Value of Off-bottom Oyster Aquaculture Baskets as Habitat for Juvenile Blue Crab

by

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Abstract

The potential of the adjustable long-line system (ALS) oyster aquaculture baskets as habitat for juvenile blue crab was investigated in Portersville Bay, AL, during Summer/Fall 2014. The density of juvenile blue crabs associated with baskets containing single shells oysters was not significantly greater than the density of juvenile blue crabs on/in baskets without single shell oysters. This suggested crabs were primarily attracted by the physical structure of the baskets, rather than the additional structure provided by the presence of oysters within the ALS baskets. The baskets as habitat appeared to be degraded by the presence of large blue crabs among the baskets. The potential of visible implant elastomer (VIE) tags as a tool to assess habitat quality in terms of juvenile crab site fidelity, survivorship, and growth was also assessed. The effect of VIE tags on survival was negligible. Crabs had an 85% tag retention rate after 16 weeks, validating VIE as a suitable tag for mark-recapture.

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˚C	Degrees Celsius
AIC	Akaike Information Criterion
AL	Alabama
ALL	Adjustable Long Line
AUORDF	Auburn University Oyster Research and Demonstration Farm
AUSL	Auburn University Shellfish Laboratory
CL	Carapace Length
CW	Carapace Width
GLM	Generalized Linear Model
KS	Kolmogrov-Smirnov
L	Tag Location Score
SAFRS	South Auburn Fisheries Research Station
SE	Standard Error
V	Tag visibility score

Chapter 1: Factors affecting the value of off-bottom oyster aquaculture baskets as habitat for juvenile blue crab

1.1 Introduction

Eastern oyster, *Crassostrea virginica* (Gmelin 1791), populations have dramatically decreased over the past 20 to 130 years globally (Beck *et al*. 2011; zu Ermgassen *et al*. 2012; Kroeger 2012). Beck *et al*. (2011) estimated that 70 % of all bays have declined to 10 % of their earliest recorded population, while 37 % of all bays globally have declined to less than 1 % of their earliest recorded population. These dramatic declines have been attributed to many factors, including habitat degradation, overharvesting, eutrophication, pollutants and freshwater runoff (Kirby 2004; Mackenzie 2007; Eastern Oyster Biological Review Team 2007; Beck *et al*. 2011). Since the 1980s in the United States, when oyster populations first began to plummet in the north east, the Gulf of Mexico states have been the largest contributor of oysters to national landings (Kirby 2004; VanderKooy 2012). The northern Gulf of Mexico now has some of the highest native oyster stocks in the world with a 'fair' rating compared to other ecoregions (Beck *et al*. 2011).

Gulf of Mexico oyster population declines have primarily been attributed to habitat degradation (Dugas *et al*. 1997; VanderKooy 2012). The Gulf faces both anthropogenic and natural sources of habitat degradation (Dugas *et al*. 1997). For example, cultch is removed during both dredging and filling, as well as during hurricane and storm surge events (Berrigan 1988; Dugas *et al*. 1997; VanderKooy 2012). Although substrate restoration is practiced in the Gulf, competition for shell limits the amount of cultch that is replenished (Berrigan, 1990; Dugas *et al*. 1991; Dugas *et al*. 1997; VanderKooy 2012). Furthermore, changes in hydrology and

freshwater input have altered salinity regimes in many estuarine areas in the Gulf, making these areas unfavorable, even though they may have once been prime oyster habitat (Dugas *et al*. 1997). Important factors driving the decline of Gulf oyster populations include pollutants, eutrophication, siltation and other results of coastal development (VanderKooy 2012).

The Eastern oyster is prized for its ecosystem services and ecological benefits (Gutiérrez *et al*. 2003; Coen *et al*. 2007; Grabowski and Peterson 2007; Beck *et al*. 2011; Grabowski *et al*. 2012; zu Ermgassen *et al*. 2013). Ecosystem services are defined as "benefits people obtain from ecosystems", while ecological services are generally services provided from ecological functions, to animals, plants or humans (Millennium Ecosystem Assessment 2005; Wallace 2007). Total services provided by oyster reefs, including shoreline stabilization, water quality and habitat provisions, ,are valued between \$10,325 and \$99,421 ha $^{-1}$ yr $^{-1}$ with oyster reef habitat ecological services alone valued between \$880 and \$21,959 ha $^{-1}$ yr $^{-1}$ depending on the extent and health of the reef (Grabowski et al. 2012). Oysters can be used to mitigate eutrophic waters and aid in nutrient filtration (Higgins *et al*. 2011; Kellog *et al*. 2014), sequester carbon, stabilize shorelines (Lenihan 1999) and provide habitat for many species (Grabowski and Peterson 2007). According to Ehrich and Harris (2015), the Eastern oyster has a maximum filtration rate of 0.17 (±0.07) m^3 g^{-1} dry wt. per day. Zu Ermgassen *et al*. (2013) estimated that the amount of water filtered by oysters in the Gulf of Mexico has decreased by 97% from pre-1900 levels, with local Mobile Bay and west Mississippi Sound water filtered by oysters decreasing by 79 % and 92 %, respectively. Oysters are also often considered ecosystem engineers by creating a three-dimensional habitat that provides refuge for other animals (Tolley and Volety 2005; Coen *et al*. 2007; Grabowski and Peterson 2007; Higgins 2011). Three-dimensional habitat passively influences the biodiversity of an area (Raj 2008). As such, oyster

reefs create crevices for many animals to hide and forage, including other mollusks, fish and crab species (Zimmerman *et al*. 1989; Meyer and Townsend 2000). Thus, oyster reefs provide complex physical structure and thus valuable habitat for juvenile blue crabs, among other taxa.

Scientists have been investigating methods to restore the ecosystem services (including provisioning of habitat), provided by *Crassostrea virginica* reefs as oyster populations are at historic lows (Beck *et al*. 2011). This includes reef restoration, importing exotic oyster species, and aquaculture. There is evidence that restored reefs provide the same services as natural reefs; however, reef creation is a long-term investment as initial reef creation can be costly (Grabowski and Peterson 2007; Kroeger 2012). Grabowski *et al*. (2012) estimated that reef creation costs between \$52,000 and \$260,000 ha^{-1} yr^{-1}; allowing a return on investment within 2-14 years. In the Chesapeake Bay, oyster reef creation costs around \$10,000 ac^{-1} for cultch only (Henderson and O'Neil 2003). Beck *et al*. (2011) claim that the Gulf of Mexico is the only region containing oyster habitat that can still handle both oyster reef conservation and a sustainable fishery. In the northern Gulf of Mexico, reef creation can cost anywhere from \$12,000 ha^{-1} to over \$1,000,000 ha^{-1} (LaPeyre *et al*. 2014).

Another option to maintain ecosystem services is the introduction of exotic oyster species, which can withstand challenges of salinity and disease, as a way to obtain oyster reef ecosystem services (Gottlieb and Schweighofer 1996; Luo and Opaluch 2011). For example, *Crassostrea ariakensis* habitat function was evaluated against *C. virginica* in 2010 (Harwell *et al*. 2010). Harwell *et al.* (2010) found that *C. ariakensis* provided functionally equivalent habitat in intertidal areas; however, in subtidal regions, there were significantly less organisms associated with *C. ariakensis* then *C. virginica.*

Another option is private aquaculture of oysters. Using cultured oysters instead of restoring reefs could decrease fishing pressure on natural stocks, while still producing a crop and providing a variety of ecosystem services, including habitat creation. Northern Economics Inc. (2014) estimated the potential value of additional habitat created by oyster aquaculture to increase commercial and recreational fisheries by approximately $925 and $632 yr^{-1} ha^{-1} respectively.

Juvenile blue crabs, *Callinectes sapidus* (Rathbun 1986) are economically important and utilize *Crassostrea virginica* reefs for habitat. In North America, the blue crab fishery had a value of $211,942,013 in 2010, making it the fifth most valuable fishery that year (FAO 2010). In general, blue crab landings have declined since the 1980s, when populations were at their peak; however, fishery-independent estimates show high variability in population size between years often due to the differential success of brood stock (VanderKooy 2013).

C. sapidus home ranges extend from along the eastern seaboard of the United States, to Argentina, and into the Gulf of Mexico (VanderKooy 2013). Adult blue crab are typically present in coastal waters (<35m deep). Mature females spawn in the spring, summer and fall, and over mild winters (Perry and McIlwain 1986). The larvae transition through 7 zoeal stages for 31-49 days, before metamorphosing into the megalopal stage for 6-21 days (Perry and McIlwain 1986) when they move back to the nearshore. The recruitment of megalopae to the juvenile stage peaks in August and September but is continuous all year (Perry and McIlwain 1986), with recruitment regulated by physical dispersal, predator avoidance and food availability (Perry and McIlwain 1986; Morgan *et al*. 1996; Moksnes *et al* 1997; VanderKooy 2013). Crabs reach maturity within one year in the Gulf of Mexico (VanderKooy 2013). Adult crabs tend to inhabit low salinity water, with females travelling to offshore saline water to spawn, creating a

differential distribution of males and females due to a salinity gradient (Perry and McIlwain 1986).

Although there is debate (Rilov *et al*. 2007; Mattila *et al*. 2008), it is generally believed that more structure allows more refuge from predation (Moody 2003; Moksnes and Heck 2006) which is vital in the blue crabs' early juvenile stages (Orth and van Montfrans 2002). Availability of complex structure allows juvenile blue crabs to hide and seek protection from predators. Oyster reef and submerged aquatic vegetation provide the structure needed for refuge. Thus, there is often active selection for structured habitat (Moksnes and Heck 2006).

Juvenile blue crabs respond to many different cues when settling. *Callinectes sapidus* are likely able to actively select habitat within an estuary due to their sensory capabilities and swimming capabilities in the megalopae and J1 stages (van Montfrans *et al*. 2003; Mosknes and Heck 2003). When structurally complex habitat is formed from a biological organism, such as seagrass or oyster reefs, both chemical cues and physical cues are at the crabs' disposal. In the laboratory, J1 instars actively selected live *Crassostrea virginica* over oyster shell, live seagrass, *Zostera marina* and artificial sea grass, but the results were not consistent with the field data (van Montfrans *et al.* 2003). Van Montfrans *et al.* (2003) suggest that the ability to discriminate, and choose habitat substrate is mediated by a chemical cue rather than a structural cue associated with *Z. marina* in the megalopae stage, and *C. virginica* in the J1 stage, as artificial grass and bleached shell were not selected as often as their live counterparts (van Montfrans *et al.* 2003). Conversely, Welch *et al.* (1997) found no evidence to support a preferential settlement of *C. sapidus* megalopae on or near oyster reefs by a chemical odor cue only, with megalopae avoiding live *C. virginica*, and no reaction to fouled *C. virginica* shell. Few studies focus on habitat

preference of juvenile blue crabs above the first few instars, despite being highly mobile and dispersing often within the estuary.

Oyster aquaculture gear can support equivalent or higher densities of aquatic fauna, including juvenile blue crab, relative to many other types of habitat (Dealteris *et al*. 2004; O'Beirn *et al*. 2004; Erbland and Ozbay 2008; Marenghi *et al*. 2010). There is evidence that oyster aquaculture can provide habitat similar to that of restored and natural reefs (Tallman and Forrester 2007), while at the same time, generating income. One of the first studies that assessed the impact of aquaculture gear on other taxa was conducted by O'Beirn *et al*. (2004). Estimates range from 12,746 organisms to 92,602 organisms per 61 cm^2 floating bag after a 10-month grow-out cycle (O'Beirn *et al*. 2004). Dealteris *et al*. (2004) compared the habitat value of rack and bag oyster gear to submerged aquatic vegetation and non-vegetated seabed. The rack-and-bag aquaculture gear had significantly ($p>0.0001$) higher densities of organisms (>1000 organisms) than either habitat type (<500 organisms), with degree of differences varying by season (Dealteris *et al*. 2004). Similarly, Erbland and Ozbay (2008), Marenghi *et al*. (2010), and Rossi-Snook *et al*. (2010) showed total density in rack and bag aquaculture gear and floating cages was double that of the natural reef.

Previous studies along the Atlantic coast suggest that blue crabs are associated with oyster aquaculture farms (O'Beirn *et al*. 2004; Dealteris *et al*. 2004; Tallman and Forrester 2007; Erbland and Ozbay 2008; Marenghi *et al*. 2010; Rossi-Snook *et al*. 2010). There are few studies examining off-bottom oyster farming in the northern Gulf of Mexico, since the industry is new (Supan 2002; Walton *et al*. 2012; Stewart 2015). Stewart (2015) compared blue crab density among long-line (ALS) gear, bags of shell on bottom, submerged aquatic vegetation and non-vegetated bottom habitat at multiple sites in the northern Gulf of Mexico. He found that ALS

gear generally had higher densities of juvenile blue crabs than bag-of-shell on bottom and submerged aquatic vegetation, with variability between summer and fall 2013. He noted, however, that this may not be due to changes in habitat complexity and structure. Bag-of-shell on bottom, while structurally similar to ALS in that they both contain oysters with similar interstitial spaces and cavities, is a benthic habitat while ALS oyster culture is not.

This study investigated whether the attractive characteristics of ALS habitat are primarily associated with the physical structure from the unique off-bottom habitat or by possible chemical cues from the oyster habitat. By bringing the 'bag of shell' or structurally similar but not chemically similar treatment (represented by empty oysters) to the same depth as ALS oyster culture, the aim was to determine if chemical or physical attributes makes ALS oyster culture habitat attractive to juvenile blue crab. The potential of ALS oyster aquaculture baskets to serve as habitat for juvenile blue crab was broken into three options represented by live single shell oysters, dead empty oysters (structural cues associated with oysters), or empty baskets (structural cues independent of live meat). It was hypothesized that blue crab juveniles recruit primarily to structure, so the presence/absence of live oysters in baskets would have little effect on juvenile crab density. The predation pressure on juvenile blue crabs and lack of refuge habitat will cause crabs to use both live oysters and empty oysters alike, despite chemical cues, which are important factors in habitat selection in the early instar and megalopae stages. It is expected that the number and size of crabs will differ between baskets with contents, and baskets without, since the structural complexity and size of the interstitial spaces change. Baskets with contents will have small interstitial space in between the oysters, creating crevices and hiding places for smaller crabs. Baskets without contents will have little refuge and, thus, be inhabited by larger crabs. With the creation of more structure from the oysters, more crabs can hide, and thus

abundance will increase in baskets with contents. Also, baskets with contents will have more surface area, and thus more epifauna for consumption and faster growth.

1.2 Methodology

Field experiments took place in Portersville Bay, AL (30°21'14.55"N, 88°11'30.06"W; Fig. 1.1). A 24-hectare Auburn University Research and Demonstration Farm (AUORDF) was established in the bay in 2011. Because the bay substrate is comprised primarily of silt/sand with no existing oyster reefs or submerged aquatic vegetation (Vittor and Associates 2009), the farm likely provides some of the only complex structure in the entire bay. The brackish water is typically polyhaline; however, in the spring when fresh water discharge is high, the system will often become mesohaline. Salinity on sample dates ranged from 19.2 ppt to 27.5 ppt, and temperature ranged from 18.1˚C in December 2014 to 32.4˚C in September 2014. The 1.5 m deep bay has diurnal tides that fluctuate, on average, around 0.5 m.

The aquaculture gear used was BST^{TM} 12-mm hexagonal mesh baskets on an adjustable long-line system (ALS). The inter-lock baskets were secured in an in-line fashion via a BST^{TM} Tee clip to a 5-mm diameter Bayco^{TM} wire covered with dripper tube that suspended the baskets approximately 1-m off the bottom. Each 'run' (row) consisted of two paired parallel 92--m long cables tied between piling pairs. The cable was tensioned, with riser posts every 2.5-m, allowing adjustment of the height of the suspension lines. The distance between riser posts created one 'bay', holding three baskets per line, with six baskets total per bay (Fig. 1.2). Cables, and the associated baskets, were generally raised once per week to remove the oysters from the water allowing them to desiccate for 24 hrs. This 24-hr. desiccation regime limits fouling on the oysters, and is part of routine oyster farm maintenance. Oysters can withstand 24 hours out of water, while many newly settled fouling organisms cannot.

Every other bay was set up with one treatment, with a total of five replicate bays each with six baskets per bay and five complete blocks with three bays each (Fig. 1.3). Baskets were deployed July 22, 2014. Treatments were live oysters (L), empty oysters (S) and empty baskets (E). Within each block, treatment order was randomized. Empty oysters did not have meat in them, to mimic the structural component provided by the shell without any of the chemical factors associated with live oysters. Empty oysters were created by shucking 2,100 oysters to rid the oyster of the meats, and letting the shell pairs dry for 2 days (Fig. 1.4). Using GOOP™ plumbing epoxy, the shells were glued together and allowed to cure for a minimum of three days. Since GOOP™ contains toluene that may have adverse effects in crustaceans (Amazing GOOP), the shells were placed in a tank with flow-through seawater for 24 hours to flush out any residual chemicals. Empty oysters and live oysters were stocked at 70 per basket. All baskets were constructed of 12-mm plastic mesh (interior dimension). Spaces, or empty bays, were kept between each treatment bay, so bays were assumed to be independent for sampling (Fig. 1.3). When one bay was sampled, it was not observed to disturb the neighboring bays and any crabs present, as the baskets and lines did not move. Bay 30 was skipped because the bay was too narrow. However, since each bay is independent, this allowed moving this replicate to bay 31. Care was taken when sampling, so that the boat and all parties involved moved from north to south, keeping at least three bays distance from the bay being sampled.

Sampling began August 12, 2014, and continued approximately every 3 weeks until December 2, 2014, for 6 sampling events. Crabs associated with individual baskets were collected by first enclosing the basket with a 500-µm mesh net (Fig. 1.5), and then unhooking the basket from the in-line cable, and finally lifting the mesh bag, basket and contents from the water. This methodology captured crabs inside, and on the outer surfaces of the basket. Each

enclosed basket sampled was emptied out into a bin. Any *Callinectes spp*. found within the basket or captured in the net were dropped onto a 6.5-mm plastic mesh screen, but it was unknown which crabs were from the inside of the basket, and which were from the outside, enclosed in the net. Crabs that retained on the screen were measured for carapace width (CW) and carapace length (CL) using digital calipers to the nearest hundredth mm.

