after bivalve soft tissue was placed in a 68°C drying oven for 48 h, cooled, and weighed.

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th><em>C. virginica</em> (<em>Cv</em>)</th>
<th><em>S. solidissima</em> (<em>Ss</em>)</th>
<th><em>M. mercenaria</em> (<em>Mm</em>)</th>
<th><em>M. arenaria</em> (<em>Ma</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell length (mm)</td>
<td>19.62 ± 1.73</td>
<td>24.46 ± 1.64</td>
<td>18.94 ± 1.34</td>
<td>18.20 ± 1.44</td>
</tr>
<tr>
<td>Shell height (mm)</td>
<td>16.95 ± 1.68</td>
<td>18.07 ± 1.06</td>
<td>16.93 ± 1.51</td>
<td>11.84 ± 0.91</td>
</tr>
<tr>
<td>Shell width (mm)</td>
<td>4.32 ± 0.38</td>
<td>9.63 ± 0.62</td>
<td>9.93 ± 1.02</td>
<td>7.01 ± 0.55</td>
</tr>
<tr>
<td>Whole wet weight (g)</td>
<td>0.822 ± 0.139</td>
<td>2.161 ± 0.390</td>
<td>2.152 ± 0.595</td>
<td>0.926 ± 0.210</td>
</tr>
<tr>
<td>Dry tissue weight (g)</td>
<td>0.009 ± 0.003</td>
<td>0.073 ± 0.018</td>
<td>0.042 ± 0.014</td>
<td>0.029 ± 0.006</td>
</tr>
</tbody>
</table>
Table 6-S2. Initial morphometric data of bivalves used in the productivity experiment. These values represent mean ± standard deviation and were used to calculate the initial tank-level clearance capacity of each mesocosm. Shell length represents the length of the anterior–posterior axis on the clams and the dorsal–ventral axis on the oysters. Shell height represents the dorsal–ventral axis on the clams and the anterior–posterior axis of the oysters. Shell width represents the dextral–sinistral axis of all bivalves. Whole wet weight is defined as the weight of the whole living organism with its shells and any external tissue patted dry. Dry tissue weight was determined after bivalve soft tissue was placed in a 68°C drying oven for 48 h, cooled, and weighed.

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>C. virginica (Cv)</th>
<th>S. solidissima (Ss)</th>
<th>M. mercenaria (Mm)</th>
<th>M. arenaria (Ma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell length (mm)</td>
<td>27.69 ± 2.53</td>
<td>22.40 ± 0.71</td>
<td>17.47 ± 2.21</td>
<td>17.18 ± 2.01</td>
</tr>
<tr>
<td>Shell height (mm)</td>
<td>20.01 ± 1.18</td>
<td>16.81 ± 0.58</td>
<td>15.23 ± 1.79</td>
<td>11.33 ± 1.53</td>
</tr>
<tr>
<td>Shell width (mm)</td>
<td>6.71 ± 0.55</td>
<td>8.21 ± 0.28</td>
<td>8.92 ± 1.36</td>
<td>6.23 ± 0.63</td>
</tr>
<tr>
<td>Whole wet weight (g)</td>
<td>1.911 ± 0.132</td>
<td>1.526 ± 0.126</td>
<td>1.618 ± 0.630</td>
<td>0.709 ± 0.198</td>
</tr>
<tr>
<td>Dry tissue weight (g)</td>
<td>0.077 ± 0.020</td>
<td>0.088 ± 0.016</td>
<td>0.055 ± 0.023</td>
<td>0.045 ± 0.017</td>
</tr>
</tbody>
</table>
Chapter 7: Concluding Remarks

7.0 Conclusion

The goal of this dissertation was to investigate ways to expand aquaculture opportunities in the Northeast United States through ecologically-sound diversification (ESD). I achieved this by conducting numerous experiments that tested different ways that ESD could be implemented in the region.

In Unit 1, I examined multiple aspects of Atlantic surfclam (*Spisula solidissima*) husbandry and assessed its potential as an ecologically-sound alternate species that Northeast aquaculturists could add to their farm plans. Recently, surfclam aquaculture production is gaining momentum around the Northeast (Zemeckis, 2020); however, commercial-scale production remains in its infancy. The research presented in Unit 1 contributes to the surfclam husbandry literature and supports the growing industry by providing information about surfclam nursery rearing temperature, nursery gear type, and grow-out performance across several locations in New Jersey.

