

2010

Cooking times and temperatures for safe consumption of Louisiana blue crabs (*Callinectes sapidus*)

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**COOKING TIMES AND TEMPERATURES FOR SAFE CONSUMPTION
OF LOUISIANA BLUE CRABS (*Callinectes sapidus*)**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
Requirements for the degree of
Master of Science

In

The Department of Food Science

By

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December, 2010

DEDICATION

To my family: without their love and support, I would be nothing.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Marlene Janes, for her patience and guidance. She is truly one of the most brilliant people I have been lucky enough to learn from in my academic career and I will always be grateful to her for this opportunity. My committee members, Dr. Beilei Ge and Dr. Lucina Lampila were constant sources of knowledge, support, and levity and I am thankful to have had such amazing scientists help me through this endeavor. I would also like to thank the professors in the department of Food Science for expressing interest and advice in my projects; these professors were open, friendly and very approachable with any question or problem and I found this to be the most important asset of the Food Science department. The almost family-like atmosphere contributed heavily to my success in this project. Furthermore, I would like to thank Mr. Gary Bauer and the entire staff at Pontchartrain Blue Crab, Inc. for their kindness in donating my research materials and LSU Ag Center and Sea Grant for partially funding my project.

In addition, I would also like to thank my lab mates for contributing to such a positive work environment – it was really a pleasure to work with you all. So, a big thank you to: Miguel Gutierrez, Ravirajsinh Jadeja, Reshani Nisansala Senevirathne, Sailaja Chintagari, Shreya Datta, and Jiemin “Dove” Chen for all of the help, knowledge, encouraging words and wisdom. Super special thanks to my “plating buddies” Genevieve Edwards and Danielle Johnson – I’m not sure there are enough thank you’s to express my gratitude for your help. I would be remiss if I didn’t thank my other sources of support: the sushi crew of Lauren Chauvin, Julie Amador, and Tristan Colonius for food therapy and sanity; Alisa Todd, for letting me complain endlessly and for always keeping our goal in focus; David Lando for online Zen; Dr. Jack Losso, for all of the little details necessary to be a successful food scientist and human being; Mrs. Laura Savage and Mrs.

Stephanie Jones, for always helping me with the confusing travel forms; Mrs. Terri Gilmer and Mrs. Bonnie Mann, for all of their help and humor and for the temporary Danielle loan. Dr. Witoon Prinyawiwatkul and his lab for letting me use their spectrophotometer. Lastly, Drs. Robert and Mary Grodner – it was truly an honor to receive such a generous scholarship.

However, none of this would have been possible without the loving support of my family. They believed in me and encouraged me throughout the entire process and it is humbling to know how much they have sacrificed for me to pursue this degree. There are no words to express my gratitude for the gift they have given to me. To my mother, Dolores Dyess for showing me limitless optimism, unfailing love and for Friday night decompression time; to my sister Monica Brittain and my niece Elizabeth Lowe for being my greatest cheerleaders and for always forgiving my negligence as a sister and an aunt; and finally, to my amazing husband Stanley Hazard, who has worn the most hats during the last two years: editor extraordinaire, head chef, computer tech, bodyguard during the late night trips to the Ag Chem building, nurse, and best friend – you give me the strength to be the best I can be. And last of all, God, for well – everything.

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ABSTRACT

Blue crabs (*Callinectes sapidus*) play an important role in Louisiana's economy. The increase in consumer popularity coupled with the ambiguity of cooking instructions available is portentous because of the tendency that some pathogenic bacteria have for sharing the natural habitats of the Louisiana blue crab. While all seafood has the potential of being associated with foodborne illness, blue crabs are environmentally exposed to *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Listeria monocytogenes* and *Salmonella* species. This study was designed to determine the least amount of time and temperature needed to reduce or eliminate each of the aforementioned bacteria from a single Louisiana blue crab with either boiling or steaming heat treatments. Once the single crab heat treatment studies were completed, the bacteria that showed the greatest thermal resistance, *Listeria monocytogenes*, and the bacteria most associated with foodborne illness in Louisiana blue crabs, *Vibrio parahaemolyticus*, were inoculated into a serving size of crabs and subjected to heat treatments. The results were based on the amount of bacterial log reduction of each heat treatment time point. The recommendations for safe cooking times were determined by the abundance of below detection limit or non-detectable level results for each bacterium tested and the temperature was determined by the lowest temperature needed to achieve these conditions. Results of the heat treatment experiments were: boil one crab four minutes and cool one additional minute for an internal temperature of at least 79.5° C and a total cooking time of five minutes; steam one crab for five minutes and cool two additional minutes for an internal temperature of at least 57° C and a total cooking time of seven minutes; boil four crabs for 10 minutes and cool five additional minutes for an internal temperature of at least 85° C and a total cooking time of 15 minutes; steam four crabs for 15 minutes and cool five additional minutes to reach an internal temperature of at least 85° C with a

total cooking time of 20 minutes. These results will be presented to consumers as easy, concise instructions for safe preparation of Louisiana blue crabs.

CHAPTER 1 – INTRODUCTION

Louisiana blue crabs (*Callinectes sapidus*) are gaining popularity as a consumer product. Formerly, Louisiana blue crabs were intimidating Gulf Coast delicacies that were only prepared by trained chefs or experienced locals. Now, through the reliability of shipping, blue crabs are rapidly becoming one of the most profitable industries for Louisiana fisheries [3]. Louisiana is the number one blue crab supplier in the country, with crab fishermen landing approximately 40 million pounds per year [4]. Primarily, Louisiana blue crabs are shipped domestically to restaurants. In 2001, Louisiana was the largest contributor of blue crabs to the U.S. market at 22% with North Carolina coming in second at 17% [5]. In 2009, Louisiana Department of Wildlife and Fisheries stated that the blue crab industry had “a total economic effect of more than \$290 million for the state of Louisiana” [6]. This industry significantly contributes to Louisiana’s state economy and is on the rise due to declining populations of blue crabs in the Chesapeake Bay area and the extensive natural estuarine habitat that is unique to Louisiana’s coastal area [5].

While blue crab habitat exists along the coastal areas from Nova Scotia all the way down through South America, there are specific environmental requirements that are needed for blue crabs to thrive [7]. Blue crabs require estuaries with their nearby wetlands to complete their life cycles. Louisiana has a great deal of land that is classified as estuarine – approximately 40% of all of the wetlands located in the lower United States are in Louisiana [8]. An estuary is a coastal region where saltwater ocean and freshwater river meet and mix. In additions, estuaries provide a unique eco-system that is rich in nutrients and has several regions of water with different concentrations of salinity, which is necessary for a blue crab to complete its life cycle [5, 9]. This

gives Louisiana crab fishermen the means to create a profitable and sustainable blue crab industry.

Since the blue crab industry is so important to Louisiana's economy, it is necessary to protect the consumers' confidence in the product. It is difficult to regain a consumers' trust in a product once an outbreak of foodborne illness is associated to it. Every food product has the potential for pathogen contamination and seafood is no exception. In general, Louisiana blue crabs are no more prone to bacterial contamination than shellfish in any other region of the country [10]. Autochthonous or naturally occurring bacteria that can sometimes be pathogenic to humans, such as some members of the species *Vibrio*, simply co-exist in the same habitat as blue crabs [10-17]. Blue crabs are omnivorous and can bio-accumulate bacteria in their gills as well as having it on their shells from the environment – they are basically surrounded externally and internally with bacteria [12, 13, 18-22]. Since there is not much that can be done about changing estuarine microbial ecology without major biological ramifications, it is much easier to give consumers safe preparation instructions to eliminate bacterial flora [23].

After undertaking this project, the problem that became apparent after researching the background and epidemiology of foodborne illness associated with Louisiana blue crabs was: people were still occasionally becoming ill after eating blue crabs although the crabs are always consumed cooked [15, 24-26]. The two main causes of foodborne illness of any etiology are cross-contamination and improper handling [10, 27]. Certainly, cross-contamination could be a contributing factor, as it is in any study involving foodborne illness but investigations into these outbreaks could not exclude improper preparation as the causative reason [10, 23, 27]. Yet, trends are emerging that show private residence as a major location for foodborne illness [28, 29]. For example, in 2007, the greatest number of bacterial-associated foodborne illnesses

investigated by the CDC was the private residence location not the restaurant/deli location [28]. In addition, approximately 19% of foodborne illness outbreaks reported during 1993-1997 were in private residences [22]. These factors led to the conclusion that natural bacterial contaminants and private residence were going to play major roles in this study.

The next avenue to investigate was preparation instructions for blue crabs. This is where there is a distinct lack of information. Cooking times and temperatures provided by the Food and Drug Administration (FDA) were not applicable because this information was oriented toward industrial settings. General advice is abundant online but vague and this is where the problems are originating [17]. Cooking instructions available to consumers typically include “cook until red” or “crabs will be red...” [30, 31]. A key factor that most instructions are missing is the quantity of crabs added to a boiling pot or a steamer. This is crucial, as overloading the cooking apparatus with “a batch of crabs” is a significant issue influencing the time and temperature needed to safely reduce any bacteria present in the blue crabs [17, 32]. There is no way to present safe cooking recommendations without quantifying the amount of crabs to be cooked for that time and temperature.

There is a need for a scientifically proven, consumer-focused study of minimum safe cooking times and temperatures for Louisiana blue crabs [17]. This study was designed to mimic consumer cooking conditions, using the same items available to the average cook. It was also customized to target *Listeria monocytogenes* 1/2b, *Salmonella typhimurium*, *Vibrio cholerae* O1, *Vibrio parahaemolyticus* (tdh+), and *Vibrio vulnificus*; the pathogens which are the most realistic threat to blue crab consumers [17, 21, 29, 33-35]. In addition, it was theorized that no one would go to all the trouble of preparing just one blue crab, therefore a serving size study was added. It was determined that the most heat resistant pathogen, *Listeria monocytogenes*, and the pathogen

most associated with blue crab-linked foodborne illness, *Vibrio parahaemolyticus*, would generate the most useful results for consumers from the serving size study [13, 17, 34]. Lastly, spectrophotometric studies of the changes in the color of a cooked crab to indicate “doneness” were conducted to determine if following the expression “cook until its red”, which is frequently the instructions for cooking blue crabs, was a reliable method to safely cook blue crabs [17, 30, 31]. These studies were intended to produce results that would not only contribute knowledge to the scientific community but also be of practical consumer use as guidelines for safe preparation and consumption of Louisiana blue crabs [17].

CHAPTER 2 – LITERATURE REVIEW

2.1 Introduction

Foodborne disease is a continuing battle in the United States. It was conservatively estimated in 1999 by Mead et al. that foodborne illness caused approximately 76 million cases of illness, 325,000 hospitalizations, and 5,000 deaths per year [36]. In 2001, Rose et al. stated that “Foodborne diseases may be one of the most significant contemporary public health problems, not only because of the large number of cases reported and the associated economic costs, but also because many of the causative agents are newly recognized.” [12]. Recent sensationalized reports have listed the cumulative costs of foodborne illness to be \$152 billion dollars per year [37]. This is more than the earlier estimated \$35 billion dollars per year [36]. These may sound like outrageous estimates but the total cost of foodborne illness is very expensive. The absolute economic impact of foodborne illness would probably exceed the \$152 billion estimate if all costs were considered, such as the amount spent on researching foodborne pathogens or the actual cost of a product recall. These are relatively new issues to the public as we become more aware of the realities caused by foodborne illness due to heightened media coverage.

The Center for Disease Control (CDC) is the governmental body responsible for monitoring and investigating foodborne illnesses. The CDC listed a total of 25,659 cases of foodborne disease outbreaks in 2006 with 6,872 (23%) suspected or confirmed cases due to bacteria [29]. Although this does not sound considerable, it is still a significant figure that is showing a trend toward consistency as seen represented in Figure 1[2]. In general, while viruses caused more foodborne illnesses, bacterial infections associated with foodborne illnesses were more likely to require hospitalization or result in death (Figure 2) [34].

