FOOD SAFETY FOR NEW HAMPSHIRE OYSTERS:
A MULTIDISCIPLINARY PERSPECTIVE

BY

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ABSTRACT

FOOD SAFETY FOR NEW HAMPSHIRE OYSTERS: A MULTIDISCIPLINARY PERSPECTIVE

by

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Oyster aquaculture in New Hampshire is a relatively new industry that has emerged in the last decade. Management of food safety is an integral part of the oyster growing process in this small community. In particular, Vibrio parahaemolyticus is a bacterium that can cause gastrointestinal illness in people who eat raw or undercooked seafood. Recently, New Hampshire created a policy to manage importation of oyster seed for V. parahaemolyticus-related human health concerns. This highlighted a need for data on V. parahaemolyticus in oyster seed. Therefore, the objectives of this research are to examine both social and microbiological aspects of food safety management in the New Hampshire oyster aquaculture community. From a social science perspective, interview and survey data document the experience of industry participants and describe their perspectives on the efficacy and process of food safety management. From a biology perspective, data on V. parahaemolyticus concentrations in juvenile oysters and 16S sequencing data on the microbial community expand our understanding of the microbial implications of oyster importation. A most probable number pipeline (Kaysner and DePaola, 2004) in combination with polymerase chain reaction was used on oyster samples collected over 3 months to compare V. parahaemolyticus concentration in adult vs. juvenile oysters. Samples from the same collection were then used for 16S rRNA sequencing to assess differences in associated microbial communities between age groups and across sample dates. In combination,
these multidisciplinary facets are intended to provide managers and industry participants with an analysis of concerns related to water quality and food safety management, a record of interaction experiences between growers and managers, and new microbial data that may inform the management of oyster seed. The results from growers indicate they are mostly satisfied with the effectiveness of food safety management, mostly support a new oyster importation policy, and have positive interactions with state regulators. Microbial results support the scientific underpinnings of the importation policy as a precautionary measure as *V. parahaemolyticus* was present in juvenile oysters and juvenile microbiomes remained distinct from adult microbiomes for months. Together, these results describe an industry that is operating a management model that minimizes food safety risks, utilizes scientific data, and satisfies the needs of industry members. Elements of this model may be useful to aquaculture industries in other areas looking to develop or improve management strategies.
1. INTRODUCTION

1.1 Rationale

Oyster aquaculture is an excellent example of an industry operating within a coupled human and natural system (Liu et al., 2007a, 2007b). Great Bay Estuary in New Hampshire, as with other oyster-producing regions, consists of an ecological community that is interconnected with human activities. Undertaking decision-making with a perspective that incorporates both human and biological systems allows for adaptive management (Armitage et al., 2009; Butler et al., 2001; Folke et al., 2005) that addresses multiple values.

One important aspect of oyster management is the regulation of food safety. As consumers do not have total control over the safety of their seafood, especially for raw products, they depend on food policy to guide production of safe seafoods. The US keeps extensive records of foodborne illness through the Centers for Disease Control and Prevention (CDC), analyzed by both pathogen (Scallan et al., 2011) and food commodity (Painter et al., 2013). For seafoods as a group, most outbreaks are caused by bacteria, with *Vibrio* species as the most commonly reported cause (Iwamoto et al., 2010). CDC disease surveillance through the COVIS and FoodNet systems indicate that rates of reported vibriosis illnesses are increasing (Newton et al., 2012), and have increased in the northeast in recent years (Newton et al., 2014). Vibriosis illnesses also bring high costs to society – Ralston et al. (2011) estimated that health consequences from direct exposure to *Vibrio* species cost $30 million annually. With this in mind, research on *Vibrio* bacteria and minimization of vibrio-related illness risk is important.

Management of seafood safety rests within a larger context of seafood production that impacts both biological and social structures. In recent decades, multidisciplinary research has
expanded attention to human dimensions in natural science fields that were historically kept separate. For example, human dimensions have been applied to expand our understandings of long-term ecological research (Redman et al., 2004), resilience to environmental stress (Stokols et al., 2013), management of pests and wildlife impacts (Enck et al., 2006; Flint et al., 2009), conservation (Bennett et al., 2017), and management of marine reserves (Pollnac et al., 2010) and recreational fisheries (Hunt et al., 2013). Specifically, researchers have also applied human dimensions to shellfish research, such as studying the effects of climate change on the Mid-Atlantic Bight surf clam fishery (McCay et al., 2011) and the social impacts of mass bivalve mortality events (Guillotreau et al., 2017).

In this vein, research and knowledge in multiple disciplines is required to fully understand safe oyster production. Research on oyster population trends (Mann et al., 2009; Southworth et al., 2010) and changes in oyster growth over time (Harding et al., 2008) help us assess the status of wild oyster beds and the viability of wild oysters as a natural resource. Mapping of oyster beds has been performed in New Hampshire (Grizzle and Ward, 2013) and area scientists indicate a negative trend in oyster population after huge population losses in Great Bay (Barber et al., 1997; Piscataqua Region Estuaries Partnership, 2018). This loss is consistent with trends worldwide; researchers estimate as much as 85% of oyster reefs have been lost globally (Beck et al., 2011).

Loss of oyster reefs is an important issue for coastal communities, and the connection between oysters and ecosystem services was brought up by growers in interviews. Oyster reefs provide a variety of ecosystem services (Coen et al., 2007), such as water filtration (Ermgassen et al., 2013), excess nutrient removal (Pollack et al., 2013), shoreline stabilization (Scyphers et al., 2011), and enhancement of fish and crustacean abundance that supports fisheries (Peterson et
al., 2003). Economic analysis can put these services into perspective by estimating their value, and researchers estimate the value of oyster reef services, excluding harvesting, to be between $5,500 and $99,000 per hectare per year (Grabowski et al., 2012). Due to this high value, oyster reefs are often a target for restoration activities.

In addition to the challenge of harvest, ecosystem services provided by oyster reefs are threatened by other environmental variables such as ocean acidification (Lemasson et al., 2017). However, research has indicated that many ecosystem services provided by oyster reefs can also be provided by aquacultured oysters (Coen et al., 2011; Ferreira and Bricker, 2016). For example, farmed oysters and their associated microbial community remove excess nitrogen and other nutrients at levels comparable to natural reefs (Higgins et al., 2011; Humphries et al., 2016). Additionally, the gear used in shellfish aquaculture creates valuable habitat for other species, contributing to local fisheries (DeAlteris et al., 2004; Marenghi et al., 2010). Thus, when we study aquaculture, we study not just a human endeavor, but a living component of the ecological community.

In order to maximize aquaculture-based productivity, extensive research has been conducted on the methods and conditions to best grow a variety of oyster species. Examples of this research include study of the microalgae used to feed oysters (Muller-Feuga, 2000), the effects of water salinity (Nell and Holliday, 1988) and diet (Berntsson et al., 1997) on growth rate, optimum stocking density (Holliday et al., 1991), and methods for pest management (Dumbauld et al., 2006). Researchers have studied selection of oysters with strong growth to breed (Langdon et al., 2003) as well as the exchange of genetic resources for goals such as developing disease-resistant strains (Brown et al., 2005; Guo, 2009) and non-reproductive triploid oysters (Allen and Downing, 1986). From a human dimensions perspective, social
research has augmented this growing knowledge, such as in studies that identify the best sites for aquaculture in a given area with attention to social constraints like proximity to boat mooring fields, conflict with commercial shipping operations, and legal restrictions (Grizzle and Ward, 2012; Silva et al., 2011).

On the consumer side, demand for oysters is dynamic. Research into demand and willingness to pay shows evidence for consumer preference for farm-raised oysters and FDA inspection (Manalo and Gempesaw, 1997), preference for native species over non-native species of oysters (Grabowski et al., 2003), and preference for the better taste of non-postharvest processed oysters despite awareness of health risks (Bruner et al., 2014). However, preferences are not universal; research reports differences in preferences between first-time and experienced oyster buyers (Kecinski et al., 2017), and the consumer’s income, age, and selectivity in oyster attributes affect their purchasing (Li et al., 2017a). Beyond personal preferences, demand is also influenced by policy. For example, after policy required oysters from certain areas to be labeled with information about *Vibrio vulnificus* disease risk, demand for those oysters decreased, leading to lower prices for producers (Dedah et al., 2011; Keithly Jr. and Diop, 2001). This reminds us how multifaceted oyster production is, and how interconnected the regulatory, scientific, and economic dimensions of the industry are.

Management of food safety for shellfish is overseen on a national level by the National Shellfish Sanitation Program, a federal/state cooperative program recognized by the US Food and Drug Administration and the Interstate Shellfish Sanitation Conference. Food safety management is part of broader fisheries management. Historically, fishers and policymakers have often been in conflict about many aspects of fisheries management, and this has been observed in American oyster fisheries as well (Kennedy and Breisch, 1983; McCay, 1984). In
recent decades, fisheries in locations across the world have made increasing efforts to shift
towards co-management (d’Armengol et al., 2018; Gutiérrez et al., 2011; Hartley et al., 2008;
Jentoft, 1989; Sen and Raakjaer Nielsen, 1996). Similarly, cooperative research endeavors aim to
involve fishers in research projects and incorporate local and traditional knowledge into
scientific understanding (Hartley and Robertson, 2006; Johnson, 2011; Johnson and van Densen,
2007; Karp et al., 2001; Thornton and Scheer, 2012). These efforts strive to reduce adversarial
interactions, promote cooperation among stakeholders, and increase confidence in the science
used to inform policy.

The increase in aquaculture production in recent years adds complexity to the
management of seafood production. Aquaculture and wild fisheries interact on the global market
(Natale et al., 2013), yet experience different conflicts. For example, oyster aquaculturists deal
with pest management (Feldman et al., 2000), carrying capacity of their system (Byron et al.,
2015), and legal constraints (Duff et al., 2003) in different ways than wild harvesters do. This
makes social research on oyster farming particularly interesting as a contrast to wild fisheries.

Although harvest and consumption of oysters has a long history in New England, the
oyster aquaculture community in New Hampshire emerged relatively recently, with the first
permit issued in 1996 and increase in activity beginning around 2011, so its policy landscape is
fairly new. This contrast to long-lived fishing communities provides a different situation and
opportunity for collaboration between producing, managing, and researching bodies. This
research intends to continue building connections between these interests and increase our
understanding of the biological and human dimensions of New Hampshire oyster aquaculture.
1.2 Research Context

*Oyster production in New Hampshire*

In New Hampshire, historical commercial harvest of wild oysters ended in 1938 in response to declining oyster populations in Great Bay (Mariano), so all commercial oysters are produced through aquaculture. Most oyster farm licenses in the state are for bottom culture, but there are also some surface floats in use as well as four upweller units. Upwellers are a type of gear that houses very small oysters before they are later transferred to grow-out sites. Since there are no oyster hatchery facilities in New Hampshire, oyster growers must buy their seed from out-of-state facilities. After applying for a permit with the New Hampshire Fish and Game Department, they import their live seed and put it into New Hampshire waters to grow. The methods used by growers include oyster cages, condos, trays, mesh bags, and bottom seeding. As of 2019, twenty-three oyster aquaculture sites are located in Little Bay, with two more sites in the Hampton-Seabrook estuary (Figure 1a&b).

![Maps showing the locations of 2018 licensed oyster aquaculture sites in Little Bay (a) and Hampton-Seabrook estuary (b). Red area is closed to aquaculture. Yellow polygons are site locations. Solid yellow is suspended culture. (Figures from NH Fish and Game Marine Aquaculture Compendium).](image)

Throughout the oyster growing process, policies created by state and federal agencies guide growers’ practices so that the final product meets standards for human consumption. Food
safety management is particularly important for oysters for multiple reasons. First, oysters are filter feeders, which means they are capable of picking up particles and microorganisms in the water. Additionally, oysters are often eaten raw, so any pathogenic microorganisms that may be in the oyster are still alive rather than having been killed through cooking. With these issues in mind, aquaculture policies designate areas with high water quality as safe harvest areas and make rules about handling and transport to keep microorganism levels low. These policies directly affect the way oyster growers work to produce their product. The primary state agencies involved in regulating aquaculture are the Department of Environmental Services, the Department of Health and Human Services, and the Fish and Game Department.

The community of New Hampshire oyster growers is small and has emerged relatively recently, making it a unique community that is different from the oystering communities of neighboring states. Oyster industries in other states in the northeast region are larger (Table 1) and have longer history. In Massachusetts, sites that were historically used for growing oysters by transplanting seed from wild beds are now in operation as farms growing hatchery-raised seed; for example, the Cotuit Oyster Company dates back to 1857 (Cotuit Oyster Company). Maine had similar historical activities and issued its first official aquaculture lease in 1973 (Maine Department of Marine Resources). Farms in Maine’s Damariscotta River have been operating since the 1980s. Rhode Island was harvesting over 1 million oysters per year by 2003 (Coastal Resources Management Council Aquaculture in RI report).
<table>
<thead>
<tr>
<th>State</th>
<th>Permits</th>
<th>Acres</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Hampshire</td>
<td>21</td>
<td>56</td>
<td>329,156</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>390</td>
<td>1300</td>
<td>47,849,698</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>73</td>
<td>296</td>
<td>8,434,541</td>
</tr>
<tr>
<td>Maine</td>
<td>82*</td>
<td>676*</td>
<td>10,716,197</td>
</tr>
</tbody>
</table>

Table 1: Comparison of oyster aquaculture industries in New England states, measured by aquaculture permits issued, acreage of aquaculture sites, and oysters harvested. Data are for 2017, from NH Fish and Game Department, MA Division of Marine Fisheries Annual Report, RI Coastal Resources Management Council Aquaculture in RI report, and Maine Department of Marine Resources. Data marked with * represent all types of shellfish, not specifically oysters.

The New Hampshire oyster industry has grown rapidly in recent years. The first aquaculture license in New Hampshire was issued in 1996, and there was little change until 2011. In 2018, there were 25 licensed aquaculture sites with a total area of 71.7 acres (Figure 2). Since some oyster farm businesses have multiple sites, the number of farms in the state is represented separately in Figure 3, reaching 16 independently owned farms in 2018.

Figure 2: Licenses and acreage for oyster aquaculture in New Hampshire. (Figure from NH Fish and Game Marine Aquaculture Compendium).
An increase in harvested oysters has accompanied the increase in aquaculture sites.

Figure 4 shows the increase of over 350,000 oysters in annual harvest in just a six-year span. (Data are unavailable for years prior to 2013 for confidentiality reasons). Though this harvest is small compared to states that have more coastline and larger industries, the growth is remarkable.

To support the growth and harvest of these oysters, the amount of seed oysters imported into New Hampshire has changed over this time period (Figure 5). These data are collected from the importation permits growers apply for to buy their seed.
First described in the literature in 1953 (Fujino et al., 1953), *Vibrio parahaemolyticus* is a gram-negative halophilic bacterium that has been observed across the world. It is naturally occurring in saltwater environments, though its abundance is highly variable. *V. parahaemolyticus* abundance correlates most strongly with water temperature (Takemura et al., 2014), with low abundance in cooler temperatures and increasing abundance with warmer temperatures above 15°C (Bartley and Slanetz, 1971; Cox and Gomez-Chiarri, 2012; Kaneko and Colwell, 1973; Urquhart et al., 2016). Salinity also correlates with *V. parahaemolyticus* abundance, but the strength of the correlation is more variable, with some studies finding a strong relationship while others do not (Takemura et al., 2014). This may be because *V. parahaemolyticus* tolerates a wide range of salinity, primarily from 5-25ppt, and with lower abundance at very low and very high salinities (DePaola et al., 2003; Johnson et al., 2012; Urquhart et al., 2016; Zimmerman et al., 2007). Additionally, *V. parahaemolyticus* is known to associate with copepods and other plankton (Colwell and Kaneko, 1975; Kaneko and Colwell, 1973), which can make chlorophyll *a* a useful addition to abundance modeling (Urquhart et al.,
2016). Because *V. parahaemolyticus* thrives in warm brackish waters, high abundance is often observed in estuaries during summertime.

