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DEVELOPMENT OF AN ALTERNATIVE BAIT FOR THE LOUISIANA COMMERCIAL BLUE CRAB (*CALLINECTES SAPIDUS)* FISHERY

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 School of Renewable Natural Resources

by
Angelle Nicole Anderson
B.S., Louisiana State University Agricultural and Mechanical College, 2008
May 2014

To the man who cultivated my love for and understanding of the natural world, my grandfather, Edward August Anderson Sr., whose love and guidance will always be remembered. He is missed each day but will forever be carried in my heart.

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ABSTRACT

The blue crab (*Callinectes sapidus*) is a commercially, recreationally, and ecologically important species in Louisiana coastal waters. Louisiana landings account for more than 80% of Gulf of Mexico hard crab landings. In 2012, over 24 thousand metric tons of blue crab were landed in Louisiana with an economic value over $52 million. The blue crab fishery in the northern Gulf of Mexico relies heavily on Atlantic menhaden (*Brevoortia tyrannus*) for bait, which is a species with stock concerns resulting in approximately a 20% decrease in total allowable catch beginning in 2013. Decreased landings results in increased cost of *B. tyrannus* for industrial and bait uses, demonstrating a need for new cost-effective alternative bait. Large amounts of waste are produced from processing over 40 thousand metric tons of penaeid shrimp annually landed in Louisiana. Shrimp carapace accounts for approximately ½ to ⅔ of the total biomass and can be used an attractant in alternative bait. The objectives of this study are: 1) determine a feasible attractant utilizing seafood processing waste; 2) determine if alternative bait soak time, diffusion, and catch will perform similar to *B. tyrannus*; and 3) Determine fishermen opinions and preferences for an alternative bait. Crabs responded favorably to bait created with shrimp waste as the attractant in laboratory choice bioassays. Field trials in three temperature regimes demonstrate soak time of alternative bait is comparable to the current natural bait used by the industry. Additionally, the alternative bait diffuses proteins, which stimulate feeding in crabs, at levels similar to or higher than natural bait. Paired-trap field trails demonstrated alternative bait produces catch rates comparable to natural bait; shrimp alginate accounted for 41% of total catch. Additionally, reduced rates of bycatch were seen in traps baited with alternative bait. Interviews conducted with commercial fishermen indicate the industry is willing to use alternative bait. Current results show a bait created with a waste product as an attractant is

feasible. Alternative bait would benefit blue crab fishermen, *B. tyrannus* stocks by reducing fishing pressure, and processors by creating a value added product.

CHAPTER 1. INTRODUCTION

1.1 THE BLUE CRAB (*CALLINECTES SAPIDUS*)

1.1.1 Ecology

The blue crab (*Callinectes sapidus* Rathbun 1896*)* is a commercially, recreationally, and ecologically important species in the family Portunidae, a family of marine swimming crabs, that contains approximately 300 extant species. The genus *Callinectes* consists of fifteen species distributed in the Pacific (three species) and Atlantic (twelve species) oceans. Members of this genus typically have a short, wide, flat body with five sets of paired appendages. In all members of the family, the fifth pair of appendages is modified into swimming legs with the propodus and dactyl being paddle-like. In many decapod crustaceans the first pair of appendages, the chelipeds, are differentiated morphologically into a crusher and a cutter claw (Mariappan et al. 2000). The larger chela, the crusher claw, typically is used for defense while the smaller claw, the cutter claw, functions in prey capture and movement of food into the maxillipeds. Some species in the genus *Callinectes* exhibit sexual dimorphism in the chelipeds. For example, male *C. sapidus* chelae are blue tipped, and female chelae are red tipped (Schenk and Wainwright 2001). Of the fifteen recognized species, eight are known to occur in the Gulf of Mexico: *C. bocourti, C. danae, C. exasperates, C. marginatus, C. ornatus, C. rathbunae, C. sapidus,* and *C. similis* (Williams 1974). Of the eight species occurring in the Gulf of Mexico, only *C. sapidus* is commercially fished and economically important.

C. sapidus is widely distributed in benthic and estuarine habitats in the western Atlantic from Nova Scotia to northern Argentina and in the Gulf of Mexico. The northern Gulf provides important habitat needed for blue crabs to carry out their life cycle. Blue crabs are able to tolerate temperatures from approximately 5 to 33 °C, however growth only occurs from 15 to 30

°C, and prolonged temperatures over 33 °C are lethal. In the northern Gulf of Mexico, water temperatures range from 14 to 21 °C in the winter and 28 to 32 °C in the summer. Over 33 rivers flow into the Gulf of Mexico influencing sediment composition, nutrient load, and to some extent local salinity. Blue crabs are found in a range of salinities depending on stage of life from freshwater (0.0 ppt) to oceanic water (over 30.0 ppt) (Swingle 1971, Christmas 1973, Perry and Stuck 1982). The combination of temperature and salinity may be more important than either factor alone since osmoregulation efficiency decreases with decreased salinity and temperature (Tagatz 1971, Rome et al. 2005).

Blue crabs are primarily found and fished in the large shallow and intertidal areas of the Gulf of Mexico, at depths less than 20 m deep. These areas account for the largest total area of the Gulf (38%) (Darnell and Defenbaugh 1990, Gore 1992). In addition to temperature and salinity, submerged aquatic vegetation (SAV) is important for blue crabs. Submerged aquatic vegetation provides critical nursery areas, foraging grounds, habitat, and protection from predators (Couvillion et al. 2011). Louisiana coastal marshes support the largest blue crab fishery in the northern Gulf of Mexico.

An adult blue crab will spend its entire life in one estuary system with the exception of females that migrate to higher salinity estuarine waters for spawning (Guillory et al. 2001). Blue crabs have been found in freshwater systems where they experience greater incremental growth during molting (Mangum and Amende 1972, Neufeld et al. 1980). In the waters of the northern Gulf, males typically reach sexual maturity at 110 mm carapace width (CW) and females at 125 mm CW (Guillory et al. 2001). One hundred percent of the population reaches sexual maturity at 130 mm CW and 160 mm CW for males and females, respectively. The blue crab commercial fishery regulation for minimum legal landing size in Louisiana (127 mm) corresponds to the CW

of sexually mature females. Within one year in the Gulf of Mexico, most blue crabs reach sexual maturity and recruit to the fishery due to high growth rates (Guillory et al. 2001). The blue crab population in the northern Gulf of Mexico is not distinctly bimodal in age classes due to egg production occurring for longer periods because of warmer temperatures (Perry et al. 1998). Increased egg production leads to continual recruitment and a longer season for juvenile growth. *C. sapidus* exhibit the classic characteristics of an R-selected species: small body size, fast growing, early maturity, high fecundity, short generation time, and the ability to widely disperse offspring with no parental care required. Similar to other R-selected species, blue crabs are able to sustain a high level of exploitation and can recover quickly if overfishing occurs (Guillory et al. 2001).

Blue crabs typically mate in vegetated, low salinity estuarine waters. Female blue crabs can only mate at one point in their lifetime during their terminal molt. However, courtship begins two to seven days prior to mating (Hay 1905). During the courtship process a male will "cradle carry" (female is right side up facing forward) a female to protect her from predators and ensure he is able to mate (Chidester 1911). The male continues to protect the female during ecdysis. Immediately following the molt, the male picks up the female (abdomens touching) and mating occurs (Hay 1905). After mating, the female is cradle carried for up to 48 hours while her carapace hardens to guard against other male inseminations (Hay 1905, Jivoff 1997). Guarding is not always successful as multiple cases of multiple paternity are documented (Jivoff 1997). Once the shell has completely hardened, the female will be released and will migrate to higher salinity waters to spawn. Females spawn more than 2 million eggs at a time depending on the size of the female (Hines et al. 2003). Females are able to retain sperm to produce multiple

broods for one to two reproductive cycles for later spawning (Darnell et al. 2009, Wolcott et al. 2005).

Water from the Caribbean Sea enters the Gulf of Mexico via the Yucatan Strait. The water then circulates through a clockwise Loop Current, exits the Gulf through the Florida Strait, and enters the Atlantic Ocean eventually forming the Gulf Stream. The Loop Current affects regional circulation patterns while nearshore environments are affected by local conditions such as bottom topography, wind patterns, and shoreline orientation. These currents contribute to the widely dispersed offspring. The Loop Current carries larvae offshore throughout the northern Gulf. In nearshore environments, tidal cycles and wind patterns influence recruitment and settlement of megalopal larvae into Gulf estuaries. Dispersal, settlement, and recruitment affect the population structure of blue crabs. The population in the northern Gulf is believed to be mostly of Louisiana origin (Guillory et al. 2001).

Planktonic zoeae larvae disperse offshore throughout the northern Gulf where the larvae undergo eight molt stages. Development from zoeal to megalopal stage is salinity and temperature dependent, with the highest survival and development at 23 to 30 ppt and 19 to 29 °C (Sandoz 1944). After 31 to 49 days, zoeae metamorphose into megalopae. In nearshore environments, tidal cycles and wind patterns influence recruitment and settlement of megalopal larvae into Gulf estuaries. Six to 20 days later megalopae will molt into the first crab stage and begin to settle in lower salinity estuaries where growth to adulthood occurs. Blue crabs are eurythermal and after the megalopal stage euryhaline. Dispersal, settlement, and recruitment affect the population structure of blue crabs. The population in the northern Gulf is believed to be mostly of Louisiana origin (Guillory et al. 2001).

1.1.2 Diet and Foraging

Blue crabs forage in benthic environments on a wide range of prey species. *C. sapidus* are detritivores, omnivores, cannibals, scavengers on carrion, and carnivores on invertebrates, motile crustaceans, and fish (Darnell 1958, Darnell 1959, Odum and Heald 1972, Laughlin 1979, Laughlin 1982, Hsueh et al. 1992). Four phyla make up the 99 species found in the diet of blue crabs: Mollusca (20 to 40%), Arthropoda (10 to 26%), Chordata (primarily fish 5 to 12%), and Annelida (primarily polychaetes 1 to 7%) (Kennedy and Cronin 2002). Juveniles (< 60 mm CW) feed primarily on smaller, shallow epibenthic organisms whereas adults (> 60 mm CW) feed on larger epibenthic organisms (Laughlin 1982, Stoner and Buchanan 1990, Mansour 1992). Juvenile crabs feed primarily on bivalves, plant material, detritus, amphipods, foraminiferans and algae while adult crabs feed primarily on fish, bivalves mollusks, and other crustaceans (Laughlin 1979, Laughlin 1982, Alexander 1986, Stoner and Buchanan 1990). Bivalve molluscs are an important food source for blue crabs and comprise the largest percentage of total prey consumed. Darnell (1958) and Laughlin (1982) found bivalve molluscs made up 35 to 40% of the blue crab diet followed by crustaceans at 16 to 24%. Bivalves include Eastern oyster, and crustaceans include penaeids (Laughlin 1979, Eggleston 1990, Abbe and Breitburg 1992, Micheli 1997, Fantle et al. 1999, Ghisalberti 2004, Newell et al. 2007, O'Connor et al. 2008).

In addition to ontogenetic shifts, blue crabs also show temporal and spatial variations in prey consumption (Laughlin 1982). However, blue crabs do not show variations in prey selection during the diel cycle (Laughlin 1982, Ryer 1987). Blue crabs feed four to seven times a day showing specific crepuscular feeding peaks within a twenty-four hour period (Nye 1989, Wolcott and Hines 1989). Temporal variation may reflect changes in availability of prey during tidal changes. In areas with large tidal cycles, higher tides allow blue crabs to access previously

unavailable prey, during low tides other prey such as fish become more concentrated and more available (Lin 1989). Water temperature also affects feeding activity in blue crabs. Consumption rates of blue crabs increase with water temperature (Landers 1954, Wallace 1973, Whetstone and Eversole 1978, Eggleston 1990). At low temperatures, feeding rates decrease and cease at temperatures below approximately 8 °C. Blue crabs forage along vegetated marsh edge, oyster reefs, and in sediment. Eggleston (1990) describes foraging behavior in blue crabs. Initial foraging begins with an increase in gill bailing rates and antennule flicking. Next, the mouthparts begin to move vigorously. The chelipeds and dactyls of the first and second anterior walking legs are then used to probe and manipulate prey. The chela, maxillipeds, and mandibles are used to disassemble prey during feeding.