To determine whether the dimensions of empty oysters remained similar to those of live, growing oysters during the course of the study, before returning the sample, the first six live oysters from each live oyster treatment were measured for shell metrics (shell height (SH), shell length (SL) and shell width (SW)) using digital calipers and the number of live and dead oysters were recorded, as well as damaged empty oysters. A damaged empty oyster was broken and no longer closed. All oysters, live or empty, were then returned to the basket. The crabs alongside the basket were placed back into the mesh net which was returned to its original position for the remainder of the study. Environmental data including water salinity and temperature were recorded on each sampling day using YSI-85.

All statistical tests and graphs were completed with JMP 11.0 and Excel 2010. Shell length violated the parametric assumption for normality (Appendix A, Table 1), and all dimensions (SH, SW, SL) had unequal variances (Appendix A, Table 2). Therefore, a non-parametric test, the Wilcoxon Test, was used to test for significant differences in average shell metrics between live and dead shells for the duration of the study (Appendix A, Table 3). Oyster shell volume was calculated (Equation 1) and used to compare average empty oyster size with the average live shell size.

$$Shell\ Volume(SV) = Shell\ Length(SL) * Shell\ Height(SH) * Shell\ Width(SW)$$

Equation 1.1 Estimation of oyster volume used to compare empty oysters and live oyster structure over five-month experiment.

This assumes the oyster is a box, but accounts for variation in SL and SW (*e.g.* flat, wide oysters or deep, skinny oysters). Although the shell volume data were normal (Appendix A, Table 4), equal variances weren't (Appendix A, Table 5); so the variability in shell shape and size was greater in the live oysters than in the empty oysters. Shell volume was compared with Students T-test.

Response variables of average crab size (CL and CW) and crab density were compared within and among treatments to compare preferences for live oysters, empty oysters or empty baskets. The parametric assumption of normality was tested using Shapiro-Wilks W test for both crab density and size. This test is robust with small and medium sample sizes, and preferred over a Kolmogorov-Smirnov D test or K-S Lilliefors test, which are better for larger sample sizes ($n>2000$). Both density of crabs (Appendix A, Table 8) and average crab size were not normal (Appendix A, Table 9). Square root and log transformations did not result in a normal distribution. Unequal variances were tested using Bartlett's, O'Brien, Brown-Forsythe, Levene, and Welch's methods for average crab size (Appendix A, Table 10) and crab density (Appendix A, Table 11). Since the data were not normal, non-parametric multiple comparisons tests were used. A Kruskal-Wallace test was applied to determine if there were significant differences between the average number of crabs in each treatment (Appendix A, Table 12) and across sample dates (Appendix A, Table 13), as well as to determine if there were any significant differences between the density of crabs in each treatment (Appendix A, Table 14) and samples dates (Appendix A, Table 15). If significance was detected, then to determine which treatments were significantly different, a Wilcoxon post-hoc test was applied to each pair (Appendix A, Table 16; Table 17). A Wilcoxon test was also applied for each pair to determine which dates

were significantly different from each other (Appendix A, Table 17). A general linear model was used to model patterns between large and small crabs among baskets, comparing models with AIC scores (Appendix A, Table 18).

1.3 Results

The empty oysters were approximately equal in shell metrics (SH: 63.6 ±1.0; SW: 48.3 ±0.6, SL: 19.8 ± 0.3) to the live oysters during the middle of the sampling period (SH: 63.8± 1.2 on 9/24/2014; SW: 48.4± 0.8 on 11/5/2014; SL: 19.7± 0.6 on 9/3/2014), and thus were judged to have been an acceptable representation of the structure provided by the live oysters (Fig. 1.6; Appendix A, Table 19). While mean SH was not significantly different ($p=0.593$), SL and SW were significantly different between live oysters and empty oysters (SL: $p=0.002$; SW: $p<0.001$; Appendix A, Table 3). There was greater mortality among live oysters than empty oysters (Appendix A, Table 20), where an empty oyster was considered dead if the pair is broken and does not remain closed. There was no significant difference between the live oyster average volume, and empty oyster volume when compared with a Student's T test (Appendix A, Table 6).

Mean crab carapace length and carapace width increased over time (Appendix A, Table 21). Crab density decreased with time (Appendix A, Table 22). There were no significant differences in mean carapace length of juvenile crabs between empty oyster, live oyster and empty basket treatments ($p=0.421$); however, there was a significant difference in crab carapace length by date ($p<0.001$). Blue crabs were significantly smaller (carapace length) on the August 12, 2014 sampling date than dates sampled after September 24, 2014. Likewise, September 3, 2014 had significantly smaller sized crabs than all later sampling dates (September 24, 2014 through December 2, 2014). August 12, 2014 and September 3, 2014 were not significantly different (Fig. 1.7). Carapace width showed a similar pattern through time (Appendix A, Table 23, 24, 25).

There was no significant difference in the number of crabs found among basket treatments (live oysters, empty oysters or empty baskets) ($p=0.466$; Appendix A, Table 14). There was a significant difference in the density of crabs by date ($p<0.001$) (Appendix A, Table 15). There was no significant difference in crab density among September 24, 2014, October 14, 2014 and November 5, 2014 sampling dates. August 12 and September 3 sampling dates had significantly more crabs while September 24 through December 2 sampling dates had significantly lower densities of crabs than those of other sampling dates (Fig. 1.8; Appendix A, Table 17).

The number of crabs per basket declined with the size of the largest crab associated with the basket, creating a heteroscedastic pattern which was modeled with a generalized linear model (Fig. 1.9). The data fit a negative binomial generalized regression, with the best AIC score estimated from Maximum likelihood (Appendix A, Table 18).

1.4 Discussion

To compare the attractive value between live and empty oyster treatments, one must first make sure the structural complexity is comparable. The intended difference between the two treatments was the presence of live, oyster meat. However, there were differences in shell length and shell width between empty oysters and live oysters. Differences in shell length or height could vary the size of the cavities created between the oysters. Empty oysters tended to have less of a cup (shell width) but a larger shell length than live oysters, so both oysters filled approximately the same amount of space. Since there was no significant difference in shell height or estimated oyster volume between empty oysters and live oysters, it can be concluded that the empty oysters were suitable proxies for the structure of the growing, live oysters.

The data support the hypothesis that the presence of live oysters does not enhance habitat value of baskets, as indicated by crab density but does not support the hypothesis that crab size and number will vary with respect to the presence absence of basket contents. There were no differences in density or average CL of crabs associated with the different basket treatments: live oysters; empty baskets; or empty oysters. The lack of a significant difference could be because crabs primarily seek habitat with refuge (basket with contents), before considering chemical composition.

While live oysters are a food item of *C. sapidus,* the size of the oyster is vital to prey determination. Menzel and Hopkins (1955) suggested that blue crabs prey on spat and small oysters that they can efficiently open, and tend to 'scavenge' adult oysters, testing oysters until they find a weak individual, since they cannot easily open larger oysters. The live oysters used in this study had a mean shell height of 47 mm at the August 12th sampling period. The largest crab

sampled was 14.36 mm carapace length on August 12th, while on Dec 2, the largest crab sampled was 39.75 mm, and the average oyster was 75.5 mm. It is unlikely that a small crab could effectively open an oyster of that size. In a 1990 study, crabs that were over 135 mm carapace width, could not crush oysters greater than 45 mm shell height (Eggleston 1990), and in a 1978 study blue crabs between 65 and 80 mm carapace width could not consume oysters with 25 mm shell height (Seitz *et al*. 2011). Oyster mortality, which increased from a mean percent mortality in August of 0.57% to 7.71% in December, could not be attributed to the presence of blue crabs, since the oysters were much too large for the crabs to consume.

Crabs entering the basket are far too small to consume the oysters, which was designed *a priori* in the experiment so that the structural integrity of the treatments would remain near constant throughout the experiment. However, this might have caused an unintentional artifact, as the empty oysters and live oysters as well as ALS baskets all contained a food resource, providing substrate for tunicates, algae, barnacles and some oyster spat. Larger juvenile blue crab (<60 mm) have a diet that is variable with habitat type, but often includes epifaunal invertebrates, bivalves, amphipods, isopods, polychaetes and gastropods (Eggleston 1990; Seitz *et al*. 201). There were no significant differences detected in density or average size (CL mm) of crabs between empty oyster and live treatments, since the crabs may have been primarily feeding on epifaunal invertebrates growing on the oyster substrate rather than the live oysters. These results are in agreement with Tolley and Volety (2005) who found that community assemblages on live oyster and shell substrate in Tarpon Bay did not indicate active selection for habitat with live oysters among decapod species. When specifically looking at *Callinectes sapidus,* Tolley and Volety (2005) did not collect any crabs on sand bottom or live oyster clusters, but collected 5 crabs among clean articulated shell.

Epifaunal fouling organisms (barnacles, polychaetes, amphipods, etc.) could grow and thrive on the ALS gear (empty basket) as well as the empty oysters and live oysters, despite weekly desiccation efforts to control fouling. More frequent, or longer durations of desiccation could be used to minimize fouling organisms. This may have separated the food resource services and thus chemical cues from structure/refuge services of oyster aquaculture by using empty oysters and live oyster treatments; however, it is unknown whether other fouling organisms were used as a food resource. Nevertheless, it can still be determined if the added structure of basket contents enhances habitat value, by comparing baskets with contents to empty baskets.

In some habitats, crab size and density vary with structure characteristics. Heck and Spitzer (2001) concluded that smaller crabs (3.1-16.1 mm CW) survive best in low density vegetation, while larger crabs (11.7-34.8 mm CW) survive best in high density vegetation, often driven by predation. This conflicts with a laboratory/field study at nearby Ono Island, AL that concluded that smaller crabs (18.3 mm CW) preferred higher density vegetation over lower density vegetation habitats and larger juvenile crabs (24 mm CW) preferred the lower density vegetation over the higher density (Williams *et al*. 1990). If this pattern of small crabs preferring high density vegetation held true in this study, it would be expected to find larger mean sizes of juvenile blue crabs in empty baskets (low structural complexity) compared to baskets with contents, empty oysters or live oysters (high structural complexity) in the early sampling events, which was not the case.

Contrary to expectations, the number and size of crabs did not significantly differ between baskets with contents and empty baskets. Similar density and mean crab CL in the basket treatments suggest that the added structural complexity of the oyster or empty oyster

contents to the ALS basket did not enhance the habitat value. Crabs appeared to be attracted to the cages itself. Our empty baskets hosted crabs with an average size of 31.62 mm CW ±14.83, while live oysters and empty oyster treatments hosted crabs at 28.98 mm CL ±16.11 and 33.68 mm CL ±20.08, respectively. A Kruskal-Wallis test did not detect any significant differences between basket contents. It is important to remember that although habitat complexity may be an integral part of habitat selection, it is not the only factor influencing habitat selection. Within grass beds and oyster substrate, densities and dispersal are often associated with mortality and predation (Spitzer *et al*. 2003; Moksnes and Heck 2006).

Observed decreases in crab density from August to December were likely due to changes in larval supply. While larvae are present year round (Morgan *et al*. 1997; Perry and McIlwain 1986; Spitzer *et al*. 2003), recruitment of megalopae peaks in the spring and fall months and decrease in the summer months and colder winter months (Perry and McIlwain 1986; Morgan *et al*. 1996; Flaherty and Guenther 2011). Due to the decrease in recruitment in winter, it is expected that the number of juveniles are expected to decrease as water temperature drops. Density decreased significantly between August 12th and December 2nd ($p < 0.001$), November 5th ($p < 0.0001$), October 14th ($p < 0.001$) and September 24th ($p = 0.001$). These results are in agreement with Stewart (2015). Since recruitment is regulated by predation as well as larval supply, predator-prey interactions may also be a reason for declining density over time.

While this study compared chemical (live oyster and empty oysters) and physical habitat parameters, no control was in place for predation. This could include predator avoidance, predation inside the basket, or predation from outside of the basket. Tapia-Lewin and Pardo (2014) suggested that crab megalopae respond strongly to predation risk by aversion, and food is not a high enough trade-off. This is consistent with Diaz *et al.* (2003), who tested IV and V

instar (8-10 mm CW) responses to a combination of external cues such as current flow, chemical cues of habitat and predators, and visual cues. They determined that crabs responded differently in offshore and estuarine water, with visual targets having a much greater influence on movement in estuarine waters. When given the choice of offshore water with a predator 'target' (black rectangle) and crushed *Callinectes* (predation) odor water, 50% of the crabs moved towards the *Callinectes* water, with almost 50% showing no response, and less than 10% of crabs moving towards the target (Diaz *et al.* 2003). In estuarine environments, visual cues become extremely important to juvenile crabs, with predator avoidance behaviors trumping responses to habitat and predation odors.

Previous studies suggested that small crabs might avoid baskets with large crabs (Stewart 2015). Large crab avoidance would result in a decrease in crab density as a function of the size of the largest crab. A study comparing fight-flight responses of crabs concluded that aggressive behavior by one crab reduced the rate that crabs entered baited traps in legal size crabs (130 + mm) (Reichmuth *et al*. 2011). If the basket is being avoided when larger crabs are present, it is expected that the size range of crabs to move towards a larger crab, and no longer include smaller crabs. However, the standard error (0.48mm to 3.52 mm CL) and range (7.07 - 14.36 to 8.39 - 39.75 mm CL) increased with sample dates (August 12 to December 2), and continued to include the smaller crabs (< 20 mm CW) as well as the larger potentially trapped crabs. Thus, it is not likely that the smaller crabs are actively selecting against baskets with large crabs. The added structural complexity of the oyster or empty oyster contents to the ALS basket might have enhanced habitat value for juvenile blue crabs, attracting more predators to the basket edge, resulting in no net gain for juvenile crabs. Juvenile crabs are extremely vulnerable, which is why nursery habitat for many taxa is typically structurally complex. Juvenile crabs do not have

any way to protect themselves during the 18-20 molts to their adult size (Vanderkooy 2013). Spitzer *et al.* (2001) suggested that in Alabama, 80% of young crabs die due to predation per day, especially by larger blue crabs (Tagatz 1968). Many studies have suggested that predator density increases within refuge habitats (Spitzer *et al*. 2003; Heck *et al*. 2001; Pile *et al*. 1996). This may be the case for habitat edge, or in this case, the outside of the basket.

The inside of a basket containing oysters, as long as there is not a larger crab inside, may still be valuable habitat. Stewart (2015) found in a tethering study that survival of juvenile blue crabs were highest in stocked ALS baskets in comparisons to bags of shell on bottom, vegetated and non-vegetated habitat. Crabs that were small enough to pass through the mesh were tethered inside of ALS baskets that did not contain larger crabs and were deployed for 24-72 hours. As Stewart (2015) points out, the basket is effectively a cage, providing refuge from predators that cannot pass through the mesh. Larger crabs can still prey on a smaller crab that is on the interior of the basket. Crabs do not stay in the middle of the basket swimming, and thus crabs settle on the edges of an empty basket (personal observation). However, when contents are added to the basket, the crabs have substrate to grab onto, and can effectively stay in the center of the basket, out of the larger crab's grasp. If the smaller crabs were being preyed on from outside the basket, one would then expect differences in empty baskets and baskets with contents, as the crabs in empty baskets would be within reach of predators, and on the basket edge. Since a decrease in density was observed across treatments with more structure and empty baskets alike, increased predation from outside the basket was probably not the case.

It is possible for juvenile crabs to become trapped in a basket. Moksnes and Heck (2006) suggested that habitat is selected more for refuge, than for food resources. Baskets were not opened from the time of deployment to the time of sampling. Crabs that use the basket as a

small juvenile (<12mm CL), molt frequently, and need refuge during this time. Often, they will molt inside the basket, and be too large to escape. Thus, the individual is trapped inside the basket until one of the doors is opened. These large crabs, still within the protection of the ALS basket, have very few predators, and are probably not food limited in the form of epifauna or other small prey items. Increased structure may not have same effect in the basket as in nature because big crabs are free of predators and have plenty of food resources. The crabs are free of the fear of predation, and may not behave as they would under natural, non-protected situations. If true, this allows for a comparison of baskets with larger crabs, to baskets without large, crabs.

Baskets, no matter the contents, that were associated with larger blue crabs had a lower total density of crabs than those with smaller crabs. Crabs on the outside of the baskets would not be distinguished from the crabs on the interior; however, in general, the larger crabs were contained within the basket (personal observation). When the largest crab in a basket is small (< 20mm CL), there is a greater chance of the basket density being high (4+ other crabs). If the largest crab in a basket is large (>30mm CL), the chance of the basket containing many additional crabs is low (<2 other crabs) (Fig. 1.9).

It is possible that larger crabs reduced the habitat value by preying on smaller crabs. Kilbane (2003) showed that juveniles (30 – 90 mm CW) consumed 20% more first instars (2.2-3.0 mm CW) than adults (111 – 130 mm CW) consumed, suggesting blue crabs are more likely to consume crabs smaller than themselves. This would result in predation from inside the basket since crabs less than 12 mm CL can enter the basket, and a crab just a couple molts ahead, now trapped inside the basket, is a likely predator. If this was the case, then the interstitial spaces provided by oyster shells would not likely have provided effective predator shelter since the "prey" could have been easily pursued by a "predator" only slightly larger than itself. This

would result in a bimodal size distribution and in a relatively small increase in average size with time accompanied by an increase in variance. The data were consistent with this scenario. There was a negative relationship between crab density and size of the largest crab associated with a given basket, but no relationship between density and presence/absence of oyster shells, suggesting oyster shells did not provide protection from predation. Average size initially increased, but quickly reached an asymptote. However, maximum size (CL) increased from 14.36 to 39.75 mm over the course of the experiment while minimum size remained relatively constant (7.07 to 8.39 mm) – suggesting the smallest size classes were not heavily preyed upon. These data suggested that big crabs are primarily preying on similar sized crabs since there are few crabs below 12 mm CL (escapement size) by end of study.

Thus, an ALS basket that is not properly maintained by releasing crabs periodically has the potential to become an ecological trap for juvenile crabs. Similar to type II error in statistics, when an individual chooses a low quality habitat when they should have avoided it the habitat is considered a trap (Schlaepfer *et al*. 2002; Kristan 2003; Battin 2004). "Ecological trap" was first mentioned in 1972 when scientists observed gulls protecting nesting waterfowl from egg predation, but then eating the newly hatched ducklings (Dwernychuk and Boag 1972), and later in a habitat suitability study on fledging success (Gates and Gysel 1978). At minimum, the singular large crab that is trapped in the basket cannot find a mate to reproduce and its fitness is zero. The worst case scenario would be if ALS baskets that are not properly maintained retain one crab that grows and consumes smaller juveniles, resulting in a large crab that cannot contribute reproductively to the populations but causes mortality in the many juvenile crabs. Thus, the ecological trap is dependent on the predator presence (trapped blue crab). Leighton *et al.* (2008) discovered a similar pattern with Hawksbill sea turtles. Mongoose, which live in

vegetated areas bordering Caribbean beaches, prey on the turtles' eggs, which are laid in nests on the beach and vegetation edge habitat. The turtles' preference for the edge habitat becomes a trap, when the mongoose abundance is high.

