**Effects of basket arrangement and stocking density when using the adjustable long-line system for oyster grow-out**

by

Julie Elizabeth Davis

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Approved by

William C. Walton, Chair, Assistant Professor of Fisheries and Allied Aquacultures
Donald A. Davis, Professor of Fisheries and Allied Aquacultures
John E. Supan, Research Professor, Louisiana State University Agricultural Center

## Abstract

In recent years, as demand has risen, interest has grown in diversifying Alabama's oyster harvest to include oysters for the premium half shell market. The adjustable long-line system (ALS) can be used to grow oysters with the attributes necessary to compete in this high-value market. ALS baskets can be configured in two ways, cross-line or in-line. The cross-line arrangement allows 33% more baskets to fit within the same space. In conjunction with a test of stocking density (75, 90, or 105 oysters per basket), we tested the effects of these two basket arrangements on product quality. Lower densities and cross-line arrangement yielded oysters with more desirable product attributes (i.e. cup, fan, meat quality). Basket arrangement and stocking density did not significantly effect *Polydora websteri* infestation or shell strength. Placing nine baskets, each with 75 oysters, per bay appears to be the best way to optimize ALS production.

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*This thesis is dedicated to my grandfather, Russell Davis, a high school science teacher who, with my grandmother, farmed and fished and made the most out of rural life in coastal Nova Scotia. Also, to my parents, who cared enough and were smart enough to realize the importance of carrying on that way of life.*

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# Chapter 1. Introduction to off-bottom oyster farming and Alabama's oyster industry

Due to a decline in shellfish harvest from wild sources and better technologies for growing shellfish, farmed shellfish production has increased rapidly in recent years (USDA, 2006; FAO, 2011). Oysters, which are commonly found in coastal estuaries, have been a popular food item for centuries (Kurlansky, 2006). Resources quickly became depleted, however, as more people began to inhabit coastal areas and an alternate means of meeting the demand for oysters needed to be found (Beck et al., 2009). Off-bottom oyster farming was viewed as a viable means of doing so because of the increased market value of oysters and increased commercial interest in seed production (Shaw, 1969).

*What is Off-Bottom Oyster Farming?*

Off-bottom oyster farming is the rearing of oysters in containers suspended, or floating, above or off the substrate. Containers are in the form of mesh baskets or bags that can be used on their own or held together in an outer housing. Off-bottom oyster farming, like other bivalve shellfish aquaculture, is an attractive method of seafood production because, unlike most other forms of aquaculture, it requires no addition of artificial food (Garen et al., 2004). In addition, bivalve shellfish, by nature of being filter feeders, improve water quality (Gibbs, 2004; Newell, 2004; Ferreira et al., 2009). Lifting oysters off the bottom dramatically increases growth rates and enclosing oysters within a cage reduces mortality (Truitt, 1931; Wisely et al., 1979; Paynter and DiMichele, 1990; Moroney and Walker, 1999; Kraeuter et al., 2007; Creswell et al., 2008). Together, these factors allow high stock turnover, thereby increasing the profitability of the business (Paynter et al., 1992).

Off-bottom oyster farming is practiced in coastal areas world-wide using methods tailored to local conditions.  Several social, regulatory, and environmental factors are considered in selecting a site for oyster farming (Silva et al., 2011).  In order to optimize growth, culture methods must be adapted to the farm's hydrodynamic conditions (Claereboudt et al., 1994b).  Some common culture methods include: rack and bag, floating raft, floating bag, and adjustable long-line systems (Appukuttan and Muthiah, 1996; Lavoie, 2005; Mallet et al., 2009).  The adjustable long-line system, developed in Australia, is used for oyster culture in Canada, Australia, France, and the United States (L. Stott pers. comm., 2011).  It is well suited to shallow and sheltered coastal areas. The system's durability and ease of handling make it an attractive option for farmers of all abilities (BST Oyster Supply Pty., 2013; Davis et al., 2012a; SEAPA, 2013).

Typically, seed oysters used in off-bottom oyster farming are single-set oysters procured from a local shellfish hatchery or wild seed supplier (Supan, 2002; Walton et al., 2012c). There are several options for raising seed: a) a floating up-weller system (FLUPSY), which is anchored in a sheltered coastal area (Davis et al., 2000; Ralonde, 1998); b) fine mesh floating bags or cages (Landry et al., 2013);  or, c) a land-based up-weller system (Wallace et al., 2008).  In Alabama, the latter two methods are used.  In areas where larvae are plentiful, seed is procured by deploying collectors upon which new recruits settle (Burke et al., 2008; Léger and Maillet pers. com., 2012).  Regardless of procurement method, farmers can buy seed at any size.  The size of the seed purchased and techniques used to grow seed up to final grow-out (9-12 mm mesh) varies depending on the farmer's needs and site conditions.  Oysters grow rapidly at the seed stage; requiring frequent maintenance to avoid overcrowding.

Stocking density is an important determinant of product quality and there is a general one third volume rule of thumb practiced by most oyster growers to avoid overcrowding (Galtsoff, 1964; Holliday et al., 1993; Comeau et al., 2011).  This rule means the container is initially filled with seed until it is 1/3 full. As the oysters grow they would fill the container. To avoid stunting and misshapen oysters, the volume typically is again reduced to 1/3 when the oysters have filled the container to ¾ of its total volume. Use of single oysters coupled with timely density reductions produces a market oyster that is more uniform in shape than oysters grown in clumps (Adams et al., 1995; Honkoop and Bayne 2002; Brake et al., 2003). When given adequate space the growing edge of oysters can be trimmed of by tumbling around in the basket with wave action. This pruning produces a nicely cupped oyster; a desirable attribute in the half shell market (Brake et al., 2003).

Oysters grown using off-bottom methods are tended on a regular basis (i.e. as frequently as weekly in highly productive waters) to control bio-fouling both on the gear and on the oysters (Taylor et al., 1997; Mallet, 2009; Walton et al., 2012c). This practice is imperative because fouling on the gear reduces water flow to the oysters and reduced water flow means reduced delivery of food and impaired waste removal (Wallace and Reisnes, 1985; Mouland and Parsons, 1999; Claereboudt et al., 1994b). The frequency and timing of fouling control can be a major determinant of the profitability of a farm (Pit and Southgate, 2003).

*Off-Bottom Oyster Farming Techniques Used in Alabama*

Several off-bottom oyster farming gear types have been experimented with in the northern Gulf of Mexico (Maxwell and Supan, 2010; Coddington-Ring, 2012; Walton et al. in press, 2012d). Currently, the floating cage system (FCS) and adjustable long-line system (ALS) appear most feasible for off-bottom oyster farming in coastal Alabama (Davis et al., 2012ab). Other systems have been tried, but either lack a hurricane plan for shallow water sites or have experienced high levels of predation and/or low growth rates (Walton et al., 2012ab). The FCS and ALS systems, as implemented in the coastal waters of Alabama, are described as typically implemented below.

The floating cage system (e.g. OysterGro™) is comprised of an outer housing of plastic coated wire mesh that has multiple compartments for plastic mesh bags, containing oysters, inside of it (Fig. 1.1).

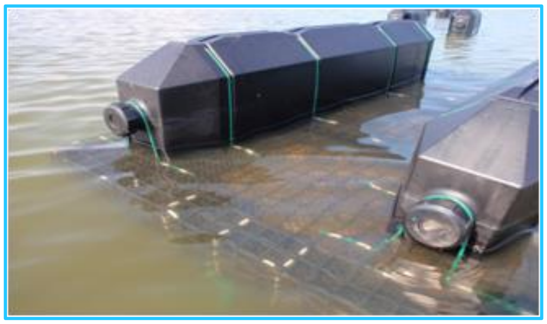

Fig. 1.1. OysterGro™ floating cage system.

Attached to the outer housing are two air-filled pontoons that allow the cage to float at the water's surface with the oysters suspended within the food-rich top 15-20 cm of the water column. Twenty cages, containing 900 oysters each at grow-out density, are tethered to a central 100 m long-line. Fouling control is accomplished by flipping the cage over, allowing the pontoons to support the cage and oysters out of the water, once a week for 18-24 hours. The floating cage system, with adaptations to the anchoring and tethering systems, can be used in any depth of water.

The adjustable long-line system is comprised of a 100 m tensioned monofilament line sleeved with rigid plastic tubing strung between two pilings (Fig. 1.2). At uniform intervals (approximately every 3 meters) along the line a riser post is driven into the substrate. The space between riser posts is called a 'bay'. Riser posts can be made of 5 cm diameter polyvinyl chloride (PVC) pipes. Riser posts have clips on them that allow the grower to adjust the position of the line, and thus the position of the baskets, in the water column. Lines are typically deployed in pairs with the pair termed a 'run'.

Fig. 1.2. Adjustable long-line system with BST-brand baskets. Baskets in drying position for fouling control.

Depending on the arrangement of baskets in each bay, a run can hold between 15,300 and 22,950 oysters at grow-out density. Fouling control is accomplished by raising the line, and thus the baskets, from their growing position riser clip to the top riser clip to locate the baskets above the mean high water mark. After 18-24 of air drying, the line is lowered back into its growing position riser clip (roughly 45-60 cm above the substrate). The ALS typically is used only in shallow waters (< 2 m deep at mean high water).

*Alabama's Oyster Industry: Traditional On-Bottom Harvest*

Oysters have been harvested from wild populations in the northern Gulf of Mexico for centuries (Dugas et al., 1997). The northern Gulf of Mexico is among the leading producers of oysters in the United States, with the majority of the supply originating from on-bottom leases in Louisiana and Florida (USDA, 2006). The majority of oysters harvested in Alabama are sold to shucking houses that pack the meats in quart or gallon containers after removing them from the shell. A select number of oysters are culled from the harvest, based on their size and physical appearance, for sale to the half-shell market (A. Sunseri pers. comm., 2011).

Harvesting continues today, using similar methods as decades past. The majority of oysters from the region are harvested from extensive on-bottom privatized leases or public reefs (Supan, 2002; Wallace, 2003). On public reefs in Alabama, oysters are raked off the reef using a manually operated set of tongs. Typically, crews of two or three people work in a small skiff to harvest oysters (Fig. 1.3).

Fig. 1.3. Tonging, the traditional method of oyster harvesting, at Cedar Point Reef in Alabama (November 2011).

To encourage oyster recruitment on on-bottom leases, cultch material (e.g. oyster shell) is spread over the area on a regular basis (Supan, 2002; Soniat and Burton, 2005; Herrmann, 2010).

During the 2011 commercial harvest season, 4,161,890 lbs. of oysters were harvested from four public reefs in Alabama. This represents 299,299 lbs. of meat and 3,862,591 lbs. of shell and a total dock-side value of $1,339,058. During the three month long season, 18-24 dealers were purchasing oysters (J. Herrmann pers. comm., 2013[1]). Oysters must be three inches in shell height when harvested (Wallace, 2003). In Alabama, oysters are harvested primarily from public reefs that are monitored by the Alabama Department of Conservation and Natural Resources, Marine Resources Division.

[1]Data courtesy of Alabama Marine Resources Division, Alabama Trip Ticket Program. Weights are calculated using an average monthly conversion derived using monthly yields collected from Alabama dealers for Alabama oysters. Data was provided February 8, 2013 and is preliminary and subject to change.

In recent years, Alabama's oyster industry has been challenged by a combination of natural and man-made disasters that lead to a decrease in wild harvest of oysters. Major hurricanes and several consecutive years of drought increased mortality on the reefs, due to predation by the Southern oyster drill (*Strominata haemostoma*) (Herrmann, 2010). In 2010, the Deepwater Horizon Oil Spill caused closure of all fishing zones, further impacting the industry (OSAT, 2010). Due to a decrease in wild supply (and an increase in wholesale prices), interest has risen in alternative methods, namely off-bottom methods, of meeting the demand for fresh oysters. Off-bottom farming techniques can be used to supply premium Alabama oysters to the half shell market, alleviating some of the pressure on wild fisheries and providing entrepreneurial opportunities for rural Alabama communities.