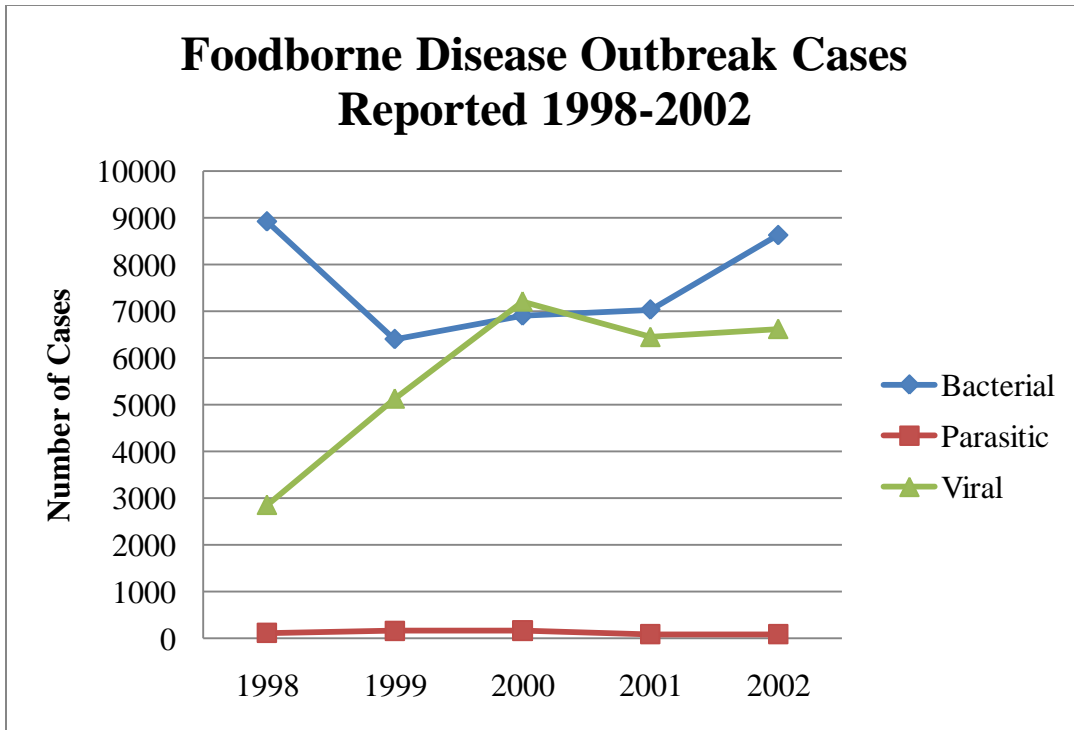


Figure 1: Graphical representation of foodborne disease outbreak cases sorted by etiological causes as reported by the CDC [3]

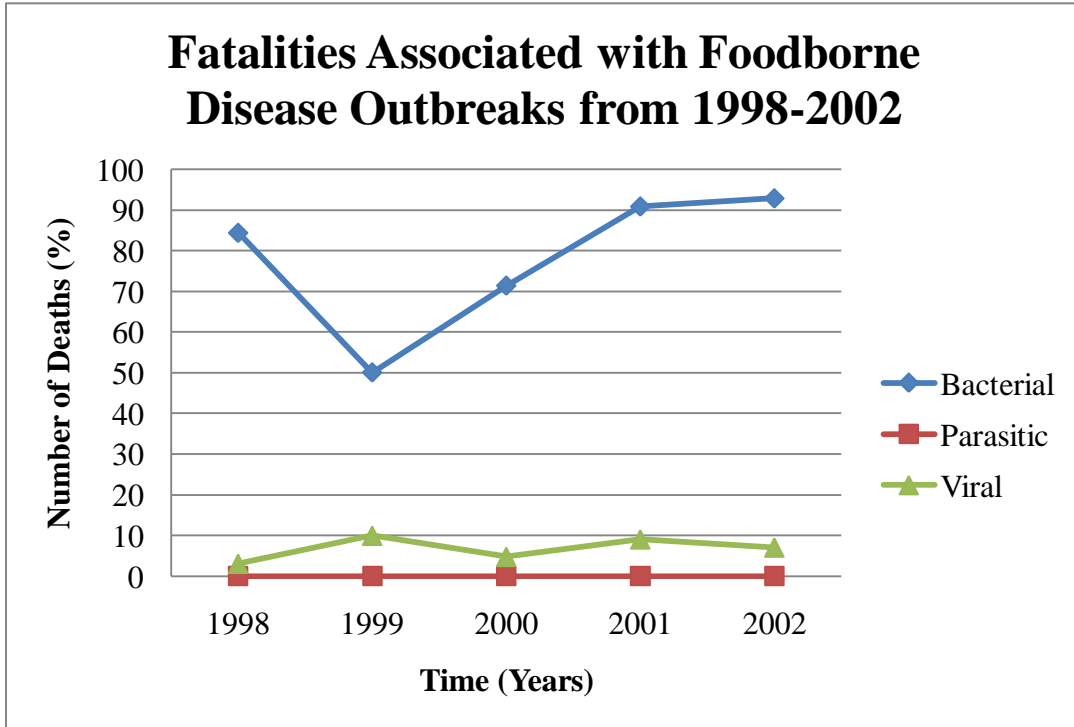


Figure 2: Graphical representation of fatalities associated with foodborne disease outbreaks sorted by etiological causes as reported by the CDC [3]

Crustaceans contaminated with bacteria are often the culprit in foodborne disease outbreaks. Many of these cases are due to improperly prepared or mishandled shellfish [2]. In 2006 alone, the FDA issued six warnings regarding shellfish consumption including a seizure of 31,800 pounds of improperly refrigerated crabmeat [38]. Many of the detailed investigations of bacterial-related foodborne illnesses have focused on oysters, since they are often consumed uncooked [34]. However, a significant number of bacterial foodborne disease outbreaks cases are caused by eating crab (Figure 3). There is usually no apparent reason for crabmeat to cause foodborne illness, since it is typically cooked before it is consumed [24]. That is why a detailed investigation into the cooking times and temperatures for safe consumption of *Callinectes sapidus* or Louisiana blue crabs are necessary, not only for industry but more importantly, for consumers [17].

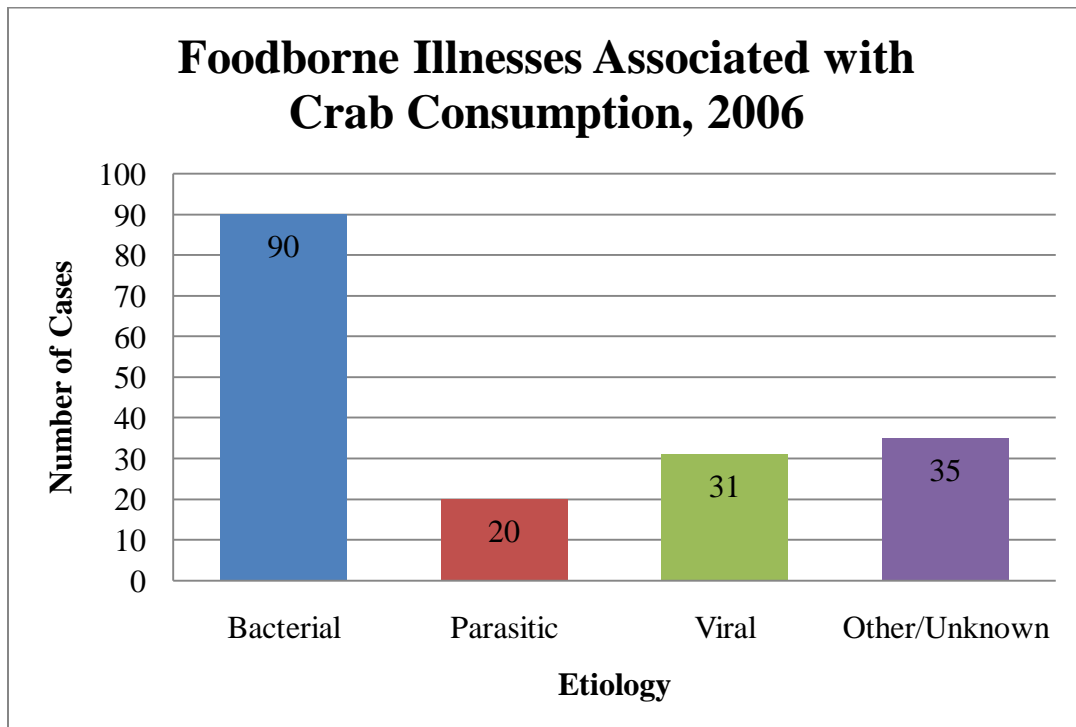


Figure 3: Foodborne illness cases associated with crab for the year 2006 as reported by the CDC [2]

2.2 Outbreaks

Outbreaks associated with the consumption of tainted seafood have been casually documented for many centuries. However, in the early 20th century, some major outbreaks got the attention of public officials. One such outbreak occurred in 1925 on the East Coast. An outbreak of typhoid fever was eventually traced back to oysters contaminated by sewage [39]. It was so severe, that it prompted officials to petition the Surgeon General of the United States to draw up formal guidelines for the safety of the public to replace the loose recommendations the shellfish industry followed at the time. Over time, small outbreaks continued around the country, but there were still many advances in sanitation, hygiene, and general processing that had yet to be discovered or implemented as regular practice. Things started changing in the 1970's with advances in fields such as bacteriology and microbiology. For example, in 1978, when over 1,100 people became ill with *Vibrio parahaemolyticus* at a shrimp dinner in Port Allen, LA, a thorough investigation of this foodborne disease outbreak was conducted. Upon further examination, it was discovered that the food was grossly mishandled – not only were the shrimp cross-contaminated after cooking, but they were also held unrefrigerated for eight hours in the middle of the Louisiana summer before being served [40]. Those were two extreme examples and since then, federal agencies such as the FDA and the CDC have instituted strict shellfish industry regulations, have developed sanitization practices that minimize cross-contamination, and in general, have tried to ensure safe food handling and consumer safety. However, recent statistics have shown that foodborne disease outbreaks due to shellfish is still a concern for consumers especially in the Gulf Coast states [2].

Bacterial contamination of crab has caused several outbreaks. In 1926, Chicago recorded an outbreak of *Salmonella* in crabmeat [41]. The product was traced back to the original

company where severely unsanitary conditions were discovered. In 1932, there was another outbreak of crabmeat-associated illness along the East Coast [41]. These events, along with the ones previously described, heralded the new regulations for seafood processing. For instance, random inspections and surveillance were instituted along the East and Gulf Coasts to periodically monitor the crab processing plants for compliance with the regulations [13]. These inspections led to better regulations and sanitation guidelines. Currently, conditions in the processing plants are very strict; however, there is still typically at least one outbreak per year that leads to a product recall. Recently, crab-associated bacterial illnesses have typically been caused by either cross-contamination [42, 43] or mishandling, either in private residences or in restaurant/delicatessen settings [2, 44, 45].

For example, ten people became ill with *Bacillus cereus* after eating crab at a home in Florida; eighty people became ill with *Vibrio parahaemolyticus* after eating crab at a restaurant in New York [2]. Also, *Vibrio cholerae* has continued to infect people who eat crabs that were improperly handled, such as the five people from New Jersey in 1991 who became infected with toxigenic *V. cholerae* O1, serotype Inaba, biotype El Tor from eating crabmeat brought back in a suitcase from Ecuador – which happens to be an epidemic area for Cholera [27]. Eleven people in Louisiana in 1978 came down with hemolytic Cholera biotype El Tor serotype Inaba after eating allegedly undercooked crabs [24, 25]. Products commonly mixed with crabmeat are also a vehicle for foodborne illness. Eggs mixed with crab to make crab cakes caused two separate outbreaks of *Salmonella* serotype Enteritidis in restaurants during 2001, a total of twenty cases in both outbreaks, with nine people hospitalized [46].

Outbreaks that are occurring at private residence setting are becoming a very common route of foodborne disease outbreaks and foodborne illness infections. At home, cooks do not

have easy guidelines for preparing crab. Many use the “cook until it’s red” technique [17, 30, 31]. However, this is inadequate, as shellfish usually appear red within ten seconds after the start of cooking [32, 47]. Also, this cooking technique may kill the bacteria, but not the spores or the heat-stable toxins [33], such as in the case of *Clostridium botulinum* or *Vibrio cholerae*, both of which are frequently isolated from the gills of crab [18, 20] . An experiment was performed that showed *V. cholerae* 01 was still isolated from crabs that had been boiled for eight minutes or steamed for twenty-five minutes – the crabs were red, the meat was opaque and firm, yet Cholera was still cultured [24, 25, 48]. The experiment reveals that appearances are deceiving and that proper cooking time and temperatures are necessary since these experimental parameters exceed the typical cooking time done in a home.

With the latest news reports on choosing healthy, low calorie, low fat, low cholesterol, high protein foods to eat, crab and other seafood are becoming a popular item for today’s health-conscious cooks. However, many instructions for cooking crab are complex or ask the cook to use equipment they may not have on hand, like a thermometer that can fit well into a large boiling pot. Simple, concise instructions, such as “bring water to a boil, toss in x amount of crabs, rapidly boil for x amount of minutes, use a different receiving tray for the cooked crab than for the raw crab” are needed for consumer ease. The simpler the instructions, the more likely the home chef will follow them, and therefore, the less likely the chef and guests will become statistics for the CDC.

Many foodborne disease outbreaks are classified as caused due to “contributing factors” such as inadequate refrigeration, inadequate cooking or cooling, inadequate “hot-holding”, and cross-contamination [49]. An actual determination of the contributing factor is often impossible due to a lack of samples of the contaminated food, which is a major problem associated with

foodborne disease outbreak investigation. Because of this, deduction is regularly employed to figure out the best scenario to fit the outbreak circumstances [25]. By the time the victim is displaying symptoms, the food is usually gone – either consumed or discarded [45]. Most bacterial pathogens associated with foodborne illnesses have an incubation time in the gut between six hours and fourteen days. Also, many foodborne illnesses are self-limiting and people only seek medical help if necessary, such as discovering the presence of blood in their diarrhea from a hemolytic strain of a pathogen, like *Vibrio cholerae* 01, for example [10, 50]. Stool and serum samples are usually the only evidence left of the “crime” [10].

CDC and FDA are depending on the hospital staff to recognize an illness as potentially being foodborne and relying on them to gather as much epidemiological information as possible to correlate to the laboratory results. However, with hospitals typically overwhelmed and short-staffed, an epidemiological report may not be a priority [50]. Even with the advances in foodborne disease outbreak surveillance, the general feeling is that all foodborne disease outbreaks are underreported due to incorrect diagnosis at the hospital, failure to report, or any number of factors [37]. In addition, foodborne disease outbreaks are difficult to track since some foodborne pathogens only affect certain populations, such as the immune compromised. Unfortunately, if only one family member becomes ill after a meal, the food is not suspected. Instead, the illness may be attributed to the “flu” or a “bug”. However, there is a need for diligence since the number of susceptible people in our population, such as the elderly and immune-compromised, is steadily growing [12, 22].