Many environmental strains of *V. parahaemolyticus* do not appear to be harmful to humans, whereas a few strains have been implicated in causing human disease known as vibriosis. The most common route of infection is through ingestion of raw or undercooked seafood, although infection can also occur through open skin wounds during swimming in salt water or through handling raw seafood. Usually, the symptoms of gastroenteritis resulting from foodborne vibriosis do not require medical care. Occasionally infected people require hospitalization, and very rarely, deaths occur (Newton et al., 2012).

Vibriosis outbreaks have occurred across the globe. The first recorded outbreak caused by *V. parahaemolyticus* in the US occurred in Maryland in 1971 (Dadisman, 1973). Since then, surveillance has increased to get a fuller understanding of vibriosis incidence. In the US, the Centers for Disease Control and Prevention (CDC) runs a passive surveillance system called the Cholera and Other Vibrio Illness Surveillance System (COVIS). Health centers across the country can voluntarily report vibriosis cases to generate national reporting data. COVIS began in 1989 with four Gulf Coast states, but now includes all US states and territories as vibriosis became nationally notifiable in 2007. Additionally, CDC works collaboratively with state health departments and the Food and Drug Administration (FDA) to record vibriosis data through the FoodNet system. This system performs active surveillance of foodborne illnesses in select areas of the US. Both of these surveillance systems indicate that US vibriosis incidence has increased since 1996 (Newton et al., 2012). In particular, northeastern US states have reported increased numbers of vibriosis cases in recent years (Figure 6, as in Urquhart et al. (2016)). (These reports include all *Vibrio* species except toxigenic *Vibrio cholerae*).
Figure 6: Reported vibriosis illnesses in northeastern US states since 2000. Numbers are approximate as health departments may revise reports upon new investigations. Data gathered from state health department records, except: NH 2000-2004 data from COVIS; CT 2016-2017 data from CDC FoodNet Fast. Data includes all non-toxigenic *V. cholerae Vibrio* species, except: RI 2000-2011 data included only *V. parahaemolyticus* and *V. vulnificus*.

Vibriosis illnesses caused by *V. parahaemolyticus* have been linked to multiple types of seafoods, such as shrimp, octopus, crab, lobster, clam, and oyster (Daniels et al., 2000). However, oysters are the most commonly implicated food. The most recent report published by COVIS in 2014 indicated that about 84% of cases caused by *V. parahaemolyticus* in 2014 were foodborne. Of those patients who reported eating a single seafood item before getting sick, 69% reported eating oysters, and of those, 89% ate the oyster raw (Centers for Disease Control and Prevention, 2014). This illustrates that raw oysters are of particular concern when analyzing vibriosis infection routes.

Vibriosis incidence can also be affected by climate change. Due to its preference for warm water temperatures, *V. parahaemolyticus* has traditionally been limited in its range by cold...
temperatures at higher latitudes. Connections have been drawn between rising sea surface
temperatures and warm water events, such as El Niño events, and the timing and location of
vibriosis diseases (Baker-Austin et al., 2013, 2016; Martinez-Urtaza et al., 2008, 2010; Vezzulli
et al., 2016a). *V. parahaemolyticus* outbreaks during warm summers in areas that had previously
not had *V. parahaemolyticus* illnesses, such as Alaska in 2004, indicated a poleward expansion
of the recorded range of pathogenic *V. parahaemolyticus* in oysters (McLaughlin et al., 2005).
These same trends have been observed in the northeastern US, where the Gulf of Maine is the
fastest-warming body of water in the world (Pershing et al., 2015). Increasing numbers of
reported vibriosis illnesses and detection of new pathogenic *V. parahaemolyticus* strains indicate
the emergence of *V. parahaemolyticus* as a public health concern in the region (Martinez-Urtaza
et al., 2013; Newton et al., 2014).

When researchers study *V. parahaemolyticus* bacteria, genetic analysis can identify
characteristics that allow the researcher to recognize individual strains. The Northeast Center for
Vibrio Disease and Ecology at UNH uses a system of multilocus sequence analysis that analyzes
seven housekeeping genes to identify sequence types (Jolley, 2010; Jolley et al., 2004; Xu et al.,
2015a). Additionally, characteristic genes are useful to analyze the potential pathogenicity of the
bacteria. The *tlh* gene, for thermolabile hemolysin, is used as a species marker for the *V.
parahaemolyticus* species. The *tdh* and *trh* genes, which encode thermostable direct hemolysin
and thermostable direct hemolysin-related hemolysin, are used as putative pathogenicity
markers. Whereas not all pathogenic strains of *V. parahaemolyticus* are positive for *trh* or *tdh*,
and some strains harboring these markers have not caused human infections, it is more common
for clinical strains to have these genes than it is for non-pathogenic environmental strains (Cox
and Gomez-Chiarri, 2012; Mahoney et al., 2010; Robert-Pillot et al., 2004; Xu et al., 2015a). Therefore they are the most widely-used markers of pathogenicity that have been identified.

Researchers use genetic analysis to identify *V. parahaemolyticus* strains that have caused significantly high numbers of human diseases. ST3 caused the largest historical outbreaks. This strain emerged in 1996 and was identified on five continents as a global pandemic (Matsumoto et al., 2000; Nair et al., 2007; Okuda et al., 1997). ST36 originated in the Pacific Northwest, but since its arrival now causes the most reported diseases in the northeastern US (Martinez-Urtaza et al., 2017; Xu et al., 2015b). This strain had not been associated with shellfish outside the Pacific Northwest before 2012, and was implicated in outbreaks on the US east coast in 2012 and 2013 (Newton et al., 2014). ST631 is a strain native to the northeastern US that was first identified in 2007 and is second to ST36 in prevalence (Xu et al., 2015a, 2017). The public health impacts related to these pathogenic strains have led to new policy in the state of New Hampshire (see “Importation Policy” section below).

**New Hampshire aquaculture policy**

New Hampshire aquaculture is primarily governed by three state agencies: the Fish and Game Department, the Department of Environmental Services, and the Department of Health and Human Services. Each department has different responsibilities that support and monitor the aquaculture industry. Broadly, Fish and Game (F&G) handles licensing, Department of Environmental Services (DES) classifies shellfish harvest areas and open/closed status, and Department of Health and Human Services (DHHS) handles food safety certification. The Shellfish Program, which is the most frequent point of contact for growers, is housed within DES.
Fish and Game Department

The State of New Hampshire Office of Legislative Services lists Administrative Rules for state agencies. For F&G, “Chapter Fis 800: The Importation, Possession, and Use of All Wildlife” describes permitting procedures and requirements for importing, possessing, releasing, and propagating wildlife. Part of this chapter, “Part Fis 807: Aquaculture – Inland and Marine,” lists requirements specifically applicable to oyster aquaculture. People who want to begin aquaculture must submit an application to F&G, which includes complete information about the species raised and methods used. Staff from F&G will perform a site assessment and arrange a public hearing. The applicant must notify all abutters and provide them with information, and the public can speak at the hearing and provide written comments about the proposed project. After this, F&G will decide whether to approve the aquaculture project. F&G will issue a license if they conclude the project does not pose unacceptable risk, conflict with other use in the area, or adversely impact private property in the area.

Further, this chapter states that inland of the Sullivan Bridge (i.e. in Little Bay), sites have a maximum size of 4.5 acres and must be at least 150 feet apart from each other. Aquaculturists must submit monthly and annual reports to F&G summarizing their sales and activities. The rules list annual fees for aquaculture, but oyster aquaculture operations in Great Bay Estuary can also get 5-year licenses. Additional fees include a $.015 fee per oyster, paid at the end of the year. F&G’s power to adopt these rules for aquaculture is described in “Chapter 211: Fish, Shellfish, Lobsters and Crabs” within New Hampshire Statutes in “Title XVIII: Fish and Game.”

In addition to the Administrative Rules, F&G writes conditions into issued aquaculture licenses to address specific issues. Under the F&G rules, eastern oysters are a non-controlled
wildlife species, which means people would not need permits to import them. However, F&G writes conditions into aquaculture licenses that require importation permits to acquire oyster seed. These imports must also be certified free of oyster diseases like MSX (*Haplosporidium nelsoni*) and Dermo (*Perkinsus marinus*). License conditions require aquaculturists to obtain proper permits, such as permits from the Wetlands Bureau and Army Corps of Engineers.

Similarly, F&G can write conditions into aquaculture licenses to address concerns raised by DHHS or DES. For example, conditions written into licenses for upwellers set a maximum size for oysters in upwellers located in prohibited harvest areas, so that most of the oyster biomass growth will occur after the oysters have been transferred to a conditionally approved area. If the conditions of the license are violated, F&G can revoke the license.

*Department of Environmental Services*

In “Chapter 143: Sanitary Production and Distribution of Food” of the New Hampshire Statutes, DES is designated as the authority that determines where shellfish may be harvested for food, and DHHS is designated as the sanitation control authority for the commercial sale and processing of shellfish. In Statutes “Chapter 487: Control of Marine Pollution and Aquatic Growth,” a fund is established within DES for the Healthy Tidal Waters and Shellfish Protection Program. The purpose of this program is to “ensure that water quality in coastal waters supports the propagation, conservation, and harvest of shellfish,” and allows the department to classify harvest waters, work to mitigate water impairments, educate citizens, and conduct strategic planning to enhance shellfish harvest and aquaculture. On a daily basis, DES monitors water quality and other conditions such as wastewater treatment plant performance and weather conditions, and decides whether shellfish harvest is open or closed.
DES also has a role in permitting aquaculture operations through the Wetlands Bureau under Statutes “Chapter 482-A: Fill and Dredge in Wetlands.” In 2019, the Wetlands Bureau is undergoing a revision to its Administrative Rules that is expected to recognize similarities between the requirements for DES Wetlands Bureau permitting and F&G aquaculture licensing. If adopted, the new rules will streamline the permitting process for oyster aquaculture operations that meet certain conditions.

Department of Health and Human Services

For the certification and control of food safety, Administrative Rules “Part He-P 2150-2159 New Hampshire Shellfish Sanitation Rules” describe procedures for DHHS regulation. Most importantly, Section He-P 2152.01 incorporates the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish so that all aquaculturists in New Hampshire must comply with the guide. The National Shellfish Sanitation Program (NSSP) is a federal/state/industry cooperative program recognized by the US Food and Drug Administration and the Interstate Shellfish Sanitation Conference (ISSC) for the sanitary control of shellfish. Participants in the NSSP include industry members, state agencies, and federal agencies like the Food and Drug Administration, the Environmental Protection Agency, and the National Oceanic and Atmospheric Administration. The program creates a model ordinance that promotes interstate commerce for shellfish, creating common standards for states that have varying individual policies.

Aquaculturists need to be certified by DHHS to sell shellfish. To achieve this, they must submit an application to DHHS that includes information about their operation and a Hazard Analysis and Critical Control Points (HACCP) Plan. Certificates must be renewed annually. When DHHS issues a certificate, they notify the FDA so the aquaculturist is added to the
Interstate Certified Shellfish Shippers List. There are five classifications of certificate that can be issued, but New Hampshire oyster growers are typically certified as shellstock shippers, which means they can grow, harvest, pack, and ship shellfish.

The HACCP Plan is a key element of certification. This document lists food safety hazards that may occur and measures for monitoring and preventing hazards. Specifically, it identifies critical control points, which are steps in the process where control can be applied to reduce a food safety hazard. At each of these, a critical limit lists the value at which the parameter must be controlled in order to reduce the food safety hazard. Additionally, aquaculturists must do DHHS-approved training on processing, handling, and transport practices every other year.

To enforce good food safety practices, DHHS can inspect aquaculture operations at any time. There is a minimum of one inspection per year, and the department may perform additional surprise inspections. The inspector records any deficiencies, or conditions that pose a health threat. If deficiencies cannot be corrected during the inspection, DHHS will make a compliance schedule for the aquaculturist to make required changes. If an operation has a strong safety record, defined by their lack of specific deficiencies in past years, they can be in the performance-based inspection program that has just one inspection per year. DHHS can perform enforcement actions such as revocation of certificates, imposition of fines, embargo of contaminated products, or cessation of production in cases of noncompliance.

Additionally, DHHS is the point of authority in emergency and recall situations. If emergencies like fire or chemical exposure present a health hazard, aquaculturists notify DHHS and suspend operations. DHHS can order a product recall when any dangerous circumstances
threaten public health. The dealer must use their records of sale to notify their wholesale and retail outlets.

**Vibrio Control Plan**

New Hampshire created a Vibrio Control Plan in 2015. This document created guidelines for aquaculture practices to reduce risk of vibrio illnesses. Updated each year, the 2019 version of the New Hampshire *Vibrio parahaemolyticus* Control Plan comes from DHHS in cooperation with DES and F&G. The Plan applies to oysters and hard clams harvested between May 1 and September 30. Proper labeling on harvest bag tags, log books, and invoices all must document compliance with the plan by showing harvest time and area, type and quantity of shellstock, and a time to temperature control statement. Temperature control is a key aspect of the Plan: time from harvest until shellfish enter temperature-controlled storage cannot exceed 4 hours in June/July/August and 5 hours in May/September. Once in temperature control, shellfish must reach an internal temperature of 50°F within 4 hours. Dealers can perform a cooling study to demonstrate that their practices accomplish this cooling rather than measuring temperature each time. Shellfish must be adequately iced and shaded, and cannot be kept in standing water, with the exception of ice slurry. Cooling shellfish in an ice slurry of less than 45°F is recommended, but is not required. Measures to reduce vibrio risk must be part of the HACCP plan.

**Comparison to other northeastern states**

Each state in the northeast region has its own aquaculture rules, and New Hampshire has similarities and differences from nearby states. Andrews et al. (2015) described the aquaculture policy structures in Massachusetts, New Jersey, and Maine. In Massachusetts, each municipality develops its own permitting requirements within a broader state-level framework overseen by the
Division of Marine Fisheries; this structure results in non-uniform procedures throughout the state. Like New Hampshire, New Jersey and Maine both use state agencies to manage aquaculture. New Jersey recently piloted a program that designated certain areas as Aquaculture Development Zones that are pre-approved and therefore require acquisition of fewer permits. Additionally, New Jersey does not hold public hearings about aquaculture sites like New Hampshire and Massachusetts do. Maine has different levels of permits (Limited Purpose Aquaculture License, Experimental License, and Standard License) with different requirements and public involvement for each, which New Hampshire does not have. However, New Hampshire did change its rules to allow for permits that last five years, rather than the single-year permits used previously.

In 2019, all northeastern coastal states have Vibrio Control Plans. In some states these are required by NSSP because of illness outbreaks that have occurred, but other states have created plans voluntarily. Similarities between the states’ plans include adequate icing, adequate shade, not keeping shellstock in standing water unless it is an ice slurry, and recording and tagging product to demonstrate compliance with the plan. Differences arise from each state’s regulatory structure and particular experiences with vibrio. For example, some states have specifications about deliveries to dealers that do not apply to New Hampshire, where the harvester and dealer are the same person. The states also differ in the dates during which the plan applies; for example, Massachusetts’ plan extends into October while New Hampshire’s ends in September. Though all of the plans have requirements for cooling shellstock, the specifications for this cooling have variation in the desired temperature (45°F or 50°F), how many hours may pass before shellstock enters mechanical temperature control (2-7 hours), and how many hours may then pass before the desired temperature is reached (4-10 hours). The plans also reflect different
management mechanisms, such as Maine’s use of 80°F ambient air temperature as a boundary that dictates how many hours the harvester has before delivering to a dealer. With the same goals and overall procedures, the minor differences between state plans reflect different industry and environmental conditions in each area.

Importation policy

In January 2018, New Hampshire created a new policy regarding the importation of oysters, which primarily applies to oyster seed. Seed shipments were already managed for oyster diseases such as MSX and Dermo, but were not regulated for any diseases that affect humans. New Hampshire became the first state to create such a policy that focuses on the human health impacts of transporting live oysters. F&G has primary control over wildlife importations, and worked cooperatively with DES, DHHS, and scientists from UNH to create the policy.