1.1.3 Chemical Cues and Foraging

Chemical signals, often natural metabolites, provide information to organisms, are ubiquitous in aquatic environments, and mediate biotic interactions (Ferrari and Targett 2003, Weissburg et al. 2003). Chemical information is often transmitted through water in a plume or cue when substances are unintentionally or intentionally released from an organism (Dusenbery 1992, Breithaupt and Thiel 2011). Chemical signals are used to deter predators, locate prey, find mates, or identify suitable habitats (Weissburg et al. 2002, Breithaupt and Thiel 2011). Chemical cues released from a transmitting agent travel through a fluid medium to a receiving agent which responds by orienting and navigating toward or away from the cue source (Breithaupt and Thiel 2011).

Chemo- and rheotaxis are important in the foraging behavior of blue crabs (Zimmer-Faust et al. 1996, Weissburg 2000, Webster and Weissburg 2001, Weissburg et al. 2002, Westerberg and Westerberg 2011). The combination of the two stimuli allows blue crabs to

track an odor plume in low (1.0 cm s^{-1}, depth 15 cm), intermediate (3.8 cm s^{-1}, depth 15 cm) and high (14.4 cm s^{-1}, depth 15 cm) flow conditions (Weissburg and Zimmer-Faust 1993, Weissburg and Zimmer-Faust 1994). In no flow and turbulent systems, the ability of blue crabs to track an odor plume decreases (Weissburg and Zimmer-Faust 1993, Weissburg et al. 2003). Fluid dynamics of the odor plume affects blue crab olfactory navigation ability; however it should be noted that body angle of the individual relative to the plume also affects tracking ability (Weissburg and Zimmer-Faust 1993, Weissburg and Zimmer-Faust 1994, Zimmer-Faust et al. 1995, Westerberg and Westerberg 2011). For example, the body angle of a blue crab changes to increase drag in low flow conditions, whereas in high flow conditions, drag minimizing body angles are assumed in order for an individual to more effectively make contact with the odor (Weissburg et al. 2003).

Blue crabs use cephalic and thoracic appendages when orienting to odor plumes for olfactory foraging. The combination of appendages may allow crabs to sense chemical signals more effectively when chemical levels are low or in high flow (Keller et al. 2003). Increased movement of chemosensory structures such the scaphognathite and antennule allow crabs to detect and orient to prey that are located long distances away and/or in currents (Hazlett 1971, Hazlett 1999, Eggleston et al. 1990a , Eggleston et al. 1990b, Eggleston 1990, Keller et al. 2003). Chemoreceptors on the antennules allow blue crabs to detect small amounts of dilute chemicals in the environment increasing the possibility of prey tracking and location (Pearson and Olla 1977, Robertson et al. 1981).

Chemical signals in water tend to be detectable over longer periods of time and space, disperse slower, and be five times stronger than in air (Westerberg and Westerberg 2011). In aquatic environments, chemical cues are a more common mode of attraction than vision due to

distance, availability of light, and turbidity (Westerberg and Westerberg 2011). The Mississippi River deposits large amounts of sediment annually into the northern Gulf of Mexico that increases turbidity. This increases the importance of an organism's ability to utilize chemical signals to locate prey. In water, soluble substances have the ability to travel long distances, stimulate olfactory senses and cause organisms to orient toward or away from chemical stimuli (Westerberg and Westerberg 2011). Commercial trap fisheries take advantage of chemically mediated feeding behavior in fish and crustaceans.

Olfactory stimulation provides an effective mechanism to attract target organisms to traps. For these fisheries to be economically beneficial, the target organism must encounter the gear and be caught. In order to increase efficiency, catch, and economic returns, bait is used to attract an organism to a trap or pot. Tissues of bait species contain water-soluble compounds such as amino acids, organic molecules, and peptides that can act as a chemical attractants (Westerberg and Westerberg 2011). Thus, effective bait transmits a chemical signal from the trap which the target organism can follow, and subsequently be landed (Westerberg and Westerberg 2011). Chemical signals emitted by natural bait can be used to create an alternative bait for the blue crab commercial fishery.

1.2 BLUE CRAB FISHERY

1.2.1 History of the Blue Crab Fishery

For more than a century, blue crabs have been exploited in the western Atlantic and the Gulf of Mexico (Perry et al. 1984, Guillory et al. 2001, Kennedy and Cronin 2002). While first reported landings of blue crab in Louisiana occurred in 1880, little documentation exists of early landing totals (Perry et al. 1984). New Orleans, Louisiana was a center of the original fishery and supplied large cities along the Gulf coast (Perry et al. 1984). Historically, the gear used to

harvest blue crabs varied by fisherman preference and location until the introduction of the crab trap (Kennedy and Cronin 2002).

In Louisiana, commercial crab traps account for 99.5% of landings (Guillory et al. 2001). Increased efficiency and landings resulted in approximately a 400% increase in licensed fisherman in Louisiana during the 1980s (Guillory et al. 2001). The number of licenses issued by the Louisiana Department of Wildlife and Fisheries (LDWF) continued to increase through the 1990s resulting in increased effort, decreased catch per unit effort (CPUE) and decreased landings. During the 1990s, the most recent data available, landings have averaged 21,092 MT, and license sales have stabilized at approximately 3,000 licensed fishermen (Guillory et al. 2001). According to trip tickets reported to LDWF, approximately 50% of license holders actively fish. There are several potential reasons for the differences between license holders and the number of active fishermen reported including the perception by shrimp fishermen that a license is needed to temporarily have traps due to gear interaction, concern over a potential future moratorium for renewed licenses, delinquency in trip ticket reporting, and recreational fishermen who wish to possess more than the ten traps allowed by a recreational license.

1.2.2 The Current Fishery

Approximately 82,000 MT of blue crab were landed in the United States in 2012 with a dockside value over $190 million (National Marine Fisheries Service 2013). In the Atlantic, crabs are fished in Connecticut, Delaware, Maryland, New Jersey, New York, North Carolina, South Carolina, Virginia, Georgia, and the east coast of Florida. Of the approximately 56,000 MT landed in the Atlantic in 2012, the Chesapeake Bay contributed 59% (over 33,000 MT) of total landings. In the Gulf of Mexico, blue crabs are commercially fished in five states: Florida, Alabama, Mississippi, Louisiana, and Texas. In 2012 the Gulf contributed 30% (> 24,000 MT)

to the total US landings with a value of over $52 million (National Marine Fisheries Service 2013). Louisiana landings account for approximately 70% or more of total Gulf landings depending on yearly fluctuations. Louisiana accounted for the highest landings, over 20,000 MT (83.3%), and dockside value, over $43 million (81.5%), of all the Gulf States in 2012 (National Marine Fisheries Service 2013). The second highest landings and value are from the west coast of Florida, followed by Texas, Alabama, and Mississippi.

In Louisiana, blue crabs are primarily fished in shallow estuaries and in offshore state waters, across the coast year round. The fishery consists of licensed fishermen, wholesale dealers, and commercial buyers. Commercial landings, which vary annually, seasonally, and geographically are sold live or for processing. The highest landings occur from May through August, with a second peak in October, and the lowest landings in February and March (Guillory et al. 2001). On average, the Pontchartrain, Terrebonne, Barataria and Atchafalaya/Vermilion-Teche Basins account for approximately 90% of blue crabs commercially landed since 1999 in Louisiana (DeAlteris et al. 2012). The Calcasieu, Mermentau, and Mississippi Basins account for the remaining 9% with the lowest annual landings (< 1%) from the Sabine River Basin (DeAlteris et al. 2012).

The commercial blue crab fishery in Louisiana is under the regulation of the Louisiana Wildlife and Fisheries Commission, LDWF, and the State of Louisiana legislature. Currently, the fishery is regulated primarily by gear restrictions and minimum size limits. Gear regulations include prohibited use of trawls and dredges and specifications on trap design (LDWF Commercial Fishing Regulations 2013). In March 2012, the commercial blue crab fishery in Louisiana was certified as sustainable by the Marine Stewardship Council (MSC). MSC works to ensure the long-term sustainability of marine fisheries and the associated habitats. The fishery

is operating at a level where recruitment is not impaired and at its target reference point (DeAlteris et al. 2012). The Louisiana fishery was the first crab fishery in the world to gain this designation.

1.2.3 Current Bait

In the Gulf of Mexico and along the East Coast of the U.S. traps are typically baited with Atlantic menhaden (*Brevoortia tyrannus*), catfish (order Siluriformes), stripped mullet (*Mugil cephalus)* and other boney fishes (DeAlteris et al. 2012). The blue crab fishery in the northern Gulf of Mexico relies heavily on East Coast caught Atlantic menhaden (>50%) as bait for commercial traps. Catfish waste from aquaculture (35%), shad (<10%) and stripped mullet (< 5%) make up the other approximately 50% (DeAlteris et al. 2012).

For the last ten years, over 350,000 MT of Gulf menhaden (*Brevoortia patronus)* have been landed annually in the Gulf of Mexico (National Marine Fisheries Service 2013). Louisiana landings account for approximately 92% of total Gulf landings (Vaughan et al. 2007). However, a small percentage of total landings are utilized as bait, approximately 1%, most are sold for reduction purposes (Vaughan et al. 2007, Smith and Vaughan et al. 2011). Gulf menhaden are not commonly used as bait in Louisiana. The fish are more valuable when sold for reduction fishery, processed or reduced and not sold in the original form, rather than as bait. In addition to low bait landing, tropical storms and the Deep Water Horizon Oil Spill have caused decreased landings in the last ten years from 579,000 MT and 0.7 MT to 379,000 MT and 0.1 MT in the reduction and bait fishery respectively (Smith and Vaughan 2011, Vaughan et al. 2011).

B. tyrannus is harvested by purse seines in almost every East Coast state as a reduction (80%) and bait (20%) fishery. In addition to being used as blue crab bait, menhaden are

commonly used in commercial and recreational hook and line fisheries, and as bait in lobster pots on the Atlantic Coast (Smith and O'Bier 2011). The highest menhaden bait fishery landings are from the Chesapeake Bay, approximately 80%, and New Jersey (Vaughan et al. 2010). Ecological concern is growing over the depletion of the Chesapeake Bay Atlantic menhaden stocks as *B. tyrannus* landings have decreased 36.5% over the last 20 years while blue crab landings have remained consistent (Figure 1.1). According to the Atlantic States Marine Fisheries Commission's Atlantic Menhaden Stock Assessment (2010), overfishing was occurring as of 2008. As landings have decreased, the ex-vessel price per pound for industrial and bait uses of *B. tyrannus* has increased over the last 50 years from \$0.01 per lb. in 1950 to an all-time high of \$0.068 per lb. in 2008 (National Marine Fisheries Service 2013). In addition to increases in price per pound, shipping costs have increased over 300% in the last 20 years (U.S. Energy Information Administration 2012). Decreased bait landings, increased price per pound, and increased shipping cost translates into higher costs for fishermen. However, dockside for crab has remained fairly stable, demonstrating a need for new alternative bait.

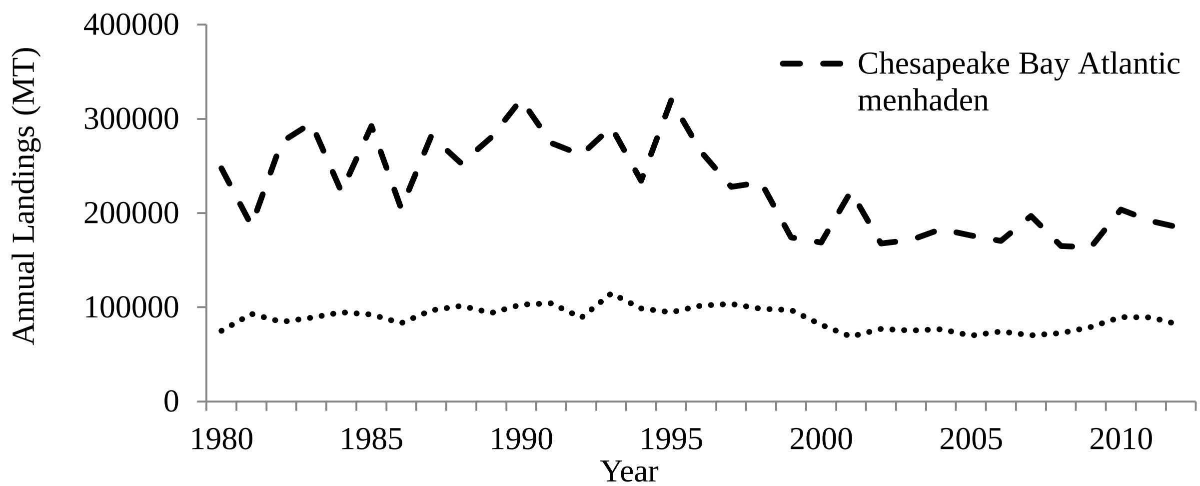

Figure 1.1: United States hard blue crab (*C. sapidus*) and Chesapeake Bay Atlantic menhaden (*B. tyrannus*) annual landings (MT). Data Source: (National Marine Fisheries Service 2013).

