Study and Evaluation of Bigano Snail (*Stramonita haemastoma*) as a Marketable Seafood Species

by

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Abstract

To evaluate whether a species is potentially edible, marketable and sustainable, we need to answer quite a few questions about what, why, where, who, when and how. This dissertation consists of five chapters. The basic question including what is bigano snail, why do we want to harvest and utilize bigano snail, where can they be found, who will harvest them and how to utilize them were answered in the literature review. The properties of fresh bigano snail were evaluated in the second chapter. The quality and marketability standards were established in the third chapter. The case studies of dry cold storage methods on the two subspecies of bigano snails over the storage time were evaluated in the fourth and fifth chapters.

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CHAPTER ONE: REVIEW OF LITERATURE

In this review of literature, the following basic questions will be answered: what is bigano snail, why do we want to harvest and utilize bigano snail, where can they be found, who can harvest them and how can we utilize them.

What is bigano snail?

Distribution and Taxonomy

Bigano snail (Stramonita haemastoma), also commonly called oyster drill and red-mouthed rock whelk, is a predatory gastropod of oysters, barnacles and other gastropods (Rilov et al. 2004, Butler 1985, López et al. 2010). This species is widely distributed in tropical and warm water regions including Gulf of Mexico, Caribbean Sea, western and southwestern Africa and Mediterranean Sea (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). It is under subfamily of Rapaninae and Genus Stramonita. The Genus Stramonita was separated as Purpura and Thais a decade ago and combined recently, but the classification is still under developing (Claremont *et al.* 2013b). In the southeastern United States, two subspecies are general recognized-Stramonita haemastoma floridana and Stramonita haemastoma canaliculata (Harding and Harasewych 2007, Walker 1982).

Traditionally, the two subspecies are separated by distribution, shell length, number and size of spine, and depth of the suture (Abbot 1974). Stramonita haemastoma floridana is generally ranged along the Atlantic coastline, and Stramonita haemastoma canaliculata is mainly from the Gulf of Mexico, larger in size and has strong, rugose shoulder nodules and deep-channeled suture. However, the recent observation and genetic based researches is still challenging the classification (Claremont *et al.* 2011, Claremont et al. 2013b, Abbot 1974, Walker 1982).

Figure 1. Typical picture of bigano snail (Stramonita haemastoma)

Population density in different regions

S. haemastoma is considered in a severe invasive species in some regions due to the rapid expended population and threaten to the native species (López et al.

2010). *S. haemastoma* can be commonly found in rocky littoral habitats in the regions mentioned earlier (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). And it is a keystone species in many of these regions, which is considered to be able to determine the structure and organize of the intertidal communities (Issaris et al. 2012). Rilov et al. (2001) studied the densities of S. haemastoma population along the Israeli Mediterranean coast, they observed very low (< 0.5 individual/m^2) density in most habitats and locations. And they expected even in the mid-littoral habitats where food and shelter are abundant the density will be around 7 snails/m^2). They concluded the possible causes to be salinity reduction, pollution from organotin material and predator of the larvae.

At the Cabo Frio upwelling tropical region on the southeastern Brazilian coast, S. haemastoma was the most abundant benthic predator, its density was 11.0±0.92 individual/m^2 (Rilov *et al.* 2002). In another literature, in the Ubatuba region also on southeast Brazil coast, the density of *S. haemastoma* was 3.4±7.8 individual/m^2 to 4.4±10.3 individual/m^2 on exposed shore and 0.1±0.6 individual/m^2 to 0.8±3.1 individual/m^2 on sheltered shore (Christofoletti et al. 2011). All literature above indicated a relatively low threat from bigano snail.

However, the historical record of population density of S. haemastoma can be as high as 150 individual/m^2 in western Mississippi Sound (Gunter 1953), 222

individual/m^2 in Santa Rosa Sound, FL (Butler 1985) and 1290 individual/m^2 in lake Maracaibo, Venezuela (Rodriguez 1963).

Life History and Anatomy

S. haemastoma can live up to 20 years in Florida with a generation time of 12 month respectively (Butler 1985). It is also a teleplanic (long-distance) species, when growing at comparable temperatures (24 ^{o}C), its larvae can remain planktonic for more than 90 days (Dobberteen and Pechenik 1987). Larvae of *S. haemastoma also* produces copious amounts of mucus, which may assist in the elimination of rejected food particles or in food collection activities (Dobberteen and Pechenik 1987).

When a dramatic change in the character of the shell occurs with a thin, knifelike varix formed that separates the smooth larval shell from the granulated adult shell, the larvae stage ends (Butler 1985). This usually occurs in month old drills of about 10 mm length, while shell still holds 75 to 80% of the total wet weight (Butler 1985). Juvenile snails grow rapidly. Drills with a mean length of 35 mm can grow about 12.2 mm after 3 month in south Florida (Ingle 1953). However, most drills die when reach 2 years old after only spawning once (Menzel and Nichy 1958) and the growth vary individually.

In extreme case of protected habitat, some drills could reach a length of 55 mm at 6 month and were larger than other drills known to be 3 years old (Butler 1954). However, male and female drills grew at similar rates from above experiment. Due to the complexion of classification, the maximum shell length from historical research may not be accurate anymore. However, *S. h. floridana* traditionally can reach 75 mm in length and *S. h. canaliculata* can reach 105 mm in length (Abbot 1974).

The spawning of *S. haemastoma* is temperature dependent and initiated at about 21°C. Depending on water temperature in the region, spawning may occur as early as January and continue sporadically through October (Butler 1954). Spawning usually occurs at salinity levels above 20 ppt. A salinity drop from 27 ppt to 13 ppt for several days did not appear to damage the embryos, a change from 27 ppt to 4 ppt would kill the embryos in 20 min (Butler 1985).

Female *S. haemastoma* normally lays fertile eggs in elongated, conical shaped capsules (Lahbib *et al.* 2011). The capsules are then attached to a hard substrate by a short stem (D'Asaro 1966). According to Brown et al. (2004), *S. haemastoma* at the subtidal, estuarine oyster reef can produce on the average 2,021 embryos per capsule around New Orleans area in Louisiana. However, the number per capsule can vary from hundreds to more than 6000 (Butler 1985, Lahbib et al. 2011). The eggs are fertilized in the oviduct at some point near the albumen gland before capsule formation ((D'Asaro 1966). Copulation may be observed in early spring

when mass spawning occurs (Butler 1985). The egg capsules are creamy yellowish first deposited then become brown when the eggs mature, but turn purple only when empty or when the developing embryo died in the capsule. At a mean temperature of 25°C, the larval development requires about 2 week after the formation of the first polar body (D'Asaro 1966).

Although I am unable to find an overall description of the gross and microscopic anatomy of S. haemastoma, I found figures for close related species (Figure 2 and Figure 3) and a generalized scheme of the anatomy of a male bigano snail (Figure 4).

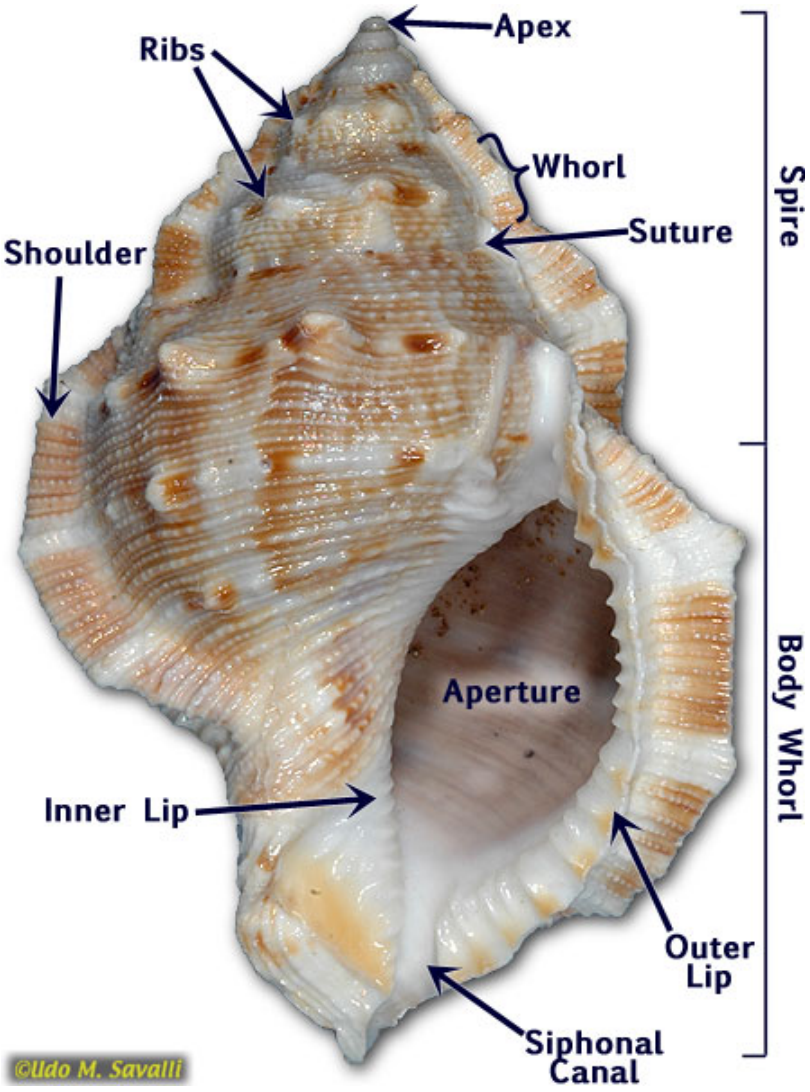

Figure 2. Shell structure of Gastropod *Bursa californica* (Hinds, 1843)

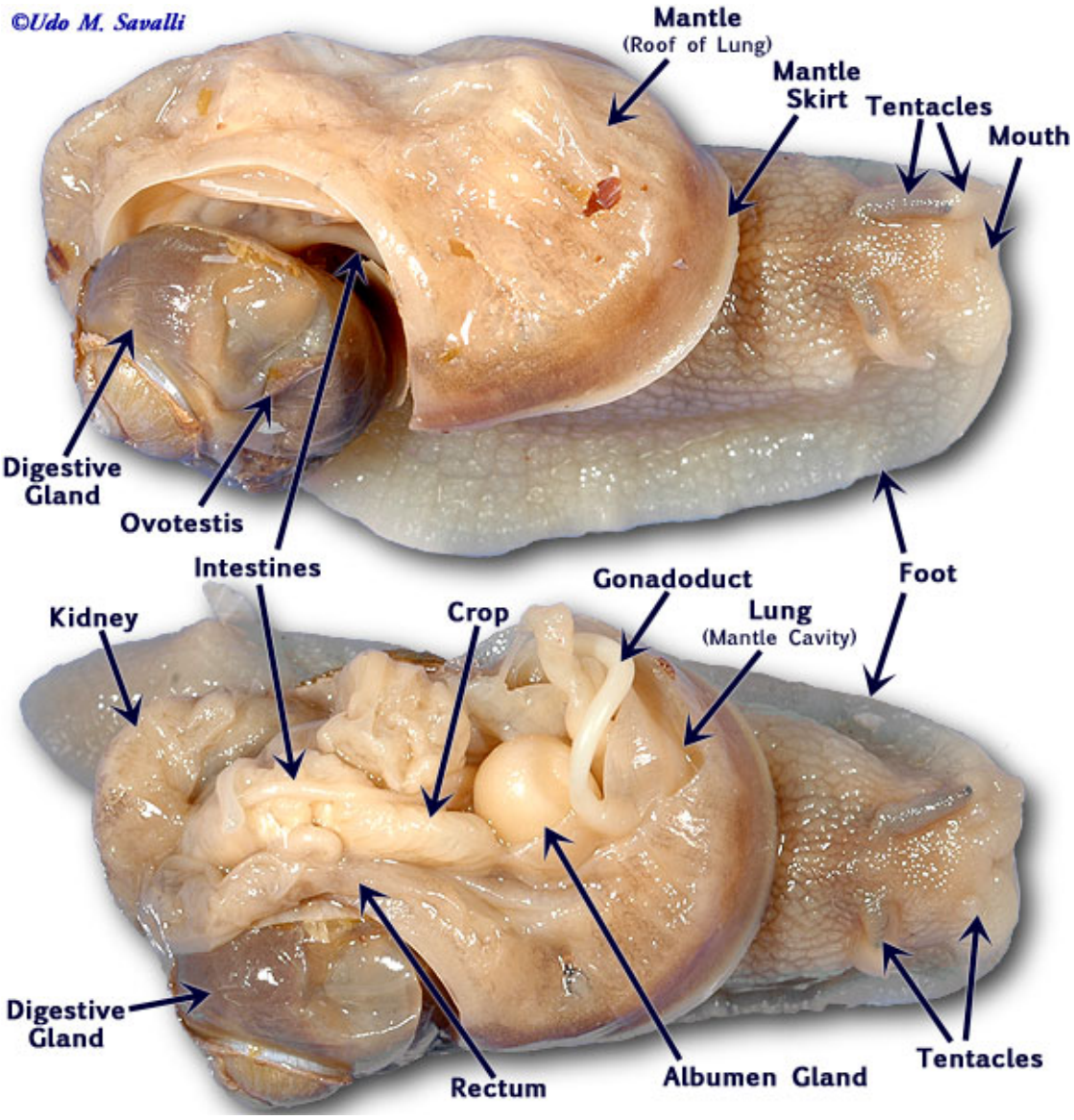

Figure 3. Dissection of Gastropod Helix aspersa (O. F. Müller, 1774)

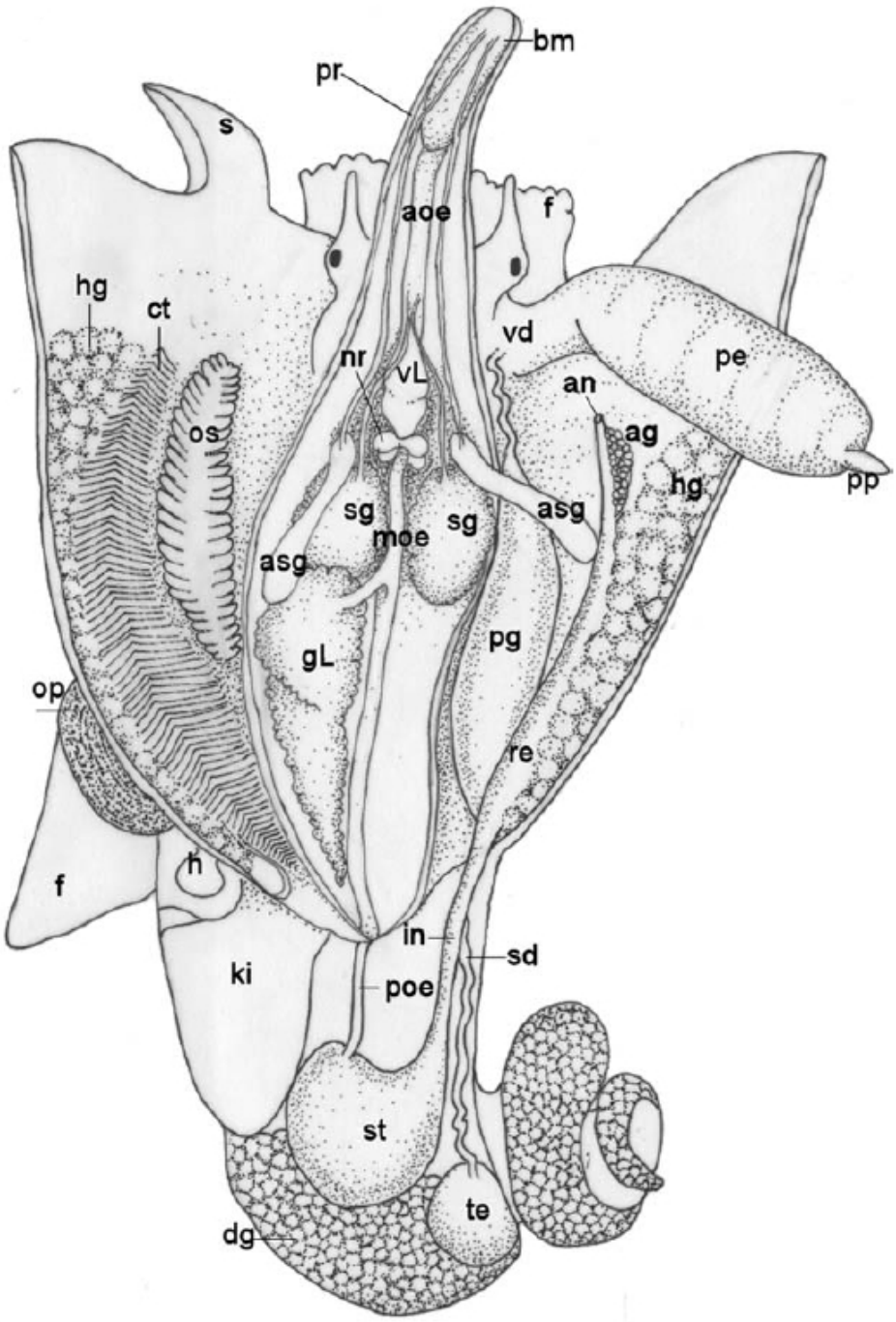

Figure 4. A generalized scheme of the anatomy of a male bigano snail
Mantle longituainally dissected, body wall not shown. ag, anal gland; an, anus; aoe, anterior oesophagus; asg, accessory salivary gland; bm, buccal mass; ct,

ctenidium; dg, digestive gland; f, foot; gL, gland of Leiblein; h, heart; hg, hypobranchial gland; in, intestine; ki, kidney; moe, middle oesophagus; nr, nerve ring; op, operculum; os, osphradium; pe, penis; pg, prostate gland; poe, posterior oesphagus; pp, penial papilla; pr, proboscis; re, rectum; s, siphon; sd, spermiduct; sg, salivary gland; st, stomach; te, testis; vd, vas deferens; vL, valve of Leiblein. Figure is modified from Hershler and Ponder (1998) and Cioni *et al.* (2012).

Foraging Behavior and Boring Motion

Bigano snails first locate prey using waterborne chemical cues, then attack bivalve prey at the shell margins by repeated alteration of radular scraping and acid secretions from an accessory boring organ on the foot (Byron and Smee 2012, Butler 1985, Brown 1997). When a large enough hole is created between the valves, the snail inserts the proboscis in and injects a toxin and proteolytic enzymes to paralyze the oyster tissue. Oyster tissue is then consumed through the proboscis until oyster adductor muscles tire, and then the oyster gapes while all the remaining tissue is lost (Brown and Alexander Jr 1994).

Although traditionally *S. haemastoma* was considered to boring holes on its prey and extract the muscle part inside the shell as explained above, several researches show it has a high ability to adapt its foraging behavior to the environment by alternating the prey, changing the prey strategy and using different technique. López et al. (2010) found *S. haemastoma* selectively preyed on exotic bivalve which takes shorter handling time. It even chipped the small preys (<25 mm) to insert proboscis and open the valves without the drilling.