The context of this policy rests in vibriosis epidemiology. *Vibrio parahaemolyticus* sequence types 36 and clade II 631, which are identified using genetic markers, are virulent strains that have caused many illnesses in the northeast region. However, neither of these strains has ever been identified in New Hampshire waters or shellfish. The purpose of this policy is to prevent the introduction of these *V. parahaemolyticus* strains into New Hampshire, and therefore prevent illnesses that they could cause here.

To accomplish this, the policy specifies that oysters and hard clams, including seed sized animals, cannot be imported into New Hampshire from areas that have had illnesses caused by *V. parahaemolyticus* ST36 or clade II 631 or had a harvest closure due to multiple *V. parahaemolyticus* illnesses. The policy contains a list of locations that are currently excluded from importation, and a stipulation that the list will be reviewed and updated each year before
the oyster farmers order their seed in the winter. Currently, virtually all seed is imported from Maine, which is permitted under the policy.

Additionally, the text contains language that the policy may be changed if conditions change, such as if allowable nurseries experience closures or the pathogenic strains are detected in New Hampshire. Thus the policy is not viewed as permanent, but as a precautionary measure that may be changed when it is no longer effective.
2. RESEARCH APPROACH AND METHODS

2.1 Approach and context for study

The researchers took a pragmatic approach that allowed utilization of multiple complementary methods. A variety of data sources were used (data triangulation) that included previously collected data as well as new data.

To become familiar with the study area and the oystering community, public meetings hosted by New Hampshire DES were attended. Researchers at UNH who study aquaculture-related topics often attend meetings with industry members to communicate their research and participate in conversations about new research and management ideas. Observations at three meetings identified key individuals and current issues and concerns in the community.

Two of the meetings attended were annual New Hampshire marine aquaculturist meetings. At these gatherings, staff members from state and federal agencies, growers, representatives from industry groups, distributors and restauranteurs, and scientists meet to discuss items that affect aquaculture. For example, at the 2018 meeting topics included changes in regulations about wetland permitting, wet storage requirements, the Vibrio Control Plan, and a wintertime closure due to elevated sewage discharge concerns. At the 2019 meeting, attendees discussed changes to harvest area classifications, changes to staffing in state agencies that would lead to altered inspection procedures, oyster restoration projects in the bay, changes to the NSSP model ordinance, and an upcoming project where Eversource planned to lay a cable across the bottom of the bay. The afternoon segment of this meeting was spent in a roundtable discussion about the future of New Hampshire aquaculture, such as possibilities for relay and depuration,
which currently are not allowed in New Hampshire. The primary structure of these meetings was a series of presentations, with opportunities for questions and comments from the audience.

The third meeting attended was a special meeting called to discuss the new importation policy. A working group comprised of DES staff, DHHS staff, F&G staff, and UNH scientists had met previously and drafted the policy, and this meeting’s goal was to introduce the policy to the growers and receive their input before finalizing the policy. State agency staff presented the policy and the scientific findings that had led to the policy’s creation. Extended conversation followed, with many questions from growers and industry representatives. While the tone of the meeting never became adversarial, the depth of engagement indicated this policy was of great interest to industry members. Growers expressed concerns about how this would affect their businesses and already limited seed sources, and also asked questions about what kinds of new research could deepen our understanding of vibrio in oyster seed and support future alternative management strategies. Growers also had the opportunity to make written public comments, which five businesses chose to do. These comments delineated suggested changes to the draft and called for additional research. As a result, changes were made to the policy, such as setting a timeline for each year that would not cut off a grower’s seed supply after they had already placed their order.

After observing the process surrounding the importation policy, it was clear that this was an important current issue in the oyster industry that offered an opportunity for cooperative research (Hartley and Robertson, 2008; Karp et al., 2001). The cooperative nature of discussion between growers and state agencies was intriguing. The conversation was also centered around scientific findings and a value for further research, indicating that research on $V$. 
parahaemolyticus in oyster seed would be valuable to the industry. For these reasons, multidisciplinary research on food safety management seemed important and timely.

2.2 Objectives

The overall goal of this research was to examine and integrate the social and microbiological aspects of seafood safety management in the New Hampshire oyster aquaculture community. From a social science perspective, the New Hampshire oyster industry is new and growing, so this research aimed to document the experience of industry participants and understand their perspectives on the efficacy and process of food safety management. Data from the interviews and survey were used to identify successes and challenges in food safety management and its future.

From a biological perspective, as an investigation of a current issue in New Hampshire, additional objectives were to establish methodology for processing seed oysters for V. parahaemolyticus analysis and to understand the dynamics of V. parahaemolyticus concentrations in oyster seed. The goals were to understand whether V. parahaemolyticus concentrations in juvenile versus adult oysters were different, observing for changes over time throughout the 3-month sampling period and for seasonal changes related mostly to water temperature changes. Analysis of 16S DNA sequencing data from these same samples allowed for determining changes in microbial communities in seed oysters imported into New Hampshire, and comparing the microbiomes of adult and juvenile oysters to understand whether they became more similar once in the same ecosystem. Additionally, linear models using oyster size measurements were intended to aid future lab work on oyster seed by demonstrating how many animals are necessary to reach a required biomass.
In combination, these multidisciplinary facets created a fuller understanding of seafood safety management in New Hampshire oyster aquaculture. Building upon observations from public meetings, this research aimed to capture current issues in New Hampshire and produce data that may aid managers in effectively dealing with these issues. With the findings from this research, managers and industry participants received an analysis of concerns related to water quality and food safety management, a record of interaction experiences between growers and managers, and new microbial data that may inform the management of oyster seed.

2.3 Social science methods

Observation at industry meetings

To understand the context of the research, three meetings hosted by New Hampshire Department of Environmental Services were observed. Notes were taken on content that was discussed, the group affiliations of attendees, and issues raised by different groups.

Interviews

To learn about the experiences and perspectives of New Hampshire oyster growers, a collaboration with UNH sociologists was formed to use previously collected interview data. Interviews were conducted in 2016 as part of the New England Sustainability Consortium (NEST) project (Whitmore, In review). With a semi-structured interview approach, interviewers asked questions from an interview guide but also allowed conversation on additional topics. Ten growers from New Hampshire, recruited via email, were interviewed in-person or over the phone. The interviews were recorded and later transcribed. These interviews were exploratory in nature due to the small size of the New Hampshire oyster industry.
The interviews were coded in NVivo software (Bazeley and Jackson, 2013; Emerson et al., 2011; Miles and Huberman, 1994; Weiss, 1995). Based on the known structure of the interviews and access to the interview guide, a priori codes were used to find general themes of water quality management and food safety management. Through reviewing the transcripts, interactions with regulators emerged as a major theme. Within these broad categories, grounded theory (Corbin and Strauss, 2014; Glaser and Strauss, 1967) was used to identify emergent themes. All participants will be given the gender-neutral pronoun “they” in this paper.

Survey

To augment the interview data, a brief survey was conducted (Dillman et al., 2009). Participants were identified through a publicly available list of aquaculture licenses, and therefore all oyster aquaculture principals who were registered in New Hampshire in 2018 were invited to participate. Eight of these invitees had previously participated in the 2016 interviews. After consultation with experts at the UNH Survey Center as well as with staff in the Department of Environmental Services, participants were recruited via email and the survey was distributed using Qualtrics software (Qualtrics, Provo, UT). The survey totaled 11 questions that were a mix of multiple-choice and open-ended questions (Appendix 2). Due to the small sample size, this survey was exploratory in nature and analyzed in a qualitative way. Survey results were analyzed as a dataset separate from the interview dataset because mostly different individuals participated in each.
2.4 Microbiology methods

*V. parahaemolyticus sample processing and quantification*

This study was performed in partnership with a local New Hampshire oyster farm to source the oyster samples. The farm is located in Little Bay, NH and uses bottom culture. An oyster condo unit was deployed on the farm site at a location such that all oysters remained submerged even at low tide. The farmer stocked the unit with bags of adult and juvenile oysters. The juvenile oysters were from the farm’s seed shipment, which was delivered on July 20, 2018 when the oysters were 9-13mm long. After the farmer received the seed, the animals were kept in a cold cooler until they were brought to the Jackson Estuarine Laboratory on the same day. The adult oysters were from the 2016 year class and had been growing at the farm site for the past 2 years. All oysters were disease-resistant *Crassostrea virginica*.

Five sample collections were performed between July and October 2018, according to the schedule in Table 2. Each sampling date included triplicate samples of both juvenile and adult oysters. The number of animals in the juvenile samples was higher than the typical 12 oysters used for adult samples due to the small amount of meat in each juvenile. The number of juveniles per sample decreased as the oysters grew bigger and enough material to perform lab analyses could be gathered from fewer animals. The first sample of juvenile oysters was taken directly from the seed shipment on the same day that the farmer received the seed, while all other samples were collected from the condo at the farm site during low tide. Additionally, a HOBO temperature logger deployed at the farm site collected water temperature data at 15-minute intervals throughout the study. All oyster samples were placed in a cold cooler and brought back to the Jackson Estuarine Laboratory for analysis on the same day.
Table 2: Record of sampling dates and number of oysters collected. Sampling was performed in triplicate – for example, the October 15 sampling date used 3 samples of 12 adult oysters each and 3 samples of 24 juvenile oysters each.

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Number of oysters in each sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
</tr>
<tr>
<td>July 20, 2018</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>August 6, 2018</td>
<td>12</td>
</tr>
<tr>
<td>August 20, 2018</td>
<td>12</td>
</tr>
<tr>
<td>September 4, 2018</td>
<td>12</td>
</tr>
<tr>
<td>October 15, 2018</td>
<td>12</td>
</tr>
</tbody>
</table>

The adult oysters were processed and analyzed using established procedures as described in Urquhart et al. (2016). For each sample, 12 oysters were scrubbed and aseptically shucked with an oyster shucking knife and the meat was homogenized in a hand blender. The homogenate was diluted with alkaline peptone water, and then incubated overnight in 3-tube serial 6-fold decimal dilution tubes that began with 1mL homogenate and continued with decimal dilutions to 0.000001mL homogenate.

For the samples of juvenile oysters, the established procedure for shucking adult oysters with a shucking knife and homogenizing them in a hand blender was modified. Juvenile oysters were shucked with a razor blade, inspired by procedures used by the Haskin Shellfish Research Laboratory at Rutgers University (personal communication). Because of the small volume of homogenate, the spinning blades on the blenders could not be submerged and therefore would not have been effective. Instead, the juvenile oyster meats were homogenized by magnetically spinning a razor blade in the bottom of a flask at 1200 RPM for 3 minutes (Figure 7). The homogenate was then incubated in 3-tube serial dilution tubes at the same dilutions used for the adult samples, with the exception that for the first three sampling dates when the oysters were especially small, there was not enough material to create the highest concentration dilution of 1mL homogenate. The success of the analytic procedure in detecting *V. parahaemolyticus* after
this processing indicates that known lab tests can be used to analyze *V. parahaemolyticus* in oyster seed with minimal modification to the sample processing steps.

![Image](image.png)

Figure 7: Juvenile oysters were shucked with a razor blade (a) and homogenized by magnetically spinning a razor blade in the bottom of a flask (b).

While shucking the oysters, 20 juvenile oysters were randomly selected to use for additional measurements. Shell length was measured with calipers and a balance was used to measure total mass and meat/liquor mass. Additionally, for all oyster samples, a portion of the homogenate was frozen in a 50mL conical tube at -80°C for later microbial community analysis.

According to the FDA Bacteriological Analytical Manual (Kaysner and DePaola, 2004), a 3-tube most probable number (MPN) culture procedure in combination with polymerase chain reaction (PCR) was used to enumerate the *V. parahaemolyticus* concentration in the samples. After 18 hours of incubation at 37°C, turbid tubes were recorded positive for growth. A sterile 1uL loop was used to streak the turbid tubes onto CHROMagar, a selective media on which *V. parahaemolyticus* colonies appear purple. After incubating overnight at 37°C, purple colonies were selected to further isolate onto tryptic soy agar (TSA) plates using sterile toothpicks. After the TSA plates grew overnight at room temperature, isolated colonies were selected to inoculate
into heart infusion (HI) broth in a 96-well culture plate. The bacteria were incubated on a shaker at 37°C for 2 hours, centrifuged and resuspended in nuclease free water, and then lysed in a thermal cycler set to 100°C for 10 minutes.

The DNA obtained after centrifuging was used to perform multiplex PCR to look for the presence of \( tlh \), \( tdh \), and \( trh \) genes. The PCR amplification was performed using a BIO RAD T100 thermal cycler and the published primers and cycling conditions (Panicker et al., 2004; Whistler et al., 2015). The 10uL reaction contained 5uL 2x Quanta Accustart supermix, 0.2uL of 10uM \( tlh \) primers, \( trh \) primers, and \( tdh \) primers, 2.8 uL nuclease free water, and 1uL template DNA. Cycling parameters included 3 minutes at 94°C, and then 30 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C, followed by 5 minutes at 72°C.

Statistical analyses were performed in R version 3.6.0 and JMP Pro 14. Geometric means were calculated using the non-transformed MPN data. There were no analyses that resulted in no detection of \( V.\ parahaemolyticus \). The MPN data were normalized with a natural log transformation, and a Shapiro-Wilk test determined that the data were successfully normalized. The transformed data were used for one-way and two-way analyses of variance (ANOVA) and analysis of covariance (ANCOVA).

Data for oyster measurements (shell length, total mass, meat mass) were also natural log transformed for normality, but Shapiro-Wilk tests did not indicate these transformed data were completely normalized. However, the p-value improved after the transformation so the transformed data were used for linear regression.

**Marker-based 16S community analysis**

The frozen homogenate samples were processed for 16S microbial community analysis following Earth Microbiome Project protocols (Caporaso et al., 2011, 2012; Parada et al., 2016;
Quince et al., 2011). DNA was extracted from the oyster samples using a Qiagen DNeasy PowerSoil kit according to manufacturer instructions. The purity of the samples was analyzed with a Thermo Scientific NanoDrop 2000c spectrophotometer and the concentration of DNA in the samples was analyzed with an Invitrogen Qubit fluorometer, according to manufacturer protocols. To prepare the samples for sequencing, the 16S rRNA V4-V5 region was amplified through PCR. The 12uL reaction contained 6uL 2x Quanta Accustart supermix, 0.7uL of 5uM 515F and 926R primers with linkers, 2.6uL nuclease free water, and 2uL template DNA. The cycling conditions included 3 minutes at 94 °C, then 35 cycles of 30 seconds at 94 °C, 30 seconds at 50 °C, and 30 seconds at 72 °C, followed by 7 minutes at 72 °C.

The UNH Hubbard Center for Genome Studies performed the second-round PCR to attach adapters and barcodes according to Miya et al. (2015). The 12uL reaction contained 6.0uL 2x KAPA HiFi HotStart ReadyMix, 0.7uL each primer (5 μM), 3.6uL sterile distilled H2O and 1.0uL template. Cycling conditions included 3 minutes at 95 °C, then 12 cycles of 20 seconds at 98 °C and 15 seconds at 72 °C, followed by 5 minutes at 72 °C. The PCR products were pooled and cleaned with a Qiaquick PCR product cleanup kit. The samples were analyzed with Qubit and diluted to a 1.1nM portion of a total sequencing lane. 250 bp paired-end reads were sequenced on an Illumina HiSeq 2500 instrument. Raw data were demultiplexed using the Illumina bcl2fastq Conversion Software v1.8.4.

The QIIME2 2019.1 platform (Bolyen et al., 2018) was used to analyze the 16S sequencing data. The DADA2 plugin (Callahan et al., 2016) was used for sequence quality control, trimming 25 bases from the front on the forward reads and 26 bases from the reverse reads to remove primers. DADA2 performs read joining, denoising, filtering of chimeras and singletons, and dereplication steps. Samples with low sequence counts were filtered out using
q2-feature-table, which left 24 samples to use for analysis. Also filtered out were chloroplast DNA, unassigned sequences, and sequences that were only identified to the kingdom bacteria. For relative abundance of taxa, the q2-feature-classifier plugin with a Naïve Bayes classifier trained on the Greengenes 13.8 99% OTUs full-length sequences was used. The q2-diversity plugin to ran core diversity metrics at a sampling depth of 1721. This provided alpha diversity measures of Pielou’s evenness, Shannon diversity index, and Faith phylogenetic diversity, permutational multivariate analysis of variance (PERMANOVA) for beta diversity, and principal coordinates analysis (PCoA) based on Bray-Curtis distances. Within this plugin pcoa-biplot was used to create a biplot, and the q2-gneiss plugin was used for differential abundance analysis. Balances were calculated (Morton et al., 2017) to compare abundances of important taxa, using the target taxon in the numerator (i.e. Brachyspiraceae or Synechococcus) and all other taxa present in the sample in the denominator. A pseudocount of 1 was used for samples where the target taxon Brachyspiraceae was not present.