By being a viable business, off-bottom oyster farming could allow coastal Alabama citizens to earn a living on the water and in the seafood industry; both of which are culturally significant in the region. Off-bottom farmed oysters are destined for the premium half-shell market. Currently, the majority of oysters harvested in Alabama are sold to the shucked meat market. So, off-bottom oyster farming provides an opportunity for Alabama to diversify its offering to the region and the nation.

*The Market for Alabama Farm-Raised Oysters*

Farmed raised oysters are one of the healthiest seafood items in the world (McNevin, 2007). The Monterey Bay Aquarium's Seafood Watch program, a highly recognized program that evaluates the ecological sustainability of seafood, has assigned farm raised oysters their highest honor of 'Best Choice' and placed them on their 'Super Green' list (McNevin, 2007). To demonstrate credibility, Whole Foods, the largest organic retailer in the US, partners with Seafood Watch to source sustainable non-finfish and shrimp seafood products (Whole Foods, 2013). Farmed oysters are recognized by these organizations because farmers are held to high standards by all levels of government throughout the growing and harvesting processes. In many cases, including in Alabama, farmers have implemented best management practices that go above and beyond what is required by local laws to ensure the consumer receives a safe, top quality product (S. Crockett pers. comm., 2012).

The premium half shell market is a high value market that demands a top quality product (Jacobsen, 2007; FAO, 2011). Shell shape and meat quality are very important factors influencing a consumer's decision-making process (Brake et al., 2003, Kow et al., 2008; Broken Bay Oysters, 2013). A well-proportioned oyster with a deep cup and broad fan will be given a higher grade than a long, skinny oyster (CFIA, 2012; Broken Bay Oysters, 2013) (Fig. 1.4). The exterior of the shell of a premium quality oysters should be devoid of fouling such as barnacles, overset of oysters, and algae and the interior should be free of blemishes from pests such as mud worms.

Fig. 1.4. Exterior (left) and interior (right) appearance of Alabama off-bottom farm-raised oysters. Meat quality of this oyster is good but the shell is slightly marred by mud worms potentially reducing its quality.

Meat quality is graded by fullness and taste, which can range from metallic to earthy to sweet (McMurray, 2007). The liquor contained within the oyster is a major contributor to its taste and varies depending on the salinity of the water in which the oyster is grown. Meat quality depends most on the reproductive state of the oyster. When an oyster has recently spawned the meat appears watery and of poor quality.  Disease, such as *Perkinsus marinus*, can also impact meat quality (Paynter and Burreson, 1991; LaPeyre et al., 2003). In Alabama, meat quality is best November through April, when oysters have maximum glycogen stores, resulting from cooler water temperatures and are not spawning (Supan, 2002; Wallace, 2003).  This positions Alabama farm-raised oysters well in the market because competitor's oysters may not be at their peak during this time due to ice coverage in northern regions.

Ultimately, off-bottom oyster farming in Alabama will only be successful if all members of the supply chain can profit from the product.  Alabama farm-raised oysters have a farm-gate value of approximately $0.50 a piece (S. Crockett pers. comm., 2012). This price allows the farmer to operate at a greater than 45% gross margin, a distributor to mark-up 30%, and a chef to make a 70% margin on the product while still placing Alabama farm-raised oysters competitively in the eyes of consumers.  These wholesale margins are comparable to those for Wellfleet oysters that are farm-raised on Cape Cod, Massaschusetts, USA (D. Hale pers. comm., 2011).

*Status of Off-Bottom Oyster Farming in Alabama*

There are currently three private off-bottom oyster farms in Alabama: Point-aux-Pins Oyster Farm, Mobile Bay Oyster Company, and Navy Cove Oyster Company.  All are very small operations, two-acres or less.  Point-aux-Pins Oyster Farm has been harvesting oysters for two years and has provided oysters to restaurants and retailers throughout the southeastern United States. Point-aux-Pins Oyster Farm was developed as a joint effort with Auburn University for research and proof-of-concept of commercial oyster farming in Alabama.  Point-aux-Pins Oyster Farm uses an adjustable long-line system for grow-out of oysters.  Mobile Oyster Company and Navy Cove Oyster

Company rely on the OysterGro™ floating cage system for the entire culture period, with the former just beginning production this year and the latter working on obtaining commercial permits.

In addition to private farms, Alabama's first aquaculture park has been established in Portersville Bay. This park, developed by Auburn University, will serve as an area for research and development of the off-bottom oyster farming industry. The concept of state aquaculture parks was proposed in March 1989 before the National Research Council's Committee on Assessment of Technology and Opportunities for Marine Aquaculture in the U.S.:

> *"Entrepreneurs could lease space, and infrastructure and be covered by an umbrella permit. Such parks would foster commercial operations, but even more importantly, would foster commercialization (i.e., parks could play an important role in technology transfer). A planned linkage between the technology centers and such aquaculture parks would facilitate the deployment of new technology"* (NRC, 1992).

Aquaculture parks, like industrial parks in urban areas, simplify permitting and regulatory hurdles for individuals, and minimize user conflicts. The Portersville Bay Oyster Park is divided into a two-acre block grid with areas for training beginning farmers and areas for farmers to sub-lease. The Portersville Bay Oyster Research and Demonstration Farm, established with funding from National Sea Grant, occupies an 8-acre block within the Park. This area will be used by the Auburn University Shellfish Lab for on-going research into farming methods and for training new farmers. This approach is analogous to the long established concept of experimental farms and demonstration plots in land-based agriculture. The Organized Seafood Association of Alabama will be the administrative body for the Park. A similar aquaculture park concept is being applied in Grand Isle, Louisiana, where the Grand Isle Port Commission will be the administrative body for the Park.

There are several prospective farmers interested in farming oysters using off-bottom culture techniques. Many of these people have access to large riparian rights or on-bottom leases that could produce a significant number of oysters. It is expected that as the industry gains exposure through development of the Portersville Bay Park and private farms, interest will rise in off-bottom oyster farming not only in Alabama, but throughout the Gulf region.

*Project Goals and Objectives*

The experiments presented here were prompted by beginning commercial oyster farmers in the north central Gulf of Mexico using the adjustable long-line system, faced with decisions about specific culture practices: basket alignment and stocking density. Arranging baskets cross-line in the system allows space for 33% more baskets on a run than arranging the baskets in-line, thereby increasing production within the same space. We sought to determine if arranging baskets cross-line versus in-line was detrimental to the quantity and quality of oysters produced. Product quality, including both shell and meat quality, was measured as shell shape, dimensions, weight and cosmetic damage and meat quality was quantified by meat weight and condition.

Stocking density using the ALS in the northern Gulf of Mexico had been examined economically using densities of 50, 75, and 100 oysters per basket (Maxwell and Supan, 2010), with a recommendation of 75 oysters per basket. Here the goal was to examine stocking density on a finer scale. The goal was to determine how varying stocking density by just 15 oysters per basket would affect product quality when considered independently and in conjunction with basket arrangement. We quantified product quality in the same ways as described above for basket arrangement.

## Chapter 2. Effects of basket arrangement and stocking density when using the adjustable long-line system for grow-out of oysters, *Crassostrea virginica*

### Introduction

Off-bottom oyster farming, an emerging industry in the northern Gulf of Mexico (GOM), is working towards providing a premium oyster for the half shell market (Walton et al., 2012c). Culture gear that has worked effectively in other parts of the world has been tested in both Alabama and Louisiana (Maxwell and Supan, 2010; Walton et al. in press, 2012d). Control of bio-fouling and predator exclusion are critical to producing a premium oyster for the half shell market (Michael and Chew, 1976; Claereboudt et al., 1994a; Taylor et al., 1997; Pitt and Southgate, 2003). The adjustable long-line system (ALS), developed in southern Australia, has shown promise in Alabama because its design allows for control of bio-fouling through routine air drying and oysters are protected from predators by being suspended in mesh baskets (BST Oyster Supply Pty, 2013). In addition, the system is durable enough to withstand tropical storm events (J. Supan, pers. comm., 2011; Davis pers. obs.).

In order to improve shellfish productivity, culture equipment and husbandry practices must be optimized (Robert et al., 1993; Handley, 2002; Louro et al., 2007). ALS manufacturers recommend two configurations for the system (BST Oyster Supply Pty, 2013) with the cross-line arrangement capable of holding 33% more baskets in a 100 m run than an in-line basket arrangement. This is enticing to farmers because it potentially represents a 33% increase in production within the same space.

Maintaining optimum densities in shellfish culture is imperative to producing a consistent, high quality product (Holliday et al., 1993; Comeau et al., 2011). Several studies have investigated optimum stocking densities for off-bottom oyster farming (Rheault and Rice, 1996; Holliday et al., 1991; Handley, 2002; Honkoop and Bayne, 2002). In addition to business models, which rely on production assumptions to estimate profit, a number of ecological models rely on this farming fundamental to be entered into the system to calculate ecological benefits and/or impacts (Ferreira et al., 2009; ShellSIM, 2012). Such information can benefit a farmer in many ways from improving product quality to negotiating regulatory hurdles. Economic models developed for the ALS in the northern GOM set stocking density at 75 oysters per basket (Maxwell and Supan, 2010).

The purpose of this study was to determine how product quality and production is impacted by basket arrangement and stocking density when using the ALS for final grow-out of oysters, *Crassostrea virginica*. We hypothesized that product quality would be negatively affected at higher stocking densities and also when baskets were arranged cross-line due to the cascading effect this has on density.

## Materials and methods

### *Site Description*

The Auburn University Oyster Research and Demonstration Farm (AUORDF) is a 60-acre submerged lands riparian rights lease located in Portersville Bay, Alabama, which is in the Mississippi Sound southwest of the western mouth of the Fowl River (30º21’11.56”N 88º11’28.45”W, Fig. 2.1). The AUORDF was established in 2011 in order to foster development of an off-bottom oyster farming industry in Alabama. The Auburn University Shellfish Laboratory maintains a portion of the lease to conduct research on new growing methods with the remainder of the lease dedicated to training beginning oyster farmers.

Fig. 2.1. Location of Auburn University Oyster Research and Demonstration Farm in coastal Alabama.

The site is well protected by barrier islands and coastal wetlands and experiences diurnal tides. Water depth at mean high water is approximately 1.5 m with a 0.6 m tidal range. The lease was historically cultivated for on-bottom oyster harvest and, as such, has a semi-firm sand substrate due to years of cultch planting. There is no submerged aquatic vegetation present within the lease (Walton, 2011).

Environmental conditions at the site were recorded using in-water loggers and measurements taken during site visits. An Onset HOBO® Water Temp Pro v2 Logger, positioned approximately 50 cm above the substrate, recorded water temperature every 10 minutes for the duration of the study (Onset Computer Corporation Inc., Bourne, MA). A Star Oddi DST CTD recorder, similarly positioned, measured and logged salinity every 10 minutes from May through September (Star ODDI, Iceland). Dissolved oxygen was measured using a YSI Model 85 Dissolved Oxygen and Conductivity Meter (Yellow Springs International, Yellow Springs, Ohio). A SeaHorse tilt current meter was deployed at the site for one 18 hour period, positioned 50 cm above the substrate and logging tilt on three planes every two minutes (Sheremet, 2010). Tilt data were translated to current speed by the manufacturer using Python® software (Python Software Foundation, Beaverton, OR).