1.3 ALTERNATIVE BAIT

Research into the development of alternative bait for commercial fisheries has been ongoing for over 40 years. Attempts incorporating synthetic and natural chemicals as attractants have spanned numerous fish and invertebrate species around the globe such as Japanese mitten crab (*Eriocheir japonica*) (Wada et al. 2000), palaemonid shrimp (Nakata et al. 2005), crawfish (Cange et al. 1986, Burns and Avault 1991), cod (*Cadus morhua)* (Lokkeborg 1990), premolt female blue crabs (Rheo and Dough 2004), American eel (*Anguilla rostra*) and conch (Ferrari and Targett 2003, Rager 2007), Dungeness crab (*Cancer magister*) (Allen et al. 1975), American lobster (Chanes-Miranda and Viana 2000, Crowley 2007),western rock lobster (*Panulirus cygnus*) (Ghisalberti 2004) and others (Carr and Derby 1986, Januma et al. 2003, Kasumyan and Døving 2003).

Use of alternative baits is common in the crawfish industry. Commercial production of an alternative bait for the crawfish industry began in the early 1980s, and currently there are several successful formulations commercially available. The bait is composed of cereal grains, grain by-product, flavoring, and binder. The bait is most effective beginning late March/ early April, in water temperatures of 21.1 to 23.8 °C, when crawfish ponds become forage deficient (Burns and Avault 1991). However, the bait is less effective in colder water, and crawfish farmers use *Clupeid* fish, the traditional trap bait.

Trying to develop alternative baits for blue crabs is not a novel concept. Rittschof and Osterberg (2002) developed eight experimental baits utilizing combinations of seasonal fish, beef stock, pig blood, duck weed, and chicken. Field tests of all eight bait types versus current bait, a seasonal mixture of fish, were conducted. Results of the study showed poultry byproducts might have potential when used as an attractant in an alternative bait (Rittschof and Osterberg 2002).

In 2007, Lynn Haynie and American Proteins Inc. developed three alternative bait formulations utilizing poultry byproduct as the attractant. The bait failed to break down and attract crabs in cold water (Seling 2007). However, Mark Peterman at Mississippi State University tried byproducts of seafood processing instead of poultry with promising results. To date, all of these results remain unpublished (Mississippi State University Coastal Research and Extension Center 2012).

Other crab bait research has focused on attracting peeler crabs (Newman and Rittschof 2004, Rheo and Dough 2004). Rheo and Dough developed an alternative bait to attract premolt female blue crabs for the soft shell industry. The cost of the bait is slightly less than the cost of a male crab, and in field trials the alternative bait performed comparably to the use of a male crab. An application to patent the chemical was submitted in 2004 with plans to produce the bait commercially (Rheo and Dough 2004). The bait is to be produced by Shure Shedders but is currently not on the market.

To date, a widely used artificial bait for blue crab commercial fisheries does not exist. Studies suggest artificial baits are more successful when natural substances are incorporated in a carrier matrix which diffuse a chemical attractant gradually through time (Adams and Johnsen 1986, Daniel and Bayer 1987, Rach and Bills 1987, Daniel and Bayer 1989, Middleton et al. 2000). Previous bait attempts have largely focused on poultry byproducts, not natural prey items that can be easily incorporated into a carrier matrix.

In previous laboratory studies, alginate has proven to be a successful matrix to which an attractant can be added (Ferrari and Targett 2003, Rager 2007). Alginate is a natural polymer obtained from brown seaweed. The addition of Ca^{2+} or other di- and trivalent cations to alginate causes an instant gelling to occur (Lyn and Ying 2010). An attractant from natural sources, such

as waste products of natural diet items, can be incorporated into the alginate creating alternative bait for commercial fisheries.

Waste products produced from the processing of Eastern oyster (*Crassostrea virginica*), brown (*Farfantepenaeus aztecus*) and white (*Litopenaeus setiferus*) shrimp can potentially be used as an attractant in an alternative bait. These waste products from known prey species contain metabolites that stimulate foraging and feeding behavior in blue crabs. An attractant that stimulates such behavior can attract a target organism to a trap where it subsequently can be landed.

More than 250,000 MT of Eastern oyster were landed in the United States over the last 20 years (National Marine Fisheries Service 2013). Louisiana lands over 5,000 MT annually, which translates into large amounts of waste produced and a potential economic profit that currently is not exploited (National Marine Fisheries Service 2013). In Louisiana, oysters are shucked in a shucking house, typically over a table or floor drain. During the shucking process, the hemolymph contained within the oyster drains out and becomes a waste product. This hemolymph could be a feasible attractant that can be added to the alginate bait matrix in the creation of an alternative bait for commercial blue crab fisheries in the northern Gulf of Mexico. In the northern Gulf of Mexico, brown and white shrimp are commercially harvested. In 2012, more than 102,000 MT of shrimp were landed in the US, with 90% (> 47,000 MT) and 97% (> 47,000 MT) of white and brown shrimp respectively, landed in the Gulf of Mexico (National Marine Fisheries Service 2013). Louisiana landings accounted for approximately 47% of total Gulf of Mexico landings in 2012 (National Marine Fisheries Service 2013). When shrimp are processed and sold as headless, the cephalothorax is discarded as waste, approximately ½ to ⅔ of the shrimp. This waste can be used as an attractant and incorporated into an alternative bait.

1.4 OBJECTIVES AND RATIONALE

Waste products from seafood processing can be easily collected and added to alginate to create an alternative bait. The average amount of fish bait used per trap in Louisiana is 0.27 kg, translating to over 8,000 MT of bait used per year in Louisiana alone (DeAlteris et al. 2012). Given the amount of bait used across the Atlantic and Gulf coasts in blue crab fisheries, a large market for an alternative bait exits. The development of a new bait would add value to current waste products, be more cost effective for commercial fishermen due to local production, be available year round, and decrease fishing pressure on Atlantic menhaden. This project aims to:

1) Determine a feasible attractant utilizing seafood processing waste;

2) Determine if alternative bait soak time, diffusion, and catch will perform similar to *B. tyrannus*;

3) Determine fishermen opinions and preferences for an alternative bait.

1.5 LITERATURE CITED

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CHAPTER 2: DETERMINING A SUITABLE ATTRACTANT FROM PROCESSING WASTE FOR AN ALTERNATIVE BAIT.

2.1 INTRODUCTION

Blue crabs (*Callinectes sapidus*) are commercially fished in the Atlantic and Gulf of Mexico. Approximately 82,000 MT of blue crab were landed in the United States in 2012, with a dockside value over $190 million (National Marine Fisheries Service 2013). In 2012 the Gulf contributed 30% (> 24,000 MT) to the total US landings with a value of over $52 million (National Marine Fisheries Service 2013). Louisiana landings account for approximately 70% or more of total Gulf landings depending on yearly fluctuations. In Louisiana, blue crabs are fished by trap in shallow estuaries and in offshore state waters, across the coast year round.

The blue crab fishery in the northern Gulf of Mexico relies heavily on East Coast caught Atlantic menhaden (>50%) as bait for commercial traps. The highest menhaden bait fishery landings are from the Chesapeake Bay, approximately 80%, and New Jersey (Vaughan et al. 2010). Ecological concern is growing over the depletion of the Chesapeake Bay Atlantic menhaden stocks as *B. tyrannus* landings have decreased 36.5% over the last 20 years while blue crab landings have remained relatively stable. According to the Atlantic States Marine Fisheries Commission's Atlantic Menhaden Stock Assessment (May 2010) overfishing was occurring as of 2008. Gulf menhaden (*Brevoortia patronus)* are commercially landed in Louisiana but are not used as bait because the fishery is more valuable when sold as a reduction fishery rather than a bait fishery. Depletion of Atlantic menhaden stocks demonstrates a need for a new alternative bait. Studies suggest artificial baits are more successful when natural substances are incorporated (Adams and Johnsen 1986, Daniel and Bayer 1987, Rach and Bills 1987, Daniel and Bayer 1989, Middleton et al. 2000). An attractant from created from natural diet items can be used to create an alternative bait for commercial fisheries.

It is well documented that blue crab are a major predator on Eastern oyster and have the ability to cause local extinction in oyster reefs (Eggleston 1990, Abbe and Breitburg 1992, Micheli 1997, Fantle et al. 1999, Ghisalberti 2004, Newell et al. 2007, O'Connor et al. 2008). More than 250,000 MT of Eastern oyster were landed in the United States over the last 20 years (National Marine Fisheries Service 2013). Louisiana lands over 5,000 MT annually, which translates into high amounts of waste produced and a potential economic profit that currently is not exploited (National Marine Fisheries Service 2013). In Louisiana, oysters are shucked in a shucking house, typically over a table or floor drain. During the shucking process, the hemolymph contained within the oyster drains out and becomes a waste product. Hemolymph could be a feasible attractant that can be added to the alginate bait matrix in the creation of an alternative bait for commercial blue crab fisheries in the northern Gulf of Mexico.

Penaeids are also a common diet component of adult blue crabs (Laughlin 1979). In the northern Gulf of Mexico, brown (*Farfantepenaeus aztecus*) and white shrimp (*Litopenaeus setiferus)* are commercially harvested. Of the more than 102,000 MT of shrimp landed in the US in 2012, 90% (> 90,000 MT) were landed in the Gulf of Mexico (National Marine Fisheries Service 2013). Gulf landings accounted for 97% (> 47,000 MT) of total US brown shrimp landings, and 90% (> 47,000 MT) of total US white shrimp landings (National Marine Fisheries Service 2013). Louisiana landings accounted for approximately 48% of total Gulf of Mexico shrimp landings in 2012 (> 45,000 MT) (National Marine Fisheries Service 2013). When shrimp are processed and sold as headless, the cephalothorax is discarded as waste, approximately ½ to ⅔ of the shrimp. This waste could be used as an attractant and incorporated into an alternative bait.

The development of a new bait would add value to current waste products, be more cost effective for commercial fishermen due to local production, be available year round, and decrease fishing pressure on Atlantic menhaden. The goal of this research was to develop a bait that was equally as effective as menhaden. The objectives were to: 1) compare the diffusion rate of protein from alternative and natural baits; 2) determine the soak time (in number of days) of alternative bait; and 3) compare catch rates of alternative and natural baits. Laboratory trials were conducted to compare the diffusion rate of the attractant from the alginate carrier matrix to the current natural bait. Field trials were conducted to determine soak time and catch rate of alginate bait containing shrimp waste as the attractant.

2.2 METHODS

Laboratory choice bioassays were conducted with processing waste. The bioassays were designed to determine a suitable attractant that can be added to the carrier matrix to produce an alternative bait for the Louisiana commercial blue crab fishery.

Crabs were purchased from commercial fishermen located in Lafourche and St. Charles Parishes, LA, USA and a commercial processor located in St. Tammany Parish, LA, USA in 2013. Crabs were transported back to the laboratory in an ice chest containing ice and wet burlap to minimize stress. All experimental crabs purchased corresponded to the minimum size required for commercial landing in Louisiana, 12.7 cm carapace width (CW) or larger.

Forty-two individuals were sampled without replacement. Of the 42 individuals tested (mean CW = 156.83 mm), 14 crabs (13 females, 1 males) were purchased from a blue crab processor located in St. Tammany Parish, LA, USA. The remaining 28 crabs (21 females, 7 males) were purchased a commercial fishermen located in Lafourche, and St. Charles Parishes, LA, USA.

In the laboratory, specimens were held in individual cages to prevent cannibalism in a 1,135 L artificial saltwater recirculated system under constant conditions (salinity of 13.4 ± 1.4, 24 °C ± 2 °C, and 12:12 h light: dark photoperiod). Artificial saltwater (ASW) was created with deionized water and Instant Ocean (Spectrum Brands Inc., Blacksburg, VA., USA). Crabs were fed freshwater drum (*Aplodinotus grunniens*) to satiation three times a week during the laboratory acclimation period. *A. grunniens* was used to prevent a feeding bias as it was not a potential attractant to be tested. Water quality was monitored with colorimetric tests and maintained by weekly partial water changes. Crabs were allowed to acclimate a minimum of seven days to ensure feeding, and all experiments were conducted within 30 days following the acclimation period. Crabs were starved for 36 to 48 h prior to laboratory bioassays.