The q2-diversity alpha-rarefaction was used to create an alpha rarefaction curve (Figure 8). The sampling depth used for diversity analyses (1721) is lower than the depth at which most samples plateau. Therefore datasets did not likely capture the true diversity of the samples and interpretations are made cautiously based on this limitation.
Figure 8: Rarefaction curve for all oyster samples.
3. SOCIAL SCIENCE RESULTS AND DISCUSSION

3.1 Observation at industry meetings

The results from observation at industry meetings were used as context for this research. See the above section 2.1 Approach and context for study for results.

3.2 Interviews

Water quality

Oyster farms are rooted in their water bodies, and ecological conditions impact a business’s ability to succeed. As farmed oysters grow, conditions in the surrounding environment affect not only survival of the animal, but also the quality and safety of the oyster as seafood. Maintaining high water quality is a key factor in producing safe, high-quality oysters.

When New Hampshire oyster growers were asked to rate the water quality in Great Bay on a scale of “good”, “fair”, “poor”, or “I don’t know,” most of them indicated that water quality is good. No growers choose “poor” (Figure 9). When discussing why they chose their answer, growers were careful to differentiate between water quality issues that are dangerous to humans, versus issues that are harmless to humans. This distinction is important for the reputation of oysters as quality seafood. For example, one grower said:

It makes me nervous when I hear people talking about water quality in the bay because I think there’s this misconception that it’s contaminated with things that are directly harmful to human health. I’ve heard people say that the sediments are contaminated with oil and heavy metals and all these terrible toxic chemicals, and the reality is that from that perspective the bay is relatively clean. The contamination issue is eutrophication, which is just excessive amounts of nutrients and algae in the bay… I just kinda keep my fingers crossed that [local NGOs] are careful to make the distinction between water quality issues that can be related to human health issues that could potentially affect my market versus water quality issues that are more of an ecosystem quality kind of an issue, like eutrophication.
These comments suggest that imprecise public communications about water quality could negatively impact oyster businesses by giving the impression that local water quality is dangerous to human health, when it really is not. Other growers shared the view that Great Bay has high water quality in terms of human health. Particularly in comparison with other bays in the US, growers agreed that Great Bay waters are fairly clean. To explain why, one grower said, “Look out the window, there is no industry here…this is still as pristine as it’s gonna get, from that perspective.” Although Seacoast New Hampshire has experienced increased development recently, much of this has been residential development rather than industrial (Brickner-Wood and Wellenberger, 2006), maintaining the absence of industrial waste-related contaminants that have caused problems in areas with more industrial development.

However, residential development has its own impacts on water quality. When discussing what types of water quality threats are present in Great Bay, growers mentioned contaminants such as oil spills, chemical runoff from lawn treatments, fuel leaks from boats at the marina, and other particulates that affect water clarity. The most frequently mentioned concerns were issues associated with wastewater and elevated nitrogen levels that lead to eutrophication. At the time the interviews were conducted, Portsmouth, NH was in the planning stages of upgrading its wastewater treatment facility. Many growers expressed concern about the output from the facility in its current state, as well as other facilities in the region, and how the effluent affected water quality. One grower said, “The sewage treatment plants that are all emptying into the bay, you know they’re not all up to par…there’s three levels to a sewage treatment plant, and only one of them is at level three and there’s several at level two and then Portsmouth’s at level one or even less right now.” This grower is referring to the different treatments that wastewater treatment facilities can be designed to execute. Primary treatment removes solids from the
wastewater and disinfects with chlorine. Secondary treatment adds removal of organic matter, and tertiary treatment further removes phosphates and nitrates (United States Environmental Protection Agency, 2004). Since 1972, the EPA has required secondary treatment except in cases where a waiver has been issued. Portsmouth had a waiver to allow only primary treatment until 2007, when the waiver was not renewed. Portsmouth had to begin planning for a wastewater facility upgrade, which is scheduled to be completed in 2020 (City of Portsmouth, 2019).

The growers expressed concern about local wastewater facilities that performed only primary treatment, and thus were adding nutrients into the bay though their effluent. This is a particular concern for nitrogen, which was mentioned frequently as an issue for Great Bay. As one grower said, “I can agree with the majority here that the [biggest] threat now, and the most likely in the future is eutrophication.” This nitrogen issue was posed as a contrast to strong human health, as growers said ecosystem health was less strong in Great Bay.

However, growers agreed that farmed oysters help combat nitrogen enrichment issues. They described the filtering activity of oysters as a “win-win” for the water that removes excess nitrogen. Growers also noted other ways that oysters help the ecosystem. When the adult oysters on farm sites spawn, the larvae are not collected by farmers, so the larvae can be distributed throughout the ecosystem and add to the wild oyster population. Oyster aquaculture gear also creates habitat for other species, and growers described seeing more organisms living near their gear.

These feelings that farmed oysters are positive impacts on the ecosystem are part of why the growers considered oysters to be sustainable seafood. In the interviews, growers were asked, “What does sustainable seafood mean to you?” and nearly all of them explained the sustainability of oysters, specifically. One grower succinctly described why, saying:
I think anything that you can regenerate that doesn’t make an impact on the environment is the way to go. Oysters are a perfect example because we actually bring them into the bay. So we buy them as spat, or as seed…they’ll spend three years in Great Bay filtering, taking out all sorts of nitrogen and things like that, and clearing up the bay, and in the end we harvest them and make a profit on it…To me that’s the picture of sustainability.

In this response, part of the key to the sustainability of farmed oysters is that the process does not remove any oysters from wild populations, while adding positively to wild populations through spawning and improving ecosystem health by filtering water. Additionally, growers contrasted the process of farming oysters to the processes surrounding finfish because oysters do not require the input of feed. Because oysters feed on what is naturally in the water, they considered them more sustainable. The growers discussed the sustainability of their farms with pride and with vested interest in maintaining ecosystem health.

In sum, the growers thought Great Bay water quality was safe for their seafood production, but faced issues with elevated nitrogen and eutrophication. They thought their oyster farms help deal with nitrogen and make multiple positive impacts on the ecosystem health of Great Bay.

**Management of water quality**

When growers were asked to rate the management of water quality in Great Bay, opinions were more divided (Figure 9). Responses ranged from “good” to “poor.” Two growers also responded “I don’t know,” and this reflects an issue raised by several growers that they were not sure who was responsible for which activities. They found it difficult to assess which municipalities were managing water quality well, or whether certain outcomes were related to the work done by governments, NGOs, or UNH. Nonetheless, there was strong agreement that the Portsmouth Peirce Island wastewater treatment facility should have been upgraded sooner. To combine responses from multiple growers, they expressed that it was “really freaking
annoying” that the government had “procrastinated” in making the upgrades, and that the government “ought to be ashamed of themselves.”

Despite this frustration, growers also acknowledged that New Hampshire lacks resources to implement things as quickly as may be desired. Even if the technology is ready, upgrades cost money, as one grower described:

It’s frustrating and it’s always been frustrating throughout my career that we have the technology to really mitigate some of these discharges, and the can has just been kicked down the road literally for decades…I realize that money doesn’t grow on trees. Or there isn’t an infinite amount of money to be allocated. But I just feel like Peirce Island should have been taken care of a long time ago and there are some legitimate threats to my business from an unmitigated Peirce Island discharge. So you know, we should be far beyond primary treatment at this point.

Throughout this discussion, though, was a belief that the government had the necessary knowledge and information to deal with water quality issues. The problems lay more in the execution of solutions, which were mired in issues of public understanding and funding. The growers expressed confidence in the data-collecting capabilities of government agencies like DES, noting that they are constantly monitoring water conditions. Many growers also brought up a dye study that had been performed by DES, and accepted it as reliable data.

Throughout the interviews, growers expressed value for scientific data. Many of the growers are scientists professionally or by undergraduate training, and they use scientific information in many aspects of their aquaculture work. As scientists, they also discussed findings with a healthy skepticism. One grower examined the dye study with a critical eye, saying, “Dye tests will show the spread but they don't necessarily note the spread of what. If things are killed before they're discharged then the dye test doesn't necessarily tell anyone the whole story.” Another grower referenced studies on nitrogen in the bay and the municipal policy that was based on those studies: “They just didn’t pull in enough of the complexities…they really didn’t
have anything in the various assessment processes that considered the water flow, hydrodynamics of the system.” These comments demonstrate a high-level engagement with research surrounding aquaculture, and a value for continued research to gain fuller understanding of pressing issues. They stress that understanding the dynamics of the bay may be more complicated than results from a single study indicate. Further, these comments underscore how interrelated the work of researchers, growers, and managers is.

When asked about their personal involvement in work surrounding water quality management, most growers indicated they did not attend public hearings that were held to discuss the Portsmouth wastewater facility. One said they lacked resources and time to spend on attending hearings. However, several noted that they were aware of the process through the newspaper.

Instead of engaging individually, some growers said they used DES as a resource. They indicated that DES called a meeting to discuss plans for upgrading the Portsmouth wastewater facility: “They wanted us to be aware of the two different plans and while they weren’t encouraging us to look at it one way or the other, I think they wanted us to be aware so we could do it personally if we wanted to.” Another grower valued this meeting because the growers had not been contacted as a stakeholder group prior to this. Through the meeting with DES, they learned about their options for becoming involved, such as attending larger meetings, replying to websites, or giving public comment at forums. While the agency did not engage in activism on the growers’ behalf, they provided resources for the growers to become involved if they chose.

Overall, growers had mixed opinions on how well water quality is managed in Great Bay. The delay in upgrading Portsmouth’s wastewater treatment facility was a major frustration, but the science and knowledge of people working on the issue was highly regarded. Growers tended
to be involved and informed on water quality management activities as a group through DES meetings rather than as individuals at the public hearings.

**Food safety**

In addition to water quality in the surrounding environment, the processes used to grow oysters affect the product’s food safety. Aquaculture policy sets standards for harvest, handling, and transport procedures that aim to reduce disease risk associated with the product. These standards directly shape the practices used by growers.

In discussing food safety for oysters, growers stressed the importance of place-based reputation in oyster marketing. As Whitmore (In review) described in her work on this same community, growers think that maintaining a disease-free reputation for New Hampshire oysters is important. At the time of the interviews in 2016, there had never been a case of vibriosis associated with New Hampshire oysters; since then, there has been a single case reported. Growers were honest in acknowledging the health threat posed by vibrio and other pathogens, and expressed that risk-reduction practices were important. Although it may not be possible to prevent vibrio illnesses entirely, it is the growers’ responsibility to minimize the risk, as one grower described:

> Oyster disease has been prevalent up and down the east coast and that's always hanging over our heads as something that could be a significant problem in the future. But I think keeping that stigma that New Hampshire is disease free in terms of the health of the people that are eating our oysters, and making sure we want to police each other as much as the government does because we don't want someone to screw up and leave their oysters out on the deck too long and get someone ill because then we can't say New Hampshire never had a case of shellfish poisoning.

This response also alludes to uncertainty about the future. With warming waters and an expanding industry, it is possible that local conditions could change and more illnesses could
emerge. Facing these possibilities, this grower stresses the importance of proper practices to keep disease risk low.

A few growers said the procedures for reducing disease risk were overcautious, or made their work more difficult. However, some of these growers added that they understood the necessity of the rules and accepted them as an effective way to prevent disease, as this grower said: “We may grumble about the regulations but the truth of the matter is if there's an outbreak associated with bad oysters we’re all gonna suffer for it, so by following these regulations there's enough safety built in that that’s not likely to happen.”

A few growers also noted that the safe reputation associated with New Hampshire oysters gave them an advantage abroad. They said that American food standards are higher than standards in other countries, and that consumer markets therefore prefer American oysters. None of the growers were making major sales to foreign markets, but some were thinking about doing so in the future.

When asked to rate the management of food safety in New Hampshire oysters, most growers chose “good,” and none chose “poor” (Figure 9). Overall, growers thought that New Hampshire was effective in managing food safety. They said the monitoring performed to enforce safety compliance was “very thorough” and that policy ensured that the oysters that made it to market were safe. One grower described, “You can’t get any healthier than that, so [DES is] monitoring water quality, and then [DHHS] comes out twice a year and they monitor our paperwork, I mean, there’s so much monitoring going on and they check the boat, and they check everything... It’s really really really well taken care of, I think.” In this response, the grower expresses confidence in the completeness of state monitoring. None of the growers viewed inspections negatively, or said they thought safety monitoring should be done differently.
They seemed to have faith that the monitoring systems in place were effective in producing a high quality product.

However, the written policies themselves were confusing for a few growers. In particular, the NSSP Guide is an extremely long document, and one grower described their difficulties in understanding it: “They give you this giant booklet of model ordinance, you have to read it, it’s just very confusing, and then trying to figure out all the reporting requirements and all the things you have to have on your invoice and now they just implemented a vibrio control plan as well.” This grower went on to suggest that it would be helpful for state agencies to create short cheat-sheets for required practices that were easier to understand.

Overall, growers thought food safety monitoring was comprehensive and effective. Required procedures for risk reduction were sometimes unwieldy, but ultimately necessary and valuable to maintain the disease-free reputation of New Hampshire oysters. Since the written protocols could be difficult to understand, working with people in state agencies was more effective.

**Interactions with regulators**

Throughout the farming process, growers interact with government staff who regulate aquaculture and enforce management policies. In New Hampshire, the most frequent type of interaction is when growers get approval to harvest from DES staff. Before each harvest, growers must contact DES to check that harvest is open. Most growers indicated they did this by texting a particular staff member. This system allows DES to control temporary closures, such as preemptive closures when there is more than 1.5 inches of rain, or reactive closures to dangerous water quality situations like red tide blooms.
Additionally, growers interact with DHHS staff at least once a year for inspections, which may be planned or a surprise. DHHS also meets with growers to review their practices and HAACP plans. As a group, the growers gather once a year for the marine aquaculturists meeting, which includes staff members from all relevant agencies, such as DES, F&G, DHHS, and FDA. Additional meetings are called for special events that are important to New Hampshire aquaculture. For example, special meetings were held about the creation of the Vibrio Control Plan, the creation of the new importation policy, plans for upgrading the Portsmouth wastewater facility, and plans for Eversource, the state’s major electrical utility, to undertake a major dredging project to bury new electrical cables under the Little Bay seabed.

Throughout discussion of these interactions, growers expressed trust that governmental agencies were a reliable source of information. At meetings, agencies present the results of studies that growers described as “pretty impressive.” Agencies are also a source of information that growers are incapable of collecting themselves, such as continuous water quality data that is integral to farming. Growers indicated that DES was very willing to share data, as one grower described: “[They] will give us pretty much anything we need to know from the salinity of the water, to any kind of outbreaks when they happen. They have charts on everything, super free with their information…They’ve been really cool about that.” Here, a state government agency was a provider of critical information.

Sometimes information from state agencies leads growers to change their practices. For example, when the Vibrio Control Plan was being discussed, staff from DHHS showed data to growers that demonstrated how icing oysters after harvest can drastically reduce vibrio levels. This type of interaction shows that state agencies are rooting their management decisions in scientific findings, and are communicating these findings to growers to explain their decisions.
Additionally, growers relied on DES to notify them of important circumstances. For small issues, such as temporary rainfall harvest closures, growers accepted DES decisions as the right call and believed DES staff “definitely go to bat for us” to avoid unnecessary closure when possible. For larger issues like the wastewater upgrades, DES kept farmers updated. One grower said, “We've been notified when there's public information and opportunities for comment…and it's the same guy at DES who does it all, who sends you an email or something about what's going on. If it's a key moment that you should be providing input he’ll let you know.” This response demonstrates belief that DES staff are keeping an eye on current happenings, and will dependably relay information to growers and tell them how and when to advocate for themselves. The grower trusts that DES is providing these communications in the best interest of the growers.