*Adjustable Long-line System Components and Construction*

The adjustable long-line system (ALS) (BST Oyster Supply Pty., Cowell, Australia), developed in Australia, was designed for growing oysters in shallow, coastal waters. At AUORDF, the ALS was comprised of a 5 mm diameter tensioned monofilament line sleeved with rigid plastic tubing strung between two pilings. Four lines were assembled on land by tensioning the monofilament and sliding on the tubing. Lines were installed in pairs, termed a 'run'. Two runs, each 91 m long and spaced 4.7 m apart, were used in this experiment. In October 2011, treated timber pilings 30 cm in diameter and 5 m long were driven 2.5 m into the substrate using a hydraulic hammer. Pilings were spaced 76 cm apart from center to center. Pilings were allowed six weeks to settle to minimize movement when a load was applied.

The remaining components of the ALS were installed in December 2011. Pre-assembled lines were installed one-by-one by securing them below the low water mark to one piling and using a manual winch to tension them on the opposite end. Once tensioned the line was then secured, below the low water line, to the opposite piling. Riser posts, with five clips pre-installed at 9 cm intervals beginning at the top of the pipe, were then manually hammered into the substrate at 2.5 m intervals along the line. The space between riser posts was termed a 'bay'. When installing riser posts, one line was installed and then used to guide the installation of the second line in the run. A level was used to ensure each pair of riser posts was vertically true. Line of sight was used to ensure each pair was horizontally true. Ensuring the lines were vertically and horizontally true was important because some baskets were going to be arranged in the cross-line configuration (Fig. 2.2). Baskets, fitted with end-cap clips, were placed in each bay to ensure identical spacing between the lines throughout the run. Riser posts were made of 3 m lengths of 5 cm diameter polyvinyl chloride (PVC) pipe. We chose grey ultraviolet resistant electrical conduit for two reasons: 1) to blend with the surroundings and; 2) it was belled at one end which would allow the height of the riser post to be easily extended should it experience excessive settling. Riser posts were allowed two weeks to settle before applying a load (i.e. placing baskets on the line).

On January 5, 2012, baskets, containing oysters, were placed on the ALS. Baskets used for this study were procured from BST Oyster Supply Pty, Australia and assembled at the Auburn University Shellfish Lab. Baskets were 71 cm long by 20 cm wide triangular cylinders made of 12 mm hexagonal plastic mesh (Fig. 2.3). Since lines were installed in pairs (Fig. 2.2), baskets could be hung parallel to the line (termed 'in-line' subsequently) or between the lines (termed 'cross-line' subsequently). Baskets arranged in-line were secured to the line by clips located on the top of the basket, whereas, baskets arranged cross-line were secured between the lines using clips located on the end-caps of the basket (Fig. 2.3). With the exception of routine maintenance events, lines were secured in the bottom riser clip leaving the bottom of the basket resting approximately 50 cm above the substrate.

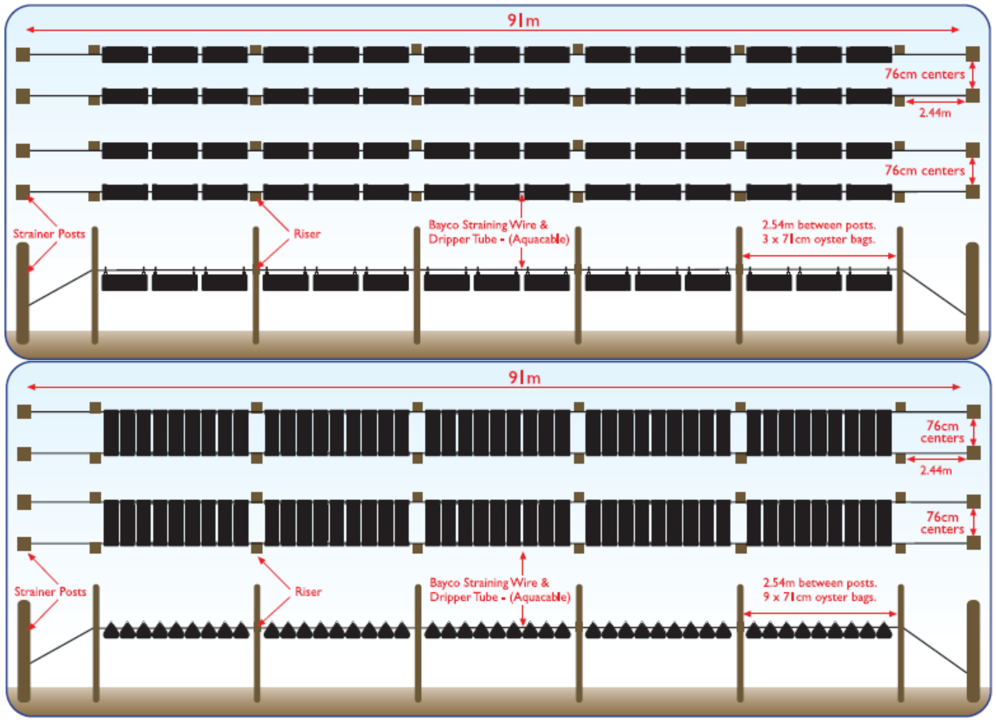


Fig. 2.2. Schematic of in-line (top) and cross-line (bottom) basket arrangements depicted as they would be deployed in a commercial production setting. Note that cross-line allows a greater number of baskets per bay. (BST Oyster Supplies Pty Ltd. 2012).

Fig. 2.3. Location of clips used to secure ALS baskets to the line depending on basket arrangement. Left: In-line arrangement. Right: Cross-line arrangement

*Husbandry Practices to Control Bio-fouling*

The ALS was designed to allow the position of the baskets, and therefore oysters, in the water column to be adjusted depending on the needs of the grower. This is accomplished by manually raising or lowering the line into one of five riser clips that are fixed to each riser post. This design also allows the baskets to be routinely lifted out of the water for air drying. Routine desiccation controls bio-fouling on baskets, lines, and oysters (Maxwell, 2007). The position the oysters were in when they were submerged was referred to as the ‘growing position’; the position they were in for air drying was called the ‘drying position’. Our growing position was the lower-most clip; the uppermost clip served as the drying position (Fig. 2.4).

Fig. 2.4. Growing position (foreground) and drying position (background) of the ALS. This photo was taken during a spring low tide where the baskets in the growing position were partially exposed.

Our air drying routine, dictated by local farming practices, included air drying for 24 hours once per week during the warmer months (in this study from March 2012 through August 13, 2012 near the project conclusion). We did not desiccate for the first month (January 2012) of the study due to extremely low winter tides that provide some natural desiccation. Our desiccation routine increased to every other week during February. During the summer months when air temperatures can exceed 32$^{o}$C, oysters were exposed overnight for only 18 hours to avoid heat-induced mortality (Coddington-Ring, 2012). Best management practices for shellfish harvesting recommend that oysters remain submerged for at least seven days prior to harvest (Leavitt, 2009;

Oesterling and Luckenbach, 2008). Our oysters remained submerged (i.e. not lifted for air drying) for 11 days prior to harvest sampling.

The in-line basket clip was approximately 20 cm higher than the cross-line basket clip (Fig. 2.3), therefore the in-line baskets hung lower in the water column than cross-line baskets. To ensure all treatments received equal desiccation regardless of basket arrangement, we affixed end-cap clips to the in-line baskets to be used only during air drying. The line was raised and then in-line baskets were detached one-by one from the line, turned cross-line and secured. The next day, prior to lowering the line, the in-line baskets were removed from their cross-line drying position and re-secured in their in-line growing position.

*Experimental Design*

This study was a two factor (basket stocking density and basket configuration) completely randomized design. Basket stocking density was tested at three levels, with treatments of 75, 90 and 105 oysters per basket. These levels were chosen based on the current practice of local farmers and economic feasibility (Maxwell and Supan, 2010). The basket stocking density factor was crossed with basket arrangement, with three levels: cross-line with 9 baskets per bay, cross-line with 6 baskets per bay or in-line with 6 baskets per bay (Table 2.1). Note that 6 baskets per bay is the maximum bay capacity for the in-line orientation (Fig. 2.1). We included the 6 baskets per bay cross-line treatment to provide a test of the effect of overall density of oysters within each bay. Replicates (n = 4, with the exception of two treatments where n = 3) were assigned at the level of bay (e.g. 6 baskets stocked at 75 oysters). From this design, we had a test of the effect of basket stocking density, basket arrangement, and any interaction between these for any of the response variables. In its entirety, the experiment included

240 baskets in 34 bays on two runs and a total of 21,690 seed oysters averaging 50.5 ± 8.23 mm in shell height.

Table 2.1.
Experimental design to test for effects of basket arrangement and stocking density when using an adjustable long-line system for oyster grow-out.

| Basket arrangement | Number of baskets/bay | Basket stocking density (oysters/basket) | Number of oysters/bay | Number of replicates |
|---|---|---|---|---|
| Cross-line | 9 | 105 | 945 | 4 |
| Cross-line | 9 | 90 | 810 | 4 |
| Cross-line | 9 | 75 | 675 | 4 |
| Cross-line | 6 | 105 | 630 | 4 |
| Cross-line | 6 | 90 | 540 | 4 |
| Cross-line | 6 | 75 | 450 | 3 |
| In-line | 6 | 105 | 630 | 4 |
| In-line | 6 | 90 | 540 | 3 |
| In-line | 6 | 75 | 450 | 4 |

*Data Collection*

A haphazard sample of 300 seed oysters were bagged and frozen at -16$^{o}$C in a Frigidaire® Gallery™ drop freezer (Frigidaire, Augusta, GA) for at least one week for determination of condition index and shell metrics, as described below, at the outset of the experiment.

A sub-sample of oysters from each treatment replicate was measured every other month to determine growth rates and allow us to predict harvest time. Five haphazardly selected oysters from three randomly selected baskets per bay were measured to the nearest 0.01 mm using Mitutoyo Digimatic Electronic Digital calipers (Mitutoyo America, Aurora, IL) every other month for the duration of the study. Shell height, shell length, and shell width were recorded during each measuring event (Fig. 2.5). The end point of the study was designated at the point when the sample of oysters from six baskets strung cross-line and stocked at 90 oysters per basket were, on average, ≥75 mm in shell height.

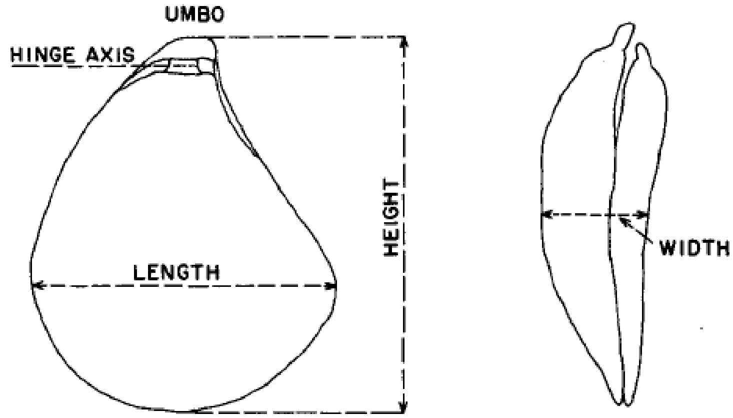


Fig. 2.5. Location of measurements recorded for each oyster (from Galtsoff, 1964).

Due to the threat of Hurricane Isaac, harvest sampling, in which five live oysters were haphazardly selected from each basket (240 baskets total), took place on August 26, 2012. In order to best manage the risk of loss presented by the storm we decided to collect the sample ahead of the planned harvest date. Oysters were bagged into one quart Ziploc™ bags and placed in a Frigidaire® Gallery™ drop freezer (Frigidaire, Augusta, GA) at -16$^{o}$C for at least one week prior to processing. Each bag was labeled according to the basket number and bay number from which the oysters were taken. Each oyster was processed at a later date to determine shell metrics, fouling, and condition index.