Best management practices can help to minimize large-small crab interactions. Regular release of crabs in baskets will help prevent the creation of 'ecological traps', and help minimize crab-induced mortality on oyster seed. Further, use of hexagonal mesh instead of square mesh may minimize crab retention. Newer BST ™ models use the hexagonal mesh, used in this study, while older styles use a square mesh. A Louisiana study concluded that hexagonal mesh minimized sub-legal harvest in commercial crab traps (Guillory and Prejean 1997). A study could determine if the hexagonal mesh in ALS baskets functions the same way for juveniles as legal crabs.

1.5 Conclusion

Habitat value was not enhanced with the added structural complexity of single oysters, nor was there a preference for live single oyster habitat. Our data suggest that cannibalistic predation greatly impacts the value of ALS aquaculture gear as habitat for juvenile blue crab and is therefore heavily dependent on best management practices and regular release of larger crabs.

Chapter 2: Using visible implant elastomer tagging in juvenile blue crab to determine the habitat quality of oyster adjustable long-line systems.

2.1 Introduction

In the north central Gulf of Mexico (GOM), there are three species of *Callinectes:* the blue crab (*C. sapidus*), the lesser blue crab (*C. similis*), and the Bocourt swimming crab (*C. bocourti*). Of these three, the blue crab is the most important ecologically and economically (FAO 2010). It plays an important role in population regulation of other estuarine invertebrates via predation (Eggleston *et al.* 1992; Seitz *et al.* 2011). The blue crab fishery is one of the most valuable in North America (FAO 2010). However, landings of blue crab have declined since the 1980's when populations were at their peak (VanderKooy 2013). An understanding of the habitat needs of blue crab is vital to development of crab enhancement and restoration programs.

Callinectes sapidus is primarily an estuarine species (Perry and McIlwain 1986). However, adult females migrate to offshore saline (>20 ppt) waters when berried (Hay 1905; Darnel 2011). Newly hatched *C. sapidus* are planktonic and metamorphose multiple times through zoeal and megalopal stages, migrating back to estuaries (Darnell 2011). Once they reach the juvenile stage (~2.2 mm carapace width; Pile *et al.* 1996), *C. sapidus* seek nursery habitat for protection while rapidly growing and molting frequently. Numerous studies have identified seagrass, marsh grass, and oyster reefs as important blue crab nursery habitat (Williams *et al.* 1990; Pile *et al.* 1996; Morgan *et al.* 1996; Moksnes and Heck 2006; Etherington and Eggleston 2000; Johnson and Eggleston 2010; Stewart 2015). However, these habitats are all in decline. Since 1980, 29% of seagrass has disappeared globally (Waycott *et al.* 2009) and Eastern oyster (*Crassostrea virginica*) reef has also declined dramatically in the past 20-130 years (Beck *et al.* 2011) due to many factors, including habitat degradation, overharvesting, pollution, and altered

salinity regimes (Kirby 2004; Mackenzie 2007; Eastern Oyster Biological Review Team 2007; Beck *et al.* 2011).

Off-bottom oyster aquaculture has been proposed as a possible way to restore lost blue crab nursery habitat. Oyster aquaculture may also decrease fishing pressure on oyster reefs, resulting in more healthy, self-sustaining reefs (Beck *et al.* 2011). The baskets used in oyster aquaculture may serve as valuable nursery habitat for juvenile blue crabs, providing ecosystem services similar to that of seagrass and restored/natural oyster reefs. For example, Dealteris *et al.* (2004) showed that rack-and-bag oyster gear supported higher densities of crustaceans than seagrass habitat, with about 100 additional organisms per square meter found among aquaculture gear than in submerged aquatic vegetation. Other studies have shown that rack-and-bag gear support higher total invertebrate densities than oyster reefs, with blue crabs only being present among aquaculture rather than reefs (Erbland and Ozbay 2008; Marenghi *et al.* 2010).

The habitat value of adjustable long-line aquaculture gear has received little attention. Parameters indicating the "value" of a given habitat include site fidelity, survivorship, growth and successful migration to the adult habitat (Beck *et al.* 2001). One method to quantify these parameters is to track populations over time via cohort identification and/or mark-recapture studies. Johnson and Eggleston (2010) utilized coded micro-wire tags to conduct a mark-recapture study assessing the value of a salt marsh as nursery habitat for *C. sapidus*. Davis *et al.* (2004) compared Visible Implant Elastomer (VIE) tags to coded microwire tags for short and long term (40 days) mortality and retention, with advantages to both coded microwire and Visible Implant Elastomer tags.

VIE tags are commonly used with fish species (Bailey *et al.* 1998; Astorga *et al.* 2005) and crustaceans, such as lobsters (Uglem *et al.* 1996; Linnane and Mercer 1998), crayfish (Jerry *et al.* 2001) and shrimp (Godin *et al.* 1997), and VIE tags have been successfully used with juvenile mud crabs as small as 15.7 mm CW (Liu *et al.* 2011), and with blue crabs less than 25 mm CW (Davis *et al.* 2004).

The practicality of using VIE tags was investigated to assess the value of long-line aquaculture gear as habitat for juvenile *C. sapidus*. In order for VIE tags to be useful, they must cause minimal mortality to blue crab juveniles, and remain visible at the initial site of injection for several months. Therefore, the main objectives were to assess:

the effect of VIE tags on short-term survival of juveniles

the effect of time on VIE tag visibility and migration within blue crab abdominal muscle tissue.

the recapture rate of tagged blue crab juveniles

2.2 Methodology

2.2.1 Suitability of VIE Tags for Mark-Recapture

Visible implant elastomer (VIE) tags (Northwest Marine Technology Inc., Shaw Island, WA) were utilized because they are internal, and not shed during ecdysis, and do not require animal sacrifice and/or specialized readers to be detected. VIE tags are a two part 10:1 mixture that are injected in liquid form into the organism with a BD™ 0.3-ml syringe and a U-100 gauge needle. The mixture has a working time of approximately 2 hours before curing as a flexible solid, dependent on temperature. They come in multiple colors and visibility can be enhanced under a purple VI light. Tags are biocompatible and have no known human health hazards (Northwest Marine Technology 2008).

This study injected VIE tags into the proximal basal segment of the fifth periopod of hatchery-raised *Callinectes sapdius* (Davis *et al.* 2004). Due to the cannibalistic nature of *C. sapidus* and high handling rate over the experiment, it was decided to tag the ventral sternite. This way, if a limb was lost, the tag was not. Juvenile mud crabs, *Scylla paramamosain*, were also successfully tagged ventrally (Liu *et al.* 2011). A ventral tagging location also allows for up to 6 different tagging locations, with increasing unique combinations for individual tagging.

2.2.2 Short term tag retention and effects on juvenile survivorship and growth

Ninety six blue crabs (10-70 mm CW; < 32 mm CL) were collected in Portersville Bay at the Auburn University Oyster Research and Demonstration Farm (AUORDF), and transported to the South Auburn Fisheries Research Station (SAFRS) in a cooler with bio-balls to reduce

cannibalism, icepacks, and a bubbler on April 11, 2014. At SAFRS, crabs were placed in individual pouches made of 7.5mm plastic mesh (Fig. 2.1) to prevent cannibalism, and held in an outdoor 950-L fiberglass tank filled with ~19 ppt artificial saltwater (Instant Ocean^{TM}). A pump and baffle were used to create circular flow (~0.5 m/s). Bio-balls and shredded PVC were placed in plastic mesh baskets to serve as biofilters (Fig. 2.2). Crabs were fed approximately 0.5 g of a sinking commercial shrimp feed every other day. Ammonia, nitrite, nitrate and pH were monitored with Tetra Easy Strips every other day. Dilutions of fresh water were used to maintain salinity between 17.6 and 19.8 ppt, and mitigate nitrites and ammonia when the biofilter could not keep nitrites below 1 ppm NO_2^-. Temperatures ranged from 14.4 to 19.8 °C.

On April 18, 2014, after 5 days of acclimation, 50 crabs (M:F ratio = 2:3) received four tags, accessing the pereiopodal muscles by inserting the needle in between the thoracic sternite and coxa basis into the tissue behind the thoracic sternite, so that one tag was injected behind the 5th or 6th sternite on each side, and one behind the 7th or 8th sternite on each side (Fig. 2.3). 50 crabs (M:F ratio = 1:1) were held as controls, receiving no tag, but being handled similarly to prevent any handling bias. The carapace width of all crabs was measured at the start (April 12, 2014) and end (April 29, 2014) of the 17-day study period. Crab molting and mortality was noted on a daily basis. A Kolmogrov-Smirnov (KS) test was used to compare differences in final and initial CW distributions of tagged and untagged crabs, while a Before-After Control Impact (BACI) test was used to compare influences of tags on growth between tagged and untagged crabs. Survivorship post-tagging (11 days) was compared using a Chi-square test.

2.2.3 Long-term tag retention, migration, and impact on growth

On June 2, 2014, 101 blue crabs were collected at the same site and in the same manner as for the short-term study. Crabs were transported to the Auburn University Shellfish Laboratory (AUSL), Dauphin Island, AL, and held in individual mesh bags (Fig. 2.4) in a 950-L tank supplied with aerated, flow-through sea water (salinity = 6.9-32.3 ppt; temperature = 26.1-33.1 ^{o}C). Tanks were cleaned daily, prior to feeding. Crabs were fed approximately 0.5 g of a commercial shrimp feed (36% protein menhaden based sinking pellet) every other day for the first two weeks, then daily for the duration of the study. Water quality was monitored in the same manner as for the short-term study.

Upon arrival at AUSL, crab CW and CL were measured and crabs divided into two groups containing similar size distributions. One group was assigned to the control (untagged) treatment while the other group was assigned to the tagged treatment. After a 2-day acclimation period, crabs in the tagged treatment were tagged in the same manner as for the short-term study; however, each of the four tags that a crab received was a different color so tag migration could be easily detected (Fig. 2.5).

Tagging locations remained the same, and 50 control crabs and 50 tagged crabs were selected at the time of tagging. Control crabs received similar handling as the tagged crabs to eliminate handling bias, without an injection. A picture of each tagged crab was taken every two weeks. Carapace width and length were recorded for all crabs at weeks 8, 12, and 16 using digital calipers. Tag retention was quantified in terms of visibility and location scores (Table 2.1), similar to Liu *et al.* (2011).

Tag visibility was rated on a 3-0 scale, with 3 being a clear, intact tag and 0 being not present or recognizable. Tag location was rated on a 2-0 scale, with 2 being in the original

location, and 0 with the tag moving quadrats (leaving original sternite). Visibility and location scores were rated at the same time as the picture in ambient light, so that picture quality did not influence the tag score.

The effect of tagging on survival was compared using a Chi^2 test. To quantify the effect of the tag on crab growth over time a BACI design was used. To compare how molting, time, and size at tagging impacted tag movement (L), visibility (V) and retention, multiple regressions were used. Since ecdysis takes a few hours (Hay 1905), it is possible that crabs consumed their molts between observations. The tanks were checked once daily and post-ecdysis stage was not monitored. Since daily observations were deemed unreliable as some molts were not observed, molt times were estimated from the carapace length measurements taken on June 10 (day 0), August 5 (day 56), September 2 (day 84) and September 30 (day 112). Linear growth was assumed in-between measurements, such that the carapace length was dependent on the original size plus the daily growth rate multiplied by *n* days (Equation 2.1).

$$When\ 0 \leq n \leq 56, CL_n = CL_{n-1} + (\frac{CL_{56} - CL_0}{56\ days})$$

$$When\ 56 \leq n \leq 84, CL_{n-1} + (\frac{CL_{84} - CL_{56}}{28\ days})$$

$$When\ 84 \leq n \leq 112, CL_{n-1} + (\frac{CL_{112} - CL_{84}}{28\ days})$$

Equation 2. 1: Estimate of *C. sapidus* growth over 112 days.

Then, once the daily assumed size was calculated for all 33 crabs, the expected size at molt could be calculated, assuming a 25% increase in CL (Equation 2.2).

$$(CL_0 * 0.25) + CL_0 = CL_{Molt^1}$$

$$(CL_{Molt^{n-1}} * 0.25) + CL_{Molt^{n-1}} = CL_{Molt^{n}}$$

Equation 2.2: Estimate of *C. sapidus* molt over 112 days.

When $CL_{Molt^{n}}$ is equal to CL_n, n becomes the estimated day of molting. Then, the nearest tag visibility and location scores were used for estimations on tag retention and growth. For instance, if the estimated $CL_{Molt} = CL_{22}$, then 22 days was converted to 3.21 weeks, and use tag retention scores from week 4 for that particular crab.

To compare the effect of the initial size at tagging on tag retention, tag location, and tag visibility, crabs were grouped into 2.5 mm CL classes (Sturges 1926). Full tag retention requires crabs to have four tags that score above 0 in both visibility and location. A location score of 0 indicates that the tag fell out, is no longer in the original quadrat, or has disappeared. Therefore, the animal would not be distinguishable. Sixteen weeks after tagging, the average number of tags per crab that scored above 0 in tag visibility and location were compared to the initial size at tagging and the number of molts using a regression. Similarly, the number of tags that remained in 'perfect' condition, that held their score over the entire 16 weeks, was compared over the initial size at tagging and the number of molts using a regression.

2.2.5 Study site

The study was conducted at the Auburn University Oyster Research Demonstration Farm (AUORDF) in Portersville Bay, AL (30°21'14.55"N, 88°11'30.06"W) (Fig. 2.6). The AUORDF utilizes Oyster-Gro culture systems, and 13 adjustable long-line runs positioned in both inline and cross-line fashion. The aquaculture gear used for the mark-recapture was BST™ 12-mm hexagonal mesh baskets in an inline fashion on an adjustable long-line (ALL). The inter-lock baskets suspended off the bottom from a 5-mm Bayco Wire covered with dripper tube via a T-

clip. Each run consists of two 92-m Bayco cables tied between piling pairs. The cable is tensioned, with riser posts every 2.5 m creating the adjustable design. The distance between riser posts creates one Bay, holding 3 baskets per line, 6 baskets total (Fig. 2.7).

2.2.6 Mark recapture

The feasibility of a mark-recapture study at this site was assessed in terms of recapture rates and the amount of labor involved. Animals were collected from non-experimental Oyster Aquaculture gear at AUORDF, tagged, and released into 4 experimental bays that had not been collected for tagging. 300 juvenile *C. sapidus* were collected on September 22nd and 26th, 2014 by flipping the cages over and immediately grabbing the exposed crabs for a total of 600 individuals. Captured crabs were held in buckets with battery-pack air bubblers and immediately measured (CL and CW) and tagged aboard the boat. Each crab < 30 mm CL was injected with two VIE tags, one on each side into the ventral sternite tissue. On the 22nd, crabs were injected with red tags, and, on the 26th, yellow tags were used to differentiate between release dates. Tagged crabs were released at 150 individuals per bay (2 bays per release date) within 1 hour of initial capture. To minimize time on-board, crabs were released into the bay in batches of 50 tagged and measured individuals. They were released by carrying the bucket of tagged crabs over to the bay, and emptying so that the closest structure was the oyster baskets. To get the most conservative recapture estimate, crabs were offered alternative habitats next to the release site. In order to make sure that this is a conservative recapture estimate, crabs were released on a central run, with similar 12-mm inline ALS oyster baskets on the runs to the right and the left (5 m apart) with plenty of alternative structure. Inline baskets were stocked with oysters of varying sizes and densities. The study used 6 bays on the southern end of the runs (Fig. 2.8). Each week

after deployment, one basket from each bay was randomly sampled daily. Sampling was continued until no animals were captured for two consecutive weeks.

2.3 Results

2.3.1 Short term tag retention and effects on juvenile survivorship and growth

Survivorship was 100% 11 days post-tagging for tagged and untagged crabs, resulting in no significant difference between treatments (p=1.0, Appendix B, Table 1). Initial CW ranged from 10.5 – 71.5 mm at the time of tagging (Fig. 2.9).

There was no significant difference in initial CW size-distribution between treatments (Fig. 2.10A; KS test, $D < D_{critical}$, $0.007 < 0.192$), nor was there a significant difference in final CW between treatments (Fig. 2.10B; KS test, $D<D_{critical}$, $0.013 < 0.192$). Similarly, a separate analysis showed no influences of tagging on growth (Fig. 2.11; BACI, p=0.95; Appendix B Table 2). Thus, there was no evidence that tagging had short-term (< 11 days) impacts on growth or survival.

2.3.2 *Long-term tag retention, migration, and impact on growth*

Out of the 33 crabs that survived 16 weeks, 61% retained all four tags, 21% retained two tags, 15% retained only 2 tags, and 3% retained no tags (Fig. 2.12A). 78% of tags remained in their original location (score: L=2) while 7% dispersed but remained in the same general area (score: L=1) and 15% exhibited high dispersal or disappeared altogether (score: L=0) (Fig. 2.12B). Almost half of the tags were reduced to low (31%) or no (16%) visibility by the end of the study (Fig. 2.12C).

Crab mortality was high in July, with less frequent death towards the end of the study. There was no significant difference in survivorship of tagged and control crabs (P=0.069, Appendix B, Table 3).

The size at tagging marginally improved the ability to predict tag retention (p=0.054; R^2=0.65, Appendix B, Table 4c) or tag visibility (p=0.053, R^2=0.65, Appendix B, Table 4a), which is apparent in the figure below (Fig. 2.14). It is important to note that the design has an unbalanced sample size. There was a significant positive relationship between the size at tagging and tag migration (p=0.042, R^2=0.68, Fig. 2.14, Appendix B, Table 4b). Less tags moved out of the original quadrat (score: L>0) when crabs were tagged at larger sizes. Similarly, the number of tags that remained in perfect condition (score: L=2) throughout the study increased with increasing carapace length (p=0.036, R^2 = 0.71, Fig. 2.14, Appendix B, Table 4b).