Brown and Alexander Jr (1994) studied the group foraging behavior of *S.*

haemastoma and found snails would join oysters already under attack rather than they initiated an attack, joined with others to initiate an attack, or fed alone. The appearance of predator species also affects the foraging behavior of *S. haemastoma* (Fodrie *et al.* 2008, Fodrie *et al.* 2012). When stone crabs (*Menippe adina*) and bigano snail foraging together generated higher than expected oyster mortality based on each species operating independently (Fodrie et al. 2008, Fodrie et al. 2012).

S. haemastoma adapts its feeding habit to worm reef also. In Walton Rocks Beach, Florida, S. haemastoma feeds on P. lapidosa by inserting the proboscis deep into a worm's tube and developed a longer proboscises than normal bivalve-feeding conspecifics (Watanabe and Young 2006).

S. haemastoma often determines the size of the prey bases on its own size. Juvenile S. haemastoma feed on small prey such as bryozoans, hydroids, barnacles and oyster spat (Fodrie et al. 2008, Rilov et al. 2001). And as they grow, large S. haemastoma prefers large mussels over small ones and is able to completely consume the soft tissue of large mussels, unlike medium-sized individuals.

Salinity and temperature tolerance

Bigano snail is most active at water temperature of 30 ^{o}C and cut off feeding when temperature low to 10 ^{o}C (Garton and Stickle 1980). This also verified the lower density and activity of the species in winter (Palmer 1990, Rilov et al. 2001)

In the northern Gulf of Mexico, the activity of bigano snail is limited by the critical temperature of 12°C, and beneath this point, feeding stops and drills tend to disappear beneath rocks and shells or bury themselves in the substrate (Butler 1954).

Larvae of bigano snails are effectively distributed by water currents, and their presumably lengthy planktonic existence facilitates dispersal of the species and repopulation of areas devastated by periodic low salinities.

Adult S. haemastoma may survive at salinities as low as 5 ppt, and the egg capsules can even survive and release viable larvae at salinities possibly low to 3.5 ppt (Stickle 1999). Planktonic larvae may survive up to 5 days when exposed to 10 ppt, but high mortality will occur when the salinity drops below 15 ppt (Wells 1961, Roller and Stickle 1989).

Tidal fluctuations of salinity also have a profound effect upon the osmotic and ionic composition of S. haemastoma (Stickle and Howey 1975). Stickle and Howey found when snails transferred to higher salinities, they will permanently lose weight even after transferring back to control salinity, the result is also confirmed by Pierce (1971b) on mussels. Pierce noticed when gastropod specimens transferred to a lower salinity extruded solute as a means of regulating cell volume, but would lose the volume after transferring back to higher ambient salinity. The source of cell solute was then found to be intracellular free amino

acids (Pierce 1971a). This result also applies to S. haemastoma (Stickle and Howey 1975).

Predators and parasites

Starved bigano snails may prey on each other, and the crown snail, Melongena corona (Gmelin), will adapt to a drill diet. Stone crabs, Menippe mercenaria (Say), will feed on them (Gunter 1968). Drills can be damaged in nature by commensals residing in and gradually eroding their hosts' shells, especially the boring sponge Cliona truitti, the boring clam Diplothyra smithii Tryon and the blister worm Polydora spp. (Butler 1985). Scyphozoan Rhopilema nomadica has been recently indicated to be the responsible for veliger mortality and reduced recruitment of the predatory whelk S. haemastoma (Rilov et al. 2001).

Why do we want to harvest and utilize bigano snail?

Economic importance

As mentioned in the review of literature, bigano snails are highly adapted to the environment and threaten native and exotic commercial bivalves. By extrapolation, an average drill can destroy 1,000 to 3,000 oysters during its lifetime (Butler 1954). It can decimate oyster beds and present an ongoing challenge to oyster farmers and oyster fishermen (Ray and Benefield 1997, Eberline 2012). This has direct impact on economic scale of coastal development (Butler 1985).

Reduce the prey/predatory tension at the intertidal area

S. haemastoma is a predator species of many filter-feeding bivalves. For conservation purpose, filter-feeding bivalves purify water column, remove superabundant nutrients and reduce the suspended solid (Nakamura and Kerciku 2000, Inoue and Yamamuro 2000). Overall, the filter-feeding improve the dissolved oxygen concentration in the water column, and reduce the risk of disease and harmful algae bloom (Ostroumov 2002, Ostroumov 2005). Thus, from food web aspect, ideally reduce and regulate the population density of the direct predator will promote the population of the downstairs level (George *et al.* 2008, López et al. 2010, Fodrie et al. 2008). But since the intertidal community is rather

complicate with multiple direct and indirect trophic level, the actually result is still uncertain.

Nutrition source, pharmaceutical use and dye production

Bigano snails are highly related to a very important commercial editable snail in Asian region *Rapana venosa* and it also produces one of the most expensive natural dyes-Royal purple (Claremont *et al.* 2013a, Koren 2008, Koren 2001, Reese 2010). The edible portion of the snail (the foot) is potentially marketable and similar species are considered delicacies in various cultures (Udofia 2009), and the lipids and amino acids are potentially beneficial for the recovery of skin burns (Badiu *et al.* 2008, Badiu *et al.* 2010). Local fisherman along the Gulf Coast have eaten the bigano snails for decades (Horst and Horst 2011), and there has been some recent experiments with serving bigano snails as sustainable seafood mainly in Houston, Texas (Cook 2011).

Where can they be found and who will harvest them?

Due to the food source, shelter and wave energy, bigano snails can be found in intertidal area with relatively more shelter place and abundant food supply. However due to the water temperature and salinity change along the season of the year, the “hotspot” place can be different. Who will harvest the bigano snail varies, and following harvest method will be slightly different also. The harvest method can be concluded into specific and general. The specific method includes hand picking and trapping. They can be use to specify the target subject to bigano snails. While the general harvest of oyster or other benthic products can potentially harvest bigano snails as by-products. At this stage, local fishermen usually harvest them as by-catch. However, any person with a valid state fishing license should be able to harvest them from public water.

How can we utilize bigano snail?

In order to answer the question how can we utilize bigano snail. I separated the overall snail into two components-shell and body. The shell is defined as the calcium carbonate based hard protection structures in this study, which includes both the outer case and operculum. And the body includes all the soft tissues in between the outer case and operculum in this study. Due to the limitation of the physical and chemical properties of the outer case, it could be utilized ideally as other calcium carbonate based structures for limestone production or raw material for glass, ceramic and construction productions (Wedepohl and Baumann 2000, Lee *et al.* 2008, Feathers 1989, Doran 1965).

The body generally includes body wall, anal gland, anus, anterior oesophagus, accessory salivary gland, buccal mass, ctenidium, digestive gland, foot, gland of Leiblein, heart, hypobranchial gland, intestine, kidney, middle oesophagus, nerve ring, operculum, osphradium, penis and/or vas deferens, prostate gland, posterior oesophagus, penial papilla, proboscis, rectum, siphon, spermiduct, salivary gland, stomach, testis and valve of Leiblein (Figure 3 and Figure 4). The recognized edible portion is the foot (Udofia 2009). However, the foot can be contaminated by secretions from the glands naturally or accidently. Thus, recognize the direct contact toxic glands to prevent the contamination become critical important to the utilization of bigano snails.

According to Carriker (1981), bigano snail presumably introduce a paralytic toxin into the mantle cavity of its prey after boring holes in the margin of the prey's shell to immobilizing the prey for predation purpose. And this toxin is suspected from primary and/or salivary or hypobranchial gland due to the studies of other muricids (Power *et al.* 2002, Endean 1972, Minniti 1986). Thus, I narrowed the answer to the question into 3 directions: 1) Are salivary glands of *S. haemastoma* edible? 2) Is hypobranchial gland of *S. haemastoma* edible?

Are salivary glands of *S. haemastoma* edible?

According to Figure 4, the salivary glands in *S. haemastoma* are relatively large and well developed. Huang and Mir (1972) found salivary gland extract of S. haemastoma was found to be a powerful vasodilator and hypotensive agent with a 43 mg/ kg LD_{50} in mice. In cats, the toxin caused a slightly pulse rate drop and an increase in the respiratory rate. These movements were also observed on rabbit duodenum and guinea pig ileum by Huang and Mir. Although no report discussed the toxicity of primary salivary glands of *S. haemastoma*, the overall salivary glands should be removed and cleaned before human consumption.

Is hypobranchial gland of *S. haemastoma* edible?

Acoording to Huang and Mir (1971), the hypobranchial gland of S. haemastoma produced a yellowish viscid slime, and this slime was found to be toxic to mice with an LD_{50} of 215 mg./kg. This toxin produced a hypertensive effect in mammals, which seems due to the ganglionic stimulation and an increased cardiac output. Roller *et al.* (1995) also conducted a light and electron microscopical study on *S. haemastoma* and found the Region II of the hypobranchial gland is the primary region for the accumulation of the secretory product prior to release (Figure 5). They also observed the undergoing fast enzyme-catalyzed and photo-oxidative reaction of the color change of the secretion as in Michel *et al.* (1992) and current study. Roller et al. (1995) pointed out the function of this hypobranchial secretion can involve an olfactory response during the feeding process. And because this secretion possess a quite strong odor after the photo-oxidative reaction which can “linger on the tissues for days”, perhaps the secretion masks the “odor” of the prey once it has been opened.

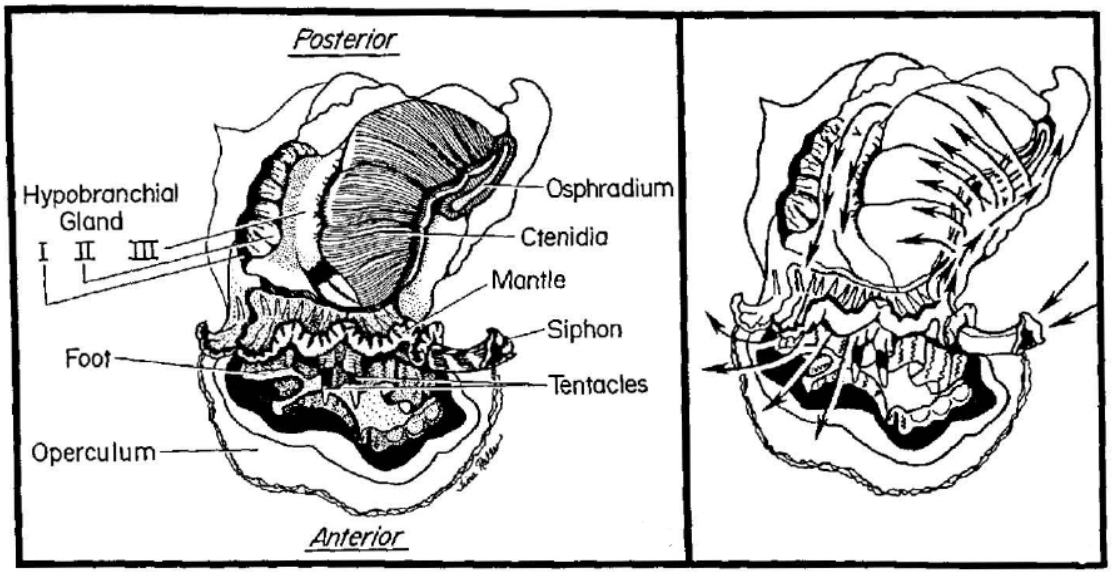

Figure 5. Diagrams of the dorsal aspect of *S. haemastoma* removed from its shell

The above information indicates the hypobranchial gland may not be a poison gland. However, it still should be removed as soon as the shell is opened to prevent and to eliminate the rapid color change of the secretion from this gland staining the edible foot portion. Further explanation of this decision will be discussion in the following chapter.

CHAPTER TWO: PROPERTIES

Introduction

Bigano snails (*Stramonita haemastoma*), also commonly called oyster drills and red-mouthed rock whelks, are a predatory gastropod. They are common predators of oysters, barnacles, gastropods, and bivalves (Rilov et al. 2004, Butler 1985, López et al. 2010). This species is widely distributed in tropical and warm water regions including the Gulf of Mexico, Caribbean Sea, and along the western and southwestern Africa and the Mediterranean Sea (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). They are under the subfamily of Rapaninae. In the southeastern United States, two subspecies are general recognized- *Stramonita haemastoma floridana* and *Stramonita haemastoma canaliculata* (Harding and Harasewych 2007, Walker 1982).

Developing bigano snails into a seafood product is based on cultural, economic and environmental aspects. First, bigano snails are closely related to a very important commercial snail that is eaten in Asian regions, *Rapana venosa.* It also produces one of the most expensive natural dyes - Royal purple (Claremont et al. 2013a, Koren 2008, Koren 2001, Reese 2010). The edible portion of the snail (the foot) is potentially marketable like similar species which are considered delicacies in other cultures (Udofia 2009). There are reports that local communities around the Gulf Coast have eaten the bigano snails for decades (Horst and Horst 2011).

There have been some recent experiments with serving bigano snails as sustainable seafood mainly in Houston, Texas.

For some *S. haemastoma* is considered a destructive invasive species due to the rapid expending populations and threat to the native species (López et al. 2010). It is a keystone species in many regions. Its large populations are considered to be able to influence the structure and organization of intertidal communities (Issaris it al. 2012). It can decimate oyster beds and present an ongoing challenge to oyster farmers and oyster fishermen (Ray and Benefield 1997, Eberline 2012). Because of their predatory nature, they have direct impacts on economic of coastal regions (Butler 1985).

Filter-feeding bivalves purify the water column, remove superabundant nutrients and reduce the suspended solid (Nakamura and Kerciku 2000, Inoue and Yamamuro 2000). They also help improve dissolved oxygen concentrations in the water column, and reduce the risk of disease and harmful algae bloom (Ostroumov 2002, Ostroumov 2005). Thus, from a food web aspect, reducing and regulating the population density of a predator, like bigano snails will promote healthy population development of other more desirable species of bivalves like oysters (George et al. 2008, López et al. 2010, Fodrie et al. 2008). Even with the complicity of multiple direct and indirect trophic levels within the intertidal

community, reducing the population of bigano snails can still temporarily reduce the predator stress of current commercial bivalve species.

Nutritional value and customer acceptance, are important for new product development, but are unknown for this species. Proximate composition, fatty acid composition, amino acid composition and mineral composition are chemical characteristics that have been used to evaluate the nutritional value of various seafood products (Sriket *et al.* 2007, Yarnpakdee *et al.* 2014, Maulvault *et al.* 2012). Color, texture and sensory evaluations have also been used in determining customer acceptance of a potential new seafood product.

In order to compare the result with other commercial seafood products, the color was measured using a CIE L*a*b system, and texture was measured by texture profile analysis (TPA). In the CIE L*a*b system, L* denotes lightness on a 0 to 100 scale from black to white; a* denotes red (+) or green (-); and b* denotes yellow (+) or blue (-) for better visualization of the reading. The chroma C* denotes the square root of ($a^{*2} + b^{*2}$)(Schubring and Meyer 2002).

TPA mimics the human bite behavior that compresses the network bonds until they reach their surface extension limits and break permanently (Wilkinson *et al.* 2000).

It can be used to indicate the tenderness and chewiness of the cooked edible muscle, and thus, to evaluate the potential customer acceptance.

Results of this test might also be useful to suggest potential processing method for the product based on the results of hardness, springiness, cohesiveness, gumminess, chewiness and resilience. Hardness is the peak force in the 1st compression curve. Springiness is the distance recovered by the sample during the time between the end of the 1st compression and the start of the 2nd compression. Cohesiveness is the ratio of the positive areas under the 2nd compression to the 1st compression. Gumminess is the product of hardness and cohesiveness, and chewiness is the product of gumminess and springiness, while resilience is the ratio of positive area under 1-2 to 2-3 in the first compression (Schubring and Meyer 2002).

Objective of this study was to use chemical and physical properties to determine the potential of bigano snails as a desirable new seafood product. Chemical properties to be analyzed include proximate composition and amino acid profile. Physical properties to be evaluated include color (shell and muscle) and texture.

Material and Methods

Sampling preparing

All samples were harvested from coastal waters of the Gulf of Mexico along the coasts of Alabama and Texas. All samples were the same species, *Stramonita haemastoma* but may have been two subspecies. Those from Texas may have been *Stramonita haemastoma canaliculata* and those from Alabama may have been *Stramonita haemastoma floridana*.

Samples from Galveston Bay, Texas (received on May 2nd, 2013 and on June 12th, 2013) were provided by personnel from Jeri's Seafood, Houston, TX. Samples from Mobile Bay, Alabama (received on May 21st, 2013 and on June 25th, 2013) were provided by personnel from the Auburn University Shellfish Laboratory, Dauphin Island, AL

After harvest, samples were shipped overnight in insulated chest with frozen gel-packs to Auburn, AL for analysis. Upon receiving they were inspected for survival. Survival was tested by evaluating the response of the muscle and mantle on each specimen. If the foot muscle or the mantle shrank back immediately after touch, the individual was considered alive. Otherwise, the individual was considered dead. Only live snails were used for further study. For each shipment, 60 specimens

were randomly selected. Each shell was measured (total length) and numbered. They were then placed in freezer bags and stored at -20 ^{o}C for additional analysis.

On the following day, each bag of snails was allowed to thaw while floating in warm water for 5 to 10 minutes. The meat (muscle) was then removed from the shell by cutting along the suture using diagonal pliers to avoid damage of body tissue. The meat portion, commonly referred to as the foot, was then separated from the rest of the body tissue and the operculum. The meats were washed in tap water, dried by paper towel and blended together to form a homogenous solution for analysis.

Proximate analysis

Moisture analysis

Moisture content of the samples was determined using a moisture analyzer, model MX-50 (AND Co. Ltd., Tokyo). The drying program was determined to be auto standard at 105 ^{o}C with raw blended sample spread upon a fiberglass pad.

Ash content

Ash content of the meat was estimated by heating the homogenized meat in a CEM Phoenix Microwave Muffle Furnace (Matthews, NC) at 550°C for 6 hours.

Lipids

Crude lipid content was determined following Folch's method using 1 part sample to 20 parts 2:1 chloroform/methanol solution in preliminary tests as descripted in (Pérez-Palacios *et al.* 2008). Because of the low lipid content (less than 3% from the preliminary result), fatty acid analysis was not included in this study..

Determine of protein content

The protein content of the meat was originally designed to be calculated from the difference out of 100% from moisture content, fat content and ash content. However, due to the difficulty of fat content measurement as mentioned above, a minimum protein content was calculated by

$$\text{Min Protein Content} = 100\% - \text{Moisture}\% - \text{Ash}\% - 3\%$$

Amino acid analysis

The complete and soluble profiles for amino acid were conducted by Advanced Analytical Testing Service (AATS, Ontario, CA). The hydrolysis procedure was modified from method 1 in United State Pharmacopeia Convention 35 Chapter 1052 protein hydrolysis (2012). The amino acid analysis procedure was modified from method 3 in United State Pharmacopeia Convention 35 Chapter 1052 appendix (2012).