The remaining sampling, which included determination of survival and sampling for intensity of *Perkinsus marinus* infection and mud worm *Polydora websteri* infestation, was conducted as planned on September 10, 2012. Baskets were transported from AUORDF to the Auburn University Shellfish Laboratory, a distance of approximately 12.5 km, where each basket was processed. The total numbers of live and dead oysters were counted in each basket (240 baskets total). Oysters sampled on August 26, 2012 were included in the live count. Four or five live oysters were then haphazardly selected for *P. marinus* and *P. websteri* studies, respectively. The remaining oysters were returned to their respective basket and returned to the AUORDF the following day.

*Determination of Fouling, Condition Index, and Shell Metrics*

Sample processing to determine the amount of fouling, shell metrics, and condition index of each oyster was a multi-step process performed on multiple days during September 2012. Seed oysters were very clean so fouling was not quantified but average initial shell metrics and condition index were determined. The first step of processing samples at harvest was to determine the amount of fouling present on each oyster. Samples were processed one bag at a time. The bag, containing five oysters, was removed from the freezer, filled half way with cold tap water, inverted twice, and then emptied. This gently loosened any oysters that had become stuck together by ice. Each oyster was then blotted on paper towel, assigned to a numbered plastic Petri dish, and weighed to the nearest 0.001 g using a Mettler Toledo PG503-S Delta Range electronic digital balance (Mettler Toledo LLC, Columbus, OH). This measurement was the ‘as harvested whole wet weight’ and included the mass of the oyster and all fouling organisms. The dominant fouling organisms on each oyster were also recorded at this time. Each oyster was then manually cleaned using a shucking knife and wire brush. Overset of oysters was removed to the maximum extent possible, though in most cases the bottom valve of the overset remained. After blotting on paper towels, each oyster was weighed to the nearest 0.0001 g using a Mettler Toledo AL204 electronic digital balance (Mettler Toledo, Columbus, OH). This measurement was the ‘clean whole wet weight’. Subtracting the ‘clean whole wet weight’ from the ‘as harvested whole wet weight’ allowed us to determine the amount of fouling, in wet weight grams, present on each oyster, which was then converted to an index by dividing this amount by the clean whole wet weight. The shell height, shell length and shell width of each oyster were then measured to the nearest 0.01 mm using Mitutoyo Digimatic Electronic Digital calipers (Mitutoyo America, Aurora, IL). These measurements were used to calculate the cup ratio (shell width/shell height), as an index of the depth of the cup of the oyster, and fan ratio (shell length/shell height), as an index of the breadth of the oyster's bill.

To determine condition index, the tissue was separated from the shell before they were both dried (Abbe and Albright, 2003). Each whole oyster was manually opened from the hinge. After severing the adductor muscle from the right (or 'top') valve it was removed and all residual muscle tissue scraped from the right valve using a knife. The meat was then removed from the left, or bottom cupped valve, and placed in a VWR 6 cm diameter aluminum foil boat labeled with a number corresponding to the number on the Petri dish in which the shell was placed. The shell, in its dish, was placed on the bench top to dry for 48 ± 2 hours at room temperature (20-23 $^{o}$C). The meat was placed in a Fisher Scientific® Isotemp drying oven (Thermo Fisher Scientific Inc., Pittsburgh, PA) at 80$^{o}$C for 48 ± 2 hours. The final step, following drying, was to weigh each dried shell and meat to the nearest 0.0001 g using a Mettler Toledo AL204 electronic digital balance (Mettler Toledo LLC, Columbus, OH). Condition index (CI) was calculated using the following formula from Abbe & Albright (2003):

CI = [(dry tissue weight in grams/(whole wet weight in grams – dry shell weight in grams)] x 100

*Dermo Testing*

We tested the effect of stocking density and basket arrangement on *P. marinus* infection intensity. We tested all three basket arrangements and the highest and lowest stocking densities (75 and 105 oysters/basket). Four live oysters were haphazardly selected from two randomly selected baskets per treatment replicate for a total of 184 oysters. The oysters were placed in a cooler and shipped overnight to Louisiana State University for whole body burden analysis.

*Perkinsus marinus* infection intensity or the number of parasites per gram of oyster wet tissue was determined using the whole-oyster procedure described by Fisher and Oliver (1996) and modified by La Peyre et al. (2003), except that oyster tissues were suspended in 25 ppt sterile seawater instead of alternative Ray's fluid thioglycollate medium (ARFTM) during the homogenization step. ARFTM was prepared using alternative fluid thioglycollate medium supplemented with 16 g/L of hw-Marine Professional sea salts (Hawaiian Marine Imports Inc., Houston, TX) and 0.5% (v/v) lipid mixture 1,000x. Homogenate tissue was incubated in ARFTM at a concentration that ranged from 30 to 60 milligrams of tissue per milliliters of ARFTM.

*Data Analysis*

There were nine treatments (three basket stocking densities x three basket arrangements) in this study replicated on the level of bay, with a total of 34 bays occupied. Data were analyzed by basket arrangement (2 df), stocking density (2 df), and any interaction between the two (4 df) for all of the response variables. Response variables included: shell height, shell length, shell width, cup ratio, fan ratio, clean whole wet weight, dry shell weight, dry tissue weight, condition index, percent survival, growth rate, and amount of fouling. The following hypotheses, and their interactions, were tested:

$H_o$: $\mu_{CL} = \mu_{IL}$; where CL = cross-line basket arrangement and IL = in-line basket arrangement

$H_a$: $\mu_{CL} \neq \mu_{IL}$

$H_o$: $\mu_{75} = \mu_{90} = \mu_{105}$; where 75, 90, and 105 refer to basket stocking densities

$H_a$: $\mu_{75} \neq \mu_{90} \neq \mu_{105}$

Systat® 12 and MINITAB® software were used to analyze the data (Systat Software Inc., Chicago, IL; Minitab Inc., State College, PA). Shapiro-Wilk test of normality and Levene's test for homogeneity of variance were used to verify the assumptions of normality and homogeneity of variance, respectively. Data were considered normally distributed and variances homogenous at $p > 0.05$. Where these assumptions were not met, data were transformed using the appropriate transformation for the type of data (Underwood, 1997); dry tissue weight data were log transformed, survival data were arc sin transformed, and *P. marinus* counts were square root transformed. We were unable to achieve homogeneity of variance in the fouling index data. A great deal of variation was experienced in the fouling data, possibly due to the ability of one animal (i.e. large barnacle or one overset oyster) to contribute a large amount of weight to a single oyster. Not all oysters in a sample were affected this way. Since our data was well balanced and we had a relatively large number of samples, we could safely interpret the analysis even with heterogeneous variances (Underwood, 1997).

Once assumptions were satisfied, a two-factor analysis of variance (ANOVA) was used to determine effects of basket arrangement, stocking density and any interaction between the two for each response variable. When no interactions were detected single factors were pooled across treatments (Appendix 1). For example, basket arrangement was pooled across densities and vice versa. All tests were performed with $\alpha = 0.05$ and means were considered significantly different if $p < 0.05$. Post-hoc pairwise comparison using Tukey's Honestly Significantly Difference Test ($p < 0.05$) was used to further investigate significant effects identified by the ANOVA.

## Results

### *Environmental Conditions*

Water temperature ranged from 8.27 to 33.4$^{o}$C, with an average of 24.2 ± 6.17$^{o}$C (Fig. 2.6). Our experiment encompassed three seasons, with the following average water temperatures: winter (January - March) 17.2 ± 3.25°C; spring (April - June) 27.0 ± 2.74°C; and summer (July - September) 29.5 ± 1.48°C.

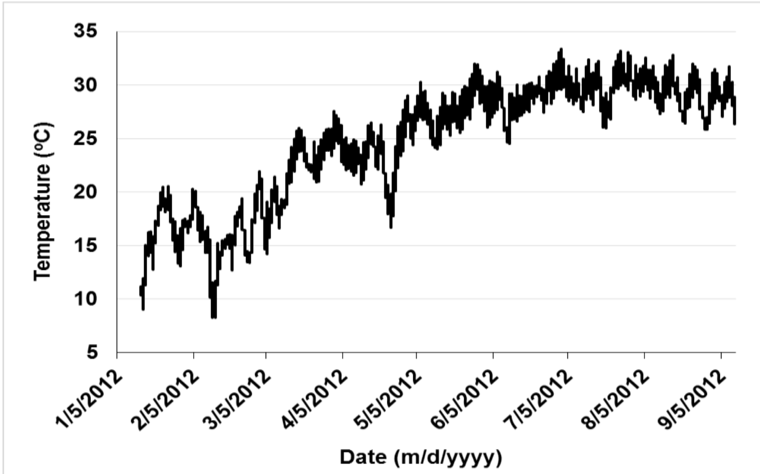


Fig. 2.6. Water temperature at Portersville Bay, AL from January 14 – September 10, 2012.

Salinity was continuously logged from May 24 – September 10, 2012 and sampled monthly from January – May 11, 2012. Salinity ranged from 8.2 to 25.7 PSU, with an average salinity of 17.9 ± 3.47 PSU (Fig. 2.7).

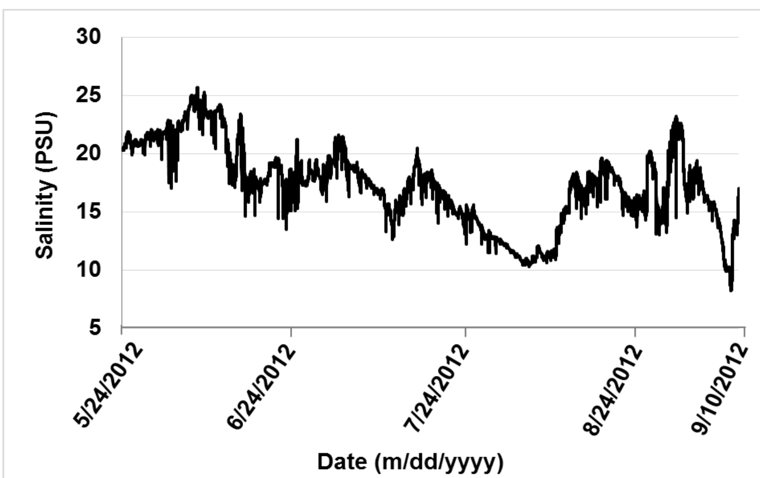


Fig. 2.7. Salinity at Portersville Bay, AL from May 24 – September 10, 2012.

Current was recorded every two minutes for 18 hours on June 21 and 22, 2012. The maximum current speed at the site was 4 cm/second, with a much lower average of approximately 1 cm/s. The meter we used was configured for much shallower waters so we did not experience enough tilt on the meter (< 10 degrees) to accurately measure low velocity currents.

*Effects of Basket Arrangement and Stocking Density*

There were no significant interactions between basket arrangement and stocking density for most of the response variables; therefore, we consider each of these factors separately, and prior to, describing the sole significant interaction between stocking density and arrangement upon the January to March growth interval.

*Basket Arrangement*

There was a significant effect of basket arrangement (Fig. 2.8) upon shell height (ANOVA, $p<0.001$), shell length (ANOVA, $p<0.001$) and shell width (ANOVA, $p<0.001$). Specifically, oysters grown in in-line baskets were larger than oysters grown in baskets arranged in either cross-line configuration for any of the three response variables (Tukey HSD, $p < 0.001$). Additionally, for any shell metric there was no significant difference between bays with nine baskets arranged cross-line versus those with six baskets arranged cross-line (Tukey HSD, $p \geq 0.761$).