2.3 Foodborne Bacteria Associated with Crab

Most bacteria associated with edible crabs are pathogenic. Due to the bacterial populations that co-exist in the same habitat that blue crabs live in, there is little possibility that

there will not be natural, environmental contamination on the crabs [22]. For example, live crabs sometimes have pathogenic bacteria such as *Clostridium botulinum* on their shells and in their gills simply due to the environment they live in [51]. It is hypothesized that blue crabs are consistently associated with foodborne bacteria because they are harvested off coastal areas – making them susceptible to land-based contamination as well as the naturally occurring estuarine bacterial populations [15, 52]. Typically with other seafood, bacteria spoil a product before pathogenic bacteria have a chance to grow in sufficient amounts to make a person ill [33]. On average, the meat of a freshly caught, healthy crab will not have a high bacteria count [52]. Therefore, most contamination of the crab and crabmeat occurs post-harvest [53]. Since crabmeat is a perfect medium for bacterial growth, dangerous concentrations of pathogenic bacteria are easily achieved [43].

2.3.1 *Listeria monocytogenes*

Listeria monocytogenes is gram-positive, non-spore forming, rod-shaped, facultative anaerobe that is ubiquitous in nature and is found in water, soil, feces, and foliage [33, 54]. *L. monocytogenes* has many serotypes associated with it, but it is serotype 1/2b that is associated with crabmeat contamination [35]. *L. monocytogenes* has unique survival properties – it is psychrotrophic (able to grow at refrigeration temperatures) [55], can survive irradiation [56], can grow in a high salt concentration, and is able to survive a wide range of pH's [35]. The greatest threat from *L. monocytogenes* is through ready-to-eat products like processed crabmeat [53]. A study by Farber in 1991 demonstrated that *L. monocytogenes* actually grows better on crabmeat than other seafood [57]. Since it can grow to high concentrations in refrigerated, vacuum packed, ready-to-eat foods that will not be subjected to further processing such as heating, there is a

serious health risk associated with this organism [58]. This has caused some safety and regulation issues, since there is no established infectious dose [35, 59].

L. monocytogenes affects susceptible populations strongly, such as pregnant women [59], or those with pre-existing conditions, like liver disease [22, 34], but otherwise it causes flu-like symptoms or is asymptomatic in non-susceptible populations [57, 60]. According to the CDC, *L. monocytogenes* accounted for the majority of deaths associated with a foodborne bacterial disease from 1998-2002 [2]. In 2005, *L. monocytogenes* had the highest incidence of hospitalizations and “the highest case-fatality rate; 12% of persons infected with *Listeria* died.” [61]. It is still a very hard pathogen to track successfully due to its elusive nature [62]. Another issue in tracking *L. monocytogenes* is the increase in the popularity of ready-to-eat products, which usually are not subjected to further heating before consumption and are the perfect culture “media” for *L. monocytogenes* to grow [53].

2.3.2 Salmonella

Salmonella spp. is a rod-shaped, gram-negative, non-spore forming bacterium [54, 63]. *Salmonella* infection presents as either enteric syndrome, also called typhoid, or as gastroenteritis, which is more common [34]. Many species of *Salmonella* can be found on live crabs because *Salmonella* is present in estuaries. However it is still under debate whether *Salmonella* is in waters because it is a part of the natural marine flora or if it is due to contamination from sewage run-off or just happen to be from both sources [21]. In addition, *Salmonella* is part of the natural flora of humans, so this presents even more difficulty when trying to determine the origin of the pathogen in a food product. When a pathogen is found commonly on humans, surfaces, the environment, and the food product, it is almost impossible to

tell if the contamination is from a processing failure, improper storage conditions, unsanitary workers, or some combination of factors [64].

It is generally believed that *Salmonella* is one of the most underreported foodborne illnesses as the gastroenteritis syndrome does tend to be a self-limiting [22]. Typically, *Salmonella* enters orally and can cause foodborne illness with as few as 15 cells [63]. *Salmonella* is implicated in many foodborne outbreaks involving crab mixtures such as crab cakes. In these cases, it is not clear which component of the crab mixture is responsible for contributing the *Salmonella*. Many outbreaks are due to eggs used as a binding agent in the crab mixture but some have no obvious cause. For example a ten person outbreak in Ohio due to crab cakes, lobster cakes, and crab-stuffed lobster served in a restaurant in July of 2001 was eventually traced back to contaminated eggs. However that same year, another ten person outbreak due to crab cakes served in a Washington, DC restaurant could not be traced to any egg contamination source [46]. Therefore, more investigation into this pathogen should be considered as it is still one of the major foodborne bacterial pathogens active today.

2.3.3 *Vibrio*

Species found in the family Vibrionaceae are gram-negative, rod-shaped or curved rod-shaped, halophilic, non-spore forming, facultative anaerobic bacteria [54]. *Vibrio* spp. has been found to be naturally occurring in temperate estuarine environments, which are commonly found in Louisiana [11, 14, 20, 54, 65]. In addition, 50% of the bacteria found in estuaries were determined to be from the *Vibrio* family [54]. The pathogenic *Vibrio* spp. associated with causing foodborne illness typically present as a gastroenteritis syndrome in people unless they have a prior health condition. People with chronic or underlying health issues, like cirrhosis or

hemochromatosis, for example, are at risk of a more severe pathology caused by a *Vibrio*-associated foodborne illness, such as primary septicemia or necrotizing fasciitis [22, 66].

Vibrio spp. is monitored by one of several specialized CDC surveillance systems. The *Vibrio* program is named COVIS which stands for the Cholera and Other *Vibrio* Illness Surveillance System. It is a multi-state program initiated in 1988 by the FDA and the CDC to work in conjunction with the Gulf Coast States most affected by these pathogens – specifically to monitor the number of *Vibrio* cases occurring in Alabama, Florida, Louisiana, Mississippi, and Texas [67].

2.3.3.1 *Vibrio cholerae*

Vibrio cholerae is a bacterium that many believe to be no longer of any threat, especially to U.S. citizens. Cholera is thought to be an issue for countries with questionable sanitation practices. Unfortunately, there are approximately one to two cases of cholera reported per week in the United States [44]. While many of these cases of cholera were travel-associated to places with endemic cholera outbreaks, it should be remembered that the Gulf Coast has a long history of cholera. The first confirmed case was in Louisiana in 1832 and the last case was in 1873. There were no reported cases of cholera in the Gulf Coast States until 1973. Then a four case cluster of eleven people infected with *V. cholerae* O1 biotype El Tor serotype Inaba was reported in Abbeville, LA in 1978 from eating contaminated crabs that had been boiled between ten and twenty minutes or steamed up to thirty-five minutes [24, 25]. *V. cholerae* has been repeatedly isolated in blue crabs from the Gulf Coast [18]. The Gulf Coast has been a reservoir of naturally occurring environmental toxigenic *V. cholerae* and the crabs harvested from that area remain a risk to consumers, especially during the warmer months [22, 48]. Even as recently as 2005 there

were two confirmed cases of toxigenic *Vibrio cholerae* O1, serotype Inaba, biotype El Tor isolated from a couple from Louisiana after eating locally caught crabs and shrimp [10].

2.3.3.2 *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is the *Vibrio* spp. most associated with blue crabs [13, 68]. Due to the halophilic nature of the *Vibrio* spp., *V. parahaemolyticus* grow very well in the same high salinity habitat necessary for blue crabs to complete their life cycles [12]. Both *V. cholerae* and *V. parahaemolyticus* have been found to bio-accumulate in the gut and gills of blue crabs, most likely due to the omnivorous diet of a blue crab [20]. *V. parahaemolyticus* was recognized as an emerging foodborne illness in 1950 [54]. In 1971, United States experienced its first major *V. parahaemolyticus* foodborne outbreak associated with crabs in Maryland. The outbreak caused approximately 425 people to become ill and was traced to improperly steamed crabs [54, 69]. In 1998, the CDC received a report that thirteen people in Florida became ill with *V. parahaemolyticus* from eating crabs [68]. Eighty people in New York in 2006 were diagnosed with *V. parahaemolyticus* after eating crab in a restaurant [29]. There are approximately 25 different serotypes of *V. parahaemolyticus* being monitored by the CDC [70]. In addition, emerging research has also determined that a specific gene, the *tdh* gene, is responsible for a virulence factor capable of causing the hemolytic syndrome when *V. parahaemolyticus* colony counts were well below the FDA accepted *V. parahaemolyticus* limits [34].

2.3.3.3 *Vibrio vulnificus*

Vibrio vulnificus is considered the most serious of all the pathogenic vibrios because it has been attributed to being the leading cause of seafood-related fatalities [54]. The infectious dose of *V. vulnificus* is only 10^3 bacteria/gram, but luckily, it is one of the more heat-sensitive bacterium and is easily destroyed with proper cooking [14]. Of the thermal death times listed

from the Seafood Network Information Center at UC Davis (<http://seafood.ucdavis.edu/Pubs/pathogen.htm>) the most virulent strains of *V. vulnificus* were much lower than other pathogenic organisms such as *E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* [1, 71]. The danger with foodborne illness associated with *V. vulnificus* is its propensity to progress into severe necrotizing wound infections or fatal septicemia in patients with pre-existing conditions such as hemochromatosis or cirrhosis [22, 58, 66]. Liver disease plays a particular factor in the virulence of *V. vulnificus* due to the availability of free iron in the patient's serum [66]. Of the *Vibrio* cases that occur, *V. vulnificus* has the highest mortality rate: approximately 50% of the cases result in death approximately 48 hours post-consumption [70, 72]. Interestingly, it is very common for only one member of a family to show symptoms of *V. vulnificus* [54]. *V. vulnificus* and *V. parahaemolyticus* are regularly isolated together in crabs sampled for bacterial titers [18].

2.4 Blue Crab Anatomy

“Beautiful, savory swimmer” is the literal translation of the blue crabs’ scientific name *Callinectes sapidus* [7, 73]. Blue crabs have a geographic range from Nova Scotia to South America, as well as France, Holland, and Denmark [8, 9]. Blue crabs do have specific habitat needs to complete their life cycle which Louisiana provides with its extensive estuarine habitat. Mating occurs in brackish waters with lower salinity (approximately <10 parts per thousand), while spawning and maturation occurs in higher salinity waters (ranging from 23 – 33 parts per thousand) in the lower parts of the estuary, closest to the ocean [7, 9, 73].

There are several distinct and immediate ways to differentiate between a male crab and a female crab. A female crab will have bright red tips on their claws, often referred to as “red fingernails” [74]. Or, for the more daring, turning a crab over will display the abdomen. Male

crabs will have a T-shaped apron while a female blue crab has a large apron that covers most of her abdomen [9, 74]. Crabs have several sets of swimming legs that allow them to move very quickly, either away from predators or toward prey [7]. Most notable on a crab are their large and powerful claws. These claws are for fighting, defense, scavenging, and posturing for females [7]. Male blue crabs have been sampled to have greater concentrations of bacterial titers in their hemolymph, possibly due to competition for food or females [18]. Most consumers eat “the claws”, which are more accurately called the chelipeds as this includes the claw (or dactyl) and the arm (the propodus) and the back meat located under the carapace (see figure 4 for detailed blue crab anatomy) [7, 74].

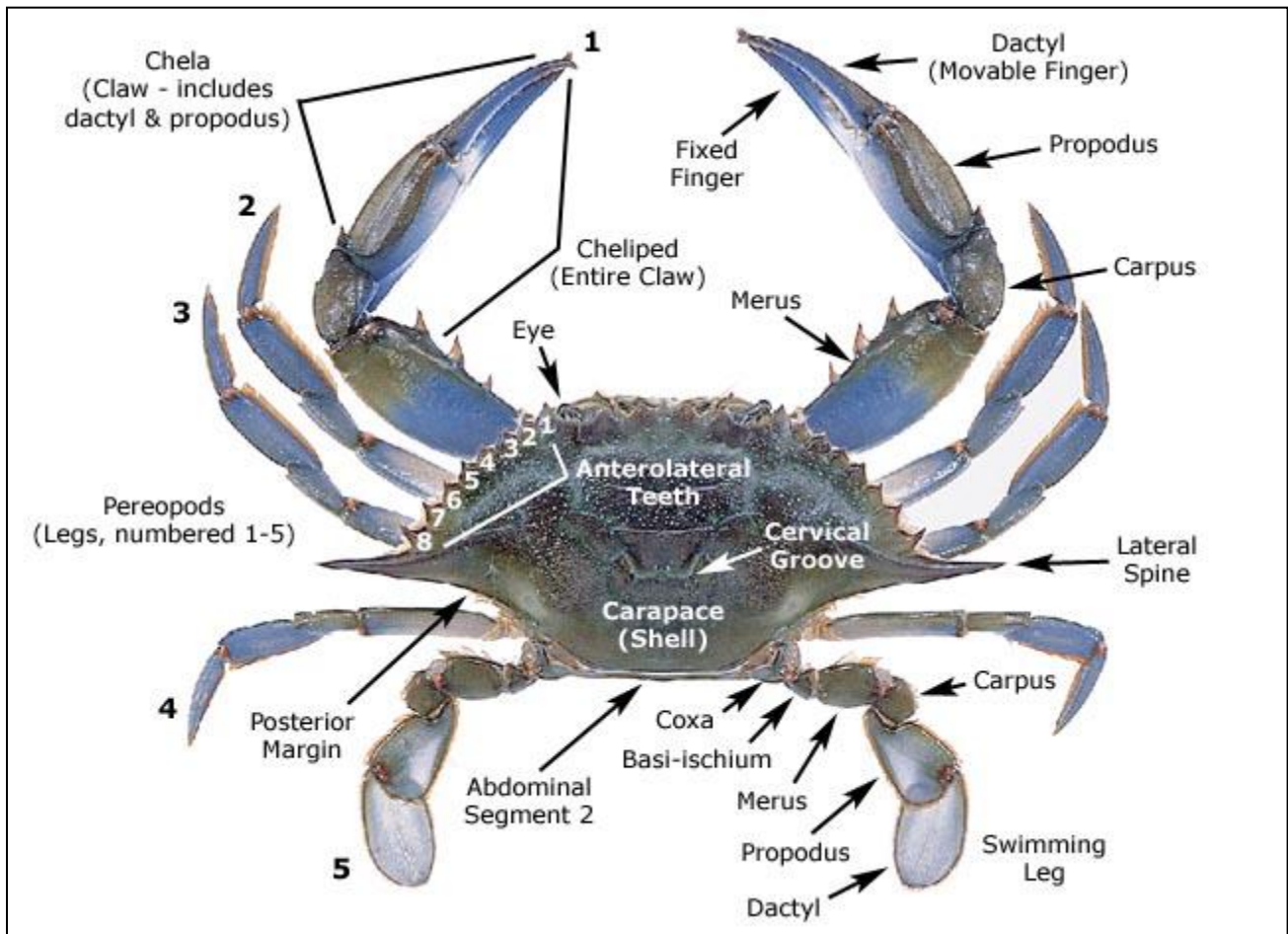


Figure 4: Anatomy of a blue crab (*Callinectes sapidus*) courtesy of Steve Zinski [74]