Color measurement

On receiving day, the color of each shell from live snails was measured on 2 points by a MiniScan XE Plus instrument from Hunterlab (Reston, VA) using a CIE L*a*b system. On the following day after deshelling and briefly cleaning, each front and backside of the meat was measured on 2 points for meat color measurements before pooling the samples for further analysis. For each replicate, L*, a* and b* was the average readings of the 2 points. C* was then calculated using the average value.

Due to the minimum capture size required for use with the MiniScan XE Plus and the sizes of the meat from the AL samples (average 2.29 gram), only the samples from TX were measured for meat color for accurate result.

Texture Profile Analysis (TPA)

Sixteen meat portions were randomly selected from the deshelled samples and cooked using a method modified from Schubring and Meyer (2002). The meat portions were cooked in 1L boiling water on medium heat for 5 min, then allowed to drain and cool to room temperature for 45 min. The meats were then put in the center of the working platform and were compressed twice to 70% of their original height for 0.50 s using a cylindrical probe 2.5 cm in diameter. Test speeds for all pre-, actual and post-test were set as 5.00 mm/s. The trigger force was 5.0 g and

triggered automatically. Hardness, springiness, cohesiveness, gumminess, chewiness and resilience were measured.

Statistical analyses

All chemical analyses were measured in triplicate from each shipment. Shell color measurements were recorded for all 60 individuals in each shipment. Color measurements of the meats were measured on individuals from TX only. The TPA was measured for 12 replicates from each shipment. Since the sampling time and locations of this study may involve different subspecies and months, the differences among the mean values of 4 shipments were pre-tested using nested two-way analysis of variance (ANOVA) with shipments nested under sampling location. Statistically differences were reported at $p < 0.05$. If the mean values of different shipments for a parameter were not different, then the means from different shipments were used as replicates and were expressed as species mean value ± standard deviation (SD) ($n = 4$) for this parameter. If the mean values of different shipments were different for a parameter, then the results from each shipment were expressed as mean value ± standard deviation (SD) and separated by student's t-test. Statistical analysis was performed using JMP 10 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

During determination of the rapid drying process for the moisture analyzer in the infrared drying process, the samples should be separated evenly and homogeneously over the entire pan to maintain the thermal energy efficiency. However, the nature of our sample could not meet the requirement if in direct contact with the pan surface. Thus, a fiberglass pad was place in between the sample and pan surface to ensure an efficient drying process.

The drying temperature was determined according to Figure 6. The standard curve was based on a constant drying process at 100 ^{o}C using preliminary deshelled raw meat to mimic the AOAC method. The end point for the standard curve was 66.85% at 80.2 min. The end point for a constant 110 ^{o}C drying program was 67.45% at 48 min and the end point for a constant 130 ^{o}C drying program was 67.45% at 28.9 min. Both methods yielded a higher than standard curve ending value, which may reflect an inaccurate measurement. The three replicates of a constant 105 ^{o}C drying program from the same sample pool yielded 65.80% at 31.8 min, 66.30% at 32.2 min and 64.75% at 33.3 min. This indicated the 105 ^{o}C drying process was accurate and precise to alternate the standard oven drying measurement in this study.

Fat extraction

Preliminary tests showed the fat content of bigano snails from AL ranged from 1.04% to 2.53%, with SD of 0.84%. This result could reflect the individual differences due to the inefficient of the homogenizing procedure, since bigano snails in this study were wild caught with a variety of sizes, ages and represented both genders. This result could also be caused by the small sample size. To ensure precision of the weight differences for sample contents around 2% fat requires at least 50 gram of raw meat. With 3 replicates for the measurement, the 150 grams of meat would require around 75 snails and at least 6.5 hour of deshelling, which would cause more moisture weight loss than actual fat extraction weight difference. Improved deshelling procedures could improve the analysis.

Proximate composition

No statistical difference was found among the 4 shipments on either chemical characteristic, thus, shipments served as replicates for overall species results. Proximate composition of bigano snails along with some other existing seafood species for comparison is presented in Table 1.

Fat content from the preliminary tests was found to be around 2%. To ensure the minimum protein content was accurate, an extra 1% was counted away due to the high standard deviation and chance of low carbohydrate content in the muscle.

Raw bigano snails have relatively low moisture content, which was only exceeded by raw hardhead catfish dorsal muscle. Protein was found as the major constituent, indicating the bigano snail meat can be a good source of protein as a food product. Bigano snails also had compatible percentages of protein, on a wet weight basis, when compared with other common seafood species (Table 1). The pre-tested ANOVA didn't detect a statistical difference between the means of proximate compositions among different shipments. Subspecies, harvesting times, age, sex and location, can still be few factors causing the high deviation of results for this wild caught species in this study as reported in other seafood species (Dal Bosco *et al.* 2012, Karakoltsidis *et al.* 1995)

Amino Acid Analysis

No statistical difference was found among the 4 shipments for amino acid analysis. Thus, shipments served as replicates for complete and soluble amino acid composition analyses. The content of each Individual amino acid was converted into percentage of total amino acids to compare with other seafood species for flavor evaluation. The amino acid composition, by percentage for bigano snail meat, along with some other existing seafood species are shown in Table 2. Amino acids were different between species; however, the most abundant amino acid in both hydrolyzed and free amino acid for bigano snails, black tiger shrimp and white shrimp was arginine. Sikorski (1990) reported the abundant of free arginine

in crustaceans enriched the sweet taste and contributed to a seafood-like flavor. He also thought the sweetness of fresh prawn and crab is due to the high content of free glycine in their muscle. Fuke (1994) also reported alanine, proline, serine and threonine as amino acids for the sweet flavor. On the down side, leucine, valine, methionine, phenylalanine, histidine and isoleucine are normally associated with bitter taste (Sikorski 1990). Thus, for bigano snails the percentages of amino acides that favor sweet seafood-like flavor was 52.16% for hydrolyzed form and 46.40% for free soluble form. These values were higher than the other 4 popular seafood products (pomfret 29.8%, black tiger shrimp 40.76%, white shrimp 41.15% and rapana whelk 41.36%)(Table 2). Arginine content for the rapana whelk was missing from the original report. Bigano snails had the lowest percentage of amino acids that contribute to bitterness (18.56% for complete hydrolyzed form and 20.62% for the soluble form) comparing to 31.03% for pomfret, 37.10% for black tiger shrimp, 36.30% for white shrimp and 26.73% for rapana whelk. Amino acid wise, bigano snails can be considered a compatible, flavorful seafood species. Some researchers have reported that nucleotides and quatemary ammonium compounds (AMP and ATP in crustaceans and mollusk) are major drivers influencing the taste of seafood (Sikorski 1990, Mendes *et al.* 2001). Thus, further study and sensory panel are needed to evaluate the customer acceptance of this species.

Color measurement

Significant differences were found among the 4 shipments of sample for shell color measurements ($p < 0.05$) (Table 3). Sampling location (potential subspecies difference) and harvesting time may affect shell color. Test results indicate the yellow color contributed more to the color intensity than the green color. Samples received in June from both locations were darker and greener in shell color than the samples in May. Shell color in samples from the Alabama site were generally greener and bluer but with less color saturation. Samples received in May from Texas and samples received in June from Alabama had more intensity and yellow shell color than the other shipments received from the sample location. Thus, the initial color measurement and size can potentially be used to identify the source of shell stock based on the result of this study.

Since the final product will be the meat, the color of the meat could influence the customer acceptance. The results of the meat color measurements from 2 shipments from Texas with comparisons to other commercial snails are shown in Table 4. Both the front- and backside of meat reflects the harvesting month difference with lighter, redder and yellower the meat sample from the June shipment than from the May shipment. Customers generally prefer whiter and brighter meat in seafood products, since the darker color is thought to be related to the poor quality from experience (Sveinsdóttir *et al.* 2009). Thus, the in-shell side of the meat should be displayed to customers, if applied, since it is lighter in all

parameters than the exposed side of the meat. Also, from the result, the meat of bigano snails was brighter but yellower than other commercial snail products. Generally customers prefers brighter color and also dislike yellow pigments, the consumer acceptance of natural bigano snails requires further study. However, food-processing technologies are available to reduce the yellow color intensity for further developing.

Texture Profile Analysis

The result of nested ANOVA for texture parameters with meat weight range plus similar data from other commercial terrestrial snails are shown in Table 5. The hardness, chewiness and resilience of bigano snails were a hundred fold higher than the data from *Helix pomatia*, *Helix aspersa*, *Helix lucorum* and *Achatina fulica* using similar cooking and testing methods. The different body components, pre-process method, weight, height and species difference could be the main cause of the differences. In our study, only the raw meat was boiled and measured, but Schubring and Meyer (2002) used the whole body of the snails he tested while Sanchez-Brambila *et al.* (2002) reported the meat portion of snails in their study is tougher than the rest of the body. Schubring and Meyer (2002) used ready-to-eat canned snails. Canned food usually requires high stress steaming procedures, which tenderizes the meat. Besides, the meat portion in our study was smaller (0.48 to 2.29 gm from AL and 1.44 to 6.72 gm from TX, after cook) but using the

same cooking temperature and time, the extra heating process could yield tougher texture. The less height of meat (8.91 to 15.13 mm from AL and 14.29 to 27.35 mm from TX) could also yield increased hardness values, due to the difficulty of the compression. Considering all these factors, the meat of bigano snails was tough and hard to chew. Thus, physical or chemical processes are recommended to reduce the firmness of the final product. These processes might include grinding, high pressure cooking or enzyme tenderizing treatments (Sanchez–Brambila et al. 2002).

Conclusion

Meat of bigano snails is a good protein resource. Its amino acid profile indicates that it may have a sweet seafood-like flavor and is acceptable for both taste and color. A negative characteristic may be its tough texture. Further processing may be useful to reduce the firmness of the meat. Further studies are needed to gain more information about the nutrition value, sensory acceptance and harvesting source identity by shell color.

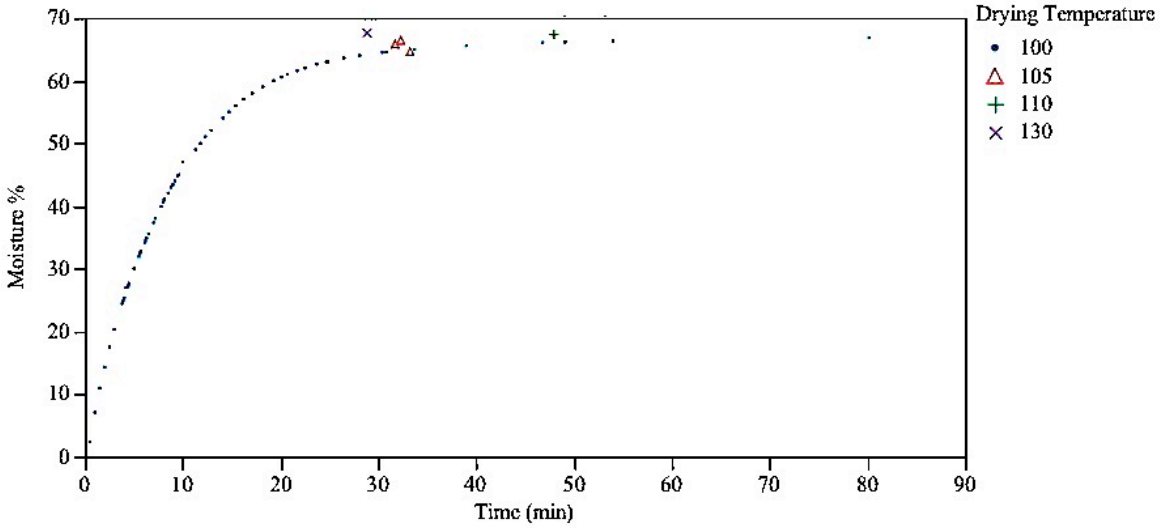

Figure 6. Moisture content over time using different drying program by a moisture analyzer

Table 1. Proximate compositions of bigano snail meat, black tiger shrimp meat, white shrimp meat, Nile tilapia dorsal muscle, broadhead catfish dorsal muscle and edible crab muscle.

Composition (% wet weight)	Bigano snail	Black tiger shrimp[1]	White shrimp[1]	Nile tilapia[2]	Broadhead catfish[2]	Edible crab[3]
Moisture	72.99 ± 1.75	80.47 ± 0.26	77.21 ± 0.18	82.1 ± 0.1	70.5 ± 3.9	76.3 ± 2.5
Ash	1.63 ± 0.42	0.95 ± 0.01	1.47 ± 0.10	1.0 ± 0.0	1.02 ± 0.1	2.2 ± 0.1
Protein	Minimum 22.38 ± 1.66	17.1 ± 0.56	18.8 ± 0.23	16.6 ± 0.1	17.5 ± 0.1	19.1 ± 1.7
Fat	-	1.23 ± 0.36	1.30± 0.09	0.2 ± 0.0	11.4 ± 0.2	0.6± 0.1
Carbohydrate	-	-	-	-	-	0.9± 0.1

Values are given as means ± SD from 4 replicate determinations.
[1] Sriket et al. (2007)
[2] Yarnpakdee et al. (2014)
[3] Maulvault et al. (2012)

Table 2. Amino acid composition by percentage of bigano snail meat, pomfret, black tiger shrimp, white shrimp and Rapana whelk.

Amino Acid	Bigano snail complete (%)	Bigano snail soluble (%)	Pomfret[1] (%)	Black tiger shrimp[2] (%)	White shrimp[2] (%)	Rapana whelk[3] (%)
Nonessential amino acid						
Cysteine	0.36	1.41	0.18	1.77	1.88	-
Serine	3.23	4.38	4.47	3.59	3.53	4.1
Tyrosine	1.79	2.07	4.21	6.56	6.75	2.65
Proline	5.12	6.11	3.07	9.69	13.26	4.66
Aspartic acid	7.86	7.78	9.14	4.88	5.85	3.8
Glutamic acid	12.44	8.65	13.7	6.22	5.16	11.8
Glycine	8.07	10.94	4.82	3.97	2.99	3.07
Alanine	0.36	0.42	5.93	5.12	5.50	22.76
Arginine	31.99	19.36	6.68	14.33	12.00	-
Essential amino acid						
Histidine	0.74	0.81	2.4	2.24	2.29	-
Methionine	1.41	2.06	2.8	4.68	4.46	2.67
Phenylalanine	2.34	2.51	5.12	7.64	6.75	7.78
Isoleucine	2.89	3.09	5.31	8.67	8.28	4.9
Leucine	7.09	7.71	9.21	9.98	10.83	6.6
Lysine	8.14	8.65	9.94	2.19	2.16	8.98
Threonine	3.39	5.19	4.83	4.07	3.88	6.77
Valine	4.09	4.44	6.19	3.89	3.70	4.78
Tryptophan	0.39	0.62	2	-	-	2.67

[1] Zhao *et al.* (2010)
[2] Sriket, Benjakul, Visessanguan, and Kijroongrojana (2007)
[3] Badiu et al. (2010)

Table 3. Color measurement and size of bigano snail shell from different sampling location and time on the receiving day.

	Shell Length (mm)	L*	a*	b*	C*
Samping Location(AL vs TX)		NS	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
AL (May vs June)		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
TX (May vs June)		$p = 0.0127$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
AL May	34.49-61.47	40.88 ± 0.48^{c}	$-0.73 \pm 0.15^{b,c}$	$8.34 \pm 0.27^{b,d}$	$8.47 \pm 0.28^{b,d}$
AL June	51.67-70.55	36.48 ± 0.48^{d}	$-1.62 \pm 0.15^{b,d}$	$10.44 \pm 0.27^{b,c}$	$10.61 \pm 0.28^{b,c}$
TX May	55.06-89.73	39.57 ± 0.48^{c}	$4.70 \pm 0.15^{a,c}$	$15.04 \pm 0.27^{a,c}$	$15.78 \pm 0.28^{a,c}$
TX June	65.05-92.36	37.85 ± 0.48^{d}	$-0.54 \pm 0.15^{a,d}$	$11.85 \pm 0.27^{a,d}$	$11.90 \pm 0.28^{a,d}$

Values are given as means ± standard deviation error from 60 replicate determinations.
NS not significant
[a,b] Same letter in the mean value column shows that there was no statistically significant
difference from different sampling location ($p < 0.05$).
[c,d] Same letter in the mean value column shows that there was no statistically significant
difference from the same sampling location but different time ($p < 0.05$).

Table 4. Color measurement of bigano snail meat from Galveston Bays, TX in 2013 May and June with terrestrial snails (*Helix pomatia*, *Helix aspersa*, *Helix lucorum* and *Achatina fulica*)

	Front				Back			
	L*	a*	b*	C*	L*	a*	b*	C*
TX May	52.1±0.48[b]	4.34±0.13[b]	15.44±0.34[b]	16.05±0.36[b]	67.14±0.49[b]	1.89±0.09[b]	9.43±0.23[b]	9.62±0.24 b
TX June	58.70±0.69[a]	5.16±0.19[a]	18.26±0.50[a]	18.98±0.52[a]	73.52±0.71[a]	2.84±0.13[a]	12.50±0.33[a]	12.84±0.34a
H. *pomatia*[1]	39.01	1.85	7.77	7.99	-	-	-	-
H. *aspersa*[1]	30.98	4.02	7.51	8.52	-	-	-	-
H. *lucorum*[1]	39.59	2.36	7.43	7.8	-	-	-	-
A. *fulica*[1]	40.31	2.97	6.27	6.94	-	-	-	-

Values are given as means ± standard deviation error from equal or less than 60 replicate determinations.

[1] Schubring and Meyer (2002)

[a,b] Same letter in the mean value column shows that there was no statistically significant
difference ($p < 0.05$).

Table 5. Texture profile analysis of bigano snail meat with terrestrial snails (*Helix pomatia*, *Helix aspersa*, *Helix lucorum* and *Achatina fulica*)

	Hardness	Springness	Cohesiveness	Gumminess	Chewiness	Resilience
Samping Location (AL vs TX)	p < 0.0001	NS	NS	NS	p < 0.0001	p < 0.0001
AL (May vs June)	p = 0.0018	NS	NS	NS	p = 0.0022	p = 0.0022
TX (May vs June)	NS	NS	NS	NS	NS	NS
AL May	3198.18[b,d]	-0.31	0.91	0.76	2409.06[b,d]	2220.854[b,d]
AL June	8849.61[b,c]	-0.49	0.94	0.75	6649.39[b,c]	6275.55[b,c]
TX May	14594.97[a]	-0.32	0.93	0.76	11142.673[a]	10280.95[a]
TX June	15517.12[a]	-0.65	0.88	0.76	11691.833[a]	10430.93[a]
H. *pomatia*[1]	23.52	0.74	0.5	-	8.68	0.3
H. *aspersa*[1]	18.54	0.63	0.4	-	4.78	0.2
H. *lucorum*[1]	27.42	0.71	0.49	-	9.63	0.27
A. *fulica*[1]	50.23	0.7	0.5	-	17.42	0.24

Values are given as means ± standard deviation error from 18 replicate determinations.