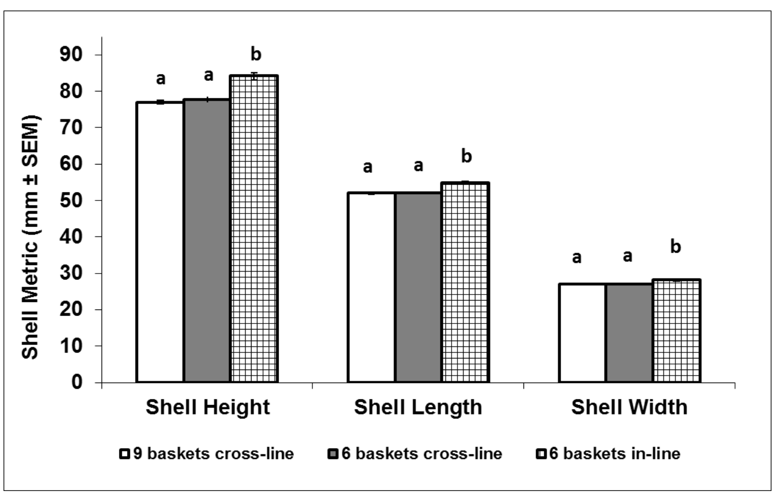


Fig. 2.8. Effect of basket arrangement upon shell metrics in millimeters (mean ± SEM, n ≥ 3). Oysters grown in baskets arranged cross-line (9 or 6 to a bay) or in-line (6 to a

bay). Different letters indicate a statistically significant difference ($p < 0.05$) within a shell metric.

There was also a significant effect of basket arrangement upon both cup (ANOVA, p<0.001) and fan (ANOVA, p<0.001) ratios (Fig. 2.9). Oysters grown in baskets arranged in either cross-line configuration were more deeply cupped relative to their length than those grown in-line (Tukey HSD, p<0.001), with no significant difference between the cross-line treatments (Tukey HSD, p=0.532). Similarly, oysters grown in baskets arranged in either cross-line configuration (Fig. 2.9) were broader across the bill, referred to as the 'fan', relative to their length than those grown in baskets arranged in-line (Tukey HSD, p≤0.002), with no difference between the two cross-line treatments (Tukey HSD, p=0.640).

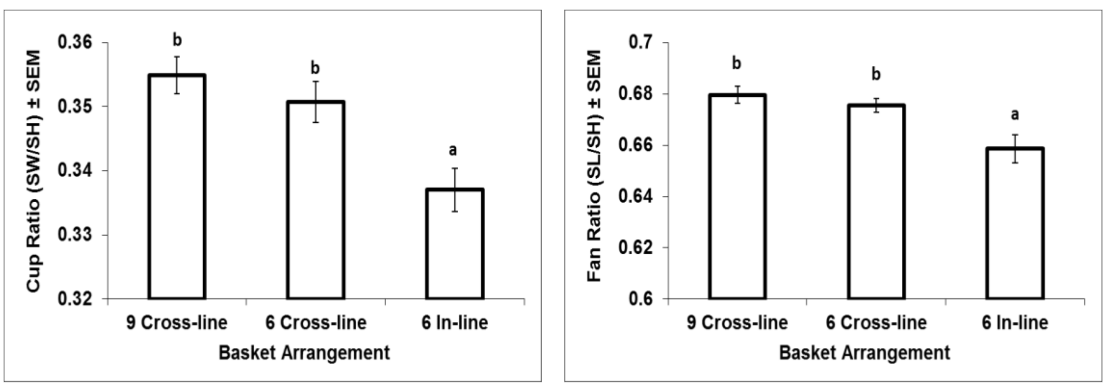


Fig. 2.9. Effects of basket arrangement (mean ± SEM, n ≥ 3) on cup ratio (ANOVA, p<0.001) and fan ratio (ANOVA, p<0.001). Different letters indicate a statistically significant difference ($p < 0.05$).

Basket arrangement had a significant effect on the clean whole wet weight of oysters (ANOVA, p=0.001). Oysters grown in baskets arranged in-line were heavier after being cleaned of fouling organisms and weighed whole than oysters grown in either of the cross-line arrangements (Tukey HSD, p=0.001)(Fig. 2.10). Whether baskets were arranged nine or six cross-line to a bay had no significant effect on whole wet weight of the oysters (Tukey HSD, p=0.756).

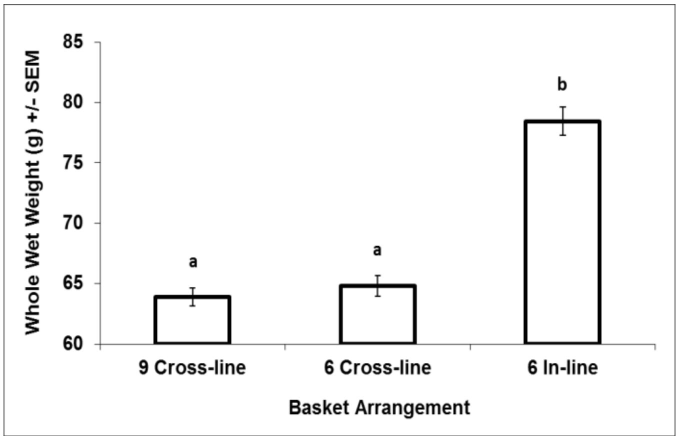

Fig. 2.10. Effect of basket arrangement on whole wet weight of oysters (ANOVA, p=0.001) (mean ± SEM, n ≥ 3). Different letters indicate a statistically significant difference (p<0.05).

Basket arrangement had a significant effect on dry shell weight (ANOVA, p<0.001) (Fig. 2.11) and dry tissue weight (ANOVA, p=0.001) (Fig. 2.12). Oysters grown in in-line baskets had heavier shells than those grown in cross-line baskets (Tukey HSD, p<0.001) baskets. Oysters grown in in-line baskets had significantly heavier tissue (or meat) than oysters grown in either cross-line arrangement (Tukey HSD, p<0.001). There was no difference in the dry shell weight or dry tissue weight of oysters grown with nine baskets cross-line versus six baskets cross-line per bay (Tukey HSD, p=0.830 and p=0.772, respectively). There was no effect of basket arrangement on condition index (ANOVA, p=0.295) (Fig. 2.13). Basket arrangement also had no effect on the intensity of *P. marinus* infection (ANOVA, p=0.837) (Fig. 2.14).

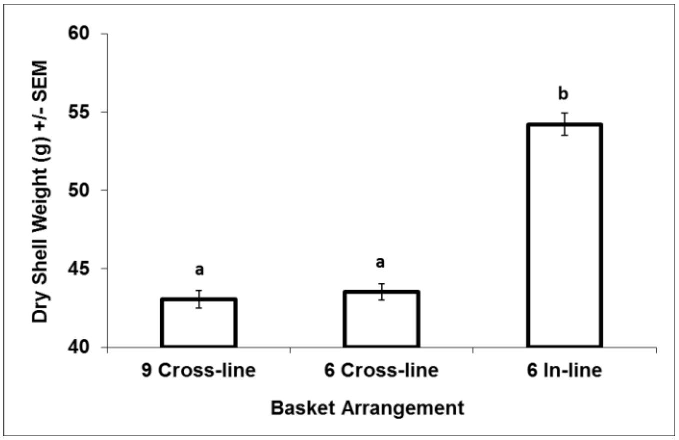


Fig. 2.11. Significant effect of basket arrangement on dry shell weight (mean ± SEM, n ≥ 3) (ANOVA, p=0.001). Different letters indicate a statistically significant difference (p < 0.05).

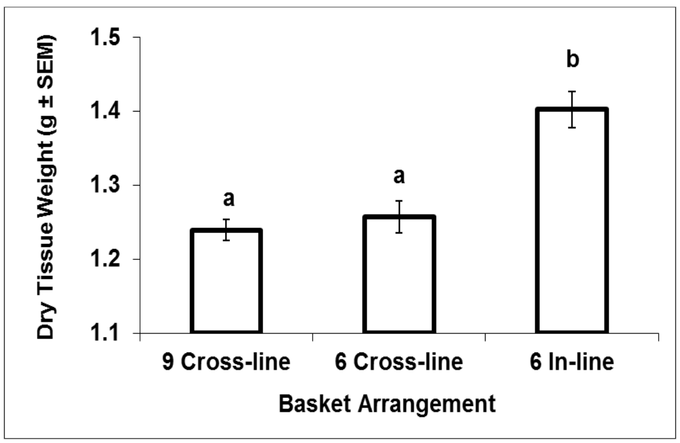


Fig. 2.12. Significant effect of basket arrangement on dry tissue mass (mean ± SEM, n ≥ 3) (ANOVA, p=0.001). Different letters indicate a statistically significant difference (p<0.05).

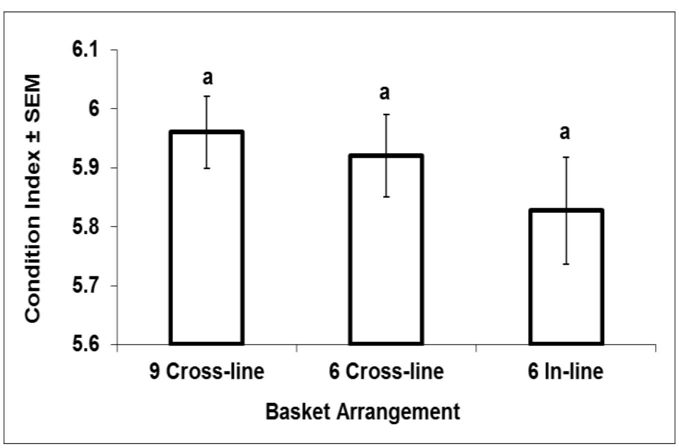


Fig. 2.13. Condition index (mean ± SEM, n ≥ 3) of oysters grown in baskets arranged cross-line and in-line. No statistically significant differences (ANOVA, p=0.295).

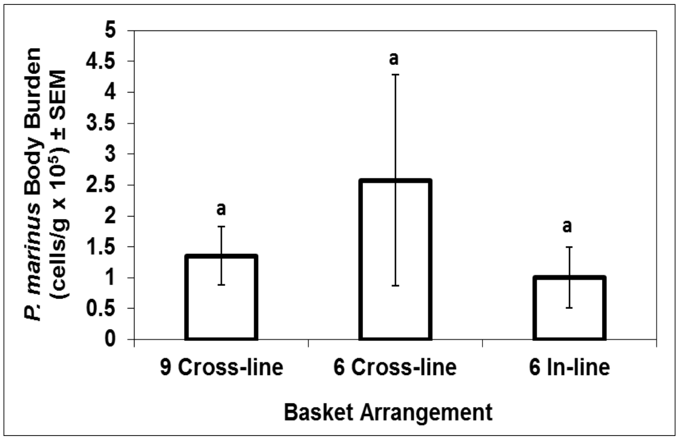

Fig. 2.14. Intensity of *P. marinus* infection in oysters grown in baskets arranged cross-line and in-line. No statistically significant differences (ANOVA, p=0.837).

In consideration of the difference in size between oysters grown in different arrangement treatments, we standardized the fouling data as a percentage of the ‘as harvested’ mass comprised of fouling, or a fouling index. Basket arrangement had a significant effect on fouling index (ANOVA, p<0.001) (Fig. 2.15). Oysters grown in in-line baskets were significantly more fouled than oysters grown in baskets arranged either nine or six cross-line (Tukey HSD, p<0.001). Whether six or nine baskets were arranged cross-line in a bay did not affect the amount of fouling accumulated on the oysters (Tukey HSD, p=0.663). These differences in fouling are equivalent to 10.8±0.92 g of fouling on an in-line oyster versus only 2.5±0.24 g and 3.0±0.24 g on nine cross-line and six cross-line oysters, respectively. This difference in fouling was visibly noticeable and more effort was required to clean oysters grown in baskets arranged in-line than those grown in baskets arranged cross-line. In addition to a thin layer of mud, fouling organisms included overset of oysters (*Crassostrea virginica*), barnacles, and bryozoans.