There were no significant relationships between the number of estimated molts, and overall tag retention, visibility (V>0) or migration (L>0) (Fig. 2.15, Appendix B, Table 5a, b, c). The migration of perfect tags (L=2) per crab increased with the number of molts, but the visibility of perfect tags (V=3) slightly increased with more molts.

Tag retention, visibility and migration significantly decreased with time (Fig. 2.16; Appendix B, Table 6a, b, c). Average retention, visibility and location were still above 3 tags per crab by week 16. Retention, visibility and location were more tightly correlated with time than either initial carapace length (R^2 = 0.64-0.71) or estimated number of molts (R^2 = 0.09-0.43).

There was no effect of tagging on growth (BACI, P= 0.572, Fig. 2.17; Appendix B, Table 7).

2.3.4 Mark-Recapture Feasibility and Suitability

No juvenile crabs were collected following the September 22nd release. Following the September 26th release, 4 juvenile crabs were captured of which one was tagged. Two weeks after the September 26th release, no juvenile crabs were captured (Appendix B Table 8). It took approximately 10 hours of labor to collect, tag, and release 600 crabs in order to recover 1 crab.

2.4 Discussion

VIE tags appeared suitable for short-term (11 days) tagging of *C. sapidus* and had no significant effect in terms of survival or growth. However, suitability was questionable since some tags were lost and tag integrity appeared to diminish with time. Molting had little effect on tag integrity, while the size at tagging had a marginal positive effect on tag integrity. If using the maximizing unique tagging patterns by varying the color, location and number of tags used, ventral tagging allows for 150,000 different tag patterns. Use 150,000 combinations of VIE tags as an identifier (unique color/location combination) requires all tags to be retained. Thus, the point at which crabs (on average) lose one tag means that unique combinations cannot be deciphered, and VIE is no longer a suitable tag. Unique identifiers should be tailored to the sample size, with the appropriate color/location combinations. If the targeted sample size is less than 150,000, a more conservative tag combinations could allow for the loss of multiple tags. If unique identifiers are not needed, successful tag retention is considered to be above 50%. Two tags would ensure that crabs could still be identified as tagged even if one was lost.

It was concluded that tagging did not have an impact on crab survival since there was no significant difference in survivorship between control and tagged crabs. This is in agreement with Davis *et al.* (2004), who reported 100% of crab survival 1 day after tagging, and 50% survivorship occurred at day 48, estimated upon recapture. Liu *et al.* (2011) found no significant effects of tagging on survival after 8 weeks. Further field tests would be needed to quantify whether a marked crab was more noticeable, and thus more vulnerable to predation. However, since the tag was located on the ventral side, little impact of the tag is expected on predation.

Given these results, a mark-recapture study could be conducted for 16 weeks. Assuming a growth rate of 19 mm CW/month in the spring/summer months (Perry and McIlwain 1986), crabs tagged at 6.5mm would reach 35mm CL after approximately 12 weeks, and thus the length of the study would be limited by the carapace losing its translucent properties (around 35 mm CL), rather than by tag retention.

Tag retention is a function of location and visibility. The most common way for a tag to receive a score of 0 in both location and visibility is to lose the tag. That is most likely why there are large consistencies in the data. A tag cannot score above 0 in location and still score 0 in visibility, since the tag is not present. However, a tag can score 0 in location and score greater than 0 in visibility if the tag moved, which occurred in only 6 tags out of a possible 132. This often resulted in a less than perfect tag, so the visibility score would be low, but still detectable. Thus, response variables were highly correlated, removing the ability to do a multi-factorial statistical analysis with multiple dependent variables.

Tag visibility, migration and retention decreased over time, as the crabs grew. Tag visibility was most affected. Tag retention on an individual tag level (n=132) was over 84% after 16 weeks. This is much higher than the retention rate of Davis *et al.* (2004) (65% of tags over 35 days), who attributed their loss mostly to loss of swimming limbs, which included the tag. Retention in blue crabs after 10 weeks (87.9%) was slightly less than crayfish and lobsters, which had retention rates of 92% (Jerry *et al.* 2011), and 99-100% retention after 3 molts (Linnane and Mercer 1998), respectively. One possible reason could be because lobsters and crayfish are typically injected in the abdominal segment which contains much more space than that of crabs' sternites (Northwest Marine Technology 2008). Another reason may have been

musculature structure. Wood and James (2003) found differences in retention when tags were injected transverse and longitudinal to the muscular structure.

VIE tag integrity remained stable across a wide size-range, 10-27 mm CL, indicating they can successfully be used on *C. sapidus* without major loss of integrity. All crabs up to 35 mm CL could be tagged with successful retention (without a 50% loss in tag retention) over 16 weeks. Size at tagging did not affect the ability to determine a tagged individual. However, if the study required unique identifiers, optimal size for tagging was above 15 mm CL. Crabs below 15 mm CL resulted in the loss of one out for 4 tags (3 retained) at the end of the 16-week study.

The initial size at tagging marginally influenced tag retention. This is similar to Linnane and Mercer (1998), who found that tags in smaller, 1.5-month juvenile *Homarus gammarus* retained 97% of tags, while 9-month juveniles had 100% tag retention over 3 molt cycles. Size at tagging influenced tag movement more than tag visibility. Crabs in the 12.5-14.99 mm CL bin had the lowest retention scores, location and visibility scores. However, it is important to know that the samples size of each bin was dissimilar, resulting in an unbalanced design. Samples sizes in the 12.5-14.99, 15-17.49, and 17.5-19.99 mm CL bins were larger than 7 each, while sample sizes from 10-12.49, 20-22.49, and 25-27.49 were less than 3 each. Smaller crabs were more difficult to tag, and may not have received a clean, neat injection. Also, it was more difficult to keep the smaller crabs from moving appendages. Once the tag was injected in the liquid form, if the crab moved its' appendages near the injection, it almost acted as a pump distributing the tag. Tags sometimes were found in the gill (Figure 2.18). It is therefore very possible for tag retention, location and visibility scores to be lower in the smaller crabs due to challenges presented during tagging.

VIE tag integrity did not seem to be strongly impacted by molting because the tag was injected into the muscle. As more time passed, more crabs molted, so there were similar patterns in tag retention, visibility and location between molt and time. Tags retained fairly well through the first and second molts, and decreased more substantially after the third molt. With *Pachygrapsus crassipes,* all tags remained visible through molting; however, after molting only 50% were deemed 'clearly visible', with 36% 'moderately visible' and 14% 'barely visible' (Spilseth and Morgan 2006). Although the tags used in this study had better visibility, some tags were completely lost, unlike Spilseth and Morgan (2006).

The time at which a crab molted varied drastically in both observation and mathematical estimates. For example, the first molt occurred in one crab after the 2nd week of tagging, and after the 14th week in other crabs. Mathematical estimates were based on multiple assumptions, since visible, observed molts were incomplete. The first assumption is that crabs increased size 25% during each molt. Tagatz (1968) estimated growth after molt to be from 7.8% to 50% carapace width. Leffler (1972) estimated that growth is highly correlated with temperature, with molt increases between 13.5% to 39.5% carapace widths from a laboratory experiment (VanderKooy 2013). Growth is also correlated with food resources. Although crabs were fed daily, they may not have been eating to satiation. In order to eliminate cannibalism, crabs were separated by mesh containers. However, due to the shape of the containers and mesh size, food can, and did fall through the mesh out of the crabs reach. Feeding amounts were increased to compensate for this; however, the amount of food the crab actually received is not exact. Effort was made to give each crab the same amount of food, especially between control and tagged crabs, but the amount of food that fell through the mesh pouch is unknown. Due to all of these assumptions, tag retention across molts may not be the most reliable. This study would benefit

from being be done again, monitoring crabs' diet and molt cycle more closely, approximately every 6 hours.

Results suggest that the feasibility of conducting a successful mark-recapture study at the aquaculture farm is very low, due to low recapture rate. It took a minimum of 20 person-hours (4 hrs x 5 people) to collect and tag 600 crabs, with only 1 crab out of 600 being recaptured across 4 bays and 5 sampling events (41% of the release area sampled). Davis *et al.* (2004) concluded that microwire tagging process is 70% faster than VIE tags in juvenile blue crab. Davis *et al.* (2004) could tag 159 crabs per hour using the hand injector with new but practiced taggers. Including only tagging time, and not crab collection, it took approximately 4.5 hours to tag 600 crabs, or 133 crabs per hour, similar to Davis *et al.* (2004).

The low recapture rate could have been due to multiple factors, such as increased mortality of tagged crabs, high mortality of juvenile crabs due to predation, or migration of juvenile crabs away from the release site. Results of the short and long term tagging trials suggest that the low recapture rate was not likely due to issues with tag integrity. Based on the lab component of the study, it is not expected that the tagging process would have resulted in low survival and thus a low recapture. It is not likely that tagging rendered crabs more susceptible to predators since tags had no effect on growth and crabs were tagged ventrally. It is also not likely for a ventral tag to make the crab more visible to predators; however, a tethering study would be needed for further investigation. Lastly, it is not likely that the tagged crabs were swamped with new recruits or the crabs emigrated, since overall capture rates of juveniles were low.

More likely, the low recapture rate was due to predation or migration. Migration of a large proportion of tagged animals away from the release site is possible, but not likely since

there is little structure away from the release site, and the predation risk is high. Crabs were deployed at high densities compared to natural levels in an attempt to have the highest probability of a recapture.

Large, adult blue crabs were not removed from baskets in experimental bays prior to releasing tagged juveniles. Based on the results from Chapter 1, juveniles may have avoided baskets with large crabs, or may have been consumed by the large crabs. Blue crabs tend to have density-dependent mortality, with large blue crabs often consuming 75-97% of smaller crabs (Hines and Ruiz 1995; Dittel *et al.* 1995). Since the experimental bays were at least 10 m from any other non-experimental bay containing gear and thus structure, migration away from the deployed bay was unlikely. A combination of high densities and the presence of large crabs make it most likely that the low recapture rate is due to predation.

Previous research suggests mortality of juvenile crabs in general is high. Heck and Coen (1995) observed 80% predation rates per day in Alabama estuaries in seagrass beds. Assuming an 80% predation rate per day, after two weeks, only 26 crabs out of the originally deployed 600 crabs would be left. If this number was spread per bay (4 experimental bays), and then per basket (6 baskets per bay), one could only expect to capture one crab maximum in each sample two weeks after release. Accounting for predation of 80% per day, the recapture rate was fairly good.

2.5 Conclusions

VIE tags are a good tagging system for mark-recapture studies because there is no effect of growth or survivorship, and tags maintain a high retention over 16 weeks. Mark-recapture studies could last up to 30 weeks; however, it is more likely that crabs will calcify before tags are lost. However, the low recapture rates observed in this study are a major hurdle that needs to be overcome in order for a mark-recapture approach to be successful in the Gulf of Mexico due to the large predation rate. Sampling one or two days after release, instead of one week, would increase the recapture rate. Likewise, removing potential trapped predators, like large blue crabs, would could increase the chance for recapture; however, more tests would need to be included to test juvenile blue crab survival under high densities, and in both survival and avoidance of juveniles in the presence of large blue crabs.

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Figures

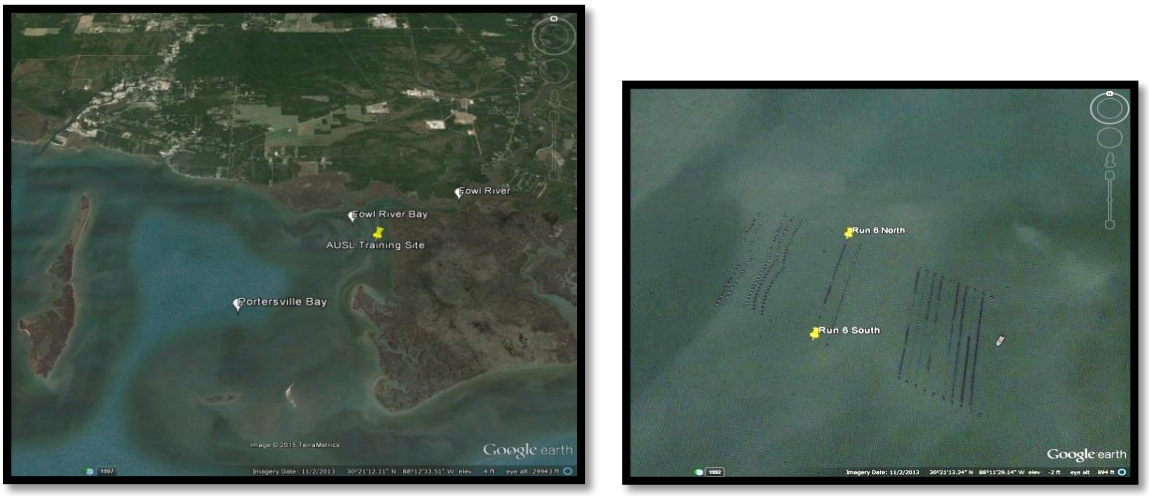

Figure 1.1 Satellite Imagery of AUORDF, Portersville Bay, AL. The experiment made use of Run 6. To the west are OysterGro™ cages, and to the east are more ALS runs (each run is approximately 92m in length).

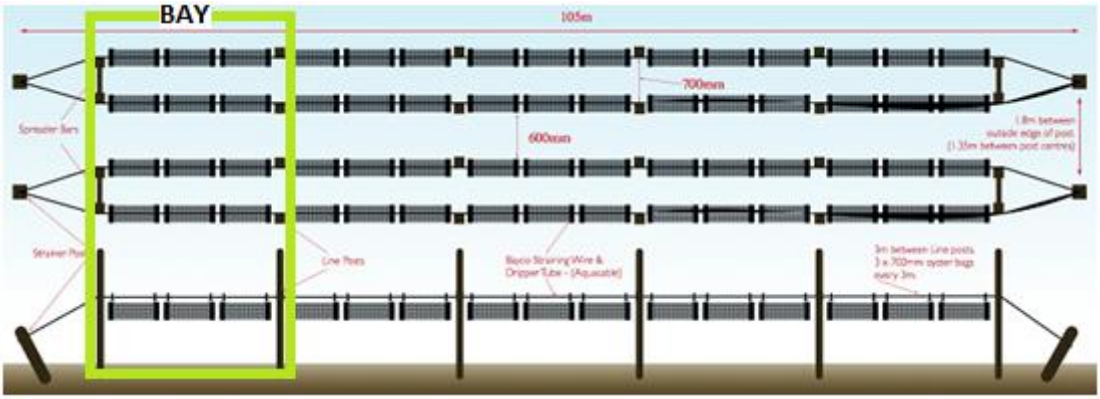

Figure 1.2 Schematic of a run: top and profile view. This diagram is amended from BST^{TM} Oyster Supply Pty. Ltd. (2009).

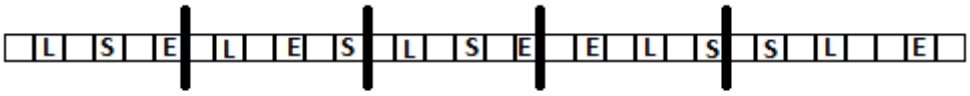

Figure 1.3 Field set-up comparing juvenile *Callinectes spp.* use and growth among ALS structure and contents. Content treatments included empty 12 mm hexagonal mesh baskets (E), oyster empty oysters in 12 mm baskets (S), and 12 mm baskets with live shells (L), with bays represented by squares, and replicate blocks separated by the black vertical bars.

Figure 1.4 Oyster pairs drying after meats were shucked.

Figure 1.5 500 µm sample net. Net was used for sampling with an empty BST™ 12 mm basket placed inside.

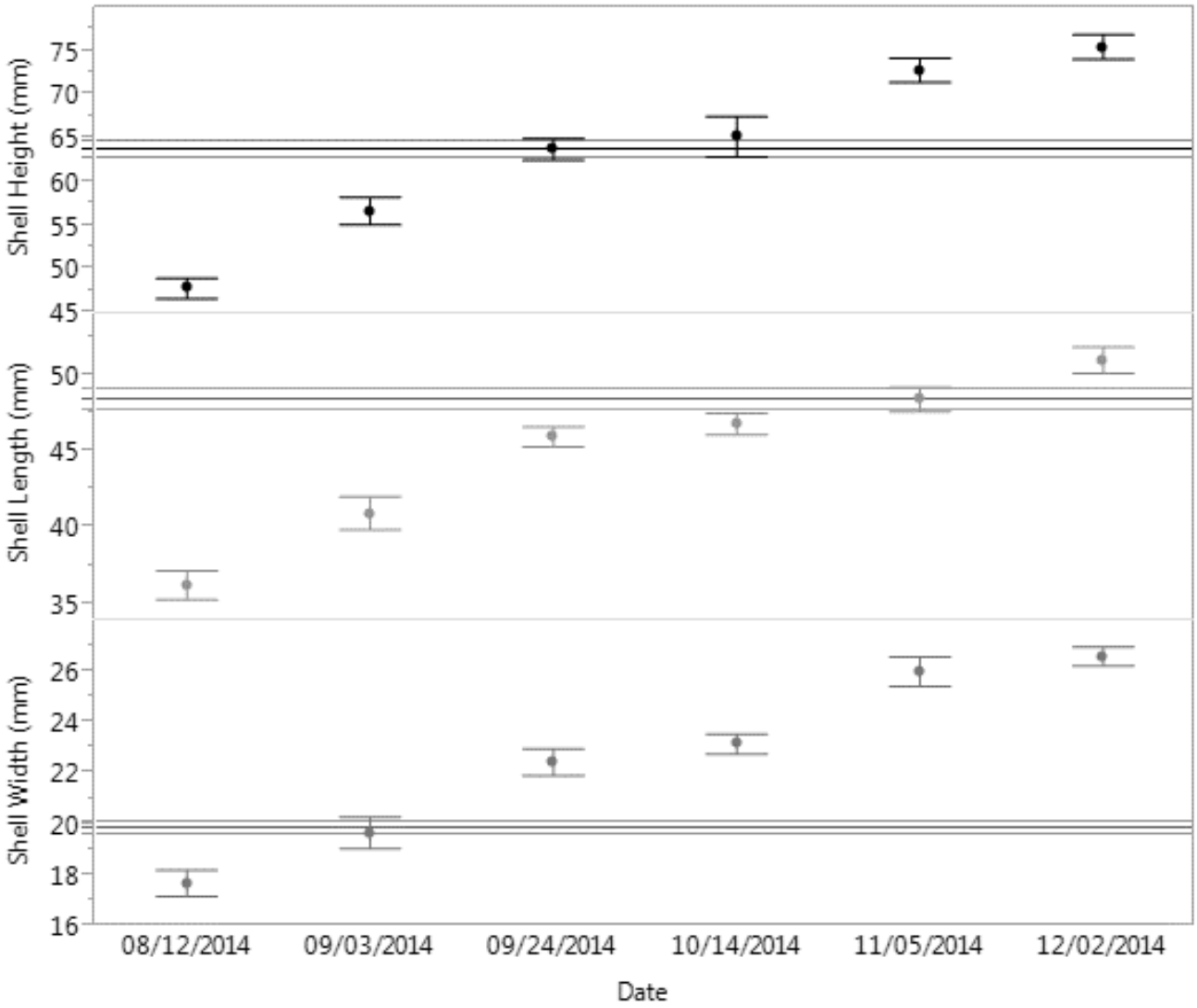

Figure 1.6. Average oyster shell metrics of live oysters (n=30) throughout the study ± SEM. The horizontal lines represent the average metric of the empty oysters (n=50) ± SEM.