[1] Schubring and Meyer (2002)

[a,b] Same letter in the mean value column shows that there was no statistically significant difference from different sampling location($p < 0.05$).

[c,d] Same letter in the mean value column shows that there was no statistically significant difference from the same sampling location but different time($p < 0.05$).

CHAPTER THREE: QUALITY CONTROL

Introduction

The marketability of a seafood product is driven by profitability. Quality and safety are the demands from the customers, while the producers consider the gap between the selling price and the cost of production. Freshness is one main quality of seafood products. Live seafood products are often an important commodity in seafood markets due to the customer demand (Christophersen *et al.* 2008). However, live seafood is also perishable, which limits the shelf life. For example, shelf life of shellfish can be influenced by harvest season, harvest method, harvest location, differences in individuals, stress from handling, stress from shipping and stress from storage before further processing or consuming (Wingerter *et al.* 2013). This is true for domestic and export markets but usually much greater in items for export because of increased transport times. Dry shipping and storage at low temperatures is the most widely used method for live shellfish since it eliminates cost of added weight from seawater and reduces stress from the accumulation of metabolic products (Wingerter et al. 2013, Lorenzon *et al.* 2007). However, dry shipping and storage can also add stress and induce unwanted symptoms leading to a degraded product. Thus, finding the maximum tolerance time with minimal degrading demerits for dry shipping and storage will provide reference for better seafood trading performance.

Several studies have been conducted on live bivalves, crustaceans and echinoderms during transportation (Ridgway 2007, Basti *et al.* 2010, Group 1999). However, few studies have been conducted on live gastropod transport. One of the reasons may be the exposed foot is easier to dry out comparing to other species. This lack of recommended transport methods bring an ongoing challenge for harvesters to reach distant locations that may provide good markets. Another reason may be the narrow tolerance range to salinity and temperature of many shellfish species. The current handling methods usually include a prewash and transportation on ice, however, the unsealed shell of gastropods may react to critically low temperatures and low salinities from melted ice than bivalve and crustacean (Stickle and Howey 1975, Pierce 1971b, Pierce 1971a).

Besides a potential quality change during live transport and storage, increased difficulties in processing can also be a limiting factor for gastropods. The more difficult the processing procedures are, the greater the final selling price has to be (Monahan 1984). Currently the most wholesome and straightforward freshness sensory assessment method is the quality index method (QIM) (Esaiassen *et al.* 2013). However, this method is based on the familiarity of the existing seafood product. It does not take processing difficulty and uncertainty of unfamiliar new seafood species into consideration. This is important since markets require new products all the time. Many segments of the industry are facing overfishing or at

least conservation problems for high demand species. Better methods for new species are needed.

The bigano snail (*Stramonita haemastoma*), also commonly known as oyster drills and red-mouthed rock whelks, are predatory gastropods (Rilov et al. 2004, Butler 1985, López et al. 2010). This species is widely distributed in tropical and warm water regions including the Gulf of Mexico, Caribbean Sea, western and southwestern Africa and the Mediterranean Sea (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). It is found in the subfamily Rapaninae and genus Stramonita. It is a predatory species of many filter-feeding bivalves such as oysters, barnacles and gastropods. In the Southeastern United States, two subspecies are general recognized- *Stramonita haemastoma floridana* and *Stramonita haemastoma canaliculata* (Harding and Harasewych 2007, Walker 1982).

This species is chosen to be a model species to develop a visual-based-marketability-index is based on following reasons.

First, developing bigano snails into a seafood product is based on cultural, economic and environmental aspects (Ray and Benefield 1997, Eberline 2012, George et al. 2008, López et al. 2010, Udofia 2009). The best current markets for

bigano snails are overseas and in domestic seafood restaurants, both requiring long distance transportation and storage comparing to off dock sales.

Second, the FAO recommends immediately cooking of whelks after capture, but excessive boiling could also make the meat very tough and harder to remove from the shell (Waterman 1983). Keeping these snails alive during transportation and storage before inspection and further processing would be desirable if a good quality product could be obtained and a higher price received (Overaa 1999). When kept alive, the snails immune system could minimize pathogen hazards (Costa *et al.* 2009). However, the time tolerance and symptoms of live bigano snails during dry, cold transportation and storage is still unknown.

Third, bigano snails are gastropods and have similar structures (shell and exposed body) as common whelks, conchs and other snail species occurring in seafood markets. They also have a color secretion from a hypobranchial gland that will potentially stain the edible parts. A fast enzyme-catalyzed and photo-oxidative reaction of the color change of this secretion has also been observed by Michel et al. (1992), the color of the secretion changes from yellow to green, then blue and finally purple. The potential staining issues are not desirable but is another reason bigano snails are a good model species to conduct shelf life studies with. If

techniques can be developed to minimize stress and staining problems, the techniques should be useful for other gastropods.

Fourth, this species is currently supplied only from wild harvest, which may have more complicated shell characteristics related to the harvest season, harvest method, harvest location and individual differences. This gives a higher variety of characteristics, which may affect the difficulty of the processing and also could be used to identify the harvesting location at this stage.

According to US fishery products hazards and control guidelines (2011), natural toxins are species specific hazards related to whelks and sea snails. The processing procedure for similar gastropods including shell removing and gutting (Temelli *et al.* 2006). Shell removal while preventing contamination from potential toxin gland to the edible meat portion becomes a real challenge.

The objective of this study was to develop a systematic evaluation method for bigano snails using a visual-based-index. A visual evaluation process will be useful for harvesters, traders and final buyer when high mortality and odor are not significant and a rapid checklist for quality is needed. Such a system could also be useful for other commercial gastropod species, when considering a new species marketability evaluation.

Method and material

To develop the marketability index, a series of group discussions were held to gather customer opinions of the product and how its appearance changed during storage time. Dry cold storage was chosen in this study, since the most concerned issue for the gastropod is the moisture loss on the edible foot part. Because of the wild harvest, 30 specimens were tested on each observation day to ensure the normality of the data. Since intense sensory testing and cold storage quickly exhaust the senses of smell and touch and potentially could yield inaccurate result, only visual based observations were performed. The scoring system was based on a scheme for catch-damage-index (Esaiassen et al. 2013).

This process included three steps: potential customer group discussion for index guidelines, observations over time from multiple trials and development of a visual-based-marketability-index (VBMI) scheme.

Potential customer group discussion for index guideline

A group of potential customers familiar with shellfish were recruited to serve as an evaluation panel. The panel was asked to record changes over time of a series of characteristics. Samples were harvested from Mobile Bay, Alabama on September 28, 2012 and shipped in chilled coolers overnight to Auburn University.

In preliminary trials, 50% of individuals died in dry cold storage before 12 days after receiving the sample package. Thus, panel members in this study were asked to randomly select 10 snails and provide opinions of selected criteria over an 11-day period. The discussion sections were held on the receiving day (day 0), day 4, day 9 and day 11 to avoid weekends. A total of 6 observation categories were selected. They were shell color, meat color, discharge color, finger feel on the shell surface, smell and mortality test.

Observations over time from multiple trials

Sampling and storage condition

Two subspecies are commonly considered to exist in North America. Thus, two sampling locations were chosen to include both subspecies. Samples from Galveston Bays, TX (received on May 2nd, 2013 and on June 12th, 2013) were provided by personnel from Jeri's seafood, Houston, TX were *Stramonita haemastoma canaliculata*. Samples from Mobile Bay, AL (received on May 21st, 2013 and on June 25th, 2013) were provided by personnel from the Auburn University Shellfish Laboratory, Dauphin Island, AL. The Alabama snails were potentially *Stramonita haemastoma floridana*. All samples were harvested live and

shipped overnight to Auburn, AL in chilled cooler for analysis. After receiving, snails were stored in an open top container in a refrigerator (6°C ± 2°C). A total of 3,661 specimens were observed and sacrificed during the study.

Observation over time

On the receiving day, 60 snails were randomly selected, numbered and evaluated for condition (exterior test on Day 0 and interior test on Day 1). Then on odd days from the day received (day 1, 3, 5, 7 and etc.) until the day the mortality rate reached 50%, 30 snails were randomly selected, numbered and examined for exterior characters. All live snails were then packed in Ziploc® bags, stored in a freezer (-20°C) overnight. The following day, they were thawed, deshelled and examined for interior characters.

Exterior characters

The shell length was measured. The mortality rate was tested by evaluating the response after stimulation of the muscle and mantle. If the foot muscle or the mantle shrank back immediately, the individual was considered alive. Otherwise, the individual was considered dead. The character of the shell, except color, the color of the discharge, the characteristics of the foot and the presence of the operculum were recorded.

Interior characters

After deshelling, the color spot of the meat, the characteristics of the shell and meat were recorded.

Development of visual-based-marketability-index (VBMI) scheme

In order to design the VBQI scheme, the different characteristics were described and registered into categories. The severity of the character was considered according to the food safety concerns and possible economical losses due to downgrading of the product, because of lower yield, higher cost of processing or total loss of the product. The characteristics were given scores according to the severity of the impact: 0 (flawless), 1 (acceptable) or 2 (severe).

Results and Discussion

Potential customer group discussion for index guideline

Since different group members repeatedly used the 18 snails of each discussion section, the stimulation response was only useful for the first few panelists. Thus, the results for the mortality test were dismissed. The color guidelines on shell and meat were failed on this group discussion, due to the individual differences on color judgment and more importantly the heterogeneous color spots on the shell

and the absent of the foot part. However, the color of the discharge was well distinguished with purple, milky and yellow holding the top three concurrencies. Since the sense of finger feel of the surface and the sense of the smell will be exhausted in a short time after intense measurements, and since this quality index is primarily designed for marketability evaluation, they were excluded from the observation category also. Thus, only visual based observation categories were carried on to later on studies.

Observations over time from multiple trials

The shell length measurements from all 4 rounds indicated all the character observations were valid in this study (Figure 7). The list of observed characters from receiving day to the day mortality rate increased to 50% is shown in Table 6. Although only the considered alive ones were deshelled for further meat examination, occasionally, dry foot/dry meat occurred due to the false responding to the mortality test.

Mortality, absence of foot/Operculum covers the aperture and dry foot

According to U. S. Food and Drug Administration Food Code (2009), dead shellfish should be discarded and not used for human consumption. The absence of the meat (foot) or dry meat, the snail was considered dead based on safety and

economic aspects. All 3 symptoms for mortality are severe and were given a score of 2.

Egg capsule and/or algae on the shell

Egg capsules only show up on the shells during the spawning season. The spawning of *S. haemastoma* is temperature dependent, usually beginning about 21°C. Depending on water temperature in the region, spawning may occur as early as January and continue sporadically through October (Butler 1954). The egg capsules are creamy yellowish when first deposited, then become brown when the eggs mature. They turn purple only when empty or when the developing embryo dies in the capsule (D'Asaro 1966). Egg capsules and algae, which occur on the shells, are organic material and can promote unwanted bacterial growth during storage and before processing. Treatment with graters as used with single oysters could be used to trim the egg capsules and other excess attachments during a prewash to force the foot to shrink in and to clean the shells. Thus, the presence of egg capsule and algae is given score 1.

Barnacle and/or mussel on the shell

Barnacles and mussels commonly coexisted in high densities on bigano snail shells (Eston *et al.* 1986, Watanabe and Young 2006). Barnacles and mussels also present a high risk of harboring bacteria like egg capsules and algae. They have

also been shown to induced shell thickening in other gastropod species (Palmer 1990). Barnacles and/or mussels on the shell can be treated by roller sorters, and were given a score of 1.

Boring sponge/boring clam

Boring sponge *Cliona truitti* and the boring clam *Diplothyra smithii,* depending on the size and density, could cause problems during deshelling when blackened bivalves and sponges are present in between shell layers (Temelli et al. 2006). Figure 8 shows examples of snails with boring sponge (a and b), with boring clam on the shell (c) with boring sponge (d) in the shell and with boring clam in the shell (e and f). Snails were given scores of 1 when boring sponges and boring clams present on the shell. When boring sponge and clam presents in the shell, as on samples from TX, a score of 2 was given.

Boring hole on the shell and shell damage

According to the U. S. Food and Drug Administration Food Code (2009), severely damaged shellfish shells should be discarded and not used for human consumption. Hungry bigano snails may prey on each other (Butler 1985). Holes in the shell could introduce pathogens into organs, which are usually protected by the shell. Thus, holes in the shell were given a score of 1 if minor and 2 if severe.

Radula out and/or foreign subject inside aperture

An extended radula (mouth) is usually a sign of impending mortality. The disposition of the radula and foreign matter inside the aperture could cause the disposition of foot, and thus the hypobranchial gland. The gland produces a yellowish viscid secretion which was found to be toxic to mice with an LD_{50} of 215 mg/kg (Huang and Mir 1971). Foreign matter in the aperture could cause problems during deshelling and gutting. This could also expose hypobranchial gland secretions, which may potentially stain the meat and cause market value loss. Both extended radula and foreign matter rarely occurred in live animals during this study. When they did occur, they were given a score of 2.

Color secretions and stained meats

The protein and moist in the discharge are sources for bacteria growth, but the immune system still defend the threatening, which is one of the great benefits for live transportation and storage, especially when the quality of the edible portion is hard to detect due to the shell as for many gastropod species. The milky and creamy color is normal color of discharge. However, for bigano snail, the secretion from the hypobranchial gland is colored and will turn darker through storage with assistance of oxygen and light. Three challenges are addressed here: first, the dry

storage or transportation could not let the snail efficiently release the secretion as in natural environment; second, the stress during the storage and transportation could potentially increase the secretion, since more observations of colored discharge were recorded as the storage days increased and third, the color can permanently stain on the meat portion. In the third situation the meat could be stained from the yellow, green, blue or purple discharge. Since the natural color of the foot is yellowish, staining of yellow or light green won't cause degrading of the meat. But blue and purple color depends on the size of the spot could cause the reduction or loss of market value, which became severe after 3 days of storage in this study. Thus, yellow discharge and color spot is considered score 1, and score 2 is set for green, blue, purple and black color on discharge and/or color spot on the meat. Normal and colored meat are illustrated in Figure 9, respectively.

Oversaturated foot edge and/or dry foot/meat

A hydrated foot may be caused by contact with low salinity water (ice) during transportation and storage. Figure 10 shows the oversaturated foot on a live bigano snail (a) and deshelled dry meat (b). Although no obvious difference was observed for the oversaturated meat comparing to the normal meat, the membrane damage can potentially promote bacteria growth. The safety concern is related to the damage; thus, score 1 is given.

Operculum missing

A missing operculum promotes bacterial growth due to the damage of foot tissue. A missing operculum is given a score of 1.

Gray meat

The whole meat portion turns gray usually a sign of mortality. Gray tissue was given an evaluation score of 2.

VBMI scheme

The final visual-based-marketability-index (VBMI) scheme developed in this study is presented in Table 7. The acceptable characteristics are listed first, while the severe characteristics are listed at the end. In this scheme, all characteristics are evaluated according to the severity to food safety, economic and processing difficulty concerns. Each characteristic is was given a 0 (flawless), 1 (acceptable) or a 2 (severe).

The scores from each category may be added up to a visual-based-marketability-index. This score will indicate the applicability for further marketing. However, if the total sum or single category reaches score 2, the marketability could be questionable. Thus, the improvements to the harvesting, transportation and storing

method that would lead to a decrease of total score for VBMI is favorable to the quality of the product and marketability of the species.

Conclusion

A Visual-Based-Marketability-Index (VBMI) scheme has been developed. The characters and symptoms that are related to food safety, processing difficulty and market value have been identified and described. The categories are given scores according to the severity of the issue: flawless is given 0, acceptable is given 1 and severe is given 2. A total sum of 2 from all categories scores will cause downgrading or decrease of marketability for bigano snail.

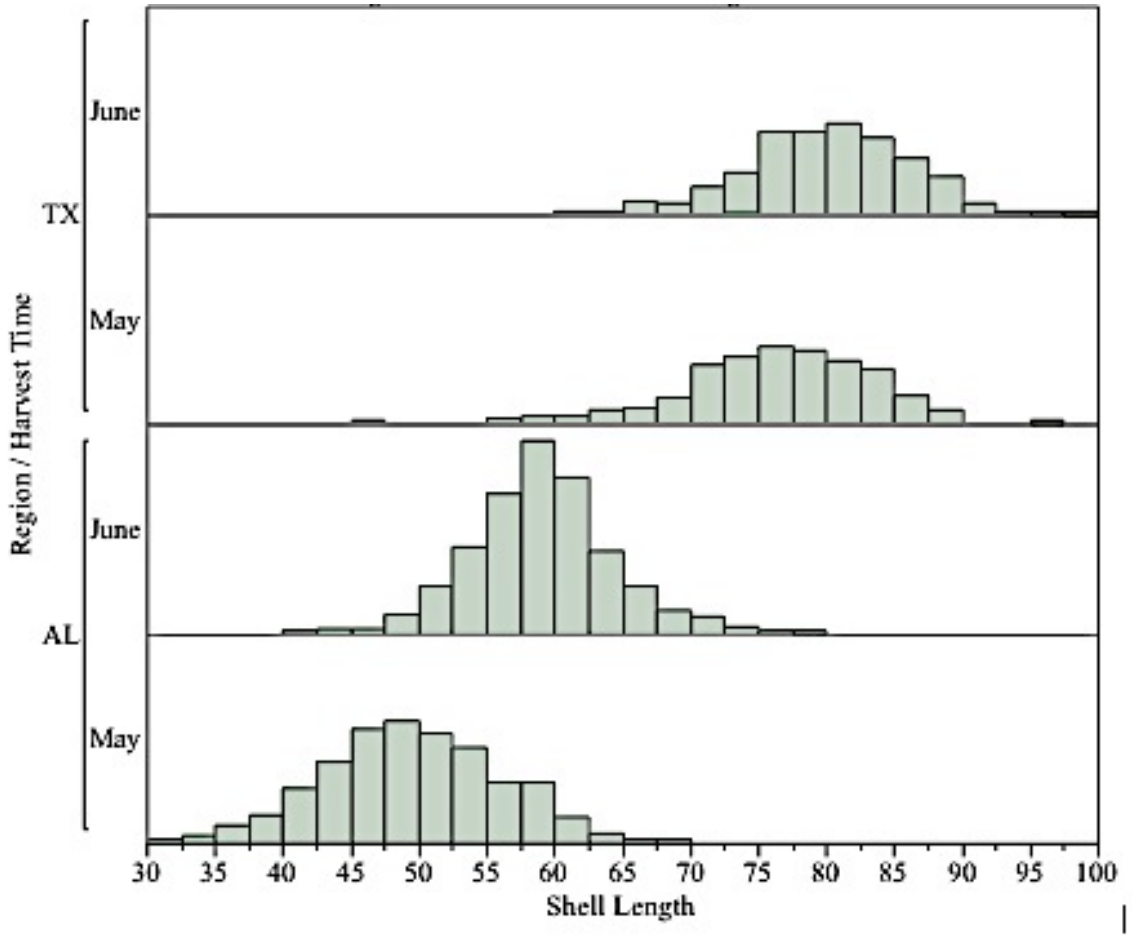

Figure 7. Distribution histogram of shell lengths of samples receiving from 4 shipments.