Similarly, growers usually trusted that government staff meant well even when they were frustrated by certain rules. When discussing some inefficient practices, one grower said of the managers, “So their hearts are in the right place but their minds are not necessarily.” Likewise, the grower who was skeptical about the application of nitrogen research said they understood that regulatory people have to make decisions as best they can, saying of one of the decision-makers, “I had high respect, he’s a very smart guy, but I think he was just caught between a rock and a hard place.” None of the growers suggested that managers were intentionally making their work more difficult; they acknowledged that with the newness of the industry rules were created “on the fly.” Overall, despite occasional frustrations, they seemed to believe that managers were making an honest effort with limited resources.

This sense of trust between growers and managers may stem from trust in individual people. One staffer at DES is the main point of contact with the growers, and several growers
spoke extremely positively about their experiences with this person. When asked why they were confident in data they received from this staffer, one grower said, “I think him, knowing him. He’s extremely thorough and he cares and he is extremely professional as well. If you’re out of line he’ll let you know, if anything needs to be done and he doesn’t know it or understand it he’ll find a way. He’s really, an incredible worker to have for us. We’re pretty lucky.” This sentiment that this staffer personally cares about oyster farms and works hard was echoed by another grower who said the staffer “goes above and beyond” to keep growers informed. Clearly this staffer has established themselves as a dependable resource for oyster growers. In explaining why this person is so important to the functioning of New Hampshire aquaculture, one grower said,

He’s in touch with the people he needs to be in touch with and he’s keeping this industry going…he samples the waters, he does everything that satisfied the National Shellfish Sanitation Program. He has to basically see that we meet all the federal guidelines so that we can be interstate shellstock shippers. So if he were to fold up, we would fold up in an instant because then we couldn’t sell our oysters outside [New Hampshire]...He makes us internationally US Food and Drug Administration capable so to speak. And he’s the main player I think so if anything comes up I go to [him] and he’ll come to us too. It’s been two ways. So I’m very pleased with that.

This response indicates that the work done by this staffer directly affects farmers’ ability to sell oysters to other states, and therefore has a huge impact on the industry in New Hampshire. The grower expresses gratitude for this staffer’s work and for the cooperative, two-way relationship they have.

This cooperative spirit was brought up by many growers. During the regular annual meetings, one grower described “a good round table discussion” about upcoming regulation that allowed growers to offer input. Several growers talked about the special meeting for the creation of the Vibrio Control Plan, and how growers and managers worked together to improve the
policy. Growers had asked for clarification on certain aspects of the document, and state agencies answered questions about these details at the meeting. In describing the meeting one grower said, “It was really interactive and you know, not hostile at all and we actually got a lot of clarification so I’ve been really pleased with that.” Another grower appreciated that this level of interaction was above and beyond what the state agencies were required to do, saying, “They chose to involve us in the process, so they get full marks in my book for that.” In addition to answering clarifying questions, state agencies sought out growers’ input to make the procedures outlined in the policy feasible. As one grower described, “They’re just like, is this working for you guys? You know, what’s working and what’s not.”

Growers seemed satisfied with their level of involvement in the process surrounding the Vibrio Control Plan. One grower expressed that they wanted this kind of cooperation to continue in the future. Growers also indicated that they thought these meetings were so successful because the industry was small, and everyone could sit in one room and talk. However, another grower mentioned that the small size of the industry limited their collective power to make infrastructure changes.

In sum, growers had mostly positive interpersonal experiences working with state agency staff. They described high respect for the individuals they work with, and trust in the information provided by state agencies. They valued the opportunity to be involved in shaping state vibrio policy and hoped for a continued cooperative relationship with state regulators.

**Frustrations**

During the interviews, growers brought up specific points that were frustrating in their aquaculture work. The first of these was the annoyance of having to call or text DES before each harvest. There had been talk in years prior to the interviews of making an online platform or app
that would allow growers to just check the status quickly, but this had still not been developed. In addition to the personal annoyance, growers thought it would make DES staff’s job easier to not be fielding phone calls at all hours.

Additionally, growers were frustrated by negative public comments during the licensing of aquaculture sites. They described abutting landowners who made ‘not in my backyard’ complaints because they didn’t want to look at an oyster farm. One grower who had this conflict noted that “It was always a visual issue for [the landowner], it was never an ethical issue about the water” and went on to explain that the public often imagines an oyster farm being much more obstructive than it really would be – with rack and bag bottom culture, only the corner buoys marking the site would be visible above the water. Growers were frustrated that complainants could leave “the process held hostage by one person” even if their complaints were unfounded, and that the regulatory staff running hearings were unwilling to move on quickly.

Finally, two growers brought up a “tax” on oysters that frustrated them. Growers pay $.015 per oyster to F&G for every oyster they sell. They thought it was unfair that this payment applies only to oysters and not to any other seafood products. They argued that they had purchased and brought the oysters into Great Bay before harvesting them, and drew a contrast to fisheries like lobster that were collecting a New Hampshire resource. They also noted that they thought the money was not spent on oyster-specific objectives, and thought that it should be.
Figure 9: Growers were asked to rate water quality in Great Bay, management of water quality, management of food safety, and management of vibrio illness risk. Management of vibrio risk data came from the 2019 survey (N=12) while the other three questions were from 2016 interviews (N=10).

**Discussion**

Literature surrounding seafood production largely focuses on wild fisheries. Many studies on wild fisheries have looked at policy processes, co-management efforts, and management success. However, fewer studies have examined management in aquaculture industries, particularly in the United States. When looking to compare the results of this research to previous findings, this leaves a shortage of adequate material for comparison. In wild fisheries, conflicts are often centered around how to allocate property rights to a common resource (Charles 1992) – and this does not apply to aquaculture settings, where growers own their own organisms. Therefore, examining the process of resolution of conflicts and the success of other management procedures in an aquaculture setting does not lend itself to direct comparison to wild fishery findings.
Yet, we may draw parallels to wild fisheries literature about the way management responsibilities are shared between the government and seafood producers. Sen and Nielsen (1996) described different co-management arrangements observed in wild fisheries. The findings in the current study suggest that New Hampshire oyster aquaculture may operate in what Sen and Nielsen describe as an instructive or consultative arrangement. In instructive management, decisions are made by the government, but mechanisms exist for the government to inform the users about decisions. This aligns with what the interviewees described for most day-to-day management activities, like open/closed harvest status decisions or health inspections, where governmental agencies were the primary decision-making bodies but growers valued receiving communications from them. In consultative management, the government consults with users, and then decisions are made by the government. Consultation was mainly described for new policies, most notably the Vibrio Control Plan, where the government was instituting something new and sought out feedback from the growers before implementation. After discussion, the government issued revised policy, but was under no obligation to incorporate every comment raised by growers. Thus, the ultimate management decision rested with the government.

Moving forward, it will be interesting to see if management continues in a more instructive or consultative fashion. Due to the way the New Hampshire oyster aquaculture industry began, with just a couple of people interested in farming at the time the state formed a regulatory framework, a co-management arrangement that used more grower involvement was probably impossible in the beginning. Now that the industry has grown and there are more individuals involved as growers, it is more feasible that the growers as a stakeholder group have enough capacity to be more involved. Presently, growers value having their input incorporated into policy development. However, many New Hampshire growers have full-time jobs and run
their farms as a part-time enterprise, so their resources to participate regularly in management discussions may be limited. The process that growers described in interviews, where DES invited them to meetings to work out specific management issues, may continue to be an effective way to consolidate the time growers spend on management involvement.

Some of the experiences documented in this study are similar to what growers elsewhere have recorded. Dewey et al. (2011) discussed the importance of water quality to Washington oyster growers, but described a more active advocacy approach than has been taken by New Hampshire growers; Washington’s industry, longer-established and with larger social clout, was involved in active lobbying. Growers in both states saw oysters as a tool to improve water quality, suggesting there may be support for involving oyster aquaculture in nutrient management strategies (Rose et al., 2014). Washington growers also faced the same struggles during the permitting process with coastal landowners making aesthetic complaints. These types of complaints have been noted in other aquaculture studies as well (D’Anna and Murray, 2015; Katranidis et al., 2003).

In contrast to the established community in Washington, Whitmore (In review) described the loose social organization of New Hampshire oyster growers as a community of practice, which is a group informally bound by what they do together (Wenger, 1998). While some growers have connections with the East Coast Shellfish Growers Association, there is not a formal group for New Hampshire growers specifically. However, the social bonds within this small community allow them to learn from each other and influence regulation. Siddiki and Goel (2017) reported on marine aquaculture groups working in partnership with government staff on aquaculture policy, and found that aquaculture groups felt they had more influence when procedures were fair and they were able to mobilize scientific and technical resources. This
aligns with what New Hampshire growers brought up as positive experiences, that they respected the data collection and research happening in New Hampshire and felt their concerns were fairly heard by government staff in management discussions. These items may be part of why New Hampshire growers felt mostly satisfied with their involvement in management decisions.

When discussing their interactions with state regulators, New Hampshire growers described a relationship with significant trust. Young et al. (2016) found that conflicts over conservation and resource use are more likely resolved when there is increased trust built through fair processes. Similarly, Turner et al. (2016) found that natural resource governance was perceived as more legitimate when users trusted information from governing bodies. These findings resonate with the trustworthy information and fair policy discussions that New Hampshire growers described, which led to satisfactory management procedures. Additionally, Gilmour et al. (2015) found that coastal stakeholders trusted information that came from someone they perceived as competent and with whom they had a positive personal history, much like New Hampshire growers’ description of why they trusted information from DES staff.

The acceptance of scientific information from government agencies by New Hampshire growers offers an interesting contrast to historic conflicts in wild fisheries. In some cases, fishers did not believe the science used to make population models that led to quota levels, which led them to challenge quotas and management decisions (Hartley and Robertson, 2006). This type of issue is still in the news today, for example in the Gulf of Maine cod fishery where fishers and scientists disagree on cod abundance (Bleiberg, 2017). In both wild fisheries and aquaculture, scientific results can affect producers’ ability to harvest; wild fishers can be limited in how many fish they can catch, while shellfish growers can have their harvest areas closed. For example, New Hampshire instituted a wintertime closure in 2018 based on the results of a study on the
spread of male-specific coliphage (an indicator of sewage-related concerns) through the estuary. While New Hampshire growers did at times want more information to complete understanding of the estuary, they did not reject science presented to them by state agencies. Continued research can help illuminate the social, historical, and economic conditions that shape these interactions and lead to acceptance or skepticism of scientific findings.

3.3 Survey

Management of vibrio risk

The registered principals of 16 New Hampshire oyster farms were invited to participate in this survey. Twelve individuals from twelve different farms responded (75% of farms represented) with a 100% completion rate.

First, the growers were asked to rate New Hampshire’s management of risk of vibrio-related illnesses in shellfish. This is similar to the question on management of food safety asked during the interviews, but more specifically targets vibrio as a single type of pathogen. This is an important distinction because risk reduction for vibrio is generally performed through post-harvest practices such as icing and shading, with harvest closures when outbreaks occur. In contrast, other food safety hazards like red tide cannot be managed with post-harvest practices. Therefore, management strategies for vibrio affect the day-to-day practices of oyster growers.

Nearly all respondents rated New Hampshire’s management of vibrio risk as “good,” indicating they thought that New Hampshire agencies are successful in minimizing risk of vibriosis illnesses (Figure 9). When asked to describe the practices they use to minimize vibrio risk in their shellfish, growers described cooling measures and said they follow HACCP guidelines set forth by state agencies. Several specified that they use an ice slurry, which is recommended but not required in New Hampshire, indicating that they are investing even more
than is required of them to reduce vibrio risk. They said they work with the oysters in the water to limit the time shellstock is out of water before harvest.

Since many bacterial pathogens thrive in warm temperatures, changes in environmental conditions could impact food safety in oysters. Most growers thought climate change could affect food safety in New Hampshire shellfish, though not all did (Figure 10). When asked what impacts climate change could cause, most respondents identified warming waters as a threat that could bring higher risk of vibrio and other bacteria. They mentioned that these could be new warm-water pathogens that have not been a concern in New Hampshire before. Two growers mentioned ocean acidification as a threat that weakens juvenile oysters. Another mentioned more frequent extreme weather events that bring increased runoff and associated risks.

![Figure 10: Grower responses to the question, “Do you think climate change could affect food safety in New Hampshire shellfish?”](image)

**Importation policy**

To understand growers’ perspectives on the new importation policy, respondents were asked to indicate their level of agreement to three statements about the policy (Figure 11). The first statement, “The importation policy is an effective way to prevent introduction of new vibrio
strains into NH waters,” was meant to assess whether growers thought the mechanism outlined in the policy would work. Simply stated, not importing oysters from areas that had certain vibrios would prevent those vibrios from establishing in New Hampshire. Respondents were primarily in agreement with the statement, and some responded neutrally. No growers thought the policy was ineffective. This indicates that there is agreement among growers that the logic behind the policy is sound and poses a viable solution.

Figure 11: Grower responses to three statements about the new importation policy.

Separate from whether the mechanism would work, is managing importations a sensible thing to do? The second statement, “The importation policy is a sensible precautionary step for New Hampshire,” was meant to assess whether growers thought the policy was a good thing to implement. Most growers agreed it was a sensible step, with one neutral response and one
disagreement. This indicates that most growers think it was appropriate to implement the importation policy to protect food safety in New Hampshire.

The third statement, “The importation policy conflicts with my business interests,” was meant to assess whether it has any associated negative impacts on oyster farms. Responses to this statement were mixed, with some growers agreeing and others disagreeing. These impacts were detailed when growers were asked what they would be doing differently if the policy did not exist. Half the respondents said they would be doing nothing differently, indicating that the policy did not limit their practices or carry associated costs for them. Other growers said they would evaluate alternative sources of seed to get different strains and availability times. Two growers said they would like to use depuration to make shellfish from restricted areas usable. These comments are similar to the concerns raised at the public meeting and in written public comments about this policy, when growers were worried about the limitation of seed sources. The split opinions suggest that not all farms felt the importation policy made much of a difference to their practices, but some growers felt limited in where they could obtain seed.

Communication of research

Finally, the last set of questions addressed how scientists can most effectively communicate vibrio research to stakeholders. The growers agreed or felt neutral that the vibrio information they have received to date was presented in a way that was easy to understand. This is encouraging that growers have not felt alienated by scientists and have been able to understand the research. To continue improving upon this communication, growers were asked in what form and from whom they would like to receive information about vibrio research. With results from these questions, we can strategically communicate in the way that growers prefer.
There were no overwhelming preferences in how growers wanted to receive vibrio information. For the form of communication (Figure 12), there were modes that growers definitely did not want to receive: social media was chosen zero times, and mail only once. For modes that were more preferred, email was most frequently chosen, followed by presentations and fact sheets at the annual aquaculturists meeting. While scientific papers are often not preferred by the public, some growers did prefer them, reflecting their scientific backgrounds.

Figure 12: Grower responses indicating in what form they would like to receive information about vibrio and related research.

For the source of information, growers did not have a strong preference for receiving vibrio information from a particular person (Figure 13). In fact, the responses seem to value a
variety of sources, as there were no options that were chosen zero times. The most frequently chosen option was DES staff, which may reflect the trust growers have for this agency as a source of information. Next was the East Coast Shellfish Growers Association, indicating value for receiving information from others in the industry, and then UNH professors, demonstrating value for receiving information from scientists. These results suggest the growers do not want one person to distill all vibrio information for them, but rather would prefer to gather information from multiple sources themselves.

Figure 13: Grower responses indicating from whom they would like to receive information about vibrio and related research.
Discussion

With pathogenic *Vibrio parahaemolyticus* strains ST36 and ST631 detected in Massachusetts but not yet in New Hampshire or northward, New Hampshire holds a unique geographic position with regard to food safety. To prevent foodborne illnesses, the state and oyster industry want to prevent those strains from establishing in Great Bay. This is a challenging objective as human activity and climate change continue to alter conditions in Great Bay.