Oyster hemolymph was obtained from a shucking house located in Golden Meadow, LA. Oysters were hand shucked over collecting containers, and hemolymph was immediately frozen to ensure water did not mix with hemolymph. *L. setiferus* cephalothoraxes, not treated with sodium sulfites, were obtained whole and frozen from Louisiana commercial processors. Cephalothoraxes were ground into a uniform paste before being used as an attractant.

C. sapidus were acclimated in ASW for 24 h in aerated individual 37.85 L opaque plastic tanks, which minimized visual stimuli under control conditions (salinity of 15.6 ± 0.8, 24 °C ± 2 °C, 12:12 h photoperiod). Bioassays were conducted with two alternative baits of similar weights (mean weight of bait 93.09 g). Alternative bait was made on the date of the t standard operating procedure in the laboratory. Sand was added to sink baits, allowing crabs to easily encounter and handle bait. In order to reduce water fouling, baits were approximately 25% of the size that will be recommended for use in commercial crab traps.

Baits of equal size containing either oyster hemolymph or shrimp cephalothorax as attractants were placed at random in each tank opposite crab location. Crabs were allowed to feed for 6 h and selection was recorded. All bait trials were conducted from 2:00 PM to 8:00 PM (± 1 hour) under white light to take advantage of crepuscular feeding behavior (Nye 1989; Wolcott et al. 1989).

2.3 STATISTICAL ANALYSIS

I used Chi Square with a Fisher's Exact Test to determine if there was a difference in CW size between males and females (PROC FREQ; SAS Institute Inc., Cary, N.C.). Logistic regression was used to determine if the probability of not selecting bait created with oyster as the attractant was affected by individual size, sex, or the combination of sex and size (PROC GENMOD; SAS Institute Inc., Cary, N.C.). Lastly, I used a generalized linear mixed model with a logit link and binomial probability distribution with attractant type as the random variable to investigate if selection of bait created with oyster or shrimp as the attractant was random (PROC GENMOD; SAS Institute Inc., Cary, N.C.).

2.4 RESULTS

Bait created with shrimp cephalothorax was selected by 100% of crabs tested, bait created with oyster hemolymph was selected by 69% (Figure 2.1). One hundred percent of both females and males selected bait created with shrimp cephalothorax, 87.5% of males and 67% of females selected bait created with oyster hemolymph. Male mean CW was 147.75 mm (139-160 mm), female mean CW was 158.97 mm (134-182 mm).

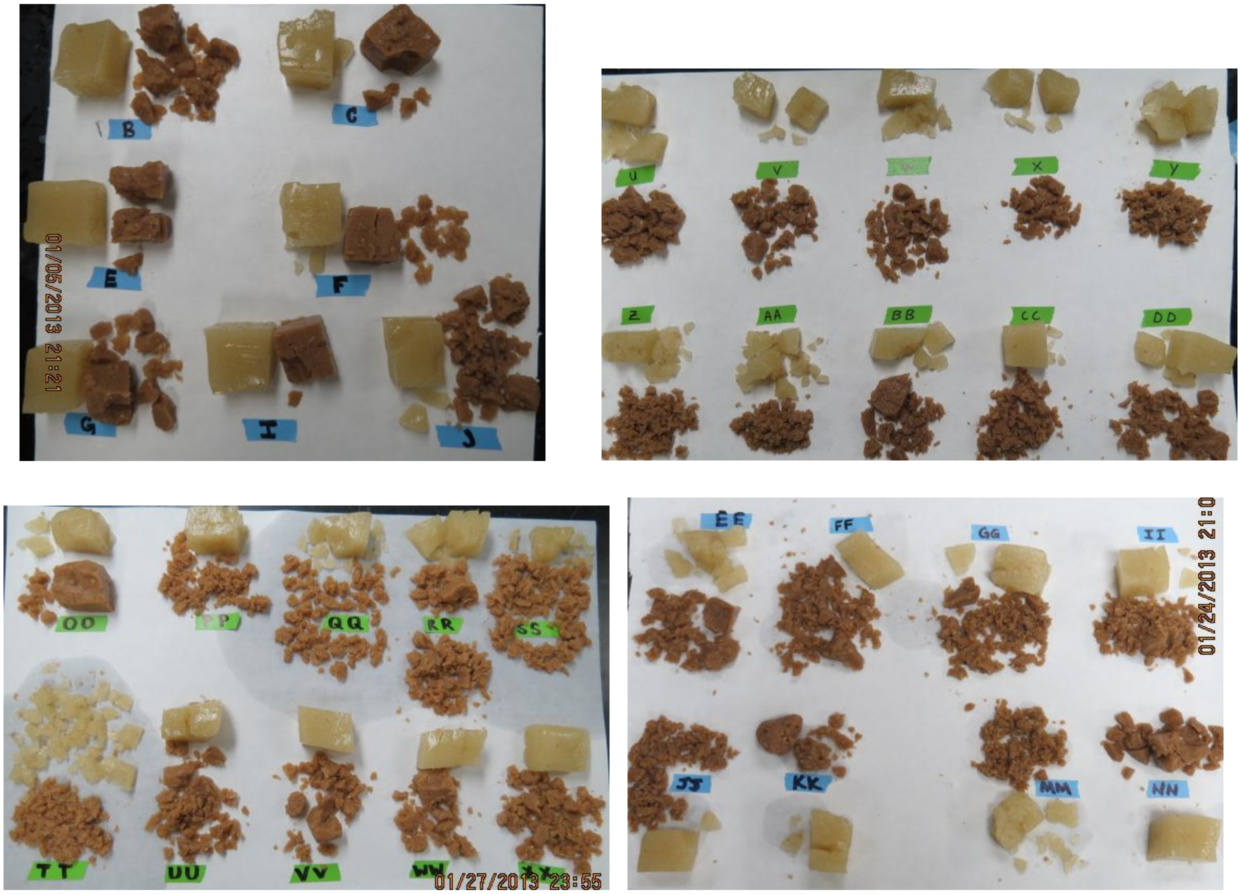

Figure 2.1: Blue crab selection of attractant source. Photographs of individual blue crab selection of bait type. Dark bait created with shrimp waste as the attractant. Light bait created with oyster waste as the attractant. (n=42).

There was no significant difference in CW size between males and females, p=0.4272. Neither sex, size, nor the combination of sex and size was statistically significant for an individual when not selecting bait created with oyster hemolymph as the attractant, $p > 0.5$ for all tests. Selection of bait was not random, bait created with shrimp cephalothorax was selected in every trial. Individual selection of bait was statistically significant and not random (p=0.0136).

2.5 DISCUSSION

Although bait created with oyster hemolymph was also selected, the higher selection rate for shrimp cephalothorax indicated it may perform better as an attractant in an alternative bait.

In addition to a higher selection rate, larger quantities of penaeid shrimp than Eastern oyster are landed in Louisiana annually, 5,000 MT and > 45,000 MT respectively, translating into more waste products available (National Marine Fisheries Service 2013). Additionally, shrimp waste products are easier to collect. Oyster processors typically shuck either by hand or with machinery, the latter becoming more common. In mechanical operations, oysters are often shucked with steam. During this process, hemolymph collection is not possible. This process can denature proteins due to high heat, potentially changing the attractive properties of the waste product. Many shrimp processors still remove the cephalothorax by hand, making separating and storing waste a less difficult process. The waste can easily be discarded into containers, as opposed to current disposal methods, and frozen for use in an alternative bait. The use of shrimp waste has many advantages: 1) easy collection, 2) easy storage, 3) inexpensive, 4) large quantities are available, and 5) study results indicate it will attract blue crabs.

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CHAPTER 3: THE DIFFUSION RATE, SOAK TIME, AND CATCH RATE OF ALTERNATIVE BAIT.

3.1 INTRODUCTION

For more than a century blue crabs have been exploited in the western Atlantic and the Gulf of Mexico (Perry et al. 1984, Guillory et al. 2001, Kennedy and Cronin 2002). More than 60,000 MT of blue crab have been landed annually in the United States for the past three decades (National Marine Fisheries Service 2013). Louisiana landings account for approximately 70% or more of total Gulf landings depending on yearly fluctuations (National Marine Fisheries Service 2013). In Louisiana, blue crabs are primarily fished in shallow estuaries and in offshore state waters, across the coast year round.

In the Gulf of Mexico and along the East Coast of the U.S. traps are typically baited with Atlantic menhaden (*Brevoortia tyrannus*), catfish (order Siluriformes), stripped mullet (*Mugil cephalus),* and other boney fishes (DeAlteris et al. 2012). Commercial crab traps account for 99.5% of landings (Guillory et al. 2001). The blue crab fishery in the northern Gulf of Mexico relies heavily on East Coast caught Atlantic menhaden (>50%) as bait for commercial traps (DeAlteris et al. 2012).

B. tyrannus is harvested by purse seines in almost every East Coast state as a reduction (80%) and bait (20%) fishery. The highest menhaden bait fishery landings are from the Chesapeake Bay, approximately 80%, and New Jersey (Vaughan et al. 2010). Ecological concern is growing over the depletion of the Chesapeake Bay Atlantic menhaden stocks as *B. tyrannus* landings have decreased 36.5% over the last 20 years while blue crab landings have remained relatively stable. According to the Atlantic States Marine Fisheries Commission's Atlantic Menhaden Stock Assessment (2010) overfishing was occurring as of 2008. As landings have decreased, the ex-vessel price per pound for industrial and bait uses of *B. tyrannus* has

increased over the last 50 years from $0.01 per lb. in 1950 to an all-time high of $0.068 per lb. in 2008 (National Marine Fisheries Service 2013). In addition to increases in price per pound, shipping costs have increased over 300% in the last 20 years (U.S. Energy Information Administration 2012). Decreased bait landings, increased price per pound, and increased shipping cost translates into higher costs for fishermen, demonstrating a need for new alternative bait.

Chemical signals, often natural metabolites, provide information to organisms, are ubiquitous in aquatic environments, and mediate biotic interactions (Ferrari and Targett 2003, Weissburg et al. 2003). Chemical information is often transmitted through water in a plume or cue when substances are unintentionally or intentionally released from an organism (Dusenbery 1992, Breithaupt and Thiel 2011). Chemo- and rheotaxis are important in the foraging behavior of blue crabs (Zimmer-Faust et al. 1996, Weissburg 2000, Webster and Weissburg 2001, Weissburg et al. 2002, Westerberg and Westerberg 2011). The combination of the two taxes allows blue crabs to track an odor plume in varying flow conditions (Weissburg and Zimmer-Faust 1993, Weissburg and Zimmer-Faust 1994). Olfactory stimulation provides an effective mechanism to attract target organisms to traps. Effective bait diffuses a chemical signal through time from the trap which the target organism can follow, and subsequently be landed (Westerberg and Westerberg 2011). In some fisheries, the chemical signal emitted by natural bait can be used in an artificial bait to create an alternative bait.

To date, a widely used artificial bait for blue crab commercial fisheries does not exist. Studies suggest artificial baits are more successful when natural substances are incorporated into a carrier matrix which diffuse a chemical attractant gradually through time (Adams and Johnsen 1986, Daniel and Bayer 1987, Rach and Bills 1987, Daniel and Bayer 1989, Middleton et al.

2000). Waste products from known prey species contain metabolites that would stimulate foraging and feeding behavior in blue crabs. An attractant that stimulates such behavior can attract a target organism to a trap.

The development of a new bait would add value to current waste products, be more cost effective for commercial fishermen due to local production, be available year round, and decrease fishing pressure on Atlantic menhaden. The goal of this research was to develop a bait that was equally as effective as menhaden. The objectives were to: 1) compare the diffusion rate of protein from alternative and natural baits; 2) determine the soak time (in number of days) of alternative bait; and 3) compare catch rates of alternative and natural baits. Laboratory trials were conducted to compare the diffusion rate of the attractant from the alginate carrier matrix to the current natural bait. Field trials were conducted to determine soak time and catch rate of alginate bait containing shrimp waste as the attractant.

3.2 METHODS

3.2.1 Bait

Four different baits were used in the experiments. Atlantic menhaden (*B. tyrannus*), the positive control, was purchased frozen in 22.6 kg boxes from blue crab processors located in southeastern Louisiana; individual mean weight 168.83 g, mean standard length 20.18 cm. Menhaden were stored in the laboratory frozen. Menhaden were used whole to mimic standard industry practice. The other three baits consisted of an alternative bait carrier matrix created with laboratory grade chemicals and industrial grade alginate. A standard amount of shrimp cephalothorax was added to the alginate to create the three other baits: 100% shrimp cephalothorax (100% shrimp bait), 50% shrimp cephalothorax with 50% deionized water (50% shrimp bait), or 100% deionized water as the control (control alginate). *L. setiferus*

cephalothoraxes, not treated with sodium sulfites, were obtained whole and frozen from Louisiana commercial processors. Cephalothoraxes were ground into a uniform paste before being used as an attractant.