Figure 8. Symptoms of boring sponge (a and b) and boring clam (c) on the shell of bigano snail and symptoms of boring sponge (d) and boring clam (e and f) in between the shell layers.

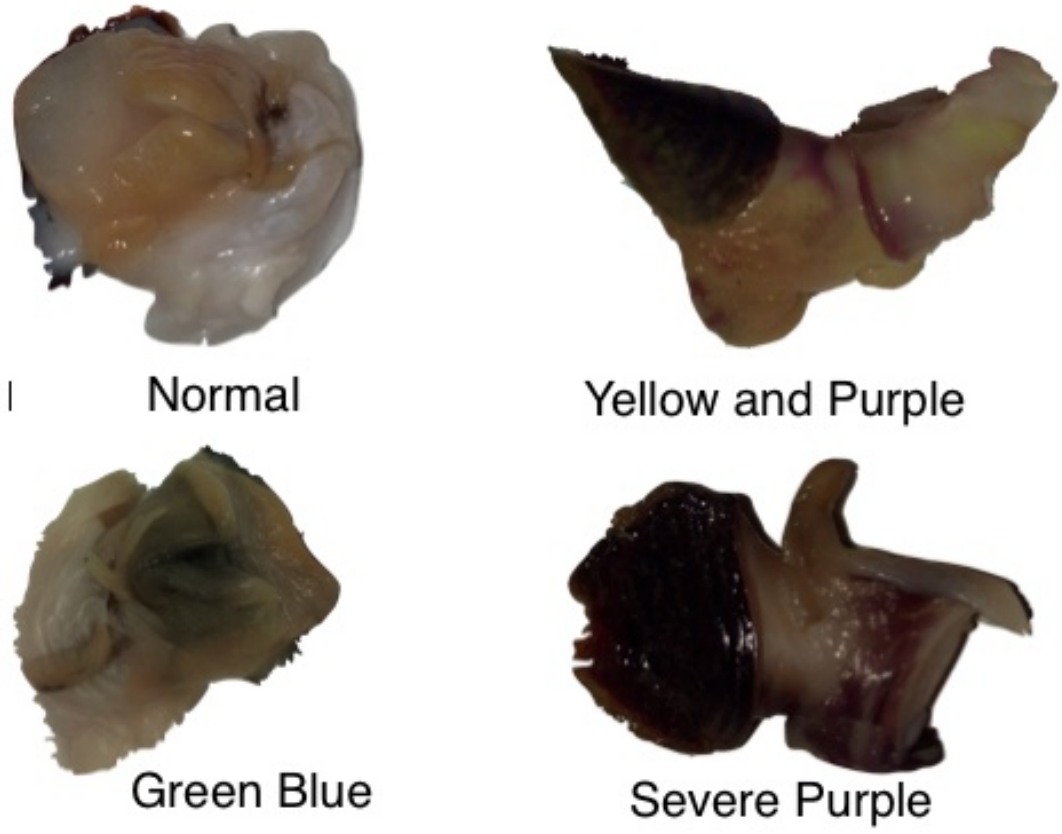

Figure 9. Meat of bigano snail with discoloration due to the secretion from the hypobranchial gland

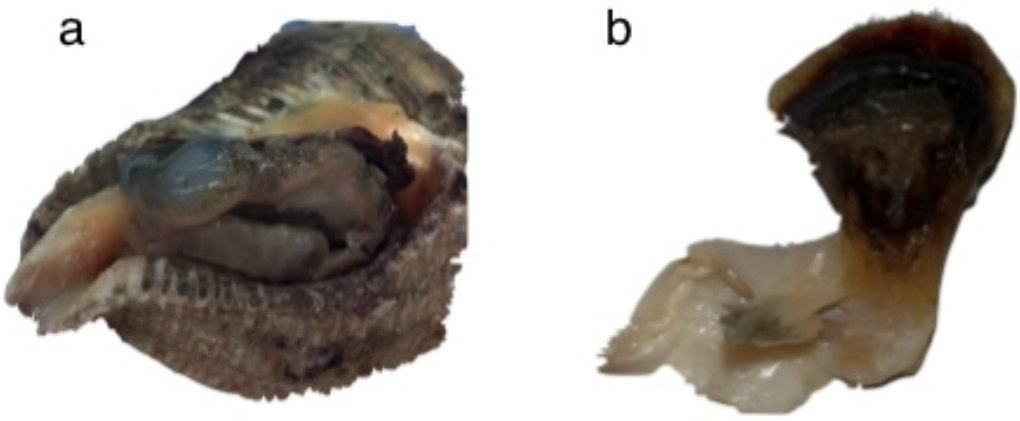

Figure 10. Oversaturated foot edge and dry foot of bigano snail

Table 6. Observed characters from receiving to cutoff day from 4 shipments.

Characters	Description
Exterior characters	
Mortality	A bigano snail is considered dead when no or slow shrinking response was observed upon stimulating on foot and mantle (Iacarella and Helmuth 2011), and/or operculum covers the whole aperture with no foot present and/or the present foot is dry and lost marketable value.
Egg capsule and/or algae on the shell	Season difference, the organic matter on the shell may cause processing difficulty and promote bacteria growth
Barnacle and/or mussel on the shell	The structure on the shell may cause processing difficulty and hide harmful pathogen
Boring sponge/boring clam on the shell, shell damage	The structure and organic matter on the shell may cause processing difficulty and promote bacteria growth
Boring hole on the shell	Potential tissue damage, promote bacteria growth
Absence of foot/Operculum covers the aperture	Considered as dead
Radula out and/or foreign subject inside aperture	The abnormal structure cause difficulty to perform mortality test and deshell procedure, which also cause potential edible tissue damage
Milky/Creamy discharge	Promote bacteria growth
Colored dye discharge	Yellow, purple and black discharge had been observed. The discharge may promote bacteria growth and the dye may stain on the meat. They general cause the concern of quality from customer.
Oversaturated foot edge	Customer quality concern, over saturation may cause membrane damage and promote bacteria growth, transportation and storage method interest
Dry foot	Customer quality concern, considered as dead, loss of marketable value

Interior characters	
Sponge and bivalve in between shell layers	Harvest location difference, organic matter in the shell may cause processing difficulty and promote bacteria growth
Operculum missing	Tissue damage may promote bacteria growth
Color spot on the meat	Yellow, green, blue and purple color spot had been observed. They cause the quality concern from the customer and economic loss by reduce or loss of marketable value
Dry meat	Customer quality concern, considered as dead, loss of marketable value
Gray meat	Customer quality concern, safety concern, loss of marketable value

Table 7. Visual-based-marketability-index (VBMI) scheme for bigano snails under dry cold storage conditions.

Categories		Description	Score
Algae on the shell	Flawless:	Absence of character	0
	Acceptable:	Algae on the shell	1
Barnacle and/or mussel on the shell	Flawless:	Absence of character	0
	Acceptable:	Barnacle and/or mussel on the shell	1
Overhydrated foot	Flawless:	Absence of symptom	0
	Acceptable:	Oversaturated foot edge	1
Operculum missing	Flawless:	Absence of symptom	0
	Acceptable:	Operculum missing	1
	Acceptable:	Operculum missing	1
Boring sponge/boring clam	Flawless:	Absence of character	0
	Acceptable:	Boring sponge/boring clam on the shell	1
	Severe:	Boring sponge/boring clam in between the shell layers	2
Shell damage and boring hole	Flawless:	Absence of character	0
	Acceptable:	Boring hole on early stage of storage (mortality rate less than 5%)	1
	Severe:	Boring hole on late stage of storage and shell damage	2
Color of discharge and color spot on the meat	Flawless:	Clear, milky or creamy discharge and no discoloration on the meat	0
	Acceptable:	Yellow discharge and/or yellow color spot on the meat	1
	Severe:	Color darker than yellow on discharge and meat	2
Gray meet	Flawless:	Absence of symptom	0
	Severe:	Whole meat portion turns gray	2
Mortality	Flawless:	Presence of moisture foot and response to stimulating on foot and/or mantle	0
	Severe:	No response to stimulating, absence or dry foot	2

CHAPTER FOUR: CASE STUDY ONE: *Stramonita haemastoma floridana* in spring

Introduction

Freshness is one main quality of seafood product, and live seafood products become more important commodity in seafood markets due to the customer demand (Christophersen et al. 2008). However, live seafood is also perishable, which has a limited shelf life from live harvesting to mortality or degrading of quality. This limited shelf life for each product can be influenced by harvest season, harvest method, harvest location, individual differences, stress from handling, stress from shipping and stress from storing before the further processing or consuming (Wingerter et al. 2013). Dry shipping and dry storing in low temperature are the most widely used methods for live shellfish due to the cost of seawater environment, and less stress and metabolic rate (Wingerter et al. 2013, Lorenzon et al. 2007). However, dry shipping and storing also distress the shipping animal and induce unwanted symptoms leading to degrading of the product. Thus, finding the maximum tolerance time with no or few degrading demerits for dry shipping and storing will provide reference for better seafood trading performance.

Bigano snails (*Stramonita haemastoma*), also commonly called oyster drills and red-mouthed rock whelks, are a predatory gastropod. They are common predators of oysters, barnacles, gastropods, and bivalves (Rilov et al. 2004, Butler 1985,

López et al. 2010). This species is widely distributed in tropical and warm water regions including the Gulf of Mexico, Caribbean Sea, and along the western and southwestern Africa and the Mediterranean Sea (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). They are under the subfamily of Rapaninae. In the southeastern United States, two subspecies are general recognized- *Stramonita haemastoma floridana* and *Stramonita haemastoma canaliculata* (Harding and Harasewych 2007, Walker 1982). And bigano snail from Billygoat Hole, AL is considered from subfamily *Stramonita haemastoma floridana*.

Developing bigano snail into a marketable seafood product has gained interests recently from cultural, economic, environmental and nutritional aspects (Ray and Benefield 1997, Eberline 2012, George et al. 2008, López et al. 2010, Udofia 2009). However, a safe, inexpensive and proper transportation and storage method has not been developed.

Thus, we aimed to evaluate different cold dry storage methods and storage time on the physical characters change of live bigano snails from Billygoat Hole, AL in 2013 May. The survival ratio, shell color, meat yield, meat moisture content and marketability concerned characters were performed for the evaluation.

Method and material

Sampling and storage condition

All samples were harvested alive and overnight gel-pack-cold shipped to Auburn, AL for analysis. Samples from Billygoat Hole, AL (received in May 2013) were provided by Auburn shellfish laboratory, Dauphin Island, AL. After receiving, snails were randomly separated into two storage treatments with an additional 60 snails for receiving condition examination for each round. No cover storage treatment was for snails had no prewash at receiving point and stored in an open top container in a refrigerator (6°C ± 2°C) after receiving point. Burlap storage treatment was for snails had no prewash at receiving point and stored in an open top container with coverage of 2-layer damp burlap in a refrigerator (6°C ± 2°C) after receiving point. Washed storage treatment was for snails briefly washed with cold tap water and at receiving point and stored in an open top container in a refrigerator (6°C ± 2°C) after receiving point. Napkin storage treatment was for snails briefly washed with cold tap water and at receiving point and stored in an open top container with coverage of 2-layer dry paper towel in a refrigerator (6°C ± 2°C) after receiving point. A total of 662 specimens were observed and scarified in the whole study.

Observation over time

On the receiving day, 60 snails were randomly selected for receiving condition examination (exterior test on Day 0 and interior test on Day 1) and sacrificed. Due to the time consuming of testing and deshelling, on odd days from receiving day (day 1, 3, 5, 7 and etc.) until survival ratio dropped below 50%, 30 snails were randomly selected only from no cover and burlap storage treatments for exterior variables tests and number marked. Then all the tested alive samples from one storage treatment of the day were packed in Ziploc® bag, stored in freezer (-20°C) overnight to sacrifice and deshelled the next day for interior variables tests). And on even days from receiving day (day 2, 4, 6 and etc) until survival ratio dropped below 50%, 30 snails were randomly selected from washed and napkin storage treatments and processed as above.

Exterior variables

The shell length and whole wet weight were measured. The survival ratio was tested by evaluating the stimulate response of the muscle and mantle on each specimen. If the foot muscle or the mantle shrank back immediately, the individual was considered alive. Otherwise, the individual was considered dead. The shell color was the average of 2 point measurement on the shell using a MiniScan XE Plus instrument from Hunterlab (Reston, VA) following CIE L*a*b system. In the CIE L*a*b system, L* denotes lightness on a 0 to 100 scale from black to white;

a* denotes red (+) or green (-); and b* denotes yellow (+) or blue (-) for better visualization of the reading. The chroma C* denotes the square root of ($a^{*2} + b^{*2}$) (Schubring and Meyer 2002).

Interior variables and yield ratio

After deshelling, the individual meat weight was measured as yield. Yield ratio was calculated using following equation:

$$\text{Yield Ratio} = \frac{\text{Individual Meat Weight}}{\text{Whole Wet Weight of the Individual}}$$

Moisture content of the meat was determined using a moisture analyzer, model MX-50 (AND Co. Ltd., Tokyo). The drying program was determined to be auto standard at 105 ^{o}C with 3 replicates from raw blended meat portion.

Visual-based-marketability-index (VBMI)

The variables listed on visual-based-marketability-index (VBMI) as following (Table 7) were also recorded during exterior and interior tests. The total score for VBMI and score excluding mortality were both calculated.

Statistical analyses

All exterior variables were measured with 60 replicates for receiving day, and with 30 replicates for each treatment after except survival ratio. The survival ratio and VBM characters were calculated from 10 random specimens of the same storage method with the same storage period. Then 3 replicates were generated for each storage method on each storage day and 6 replicates were generated for receiving day. The interior variables were recorded with 60 or less replicates for receiving day, and with 30 or less replicates for each treatment after, depends on the survival ratio. One-way analysis of variance (ANOVA) with 4 storage methods was performed by storage day (day 1 and day 2 was considered as day 1-2, etc.) for all variables. Then the mean values of all variables were plotted separately against the storage time by the storage method methods. Normality and homoscedasticity of the shell lengths were tested to ensure the validity of the tests of the day. Statistically differences were reported at $p < 0.05$. If the mean values were different for a variable, then the results were expressed as mean value and standard error of mean (SEM) and separated by Tukey HSD. Statistical analysis was performed using JMP 10 (SAS Institute Inc., Cary, NC, USA).

Results

All the data were normally distributed and homoscedastic. The results for the shelf-life trial are presented individually for each of the variables.

Survival ratio

The survival ratios by storage method on different storage day are shown in Figure 11. The burlap storage treatment had the highest survival ratios during the storage, while the washed treatment had the lowest over time. Day 4 is considered the safe day for 80% above survival ratio across all 4 storage treatments.

Shell color

Storage method affected significantly differently on reserving moisture on the shell (Table 8). Due to the prewash procedure, the washed and napkin storage treatments had generally higher lightness and lower chrome values than the burlap and no cover treatments. The burlap storage method reserved the moistest shell with the lowest lightness and the highest chrome value, while washed treatment had the most dry and fade shells on each testing period.

Meat Moisture

Although the shell color indicted the burlap storage treatment reserved more moisture than the other three storage method from the beginning of the storage, it didn't reflected meat moisture content until day 7 and day 8 (Figure 12). The meat from the burlap storage treatment on day 7 contained 77.20% moisture, which was significantly higher than the napkin treatment (75.78%, $p = 0.003$) and the no

cover treatment (75.38%), while the washed treatment discontinued because of the high mortality.

Meat yield ratio

Over the 662 specimens in this study, the shell length ranged from 31.25 mm to 69.51 mm with a mean of 49.55 mm. Over the storage time, the live ones of they yielded 0.42 gm to 3.71 gm of meat per individual. Even under the low storage temperature (6°C ± 2°C), the live animal still maintains a low metabolic rate, which may affect the meat yield besides the moisture loss and other factors. Unlike the meat moisture content, the yield ratios were affected by the storage methods at the beginning of the storage (Figure 13). In the storage day 1 and day 2 period, the napkin storage treatment had the highest yield ratio (0.110, $p = 0.004$). Although this ratio was not significantly separated from the burlap storage treatment (0.103), it was significantly greater than the no cover (0.101) and the washed (0.101) storage treatments. In the storage day 3 and day 4 period, the washed storage treatment yielded the most meat on wet weight scale (yield ratio = 0.104, $p = 0.03$), although it was not higher than the no cover (0.099) and the napkin (0.098) storage treatments statistically. The burlap treatment yielded the least meat (0.097) on day 3 in the day 3-4 testing period. After storage day 4, no significant difference of mean of yield ratio was detected among the storage treatment on the storage day, with day 5-6 period had 0.096 to 0.101 and the day 7-8 period had

0.089 to 0.097 for all 4 treatments, and the day 9-10 period had 0.086 to 0.088 for only the burlap and no cover storage treatments. The moisture content difference of the meat caused by the storage method over the storage time obviously was not the only driver for the meat yield change. Individual differences and prewash procedure might also affect meat over whole wet weight ratio in this study. The prewash might reduce the excess on the shell, thus, reduced the whole wet weight and further increased the yield ratio. The individual differences may include a combination of age, sex, genetic and other factors.

Visual-based-marketability-index (VBMI) and marketability concerned characters

The total VBMI, color and mortality (N=635, day 11 was excluded) scores by each storage method over the storage time were shown in Figure 14. Mortality score was the main contributor to the total VBMI. Severe symptom of color category and total color score over storage time by each storage method indicated the purple discharge and dark color spot (green, blue and purple) on the meat were the main score symptoms for the color category. The napkin storage method had constantly lowest color score over the storage time, but the shortest shelf life. Thus, if the expected storage time is less than 4 days, the prewashed napkin storage method has the better yield ratio and the better meat quality. However, the quality degraded dramatically after day 4 with the rise of the mortality ratio. If longer shelf

life with higher survival ratio is the goal, then the burlap storage method should be chose.