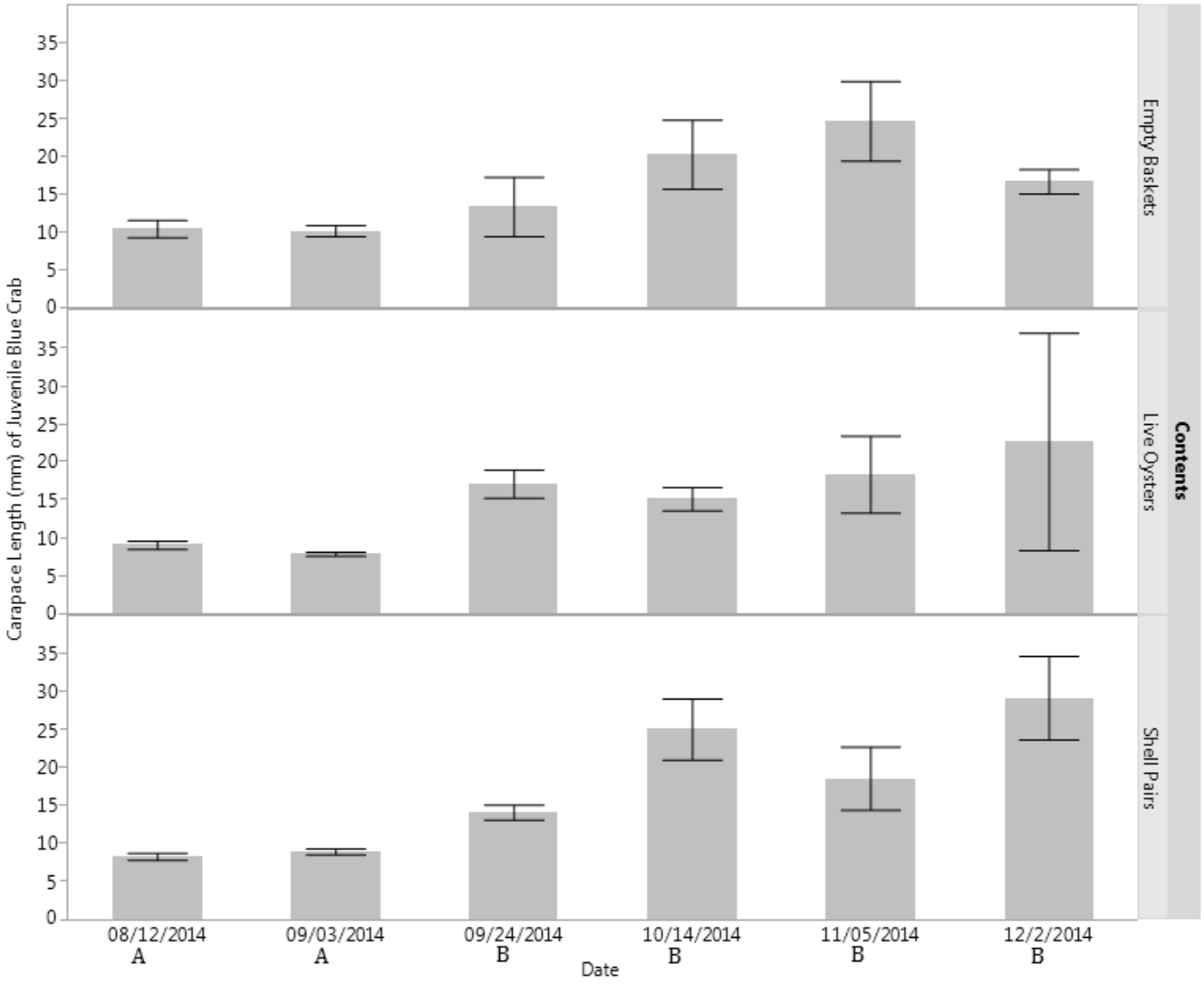

Figure 1.7: Average carapace length of crabs (mm) by basket content and date. n =5. Each error bar is constructed using 1 standard error from the mean. Significant differences from Wilcoxon Test are denoted by letters in heading. There were no significant differences between the basket contents, only by date.

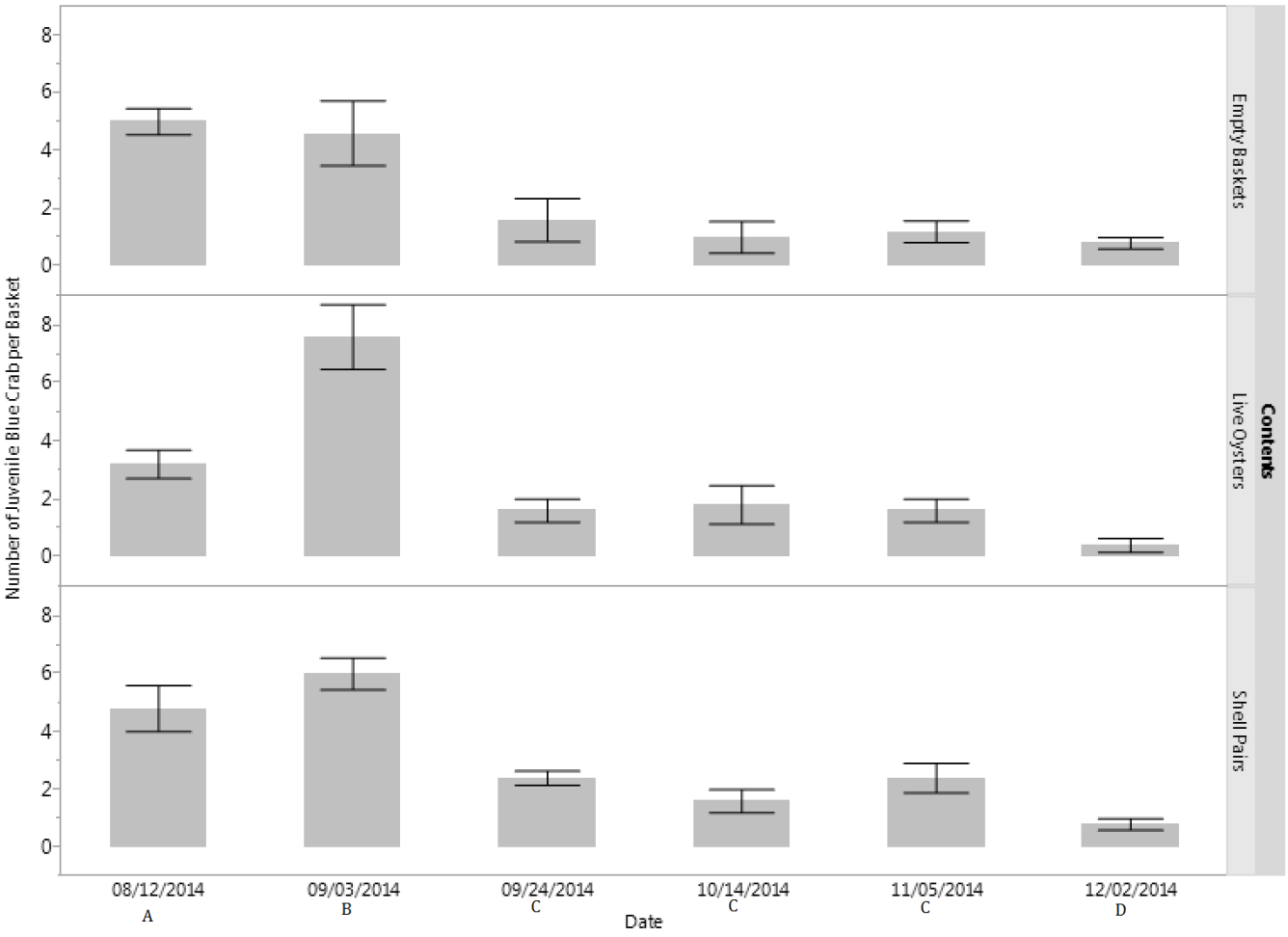

Figure 1.8: Average crab density per basket by basket content and date. n =5. Each error bar is constructed using 1 standard error from the mean. Significant differences from Wilcoxon Test are denoted by letters on the x-axis. There were no significant differences between the basket contents, only by date.

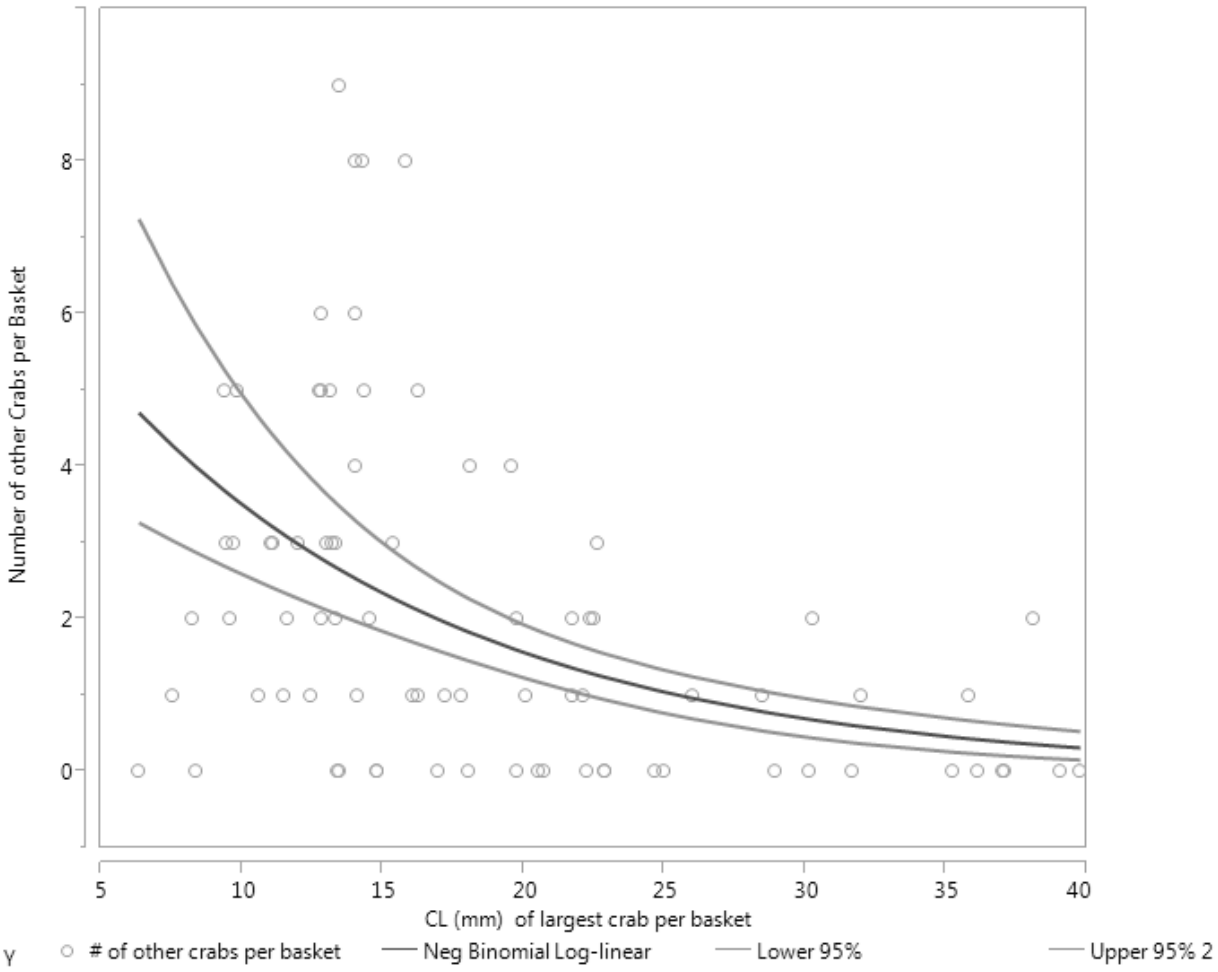

Figure 1.9: The number of other crabs in a basket in relation to the size of the largest crab in the basket, fit with a negative binomial log-linear curve ± 95% CI. Model Equation: $\#\ of\ other\ crabs = \exp(2.1 - (0.08 * size\ of\ largest\ crab\ (mm))$.

Figure 2.1: Mesh pouches for holding individual crabs constructed from plastic mesh netting.

Figure 2.2: Biofilter and tank used to house crabs at SAFRS, Auburn University.

Figure 2.3: Tagged juvenile *Callinects spp.* with VIE.

Figure 2.4: Individual holding cages were creating using 7.5 mm plastic mesh netting, and creating a cuboid with a 90˚ twist for maximum space. The mesh was secured at the bottom corners and sides with zip ties, and along the top with a PVC slide for easy access at AUSL.

Figure 2.5: Juvenile *Callinectes spp.* with fluorescent visible implant elastomer tags injected into the ventral sternite under normal (left) and purple light (right).

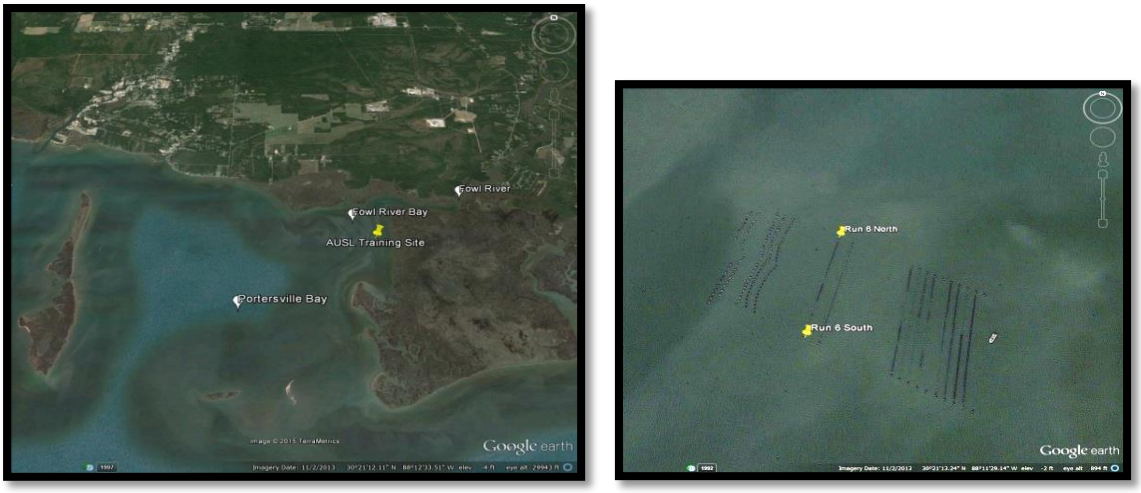

Figure 2.6. Satellite Imagery of AUORDF, Portersville Bay, AL. The experiment made use of Run 6. To the west are OysterGro™ cages, and to the east are more ALS runs (each run is approximately 92m in length).

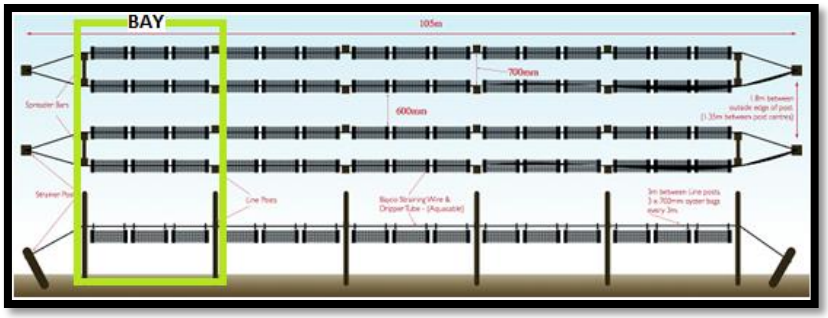

Figure 2.7: A schematic of a run, with top and profile view. This diagram is amended from BST (BST Oysters 2009).

Figure 2.8: AUORDF ALL gear. The experiment made use of Run 3. Run 3 is surrounded by more ALL runs. Each run is 100 yards.

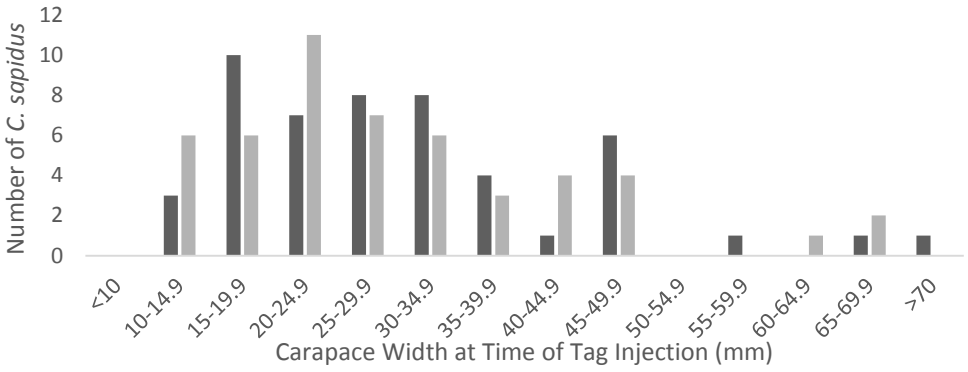

Figure 2.9: The number of individuals per size class for tagged and not tagged (control) treatments. Black bars are tagged individuals, while grey bars are control individuals.