2.5 Conclusion

Foodborne pathogens are a continuing epidemic in the world. This is not just a problem in exotic locales with questionable sanitation practices; major outbreaks occur yearly in the U.S. despite stringent regulations. Intense research into bacterial foodborne pathogens needs to continue so that outbreaks can be contained as quickly as possible. However, in addition to further research, the information gathered by this research also needs to be disseminated to the public in a user-friendly format that will help prevent common mistakes that can lead to foodborne illness. While there have been extensive scientific studies on thermal death times of different bacteria in crabs, these studies are important in industry, but not particularly useful for consumers since the charts are confusing. It is clear that there is a noticeable deficiency in the literature and translating the data into useful instructions for consumers. For example, most of the foodborne illnesses caused by consuming crabs were due to mishandling on the part of the consumer during cooking or preparation, such as undercooking the crab or allowing the crab to come into contact with raw products after it is cooked [43]. While these basic principles are emphasized in all mandatory retail and industrial courses for safe food handling practices, the average consumer is not likely to be as aware of the dangers of cross-contamination or undercooking a crab. Therefore, as cases of foodborne illness associated with crab continue to escalate in private residences, there is obviously a need for easy-to-follow instructions that are based in science. To cook crabs safely, consumers only want two numbers to remember: the amount of time and the temperature. To satisfy this need, research is required to obtain scientifically-proven times and temperatures necessary to cook blue crabs so that there is minimal bacterial threat and then these results need to be transformed into instructions that consumers would feel comfortable following.

CHAPTER 3 – MATERIALS AND METHODS

3.1 Media

Tryptic soy broth (TSB) consisted of 30 g of tryptic soy broth (Becton, Dickinson and Co., Sparks, MD) and 5 g of yeast extract (Acumedia Manufacturers, Inc., Lansing, MI) in 1 L of distilled water (di H₂O). TSB + 3% NaCl was prepared by making TSB broth with the addition of 30 g of NaCl. Brain heart infusion (BHI) broth was comprised of 37 g of BHI powder (Acumedia Manufacturers, Inc., Lansing, MI) in 1 L of di H₂O. BHI and TSB deep agar slants were prepared by making BHI broth or TSB broth with the addition of 15 g of technical agar (Becton, Dickinson and Co., Sparks, MD). In each liter of di H₂O, vibrio maintenance media (VMM) contained 8.0 g tryptone, 22.5 g NaCl, 4.0 g nutrient broth, 4.0 g technical agar, 4.0 g KCl, and 4.0 g MgCl₂*6 H₂O. Phosphate buffered solution (PBS) had 2.4 g of sodium phosphate monobasic anhydrous, 2.84 g of sodium phosphate dibasic and 8.5 g of NaCl in 1L of di H₂O. Modified Oxford Listeria agar was prepared by adding 57.5 g of Oxford Listeria agar base powder (Acumedia Manufacturers, Inc., Lansing, MI) to 1 L of di H₂O. Once the Oxford Listeria media had been autoclaved and cooled slightly, two vials of re-suspended Oxford Listeria supplement (Acumedia Manufacturers, Inc., Lansing, MI) were added and the now modified Oxford Listeria media (MOX) was mixed briefly before the agar plates were poured. Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar was prepared by adding 88 g of TCBS powder (Acumedia Manufacturers, Inc., Lansing, MI) to 1 L of di H₂O. Xylose Lysine Desoxycholate (XLD) agar was prepared by adding 55 g of XLD powder (Remel – Thermo Fisher Scientific, Lenexa, KS) to 1 L di H₂O. *Vibrio vulnificus* agar (VVA) was prepared by following the U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM) media M190

instructions [75]. All solutions except the cellbiose in the VVA, the Oxford Listeria supplement in the MOX, TCBS, and XLD agars were sterilized at 121° C for 15 minutes by autoclave.

3.2 Bacterial Cultures

All cultures were obtained as -70° C frozen stocks from the Louisiana State University Department of Food Science culture collection. One loopful (approximately 10 ul) of frozen stock was inoculated in nine mL of BHI broth and left overnight or until turbid to incubate at 37° C. Overnight cultures produce approximately $10^7 - 10^9$ amounts of bacteria after 14 hours. After incubation, cultures were streaked onto agar plates and incubated inverted at 35° C or 37° C overnight. Single colonies were picked, stabbed into deep agar slants, left at room temperature and transferred monthly to retain viability.

Cultures of *Vibrio cholerae* O1 (ATCC 14035), tdh+ *Vibrio parahaemolyticus* (ATCC 33847), and *Vibrio vulnificus* (ATCC 27562) were maintained in vibrio maintenance media deep agar slants. *Vibrio cholerae* O1 and *V. parahaemolyticus* were streaked onto TCBS plates and incubated at 37° C while *V. vulnificus* was streaked on VVA plates and incubated at 35° C. Prior to an experiment, one loopful of the maintained *Vibrio* to be tested would be inoculated in 10 mL of TSB + 3% NaCl and incubated at 37° C for approximately 14 hours.

Salmonella enterica serovar *Typhimurium*, or as it is also known, *Salmonella typhimurium* (ATCC 14028) culture was maintained in BHI deep agar slants. *S. typhimurium* was streaked onto XLD plates. Prior to an experiment, one loopful of maintained *S. typhimurium* was incubated at 37° C and then sub-cultured twice in 10 mL of BHI broth at approximately 16-hour intervals.

Listeria monocytogenes ½b (Lm F4260 CDC, Atlanta) culture was maintained in TSB deep agar slants. *L. monocytogenes* ½b was streaked onto MOX plates. Prior to an experiment,

one loopful of maintained *L. monocytogenes* ½b was incubated at 37° C and then sub-cultured twice in 10 mL of TSB broth at approximately 16-hour intervals.

3.3 Sample Preparation

Samples of *Callinectes sapidus* (Louisiana blue crabs) were donated by Mr. Gary Bauer, owner of Pontchartrain Blue Crab, Inc. 38327 Salt Bayou Road, Slidell, LA 70461. Male, medium blue crabs were blast frozen on site overnight at -80° C. Each crab weighed approximately one pound each, based on sorting standards for retail distribution. Crabs were maintained at -20° C and used no later than seven days post-procurement. Two hours prior to experiment, crabs were placed under running water and thawed to approximately room temperature prior to inoculation.

Each crab, except the negative control, was inoculated at 10 points on the claws and body with 100 µL of inoculum at each point, see table 1 for amounts recovered from each experiment. The points were chosen to target the meat typically consumed on a blue crab; the dactyls on each claw to target the propodus, each soft junction of the carpus to target the merus, and six additional points equally distributed in the back meat through a hole drilled into the carapace. After inoculation, an ACR SmartButton® temperature probe was inserted into the hole in the carapace to monitor the internal temperature in one minute increments during heat treatment. This probe is 17 mm diameter x 6 mm height, approximately the size and thickness of a typical watch battery (Figure 5). The hole was sealed with adhesive and the crab was left at room temperature for 30 minutes which allowed the bacteria to acclimate to its surroundings



Figure 5: Side and front view of an ACR SmartButton® temperature data logger.

Table 1: Log amounts of bacteria recovered from the inoculated positive control for each experiment

Positive Control Inoculation Amounts Recovered			
Date of Experiment	Bacteria	Condition	Recovery (log10)
7/15/2010	<i>L. monocytogenes</i>	Single Crab	TNTC
9/8/2010	<i>L. monocytogenes</i>	Single Crab	6.62325
9/16/2010	<i>L. monocytogenes</i>	Single Crab	X
9/17/2010	<i>L. monocytogenes</i>	Single Crab	7.25527
9/18/2010	<i>L. monocytogenes</i>	Single Crab	7.23754
9/19/2010	<i>L. monocytogenes</i>	Single Crab	7.15106
9/17/2010	<i>L. monocytogenes</i>	Serving Size (4) Crabs	7.25527
9/17/2010	<i>L. monocytogenes</i>	Serving Size (4) Crabs	7.23754
9/18/2010	<i>L. monocytogenes</i>	Serving Size (4) Crabs	7.16106
7/12/2010	<i>S. typhimurium</i>	Single Crab	TNTC
7/15/2010	<i>S. typhimurium</i>	Single Crab	6.59835
9/6/2010	<i>S. typhimurium</i>	Single Crab	6.63397
9/12/2010	<i>S. typhimurium</i>	Single Crab	6.82347
9/17/2010	<i>S. typhimurium</i>	Single Crab	7.21272
9/18/2010	<i>S. typhimurium</i>	Single Crab	6.49623
8/6/2009	<i>V. cholerae</i>	Single Crab	3.45092
10/7/2009	<i>V. cholerae</i>	Single Crab	4.51055
11/23/2009	<i>V. cholerae</i>	Single Crab	2.83197
7/6/2009	<i>V. parahaemolyticus</i>	Single Crab	4.12057
8/18/2009	<i>V. parahaemolyticus</i>	Single Crab	4.40140
8/24/2009	<i>V. parahaemolyticus</i>	Single Crab	4.33445
11/9/2009	<i>V. parahaemolyticus</i>	Single Crab	3.84926
5/3/2010	<i>V. parahaemolyticus</i>	Serving Size (4) Crabs	4.68529
5/11/2010	<i>V. parahaemolyticus</i>	Serving Size (4) Crabs	5.45117
6/8/2010	<i>V. parahaemolyticus</i>	Serving Size (4) Crabs	TNTC
8/26/2009	<i>V. vulnificus</i>	Single Crab	2.99563
9/9/2009	<i>V. vulnificus</i>	Single Crab	3.01283
12/16/2009	<i>V. vulnificus</i>	Single Crab	3.74853

3.4 Heat Treatments

3.4.1 Boiling One Crab

In a Tramontina® professional 3-piece 8 quart stainless steel pot (Wal-Mart Stores, Inc., Bentonville, AR) with the steamer apparatus removed and the glass lid on, 5 L (approximately 1 gallon) of tap water was brought to a rolling boil. One inoculated crab was added to the boiling water for a designated amount of time and the lid was replaced. The time points for boiling one crab were from 1 to 6 minutes. After the designated time, the crab was removed and allowed to cool for one additional minute. The carapace is much thicker than the shells of the chelipeds and it is harder for the heat to penetrate to the back meat. Therefore, since the chelipeds cook faster and are easier to pick, it can be assumed that a consumer would start with the claws, so the chelipeds were picked first. While the chelipeds were being picked, residual heat from boiling allowed the back meat to continue cooking. Once the carapace was removed, the ACR SmartButton® temperature probe was removed and sanitized. After the back meat was picked and added to the claw meat, it was briefly homogenized by hand to check for shell debris and to facilitate cooling. All of the meat was then placed in a tared, divided homogenizer bag which was then weighed. After the weight was recorded, an equivalent by weight in volume of PBS was added and the bag was stomached for one minute in a Lab-Blender 400 Stomacher (Tekmar Co., Cincinnati, OH) to simulate digestive break down. The total cooking time after the addition of PBS was noted; assuming that the addition of the PBS has stopped carryover cooking by cooling the meat to room temperature.

3.4.2 Steaming One Crab

Once the single crab boiling treatments were completed, the steaming apparatus that came with the pot was inserted and left to prime for approximately 30 minutes. One inoculated

crab was added to the steamer for a designated amount of time and the lid was replaced. The time points for steaming were from 3 to 9 minutes. The steaming method of picking and homogenizing was the same as for the one crab boiling method.