Conclusion

Day 4 is considered the safe day for 80% above survival ratio across all 4 storage treatments. Moisture reserving capabilities of the storage methods affected both shell colors and meat moisture contents. Shell color faded and became lighter after prewash and/or longer storage time. The burlap storage method kept the most moisture during storage time comparing to the other 3 storage methods. Meat yield ratio over the storage time was not only affected by the moisture factor, but also the prewash procedure and individual differences. If the expected storage time is less than 4 days, the prewashed napkin storage method has the better yield ratio and the better meat quality. However, if longer shelf life with higher survival ratio is the goal, then the burlap storage method should be chose.

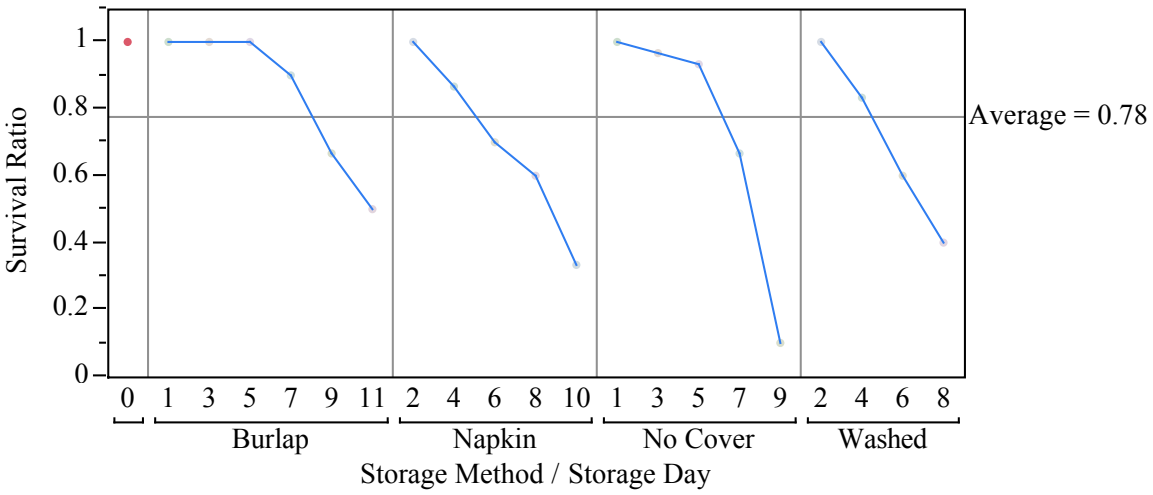

Figure 11. Survival ratio over storage day by storage method

Table 8. Effect of storage method on shell lightness (L*) and chrome (C) by storage time

		No Cover	Burlap	Washed	Napkin	SEM	p-value
Day 1-2	L*	46.33^{b}	38.05^{c}	52.48^{a}	49.01^{ab}	1.29	P < 0.0001
	C	12.35^{a}	10.70^{b}	9.92^{b}	9.59^{b}	0.37	P < 0.0001
Day 3-4	L*	48.81^{b}	40.50^{c}	60.15^{a}	50.88^{b}	1.14	P < 0.0001
	C	12.29^{a}	11.37^{ab}	10.95^{b}	9.48^{c}	0.35	P < 0.0001
Day 5-6	L*	56.14^{a}	42.05^{c}	58.48^{a}	49.76^{b}	0.96	P < 0.0001
	C	13.28^{a}	11.54^{b}	10.16^{c}	10.10^{c}	0.35	P < 0.0001
Day 7-8	L*	54.19^{b}	42.36^{c}	60.30^{a}	51.59^{b}	1.02	P < 0.0001
	C	13.53^{a}	11.00^{b}	10.46^{b}	9.93^{b}	0.31	P < 0.0001
Day 9-10	L*	52.25^{b}	41.43^{c}	-	60.25^{a}	0.81	P < 0.0001
	C	13.56^{a}	10.81^{b}	-	10.60^{b}	0.34	P < 0.0001

^{a,b,c} Same letter in the mean value row shows that there was no statistically significant
difference from different storage method ($p < 0.05$).

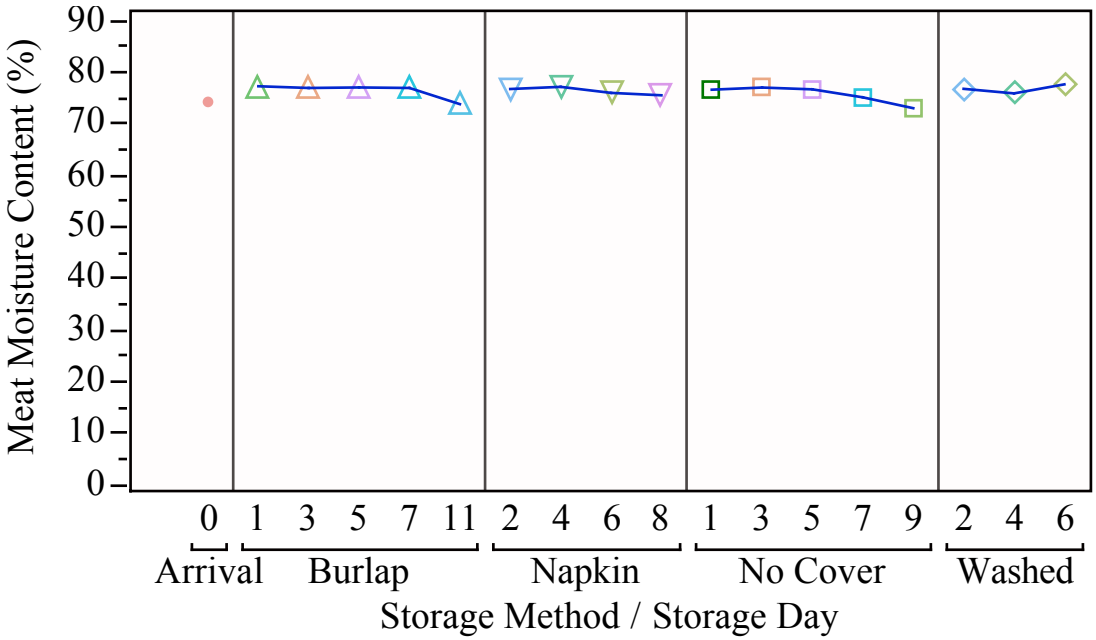

Figure 12. Mean of meat moisture content by storage method over storage day.

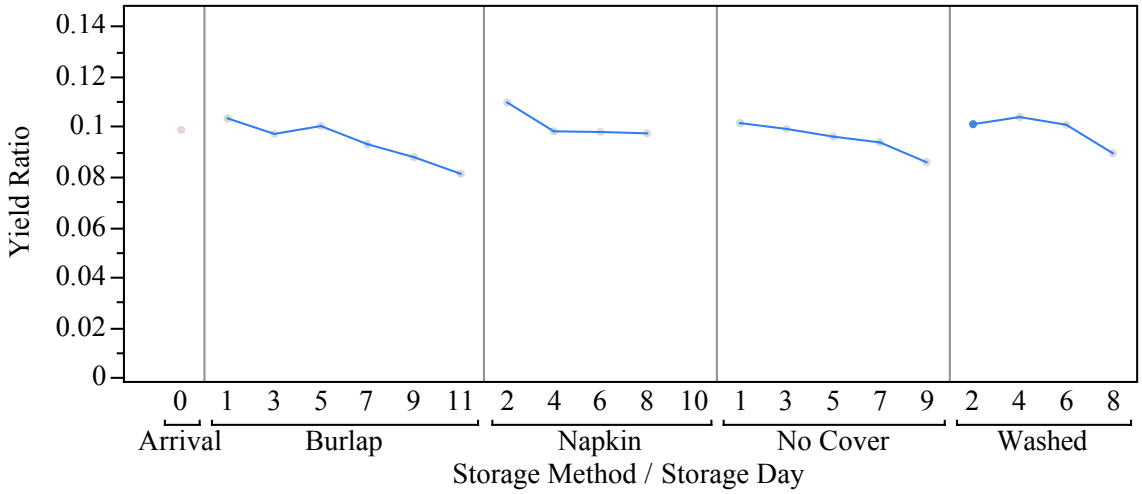

Figure 13. Mean of yield ratio by storage method over storage day.

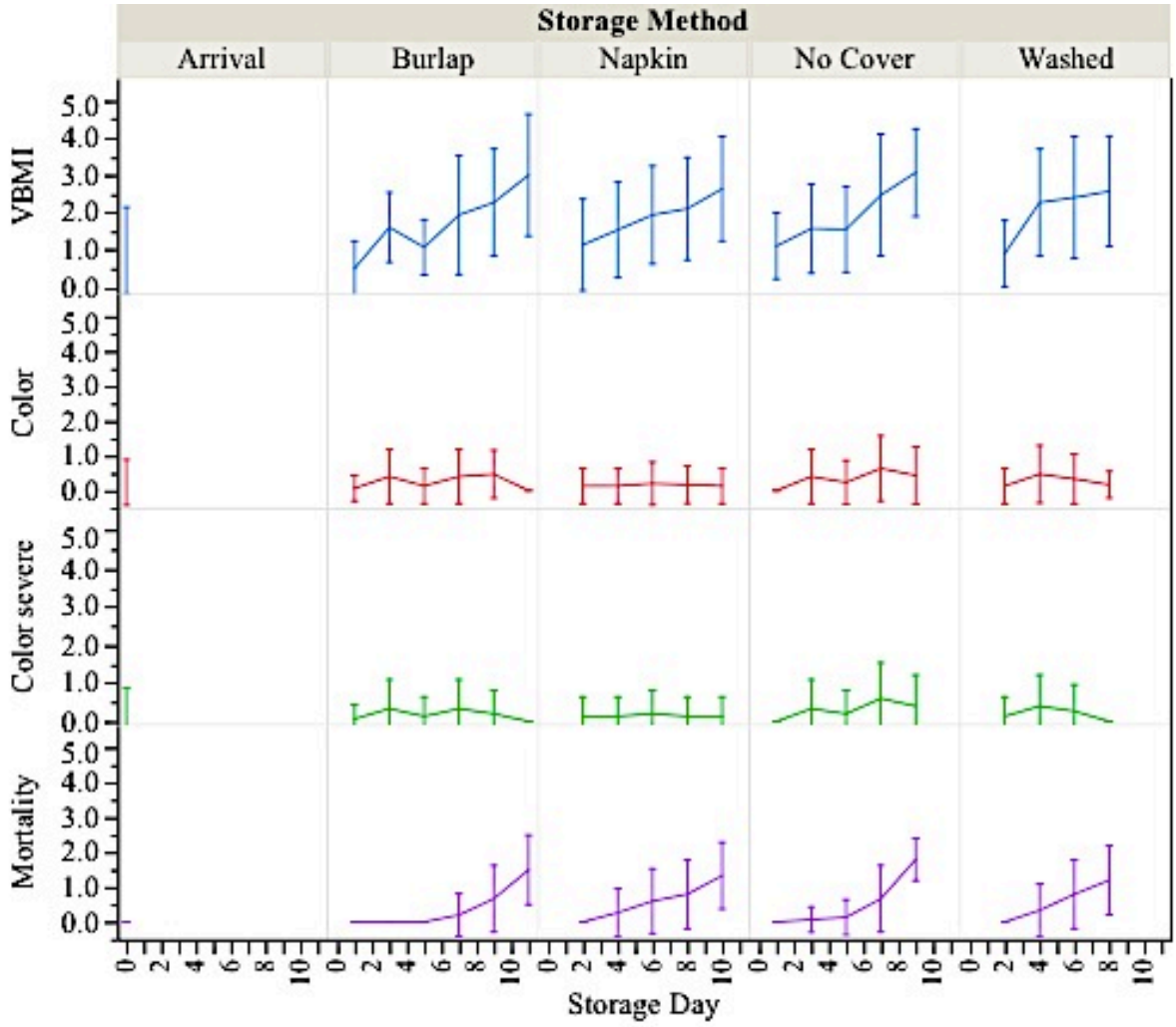

Figure 14. Means and standard deviations of visual based marketability index, color score (1 and 2), severe color score (2), and mortality (2) by storage method over time.

CHAPTER FIVE: CASE STUDY ONE: *Stramonita haemastoma canaliculata* in spring

Introduction

Freshness is one main quality of seafood product, and live seafood products become more important commodity in seafood markets due to the customer demand (Christophersen et al. 2008). However, live seafood is also perishable, which has a limited shelf life from live harvesting to mortality or degrading of quality. This limited shelf life for each product can be influenced by harvest season, harvest method, harvest location, individual differences, stress from handling, stress from shipping and stress from storing before the further processing or consuming (Wingerter et al. 2013). Dry shipping and dry storing in low temperature are the most widely used methods for live shellfish due to the cost of seawater environment, and less stress and metabolic rate (Wingerter et al. 2013, Lorenzon et al. 2007). However, dry shipping and storing also distress the shipping animal and induce unwanted symptoms leading to degrading of the product. Thus, finding the maximum tolerance time with no or few degrading demerits for dry shipping and storing will provide reference for better seafood trading performance.

Bigano snails (*Stramonita haemastoma*), also commonly called oyster drills and red-mouthed rock whelks, are a predatory gastropod. They are common predators of oysters, barnacles, gastropods, and bivalves (Rilov et al. 2004, Butler 1985,

López et al. 2010). This species is widely distributed in tropical and warm water regions including the Gulf of Mexico, Caribbean Sea, and along the western and southwestern Africa and the Mediterranean Sea (Harding and Harasewych 2007, Haupt et al. 2010, Liu et al. 1991, Rilov et al. 2001). They are under the subfamily of Rapaninae. In the southeastern United States, two subspecies are general recognized- *Stramonita haemastoma floridana* and *Stramonita haemastoma canaliculata* (Harding and Harasewych 2007, Walker 1982). And bigano snail from Galveston Bay, Texas is considered from subfamily *Stramonita haemastoma canaliculata.*

Developing bigano snail into a marketable seafood product has gained interests recently from cultural, economic, environmental and nutritional aspects (Ray and Benefield 1997, Eberline 2012, George et al. 2008, López et al. 2010, Udofia 2009). However, a safe, inexpensive and proper transportation and storage method has not been developed.

Thus, we aimed to evaluate different cold dry storage methods and storage time on the physical characters change of live bigano snails from Galveston Bay, Texas in 2013 May. The survival ratio, shell color, meat color, meat yield, meat moisture content, meat ash content, meat amino acid profile, after-cook meat texture profile and marketability concerned characters were performed for the evaluation.

In order to compare the result over the storage time from different storage method, the color was measured using a CIE L*a*b system, and texture was measured by texture profile analysis (TPA).

Method and material

Sampling and storage condition

All samples were harvested alive and overnight gel-pack-cold shipped to Auburn, AL for analysis. Samples from Galveston Bay, Texas (received in May 2013) were provided by personnel from Jeri's Seafood, Houston, TX. After receiving, snails were randomly separated into two storage treatments with an additional 60 snails for receiving condition examination for each round. No cover storage treatment was for snails had no prewash at receiving point and stored in an open top container in a refrigerator (6°C ± 2°C) after receiving point. Burlap storage treatment was for snails had no prewash at receiving point and stored in an open top container with coverage of 2-layer damp burlap in a refrigerator (6°C ± 2°C) after receiving point. A total of 420 specimens were observed and scarified in the whole study.

Observation over time

On the receiving day, 60 snails were randomly selected for receiving condition examination (exterior test on Day 0 and interior test on Day 1) and sacrificed. Due to the time consuming of testing and deshelling, on odd days from receiving day (day 1, 3, 5, 7, 9 and 11), 30 snails were randomly selected only from no cover and burlap storage treatments for exterior variables tests and number marked. Then all the tested alive samples from one storage treatment of the day were packed in Ziploc® bag, stored in freezer (-20°C) overnight to sacrifice and deshelled the next day for interior variables tests).

Exterior variables

The shell length and whole wet weight were measured. The survival ratio was tested by evaluating the stimulate response of the muscle and mantle on each specimen. If the foot muscle or the mantle shrank back immediately, the individual was considered alive. Otherwise, the individual was considered dead. The shell color was the average of 2 point measurement on the shell using a MiniScan XE Plus instrument from Hunterlab (Reston, VA) following CIE L*a*b system. In the CIE L*a*b system, L* denotes lightness on a 0 to 100 scale from black to white; a* denotes red (+) or green (-); and b* denotes yellow (+) or blue (-) for better visualization of the reading. The chroma C* denotes the square root of (a^{*2} + b^{*2})(Schubring and Meyer 2002).

Interior variables and yield ratio

After deshelling, each front and backside of the meat was measured on 2 points for the color read. The individual meat weight was measured as yield. Yield ratio was calculated using following equation:

$$\text{Yield Ratio} = \frac{\text{Individual Meat Weight}}{\text{Whole Wet Weight of the Individual}}$$

Meat properties

Moisture content of the meat was determined using a moisture analyzer, model MX-50 (AND Co. Ltd., Tokyo). The drying program was determined to be auto standard at 105 ^{o}C with 3 replicates from raw blended meat portion.

Ash content of the meat was estimated by heating the homogenized meat in a CEM Phoenix Microwave Muffle Furnace (Matthews, NC) at 550°C for 6 hours with 3 replicates.

The complete and soluble profiles for amino acid were conducted by Advanced Analytical Testing Service (AATS, Ontario, CA). The hydrolysis procedure was modified from method 1 in United State Pharmacopeia Convention 35 Chapter 1052 protein hydrolysis (2012). The amino acid analysis procedure was modified

from method 3 in United State Pharmacopeia Convention 35 Chapter 1052 appendix (2012).

For the after-cook texture profile, 8 meat portions were randomly selected from the deshelled samples and cooked using a method modified from Schubring and Meyer (2002) for after-cook texture profile analysis. The meat portions were cooked in 1L boiling water on medium heat for 5 min, then allowed to drain and cool to room temperature for 45 min. The meats were then put in the center of the working platform and were compressed twice to 70% of their original height for 0.50 s using a cylindrical probe 2.5 cm in diameter. Test speeds for all pre-, actual and post-test were set as 5.00 mm/s. The trigger force was 5.0 g and triggered automatically. Hardness, springiness, cohesiveness, gumminess, chewiness and resilience were measured.

Visual-based-marketability-index (VBMI)

The variables listed on visual-based-marketability-index (VBMI) as following (Table 7) were also recorded during exterior and interior tests. The total score for VBMI and score excluding mortality were both calculated.

Statistical analyses

All exterior variables were measured with 60 replicates for receiving day, and with 30 replicates for each treatment after except survival ratio. The survival ratio and VBM characters were calculated from 10 random specimens of the same storage method with the same storage period. Then 3 replicates were generated for each storage method on each storage day and 6 replicates were generated for receiving day. The interior variables were recorded with 60 or less replicates for receiving day, and with 30 or less replicates for each treatment after, depends on the survival ratio. The meat properties were measured with 3 replicates respectively. One-way analysis of variance (ANOVA) with 2 storage methods was performed by storage day for all variables. Then the mean values of all variables were plotted separately against the storage time by the storage method methods. Normality and homoscedasticity of the shell lengths were tested to ensure the validity of the tests of the day. Statistically differences were reported at $p < 0.05$. If the mean values were different for a variable, then the results were expressed as mean value and standard error of mean (SEM) and separated by Tukey HSD. Statistical analysis was performed using JMP 10 (SAS Institute Inc., Cary, NC, USA).