In Chapter 2, I conducted a study to assess how temperature influenced the growth and survival of early juvenile surfclams. I demonstrated that after metamorphosis, surfclams should be raised in temperatures close to 20°C for the duration of their nursery period to sustain maximum survival and high growth rates. I also found that juvenile surfclams can tolerate temperatures that deviate significantly from 20°C, although some survival and growth were compromised at temperatures too low or high. This information is important because heating and chilling seawater can be some of the highest costs associated with operating commercial bivalve hatchery and nursery facilities (Helm & Bourne, 2004). My research shows that currently, the ambient seawater available at many
locations across the Northeast United States should be sufficient for surfclam seed production at commercial scales. Not only is using ambient water more cost-effective for growers, it also reduces the energy needed to produce surfclams. Hence, this study further supports ESD.

In Chapter 3, I conducted a study that tested and evaluated various methods of surfclam rearing during the nursery and grow-out phases of production. Results from the nursery gear experiments indicate that multiple rearing methods can effectively produce surfclams. Either downwellers or bell siphon systems can be used as early nursery gear types, while upwellers were clearly the best late nursery gear type that I tested. Notably, there was no advantage to rearing surfclam seed in sediment during the nursery phase (shell length: 0.28-18.0 mm). My results also support previous work that shows that flow rate, food availability, and temperature are important factors that can limit bivalve nursery gear efficiency (Goldberg, 1980; Liu et al., 2011).

In the grow-out experiments, survival was highly variable. Survival was excellent at some locations, while at others, all clams perished. Surfclam mortality was associated with anoxic conditions, warm temperatures, aerial exposure during a harsh winter, and predation. Surfclam aquaculture also appears to be associated with a diversity of macrofauna, similar to what has been observed at other clam and oyster farms (Luckenbach et al., 2016; Mercaldo-Allen et al., 2020). Generally, growth, condition, and gonad development were consistent across farms, but varied seasonally. The greatest growth rates were achieved between the spring and summer, but considerable growth was also observed between the fall and spring. The latter period is typically a time when other commonly cultivated bivalves in the region, namely the Eastern oyster (Crassostrea
and the hard clam (*Mercenaria mercenaria*), are dormant. Data from this study suggested that the cold-tolerant surfclam can exploit the winter and spring algal blooms during the fall-spring season and achieve impressive growth. If growers were to culture surfclams in addition to one or both of these other species, they would be able to better exploit the available resources of their farm and sustain harvests throughout a longer portion of the year. In this sense, surfclam aquaculture can be considered a form of temporal ESD, in addition to spatial ESD.

However, more research is required for optimizing surfclam grow-out production. In this study, 67-98% of clams reached the pastaneck market size (31.75 ≤ x < 44.45 mm) within 9 months, notably slower than the growth observed in other studies (Goldberg, 1980; 1989; Walker, 2001). Yet unlike those studies, my research used a stocking density more similar to what the industry uses for other bivalves. Future research should be devoted towards identifying the optimal gear type for grow-out phase production as well as optimal stocking densities for commercial-scale production.

Overall, research from Unit 1 demonstrates that surfclam aquaculture is feasible in New Jersey and fits well into the Northeast region’s established shellfish farming framework. If adopted by the region’s growers, surfclam aquaculture would be a form of temporal and spatial ESD.

In Chapter 2, I discovered that different surfclam cohorts responded differently to temperature, exhibiting vastly different survival rates. In Chapter 3, I found that surfclams were particularly vulnerable to warm water conditions on shallow coastal shellfish farms. These findings laid the groundwork for Unit 2 (Chapters 4 and 5).
High temperature conditions will be exacerbated by climate change and this could have devastating impacts on surfclam aquaculture. Although surfclams are not widely cultivated yet, Chapters 4 and 5 represent the first foundational steps towards developing a breeding program for producing surfclams with greater heat tolerance. As such, the research presented in this unit takes a proactive approach towards climate-informed aquaculture. Since this research aims to help farmed surfclams stay adaptive and resilient to environmental change, it therefore supports the genetic aspect of ESD.

In Chapter 4, I explored the feasibility of breeding surfclams for greater heat tolerance. Specifically, I studied the response of adult farmed surfclams to heat stress after juvenile exposure, and I tested the ability for heat tolerance to be passed to subsequent generations. My results showed that when juvenile surfclams were exposed to prolonged lethal temperatures (Chapter 2), the adult survivors withstood subsequent heat stress for significantly longer than individuals not exposed to lethal temperatures as juveniles. I also found that selective breeding enhanced heat tolerance in first-generation surfclam progeny. Importantly, the growth of the heat-selected progeny was not significantly different from that of control clams under ambient conditions. Although more research on this topic is necessary, my findings suggest that surfclams have some capacity to adapt to some degree of future ocean warming.