3.4.3 Boiling Four Crabs (Serving Size)

In the same way as the one crab boiling heat treatment, 5 L (approximately 1 gallon) of tap water was brought to a rolling boil. Four inoculated crabs were added to the boiling water for a designated amount of time and the lid was replaced. The time points of boiling for a serving size of crabs were from 8 to 25 minutes. The serving size boiling method of picking was the same as for the one crab boiling method. Once all of the meat was picked, it was homogenized by hand to check for shell debris and to thoroughly mix all the meat from the four crabs. Subsequent to the manual homogenization of the four picked crabs, 25 g of mixed meat was removed and added to a tared, divided homogenizer bag. An equivalent of PBS was added and the bag was homogenized for one minute in the stomacher. The total cooking time after the addition of PBS was noted; assuming that the addition of the PBS has stopped carryover cooking by cooling the meat to room temperature.

3.4.4 Steaming Four Crabs (Serving Size)

In the same way as the one crab steaming heat treatment, the steaming apparatus that came with the pot was inserted and left to prime for approximately 30 minutes. Four inoculated crabs were added to the steamer for a designated amount of time and the lid was replaced. The time points of steaming for a serving size of crabs were from 10 to 30 minutes. The steaming method of picking and homogenizing was the same as for the four crabs boiling method.

3.5 Enumeration of Bacteria

Under sterile conditions, 1:10 serial dilutions of homogenate fluid in PBS were made and 100 μ L was spread plated in duplicate. Plates were inverted and incubated at either 35° C or 37° C overnight. After 24 hours, colonies were counted. As commonly accepted, only bacterial counts of ≤ 250 colonies were used in the data analysis. An exception was during the serving size experiments with *Vibrio parahaemolyticus* and *Listeria monocytogenes*, bacterial counts of ≤ 1000 colonies had to be included as positive controls. Replicate plates were averaged and used to determine colony forming units/g*mL or CFU and then mathematically transformed to log CFU.

3.6 ACR SmartButton® Temperature Data Logger Processing

Prior to the experiment, the ACR SmartButton® temperature probes were calibrated by inserting them individually into an ACR SmartButton® interface port and removing any residual readings, thus zeroing the data logger. After plating, the sanitized ACR SmartButton® temperature probes were taken to a computer with an ACR SmartButton® interface port. Each probe was individually inserted into the port and the SmartButton® Reader software transferred the internal temperature data accumulated in one minute increments during the heat treatments. This data was then exported into a MS-Windows® Excel compatible format and analyzed for peak temperatures.

3.8 Colorimeter Measurements

Changes in color of the crab carapace after different heat treatments were analyzed using a Minolta Spectrophotometer CM-508d. The colorimeter was calibrated by taking an initial measurement of the ambient surroundings. The colorimeter took three readings of each crab shell, averaged the results, and presented the data as L, a, and b measurements. All values were

recorded. However, only the “a” Value results were analyzed with respect to their position on the red – green axis of color. No further calculations were required to use the measurements. Each blue crab shell was measured immediately after the chelipeds were picked and the carapace of the blue crab was removed to begin picking the back meat.

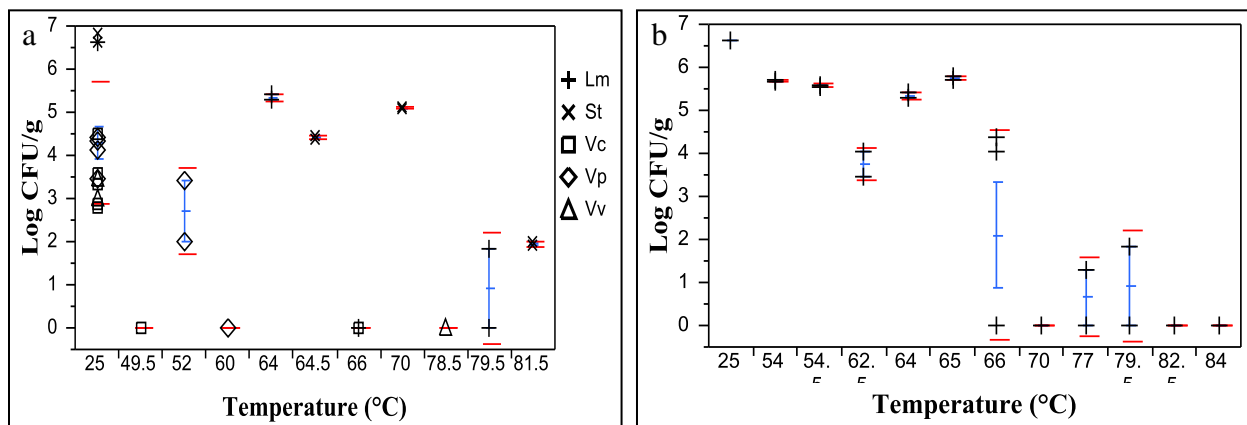
3.9 Statistical Analysis

The heat treatment data and colorimeter data were analyzed using the JMP® 8 statistical analysis software (SAS Institute Inc., Cary, NC). The heat treatment data was analyzed using one-way analysis of variance (ANOVA) with Dunnett’s test to measure each time point against the positive control. The colorimeter data was analyzed using one-way ANOVA method with the Tukey-Kramer ad hoc test measuring the variance of the amount of redness detected after each time point. Statistical significance can be implied with these tests given a p-value of <0.05. All experiments were conducted in triplicate.

CHAPTER 4 – RESULTS AND DISCUSSION

4.1 Boiling One Crab

Results of the single crab boiling experiment showed sufficient bacterial load reduction for all organisms at four minutes and at a minimum internal temperature of 78.5° C (Figure 6a). The bacterial reduction for *Listeria monocytogenes* (Figure 6b) went from 6.6 log CFU to below detection limits at an internal temperature of 77° C at the five minute time point. However, since FDA regulations require zero tolerance for *L. monocytogenes* growth on any cooked or ready-to-eat product, this time point could not be chosen due to the growth of one colony [76]. Therefore, the next time point to reach non-detectable levels was an internal temperature of 82.5° C at five minutes ($p=0.051^*$). Figure 6c shows the reduction of *Salmonella typhimurium* from 6.8 log CFU to non-detectable levels at an internal temperature of 65° C at five minutes ($p<0.0001^*$). For *Vibrio cholerae* (Figure 6d), the bacterial load was consistently reduced from 4.5 log CFU to non-detectable levels when the blue crab's internal temperature reached 58° C at three minutes ($p=0.0005^*$). In Figure 6e, *Vibrio parahaemolyticus* was reduced from 4.4 log CFU to non-detectable counts at an internal temperature of 55° C at the three minute time point ($p<0.0001^*$). Figure 6f shows the bacterial reduction of *Vibrio vulnificus* from 3.4 log CFU to non-detectable levels with an internal temperature of 78.5° C at four minutes ($p=0.0010^*$).



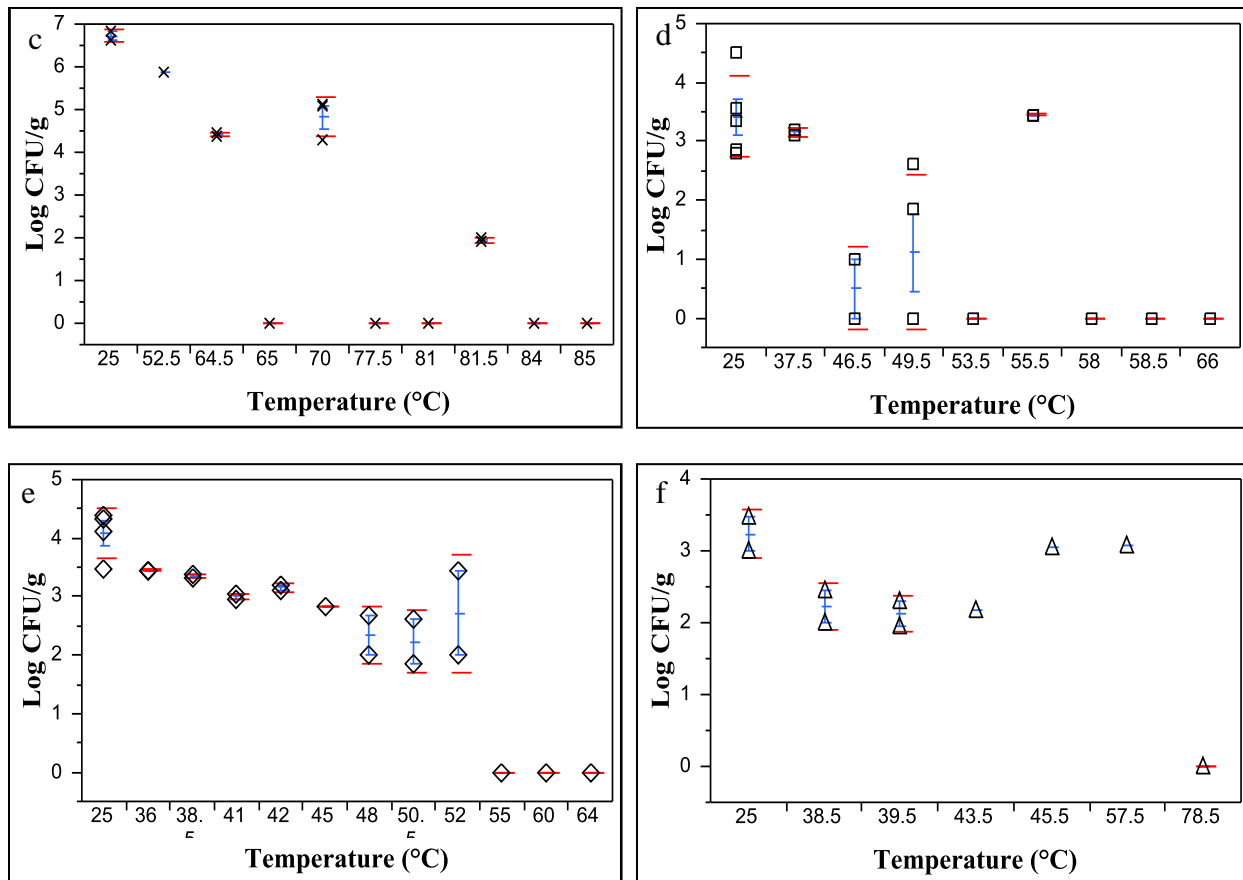


Figure 6: (6a) Results of the single crab boiling experiments showing optimum reduction of all organisms tested at four minutes after reaching an internal temperature of 79.5° C. Time point results of the single crab boiling experiments for: (6b) *L. monocytogenes*, (6c) *S. typhimurium*, (6d) *V. cholerae*, (6e) *V. parahaemolyticus*, and (6f) *V. vulnificus*.

Due to the tendency of *L. monocytogenes* to be thermally resistant [17] the time point that would have been set for boiling a single crab would have been five minutes with an internal temperature reaching at least 70° C. Similarly, the *S. typhimurium* heat treatment experiments required five minutes with the internal temperature reaching a minimum of 65° C for consistent results of non-detectable levels. The meat of a single crab boiled for five minutes is tough and feels rubbery. A contributing factor for this study is the idea that consumers do not want to overcook their blue crabs and would sometimes err on the side of undercooking the seafood rather than serving rubbery crabmeat [17]. Since four minutes boiling with an internal

temperature of 78.5° C was enough to reduce all of the *Vibrio* species tested to non-detectable levels and given that these pathogens are more likely to be the cause of a foodborne illness resulting from eating Louisiana blue crabs [18, 75], this was the best choice as a recommendation for safe cooking times and temperatures for boiling a single crab. With the addendum of an additional minute of cooling time before picking the boiled crab bringing the total heat treatment time to five minutes with carryover cooking, the most likely conditions for safe consumption can be achieved without overcooking the product.

4.2 Steaming One Crab

Results of the single crab steaming experiment showed sufficient bacterial load reduction for all organisms at five minutes at a minimum internal temperature of 57° C (Figure 7a). The log reduction for *L. monocytogenes* (Figure 7b) went from 6.6 log CFU to non detectable levels at an internal temperature of 75.5° C at the eight minute time point ($p < 0.0001^*$). Figure 7c shows the reduction of *S. typhimurium* from 6.8 log CFU to non-detectable levels at an internal temperature of 70° C at eight minutes ($p < 0.0008^*$). For *V. cholerae* (Figure 7d), the bacterial load was reduced from 4.5 log CFU consistently to non-detectable levels when the crab's internal temperature reached 57° C at five minutes ($p < 0.0001^*$). In Figure 7e, *V. parahaemolyticus* was reduced from 4.4 log CFU to non-detectable counts at an internal temperature of 53.5° C at the five minute time point ($p < 0.0001^*$). Figure 7f shows the log reduction of *V. vulnificus* from 3.4 log CFU to non-detectable levels at 46.5° C at four minutes ($p = 0.0005^*$).