Results

All the data were normally distributed and homoscedastic. The results for the shelf-life trial are presented individually for each of the variables.

Survival ratio

The survival ratios by storage method on different storage day are shown in Figure 15. The burlap storage treatment had slightly better survival ratios than the no cover storage treatment during the storage. Day 2 is considered the safe day for 80% above survival ratio for both the storage treatments.

Shell color and meat color

Storage method affected significantly differently on reserving moisture on the shell (Table 9). The burlap storage method reserved significantly more moisture on the shell with the lower lightness values over, especially from the results on Day 7 and Day 9 ($p<0.0001$). Then on Day 11, as it was the end of the experiment, the dumped burlap dried out also, which couldn't reserve more moisture than the no cover treatment. Thus, no significant differences were detected on any color measurement.

No significant difference was found between the storage methods on meat color within each sampling storage day (Table 10). However, within the no cover storage method, the means of lightness of the in-shell meat color on Day 1 and Day 11 were significantly lower than all the other storage days, which were also shown within the burlap storage method ($p<0.0001$). The meat color also faded over storage days (Figure 16).

Meat moisture

The storage treatment didn't significantly affect the moisture content in the meat except Day 1 (No cover 69.83% and Burlap 67.82%, $p = 0.013$) and Day 9 (Burlap 70.52% and No cover 69.30%, $p = 0.024$). Noticeably, the moisture content within each storage treatment started with a significantly lower value on Day 1 ($p < 0.01$). This may be caused by the long transportation from the Galveston Bay, Texas to Auburn, AL, but the time duration of the deshelling procedure could also be part of the reason caused the differences. Discarding Day 1, no significant difference was found on meat moisture content through Day 9 using burlap storage treatment. However, for the no cover storage treatment, Day 3 had significantly higher value on meat moisture content than Day 1, 5 and 9 but not was separated from Day 7 ($p=0.009$). This difference reflected on the meat color (Figure 16 and Table 10) and survival ratio (Figure 15). All color readings on Day 3 were significantly higher than nearby days (Day 1 and Day 5), and the no cover storage method yielded higher survival ratio than the burlap method only on Day 5, which was the coming sampling day after Day 3.

Meat ash and amino acid profile

The storage method and storage time did not affect the meat ash content and amino acid profile significantly ($p<0.05$).

Meat yield ratio

Over the 420 specimens in this study, the shell length ranged from 47.44 mm to 95.14 mm with a mean of 76.34 mm. Over the storage time, the live ones of they yielded 1.92 gm to 17.12 gm of meat per individual, and the average yield ratio is 0.105. The storage methods did not affect the yield ratio on storage day basis, and no difference was found among storage days for the no cover storage method. However, for the burlap storage method, there was a major decrease on yield ratio on Day 11 ($p=0.014$). Comparing to the meat yield from snails from Billygoat Hole, AL also in 2013 May, the individual differences did not show on the meat yield ratio from the snails from Galveston Bay, Texas.

After-cook texture profile

Although the storage methods did not affect the texture of the after-cook meat on storage day basis, the significant differences were found on the hardness, chewiness and resilience of the after-cook meat on the burlap storage method. The after-cook meat from snails using burlap storage method on Day 11 were significantly harder, chewier and more elastic than meats from Day 3, which as mentioned above had the highest moisture content of the meat over the storage period.

Visual-based-marketability-index (VBMI) and marketability concerned characters

The total VBM, boring sponge/boring clam, color and mortality scores by each storage method over the storage time were shown in Figure 17. Mortality score was the main contributor to the total VBM socre especially for the no cover storage treatment. Boring sponge/boring clam, color of discharge and color spot on the meat were also main contributors to the total score. The severe boring sponge/boring clam symptoms only showed on bigano snails from Galveston Bay, Texas comparing to samples from Billygoat Hole, AL in this study. Severe symptom of color category and total color score over storage time by each storage method indicated the purple discharge and dark color spot (green, blue and purple) on the meat were the main score symptoms for the color category. The burlap storage method had significantly higher mean VBM score (3.63) than the no cover method (2.53) on Day 9 ($p=0.0062$), and higher mean color score (1.20 on both Day 9 and Day 11) comparing to 0.33 on both days from the no cover method ($p<0.001$). However, the no cover storage method had significantly great mortality score on Day 11 (1.87 comparing to 0.80, $p<0.0001$), which evened up the total VBM scores from both storage methods on Day 11. Thus, if the expected storage time is less than 2 days, both storage methods could be used. However, if longer live storage time is needed, a balance between mortality and quality need to be considered, as the burlap storage method brought higher survival rate at later

storage days but also yielded tougher and chewier meat with higher chance of presence of darker color spot on the meat.

Conclusion

Day 2 is considered the safe day for 80% above survival ratio on both the storage treatments. Both shell and meat color faded over storage, however, the ash content and the amino acid profile of the meat did not change over time of storage on both the storage method. The high moisture content meat has lighter color and softer texture. Mortality, boring sponge/boring clam, color discharge and color spot on the meat were the main contributors to the total VBM score for this product during this study. If the expected storage time is less than 2 days, both storage methods could be used. However, if longer live storage time is needed, a balance between mortality and quality need to be considered, as the burlap storage method brought higher survival rate at later storage days but also yielded tougher and chewier meat with higher chance of presence of darker color spot on the meat.

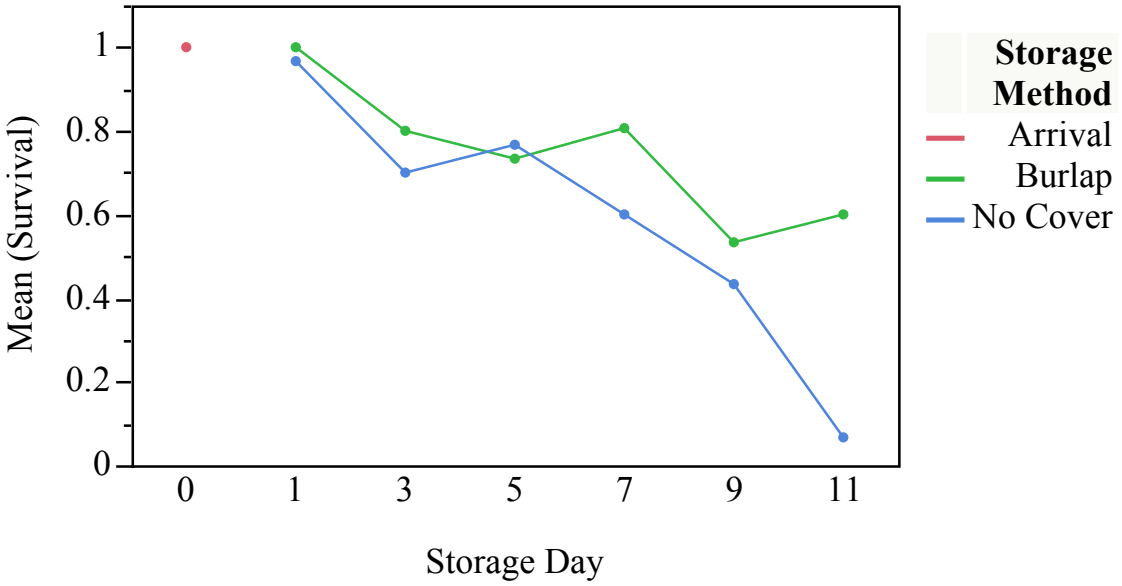

Figure 15. Survival ratio over storage day by storage method

Table 9. Effect of storage method on shell color by storage time

		Shell L*	Shell a*	Shell b*	Shell C
Day 0		39.57±0.61	4.70±0.18	15.04±0.34	15.78±0.37
	Burlap	44.15±0.80[b]	2.65±0.21	14.60±0.50	14.86±0.52
Day 1	No Cover	46.72±0.80[a]	2.79±0.21	15.22±0.50	15.50±0.52
	p-value	0.028	NS	NS	NS
	Burlap	42.65±1.03[b]	3.41±0.25[a]	16.15±0.57	16.54±0.60
Day 3	No Cover	49.32±1.10[a]	2.59±0.26[b]	15.80±0.61	16.02±0.64
	p-value	<0.0001	0.027	NS	NS
	Burlap	49.08±1.44	2.66±0.26	15.33±0.43	15.58±0.46
Day 5	No Cover	49.94±1.41	2.95±0.25	15.61±0.42	15.92±0.45
	p-value	NS	NS	NS	NS
	Burlap	43.23±0.88[b]	3.31±0.22[a]	15.72±0.56	16.09±0.57
Day 7	No Cover	56.28±1.04[a]	2.49±0.25[b]	17.13±0.65	17.32±0.68
	p-value	<0.0001	0.018	NS	NS
	Burlap	44.06±0.89[b]	2.50±0.23	14.21±0.55[b]	14.44±0.56[b]
Day 9	No Cover	59.90±0.99[a]	2.00±0.25	16.58±0.61[a]	16.72±0.63[a]
	p-value	<0.0001	NS	0.008	0.012
	Burlap	51.19±1.24	2.38±0.24	16.24±0.62	16.43±0.64
Day 11	No Cover	58.63±3.73	2.18±0.71	15.96±1.87	16.11±1.91
	p-value	NS	NS	NS	NS

[a,b] Same letter in the mean value column of the day shows that there was no statistically significant difference from different storage method ($p < 0.05$).

Table 10. Effect of storage method on meat lightness (L*) and chrome (C) on both environment exposed part (Front) and in-shell part (Back) by storage time

		Meat Front L*	Meat Front C	Meat Back L*	Meat Back C3
Day 0		52.10±0.46	16.05±0.35	67.14±0.48	9.62±0.22
	Burlap	53.20±0.66	16.61±0.66	68.04±0.35	10.43±0.34
Day 1	No Cover	54.84±0.66	15.80±0.66	69.01±0.35	10.08±0.34
	p-value	NS	NS	NS	NS
	Burlap	58.43±0.58	16.53±0.55	75.60±0.45	10.28±0.34
Day 3	No Cover	58.02±0.62	17.97±0.59	75.43±0.48	10.92±0.36
	p-value	NS	NS	NS	NS
	Burlap	54.82±0.81	14.45±0.58	72.13±0.61	10.03±0.37
Day 5	No Cover	54.94±0.83	14.85±0.59	73.31±0.62	10.47±0.38
	p-value	NS	NS	NS	NS
	Burlap	55.30±0.70	13.78±0.48	73.71±0.66	10.10±0.42
Day 7	No Cover	56.22±0.82	14.07±0.56	73.69±0.78	10.19±0.50
	p-value	NS	NS	NS	NS
	Burlap	55.91±0.84	14.24±1.05	73.49±0.71	9.83±0.53
Day 9	No Cover	56.88±0.93	15.24±1.16	75.28±0.79	10.46±0.58
	p-value	NS	NS	NS	NS
	Burlap	49.88±1.14	12.98±0.98	67.36±1.05	8.02±0.62
Day 11	No Cover	50.80±3.33	12.60±2.86	70.95±3.07	10.38±1.82
	p-value	NS	NS	NS	NS

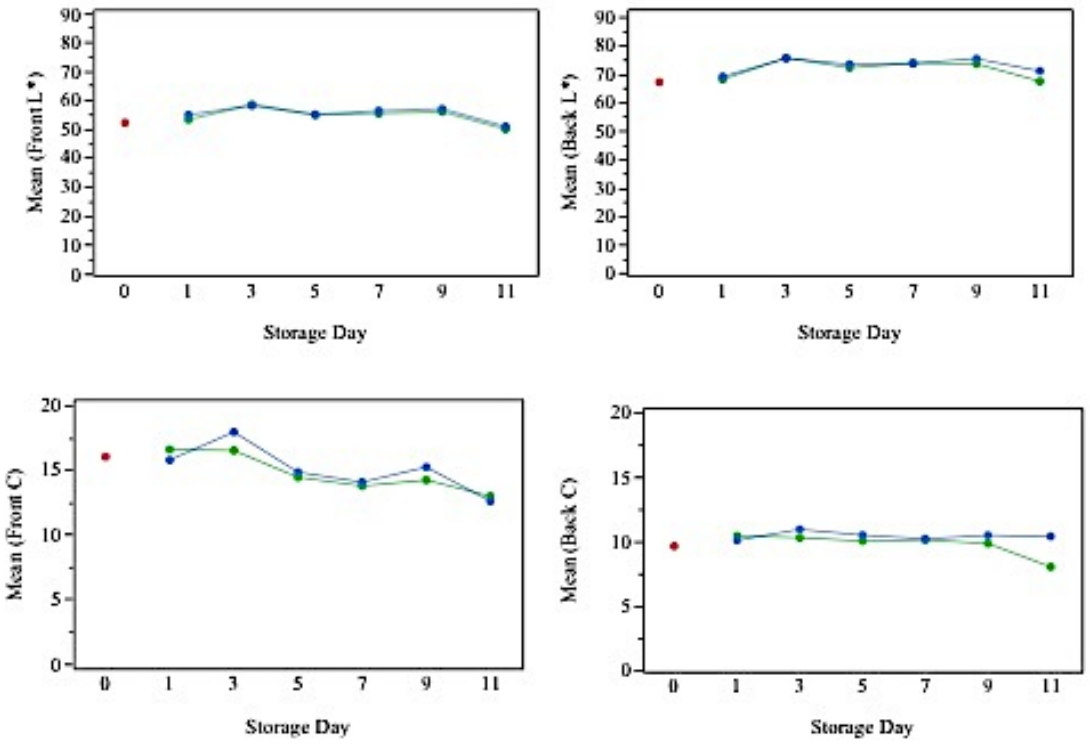

Figure 16. Meat color plotted against storage day by storage treatment (red dot-receiving condition, blue line-no cover and green line-burlap)

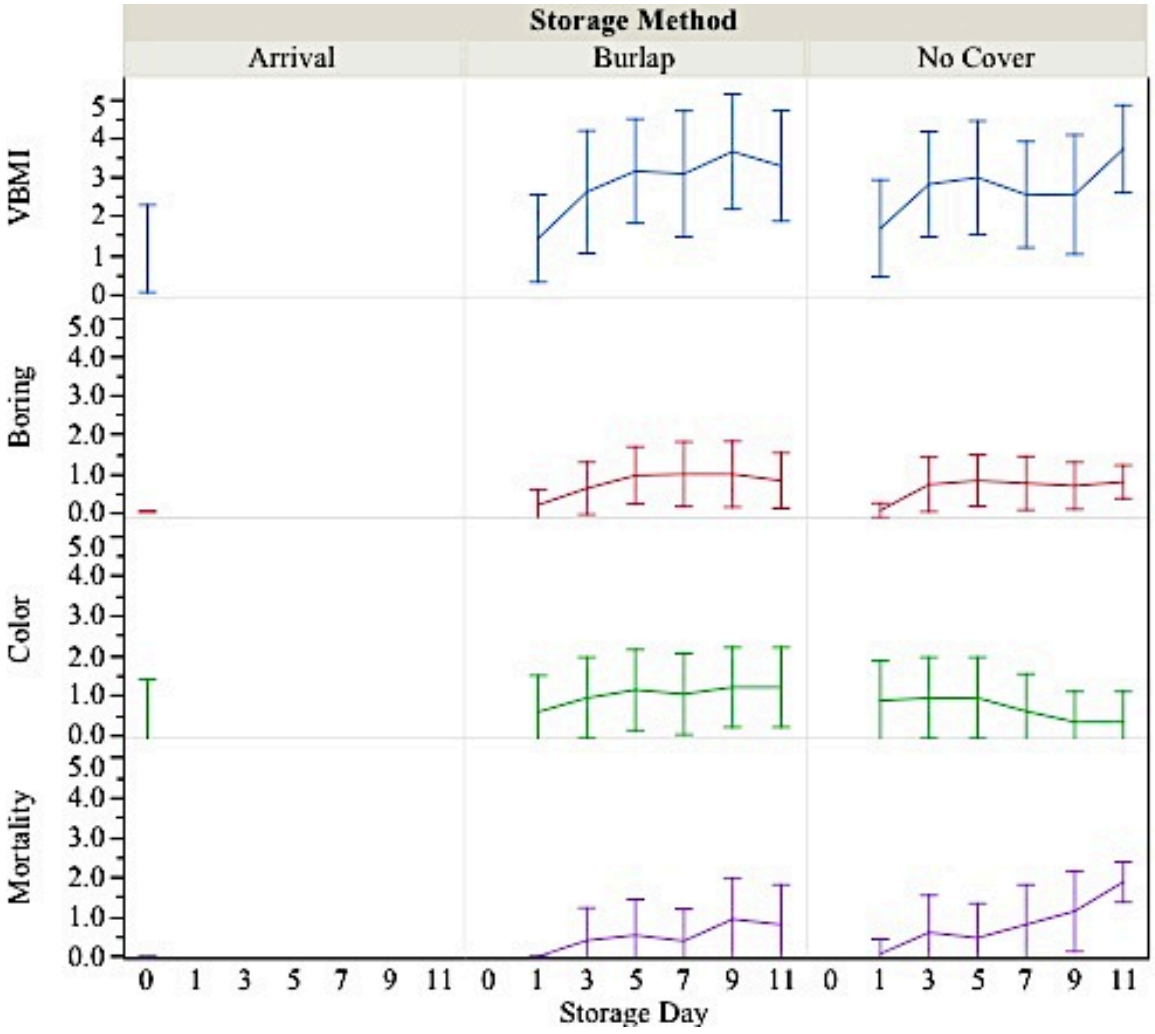

Figure 17. Means and standard deviations of visual based marketability index, boring score (1 and 2), color score (1 and 2), and mortality (2) by storage method over time.

REFERENCE

35, U. S. P. 2012. United States Pharmacopeial convention chapter <1052> Biotechnology-derived articles-amino acid analysis. 564-573.

Abbot, R. T. 1974. American Seashells. The Marine Mollusca of the Atlantic and Pacific Coasts of North America. Pages 663. Van Nostrand Reinhold Company, New York.

Badiu, D. L., Balu, A. M., Barbes, L., Luque, R., Nita, R., Radu, M., Tanase, E. & Rosoiu, N. 2008. Physico-chemical characterisation of lipids from Mytilus galloprovincialis (L.) and Rapana venosa and their healing properties on skin burns. Lipids, 43**,** 829-841.

Badiu, D. L., Luque, R., Dumitrescu, E., Craciun, A. & Dinca, D. 2010. Amino acids from Mytilus galloprovincialis (L.) and Rapana venosa molluscs accelerate skin wounds healing via enhancement of dermal and epidermal neoformation. The Protein Journal, 29**,** 81-92.