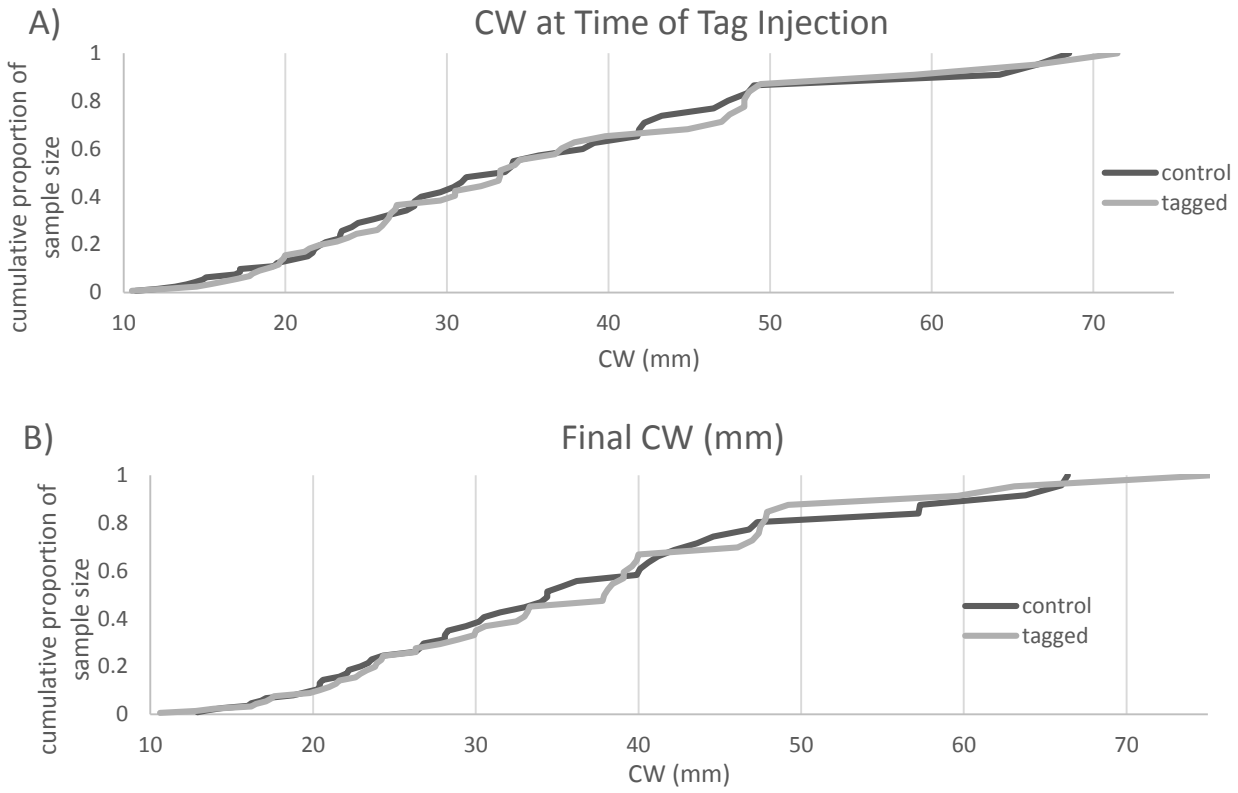

Figure 2.10: The cumulative distribution of crabs' size at tagging and end of experiment. (A) Cumulative proportion of the untagged (control) and tagged sample size, used to determine where the largest difference lies in the population. D= 0.0069, and $D_{Critical}$=0.19233. $D<D_{Critical.}$ (B) Final cumulative proportion of the untagged (control) and tagged sample size. D= 0.013159, and $D_{Critical}$=0.19233. $D<D_{Critical.}$

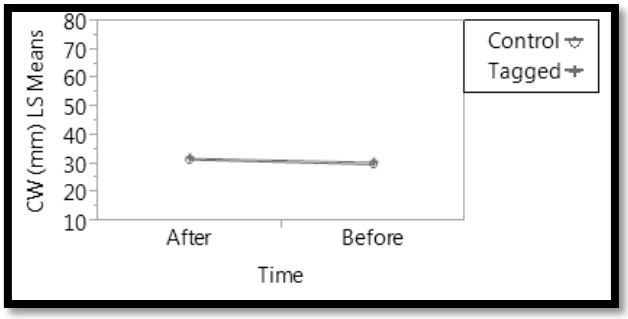

Figure 2.11: LS Means plot of BACI test comparing the impact of tags on crab growth measured by carapace width (mm). Before and after measurements were 11 days apart.

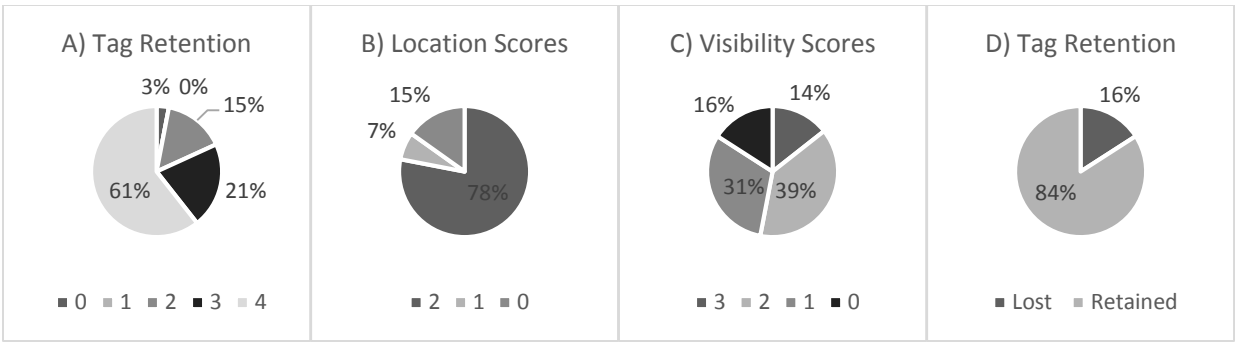

Figure 2.12: Tag integrity 16 weeks post VIE injection. (A) # of tags retained after 16 weeks per crab. n=33 (B) Location scores, (C) Visibility scores and (D) % of tags lost or retained after 16 weeks. n =132.

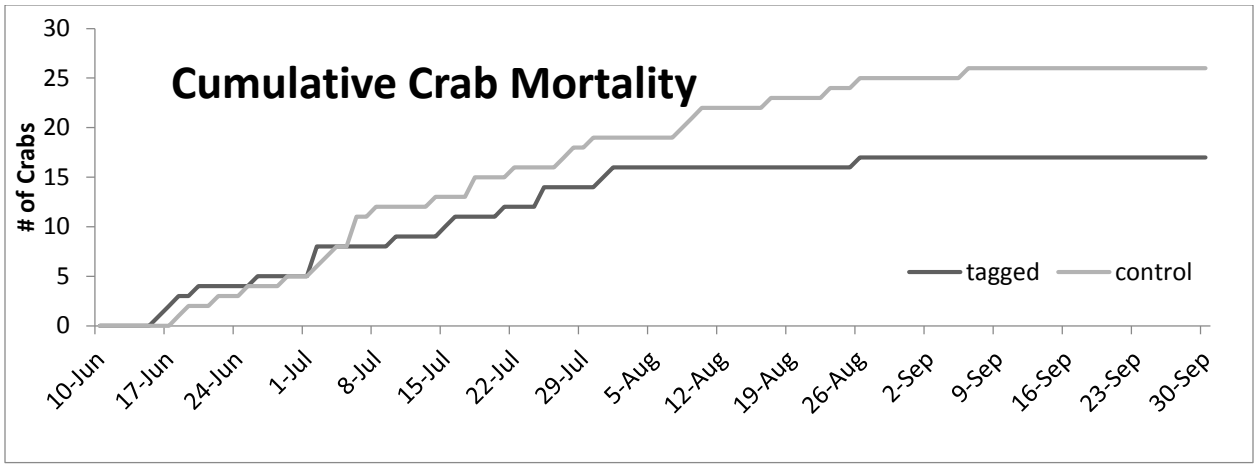

Figure 2.13: Cumulative number of crabs that died each day over time. Tagged tags are in black, while control tags are grey.

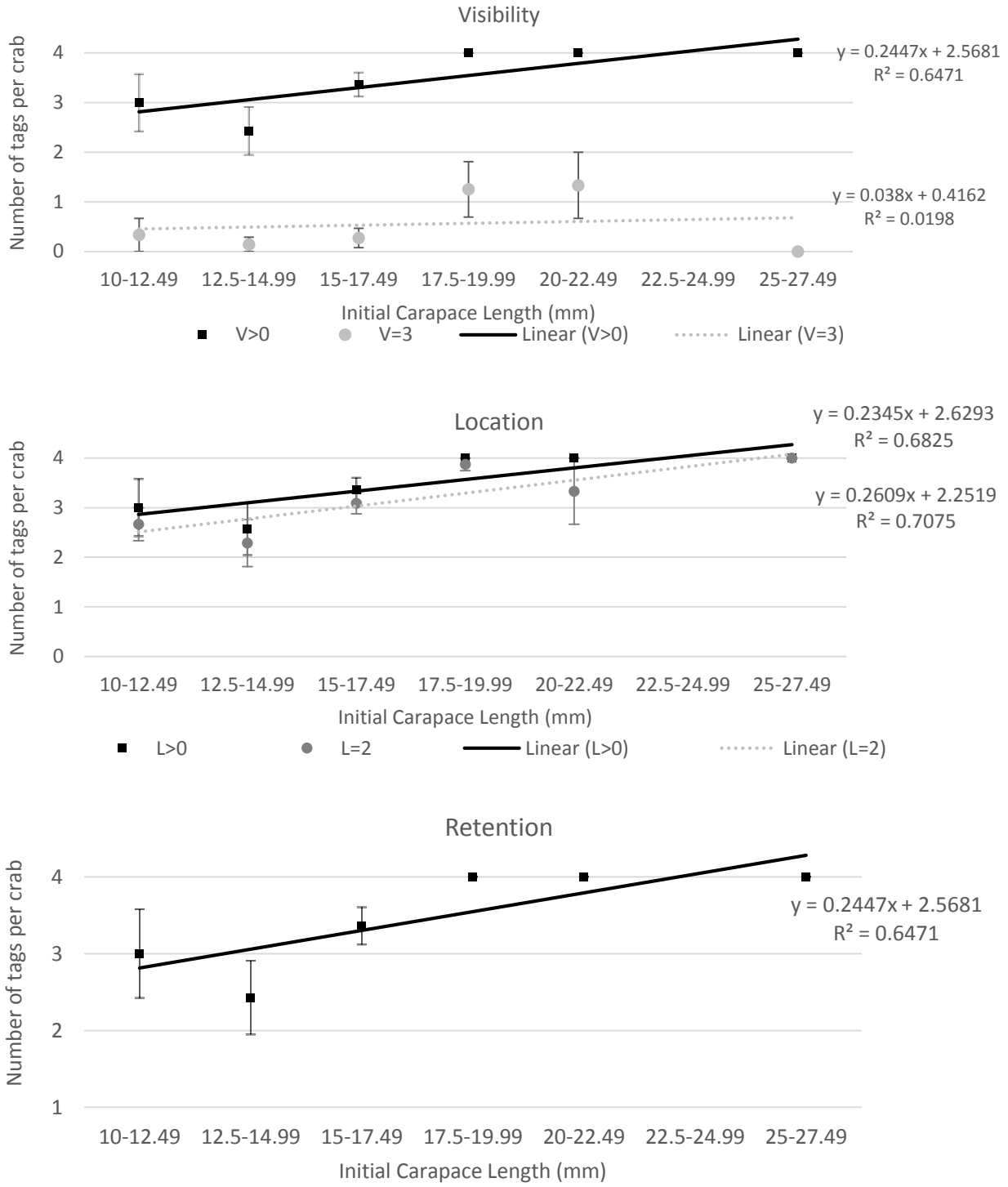

Figure 2.14: Effect of size at tagging on tag integrity. The top plot compares the average number of tags per crab that are visible (V>0) over increasing CL (black), and the average number of V=3 tags per crab (grey) over increasing initial CL at the end of the 16 week study. The middle plot compares the average number of tags per crab that did not migrate out of the quadrat (L>0) over increasing CL(black), and the average number of tags that did not move (L=2; grey). The bottom plot compares the average number of tags per crab that retained (V>0, L>0) after 16 weeks, with increasing CL at the time of tagging. Each error bar is constructed using 1 standard error from the mean.

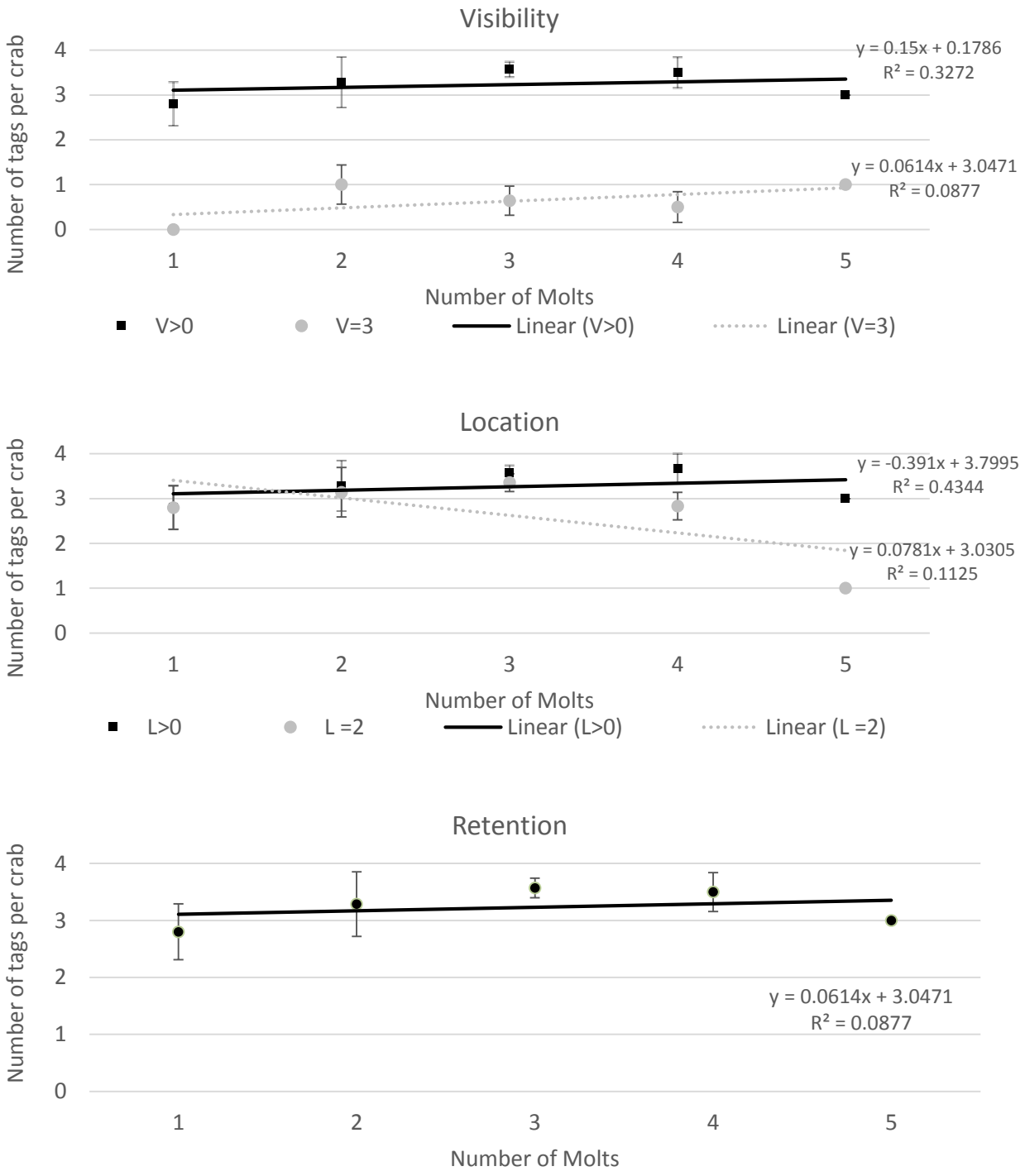

Figure 2.15: Effect of molts on tag integrity. The top plot compares the average number of tags per crab that are visible (V>0) over increasing number of molts (black), and the average number of V=3 tags per crab over increasing number of molts at the end of the 16 week study (grey). The middle plot compares the average number of tags per crab that did not migrate out of the quadrat (L>0) number of molts (black), and the average number of tags that did not move (L=2; grey)). The bottom plot compares the average number of tags per crab that retained (V>0, L>0) after 16 weeks, with increasing number of molts. Each error bar is constructed using 1 standard error from the mean.