3.2.2 Diffusion

In order to compare diffusion rates between alginate baits and menhaden, individual recirculated systems (n=12) were constructed with 37.85 L opaque plastic tanks with flow rate of 3.88 L/min in an environmental chamber in order to maintain constant water temperatures. Each system contained a total volume of 25 L of artificial saltwater (ASW), composed of aged municipal water and Instant Ocean (Spectrum Brands, Inc., Blacksburg, VA., USA). Baits were tested at all combinations of three temperatures (18, 22, 31 °C) and two salinities (5, 25 ppt), which are common in the northern Gulf of Mexico, for a total of 6 separate trials.

Menhaden, 100% shrimp alginate, 50% shrimp alginate, and control alginate of standard commercial fishing size were tested with three replicates per bait for each temperature and salinity combination trial. One 15- ml water sample was taken at 0 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h, and 72 h. ASW was completely changed after the 24 h and 48 h water samples to ensure continuous diffusion could be detected. Samples were immediately frozen at -20 °C for later analysis. A standard Coomassie Plus (Bradford) Assay micro test tube preparation (per manufacturer instructions, ThermoFisher Scientific) was used to measure protein concentration in water samples.

3.2.3 Soak Time

Two field sites were chosen to determine the soak time of alginate baits for three seasons: summer (July), spring (April), and fall (October / November). The low salinity (0.1 ppt) site was located in Lake Maurepas, LA, USA (30° 15' 38.3034"N, -90° 42' 47.4834"W); the high salinity

(> 15.0 ppt) site was located in Grand Isle, LA, USA (29° 14' 18.8514"N, -90° 0' 10.872"W). Individual cages (23.97 cm x 23.97 cm x 8.25 cm) constructed of commercial bait box mesh (1.9 cm x 1.9 cm) were used to mimic fishing conditions. The cages allowed small vertebrates and invertebrates that would normally feed on the bait access while excluding larger consumers. Five replicates of each of the two bait types, 100% shrimp alginate and the control alginate, were tested each season. Cages were suspended no less than 0.3 m from the sediment to mimic the bait box in standard commercial crab traps. All baits were randomly assigned a cage and allowed to soak for five days. Temperature (Inset TidbiT v2 water temperature data logger – UTBI-001), salinity, and presence / absence was recorded.

3.2.4 Paired Trap Field Study

In order to compare the catch rates of 100% shrimp bait and *B. tyrannus,* two field locations were chosen to represent a high and low salinity site. The low salinity (< 15.0 ppt) site was located in Cocodrie, LA, USA (29° 15' 16.056"N, -90° 39' 42.4794"W); the high salinity (> 15.0 ppt) site was located in Grand Isle, LA, USA (29° 14' 18.8514"N, -90° 0' 10.872"W). Twenty paired traps were set in each field location (Mackie et al. 1980, Rittschof and Osterberg 2002, Rittschof and Osterberg 2005, Newman and Rittschof 2004). Traps were set to mimic current commercial fishing practices for season and location. Traps were spaced a minimum of 4 m apart within and between pairs in straight rows. In each pair, one trap was baited at random with one whole uncut menhaden, and the other trap was baited with 100% shrimp alginate bait. Traps were baited and allowed to soak 48 h. After 48 h, traps were pulled and contents were removed. All crabs were removed from the system after each trial to ensure individuals were not recaptured. The number, sex and size (CW) of blue crab caught was recorded. Bycatch was identified to species level. Three replicates were conducted at each location a minimum of one

week apart during September and October 2013. Traps were moved a minimum of 90 m away from the previous set location for all three replicates conducted at each site.

3.3 STATISTICAL ANALYSIS

3.3.1 Diffusion

I used a generalized linear mixed model with a log link and poisson probability distribution with protein concentration as the random variable to investigate differences in protein concentration by experiment and temperature/salinity combination (PROC GLIMMIX; SAS Institute Inc., Cary, N.C.).

3.3.2 Paired Trap Field Study

I used a mixed linear model to investigate if the total number of crabs caught was affected by the random variables of bait type, salinity, bycatch size, bycatch type, and the interactions therein (PROC MIXED; SAS Institute Inc., Cary, N.C.). Next, I ran a nonlinear mixed model defined by the logit function removing empty traps with mean salinity and trap ID as the random variables to investigate what variables affected presence/absence and abundance (PROC NLMIXED; SAS Institute Inc., Cary, N.C.). Lastly, I used a nonlinear mixed model defined by the logit function with bait type removed with mean salinity and trap ID as the random variables to determine if catch was random (PROC NLMIXED; SAS Institute Inc., Cary, N.C.).

3.4 RESULTS

3.4.1 Diffusion

Results of the generalized mixed model show an effect of bait type on protein diffusion rate, $P = 0.0043$. According to the conservative T grouping, 100% shrimp alginate diffused proteins at a rate higher than average, followed by menhaden, and then 50% shrimp alginate

(Figure 3.1). The control alginate diffused at the lowest rate and broke out into a separate group. All baits diffused proteins throughout the 72 h period. Overall, shrimp alginate baits diffused proteins at a similar or higher rate than menhaden.

3.4.2 Soak Time

Both alginate and shrimp alginate bait lasted 5 days at the low salinity site, Lake Maurepas, LA, in all 3 seasons: summer (28 °C, 0.1 ppt), spring (16.9 °C, 0.1 ppt), and fall (19.6 °C, 0.1 ppt). Shrimp alginate and alginate lasted 5 days in the spring at the high salinity site, Grand Isle, LA (20.7 °C, 22.1 ppt). Alginate lasted 1 day at the high salinity site in the summer (32.5 °C, 27.2 ppt) and fall (23.7 °C, 22.2 ppt); shrimp alginate lasted less than 1 day (Figure 3.2).

3.4.3 Paired Trap Field Study

In total, 212 blue crabs were caught during the three replicate trials conducted at each location, high salinity, Grand Isle, LA (25 ± 3 °C, 20 ± 3ppt) and low salinity, Cocodrie, LA (26 ± 3 °C, 13 ± 2 ppt). Of the 212 total crabs landed, 72% were caught at the low salinity site, and 28% were caught at the high salinity site. Of the 153 blue crabs caught in Cocodrie, LA, traps baited with menhaden yielded a higher total blue crab catch rate of 63% than traps baited with shrimp alginate, 37% of total catch. Total catch of blue crabs in Grand Isle, LA (n= 59) was nearly equal; 51% were caught with menhaden and 49% were caught with shrimp alginate. Mean catch per trap of shrimp alginate was 0.66 males and 1.2 females at the low salinity site, and 0.36 males and 0.63 females at the high salinity site compared to menhaden which had a mean catch rate of 2.16 males and 1.06 females at the low salinity site, and 0.43 males and 0.53 females at the high salinity site (Figure 3.3).

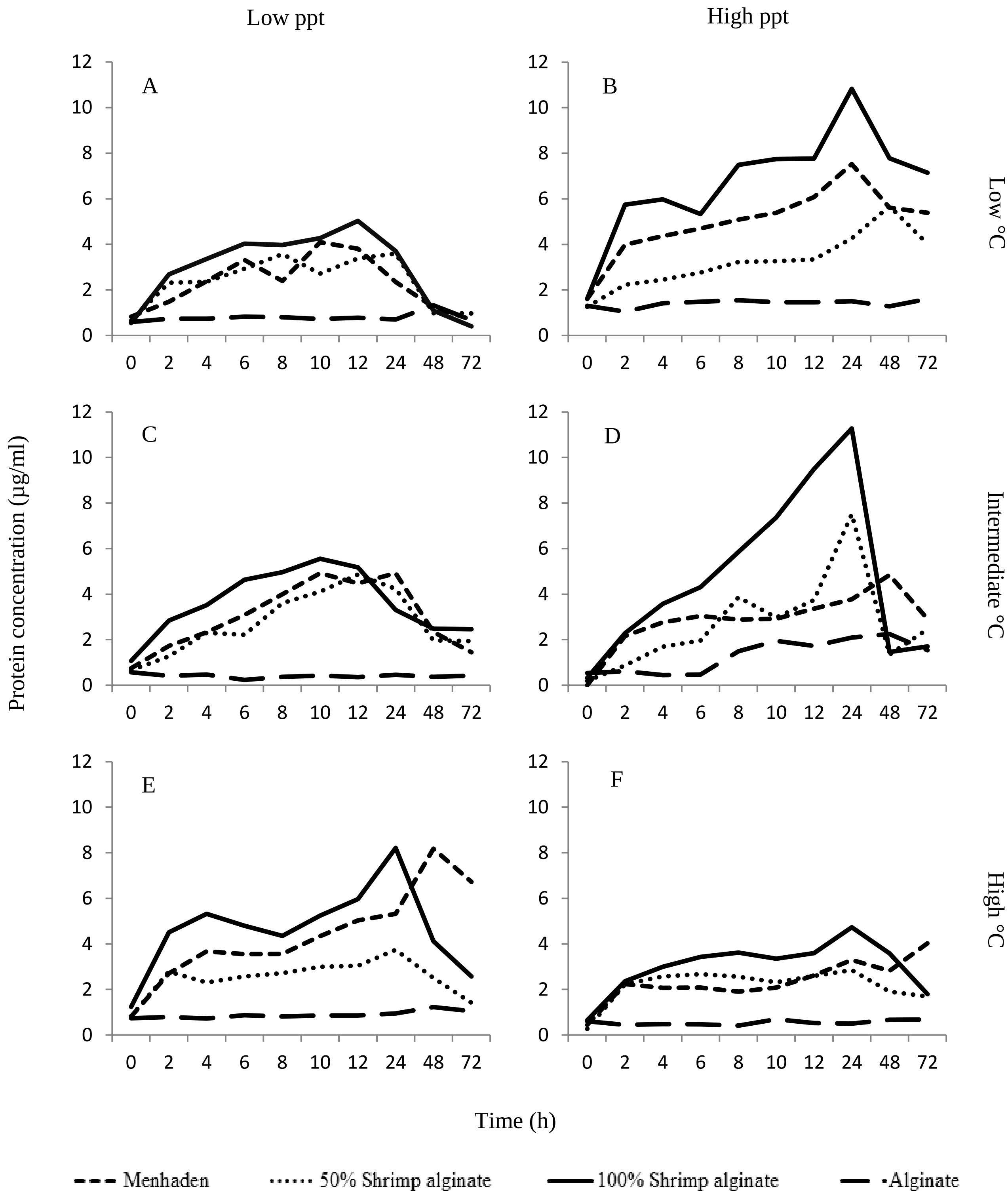

Figure 3.1: Rate of protein diffusion for the four bait types at varying temperatures and salinities. Protein diffusion (µg/ml) for A) low temperature, low salinity (18 °C, 5 ppt), B) low temperature, high salinity (18 °C, 25 ppt), C) intermediate temperature, low salinity (22 °C, 5 ppt), D) intermediate temperature, high salinity (22 °C, 25 ppt), E) high temperature (31 °C, 5 ppt) and F) high temperature, low salinity (31 °C, 25 ppt) over 72 h with water changed after 24 h and 48 h sample.