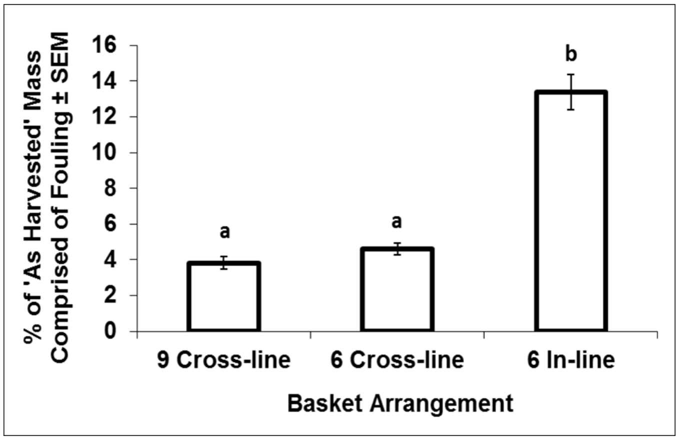

Fig. 2.15. Effect of basket arrangement on the fouling index (mean ± SEM, n ≥ 3) (ANOVA, p<0.001). Different letters indicate a statistically significant difference (p<0.05).

Basket arrangement had no significant effect on survival of oysters (ANOVA, p=0.294) (Fig. 2.16). Survival met or exceeded 97% in all treatments.

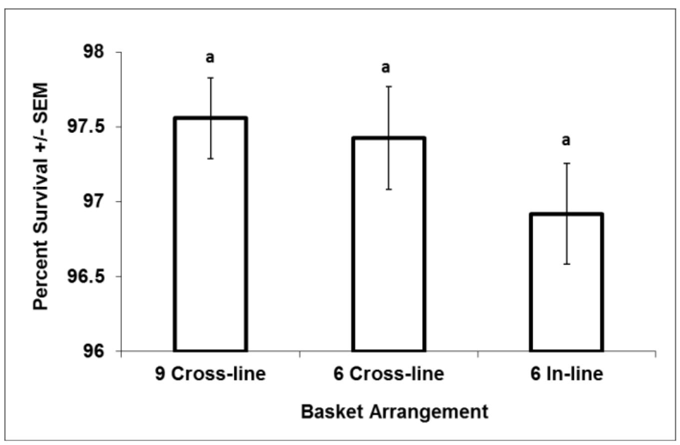

Fig. 2.16. Percent survival (mean ± SEM, n ≥ 3) of oysters grown in baskets arranged cross-line and in-line. No statistically significant effect of basket arrangement on survival (ANOVA, p=0.294).

In addition, basket arrangement had no significant effect on average growth rate during each two-month growth interval from March to harvest (ANOVA, p≥0.158) (Fig. 2.17).

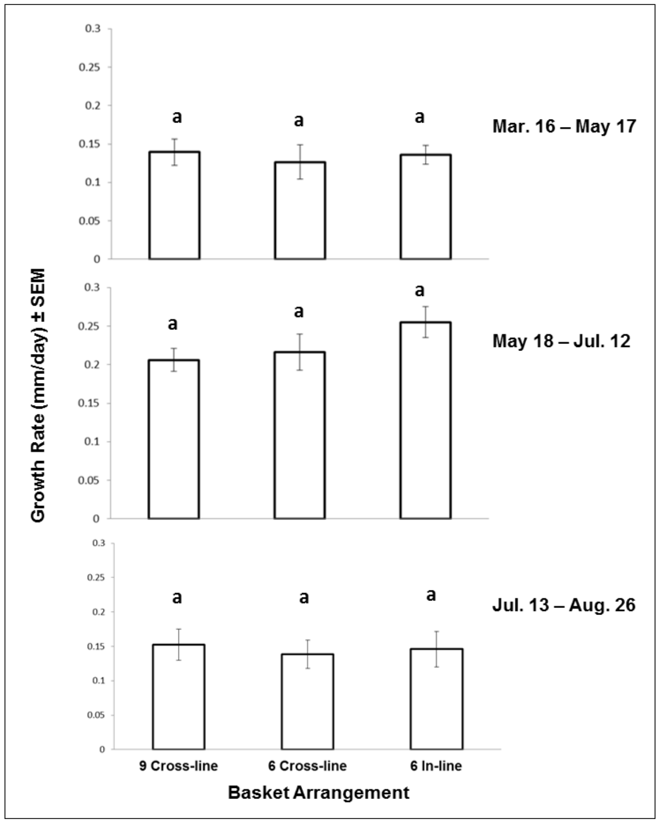


Fig. 2.17. Growth rate (mean ± SEM, n ≥ 3) at bi-monthly intervals of oysters grown in baskets arranged cross-line and in-line. No statistically significant effect of basket arrangement on bi-monthly growth rates (ANOVA, p≥0.158).

Basket arrangement, however, did have a significant effect on the overall growth rate (ANOVA, p=0.001). From January to harvest, oysters grown in baskets arranged in-line grew at a significantly faster rate than oysters in either cross-line arrangement (Tukey HSD, p<0.001). There were no significant differences between bays with nine baskets cross-line and six baskets cross-line (Tukey HSD, p=0.761). Overall, oysters in in-line baskets grew on average 0.14 mm/day versus oysters grown in cross-line baskets which grew on average 0.11 mm/day (Fig. 2.18).

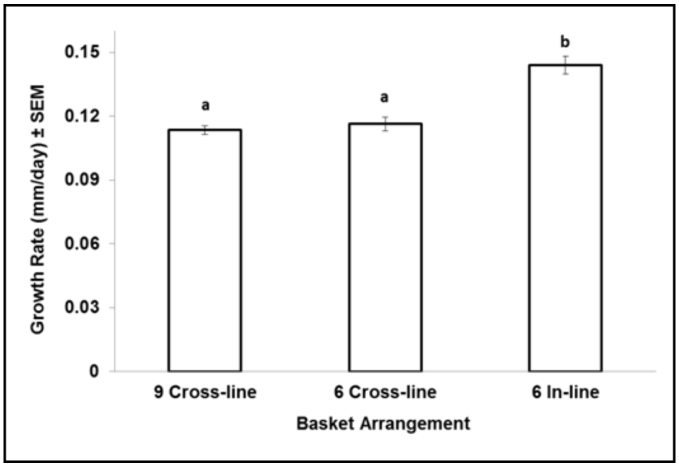


Fig. 2.18. Significant effect of basket arrangement on the overall growth rate (mean ± SEM, n ≥ 3) of oysters grown in baskets arranged cross-line and in-line (ANOVA, p≤0.001). Different letters indicate a statistically significant difference (p<0.05).

*Stocking Density*

Stocking density, the second factor tested, had no significant effect on shell length or shell width (ANOVA, p≥0.100); however stocking density did have a significant effect on shell height (ANOVA, p<0.001) (Fig. 2.19). Oysters stocked at 105 per basket were significantly larger in shell height than those stocked at 75 or 90 per basket (Tukey HSD, p≤ 0.044).

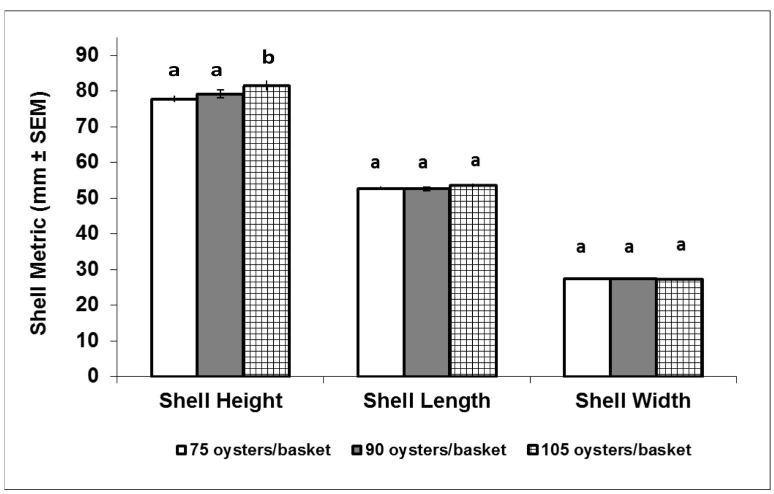


Fig. 2.19. Effect of stocking density on shell metrics (mean ± SEM, n ≥ 3) for oysters stocked at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference within the shell metric (p<0.05).

Stocking density had a significant effect on the depth of the oyster's cup relative to its length (ANOVA, $p<0.001$) and the breadth of its fan relative to its length (ANOVA, $p<0.001$) (Fig. 2.20). Oysters grown at 75 per basket had a deeper cup than those grown at 90 (Tukey HSD, $p=0.039$) and 105 per basket ($p<0.001$). Similarly, oysters stocked at 90 per basket were more deeply cupped than those stocked at 105 per basket (Tukey HSD, $p=0.033$). Oysters stocked at 75 per basket had a broader fan than those stocked at either 90 or 105 per basket ($p\leq0.012$).

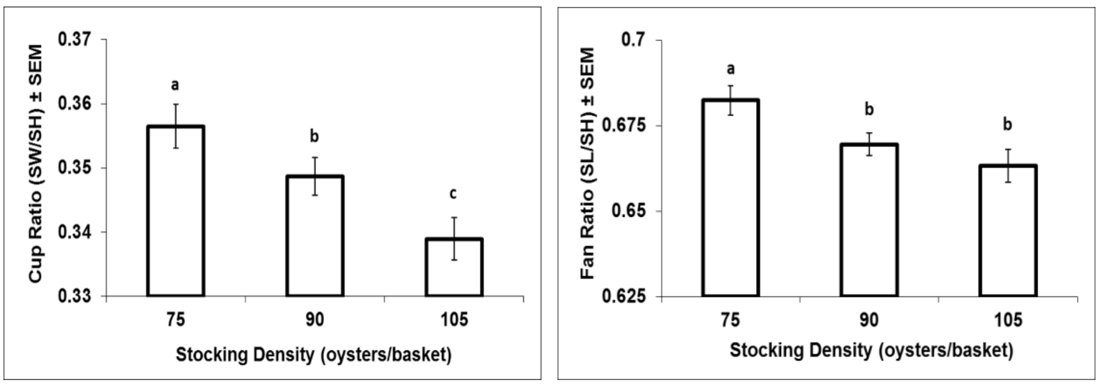


Fig. 2.20. Significant effect of stocking density on cup and fan ratio (ANOVA, $p\leq0.001$) (mean ± SEM, $n \geq 3$) of oysters stocked at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference ($p<0.05$).

Stocking density had a significant effect on the whole wet weight of oysters after they had been cleaned of all fouling organisms (ANOVA, $p=0.020$) (Fig. 2.21). Oysters stocked at 105 per basket were significantly heavier than those stocked at 75 and 90 per basket (Tukey HSD, $p=0.028$).

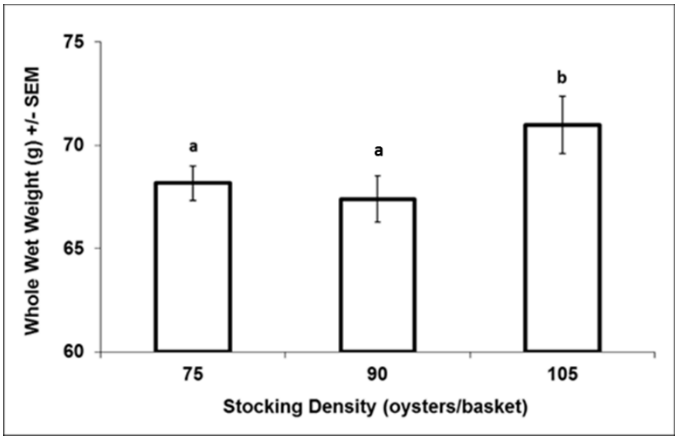

Fig. 2.21. Significant effect of stocking density on whole wet weight (ANOVA, p=0.020) (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference (p<0.05).