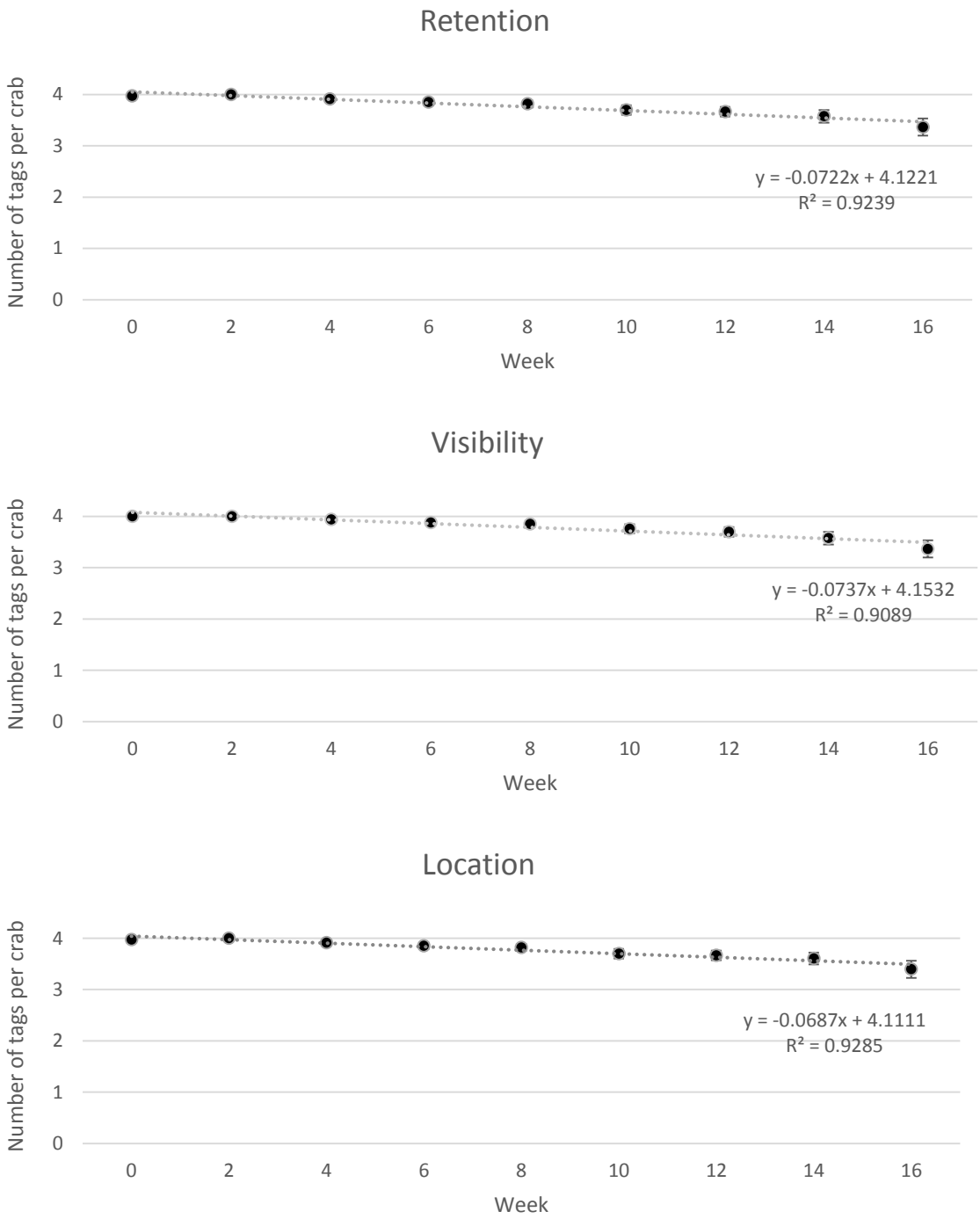

Figure 2.16: Effect of time on tag integrity. The top plot compares the average number of tags per crab that are visible (V>0) over increasing number of weeks from tagging. The middle plot compares the average number of tags per crab that did not migrate out of the quadrat (V>0) over time. The bottom plot compares the average number of tags per crab that retained (V>0, L>0) after 16 weeks, with increasing time from tagging. Each error bar is constructed using 1 standard error from the mean.

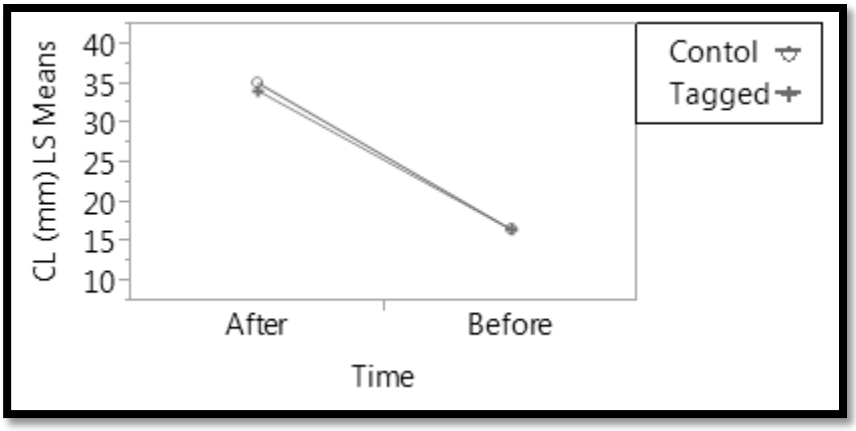

Figure 2.17: LS means plot of BACI test comparing the impact of tags on crab growth measured by carapace length (mm).

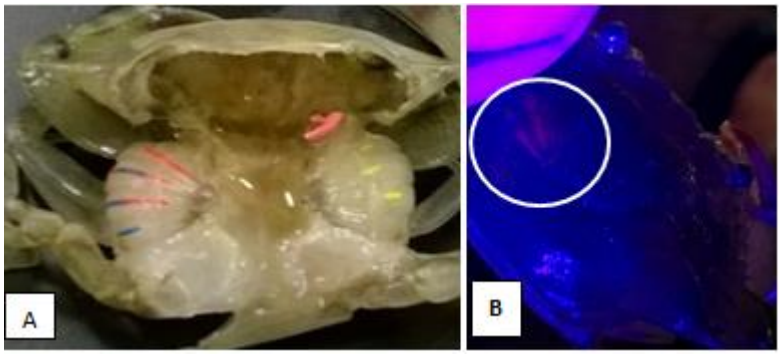

Figure 2.18. *C. sapidus* with tags that have migrated into the gills. (A) is an picture of a crab with VIE tag in the gill structure after the carapace is removed. (B) is a picture of the dorsal side of a juvenile *C. sapidus* under black light. The white circle encloses an area where the tag can be seen through the carapace, after migrating to the gills.

Tables

Table 2.1: Scoring criteria for tag visibility and tag location with examples.

Tag Visibility (V)			
3	High visibility:		Tag is clear and intact.
2	Medium visibility:		Tag is fragmented (right) or blurry (left).
1	Low visibility:		Tag is fragmented and blurry (right), or can only be seen under black light (left).
0	Unidentifiable:		Tag is absent.
Tag Location (L)			
2	No migration:		Tag is in original location.
1	Low migration:		Tag migrate, but can still be identified by general area (blue).
0	High migration:		Tag is not present (right side tags), or in another quadrat (pink tag).

Appendix A: Chapter One Supporting Data

Table 1. Test for parametric assumption of normality for oyster metrics between empty oysters (n=50) and live oysters (n=180).

Shapiro-Wilks W test for Normality by Oyster Metrics

		N	P-value
Empty oysters	Shell Length (mm)	50	**<0.0001**
	Shell Height (mm)	50	0.8496
	Shell Width (mm)	50	0.1375
Live Oysters	Shell Length (mm)	180	**0.0006**
	Shell Height (mm)	180	0.0634
	Shell Width (mm)	180	0.0209

Table 2. Test for parametric assumption of unequal variances for oyster metrics between empty oysters (n=50) and live oysters (n=180).

Unequal Variance for Shell Metrics

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	27.289	5	683	<.0001
Brown-Forsythe	41.8148	5	683	<.0001
Levene	44.3801	5	683	<.0001
Bartlett	65.0189	5	.	<.0001

Table 3: Non-Parametric Wilcoxon Test comparing oyster metrics between empty oysters (n=50) and live oysters (n=180).

Empty oyster vs Live Oyster	*Score Mean Difference*	***Non-Parametric comparisons for each pair using Wilcoxon Method***						
		Std. Err Dif.	**Z**	**p-Value**	**Hodges-Lehmann**	**Lower CL**	**Upper CL**	**Alpha**
Shell Length (mm)	33.146	10.63704	3.1161	**0.0018**	2.64	0.97	4.39	0.05
Shell Height (mm)	5.667	10.59732	0.5348	0.5928	0.8	-2.3	3.93	**q***
Shell Width (mm)	-53.015	10.63702	-4.984	**<.0001**	-3.09	-4.18	-1.97	1.960

Table 4: Test for parametric assumption of normality for oyster volume between empty oysters (n=50) and live oysters (n=180).

Shapiro-Wilks W test for Normality Oyster Volume

	N	P-value
Empty oysters	50	0.0532
Live oysters	180	0.0744

Table 5: Test for parametric assumption of unequal variances for oyster volume between empty oysters (n=50) and live oysters (n=180).

Unequal Variance for Shell Metrics

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	23.9323	1	227	<.0001
Brown-Forsythe	37.3273	1	227	<.0001
Levene	37.4198	1	227	<.0001
Bartlett	42.9058	1	.	<.0001
F Test 2-sided	6.0671	178	49	<.0001

Table 6: Non-Parametric 1-way ChiSquare for oyster volume between empty oysters (n=50) and live oysters (n=180).

1-way test, Chi Square Approximation

ChiSquare	DF	Prob>ChiSq
2.8158	1	0.0933

Table 7. Experimental design

Response Variable	***Density of Crabs (measured by number crabs per sample)***								
	Average size of crab (measured by average Carapace Length and Carapace Width per Sample								
Factor	Date						Basket Contents		
Levels	8/12	9/3	9/24	10/14	11/5	12/2	Live Oysters	Empty oysters	Empty Basket
Replicate	15 baskets sampled per date						5	5	5

Table 8: Shapiro-Wilk W Test for normality on density of crabs per basket.

Date	***Contents***	***N***	***Shapiro-Wilk W Test Goodness of Fit***
12-Aug-14		15	**0.0383**
3-Sep-14		15	0.1081
24-Sep-14		15	0.2762
14-Oct-14		15	0.0907
5-Nov-14		15	0.1104
2-Dec-14		15	**<0.0001**
	empty	30	**0.003**
	live	30	**<0.0001**
	shells	30	**0.002**

H_o= the data are from the normal distribution. P<0.05, reject H_o

Table 9: Shapiro-Wilk W Test for normality on average size of crabs per basket.

Date	***Contents***	***N***	***N w/ crabs***	*Shapiro-Wilk W Test Goodness of Fit*	
				CL	**CW**
12-Aug-14		15	15	0.0572	0.0742
3-Sep-14		15	15	0.2092	0.1330
24-Sep-14		15	13	0.7737	0.4549
14-Oct-14		15	12	0.2729	**0.0262**
5-Nov-14		15	14	0.3115	0.3811
2-Dec-14		15	10	0.1399	0.2483
	empty	30	24	**0.0028**	**0.0021**
	live	30	26	**0.0003**	**0.0008**
	shells	30	29	**0.0005**	**0.0001**

H_o= the data are from the normal distribution. P<0.05, reject H_o

Table 10: Test for unequal variances on the average size of crabs per basket.

		Carapace Length (mm)			*Carapace Width (mm)*		
	Test	**F-ratio**	**DF**	**Prob >F**	**F-ratio**	**DF**	**Prob >F**
Date	**O'Brien**	6.5282	5	**<.0001**	5.4901	5	**0.0002**
	Brown-Forsythe	8.1073	5	**<.0001**	8.1714	5	**<.0001**
	Levene	10.8624	5	**<.0001**	13.0139	5	**<.0001**
	Bartlett	13.4774	5	**<.0001**	13.3413	5	**<.0001**
	Welch's	14.1598	5	**<0.0001**	12.8419	5	**<0.0001**
Contents	**O'Brien**	0.7605	2	0.4709	0.8732	2	0.4218
	Brown-Forsythe	0.7387	2	0.4811	0.4932	2	0.6126
	Levene	0.9768	2	0.3812	0.8345	2	0.438
	Bartlett	1.0401	2	0.3534	1.2817	2	0.2776
	Welch's	0.6482	2	0.5273	0.4725	2	0.6261

*H_o= the data are have equal variances ** Welch's test: H_o = Means are equal, but SD are not*

Table 11: Test for unequal variances on the density crabs per basket.

Density of Crabs per Basket

	Test	F-ratio	DF	Prob >F
Date	**O'Brien**	8.4164	5	**<.0001**
	Brown-Forsythe	6.0596	5	**<.0001**
	Levene	7.1843	5	**<.0001**
	Bartlett	6.3824	5	**<.0001**
	Welch's	28.6677	5	**<.0001**
Contents	**O'Brien**	0.7337	2	0.4831
	Brown-Forsythe	0.0789	2	0.9242
	Levene	0.2425	2	0.7852
	Bartlett	1.026	2	0.3584
	Welch's	0.6413	2	0.5303

*H_o= the data are have equal variances ** Welch's test: H_o = Means are equal, but SD are not*

Table 12: Kruskal-Wallis Test of mean CL by basket contents.

Kruskal-Wallis Test (Rank Sums) of mean CL (mm) by Basket Contents

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Empty	24	1021	960	42.5417	0.645
Live	26	914	1040	35.1538	-1.309
Shells	29	1225	1160	42.2414	0.656

1-way ChiSquare Approximation of mean CL (mm) by date

ChiSquare	DF	Prob>ChiSq
1.7304	2	0.421

Table 13: Kruskal-Wallis Test of mean CL of crabs per basket by date.

Kruskal-Wallis Test (Rank Sums) of mean CL (mm) by date

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
12-Aug-14	15	306	600	20.4	-3.669
3-Sep-14	15	269	600	17.9333	-4.131
24-Sep-14	13	600.5	520	46.1923	1.058
14-Oct-14	12	691	480	57.5833	2.875
5-Nov-14	14	720.5	560	51.4643	2.054
2-Dec-14	10	573	400	57.3	2.543

1-way ChiSquare Approximation of mean CL (mm) by date

ChiSquare	DF	Prob>ChiSq
41.9776	5	**<0.0001**

Table 14: Kruskal-Wallis Test of crab density per basket by basket contents.

Kruskal-Wallis Test (Rank Sums) of Crab Density by Basket Contents

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Empty	30	1253.5	1365	41.7833	-0.966
Live	30	1316	1365	43.8667	-0.422
Shells	30	1525.5	1365	50.85	1.393

1-way ChiSquare Approximation of mean CW (mm) by date

ChiSquare	DF	Prob>ChiSq
1.5253	2	0.4664

Table 15: Kruskal-Wallis Test of crab density per basket by date.

Kruskal-Wallis Test (Rank Sums) of Crab Density by Date

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
12-Aug-14	15	1026.5	682.5	68.4333	3.782
3-Sep-14	15	1161.5	682.5	77.4333	5.269
24-Sep-14	15	596	682.5	39.7333	-0.947
14-Oct-14	15	482	682.5	32.1333	-2.202
5-Nov-14	15	559	682.5	37.2667	-1.354
2-Dec-14	15	270	682.5	18	-4.537

1-way ChiSquare Approximation of mean CW (mm) by date

ChiSquare	DF	Prob>ChiSq
58.6915	5	**<.0001**

Table 16: Pairwise comparisons to determine significant differences between dates of mean CL of each basket.

NonParametric Comparisons for Each Pair Using Wilcoxon Method of mean CL (mm) by date

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	
14-Oct-14	3-Sep-14	13.275	3.074085	4.31836	**<.0001**	10.4995	5.5975	15.47	++++
14-Oct-14	12-Aug-14	12.975	3.074085	4.22077	**<.0001**	10.2373	5.5883	15.165	++++
24-Sep-14	3-Sep-14	12.3487	3.117088	3.96162	**<.0001**	5.4657	3.2656	9.0956	++
24-Sep-14	12-Aug-14	11.4872	3.117088	3.68523	**0.0002**	5.5	2.88	8.4833	++
5-Nov-14	3-Sep-14	10.8405	3.164159	3.42602	**0.0006**	10.4917	2.25	19.11	++++
12-Aug-14	3-Sep-14	1.3333	3.21455	0.41478	0.6783	0.2432	-0.9494	1.4136	
14-Oct-14	5-Nov-14	0.8512	3.008915	0.28289	0.7773	1.0367	-7.7625	7.76	
14-Oct-14	2-Dec-14	-0.825	2.780388	-0.29672	0.7667	-0.9025	-12.09	6.61	
5-Nov-14	2-Dec-14	-1.6286	2.9277	-0.55626	0.578	-2.6375	-11.72	8.03	-
24-Sep-14	5-Nov-14	-3.4121	3.056681	-1.11627	0.2643	-4.345	-12.292	2.52	--
24-Sep-14	2-Dec-14	-5.0423	2.852799	-1.76749	0.0771	-5.2475	-18.63	0.59	--
24-Sep-14	14-Oct-14	-5.3686	2.946278	-1.82216	0.0684	-4.4717	-9.9	0.42	--
12-Aug-14	5-Nov-14	-10.15	3.164159	-3.2078	**0.0013**	-10.02	-17.28	-2.8325	----
12-Aug-14	2-Dec-14	-10.4167	3.004626	-3.46688	**0.0005**	-10.0993	-26.5867	-6.005	----
3-Sep-14	2-Dec-14	-10.9167	3.004626	-3.63329	**0.0003**	-10.1633	-26.9333	-6.1533	----
*q**									Alpha
1.95996									0.05

Table 17: Pairwise comparisons to determine significant differences between dates of crab density per basket by date.

NonParametric Comparisons for Each Pair Using Wilcoxon Method of Crab Density by date

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	
3-Sep-14	2-Dec-14	14.9333	3.140393	4.75524	**<.0001**	5	3	7	++++++++
12-Aug-14	2-Dec-14	14.9333	3.136731	4.7608	**<.0001**	4	3	5	++++++
12-Aug-14	5-Nov-14	12.4667	3.163368	3.94095	**<.0001**	3	2	4	+++++
24-Sep-14	2-Dec-14	9.6	3.046007	3.15167	**0.0016**	1	1	2	++
5-Nov-14	2-Dec-14	9.2667	2.939622	3.15233	**0.0016**	1	0	2	++
14-Oct-14	2-Dec-14	5.9333	2.92669	2.02732	**0.0426**	1	0	1	++
24-Sep-14	14-Oct-14	3.2667	3.112452	1.04955	0.2939	1	0	1	++
24-Sep-14	5-Nov-14	1.4	3.081275	0.45436	0.6496	0	-1	1	
14-Oct-14	5-Nov-14	-2.3333	3.07156	-0.75966	0.4475	0	-1	1	
12-Aug-14	3-Sep-14	-6.3333	3.154271	-2.00786	**0.0447**	-1	-3	0	--
24-Sep-14	12-Aug-14	-11.8667	3.158641	-3.75689	**0.0002**	-3	-4	-1	-----
14-Oct-14	12-Aug-14	-12.7333	3.174974	-4.01053	**<.0001**	-3	-4	-2	-----
24-Sep-14	3-Sep-14	-14	3.182567	-4.39896	**<.0001**	-4	-6	-2	------
5-Nov-14	3-Sep-14	-14.1333	3.180761	-4.44338	**<.0001**	-4	-6	-3	------
14-Oct-14	3-Sep-14	-14.1333	3.185095	-4.43734	**<.0001**	-4	-6	-3	------
*q**									Alpha
1.95996									0.05

Table 18: The number of other crabs in a basket in relation to the size of the largest crab in the basket, comparing a Poisson log-linear fit to negative binomial log- linear fit.