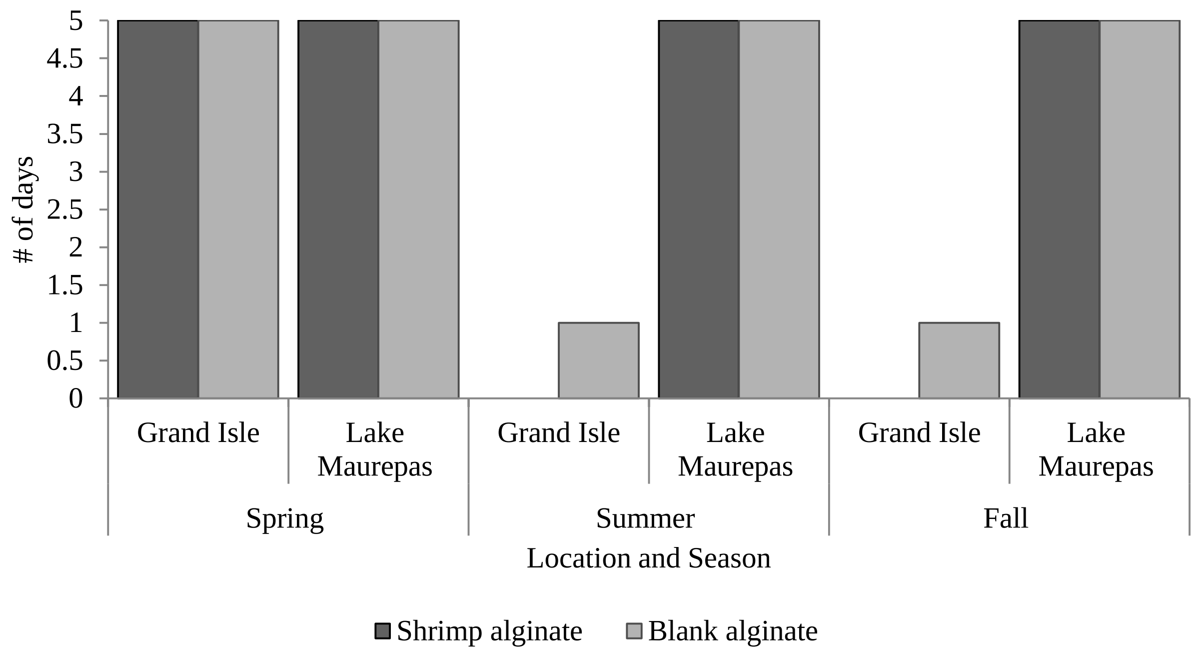

Figure 3.2: Soak time of bait across three seasons. Alginate (light grey) and shrimp alginate (dark grey) soak times in three seasons; summer, fall, and spring, in a high (Grand Isle, LA, USA) and low (Lake Maurepas, LA) salinity site (n=5 per season and site). No error bars due to no variance in the replicates.

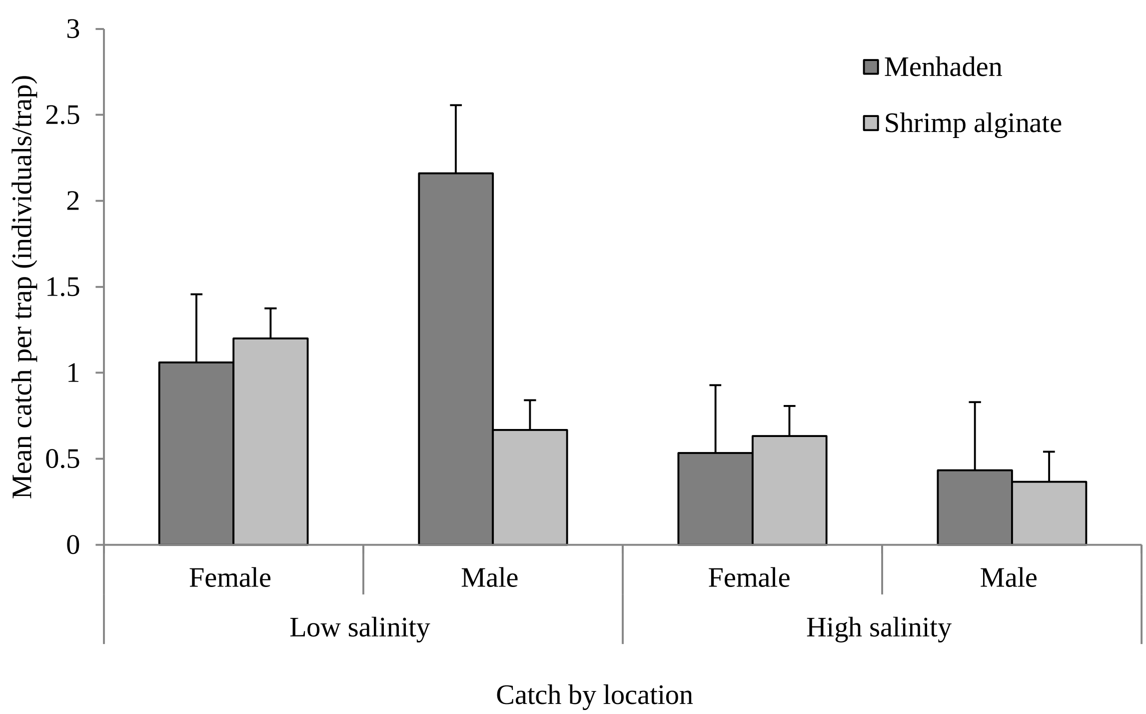

Figure 3.3: The amount of blue crab caught in the field by bait type. Mean catch of blue crab per trap (n=212) at each site by bait type (dark grey = menhaden and light grey= shrimp) and sex. Error bars represent standard error.

At the low salinity site, 44% of blue crabs caught were female and 54% of those 67 were caught with shrimp alginate. Males accounted for 56% of total catch at the low salinity site, 24% were caught with shrimp alginate. At the high salinity site, 59% of total catch was female, and 54% were caught with shrimp alginate. Males accounted for 40% of total catch, with 46% of those total 24 caught with shrimp alginate at the high salinity site.

At Cocodrie, females had a CW range of 92.68 mm to 179.4 mm, mean of 155.76 mm whereas males had a CW range of 102.28 mm to 190.01 mm, mean of 145.23 mm. At Grand Isle, females ranged slightly larger with a CW of 98.07 mm to 189.44 mm, but a smaller mean of 149.21 mm. For males at Grand Isle, CW ranged from 108.94 to 161.55 mm, mean of 132.63 mm.

Total bycatch for all three trials at both field sites was 79 total fish and two stone crabs, 17 fish and one stone cab from the low salinity site and 62 fish and one stone crab from the high salinity site. Seventy-eight percent of bycatch was landed at the high salinity site, and traps baited with menhaden accounted for 73% of total bycatch. Shrimp alginate caught a mean bycatch of 0.13 fish per trap in the low salinity site and 0.6 fish per trap in the high salinity site (Figure 3.4). Bycatch at both sites consisted of stone crab (*Menippe adina*), sheepshead (*Archosargus probatocephalus*), hardhead catfish (*Arius felis)*, pinfish *(Lagodon rhomboides*). Mangrove snapper *(Lutjanus griseus)* and spadefish *(Chaetodipterus faber)* were also caught as bycatch at the high salinity site (Figure 3.5).

A total of 60 traps were set at each site over the three replicate trials, 20 traps per trial. Of the 30 total traps baited with shrimp alginate at the high salinity site, 10 were empty and two contained bycatch only, whereas four menhaden traps were completely empty and ten contained

bycatch only. At the low salinity site, eight of the 30 traps baited with shrimp alginate, and one of the 30 traps baited with menhaden were completely empty, and zero contained bycatch only.

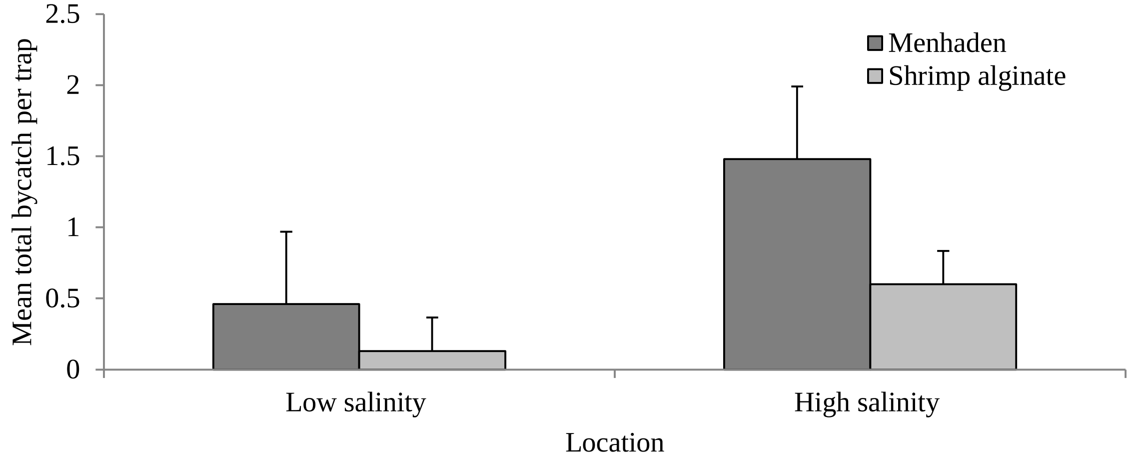

Figure 3.4: Total bycatch caught with menhaden and shrimp alginate. Mean total bycatch per trap by bait type (dark grey= menhaden, light grey= shrimp alginate) and location (n=80).

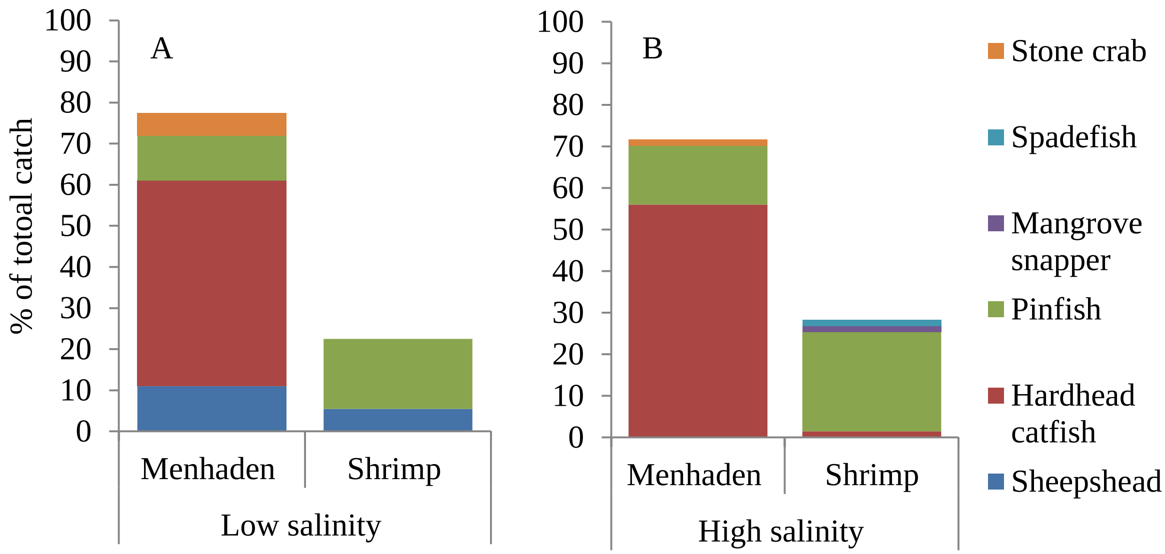

Figure 3.5: Total bycatch by type and location. The percentage of total bycatch (n= 80) by bait type (menhaden or shrimp alginate) and location: A) low salinity and B) high salinity.

The number of crabs caught was not affected by bait type, bycatch size, bycatch type, or the interactions thereof ($p > 0.05$). Results indicate the total number of crabs in a trap is influenced by salinity alone ($p = 0.0028$). Results of the nonlinear mixed model defined by the logit function indicates bait type influences the number of crabs in a trap but not the presence or absence of crabs in a trap. The model also indicates the effect of bait is negligible in determining if crabs enter a trap. Results of the second nonlinear mixed model defined by the logit function with bait type removed influenced there is a small effect of bait type on the number of crabs in a trap. Comparing the AIC values for the two models indicates if a crab entered a trap it was in favor of menhaden.

3.5 DISCUSSION

Our findings demonstrate an alginate bait can be created with seafood processing waste that 1) will diffuse at a similar or higher rate than natural bait; 2) can be fished for one to five days depending on season; and 3) produce comparable catch rates to the current natural bait utilized in the industry. The combination of attractant diffusion, soak time, and catch rates are important properties of bait that can affect bait efficiency, catch rates, and therefore economic returns for fishermen.

For a bait to be effective, it needs to not only attract but also continue to diffuse through time and catch until a trap is rebaited. The results of this study show proteins emitted by shrimp alginate diffuse at a rate higher than the current natural bait used in the industry. Additionally, the proteins continued to diffuse through time for at least 72 h. Mackie (1980) found similar diffusion rates from alternative bait for lobster. The study showed the release rates of amino acids from alternative baits composed of gelatin or agar was higher than natural baits.

Fishermen soak traps from one to five days depending on season and location. Fishermen allow traps to soak for three to five days during seasons with lower water temperatures or lower rates of catch (Guillory at al. 2001). In seasons with higher water temperatures, fishermen run traps every 24 h due to the increased rate of bait break down and higher rates of catch. Shrimp alginate bait lasted five days for all three seasons in the low salinity site. In the high salinity location shrimp alginate lasted five days in the spring. However, the bait did not last more than 24 h in the summer and fall when water temperatures increased. Our results indicate the soak time of shrimp alginate is comparable to the current natural bait used by the commercial blue crab fishery. Future research is needed to compare the soak time of shrimp alginate to menhaden across more sites and seasons.