Stocking density had a significant effect on dry shell weight of oysters (ANOVA, p=0.036) (Fig. 2.22), but no significant effect on dry tissue weight (ANOVA, p=0.494) (Fig. 2.23). Oysters stocked at 90 per basket had significantly heavier shells than those stocked at 105 per basket (Tukey HSD, p=0.044). Neither of these treatments, however, differed significantly from those stocked at 75 per basket (Tukey HSD, p≥0.104).

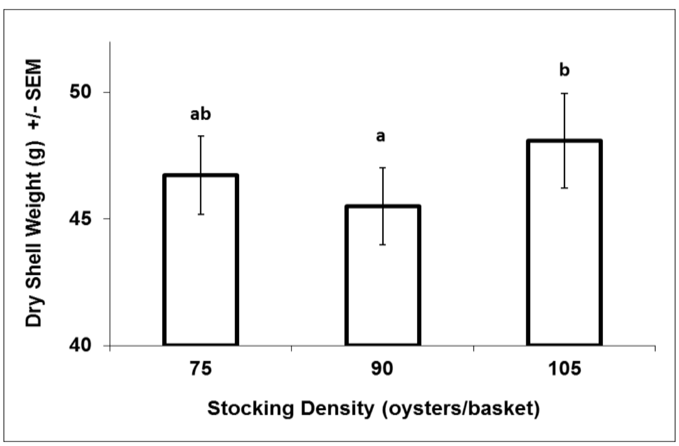

Fig. 2.22. Significant effect of stocking density on dry shell weight in grams (ANOVA, p=0.036) (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference (p<0.05).

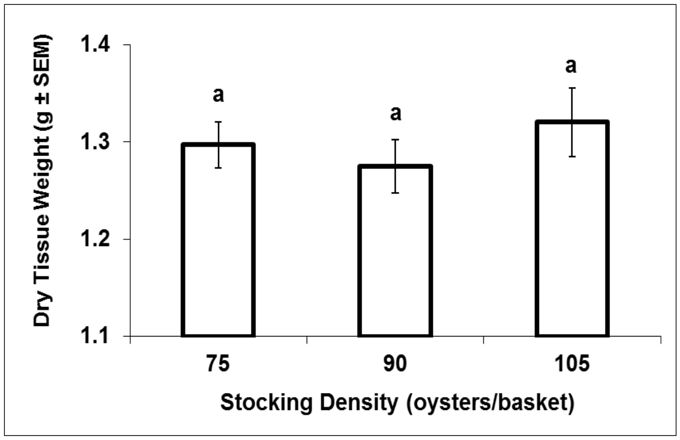

Fig. 2.23. Dry tissue weight (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. No statistically significant differences (ANOVA, p=0.494).

Stocking density had a significant effect on condition index of oysters (ANOVA, p=0.008) (Fig. 2.24). Oysters stocked at 75 per basket had a significantly higher condition index than those stocked at 105 per basket (Tukey HSD, p=0.008). However, neither treatment differed significantly from those stocked at 90 per basket (Tukey HSD, p≥0.052). The oysters stocked at 75 per basket were very close to significantly different from the 90 per basket (Tukey HSD, p=0.052), while oysters stocked at 90 per basket did not differ significantly with respect to condition index from those stocked at 105 per basket (Tukey HSD, p=0.727).

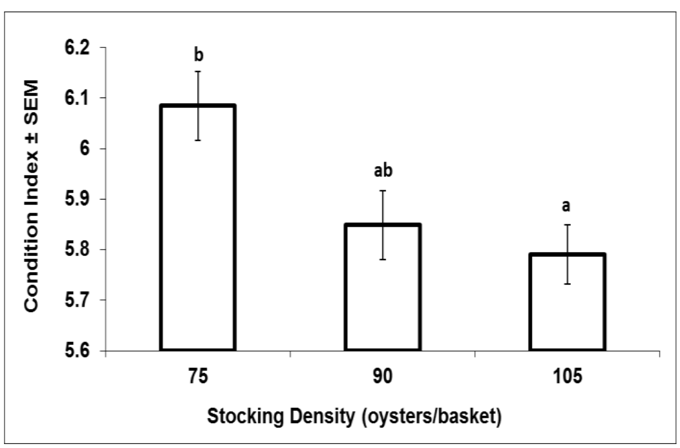

Fig. 2.24. Significant effect of stocking density on condition index (ANOVA, p=0.008) (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference (p<0.05).

Stocking density had no significant effect on the number of *P. marinus* cells present in oysters grown at either 75 or 105 per basket (ANOVA, p=0.177) (Fig. 2.25).

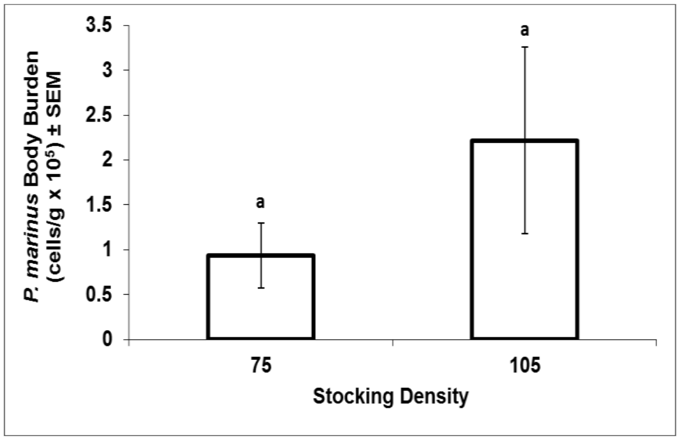


Fig. 2.25. Intensity of *P. marinus* infection in oysters grown in BST brand ALS baskets at either 75 or 105 oysters/basket. No statistically significant differences (ANOVA, p=0.177).

Stocking density did not have a significant effect on the fouling index, described above (ANOVA, p=0.276) (Fig. 2.26). In absolute values, oysters stocked at 105 per basket accumulated, on average, 6.3±1.55 g of bio-fouling when measured as wet weight versus only 4.9±1.02 g and 4.8±1.15 g accumulated on oysters stocked at 75 and 90 per basket, respectively. This difference was not visibly noticeable.

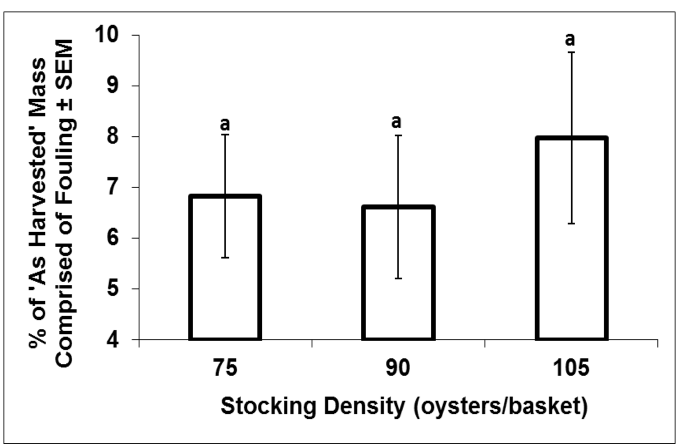


Fig. 2.26. Fouling index (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. No statistically significant differences (ANOVA, p=0.276).

Stocking density had no effect on survival (ANOVA, p=0.051) (Fig. 2.27). Survival exceeded 96.5% for all treatments. Evidence in support of this null hypothesis is weak, though, with a trend for decreased survival in the bays stocked at 105 oysters per basket.

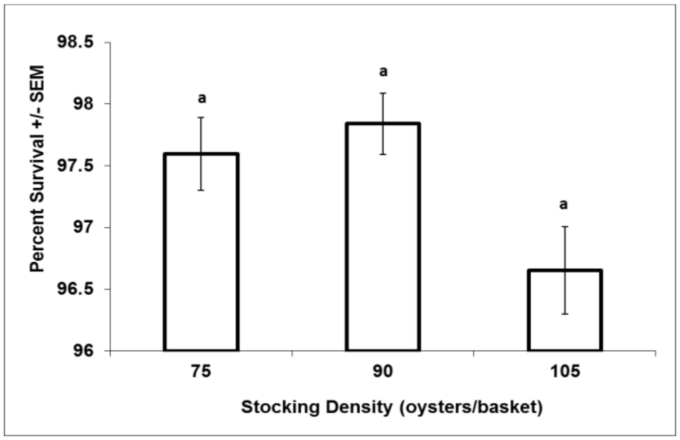


Fig. 2.27. Percent survival (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. No statistically significant differences (ANOVA, p=0.051).

All oysters, regardless of stocking density, grew at the same average rate during each growth interval from March to harvest (ANOVA, p≥0.145) (Fig. 2.28). January to harvest oysters stocked at 105 per basket, however, grew at a significantly faster rate, averaging 0.13 mm/day, than oysters stocked at either 75 or 90 oysters per basket (Tukey HSD, p≤0.044) (Fig. 2.29).

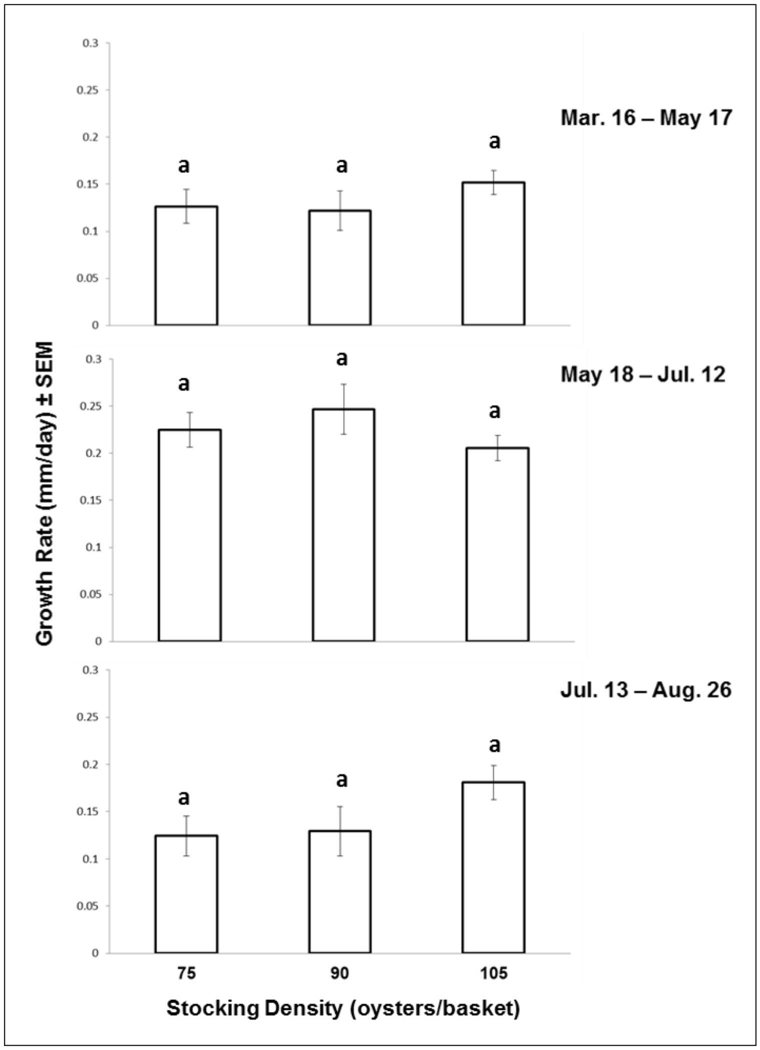


Fig. 2.28. Growth rate (mean ± SEM, n ≥ 3) at bi-monthly intervals of oysters grown at various densities in BST brand ALS baskets. No statistically significant differences (ANOVA, p≥0.145).