Distribution	***Poisson***	***Negative Binomial***
Response	# of other crabs per basket	# of other crabs per basket
Estimation Method	Maximum Likelihood	Maximum Likelihood
Validation Method	None	None
Mean Model Link	Log	Log
Dispersion Model Link		Identity
Measure		Training
Number of rows	79	79
Sum of Frequencies	79	79
-Log Likelihood	153.04142	**141.90171**
BIC	314.82174	**296.91177**
AIC	310.08284	**289.80343**

Table 19. Descriptive statistics on oyster metrics

Shell Metrics

Contents	**Date**	**Metric**	**N**	**Mean**	**SEM**
Empty oysters	prior to deployment	Shell Height	50	63.6196	0.962684
		Shell Weight		48.3288	0.645752
		Shell Length		19.8082	0.283595
Live Oysters	8/12/2014	Shell Height	30	47.78	1.167798
		Shell Weight		36.23367	0.951955
		Shell Length		17.68933	0.516977
	9/3/2014	Shell Height	30	56.67333	1.587204
		Shell Weight		40.957	1.083957
		Shell Length		19.67933	0.618991
	9/24/2014	Shell Height	30	63.75552	1.2263
		Shell Weight		45.98133	0.660263
		Shell Length		22.454	0.520652
	10/14/2014	Shell Height	30	65.211	2.306408
		Shell Weight		46.81967	0.700219
		Shell Length		23.17567	0.3914
	11/5/2014	Shell Height	30	72.83833	1.373841
		Shell Weight		48.44467	0.792302
		Shell Length		26.033	0.580152
	12/2/2014	Shell Height	30	75.52733	1.40722
		Shell Weight		51.01667	0.852805
		Shell Length		26.62167	0.377573

Table 20. Descriptive statistics on oyster mortality.

% Mortality of Live Oysters and Empty oysters

Contents	Statistic	8/12/15	9/3/15	9/24/15	10/14/15	11/5/15	12/2/15
Live Oysters	Mean	0.57	3.71	4.86	8.57	8.86	7.71
	Std Err Mean	0.57	1.16	1.67	1.56	3.08	1.90
	N	5.00	5.00	5.00	5.00	5.00	5.00
Empty oysters	Mean	0.00	0.57	0.57	0.57	3.71	4.86
	SEM	0.00	0.35	0.35	0.35	1.25	0.73
	N	5.00	5.00	5.00	5.00	5.00	5.00

Table 21: Descriptive statistics: crab size in various treatments

Date	*Contents*	*N*	*N w/ crabs*	*Carapace Length (mm)*				*Carapace Width (mm)*			
				Mean	**Min**	**Max**	**SE**	**Mean**	**Min**	**Max**	**SE**
12-Aug-14	empty	5	5	10.52	7.47	14.36	1.14	21.67	16.08	28.45	2.01
12-Aug-14	live	5	5	9.10	7.07	10.47	0.56	19.12	15.57	21.62	1.01
12-Aug-14	shells	5	5	8.33	7.17	9.59	0.47	17.52	15.21	19.33	0.86
3-Sep-14	empty	5	5	10.24	8.11	12.53	0.73	21.42	16.71	26.45	1.69
3-Sep-14	live	5	5	7.94	7.21	8.50	0.28	16.04	14.66	17.86	0.64
3-Sep-14	shells	5	5	8.97	8.08	10.05	0.38	18.31	16.38	20.73	0.86
24-Sep-14	empty	5	3	13.43	8.56	21.12	3.89	28.61	19.46	43.00	7.28
24-Sep-14	live	5	5	17.14	11.68	22.22	1.84	36.03	24.62	48.48	4.25
24-Sep-14	shells	5	5	14.13	12.27	17.51	0.97	29.23	24.94	36.56	2.19
14-Oct-14	empty	5	3	20.28	11.18	25.00	4.55	41.68	22.41	51.56	9.63
14-Oct-14	live	5	4	15.18	12.66	19.54	1.54	31.99	25.15	40.89	3.28
14-Oct-14	shells	5	5	25.04	16.77	39.04	4.03	44.72	26.81	83.83	10.25
5-Nov-14	empty	5	4	24.67	12.42	36.10	5.26	49.68	25.46	71.50	10.17
5-Nov-14	live	5	5	18.39	6.34	35.22	5.08	35.72	12.23	67.55	9.71
5-Nov-14	shells	5	5	18.60	9.34	31.64	4.16	36.62	17.95	62.93	8.23
2-Dec-14	empty	5	4	16.73	13.47	20.53	1.59	33.48	26.23	41.98	3.40
2-Dec-14	live	5	2	22.69	8.39	36.98	14.30	45.48	16.33	74.62	29.15
2-Dec-14	shells	5	4	29.17	16.94	39.75	5.51	61.21	31.42	81.33	10.94
12-Aug-14		15	15	9.32	7.07	14.36	0.48	19.44	15.21	28.45	0.87
3-Sep-14		15	15	9.05	7.21	12.53	0.37	18.59	14.66	26.45	0.85
24-Sep-14		15	13	15.13	8.56	22.22	1.16	31.70	19.46	48.48	2.44
14-Oct-14		15	12	20.56	11.18	39.04	2.30	39.71	22.41	83.83	4.90
5-Nov-14		15	14	20.26	6.34	36.10	2.68	40.03	12.23	71.50	5.24
2-Dec-14		15	10	22.90	8.39	39.75	3.52	46.97	16.33	81.33	7.32
	empty	30	24	15.44	7.47	36.10	1.53	31.62	16.08	71.50	3.03
	live	30	26	14.19	6.34	36.98	1.59	28.98	12.23	74.62	3.16
	shells	30	29	16.97	7.17	39.75	1.83	33.68	15.21	83.83	3.73

Table 22: Descriptive statistics: density of crabs by treatment and date

Date	*Contents*	*N*	*Mean*	*Min*	*Max*	*SE*
12-Aug-14	empty	5	5.00	4	6	0.45
12-Aug-14	live	5	3.20	2	5	0.49
12-Aug-14	shells	5	4.80	2	6	0.80
3-Sep-14	empty	5	4.60	3	9	1.12
3-Sep-14	live	5	7.60	4	10	1.12
3-Sep-14	shells	5	6.00	4	7	0.55
24-Sep-14	empty	5	1.60	0	4	0.75
24-Sep-14	live	5	1.60	1	3	0.40
24-Sep-14	shells	5	2.40	2	3	0.24
14-Oct-14	empty	5	1.00	0	3	0.55
14-Oct-14	live	5	1.80	0	4	0.66
14-Oct-14	shells	5	1.60	1	3	0.40
5-Nov-14	empty	5	1.20	0	2	0.37
5-Nov-14	live	5	1.60	1	3	0.40
5-Nov-14	shells	5	2.40	1	4	0.51
2-Dec-14	empty	5	0.80	0	1	0.20
2-Dec-14	live	5	0.40	0	1	0.24
2-Dec-14	shells	5	0.80	0	1	0.20
12-Aug-14		15	4.33	2	6	0.39
3-Sep-14		15	6.07	3	10	0.61
24-Sep-14		15	1.87	0	4	0.29
14-Oct-14		15	1.47	0	4	0.31
5-Nov-14		15	1.73	0	4	0.27
2-Dec-14		15	0.67	0	1	0.13
	empty	30	2.37	0	9	0.40
	live	30	2.70	0	10	0.49
	shells	30	3.00	0	7	0.38

Table 23: Kruskal-Wallis test of mean CW by basket contents.

Kruskal-Wallis Test (Rank Sums) of mean CW (mm) by Basket Contents

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Empty	24	1033	960	43.0417	0.773
Live	26	924	1040	35.5385	-1.205
Shells	29	1203	1160	41.4828	0.432

1-way ChiSquare Approximation of mean CW (mm) by date

ChiSquare	DF	Prob>ChiSq
1.5253	2	0.4664

Table 24: Kruskal-Wallis test of mean CW of crabs per basket by date.

Kruskal-Wallis Test (Rank Sums) of mean CW (mm) by date

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
12-Aug-14	15	312	600	20.8	-3.594
3-Sep-14	15	267	600	17.8	-4.156
24-Sep-14	13	625	520	48.0769	1.382
14-Oct-14	12	670	480	55.8333	2.588
5-Nov-14	14	715	560	51.0714	1.984
2-Dec-14	10	571	400	57.1	2.514

1-way ChiSquare Approximation of mean CW (mm) by date

ChiSquare	DF	Prob>ChiSq

Table 25: Pairwise comparisons to determine significant differences between dates of mean CW of each basket.

NonParametric Comparisons for Each Pair Using Wilcoxon Method of mean CW (mm) by date

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	
14-Oct-14	3-Sep-14	12.975	3.074085	4.22077	**<.0001**	15.6954	10.3357	28.095	+++
14-Oct-14	12-Aug-14	12.975	3.074085	4.22077	**<.0001**	15.6288	9.6733	27.0567	+++
24-Sep-14	3-Sep-14	12.4923	3.117088	4.00768	**<.0001**	11.2433	6.9857	19.2917	++
24-Sep-14	12-Aug-14	12.2051	3.117088	3.91555	**<.0001**	10.385	5.95	17.65	++
5-Nov-14	3-Sep-14	10.8405	3.164159	3.42602	**0.0006**	20.1283	4.9933	36.48	++++
12-Aug-14	3-Sep-14	2.2667	3.21455	0.70513	0.4807	1.0383	-1.3967	3.1625	
14-Oct-14	5-Nov-14	0.0774	3.008915	0.02572	0.9795	0.6275	-16.49	14.26	
14-Oct-14	2-Dec-14	-1.5583	2.780388	-0.56047	0.5752	-4.2133	-30.27	10.57	-
5-Nov-14	2-Dec-14	-1.9714	2.9277	-0.67337	0.5007	-6.5175	-27.58	13.285	-
24-Sep-14	5-Nov-14	-2.7445	3.057148	-0.89773	0.3693	-6.7725	-21.335	6.3183	-
24-Sep-14	14-Oct-14	-3.766	2.946278	-1.27823	0.2012	-5.3746	-15.79	3.8117	-
24-Sep-14	2-Dec-14	-4.3346	2.852799	-1.51943	0.1287	-10.2333	-36.935	4.645	--
12-Aug-14	5-Nov-14	-9.5976	3.164159	-3.03323	**0.0024**	-19.5307	-34.478	-4.91	----
3-Sep-14	2-Dec-14	-10.25	3.004626	-3.41141	**0.0006**	-20.1233	-53.0257	-10.9375	----
12-Aug-14	2-Dec-14	-10.4167	3.004626	-3.46688	**0.0005**	-19.7533	-52.725	-10.51	----
*q**									Alpha
1.95996									0.05

Appendix B: Chapter Two Supporting Data

Table 1: Chi^2 test comparing survival 11 days after tagging.

Observed	*Tagged*	*Control*	*Total*
Dead	0	0	0.00
Alive	50	50	100.00
	50	50	100.00
Expected	Tagged	Control	Total
Dead	0	0	0.00
Alive	50	50	100.00
	50	50	100.00
P	#DIV/0!		

Table 2: BACI effect test and parameter estimates comparing the impact of tags on crab growth measured by carapace width (mm). Before and after measurements were 11 days apart.

Term		*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept		31.0427	0.976253	31.8	<.0001
Treatment[Control]		-0.3313	0.976253	-0.34	0.7347
Time[After]		0.7673	0.976253	0.79	0.4328
*Treatment[Control]*Time[After]*		0.0613	0.976253	0.06	0.95
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1.000	1	21.95194	0.1152	0.7347
Time	1.000	1	117.7499	0.6177	0.4328
*Treatment*Time*	1.000	1	0.75154	0.0039	0.95

Table 3: Chi^2 test to compare control and tagged crab mortality after 16 weeks post tagging.

Observed	*Tagged*	*Control*	*Total*
Dead	17	26	43.00
Alive	33	24	57.00
	50	50	100.00
Expected	*Tagged*	*Control*	*Total*
Dead	21.5	21.5	43.00
Alive	28.5	28.5	57.00
	50	50	100.00
P	0.069079		

Table 4:

(a) Linear Regression on the average number of tags per crab that are visible (V>0) over increasing CL, and the average number of V=3 tags per crab over increasing initial CL.

V>0, CL

Regression Statistics	
Multiple R	0.804419
R Square	0.647089
Adjusted R Square	0.558862
Standard Error	0.43648
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.397297	1.397297	7.334311	0.053637
Residual	4	0.762061	0.190515		
Total	5	2.159358			

V=3, CL

Regression Statistics	
Multiple R	0.140805
R Square	0.019826
Adjusted R Square	-0.22522
Standard Error	0.644576
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.033616	0.033616	0.080908	0.790189
Residual	4	1.661912	0.415478		
Total	5	1.695528			

(b) Linear Regression on the average number of tags per crab that are still in the original quadrat (L>0) over increasing CL, and the average number of L=2 tags per crab over increasing initial CL.

L=2, CL

Regression Statistics	
Multiple R	0.841145
R Square	0.707526
Adjusted R Square	0.634407
Standard Error	0.405176
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.588552	1.588552	9.676413	0.035848
Residual	4	0.65667	0.164167		
Total	5	2.245222			

L>0, CL

Regression Statistics	
Multiple R	0.826137
R Square	0.682502
Adjusted R Square	0.603127
Standard Error	0.386309
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.283197	1.283197	8.598501	0.042715
Residual	4	0.59694	0.149235		
Total	5	1.880137			

(c) The average number of tags per crab that retained (V>0, L>0) after 16 weeks, with increasing CL at the time of tagging.

L>0, V>0, CL

Regression Statistics	
Multiple R	0.804418597
R Square	0.647089279
Adjusted R Square	0.558861599
Standard Error	0.436480404
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.397297384	1.397297384	7.334311376	0.05363743
Residual	4	0.762060574	0.190515143		
Total	5	2.159357958			

Table 5:

(a) Linear Regression on the average number of tags per crab that are visible (V>0) over increasing number of molts, and the average number of V=3 tags per crab over increasing number of molts.

V>0, Molts

Regression Statistics	
Multiple R	0.296108
R Square	0.08768
Adjusted R Square	-0.21643
Standard Error	1.743865
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.876802	0.876802	0.288321	0.628567
Residual	3	9.123198	3.041066		
Total	4	10			

V=3, Molts

Regression Statistics	
Multiple R	0.183198
R Square	0.033562
Adjusted R Square	-0.28858
Standard Error	1.794843
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.335616	0.335616	0.104181	0.768056
Residual	3	9.664384	3.221461		
Total	4	10			

(b) Linear Regression on the average number of tags per crab that are still in the original quadrat (L>0) over increasing number of molts, and the average number of L=2 tags per crab over increasing number of molts.

L>0, Molts

Regression Statistics

Multiple R	0.335413
R Square	0.112502
Adjusted R Square	-0.18333
Standard Error	0.400468
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.060989	0.060989	0.380288	0.581088
Residual	3	0.481125	0.160375		
Total	4	0.542113			

L=2, Molts

Regression Statistics	
Multiple R	0.679112903
R Square	0.461194335
Adjusted R Square	0.281592446
Standard Error	1.166180981
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.492247166	3.492247166	2.567870186	0.207379557
Residual	3	4.07993424	1.35997808		
Total	4	7.572181406			

(c) Linear Regression on The average number of tags per crab that retained (V>0, L>0) over increasing number of molts within 16 weeks.

L>0, V>0, molts

Regression Statistics	
Multiple R	0.296108
R Square	0.08768
Adjusted R Square	-0.21643
Standard Error	0.36177
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.037735	0.037735	0.288321	0.628567
Residual	3	0.392633	0.130878		

Total	4	0.430367

Regression Statistics	
Multiple R	0.961197
R Square	0.923901
Adjusted R Square	0.913029
Standard Error	0.060684
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.312963	0.312963	84.98496	3.65E-05
Residual	7	0.025778	0.003683		
Total	8	0.338741			

Table 6:

(a) Linear Regression on the average number of tags per crab that are visible (V>0) over 16 weeks.

V>0, week

Regression Statistics	
Multiple R	0.953346
R Square	0.908869
Adjusted R Square	0.89585
Standard Error	0.068359
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.326232	0.326232	69.81223	6.9E-05
Residual	7	0.032711	0.004673		
Total	8	0.358943			

(b) Linear Regression on the average number of tags per crab that are still in the original quadrat (L>0) over 16 weeks.

L>0, week

Regression Statistics	
Multiple R	0.963594
R Square	0.928514
Adjusted R Square	0.918302
Standard Error	0.055798
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.283073	0.283073	90.92135	2.93E-05
Residual	7	0.021794	0.003113		
Total	8	0.304867			

(c) Linear Regression on The average number of tags per crab that retained (V>0, L>0) over 16 weeks

(V>0, L>0)

Regression Statistics	
Multiple R	0.961197
R Square	0.923901
Adjusted R Square	0.913029
Standard Error	0.060684
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.312963	0.312963	84.98496	3.65E-05
Residual	7	0.025778	0.003683		
Total	8	0.338741			

Table 7: BACI effect test and parameter estimates comparing the impact of tags on crab growth

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	6.5183	0.3267	0.5688
Time	1	1	8584.516	430.2522	<.0001
*Treatment*Time*	1	1	6.4271	0.3221	0.5715

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	25.64769	0.434675	59	<.0001
Treatment[Control]	0.248447	0.434675	0.57	0.5688
Time[After]	9.01625	0.434675	20.74	<.0001
*Treatment[Control]*Time[After]*	0.246705	0.434675	0.57	0.5715

Table 8. Mark-recapture at AUDORF Portersville Bay, AL.

Sep 22, 2014	300	# Initially Captured
Sep 29, 2014	0	# Total Recapture
	0	# Marked
Oct 6, 2014	0	# Total Recapture
	0	# Marked
Sep 26, 2014	300	# Initially Captured
Oct 3, 2014	4	# Total Recapture
	1	# Marked
Oct 10, 2014	0	# Total Recapture
	0	# Marked
Oct 17, 2014	0	# Total Recapture
	0	# Marked

Evidence suggests that big crabs are primarily preying n similar size crabs (don't get a bunch of crabs below 12 mm (escapement size) by end of study.

Increased structure may not have same effect in cage as in nature because big crabs are free of predators – free to eat all the time.

Downplay cannibalism by trapped crabs, and upgrade main conclusion of lack of effect of shell structure – Ken gave nice explanation of why this is so.

Replace "calcification" with direct description "no longer can see through".