The results of this study demonstrate shrimp alginate produces catch rates comparable to natural bait; shrimp alginate accounted for 41% of total catch. Mackie (1980) found similar catch rates with an alternative bait created with *Sprattus* as the attractant and gypsum as the carrier matrix for the commercial lobster fishery (*Homarus hammarus*). The alternative bait was 50% to 100% as effective as natural bait over three trials conducted over two years. The author concluded the alternative bait catch rate was acceptable when compared to the natural bait used by the commercial lobster fishery. Similar results were demonstrated by an alternative bait created for the commercial blue crab fishery by Rittschof and Osterberg (2002). Of the eight baits created, one chicken bait showed a catch rate comparable to menhaden. Menhaden caught a mean of 15.5 crabs/pot and chicken caught an average of 15.3 crabs/pot demonstrating chicken waste product could be a feasible alternative to current natural bait. However, our bait utilized natural diet items for blue crabs.

Bycatch is an important consideration for trap baits. According to commercial fishermen, increased bycatch in a trap causes bait to break down more quickly and reduces the number of crabs in a trap. Our findings demonstrate reduced rates of bycatch in traps baited with shrimp alginate. Overall, traps baited with menhaden accounted for the highest percentage of total bycatch, more than 60%, for individual locations and for both locations combined. Decreased bycatch could potentially equal higher economic returns for fishermen due to decreased rates of bait break down, less time emptying bycatch from traps, and increased catch of blue crabs. Additional field testing across more season and in multiple locations is needed to ensure results translate into a larger scale more similar to a commercial fishing operation.

The average amount of fish bait used per trap in Louisiana is 0.27 kg, translating to over 8,000 MT of bait used per year in Louisiana alone (DeAlteris et al. 2012). Given the amount of bait used across the Atlantic and Gulf coasts in blue crab fisheries, a large market for an alternative bait exits. The development of an alternative bait would reduce fishing pressure on Atlantic menhaden stocks, create a value added product from a current waste product, be more cost effective for commercial fishermen due to local production, and be available year round.

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CHAPTER 4: DETERMINING FISHERMEN OPINIONS OF AND PREFERENCES FOR ALTERNATIVE BAIT.

4.1 INTRODUCTION

For more than a century blue crabs have been exploited in the western Atlantic and the Gulf of Mexico (Perry et al. 1984, Guillory et al. 2001, Kennedy and Cronin 2002). More than 60,000 MT of blue crab have been landed annually in the United States for the past three decades. Louisiana landings account for approximately 70% or more of total Gulf landings (National Marine Fisheries Service 2013). In Louisiana, blue crabs are primarily fished in shallow estuaries and in offshore state waters, across the coast year round.

In the Gulf of Mexico and along the East Coast of the U.S. traps are typically baited with Atlantic menhaden (*Brevoortia tyrannus*), catfish (order Siluriformes), stripped mullet (*Mugil cephalus),* and other boney fishes *(*DeAlteris 2012). The blue crab fishery in the northern Gulf of Mexico relies heavily on East Coast caught Atlantic menhaden (>50%) as bait for commercial traps.

For the last ten years, over 350,000 MT of Gulf menhaden (*Brevoortia patronus)* have been landed annually in the Gulf of Mexico (National Marine Fisheries Service 2013). Louisiana landings account for approximately 92% of total Gulf landings (Vaughan et al. 2007). However, a small percentage of total landings are utilized as bait, approximately 1%; most are sold for reduction purposes (Vaughan et al. 2007, Smith and Vaughan et al. 2011). Gulf menhaden are not commonly used as bait in Louisiana. The fish is more valuable when sold for reduction fishery, processed or reduced and not sold in original form, rather than as bait. In addition to low bait landing, tropical storms and the Deep Water Horizon Oil Spill have caused decreased landings over the last ten years from in the reduction and bait fishery (Smith and Vaughan 2011, Vaughan et al. 2011).

In Louisiana, commercial crab traps account for 99.5% of landings (Guillory et al. 2001). The increased efficiency and landings resulting from the introduction of the wire crab trap led to approximately a 400% increase in licensed fisherman in Louisiana during the 1980s (Guillory et al. 2001). The number of licenses issued by the Louisiana Department of Wildlife and Fisheries (LDWF) continued to increase through the 1990s resulting in increased effort, decreased catch per unit effort (CPUE) and decreased landings. Over the last decade, landings have averaged 21,092 MT, and license sales have stabilized at approximately 3,000 licensed fishermen (Guillory et al. 2001). According to trip tickets reported to LDWF, approximately 50% of license holders actively fish (Guillory et al. 2001). There are several potential reasons for the differences between license holders and the number of active fishermen reported including the perception by shrimp fishermen that a license is needed to temporarily have traps due to gear interaction, concern over a potential future moratorium for renewed licenses, delinquency in trip ticket reporting, and recreational fishermen who wish to possess more than the ten traps allowed by a recreational license (Guillory et al. 2001).

Bait choice is a combination of cost, availability, tradition, location, and fishermen preference. Important properties of bait include cost, packaging, efficient storage and handling, size, shape, soak time, time to re-bait, and effectiveness. The development of a new bait could add value to current waste products, be more cost-effective for commercial fishermen due to local production, be available year round, and decrease fishing pressure on Atlantic menhaden. In order for a new alternative bait to be effective, it must be adopted by commercial fishermen in the industry. An industry interview of blue crab commercial fishermen was conducted in order to determine what properties they consider important and their willingness to try an alternative bait.

4.2 METHODS

A twelve-question survey containing multiple-choice and open-ended questions was developed as an industry interview to gauge fishermen preference of a new bait (Appendix A). Interpersonal interviews of commercial fishermen were conducted by blue crab processors in southeastern Louisiana during the 2013 season. Interviews conducted at Luke's Seafood, located in Dulac, LA, were read to fishermen and responses recorded. At Pontchartrain Blue Crab, located in Slidell, LA, interviews were completed by fishermen.

4.3 STATISTICAL ANALYSIS

I used a generalized linear mixed model with a logit link and gamma probability distribution with fixed questions and a random location variable to investigate differences in respondent opinion based on the number of traps fished (PROC GENMOD; SAS Institute Inc., Cary, N.C.). Gamma probability distribution was used because it was the best fit among distributions in the two-parameter exponential family. I also investigated if there was a relationship between the number of traps fished, the cost of a new bait, and willingness to try a new bait with a generalized linear mixed model with a logit link and binomial probability distribution with fixed questions and a random response variable (PROC GENMOD; SAS Institute Inc., Cary, N.C.).

4.4 RESULTS

I had 45 total responses from two parishes. Of the total respondents, 30 were from Terrebonne Parish and 15 were from St. Tammany Parish. Fishermen were asked to check all types of bait used. Of the Terrebonne Parish respondents, fishermen commonly use a combination of fish types for bait, 86.67% use menhaden and catfish, while 20% use menhaden, catfish, and other fish, such as mullet (Figure 4.1). A percentage of fishermen, 13.33%, use

catfish exclusively to bait traps, however no respondents indicating exclusive menhaden use. In contrast, 100% of St. Tammany Parish respondents report using menhaden, 93.33% exclusively, and 6.67% use catfish with menhaden. From both parishes, 91.11% of respondents use menhaden as bait, 31.11% of those exclusively. Catfish and other fish are utilized by 71.11% and 13.33% respectively of fishermen in both parishes. Of the more than 70% of fishermen who utilize catfish, 60% also use menhaden, 13.33% also use menhaden and other fish, and 8.89% use the bait exclusively. None of the explanatory models assembled were statistically significant.

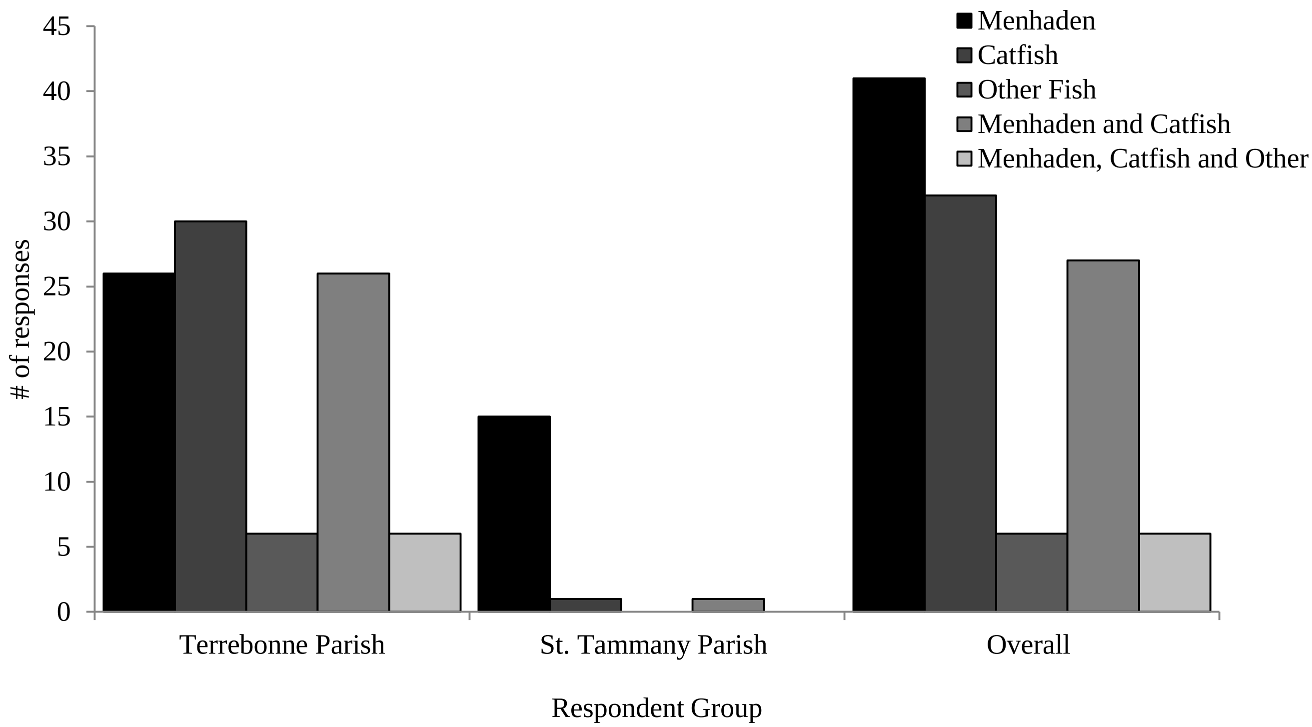

Figure 4.1: Current bait use of commercial blue crab fishermen in Louisiana. Number of respondents bait type use per parish and overall (n=45).

Individual fishermen reported running 50 to 800 traps per trip, with an overall mean of 362. In Terrebonne Parish fishermen reported the same range with a mean of 331. However, in St. Tammany Parish the range was smaller, 200 to 700 with a mean of 423 (Figure 4.2).

Monthly bait expenditure ranged from $100 to $11,000 depending on the number of traps and days fished. Terrebonne Parish fishermen reported spending a mean of $3,556 monthly on bait, whereas St. Tammany Parish fishermen spend approximately less than half of that amount, mean $1,476.