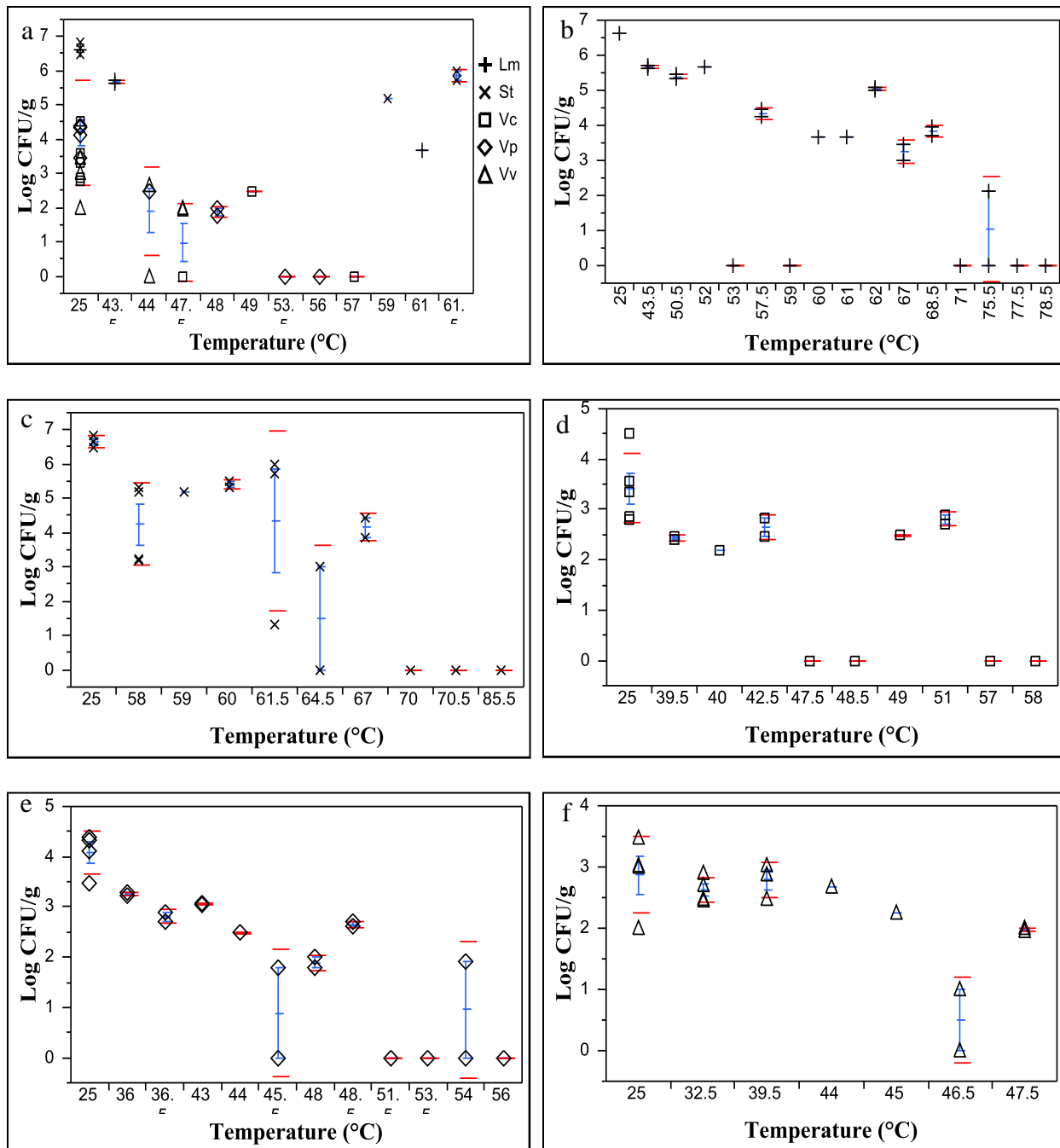


Figure 7: (7a) Results of the single crab steaming experiments showing optimum reduction of all organisms tested at five minutes after reaching an internal temperature of 57° C. Time point results of the single crab steaming experiments for: (7b) *L. monocytogenes*, (7c) *S. typhimurium*, (7d) *V. cholerae*, (7e) *V. parahaemolyticus*, and (7f) *V. vulnificus*.

As with the single crab boiling experiments, *Listeria monocytogenes* and *Salmonella typhimurium* needed extended steaming times to achieve below detectable or non-detectable levels. Both *L. monocytogenes* and *S. typhimurium* required eight minutes of steaming, with the internal temperature reaching a minimum of 77.5° C for *L. monocytogenes* and 70° C for *S. typhimurium*, to reach non-detectable levels. Again, as with the single crab boiling experiments, the extra steaming time made the blue crab meat seem visually overcooked. Both *Vibrio parahaemolyticus* and *Vibrio cholerae* were reduced to non-detectable levels after steaming for five minutes, while *Vibrio vulnificus* only needed four minutes steaming time to reach below detectable levels. Therefore, steaming one blue crab a minimum of five minutes with an additional two minutes of carryover cooking during cooling for a total of seven minutes cooking time will allow the crab to reach an internal temperature of at least 57° C and would produce maximum reduction of bacteria while conserving the desired texture of blue crab meat.

4.3 Boiling Four Crabs (Serving Size Experiments)

Figure 8a shows the results of all the time points tested in the serving size boiling experiments. Inconsistent results because of thermal resistance in *Listeria monocytogenes* caused the time for boiling four blue crabs until bacterial loads were non-detectable to be 20 minutes, with an internal temperature holding at 85° C between 13 and 15 minutes ($p < 0.001^*$). Results for *Vibrio parahaemolyticus* were more consistent with non-detectable levels being reached at 10 minutes and a minimal internal temperature of 82.5° C ($p < 0.0001^*$). Figure 8b shows the results of the 10 minute boiling time point which had non-detectable levels for both *L. monocytogenes* and *Vibrio parahaemolyticus* ($p < 0.0001^*$). Bacterial reduction for *L. monocytogenes* went from 7.2 log CFU to non-detectable levels at an internal temperature of 85° C while *V.*

parahaemolyticus was reduced from 5.5 log CFU to non-detectable levels at an minimal internal temperature of 82.5° C.

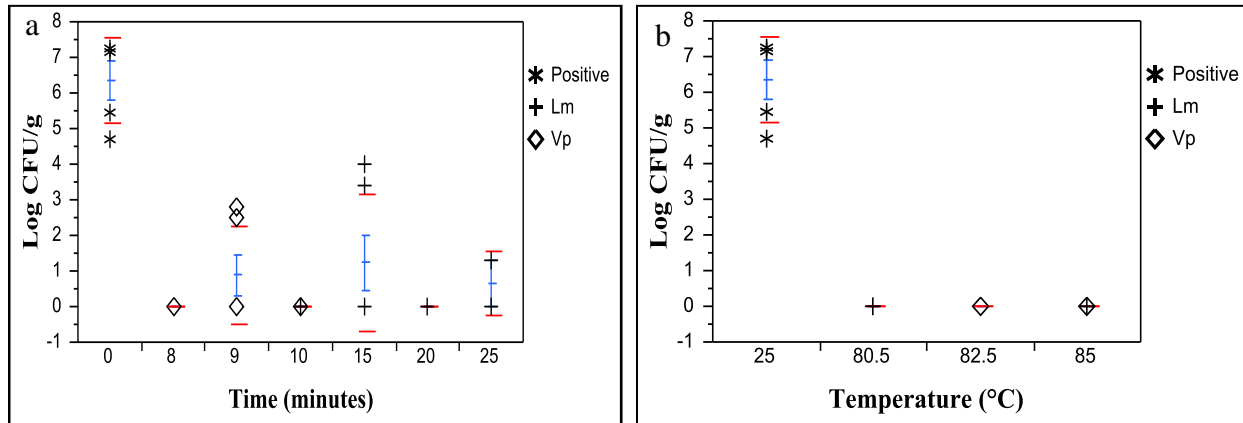


Figure 8: Boiling results for a serving size (four) of blue crabs. (8a) Time point results of four blue crabs inoculated with either *L. monocytogenes* or *V. parahaemolyticus*. (8b) Results of the 10 minute serving size time point which showed optimal bacterial reduction once an internal temperature of 85° C was reached.

While *Listeria monocytogenes* did show significant growth in one experiment at the 15 minute time point after holding an internal temperature of 85° C for six minutes, it also resulted in non-detectable levels in two other experiments after holding an internal temperature of 85° C, once for 8 minutes and once for nine minutes. Figure 8b shows that *L. monocytogenes* attained non-detectable levels twice at the 10 minute mark with internal temperatures of 80.5° C and 85° C from a bacterial load of 7.15 log CFU and 7.23 log CFU, respectively. Therefore, boiling a serving size of four blue crabs for 10 minutes reaching a minimum internal temperature of 85° C, with an additional five minutes for cooling should result in maximum bacterial reduction while conserving the texture and flavor of the blue crab meat.

4.4 Steaming Four Crabs (Serving Size Experiments)

Figure 9a shows the results of all the time points tested in the serving size steaming experiments. The recommended time for steaming four blue crabs inoculated with *Listeria*

monocytogenes was 20 minutes with an internal temperature holding at 79.5° C ($p < 0.001^*$). Bacterial loads of *Vibrio parahaemolyticus* were reduced from an average of 5 log CFU to non-detectable levels at 15 minutes with a minimum internal temperature of 79° C ($p < 0.0001^*$). Figure 9b shows the results of the 15 minute steaming time point which had non-detectable levels for both *L. monocytogenes* and *V. parahaemolyticus* ($p < 0.0001^*$). Bacterial loads for *L. monocytogenes* were reduced from 7.15 log CFU to below detection limits at an internal temperature of 83.5° C while *V. parahaemolyticus* was reduced from 5.45 log CFU to non-detectable levels at an minimal internal temperature of 79° C.

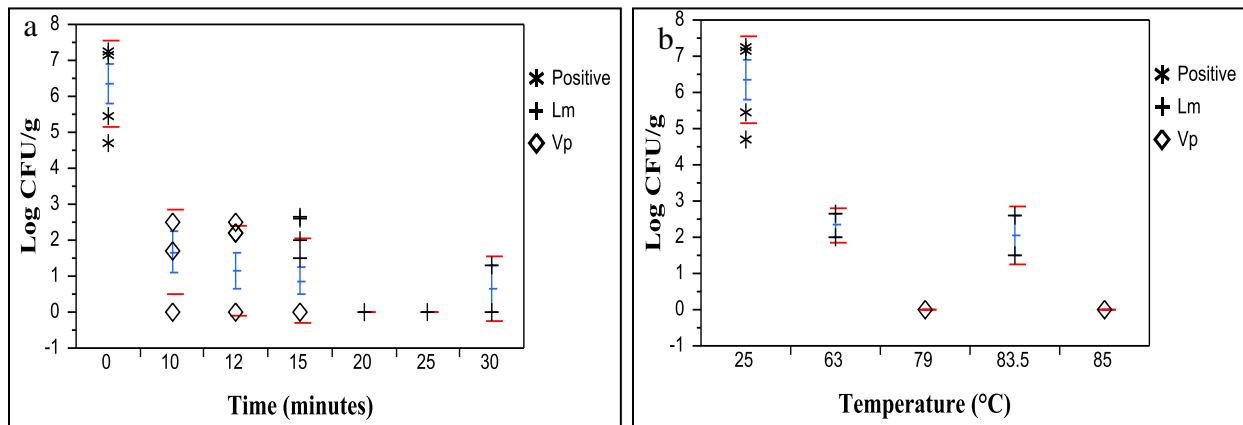


Figure 9: Steaming results for a serving size (four) of blue crabs. (9a) Time point results of four blue crabs inoculated with either *L. monocytogenes* or *V. parahaemolyticus*. (9b) Results of the 15 minute serving size time point which showed optimal bacterial reduction once an internal temperature of 85° C was reached.

Although *L. monocytogenes* was never reduced to non-detectable levels at the 15 minute steaming time point, it was reduced significantly from 7.23 log CFU to an average of 2.3 log CFU after reaching an internal temperature of 63° C in one experiment and from 7.15 log to an average of 2.3 log CFU after reaching an internal temperature of 83.5° C in a second experiment. Figure 9a shows that *L. monocytogenes* grew one colony after steaming for 30 minutes, holding an internal temperature of 85° C for 17 minutes. Therefore, steaming a serving size of four blue crabs for 15 minutes and reaching an internal temperature of 85° C, with an additional five

minutes for cooling, should result in an inferably sufficient bacterial reduction while conserving the texture and flavor of the blue crab meat. Again, the steaming results were more consistent than the boiling method and should therefore be the recommended method of preparation.

4.5 Below Detection Limits/Non-detectable Levels

Finally, the least amount of time necessary for the safest consumption of blue crab was determined by graphing all of the time points that reached below detection level (BDL) colony counts (≤ 10 colonies/plate) and non-detectable (ND) colony counts, where no growth was detected on the agar plates. The graphs were used to see how often these results were achieved on each time point, thereby giving the least amount of time required to consistently reach maximum bacterial load reduction, ergo the least amount of time for safest consumption.