Basti, D., Bricknell, I., Hoyt, K., Chang, E. S., Halteman, W. & Bouchard, D. 2010. Factors affecting post-capture survivability of lobster Homarus americanus. Diseases of aquatic organisms, 90**,** 153-166.

Brown, K. & Alexander Jr, J. 1994. Group foraging in a marine gastropod predator: benefits and costs to individuals. Marine ecology progress series. Oldendorf, 112**,** 97-105.

Brown, K. M. 1997. Size-specific aspects of the foraging ecology of the southern oyster drill (Stramonita haemastoma) (Kool 1987). Journal of experimental marine biology and ecology, 214, 249-262.

Brown, K. M., McDonough, M. & Richardson, T. D. 2004. Intraspecific life history variation in the southern oyster drill, Stramonita haemastoma: patterns and causes. Journal of Shellfish Research, 23, 149-155.

Butler, P. A. 1954. The southern oyster drill. Pages 67-75. Proc. Natl. Shellfish. Assoc.

Butler, P. A. 1985. Synoptic review of the literature on the southern oyster drill, Thais haemastoma floridana, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Byron, K. W. & Smee, D. L. 2012. Effects of Flow on the Behavior of the Southern Oyster Drill Stramonita haemastoma in Response to Exudates from Oysters and Oyster Reef Fauna. Journal of Shellfish Research, 31, 93-100.

Carriker, M. 1981. Shell penetration and feeding by naticacean and muricacean predatory gastropods: a synthesis. Malacologia: International Journal of Malacology, 20.

Christofoletti, R. A., Takahashi, C. K., Oliveira, D. N. & Flores, A. A. 2011. Abundance of sedentary consumers and sessile organisms along the wave exposure gradient of subtropical rocky shores of the south-west Atlantic.

Journal of the Marine Biological Association of the United Kingdom, 91, 961-967.

Christophersen, G., Román, G., Gallagher, J. & Magnesen, T. 2008. Post-transport recovery of cultured scallop (Pecten maximus) spat, juveniles and adults. Aquaculture International, 16, 171-185.

Cioni, C., Patti, D., Bonaccorsi, M. C., Venturini, G., Modica, M. V., Scarpa, F., Oliverio, M. & Toni, M. 2012. Cellular, biochemical, and molecular characterization of nitric oxide synthase expressed in the nervous system of the prosobranch Stramonita haemastoma (Gastropoda, Neogastropoda). Journal of Comparative Neurology, 520, 364-383.

Claremont, M., Vermeij, G. J., Williams, S. T. & Reid, D. G. 2013a. Global phylogeny and new classification of the Rapaninae (Gastropoda: Muricidae), dominant molluscan predators on tropical rocky seashores. Molecular Phylogenetics and Evolution, 66, 91-102.

Claremont, M., Vermeij, G. J., Williams, S. T. & Reid, D. G. 2013b. Global phylogeny and new classification of the Rapaninae (Gastropoda: Muricidae), dominant molluscan predators on tropical rocky seashores. Mol Phylogenet Evol, 66, 91-102.

Claremont, M., Williams, S. T., Barraclough, T. G. & Reid, D. G. 2011. The geographic scale of speciation in a marine snail with high dispersal potential. Journal of Biogeography, 38, 1016-1032.

Cook, A. 2011. http://tinyurl.com/6aqn6f8. Access 2012 April.

Costa, M., Prado-Álvarez, M., Gestal, C., Li, H., Roch, P., Novoa, B. & Figueras, A. 2009. Functional and molecular immune response of Mediterranean mussel (< i> Mytilus galloprovincialis</i>) haemocytes against pathogen-associated molecular patterns and bacteria. Fish & shellfish immunology, 26, 515-523.

D'Asaro, C. N. 1966. The egg capsules, embryogenesis, and early organogenesis of a common oyster predator, Thais haemastoma floridana (Gastropoda: Prosobranchia). Bulletin of Marine Science, 16, 884-914.

Dal Bosco, A., Mugnai, C., Mourvaki, E. & Castellini, C. 2012. Seasonal changes in the fillet fatty acid profile and nutritional characteristics of wild Trasimeno Lake goldfish (*Carassius auratus*). Food Chemistry, 132, 830-834.

Dobberteen, R. A. & Pechenik, J. A. 1987. Comparison of larval bioenergetics of two marine gastropods with widely differing lengths of planktonic life,< i> Thais haemastoma canaliculata</i>(Gray) and< i> Crepidula fornicata</i>(L.). Journal of experimental marine biology and ecology, 109, 173-191.

Doran, E. 1965. Shell roads in Texas. Geographical review, 55, 223-240.

Eberline, B. S. 2012. Population Dynamics of the Eastern Oyster in the Northern Gulf of Mexico. Thesis. Virginia Polytechnic Institute and State University.

Endean, R. 1972. Aspects of molluscan pharmacology. Chemical Zoology., 7, 421-466.

Esaiassen, M., Akse, L. & Joensen, S. 2013. Development of a Catch-damage-index to assess the quality of cod at landing. Food Control, 29, 231-235.

Eston, V. R., Galves, A., Jacobi, C. M., Langevin, R. & Tanaka, N. I. 1986. Chthamalus bisinuatus (Cirripedia) and Brachidontes solisianus (Bivalvia) spatial interactions: A stochastic model. Ecological modelling, 34, 99-113.

FDA 2009. http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm186451.htm Access on 11/13/2013.

Feathers, J. K. 1989. Effects of temper on strength of ceramics: response to Bronitsky and Hamer. American Antiquity, 579-588.

Fish, F. 2011. Fishery Products Hazards and Controls Guidance. Technical report, US Department of Health and Human Services.

Fodrie, F. J., Brodeur, M. C., Toscano, B. J. & Powers, S. P. 2012. Friend or foe: Conflicting demands and conditional risk taking by opportunistic scavengers. Journal of experimental marine biology and ecology, 422, 114-121.

Fodrie, F. J., Kenworthy, M. D. & Powers, S. P. 2008. Unintended facilitation between marine consumers generates enhanced mortality for their shared prey. Ecology, 89, 3268-3274.

Fuke, S. 1994. Taste-active components of seafoods with special reference to umami substances. Pages 115-139. Seafoods: chemistry, processing technology and quality. Springer.

Garton, D. & Stickle, W. B. 1980. Effects of salinity and temperature on the predation rate of Thais haemastoma on Crassostrea virginica spat. The Biological Bulletin, 158, 49-57.

George, G. J., Brown, K. M., Peterson, G. W. & Thompson, B. A. 2008. Removal of black drum on Louisiana reefs to increase survival of eastern oysters Crassostrea virginica. North American Journal of Fisheries Management, 28, 1802-1811.

Group, A. F. W. 1999. Air Shipment of Live and Fresh Fish and Seafood Guidelines. A manual on preparing, packaging and packing live and fresh fish & seafood air shipments along with customs and inspection guidelines for six apec member economies, 126.

Gunter, G. 1953. The relationship of the Bonnet Carre spillway to oyster beds in Mississippi Sound and the Louisiana marsh, with a report on the 1950 opening. Publications of the Institute of Marine Science University of Texas, 3, 17-71.

Gunter, G. 1968. Some factors concerning the drilling apparatus and the feeding and predation of prosobranchiate gastropods especially on other molluscs.

Pages 370-378. Proc. Symposium on Mollusca-Part I-Marine Biol. Assoc. India.

Harding, J. M. & Harasewych, M. 2007. Two new modern records of the southern oyster drill *Stramonita haemastoma floridana* (Conrad, 1837) in Chesapeake Bay, USA. Nautilus, 121, 146-158.

Haupt, T., Griffiths, C., Robinson, T. & Tonin, A. 2010. Oysters as vectors of marine aliens, with notes on four introduced species associated with oyster farming in South Africa. African Zoology, 45, 52-62.

Hershler, R. & Ponder, W. F. 1998. A review of morphological characters of hydrobioid snails, Smithsonian Institution Press.

Horst, J. & Horst, G. 2011. The Louisiana Seafood Bible: Oysters, Pelican Publishing Company.

Huang, C. & Mir, G. 1971. Pharmacological properties of hypobranchial gland of Thais haemastoma (Clench). Journal of Pharmaceutical Sciences, 60, 1842-1846.

Huang, C. & Mir, G. 1972. Pharmacological investigation of salivary gland of Thais haemastoma (clench). Toxicon, 10, 111-117.

Iacarella, J. C. & Helmuth, B. 2011. Experiencing the salt marsh environment through the foot of< i> Littoraria irrorata</i>: Behavioral responses to thermal and desiccation stresses. Journal of experimental marine biology and ecology, 409, 143-153.

Ingle, R. M. 1953. Notes on growth of Thais haemastoma floridana and thais (Stramonita) rustica. Proc Natl Shellfish Assoc*,* 42**,** 12-14.

Inoue, T. & Yamamuro, M. 2000. Respiration and ingestion rates of the filter-feeding bivalve *Musculista senhousia*: implications for water-quality control. Journal of Marine Systems*,* 26**,** 183-192.

Issaris, Y., Katsanevakis, S., Salomidi, M., Tsiamis, K., Katsiaras, N. & Verriopoulos, G. 2012. Occupancy estimation of marine species: dealing with imperfect detectability. Marine Ecology Progress Series*,* 453**,** 95-106.

Karakoltsidis, P. A., Zotos, A. & Constantinides, S. M. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and molluscs. Journal of Food Composition and Analysis*,* 8**,** 258-273.

Koren, Z. C. 2001. The first optimal all-Murex all-natural purple dyeing in the eastern Mediterranean in a millennium and a half. Archaeology*,* 10.

Koren, Z. C. 2008. Archaeo-chemical analysis of Royal Purple on a Darius I stone jar. Microchimica acta*,* 162**,** 381-392.

Lahbib, Y., Abidli, S. & El Menif, N. T. 2011. Spawning and intracapsular development of Stramonita haemastoma haemastoma (Gastropoda: Muricidae) collected in northern Tunisia. Marine Biology Research*,* 7**,** 719-726.

Lee, C. H., Lee, D. K., Ali, M. A. & Kim, P. J. 2008. Effects of oyster shell on soil chemical and biological properties and cabbage productivity as a liming materials. Waste Management, 28, 2702-2708.

Liu, L., Foltz, D. & Stickle, W. 1991. Genetic population structure of the southern oyster drill *Stramonita* (= Thais) haemostoma. Marine Biology, 111, 71-79.

López, M. S., Coutinho, R., Ferreira, C. E. & Rilov, G. 2010. Predator-prey interactions in a bioinvasion scenario: differential predation by native predators on two exotic rocky intertidal bivalves. Mar. Ecol. Prog. Ser, 403, 101-112.

Lorenzon, S., Giulianini, P., Martinis, M. & Ferrero, E. 2007. Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster< i> Homarus americanus</i>. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147, 94-102.

Maulvault, A. L., Anacleto, P., Lourenço, H. M., Carvalho, M. L., Nunes, M. L. & Marques, A. 2012. Nutritional quality and safety of cooked edible crab (*Cancer pagurus*). Food Chemistry, 133, 277-283.

Mendes, R., Quinta, R. & Nunes, M. L. 2001. Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. European Food Research and Technology, 212, 141-146.

Menzel, W. R. & Nichy, F. E. 1958. Studies of the distribution and feeding habits of some oyster predators in Alligator Harbor, Florida. Bulletin of Marine Science, 8, 125-145.

Michel, R. H., Lazar, J. & McGovern, P. 1992. The chemical composition of the indigoid dyes derived from the hypobranchial glandular secretions of Murex molluscs. Journal of the Society of Dyers and Colourists, 108, 145-150.

Minniti, F. 1986. Morphological and histochemical study of pharynx of Leiblein, salivary glands and gland of Leiblein in the carnivorous Gastropoda Amyclina tinei Maravigna and Cyclope neritea Lamarck (Nassariidae: Prosobranchia Stenoglossa). Zoologischer Anzeiger, 217, 14-22.

Monahan, J. P. 1984. A quantity discount pricing model to increase vendor profits. Management science, 30, 720-726.

Nakamura, Y. & Kerciku, F. 2000. Effects of filter-feeding bivalves on the distribution of water quality and nutrient cycling in a eutrophic coastal lagoon. Journal of Marine Systems, 26, 209-221.

Ostroumov, S. 2002. Inhibitory analysis of top-down control: new keys to studying eutrophication, algal blooms, and water self-purification. Hydrobiologia, 469, 117-129.

Ostroumov, S. 2005. Some aspects of water filtering activity of filter-feeders. Pages 275-286. Aquatic Biodiversity II. Springer.

Overaa, T. 1999. Live transport of the great scallop (Pecten maximus). Marketing and Shipping**,** 105.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk Nucella lapillus (L.). Pages 155-182. Progress in Littorinid and Muricid Biology. Springer.

Pérez-Palacios, T., Ruiz, J., Martin, D., Muriel, E. & Antequera, T. 2008. Comparison of different methods for total lipid quantification in meat and meat products. Food Chemistry*,* 110**,** 1025-1029.

Pierce, S. K. 1971a. A source of solute for volume regulation in marine mussels. Comparative Biochemistry and Physiology Part A: Physiology*,* 38**,** 619-635.

Pierce, S. K. 1971b. Volume regulation and valve movements by marine mussels. Comparative Biochemistry and Physiology Part A: Physiology*,* 39**,** 103-117.

Power, A., Keegan, B. & Nolan, K. 2002. The seasonality and role of the neurotoxin tetramine in the salivary glands of the red whelk< i> Neptunea antiqua</i>(L.). Toxicon*,* 40**,** 419-425.

Ray, S. & Benefield, R. 1997. Present and future challenges facing the Texas oyster fishery. Journal of Shellfish Research*,* 16**,** 316.

Reese, D. S. 2010. Shells from Sarepta (Lebanon) and East Mediterranean Purple-Dye Production. Mediterranean Archaeology and Archaeometry, 10, 113-141.

Ridgway, I. D. 2007. A preliminary study of the effect of meteorological factors on the live export trade of the Scottish Nephrops trawling fishery. Journal of Shellfish Research, 26, 611-615.

Rilov, G., Benayahu, Y. & Gasith, A. 2001. Low abundance and skewed population structure of the whelk Stramonita haemastoma along the Israeli Mediterranean coast. Marine Ecology Progress Series, 218, 189-202.

Rilov, G., Benayahu, Y. & Gasith, A. 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? Marine Ecology Progress Series, 282, 193-204.

Rilov, G., Gasith, A. & Benayahu, Y. 2002. Effect of an exotic prey on the feeding pattern of a predatory snail. Marine environmental research, 54, 85-98.

Rodriguez, G. 1963. The intertidal estuarine communities of Lake Maracaibo, Venezuela. Bulletin of Marine Science, 13, 197-218.

Roller, R., Rickett, J. & Stickle, W. 1995. The hypobranchial gland of the estuarine snail Stramonita

haemastoma canaliculata (Gray) (Prosobranchia: Muricidae): a light and electron microscopical study. American Malacological Bulletin, 11, 177-190.

Roller, R. A. & Stickle, W. B. 1989. Temperature and salinity effects on the intracapsular development, metabolic rates, and survival to hatching of< i> Thais haemastoma canaliculata</i>(Gray)(Prosobranchia: Muricidae) under laboratory conditions. Journal of experimental marine biology and ecology, 125, 235-251.

Sanchez‐Brambila, G., Lyon, B., Huang, Y., Santiago, J., Lyon, C. & Gates, K. 2002. Sensory and texture quality of canned whelk (Astraea undosa) subjected to tenderizing treatments. Journal of food science, 67, 1559-1563.

Schubring, R. & Meyer, C. 2002. Quality factors of terrestrial snail products as affected by the species. Journal of food science, 67, 3148-3151.

Sikorski, Z. E. 1990. The nutritive composition of the major groups of marine food organisms. Seafood: resources, nutritional composition, and preservation, 29-54.

Sriket, P., Benjakul, S., Visessanguan, W. & Kijroongrojana, K. 2007. Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food Chemistry, 103, 1199-1207.

Stickle, W. 1999. Effects of estuarine environmental factor gradients on the tolerance and physiology of the southern oyster drill Stramonita (= Thais) haemastoma; An important predator of the American oyster Crassostrea virginica. Natural system function and response to human influence, 277.

Stickle, W. & Howey, T. 1975. Effects of tidal fluctuations of salinity on hemolymph composition of the southern oyster drill Thais haemastoma. Marine Biology, 33, 309-322.

Sveinsdóttir, K., Martinsdóttir, E., Green-Petersen, D., Hyldig, G., Schelvis, R. & Delahunty, C. 2009. Sensory characteristics of different cod products related to consumer preferences and attitudes. Food Quality and Preference, 20, 120-132.

Temelli, S., Dokuzlu, C. & Sen, M. K. C. 2006. Determination of microbiological contamination sources during frozen snail meat processing stages. Food Control, 17, 22-29.

Udofia, U. S. 2009. Snail (Archachatina marginata) pie: a nutrient rich snack for school-age children and young mothers. International Journal of Food Safety, Nutrition and Public Health, 2, 125-130.

Walker, R. L. 1982. The gastropod Thais haemastoma in Georgia: T. h. floridana or T. h. canaliculata? Gulf Res. Rep., 7, 183-184.

Watanabe, J. T. & Young, C. M. 2006. Feeding habits and phenotypic changes in proboscis length in the southern oyster drill, Stramonita haemastoma (Gastropoda: Muricidae), on Florida sabellariid worm reefs. Marine Biology, 148, 1021-1029.

Waterman, J. 1983. Processing Mussels, Cockles and Whelks, Ministry of Agriculture, Fisheries and Food, Torry Research Station.

Wedepohl, K. H. & Baumann, A. 2000. The use of marine molluskan shells for Roman glass and local raw glass production in the Eifel area (Western Germany). Naturwissenschaften, 87, 129-132.

Wells, H. W. 1961. The fauna of oyster beds, with special reference to the salinity factor. Ecological Monographs, 31, 239-266.

Wilkinson, C., Dijksterhuis, G. & Minekus, M. 2000. From food structure to texture. Trends in Food Science & Technology, 11, 442-450.

Wingerter, K., Mujica, B., Miller-Morgan, T. & Grant, O. S. 2013. Development of Live Shellfish Export Capacity in Oregon.

Yarnpakdee, S., Benjakul, S., Penjamras, P. & Kristinsson, H. G. 2014. Chemical compositions and muddy flavour/odour of protein hydrolysate from Nile tilapia and broadhead catfish mince and protein isolate. Food Chemistry, 142, 210-216.

Zhao, F., Zhuang, P., Song, C., Shi, Z.-h. & Zhang, L.-z. 2010. Amino acid and fatty acid compositions and nutritional quality of muscle in the pomfret, *Pampus punctatissimus*. Food Chemistry, 118, 224-227.