In Chapter 5, surfclam heat tolerance was assessed with a different lens. Here, I examined how the surfclam transcriptome differed between ambient and heat stress conditions. I also assessed how a selective pressure—the lethal heat challenge used to produce the heat-tolerant surfclam broodstock in Chapter 4—would alter the gene expression profile of a surfclam population. The results of this study showed that when
naïve clams were exposed to warm water conditions, they differentially expressed significantly more genes compared to clams with a prior history of experiencing heat stress. However, the heat-selected clams exhibited a stronger heat shock protein response. Other genes activated during heat stress include those related to immunity, apoptosis, and remodeling membrane components to maintain cell integrity. These findings highlight specific targets for selection, which can be applied in more sophisticated surfclam breeding efforts.

Selective breeding is a critical tool for sustainable aquaculture, and it will be increasingly important in the future as climate change exacerbates food insecurity (Wheeler & von Braun, 2013). Unit 2 represents a small yet promising first step towards developing a breeding program for heat-tolerant surfclams, which in turn may help the Northeast aquaculture industry become more resilient to climate change. Future research efforts should be aimed towards understanding the genetic variation among wild surfclam populations and elucidating whether wild surfclams, throughout their geographic range, truly have the innate adaptive capacity to persist under future climate scenarios.

By evaluating various surfclam husbandry techniques in Unit 1, I laid the foundation for surfclam aquaculture to become a facet of ESD in the Northeast. In Unit 3 (Chapter 6), I expand upon this by determining whether existing shellfish growers can adopt surfclam aquaculture onto their farms without compromising the growth or survival of their existing crops. Moreover, I reached beyond surfclams and evaluated the efficacy of bivalve polyculture with three other species. I tested whether more diverse bivalve assemblages have greater clearance rates and are more productive (i.e., exhibit greater biomass growth and have higher survival) than those which were less diverse.
Specifically, I conducted a series of mesocosm experiments that simulated farm conditions and utilized all 15 combinations of the following four ecologically and economically important species: the Eastern oyster (*C. virginica*), the Atlantic surfclam (*S. solidissima*), the hard clam (*M. mercenaria*), and the softshell clam (*Mya arenaria*). With respect to clearance rates of one or two species of cultured microalgae, more diverse assemblages did not exhibit significantly greater clearance rates than those that were less diverse. Instead, the clearance rates of each species were additive across assemblages. However, when the assemblages were provided a natural array of seston, the four-species polyculture exhibited significantly greater clearance rate for particles <25 µm compared to most monocultures. Bivalve productivity was not enhanced in multispecies groups, but in almost every case, increased species richness had no negative consequences for each species’ growth.

Interestingly, surfclams were often the outlier in this study. Surfclam monoculture cleared natural seston particles as well as the four-species polyculture, and surfclam shell length was the only biomass metric of any species to decrease with increasing assemblage diversity, albeit minimally. Surfclam survival was also markedly lower and highly variable in one of the 15 combinations tested. Considering the work of Unit 1, I do not believe these results indicate that surfclams cannot be co-cultured with the other focal species. Instead, I believe the findings are suggestive of the fact that surfclam performance is influenced by environmental conditions. In this study, the clearance experiment was conducted during favorable conditions of the late fall (cool and minimally turbid), while the productivity experiment was conducted during mid-summer conditions, which tend to be unfavorable to surfclams (warm and highly turbid).
more research is conducted, growers should be aware that the strength and sign of the species interactions observed in this study might be modulated by environmental conditions. The degree of niche partitioning or interspecific competition among these bivalves might vary temporally, spatially, or across other environmental dimensions.

Consequently, not every grower will have a farm with the ideal conditions to rear each of these species, nor will every farm have the capacity to purchase and maintain the various gears needed to operate a diversified farm. Future research should be put towards understanding the economic constraints that may hinder ESD, since economic viability is key to its longevity.

Overall, Unit 3 (Chapter 6) demonstrates that some degree of niche complementarity exists among these four bivalve species. Moreover, in non-food limited systems, these species could be co-cultured without outcompeting one another. Since bivalve aquaculture is not currently close to carrying capacity with respect to food limitation in the Northeast (Dame & Prins, 1997; Byron et al., 2011; Chapman et al., 2020), growers could consider diversifying without the threat of one bivalve species overwhelming another. Notably, the other bet-hedging benefits of species diversification also still hold true, including insulating farmers from crop failures associated with disease, predation, fluctuating environmental conditions, or market forces.

In this dissertation, I argued the necessity of ESD as a critical facet of aquaculture sustainability in the 21st century. I supported this argument by demonstrating multiple ways that ESD could expand opportunities in the Northeast United States. It is my hope that other scientists continue researching ESD in this region and throughout the world.
Finally, I hope that shellfish growers in the Northeast find the information provided in this dissertation applicable to their livelihoods and are persuaded to diversify their farms.
7.1 Literature Cited