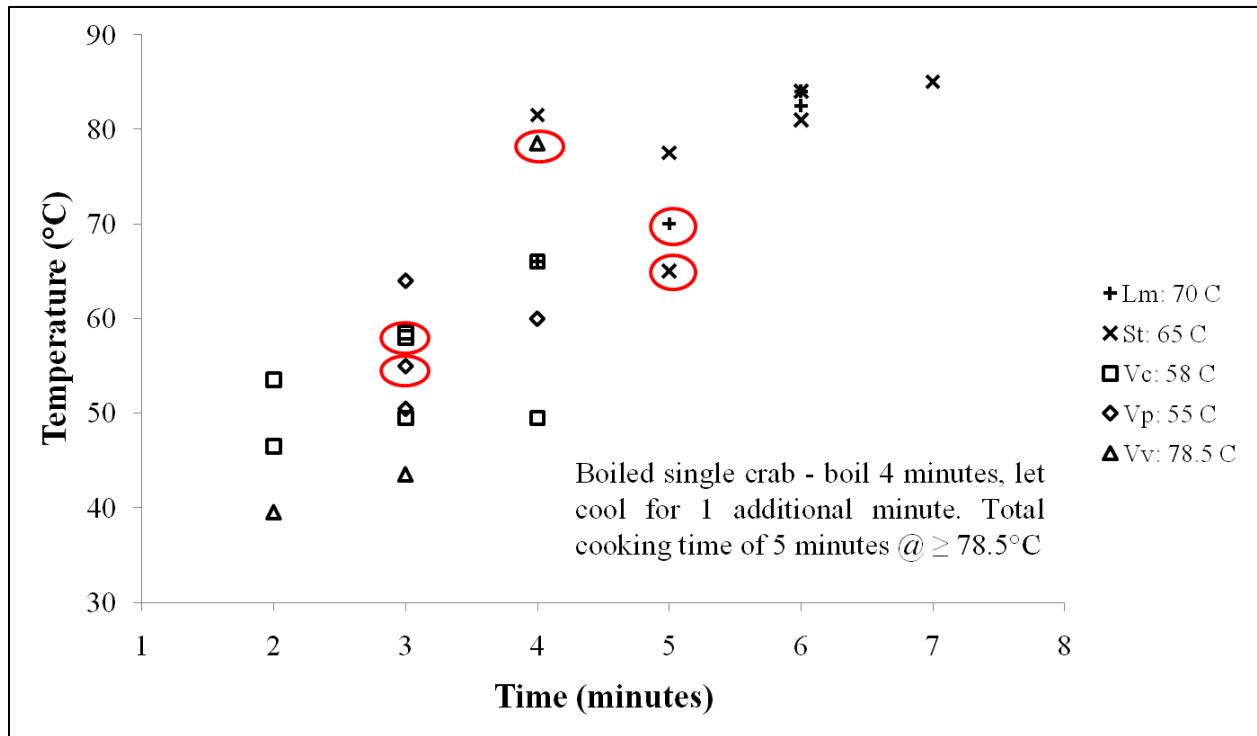


Figure 10: Graph of below detection limit/non-detectable level reached for boiling a single crab at each time point and the optimum temperature each was achieved for *L. monocytogenes* (Lm), *S. typhimurium* (St), *V. cholerae* (Vc), *V. parahaemolyticus* (Vp), and *V. vulnificus* (Vv).

For boiling a single crab, the greatest abundance of BDL/ND results are on the four minute time point. While three minutes is sufficient in eliminating the *Vibrios* tested here, *L. monocytogenes* and *Salmonella typhimurium* require an additional minute to reach BDL/ND levels. Therefore, the final determination for boiling a single crab is four minutes with one additional minute for cooling would provide maximum bacterial reduction with the least amount of cooking time.

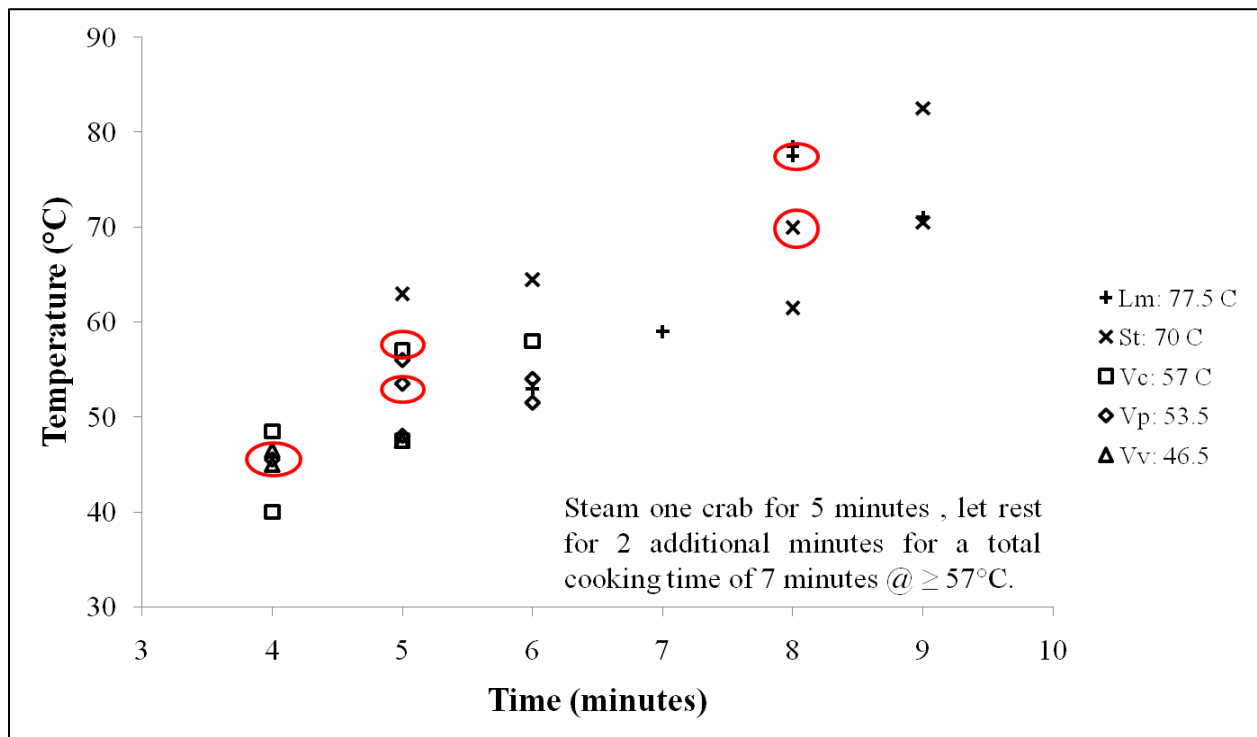


Figure 11: Graph of below detection limit/non-detectable level reached for steaming a single crab at each time point and the optimum temperature each was achieved for *L. monocytogenes* (Lm), *S. typhimurium* (St), *V. cholerae* (Vc), *V. parahaemolyticus* (Vp), and *V. vulnificus* (Vv).

For steaming a single crab, the greatest abundance of BDL/ND results are on the five minute time point. Again, the *Vibrios* tested here are eliminated sooner than the more heat resistant *L. monocytogenes* and *S. typhimurium*. Therefore, the final determination for steaming a single crab is five minutes with two additional minutes for cooling to provide maximum bacterial

reduction with the least amount of cooking time. In this case, boiling is preferred over steaming for a single crab.

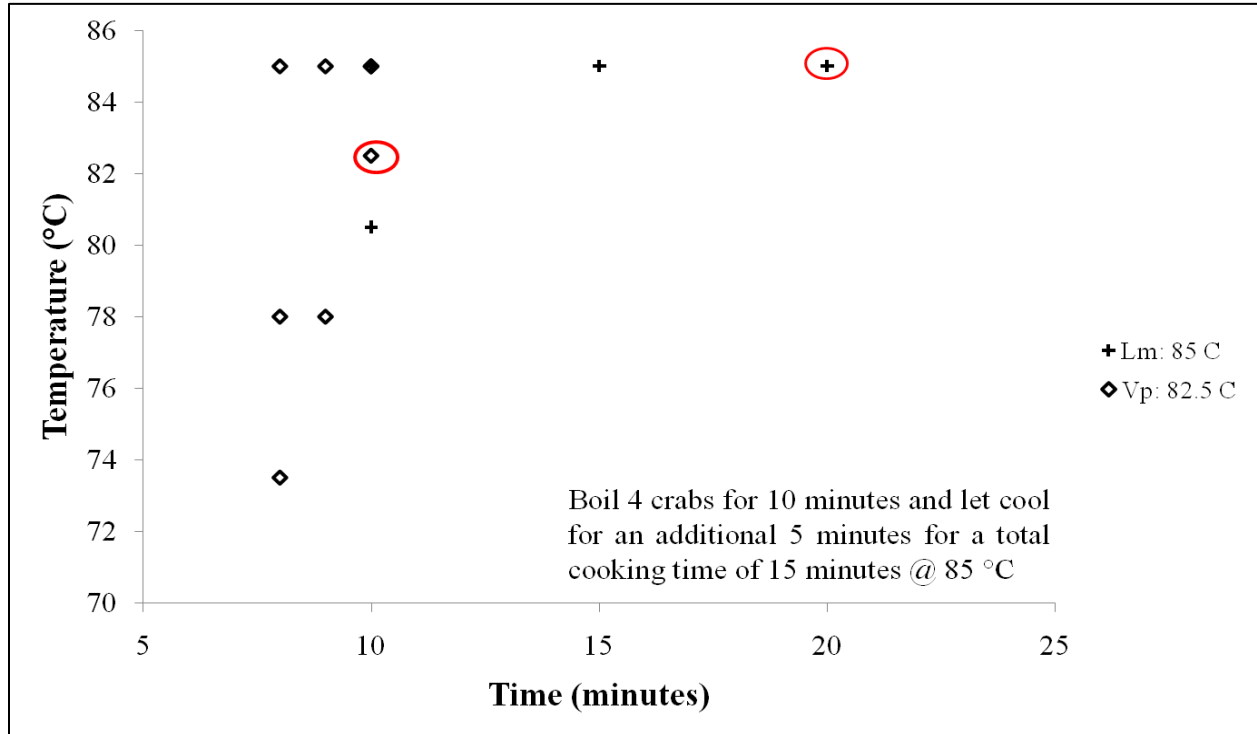


Figure 12 Graph of below detection limit/non-detectable level reached for boiling a serving size of crabs (4) at each time point and the optimum temperature each was achieved for *L. monocytogenes* (Lm) and *V. parahaemolyticus* (Vp).

Boiling four blue crabs, deemed a serving size, produced clearance of both *L. monocytogenes* and *Vibrio parahaemolyticus* at the 10 minute time point. The recommendation to allow five additional minutes for the crabs to cool will allow the heat to further penetrate into the back meat through carryover cooking. The final recommendation to consumers will suggest that they use this five minute waiting time to pick the chelipeds (the claws) as the chelipeds cook faster than the back meat due to their thinner shells. The final recommendation released to the public will also suggest the serving size method of cooking since the extended times with additional blue crabs produce a synergistic effect of more consistent clearance while preserving the sensory integrity of the blue crab meat.

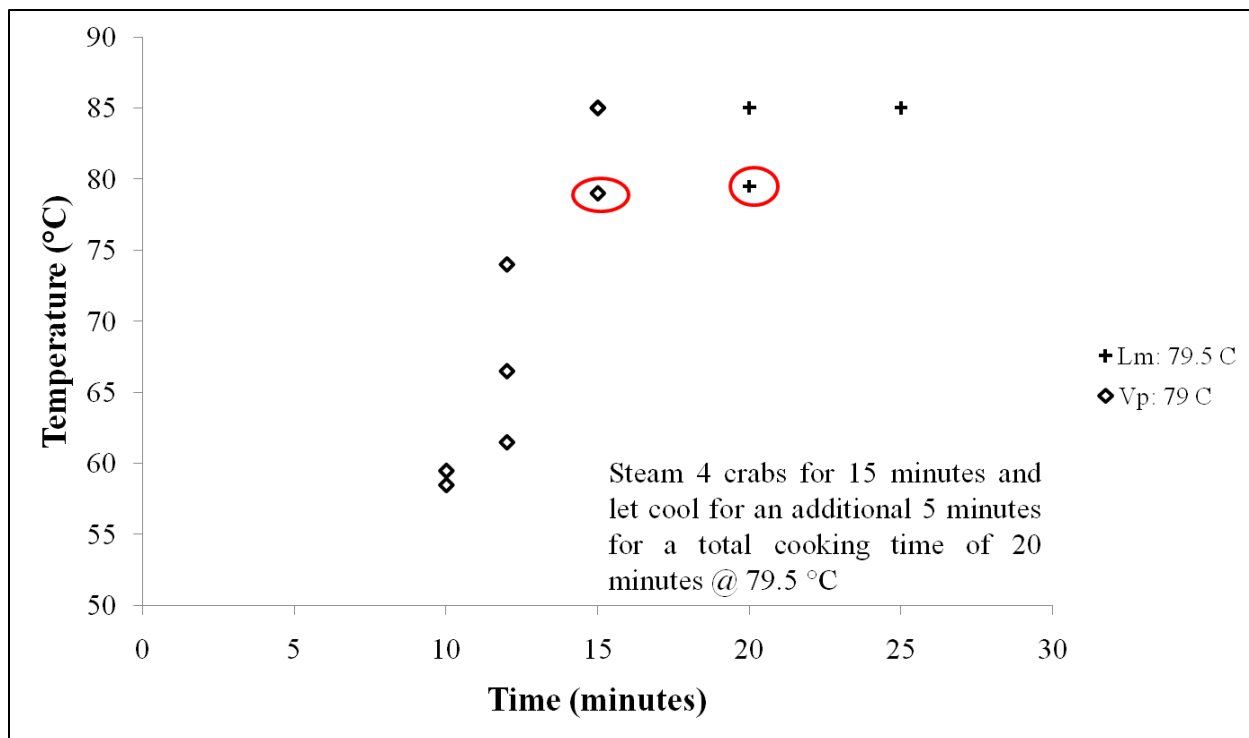


Figure 13 Graph of below detection limit/non-detectable level reached for steaming a serving size of crabs (4) at each time point and the optimum temperature each was achieved for *L. monocytogenes* (Lm) and *V. parahaemolyticus* (Vp).