The state has targeted possible *V. parahaemolyticus* transport mechanisms by managing the importation of oysters with the new importation policy. Growers’ responses to the three statements about the importation policy shed light on the priorities of oyster growers in this situation. Some growers agreed that the importation policy conflicted with their business interests, and about half of the growers said they would be doing something different if the policy didn’t exist, primarily obtaining seed differently. However, only one respondent disagreed that the policy was a sensible step for New Hampshire. This suggests that even though the importation policy brought some limitations, those growers who felt limited may prioritize food safety and still think the policy was a good idea. If this is true, the growers may be willing to accept the limitations in order to gain the prioritized goal of preventing new vibrio strains from establishing in New Hampshire. This would align with the importance placed on a good reputation as necessary for oyster marketing.

The vibrio situation is complicated by climate change. The growers thought New Hampshire’s management of vibrio risk, including the importation policy, was effective. However, most of them also thought climate change could impact food safety in their product. Specifically, they mentioned that warmer water could bring more vibrio to New Hampshire.
Research in other locations has documented poleward range expansions for *V. parahaemolyticus* (McLaughlin et al., 2005) and has associated vibriosis illness with unusually warm waters (Baker-Austin et al., 2013, 2016; Martinez-Urtaza et al., 2008, 2010; Vezzulli et al., 2016b). If Great Bay waters warm and total *V. parahaemolyticus* abundance increases, this does not necessarily mean pathogenic strains will increase in equal proportion; the dynamics between total *V. parahaemolyticus* and pathogenic *V. parahaemolyticus* is an active area of research. However, if *V. parahaemolyticus* abundance increases in New Hampshire, food safety policies may need to be modified accordingly. The state has taken precautionary steps with the importation policy, and has made the policy flexible enough that it can be changed or removed as conditions change. Growers’ involvement in forming the importation policy, combined with their awareness that climate change could impact their industry, sets them up to be ready to be involved in management discussions surrounding climate change. Their use of non-required ice slurry demonstrates their dedication to protecting food safety and maintaining the disease-free reputation of New Hampshire oysters.

Moving forward, researchers at UNH can use the data from this survey to inform the ways we communicate our research to stakeholders. Unfortunately, the growers did not have a single obvious preference for how they would like to hear about our work; however, this lets us know that we, as UNH researchers, are just one source out of many that growers use to understand vibrio. For regular updates on our research, the survey results show that an update email or a presentation or fact sheet at the annual meeting would be preferred by the growers. From the interview data, we know that growers trust state agencies as a source of information and utilize meetings with DES to stay informed about important events; this suggests that using
DES as a point of contact for pressing research concerns or unusual vibrio conditions would be an effective way to reach all the growers at once.
4. MICROBIOLOGY RESULTS AND DISCUSSION

4.1 Results

*V. parahaemolyticus quantification*

*Establishing lab procedures*

There was a total of 36 samples analyzed for *V. parahaemolyticus* concentration in MPN/g. On the first sampling date, extra samples of juvenile oysters were collected to find out if there were any significant differences in *V. parahaemolyticus* concentrations based on the number of animals (i.e. tissue biomass) in the sample. The purpose of this investigation was to inform the number of oysters that would be used for analysis on subsequent sampling dates. The goal was to be able to cost-effectively minimize the number of animals used without negatively impacting the detection ability of MPN analysis. A one-way ANOVA on natural-log transformed data determined that, although the MPN concentrations for the 24-animal samples were lowest, there was no significant difference in *V. parahaemolyticus* MPN between samples of 24, 36, and 48 oysters (Table 3, Figure 14).

<table>
<thead>
<tr>
<th>Animals in Sample</th>
<th>Geometric mean <em>V. parahaemolyticus</em> concentration, MPN/g</th>
<th>Standard deviation</th>
<th>p-value</th>
<th>Average meat mass per oyster, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>19</td>
<td>47</td>
<td>0.1612</td>
<td>0.07</td>
</tr>
<tr>
<td>36</td>
<td>188</td>
<td>107</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>94</td>
<td>191</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Statistical analyses of *V. parahaemolyticus* concentrations in juvenile oyster samples containing different numbers of oysters.
Figur 14: V. parahaemolyticus concentrations in juvenile oyster samples containing different numbers of oysters.

Data from measurements of juvenile oyster total mass, meat mass, and shell length were used to perform linear regressions. The purpose of these analyses was to inform the number of oysters that would be used for analysis in future research. In order to set up serial dilution tubes for MPN analysis, a certain amount of oyster meat biomass is required to create the correct concentrations. When using samples of 12 adult oysters there was excess biomass; however, the juvenile oysters were so small that there was risk of running out of biomass and being unable to create the higher concentration dilution tubes. Understanding the relationships between different oyster characteristics can help researchers make decisions about how many oysters to use. Additionally, as oyster characteristics often vary by location, the results of this investigation are location-specific for northern New England and can be compared to results from other areas.

The approaches presented here were used as an alternative to the commonly used Condition Index (Lawrence and Scott, 1982) because the small size of the juvenile oysters made measuring cavity volume difficult; the shells often crumbled apart upon opening. Additionally,
the relationship between shell length and meat mass is more practically useful because oysters are often sold graded by shell length. The relationship between meat mass and total mass allows for comparison to previous studies.

First a linear regression was performed on the relationship between meat mass (g) and shell length (mm). Natural log-transformed data were used to build a linear model with $R^2=0.89$ (Figure 15). The coefficients yielded by the model were converted back to the non-logarithmic equation $\text{MeatMass} = 0.0000097329g/mm \times \text{ShellLength}^{3.1298}$, which may be useful to estimate how much meat is present in a juvenile oyster of a known shell length. In Figure 16, this equation is graphed over the non-transformed data to demonstrate the relationship.
Additionally, a linear regression was performed on the relationship between total oyster mass (g) and meat mass (g). Natural log-transformed data were used to build a linear model with $R^2=0.96$ (Figure 17). The coefficients yielded by the model were converted back to the non-logarithmic equation $\text{MeatMass} = 0.22216\times\text{TotalMass}^{1.1436}$, which may be useful to estimate how much meat is present in a juvenile oyster of a known total mass. These same measurements were then used for comparison to previous studies.
To compare the current findings to previous research, a wet shell weight (WSW = \text{TotalMass} - \text{MeatMass}) was calculated to run a linear regression. The resulting power equation of $WSW = 0.000094558g/mm \times \text{ShellLength}^{2.7904}$ is somewhat different from the equations found by Southworth et al. (2010) ($WSW = 0.00017324\times \text{ShellLength}^{2.9926}$) and by Mann et al. (2009) ($WSW = 0.002374\times \text{ShellLength}^{2.21}$). When these equations are plotted over the current data, it is clear that the Southworth et al. and Mann et al. equations overestimate WSW for a given shell length (Figure 18). This indicates that these equations cannot be applied universally.
Figure 18: Curves from linear models overlaid on the current oyster data demonstrate differences between this model and models from previous research. The current equation is represented by the black line, Southworth et al. is the red line, and Mann et al. is the blue line.

To present information about juvenile oyster meat mass in a more utilitarian fashion, Table 4a&b summarizes the findings. These values may be useful to a researcher when deciding how many juvenile oysters to use in order to obtain a given amount of meat biomass.

<table>
<thead>
<tr>
<th>Shell length range (mm)</th>
<th>Average meat mass (g)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-20</td>
<td>0.88</td>
<td>0.04</td>
</tr>
<tr>
<td>21-30</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>31-40</td>
<td>0.80</td>
<td>0.36</td>
</tr>
<tr>
<td>41-50</td>
<td>1.77</td>
<td>0.24</td>
</tr>
<tr>
<td>51-55</td>
<td>2.53</td>
<td>0.15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total mass range (g)</th>
<th>Average meat mass (g)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2-0.6</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>0.7-1.0</td>
<td>0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>1.1-2.0</td>
<td>0.38</td>
<td>0.10</td>
</tr>
<tr>
<td>2.1-4.0</td>
<td>0.85</td>
<td>0.28</td>
</tr>
<tr>
<td>4.1-7.0</td>
<td>1.63</td>
<td>0.24</td>
</tr>
<tr>
<td>7.1-10.0</td>
<td>2.46</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 4: Average juvenile oyster meat mass related to shell length (a) and total oyster mass (b).

*Comparison of V. parahaemolyticus in adults and juveniles*

30 oyster samples were used to compare *V. parahaemolyticus* concentrations in adult versus juvenile oysters. The purpose of this investigation was to determine if there was any
difference in *V. parahaemolyticus* concentrations between adult and imported juvenile oysters living at the same farm site post-importation. For each of the 5 sampling dates, 3 adult samples and 3 juvenile samples were analyzed. For the July 20 sampling date, one set of triplicate samples was chosen at random out of the sets that contained 24, 36, or 48 oysters (described above).

For each sample date, one-way ANOVAs indicated there was no significant difference between adult and juvenile *V. parahaemolyticus* concentration (Table 5). As the oysters grew throughout the sampling period, the average meat mass per oyster increased over time for both juveniles and adults. Correspondingly, the average meat mass per sample increased over time as well. The consistent similarity of MPN values indicates that the earlier juvenile samples were not limited for *V. parahaemolyticus* detection ability by inadequate biomass.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Age</th>
<th>Geometric Mean <em>V. parahaemolyticus</em> Concentration, MPN/g</th>
<th>Standard Deviation</th>
<th>p-value</th>
<th>Average meat mass per sample, g</th>
<th>Average meat mass per oyster, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/20/18</td>
<td>Juvenile</td>
<td>19</td>
<td>47</td>
<td>0.5631</td>
<td>1.67</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>37</td>
<td>30</td>
<td></td>
<td>98.66</td>
<td>8.22</td>
</tr>
<tr>
<td>8/6/18</td>
<td>Juvenile</td>
<td>213</td>
<td>812</td>
<td>0.8799</td>
<td>4.58</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>177</td>
<td>202</td>
<td></td>
<td>107.26</td>
<td>8.94</td>
</tr>
<tr>
<td>8/20/18</td>
<td>Juvenile</td>
<td>123</td>
<td>228</td>
<td>0.8481</td>
<td>6.03</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>143</td>
<td>75</td>
<td></td>
<td>111.3</td>
<td>9.27</td>
</tr>
<tr>
<td>9/4/18</td>
<td>Juvenile</td>
<td>135</td>
<td>23</td>
<td>0.4188</td>
<td>12.32</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>71</td>
<td>115</td>
<td></td>
<td>118.19</td>
<td>9.85</td>
</tr>
<tr>
<td>10/15/18</td>
<td>Juvenile</td>
<td>4</td>
<td>3</td>
<td>0.9159</td>
<td>39.02</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>4</td>
<td>4</td>
<td></td>
<td>146.94</td>
<td>12.25</td>
</tr>
</tbody>
</table>

Table 5: Statistical analyses of *V. parahaemolyticus* concentrations in adult and juvenile oyster samples on 5 sampling dates.

In addition to determining differences in *V. parahaemolyticus* concentration between adults and juveniles, seasonal changes in *V. parahaemolyticus* concentration were also tracked.
The *V. parahaemolyticus* concentrations in both adult and juvenile oysters increased from relatively low levels in July (geometric mean 19-37 MPN/g) to higher levels in August (geometric mean 123-213 MPN/g), and then declined through September to much lower levels in October (geometric mean 5 MPN/g). Viewed holistically, a two-way ANOVA showed no significant difference in *V. parahaemolyticus* MPN between adult and juvenile oysters (p=0.9814), and no significant interaction between the effects of age and sample date (p=0.8748). There was a significant difference in *V. parahaemolyticus* MPN based on sample date (p<0.0001), and Tukey’s post-hoc test indicated further differences based on sample date (Figure 19). Since water temperature is known to be one among several environmental factors that influence *V. parahaemolyticus* concentration in oysters, Figure 20 depicts the water temperature at collection time. Notably, the much lower temperature in October corresponds with the lower MPN values recorded for the October samples, suggesting similar seasonal patterns in *V. parahaemolyticus* concentration between adult and juvenile oysters.
Figure 19: *V. parahaemolyticus* concentrations in adult and juvenile oyster samples on 5 sampling dates. Dates not connected by the same letter are significantly different.

Figure 20: Water temperature at the time of sample collection.

Analysis of covariance (ANCOVA) was performed to determine if the relationship between water temperature and *V. parahaemolyticus* MPN differed between adult and juvenile oysters. This was tested using temperature at sample collection, average temperature over the
previous 24 hours, maximum temperature over the previous 24 hours, and minimum temperature over the previous 24 hours. For all of these tests, significant p-values for the effects of temperature indicated that MPN was affected by temperature. Insignificant p-values for the effects of age and for the interaction between the effects of age and temperature indicated that the relationship between temperature and *V. parahaemolyticus* MPN was similar for adult and juvenile oysters. To illustrate this, Figure 21 depicts this test for temperature at collection. The effect of water temperature at collection was significant (p<0.0001), and the effect of age (p=0.4126) and the interaction between the effects of age and temperature (p=0.8442) were insignificant.

![Figure 21: Relationship between water temperature and *V. parahaemolyticus* MPN for adult and juvenile oyster samples.](image)

A total of 222 positive *V. parahaemolyticus* isolates were collected in this study. Each of these was analyzed for presence of pathogenicity marker genes *trh* and *tdh* to investigate whether there were differences in the proportions of potentially pathogenic *V. parahaemolyticus* strains in adult and juvenile oysters. There were two isolates that were positive for both genes. Both
isolates came from samples of adult oysters, one from the July 20 sampling date and one from October 15.

**16S microbial community analysis**

24 samples of oyster homogenate were used for microbial community analysis. The purpose of this investigation was to understand differences between the microbiomes of adult and juvenile oysters, observe changes over time, and determine if the juvenile oyster microbiomes became more similar to the adult oyster microbiomes after they were imported to live at the same farm site. After 6 samples were removed from analysis due to low sequence counts, there were 10 juvenile oyster samples and 14 adult oyster samples. The filtered data used for analysis contained a total of 410,664 trimmed, high quality sequences. These represented 4,004 unique features that were assigned to 748 different taxonomic identifications through the species level.

Relative abundance describes which taxa were dominant in the oyster microbiomes. The relative abundance of taxa is presented at the phylum level (Figure 22), including identified phyla that represented >1% of sequences present, as others have done in similar studies (Arfken et al., 2017; King et al., 2019). For this figure, data from samples of the same group (defined by age category and sample date) were averaged.
Figure 22: Relative abundance of identified phyla for adult and juvenile oyster samples. Phyla that represented <1% for all categories were excluded.

When averaged across all the oyster samples, the phyla with the highest average relative abundances were *Proteobacteria* (37%), *Spirochaetes* (15%), *Tenericutes* (9%), and *Cyanobacteria* (8%). The phyla with lowest average relative abundance represented less than 1% (*Fusobacteria*, *Acidobacteria*). However, differences emerged when the adult and juvenile oyster samples were analyzed separately. The largest difference was in the phylum *Spirochaetes*, which represented an average of 25% of adult samples and 1% of juvenile samples. There were also differences in *Cyanobacteria*, which averaged 5% of adult samples and 12% of juvenile samples. Additionally, there were differences in average relative abundance in oyster microbiomes from different sample dates. For example, *Proteobacteria*, which was a dominant phylum in all samples, had lower relative abundance in August (26%) and increased thereafter to its highest relative abundance in October (47%). Conversely, *Cyanobacteria* was at its highest relative...
abundance in August (19%) and its lowest in October (2%). Planctomycetes was at its highest average relative abundance in September (14%) while other dates were lower (4-7%). These observations suggest that there were microbiome differences related both to age and to ecosystem-related seasonal changes. Changes in relative abundance across sample dates that were mirrored in both adult and juvenile samples (such as Cyanobacteria, Planctomycetes, and Proteobacteria) suggest that the microbiomes of adult and juvenile oysters were similarly affected by ecosystem factors.