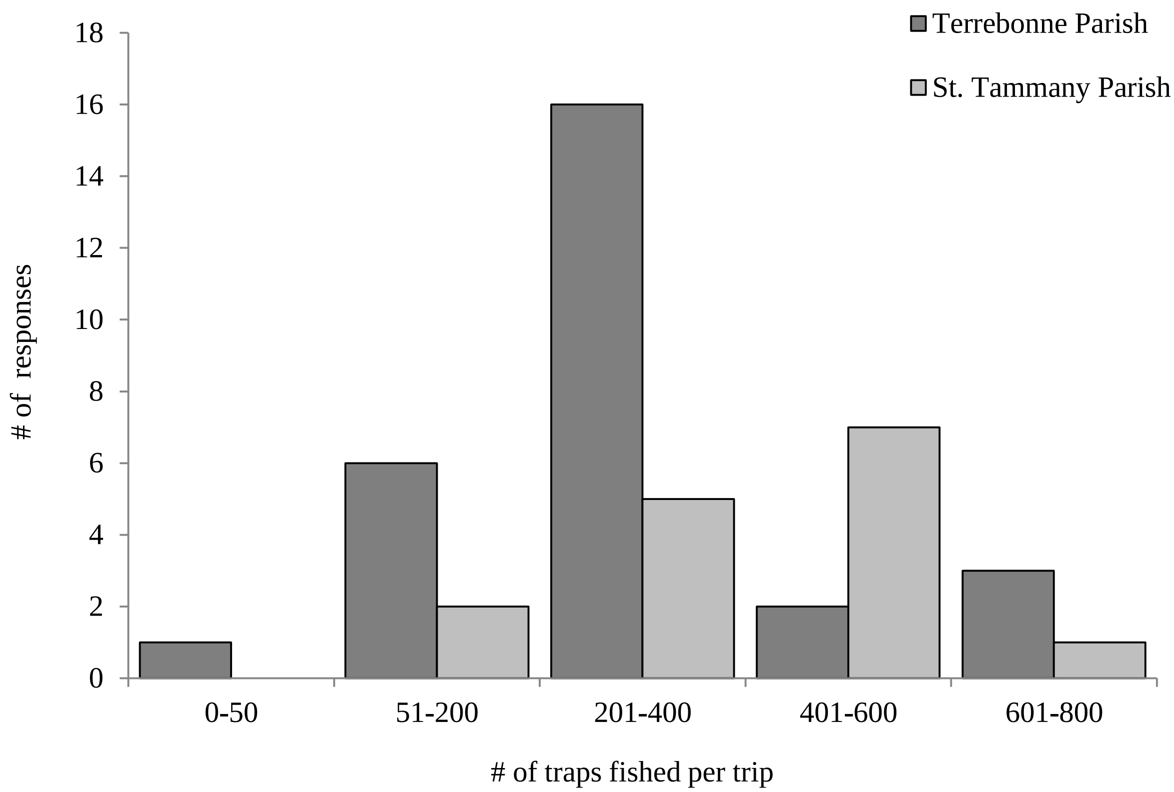

Figure 4.2: Number of traps run by fishermen. Number of traps fished by parish (dark grey = Terrebonne, light grey = St. Tammany) n=45.

Fishermen in both parishes soak traps a mean of three days, however depending on season, bait type, and catch rates traps are soaked for a range of 1 to 7 days. Fishermen in both parishes prefer to buy enough bait for one trip at a time. The majority of respondents, 70%, in Terrebonne Parish did not want bait that could be fished more than once. However, 66.67% of St. Tammany respondents would like bait that can be fished more than once. Overall,

respondents did not want bait that could be fished more than one (55.56%). The remaining respondents did want bait that could be fished more than once (42.22%), or did not care (2.22%).

Respondents preference for a bait that would float or sink was different by location. Responses from Terrebonne Parish preferred bait that would sink (96.67%) rather than float (3.33%). However, the majority of respondents from St. Tammany Parish did not have a preference (80%), while 6.67% and 13.33% preferred bait to sink and float, respectively.

The majority of respondents from Terrebonne Parish preferred bait packed in a bucket; however, St. Tammany responses favored bait packed in a box, 90% and 80% respectively. Eighty percent of Terrebonne respondents also preferred bait stored by refrigeration, whereas 93.33% of St. Tammany fishermen preferred bait stored by freezing. The combination of packaging and storage shows different preferences between the two groups of fishermen. Terrebonne fishermen prefer bait be sold in a refrigerated bucket, 70.59%, whereas 80% of St. Tammany fishermen prefer bait sold in frozen box. When asked about the costs of new bait, 95% of fishermen indicated they are willing to try an alternative bait if it costs less than current natural bait, 20% indicated they would be willing if it costs the same or less. Overall, 91% of fishermen interviewed are willing to try an alternative bait if it caught the same as the current natural bait utilized by the industry.

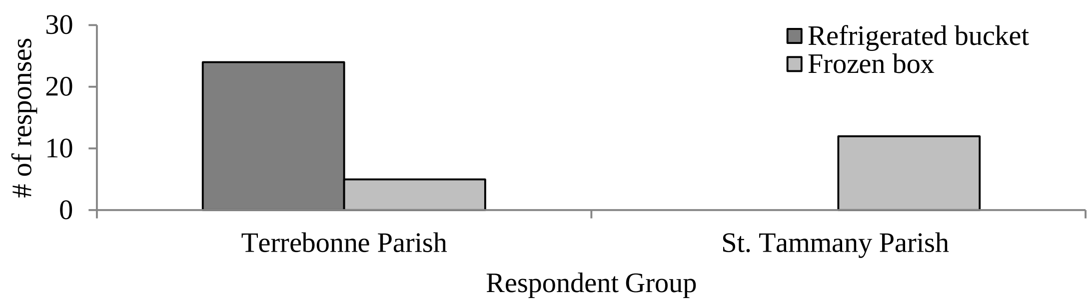

Figure 4.3: Storage and packing preference. The number of respondents by parish preferring a combination of boxes or buckets that are refrigerated or frozen (n=45).

4.5 DISCUSSION

More than 90% of commercial blue crab fishermen in Louisiana use Atlantic menhaden for bait and are willing to try an alternative bait, if it costs equal to or less than current natural bait. Each year, more than 8,000 MT of bait is used by blue crab commercial fishermen in Louisiana, more than 50% of which is Atlantic menhaden (DeAlteris 2012). Bait choice is a combination of fishermen preference, availability, cost, location, and tradition. In 2013, there were 3,667 licensed commercial fishermen in Louisiana, 60% of which were from seven parishes in Louisiana: 1) Terrebonne; 2) Jefferson; 3) St. Bernard; 4) Lafourche; 5) St. Mary; 6) Plaquemines; and 7) St. Tammany (AgCenter 2013). These seven parishes are located within the basins which account for the highest annual landings. The Pontchartrain, Terrebonne, Barataria and Atchafalaya/Vermilion-Teche Basins account for approximately 90% of blue crabs commercially landed in Louisiana since 1999 (DeAlteris et al. 2012).

This study shows an individual fishermen will run and average of 360 traps per season, with a range of 50 to 800. LDWF surveyed 211 commercial fishermen and 28 dealers to gather information on the number of traps run by individual fishermen (Guillory et al. 2001). The results of the LDWF survey are within the range of traps found in this study, although the information is reported by basin as opposed to by parish. Fishermen in the Vermilion-Teche Basin ran the most traps per fishermen, 513. Mississippi River Basin was second with 438, followed by 400 in the Atchafalaya River Basin, 282 in Barataria Basin, 274 in Pontchartrain basin, 205 In Terrebonne Basin, and 203 in Calcasieu River Basin (Guillory et al. 2001).

Currently menhaden is sold frozen boxes, but I found fishermen in Terrebonne parish would prefer to purchase bait in a refrigerated plastic bucket. Fishermen in St. Tammany preferred the current method of packing and storage. Using a plastic bucket would allow

fishermen to reuse the bucket and produce less waste than the current cardboard box used by the industry. In order for a new alternative bait to be effective, it must be adopted by commercial fishermen in the industry. Taking into consideration what properties commercial fishermen consider important will increase their willingness to try an alternative bait.

4.6 LITERATURE CITED

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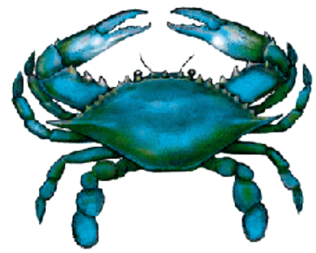
APPENDIX I: INDUSTRY INTERVIEW

WHAT WOULD YOU LIKE IN A CRAB BAIT?

We are working on a blue crab bait at LSU. We would like to know what you want!



1) What type of bait do you use? (please circle all that apply)
   a. Atlantic pogy/ menhaden
   b. Local fish
   c. Catfish (Hardhead, Gafftop)
   d. Other (Please list) ___________________________

2) How many traps do you run per trip? __________________

3) How much bait would you like to buy at one time? Enough for ________ trips?

4) If you could buy bait for more than 1 trip, how would you like it to be stored?
   a. Frozen
   b. Refrigerated
   c. Shelf
   d. Don't care

5) How would you prefer bait be packed?
   a. Box
   b. Bucket
   c. Plastic Bag
   d. Other (Please list)________

6) Would you like bait that can be fished more than once?
   a. Yes
   b. No
   c. Don't care

7) Do you want bait that _____?
   a. Floats
   b. Sinks
   c. Don't care

8) Would you be willing to use a new bait if it caught the same?
   a. Yes
   b. No
   c. Not sure

9) Would you be willing to use a new bait if it cost _____? (Circle all that apply)
   a. The same
   b. Less
   c. More
   d. Won't try

10) What range of soak times would you want a bait to have? __________________________ days

11) How much do you spend on bait each month? $_______________________________________

12) Is there anything else that you consider important?

If you have questions or want to know more contact Julie Anderson.
Phone: 225-578-0771 E-mail: janderson@agcenter.lsu.edu

APPENDIX II: IRB APPROVAL

LSU AgCenter Research & Extension

LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Human Ecology
209 Knapp Hall
225-578-1708
mkeenan@agctr.lsu.edu

Application for Exemption from Institutional Oversight

All research projects using living humans as subjects, or samples or data obtained from humans must be approved or exempted in advance by the LSU AgCenter IRB. This form helps the principal investigator determine if a project may be exempted, and is used to request an exemption.

- Applicant, please fill out the application in its entirety and include the completed application as well as parts A-E, listed below, when submitting to the LSU AgCenter IRB. Once the application is completed, please submit the original and one copy to the chair, Dr. Michael J. Keenan, in 209 Knapp Hall.

- A Complete Application Includes All of the Following:
  (A) The original and a copy of this completed form and a copy of parts B through E.
  (B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  (C) Copies of all instruments and all recruitment material to be used.
    - If this proposal is part of a grant proposal, include a copy of the proposal.

  (D) The consent form you will use in the study (see part 3 for more information)
  (E) Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
  Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: Dr. Julie Anderson **Rank:** Assistant Professor **Student?** N
Dept: SRNR **Ph:** 578-0771 **E-mail:** janderson@agcenter.lsu.edu
2) Co-Investigator(s): please include department, rank, phone and e-mail for each

- If student as principal or co-investigator(s), please identify and name supervising professor in this space

  A. Nikki Anderson, MS student

3) Project Title: Development of an alternative bait for the Louisiana commercial blue crab (*Callinectes sapidus*) fishery
4) Grant Proposal?(yes or no) NO **If Yes, Proposal Number and funding Agency** ____

Also, if Yes, either: **this application completely matches the scope of work in the grant Y/N**__
OR
more IRB applications will be filed later Y/N__

5) Subject pool (e.g. Nutrition Students) Commercial blue crab fishermen

- Circle any **"vulnerable populations" to be used:** (children<18, the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted.

6) PI signature Julie Anderson ****Date** 5/5/2014 (no per signatures)

****I certify that my responses are accurate and complete.** If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted √ Not Exempted____ **IRB#** HE14-6

Reviewer Michael Keenan Signature Michael Keenan Date 5-9-2014

Part 1: Determination of "Research" and Potential for Risk

- This section determines whether the project meets the Department of Health and Human Services (HSS) definition of research involving human subjects, and if not, whether it nevertheless presents more than "minimal risk" to human subjects that makes IRB review prudent and necessary.

1. Is the project involving human subjects a systematic investigation, including research, development, testing, or evaluation, designed to develop and contribute to generalizable knowledge?
(Note some instructional development and service programs will include a "research" component that may fall within HHS' definition of human subject research)

__X__Yes

____No

2. Does the project present physical, psychological, social or legal risks to the participants reasonably expected to exceed those risks normally experienced in daily life or in routine physical or psychological examination or testing? You must consider the consequences if individual data inadvertently become public.

____**Yes Stop.** This research cannot be exempted—**submit application for full IRB review.**

__X__**No Continue** to see if research can be exempted from IRB oversight.

3. Are any of your subjects incarcerated?

____**Yes Stop.** This research cannot be exempted—**submit application for full IRB review.**

__X__No Continue to see if research can be exempted from IRB oversight.

4. Are you obtaining any health information <u>from a health care provider</u> that contains any of the identifiers listed below?

A. Names
B. Address: street address, city, county, precinct, ZIP code, and their equivalent geocodes. <u>Exception for ZIP codes:</u> the initial three digits of the ZIP code may be used, if according to current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combining all ZIP codes with the same three initial digits contains more than 20,000 people; and (2) the initial three digits of a ZIP code for all such geographic units containing 20,000 or fewer people is changed to '000.' (Note: The 17 currently restricted 3-digit

VITA

Angelle Nicole Anderson was born.