Steaming a serving size of blue crabs produced clearance of both *L. monocytogenes* and *Vibrio parahaemolyticus* at the 15 minute time point. Again, the recommendation to allow five additional minutes for the crabs to cool will allow the heat to further penetrate into the back meat through carryover cooking. The final recommendation released to the public will also suggest the serving size method of cooking since the extended times with additional blue crabs produce a synergistic effect of more consistent clearance while preserving the sensory integrity of the blue crab meat with steaming preferred over boiling since it tends to penetrate better with steam.

4.6 Colorimeter Studies

The readings were taken approximately five to six minutes after removing the blue crabs from each heat treatment while the chelipeds were removed and picked. Figure 14a shows the results of all the time points analyzed by spectrophotometer for significant changes in shell color

of the blue crabs during each of the *V. parahaemolyticus* serving size boiling experiments ($p=0.2062$). Figure 14b is a graphical representation of the standard deviations of the a Values from Figure 14a. Figure 14c shows the results of all the time points analyzed for significant shell color changes of the blue crabs during each of the *V. parahaemolyticus* serving size steaming experiments ($p=0.6146$) and Figure 14d is a graphical representation of the standard deviations of the a Values from Figure 14c. Neither 14a nor 14c showed statistically significant differences.

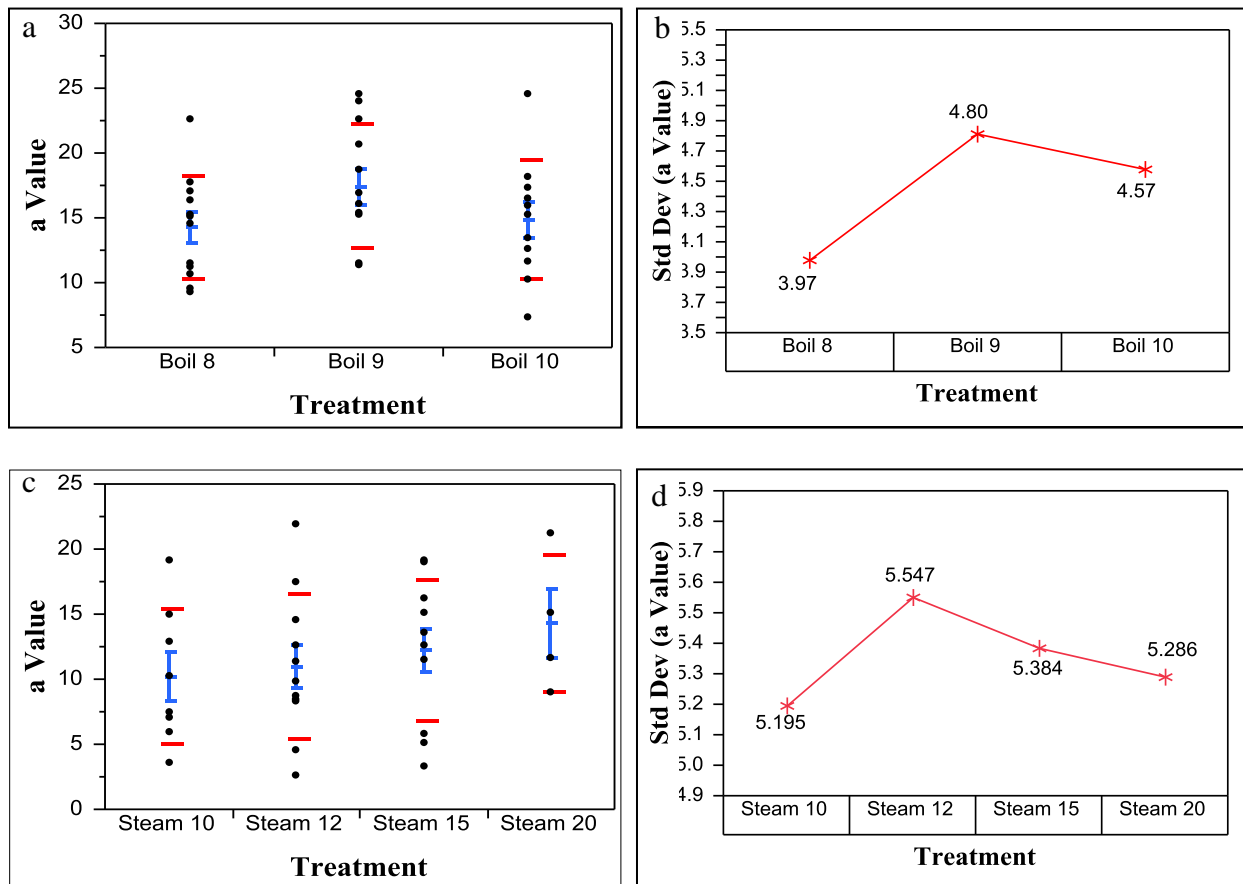


Figure 14: (14a) a Values for the serving size boiling experiment time points. (14b) Standard deviations of the serving size boiling experiment a Values. (14c) a Values for the serving size steaming experiment time points. (14d) Standard deviations of the serving size steaming experiment a Values.

The “a” Value was used to measure changes in redness intensity by a specialized spectrophotometer also referred to as a colorimeter. Figure 14a and 14c shows the results of the colorimeter reading of each shell after each boiling time point and after each steaming time point

experiment, correspondingly. Figure 14b and 14d interpret the importance of the a Value data. In graphically displaying the standard deviations of the a Values from the time points, it is clear how little differentiation there is between the redness of each time point. There is not a drastic enough change in color for a person to accurately identify a partially cooked blue crab (Figure 15a) from a fully cooked blue crab (Figure 15b) based solely on the expression “cook until it turns red”, particularly because a blue crab will turn red within 30 seconds of boiling [77]

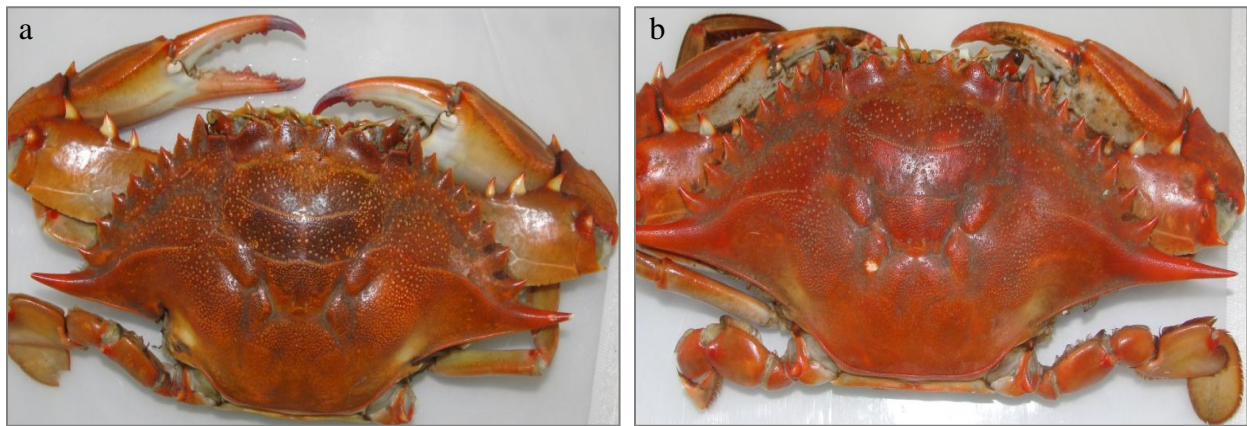


Figure 15: Boiled Louisiana blue crabs. (15a) Undercooked Louisiana blue crab boiled until red, approximately 30 seconds. (15b) Cooked Louisiana blue crab boiled, approximately three minutes

CHAPTER 5 – CONCLUSION

The purpose of this study was two-fold: to determine the safe cooking time and temperatures for Louisiana blue crabs and to see if cooking until red was a viable cooking method. Initially, the results of this project illustrated the frustration of meeting both scientific needs and consumer needs. Scientists need to have a single time and a single temperature to eliminate all pathogens in the manner established by food safety guidelines, usually using traditional thermal death time studies. The scientists need this one time and one temperature to pass onto retailers and consumers who want simplicity in their cooking instructions, not confusing D-values and Z-values of deadly pathogens. Due to the variability of the experimental conditions, the results of past scientific studies did not give a straight-forward answer that satisfied consumers [17]. Consequently, the problem was never addressed, which left a gap in both scientific and consumer knowledge possibly causing some instances of foodborne illness after eating blue crabs.

In an industry or retail setting, the FDA standards should still be practiced. Cross-contamination and proper handling techniques are just as relevant to consumers as they are for retailers and industry protocol [17]. However, the high microbial eradication standards held by the FDA, while admirable, are not necessarily applicable for consumers who purchase live Louisiana blue crabs for private consumption. The parameters of safety needed to be customized to fit the specific hazards particular to personal preparation of Louisiana blue crabs [17].

First, the spectrum of the experiment had to be defined; second, the design had to be optimized; and finally, the results had to be analyzed. After struggling to fit the results of this study to analytical methods found in literature, it was obvious that another approach was necessary to elucidate the information uncovered during this investigation. Once the data was

examined from a different angle, some trends did emerge that can help bridge the gap between scientific knowledge and consumer practicality.

Initial results for the heat treatment experiments showed that, for Louisiana blue crabs to be as safe to eat as possible, they had to be completely overcooked. However, the fear of overcooking shellfish and making the meat rubbery and unpleasant to eat was considered to be a probable causative factor of blue crab associated-foodborne illness, since some cases of foodborne illness associated with blue crabs were primarily due to consumers not wanting to overcook their blue crabs. It is not as though consumers are purposefully choosing foodborne illness over rubbery blue crab meat – it seems likely that the circumstances were a lack of familiarity with cooking this product, combined with inadequate availability of reasonable cooking instructions [17]. This is why the results were evaluated to offer cooking times that maintained the blue crabs sensory quality, while ensuring sufficient bacterial reduction.

The second purpose of this project was to determine if one could differentiate changes in the shell color to identify when a crab was safely cooked. The “cook until it is red” method is commonly used as a standard for cooking times with shellfish; this is the basis of “doneness” to most consumers. While this method works for other types of shellfish such as shrimp [Janes lab, unpublished results], cooking until the shell turns red does not adequately indicate “doneness” in blue crabs. Biochemical changes during any heat treatment cause the release of the astaxanthin, or red color, from the pigment complex of the blue crab shell. This is an acceptable indicator of “doneness” for shrimp because the shell is very thin and close to the meat. Blue crab shells are much thicker (an average of approximately 1 millimeter thick), so they require a greater application of heat to penetrate fully to the back meat. While the dissociation of astaxanthin from

the pigment complex can be accomplished fairly quickly – within 30 seconds – it does not correspond to the amount of time necessary to fully cook the crab [77].

The final recommendations for the public would not be given in precise temperatures that are impossible for consumers to measure without scientific equipment. While the minimal temperatures were discerned, solely presenting the data in those terms would not fulfill the main objective of this research project, which was to give consumers easy to follow instructions for cooking Louisiana blue crabs. The recommendations for boiling blue crabs are: bring approximately one gallon of water to a rolling boil, add crab(s), and cover with the lid. Boil one crab for four minutes and let cool for one additional minute; or boil a serving size of crabs (four) for 10 minutes and cool for five more minutes. Remove, cool, and pick the claws first. Be sure to bring water back to a rolling boil before adding the next batch of crabs. The recommendations for steaming blue crabs are: bring half a pot of water to a boil with the steamer apparatus inserted and the lid on. Steam the apparatus for about 30 minutes, add crab(s), and replace the lid. Steam one crab for five minutes and let cool for two additional minutes; or steam a serving size of crabs (four) for 15 minutes and cool for five more minutes. Remove, cool, and pick the claws first. Bring the water back to a boil with the lid on for 10 – 15 minutes, or until steam consistently escapes from under the lid, in between batches to allow the steamer apparatus to come back up to the starting temperature. Steaming is the preferred method over boiling because it is a better penetrative method.

There are several other avenues to explore in conjunction with this project. Future studies should include the use of common additives associated with cooking crabs, such as investigating if there are any thermal properties that salt or “crab boil”, a commercially available seasoning product, may contribute when added to the boiling water, and determining if the amount of time

necessary to achieve the optimum internal temperatures can be decreased due to ionic conductivity. Also, is some mixed research on certain types of herbs and spices acting as anti-microbial agents, so the use of crab boil during the cooking process should be investigated to determine if it offers any microbial protection [54].

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VITA

Nicole Watson Hazard was born and raised in Northern California. In 1993, three years after graduating high school, her family moved to Alexandria, Louisiana, where she began attending Louisiana State University at Alexandria. Working to finance college caused a two-year degree to become a six-year degree, but finally in 1999, she became the first person in her family to graduate college with an associate's degree in criminal justice. Her original goal of becoming a forensic scientist led her to continue her education in Baton Rouge at Louisiana State University and she completed her Bachelor of Science in microbiology degree with minors in chemistry and anthropology in 2003. Instead of forensics, she took a position at LSU working as Dr. Hollie Hale-Donze's research associate for three years. Following that position, she became a research associate for Dr. Zhi-Yuan Chen in the LSU AgCenter's Department of Plant Pathology and Crop Physiology for two more years. Then the timing was right to go back to school and pursue a master's degree in food microbiology with Dr. Marlene Janes in 2008 with plans on combining all of her degrees by working in a federal agency to investigate and improve food safety.