To provide an assessment of overall bacterial community differences, alpha diversity measures compared differences between samples of adult and juvenile oysters and between sample dates. Faith phylogenetic diversity, a measure of richness, was not significantly different between adults and juveniles (p=0.91) or between different sample dates (p=0.49). Similarly, Shannon diversity index, which accounts for richness and evenness of species present, was not significantly different for age group (p=0.11) or sample date (p=0.10). For Pielou’s evenness, there was no significant difference between sample dates (p=0.19), but there was a significant difference between adult and juvenile oyster samples (p=0.01) (Figure 23). Evenness, a measure of how similar in abundance all species in the sample are, was higher in juvenile oyster samples. Together these results suggest there was similar richness of taxa present in the adult and juvenile microbiomes, but there were dominant taxa in the adult microbiomes that were less dominant in the juvenile microbiomes.
Figure 23: Pielou’s evenness for adult and juvenile oyster samples.

Additional statistical methods analyzed differences between groups of samples to find out if age and sample date impacted the microbial community. Using the taxonomic data to the species level, the Bray-Curtis distance metric was used for beta diversity analysis. PERMANOVA results showed significant differences between age groups (p=0.001) and between sample date groups (p=0.002). These results indicate that there were meaningful differences in the microbial communities associated with adult vs. juvenile oysters and with different sample dates.

Principal coordinates analysis (PCoA) visualized these differences by clustering similar samples together. Using Bray-Curtis distances built from taxonomic data to the species level, the PCoA explained 44.1% of the variation in the data. Axis 1 distinctly separated the samples by age (Figure 24a). Axes 2 and 3 separated the samples by sample date, with the most distinct cluster being the October 15 date (Figure 24b). These analyses illustrate differences in the microbial community associated with oyster samples from different age and sample date groups.
Figure 24: Principal coordinates analysis showing clustering by age (a) and sample date (b).
Creation of a biplot as well as differential abundance analysis in the QIIME2 gneiss plugin identified which taxa were driving the clustering in the PCoA. Position along Axis 1, and therefore separation between adults and juveniles, was primarily influenced by the family *Brachyspiraceae*. Separation between sample dates was most strongly influenced by the genus *Synechococcus* on Axis 2, and was also influenced by the class *Mollicutes* and the order *Rhizobiales* on Axis 3.

The two taxa that were most influential in the ordination were analyzed further. Balances (Morton et al., 2017) were used to analyze differences in a target taxon between samples. When using relative abundances, if one taxon increases then all other taxa must decrease; the balance concept is intended to isolate changes in target taxa without changing the rest of the community. The balance uses a calculation to compare the abundance of the target taxon to the geometric mean of other taxa present in the sample. A value greater than zero indicates that the abundance of the target taxon is greater than other taxa, and a value less than zero indicates the target taxon is of lower abundance than other taxa. This method is used to support the findings that *Brachyspiraceae* and *Synechococcus* were driving factors that differentiated the samples.

First, balances for *Brachyspiraceae* were calculated, using a pseudocount of 1 for juvenile samples where *Brachyspiraceae* was not present (Figure 25). *Brachyspiraceae* was present in all adult samples (N=14) but was absent from 4 juvenile samples (N=10), appearing only in the final two sampling dates. *Brachyspiraceae* balance was greater in adult samples than in juvenile samples for all sample dates, supporting the finding that this taxon was a major difference between adult and juvenile samples. However, the increase in the balance in juvenile samples over time suggests an ecosystem influence as the juvenile microbiomes may have been becoming more similar to adult microbiomes.
Next, balances for *Synechococcus* were calculated (Figure 26). Balances for adult and juvenile samples were similar. The balances changed over time, with particularly lower balances on the October 15 sample date. This supports the finding that this taxon was a factor that differentiated all adult and juvenile samples by sample date.
4.2 Discussion

**Comparison of V. parahaemolyticus in adults and juveniles**

The primary question about these data was whether there was any difference in *V. parahaemolyticus* concentration between adult and juvenile oysters, and the data showed no significant difference. On the first sample date, the adult oysters and juvenile oysters were collected from different locations, so their associated *V. parahaemolyticus* levels reflected differences between the oyster hatchery and farm site ecosystems. On subsequent sample dates, the oysters were all living at the same site. If the juvenile data had showed marked differences between the first sample date and subsequent dates, this would probably have reflected changes related to the new habitat. If the juveniles, which happened to have similar MPNs on the first date, then had different MPNs from the adults later on, this may have indicated differences inherent to the age of the animals and how they interact with their environment. However, MPN values for juvenile samples were similar to those for adult samples at all sample dates, and the
first sample date was not significantly different from all other dates. This suggests there are not major differences in the *V. parahaemolyticus* concentrations associated with these samples of oysters of different ages. Whether seed oysters from other areas would have different *V. parahaemolyticus* concentrations than Little Bay adult oysters is not known, but these results are useful to confirm that one permitted source of seed did not have elevated levels of *V. parahaemolyticus*.

Additionally, juvenile and adult oysters responded to seasonal changes in the growing area ecosystem in similar ways. The significant p-value for the effects of sample date on MPN likely reflects the effects of water temperature, and probably to a lesser extent other ecosystem variables, as temperature ranged from about 24°C on the warmest sampling date (August 6) to about 14°C on the coldest date (October 15). Statistical analyses also showed that the relationship between water temperature and MPN was not different for adult and juvenile oysters. This suggests that our understanding of the relationship between water temperature-driven seasonal ecosystem changes and *V. parahaemolyticus* concentration (Cox and Gomez-Chiarri, 2012; DePaola et al., 1990; Johnson et al., 2010; Sobrinho et al., 2010; Urquhart et al., 2016), which we have gathered from data on adult oysters, is an understanding that can be applied to *V. parahaemolyticus* in juvenile oysters as well.

Since this study is contextualized in discussions of public health, information about pathogenicity is important. With only 2 isolates identified as having putative pathogenicity marker genes *trh* and *tdh*, less than 1% of the isolates collected during this study had pathogenicity markers. This is similar to other studies in this region that tested environmental isolates and found low proportions of these markers (Mahoney et al., 2010; Parveen et al., 2008; Xu et al., 2015a). From a management standpoint, it was encouraging that the initial seed
shipment did not have pathogenicity markers, because the juveniles were imported from an approved location that had not detected *V. parahaemolyticus* ST36 and ST631.

Furthermore, the relationships between different oyster measurements are useful for future research. Since *V. parahaemolyticus* analyses require a certain amount of meat mass, researchers need to know how many animals of a given size they will need to use. The differences between the relationships described in the current New England study and those described in previous Virginia studies (Mann et al., 2009; Southworth et al., 2010) suggest that the location and associated growing conditions may affect the relationship between shell length and WSW. This is consistent with previous research using Condition Index that found differences in oyster size characteristics in different locations (Chávez-Villalba et al., 2010; Mercado-Silva, 2005; Volety, 2008). Additionally, the size of the oysters used to study these relationships was different: Southworth et al. studied oysters with shell lengths from 16.5–115.6mm, Mann et al. studied oysters with shell lengths from 30–139mm, and oysters in the current study ranged from 14.1–55.1mm in shell length. The differences in these relationships suggest that the current study is useful for practical estimates of meat mass in New England oysters less than 5cm long, but may not be applicable to oysters in other locations and sizes.

Additionally, this study may inform future research by demonstrating a useful modification for processing small juvenile oysters to enable determination of *V. parahaemolyticus* concentrations. The finding that there was no significant difference in MPN based on the number of juvenile oysters in the sample indicates that the method was not limited in detection ability by the amount of biomass used for analysis. Therefore time and resources can be saved by using small numbers of animals.
The information collected in this study begins to establish our understanding of *V. parahaemolyticus* in juvenile oysters. From historical studies to the present, there is a wealth of literature on *V. parahaemolyticus* concentrations in adult oysters, but virtually no data on juvenile seed oysters. This research is a step towards fuller knowledge of *V. parahaemolyticus* in oyster seed. It is important to acknowledge that this research represents a single location and single season, and due to a limited number of samples should be interpreted as such. The results from this pilot investigation into *V. parahaemolyticus* in oyster seed are informative and can provide a foundation for further research in this area.

**Microbial community analysis**

The eastern oyster microbiomes in this study contained similar taxa found in previous studies, but with some differences in proportion. The samples were dominated by *Proteobacteria*, which is similar to findings from other oyster microbiome studies (Fernandez-Piquer et al., 2012; Green and Barnes, 2010; Lokmer and Mathias Wegner, 2015; Madigan et al., 2014; Trabal Fernández et al., 2014). According to the review by Li et al. (2017b), the most abundant phyla in the oyster microbiome tend to be *Proteobacteria, Firmicutes*, and *Bacteriodetes*. Although all of these phyla were present in the samples, they did not hold the highest relative abundance averaged across all samples; the highest overall average relative abundance phyla in the samples were *Proteobacteria, Spirochaetes, Tenericutes*, and *Cyanobacteria*. Previous studies of eastern oyster microbiota have found high proportions of *Cyanobacteria* in oyster tissues (Chauhan et al., 2014) and dominance of *Mycoplasmataceae* from the phylum *Tenericutes* in oyster digestive tissues (Arfken et al., 2017; King et al., 2012).

The greatest difference in taxa between these samples and previous studies is the phylum *Spirochaetes*. Spirochetes were first observed in oysters over 100 years ago (Certes, 1882), and
are usually associated with the crystalline style in the digestive tract (Husmann et al., 2010; Margulis et al., 1991; Noguchi, 1921; Tall and Nauman, 1981). They are considered widespread but non-essential symbionts of oysters that may play a role in digestion, but this has not been fully explored (Husmann et al., 2010).

In microbiome studies, the phylum *Spirochaetes* has been detected in low abundances in *C. virginica* (King et al., 2012; Taylor, 2017) and in varying abundances in other oyster species (Fernandez-Piquer et al., 2012; Green and Barnes, 2010; King et al., 2019; Lokmer and Mathias Wegner, 2015; Madigan et al., 2014; Trabal et al., 2012; Trabal Fernández et al., 2014; Wegner et al., 2013). Most of the observations in other oyster species are also low abundances, but Madigan (2014) found *Saccostrea glomerata* had 27% *Spirochaetes*; however, these were of the family *Spirochaetaceae*. Many papers that reported finding *Spirochaetes* in oysters identified members of the family *Spirochaetaceae* in the genus *Spirochaeta* or *Cristispira* (Fernandez-Piquer et al., 2012; Green and Barnes, 2010; Husmann et al., 2010; King et al., 2012; Madigan et al., 2014; Trabal et al., 2012). *Cristispira* in particular has been a focus of investigation in oysters. Fewer studies specifically report finding the family *Brachyspiraceae* (King et al., 2019; Taylor, 2017; Trabal et al., 2012). Notably, one of these came from the same estuary as the current study - Taylor (2017) found *Brachyspiraceae* in New Hampshire *C. virginica* oysters, but at a relative abundance of under 3%. In contrast, samples in the current study contained as much as 38% *Brachyspiraceae*.

The spirochetes may explain some of the diversity measures that indicate differences in bacterial communities between adult and juvenile oysters. The majority of reads in the *Spirochaetes* phylum were identified as the family *Brachyspiraceae*. This family made up 5-38% of the adult sample bacterial communities, but only 0-2% of the juvenile sample communities.
This dominance in the adult samples may explain why juvenile samples had higher evenness. This family was also the primary item that influenced separation of adult and juvenile samples in the PCoA.

One of the purposes of this study was to investigate whether the microbiome of the imported juvenile oysters remained different from the microbiome of the adults, or became more similar over time. The significant differences between adults and juveniles evidenced by PERMANOVA suggest that, at least within the time frame of this study, the juvenile microbiomes remained distinct from the adult microbiomes. *Brachyspiraceae* may be the key to this difference, because it was present in all adult samples at high relative abundance, but present in juvenile samples only at the last two sampling dates and even then at very low relative abundance. Previous studies that involved transplanting of young oysters (Trabal et al., 2012; Trabal Fernández et al., 2014) observed changes in juvenile oyster microbiota after they arrived at a new site, and differences at different growth stages. They also reported distinctions between grow-out sites, suggesting that the site influenced the microbiome of the juveniles, as others have reported (Marcinkiewicz et al., 2017). These studies sampled 6-12 months after transplantation, so it is possible that 3 months was not long enough to see the full effects of the new transplantation site in microbiome convergence. However, *Brachyspiraceae* first appeared in the juvenile samples in September and increased in October, and this may be evidence that the juvenile microbiomes were evolving in the transplant ecosystem toward convergence with adult microbiomes.

The observed changes in oyster microbiomes over time probably reflect differences in seasonally variable ecosystem conditions in the growing area. These changes include changing water temperature, which previous studies have found to impact the oyster microbiome (Lokmer...
and Mathias Wegner, 2015; Pierce et al., 2015; Zurel et al., 2011). The October 15 sampling date had the coldest water temperature, and this cluster is most separated from other dates in the PCoA. In particular, *Cyanobacteria* relative abundance varied by sample date, and specifically *Synechococcus* was influential in the PCoA clustering. *Synechococcus* is common in nutrient-rich, near-coastal waters, thrives in warmer temperatures, and is limited by low light (Partensky et al., 1999). These qualities are consistent with the lower levels of *Synechococcus* in October, a time of year with colder water temperature and weaker sunlight than earlier sampling dates. While published data on plankton blooms in Great Bay is lacking, Friedland et al. (2016) reported that the George’s Bank region frequently has fall blooms that are variable, but begin in mid-October. Therefore, this sampling date may have preceded the fall bloom.
5. CONCLUSIONS AND RECOMMENDATIONS

This research reports findings on various elements of food safety management in New Hampshire oyster aquaculture. In the interviews, growers thought Great Bay water quality was safe for their seafood production, but faced issues with elevated nitrogen and eutrophication, and thought their oyster farms made multiple positive impacts on the Great Bay ecosystem. The results also indicated that oyster growers were frustrated by the delay in upgrading Portsmouth’s wastewater treatment facility, but respected the data collection and science used to manage water quality in the region. Growers thought state agency monitoring of food safety was comprehensive and effective, if at times overcautious. They described positive interpersonal experiences working with state agency staff, identifying state agencies as a reliable source of information and individual staffers as trustworthy. They valued the opportunity to be involved in shaping state vibrio policy and hoped for a continued cooperative relationship with state regulators.

In the surveys, growers thought New Hampshire’s management of vibrio risk was good. Most growers thought climate change could impact food safety in their product, and specified that vibrio risk in particular could increase. While not unanimous, most growers agreed that the new importation policy was a sensible step for New Hampshire. Some felt that the policy limited their options for buying seed.

With the microbiology data, procedures were established for processing seed oysters that may be helpful for future research. There was no significant difference in *V. parahaemolyticus* concentration between adult and juvenile oyster samples on any sample date, which supports the importation policy that seed oysters from areas with no history of illness from this region’s major pathogenic strains should not increase public health risks at New Hampshire farms. The
significant difference between sample dates likely reflects the effects of water temperature, which has previously been shown to affect *V. parahaemolyticus* concentration in oysters. Adults and juveniles responded similarly to the onset of colder temperatures. The incidence (N=2) of *V. parahaemolyticus* isolates that had pathogenicity markers was very low and occurred only in adult oyster samples.

From the 16S data, there were significant differences in microbiota associated with adult and juvenile oysters, and between sample dates. The adult samples had higher relative abundance of the spirochete family *Brachyspiraceae*, which had not been reported in such high abundance in this oyster species in prior studies.

In the context of the new importation policy, these findings support the policy as an appropriate precautionary measure. Previously there was essentially no research on *V. parahaemolyticus* levels in juvenile oysters, and this research indicates that *V. parahaemolyticus* can be associated with juvenile oysters in concentrations similar to those in adult oysters. The microbiomes of the adult and juvenile oysters remained significantly different throughout this 3-month study, indicating that imported oysters did not immediately evolve to their new environment or to be like adult oysters. This suggests that if imported animals were carrying bacteria of concern that were not in adult oysters, these bacteria may take time to diminish and indefinitely remain in the juvenile oysters. Further research over longer time frames could illustrate how long microbiome differences persist after importation. Research that compares the microbiome of the oysters to their surrounding water and sediment could illuminate whether bacteria introduced by the imported animal also colonizes its surroundings. Since this study did not identify any pathogenicity markers in juvenile oyster samples, further research is needed to understand the dynamics of pathogenic *V. parahaemolyticus* associated with seed oysters.
From a human dimensions perspective, growers described mostly positive perceptions of food safety management in New Hampshire. Their biggest frustration is on track to be dealt with as the Portsmouth wastewater treatment facility upgrade is scheduled to complete in 2020. Other elements of their experience were quite positive, such as their respect for the data collection and scientific knowledge being used by state agencies, their trust in individual staff members they work with, and their value for being involved in the management process. These human elements are important to the success of the industry and effective minimization of food safety risks. Users perceiving regulation as legitimate and well-informed helps avoid adversarial interactions and fosters cooperation. From New Hampshire’s record of extremely few oyster-associated illnesses, we know food safety protocols are effective; from this research, we see that growers are mostly satisfied with management. This balance can be elusive, and New Hampshire’s ability to achieve multiple goals can be an example for other communities that are looking to develop or improve aquaculture management structures. Further research on other aquaculture communities can determine if the positive elements identified here are also successful in other places, to find common features that lead to effective management of aquaculture.

The multidisciplinary nature of this research allows us to understand food safety management in a more complete way. We can address the effectiveness of management strategies on multiple levels that examine the dynamics of the pathogens themselves as well as the people working to protect consumers from those pathogens. Oyster production is a precise operation, where practices that work in one location do not necessarily work in others; and this is true for the human element too, as each community has unique history, culture, and capacity. Research that incorporates both the biological and human elements helps us understand how multiple factors come together to shape an industry.
New Hampshire’s aquaculture industry has been growing recently, but faces constraints to continued growth. Physical space that is open to aquaculture is limited by the National Estuarine Research Reserve in Great Bay and by water quality classifications that limit areas where harvesting is allowed. The small scope of the managing state agencies and resources dedicated to managing the shellfish industry also limit what they are capable of. For example, New Hampshire does not have the resources available to support relay and depuration activities, which limits the harvest areas farmers can work in. This research adds an additional perspective to this understanding, because growers talked about their close personal relationships with individual staff members in state agencies. This raises questions about what would happen if those staffers left their jobs, whether smooth functioning of the industry depends on this trust, and if similar trust could be built with new staff. If the industry expanded and the bureaucratic support structures expanded correspondingly, this personal connection could be lost, which would diminish some of the positive experiences about working in the industry. New Hampshire’s ability to expand aquaculture agencies is a question in itself, as hiring additional staff would require the state to commit more money to aquaculture.

Overall, this research provides insight into the New Hampshire aquaculture industry and what its future may hold. Collecting baseline data about the industry can help participants make informed decisions in the future. With the results presented here, managers and industry members have knowledge that will give them the flexibility to adapt to changing conditions and continue to support a productive and safe industry.
LIST OF REFERENCES


Vibrio vulnificus in the Coastal and Estuarine Waters of Louisiana, Maryland, Mississippi, and Washington (United States). Appl. Environ. Microbiol. 78, 7249–7257.


Maine Department of Marine Resources What is Aquaculture?


APPENDIX 1: IRB APPROVAL LETTERS

University of New Hampshire
Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

12-Feb-2019

Tosiello, Lia
NREN, James Hall
College Road
Durham, NH 03824

IRB #: 8017
Study: Oyster Grower Perspectives on Aquaculture Policy and Food Safety
Approval Date: 12-Feb-2019

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved the protocol for your study as Expedited as described in Title 45, Code of Federal Regulations (CFR), Part 46, Subsection 1101(b). Approval is granted to conduct your study as described in your protocol.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the attached document, Responsibilities of Directors of Research Studies Involving Human Subjects. (This document is also available at http://unh.edu/research/irb-application-resources.) Please read this document carefully before commencing your work involving human subjects.

Note: IRB approval is separate from UNH Purchasing approval of any proposed methods of paying study participants. Before making any payments to study participants, researchers should consult with their BSC or UNH Purchasing to ensure they are complying with institutional requirements. If such institutional requirements are not consistent with the confidentiality or anonymity assurances in the IRB-approved protocol and consent documents, the researcher may need to request a modification from the IRB.

Upon completion of your study, please complete the enclosed Study Final Report form and return it to this office along with a report of your findings.

If you have questions or concerns about your study or this approval, please feel free to contact Melissa McGee at 603-862-2005 or melissa.mcgee@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

Julie F. Simpson
Director

cc: File
Robertson, Robert
15-May-2019

Tosiello, Lia
NREN, James Hall
College Road
Durham, NH 03824

IRB #: 8087
Study: Food Safety in New Hampshire Oysters
Approval Date: 13-May-2019

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved the protocol for your study as Exempt as described in Title 45, Code of Federal Regulations (CFR), Part 46, Subsection 104(d). Approval is granted to conduct your study as described in your protocol.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the attached document, Responsibilities of Directors of Research Studies Involving Human Subjects. (This document is also available at http://unh.edu/research/irb-application-resources.) Please read this document carefully before commencing your work involving human subjects.

Note: IRB approval is separate from UNH Purchasing approval of any proposed methods of paying study participants. Before making any payments to study participants, researchers should consult with their BSC or UNH Purchasing to ensure they are complying with institutional requirements. If such institutional requirements are not consistent with the confidentiality or anonymity assurances in the IRB-approved protocol and consent documents, the researcher may need to request a modification from the IRB.

Upon completion of your study, please complete the enclosed Exempt Study Final Report form and return it to this office along with a report of your findings.

If you have questions or concerns about your study or this approval, please feel free to contact Melissa McGee at 603-862-2005 or melissa.mcgee@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

[Signature]
Julie F. Simpson
Director

cc: File
  Robertson, Robert
APPENDIX 2: SURVEY QUESTIONS

Default Question Block

Consent form for participation in a research study:

You are invited to take part in a study investigating the management of food safety in New Hampshire oysters. This study is part of a master's thesis being conducted by Lia Tosiello at the University of New Hampshire. The objectives of this study are to document the experiences of industry participants, hear their perspectives on aquaculture management, and identify successes and challenges in food safety management and its future.

On the survey you are being invited to complete, you will be asked to share your opinions and experiences. The survey is designed to take approximately 7-10 minutes and will consist of both multiple choice and open-ended questions. Approximately fifteen individuals like yourself will be invited to take this survey. In the write-up of the findings of this study, the information you provide will remain anonymous.

It is not anticipated that there are risks to you by participating in this study other than those encountered in day-to-day life. However, if you feel any questions are inappropriate or may pose a risk to you or your organization you are encouraged to not answer them. Participation in the study is completely voluntary and you may choose to end your involvement at any time. Upon completion of the project, the research results will be available to you in the form of written reports.

If you would like, you can download the full-text consent form here: Survey consent form.

Click here if you consent to participate in the research study.
Click here if you decline to participate in the research study.

How would you rate the way New Hampshire agencies collectively manage risk of Vibrio-related illnesses from NH shellfish? (Agencies include Department of Environmental Services, Department of Health and Human Services, Fish and Game Department)

Good
Fair
Poor
I don’t know

Comments (optional): 

What practices do you use to minimize Vibrio illness risk in your shellfish?

Do you think climate change could affect food safety in New Hampshire shellfish?

Yes
No
What impacts do you think climate change might have on food safety in NH shellfish?

Are you familiar with the 1/29/18 policy about the importation of oysters?
(It specified that oysters, including seed, could not be imported into New Hampshire from areas that had illnesses caused by certain forms of *Vibrio*).

Yes
No
I don’t know

Please read the following statements about the new importation policy and indicate your level of agreement:

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<th>Statement</th>
<th>Strongly agree</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
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<td>The importation policy conflicts with my business interests.</td>
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<td>The importation policy is a sensible precautionary step for New Hampshire.</td>
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<td>The importation policy is an effective way to prevent introduction</td>
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</table>
of new Vibrio strains into NH waters.

If the importation policy didn’t exist, what would you be doing differently in your business?

Do you agree or disagree with the following statement:
When I receive information about Vibrio research, the research is presented in a way that is easy to understand.

Strongly agree
Agree
Neutral
Disagree
Strongly disagree

In what form would you like to receive information about Vibrio and related research?
(Select all that apply)

Web site
Scientific paper
Email
Social media
Fact sheet at annual meeting
Mail
Presentation at annual meeting

Other (please specify)
I don’t want to receive Vibrio information

**From whom** would you like to receive information about Vibrio and related research? (Select all that apply)

East Coast Shellfish Growers Association
Interstate Shellfish Sanitation Conference (ISSC)
UNH Cooperative Extension staff
US Food and Drug Administration staff
Department of Health and Human Services staff
UNH professors
Department of Environmental Services staff
UNH students

Other (please specify)

I don’t want to receive Vibrio information

Is there anything else you want to share?

Block 1

Thank you for completing this survey! Your responses will help us learn more about management of oyster aquaculture and how to communicate about Vibrio research.
APPENDIX 3: INTERVIEW GUIDE

Farmer Guide – 6-29-16 Final

“Hi, my name is ____. I am a ____ at the University of New Hampshire. Here with me today is ____ a ____ at the University of New Hampshire. As you saw from the email, I am part of a social science project that is researching the development of the seafood industry in New Hampshire. Our findings will contribute to a larger collaborative research project focused on improving the scientific and economic information available to support marine-related businesses and coastal managers in New England. Your input is very valuable and we appreciate you taking the time to talk with us. Your answers will be kept completely confidential.”

Next, provide respondents with a copy of the informed consent form. Explain / discuss the details where necessary, particularly related to recording. Ensure respondents understand that their answers will be anonymous and recordings will only be accessible to research team. Emphasize the use of their input to inform state and local coastal management as a benefit from participation. Be sure to keep signed copy and give an extra copy to those that want one for their records.

“Thanks for your willingness to participate in our study. You will be asked to answer a series of short questions, there is no right answer, please just share your thoughts on each topic. Do you have any further questions before we begin?”

1. First, could you tell me a little about yourself and how you got into oyster farming?
   Probe: Education / professional background / training (degrees), business experience?
   [Don't ask probes on education etc. if can get online prior to interview]
   Probe: Specific role in operation (manager, technician, etc.)?
   Probe: Can you talk a little about how the industry has changed over time?

2. If you had to pick one, what is the best thing about oyster farming?
   Probe: What are some other things you enjoy about oyster farming?

3. Similarly, if you had to pick one, what is the biggest challenge you face as an oyster farmer right now?
   Probe: What are some additional challenges?

4. Do you interact with or meet regularly with other oyster farmers from NH? other states?
   Probe: [If no] Why not?
   Probe: Do you think there is a sense of “community” among oyster farmers? Why/why not?

5. Now switching topics, what do you think most affects consumer’s decisions to buy or eat oysters?
   Alternate phrasing - what do you think are some of the main factors that play into customer's decisions when buying seafood?

6. Could you tell me about your customers [to whom do you sell your oysters]?
   Probe: Sell to distributors? Direct sales to restaurants / stores? Direct sales to individuals?
   Probe: Why do you choose to sell to those customers/buyers? Why do you think they want to buy your oysters?
   Probe: Do you coordinate your sales or partner with other farmers to sell your oysters?
   Probe: Are there market-related (or economic) issues that are challenges for you/your business?

7. Now, what does “sustainable seafood” mean to you?
   Probe: Does it include environmentally sustainable? | socially sustainable? | economically sustainable?
   Probe: Help environment only, communities/economies only or both?

8. Now, overall, how would you evaluate the way government officials manage or regulate the health and safety of seafood?
   Use scale: [Good, Fair, Poor, (DK)]
   Probe: What do you think they do well / not so well? What should be the priorities be in the future?
   *If necessary probe on specific agency performance: e.g. FDA / NH DHHS (Health and Human Services)
   Probe: Do you interact directly with officials managing or regulating seafood safety? (If yes - probe nature of interactions)
   Probe: Are seafood businesses sufficiently involved / informed about efforts to ensure seafood safety?
   Probe: What role should businesses / establishments like yours play in seafood safety efforts?

9. Switching topics a bit, what kinds of scientific or technical information or data is most important for your business [oyster farm]?
   Probe: Technical information about best practices for growing oysters? Shellfish diseases (e.g. Dermo, MSX)
   Probe: Economic or market data about shellfish price, business practices, etc.?
   Probe: Information about water quality - bacteria, pollutants/contaminants?
   Probe: Information about climate change? (E.g. ocean temperature change, ocean acidification)
10. How do you get or access the scientific or technical information that is important for your business [oyster farm]?

Probe: Do you call someone? Go on the web? Get email newsletters?

Probe: Who do you get it from? (If name a person) What organization are they part of? What is it about that person or organization that makes you confident in their information?

Probe: *Ask about interactions with different providers of information? (Were they positive/negative) [e.g. UNH, Sea Grant Extension, state government scientists, federal scientists]

Probe: Are you involved directly in any research or citizen science projects? What helps you work well with researchers and/or citizen scientists? [Define “citizen science project” if necessary]

11. Can you think of examples where scientific data/findings changed a decision you made related to oyster growing or your business?

Probe: What was it about the information that made you change your decision?

Probe: Did you know the person you got it from? Were you involved in the data collection? Were you a member of the organization?

12. Similarly, can you think of examples where you learned about new scientific data/findings but you chose not to make a decision related to oyster farming or your business based on those data?

Probe: What do you think it was about the information that made you not use it? Was it something about how it was shared? Who provided it? What it said?

13. Now thinking about Great Bay, if you heard people saying there are issues with the “WATER QUALITY” in Great Bay, what would you FIRST think of?

14. How would you rate water quality conditions in Great Bay? [Use scale: Good, Fair, or Poor (DK)?]

(*Allow responds to define for themselves what is meant by “water quality”)

Probe: What do you think are the most serious current and future threats to water quality in Great Bay?

15. Similarly, how would you evaluate the way the government, overall, manages water quality in Great Bay? (*Here you can clarify if necessary what is meant by water quality) [Use scale: Good, Fair, or Poor (DK)]

Probe: What do you think they do well / not so well? What should be the priorities be in the future?

Alternate phrasing: Do you think any agencies or towns are stepping out as leaders? Why?; Do you think any agencies or towns are lagging behind? Why?

Probe: What do you think governments could do to better address issues that might impact shellfish?

16. Do you attend meetings or interact with government officials managing water quality in Great Bay?

Probe: How would you evaluate those meetings / interactions?

Probe: If no, why have you chosen not to attend meetings or interact with government officials?

17. Have you heard about or been involved with the debate related to the upgrade of the Portsmouth sewage treatment facility?

Probe: What do you think about how the City approached the problem and made decisions? What information do you think they focused on?

Probe: Do you think the information used to inform these decisions was adequate and/or reliable? If not, what was missing?

Probe: Did you attend any public meetings? (Why? / Why not?) If yes, How would you evaluate them?

Probe: Do you feel oyster farmers have been adequately consulted in this process or that their input is helping inform decision making about the sewage treatment facility?

Probe: Do you feel oyster farmers concerns have been addressed?

18. Finally, what do you think are the biggest challenges for the shellfish industry /oyster industry in the future?

Probe: Locally in NH? More broadly?

19. And similarly, what are the biggest opportunities for the shellfish industry /oyster industry in the future?

Probe: Locally in NH? More broadly?

20. Add follow ups where necessary ...

21. Those are our questions. Is there anything else you’d like to share with us or are there any other issues related to the seafood industry or coastal planning in NH that we haven’t discussed?

22. Are you interested in receiving information about the results of our study? If so, what method/format is best? (one-pagers, scientific papers, summary fact sheets, workshop, call, presentation at professional group?)

“Thank you for your time. We appreciate your input.”
APPENDIX 4: SUPPLEMENTAL TABLES AND FIGURES

Supplemental table 1: Resulting statistics from DADA2 processing of 16S data.

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Supplemental table 2: Resulting statistics from 16S data filtering.

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Supplemental figure 1: Biplot for PCoA.
Supplemental table 3: *V. parahaemolyticus* MPN data

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