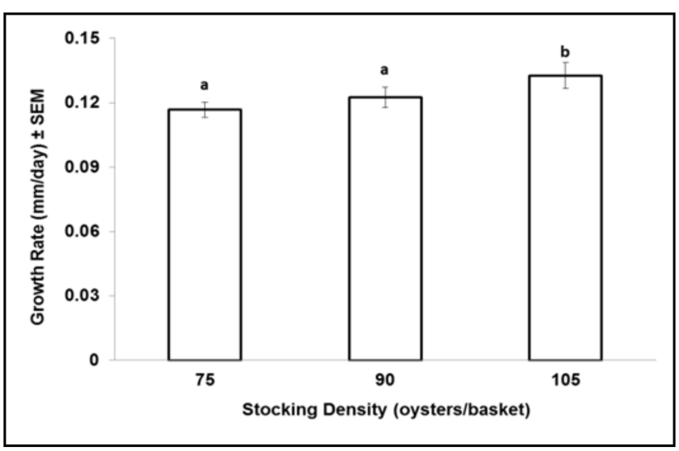


Fig. 2.29. Significant effect of stocking density on overall growth rate (ANOVA, p≤0.001) (mean ± SEM, n ≥ 3) of oysters grown at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference (p<0.05).

*Interaction of basket arrangement and stocking density during January-March growth interval*

There was a significant interaction between basket arrangement and stocking density affecting the growth rate of oysters from January to March 2012 (ANOVA, p<0.001). From January to March, oysters grown in baskets arranged in-line, six baskets per bay, and stocked at 105 per basket (6IL 105) grew faster than oysters grown, at any density, in baskets arranged cross-line with nine baskets per bay (9CL) (Tukey HSD, p=0.037, 0.001, and 0.002 for treatments 9CL 75, 9CL 90, and 9CL 105; respectively). Similarly, the 6IL 105 oysters also grew faster than oysters grown in baskets arranged cross-line, six baskets per bay, and stocked at either 75 or 105 per basket (Tukey HSD, p=0.006 and 0.028, respectively) (Fig. 2.30). Growth in all other treatments (6CL 90 and the other two 6IL treatments) did not significantly differ from 6IL 105 (Tukey HSD, p≥0.089). Negative 'average growth' in several treatments is indicative of growth being outpaced by loss of shell to handling and movement.

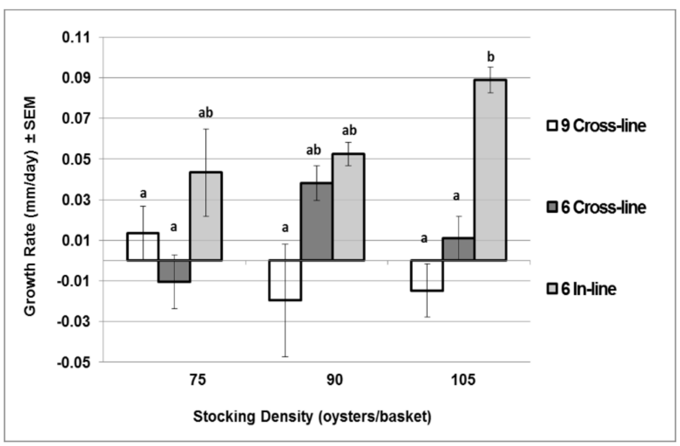


Fig. 2.30. Significant effect of stocking density on growth rate from January to March (ANOVA, p≤0.001) (mean ± SEM, n ≥ 3) of oysters grown at various densities and in baskets arranged cross-line and in-line. Different letters indicate a statistically significant difference (p<0.05).

## Discussion

*Basket Arrangement*

Oysters grown in baskets arranged in an in-line configuration grew larger than oysters grown in baskets arranged in a cross-line configuration; however, they accumulated much more fouling than oysters grown in baskets arranged cross-line. Though fouling was not associated with any declines in yield (survival, growth), fouling would be expected to increase handling time/costs at harvest and/or affect consumers' perceptions of product quality (discussed below). Qualitatively, baskets used in all treatments appeared to accumulate an equal minimal amount of fouling, suggesting that the air drying routine was sufficient to control fouling on the culture gear itself.

One hypothesis to explain the differences in fouling between the in-line and cross-line configurations is the difference in the movement of water in and around treatment bays. Bays in which baskets were arranged in-line had an empty space between the lines (Fig. 2.2), whereas baskets arranged cross-line spanned this space. This empty space may have allowed increased water movement and/or turbulence along the length of the baskets, thereby increasing the chance of encounter for competent larvae looking for suitable settlement substrate (Bushek, 1988).

Another hypothesis to explain the differences in fouling is the distance from shore: solely as a result of random assignment of treatments to bays in two runs, seven out of 11 in-line treatment replicates were assigned to bays in Run A. Run A was located 4.7 m further offshore of a *Juncus roemerianus* and *Spartina alterniflora* marsh than Run B. Bushek (1988), in a study of barnacle and oyster settlement in Texas, found that barnacle settlement, in a similar marsh environment, was greater in areas further from shore, with barnacle settlement increasing rapidly in a horizontal space of only five meters. Conversely, oyster settlement was greater near shore and showed the same pattern of a rapid decrease within a short horizontal distance (Bushek, 1988).

A third hypothesis to explain the differences in degree of fouling between the two basket configurations is the depth at which the baskets were suspended. Marine invertebrate larvae settle in response to a variety of cues, including light, temperature, salinity and chemicals (Ritchie and Menzel, 1969; Crisp, 1967; Turner et al., 1994). *Crassostrea virginica* larvae are negatively phototaxic (Ritchie and Menzel, 1969), meaning they move away from light to settle. Although the line was placed in the same riser clip for all treatments, due to placement of the clip attaching the basket to the line, in-line baskets rode 20 cm lower in the water column than cross-line baskets. This may have led to oysters in in-line baskets being chosen as a preferential settlement substrate for *C. virginica*, and perhaps other invertebrate larvae. If this experiment were to be repeated, the riser clips should be positioned on the posts such that the bays containing in-line treatments could have the line placed at a level that would let the in-line basket ride at the same height in the water column as the cross-line baskets.

Excess accumulation of fouling impacts a farmer's profit by requiring additional cleaning time at harvest to prepare oysters for market (Young-Lai and Aiken, 1986; Gribben et al., 2006) and may impact the longevity of the culture gear, due to the increased weight of the baskets. Minimizing accumulation of fouling organisms also decreases potential environmental impacts due to biodeposition (Stenton and Dozey, 2001). Additionally, Taylor et al. (1997) determined a greater number of oysters would have deformed shells when heavily fouled, potentially affecting consumers' perceptions of the quality of the oysters.

Accumulation of fouling organisms on oysters can create competition for space and/or food within the basket that, in turn, affects the growth of the cultured oysters. Lesser et al. (1992) found that commercial yield of mussels could be significantly impacted by growth and competition for food by fouling communities. Food availability is not believed to be a limiting factor at the Portersville Bay Farm (Davis, unpublished data). The waters are very food rich and there were no other oysters present with ± 200 m of the site. Dayton (1971) and Harger (1972) suggest that space is the major limiting resource for sessile suspension-feeding invertebrates. Dubois et al. (2007) showed through use of isotopic signatures that co-occurring suspension feeders are not necessarily competing for food because they consume the food items in different proportions. Competition for food and space are linked, and often difficult to describe separately for suspension feeders, because when space is limiting procurement of food becomes difficult (Sebens, 1986; Frechette and LaFaivre, 1990). Competition for space between oysters and fouling organisms in the basket may have caused oysters to grow larger in height in order to procure food. This is similar to hypotheses that "long and skinny" oysters are the result of competition for food and space on natural dense oyster reefs (Galtsoff, 1964; Manley, 2009).

In addition to differences in fouling among basket arrangements, there were differences in the shape of the oyster, and increased growth in height came at a cost to product quality with respect to shell shape (Fig. 2.9 and 2.20). A well cupped oyster is a desirable attribute of oysters destined for the half-shell market (Brake et al., 2003). An oyster that grows rapidly in shell height often does so at a cost to shell width, which in turn results in a shallow cup (Carriker, 1996; Brake et al., 2003). This was the case with oysters grown in baskets arranged in-line when compared to those grown in baskets arranged cross-line.

In this study, basket arrangement did not affect condition index, *P. marinus* infection, or survival. Additionally, there were only minor differences, none statistically significant, observed between the two cross-line treatments. This suggests that there was no significant detriment of placing nine baskets cross-line per bay (up to 945 oysters/bay) relative to six baskets cross-line per bay (up to 630 oysters/bay). This represents a potential 33% increase in production within the same space with no significant decline in product quality.

*Stocking Density*

Growth is density dependent in many populations of benthic suspension feeders, including when these animals are transitioned to the culture environment (Frechette and LeFaivre, 1990; Heral, 1993). In this study, oysters stocked at 105 per basket grew larger in shell height that the two lower stocking densities; however, oysters stocked at 75 per basket had more desirable product attributes than those stocked at higher densities. As with basket arrangement, additional growth in shell height was associated with a cost to cup depth and fan breadth.

Stocking oysters at a lower density resulted in better condition index, improved shell shape, and oysters with a shell weight similar to oysters stocked at 105 per basket. These are all attributes that are expected to fetch higher prices on the half shell market (Brake et al., 2003). Oysters stocked at 75 per basket had more room in the basket to be physically moved around by wave action or when disturbed during air drying. Frequent physical movement ('rumbling' or 'tumbling') of oysters chips off the thin growing edge at the bill and encourages deepening of the cup; this is referred to as 'pruning' (Brake et al., 2003). Oysters stocked at 75 per basket were similar in shell weight to oysters stocked at 105 per basket, demonstrating that even though the oysters were growing at different rates with regard to shell height they were accumulating the same amount of shell resulting in a smaller but more thickly shelled oyster.

These contrasting differences among stocking densities (increased shell height at higher densities and improved product attributes at lower densities) can be explained by two potentially interacting hypotheses. First, the increase in shell height may be attributed to crowding and resulting intraspecific competition for space within the basket, where more crowded oysters put relatively more energy into shell height. Second, the oysters stocked at lower densities may have been ‘tumbled’ more due to the relatively open space. There were no drastic differences in the amount of fouling accumulated on oysters among density treatments, suggesting that this was not an important factor. Regardless of the cause, in this study, a lower stocking density led to oysters with a more desirable shell shape.

Condition index is a valuable tool for comparing oysters of a similar size, however, it can vary depending on a number of factors, such as season, reproductive output, and physiological state (Rheault and Rice, 1996). We found condition index decreased with increasing stocking density. Rheault and Rice (1996) reported a similar result for *C. virginica* in Rhode Island. Oysters stocked at 105 per basket were growing faster and potentially subject to more competition; this may have affected their physiological state and, in turn, their condition index.

Importantly, stocking density did not affect a number of response variables. We did not observe any effect of density on survival or *P. marinus* infection. We were seeking to fine tune stocking density (density treatments differed by only 15 oysters) so the baskets were not filled to a point where flow would be severely restricted, resulting in mortality, hence our low mortality rates (≤ 3%).

*January to March Growth Rate*

The interaction of basket arrangement and stocking density affected the shell height growth rate of oysters only from January to March, presumably because this was a relatively poor growing period. During this period, many treatments experienced very little or no growth, while the six in-line arrangement and 105 oysters per basket stocking density treatments combined to produce the highest growth (although this was still relatively slow growth compared to later periods). Due to the relatively small differences, in the future, individual oysters should be tagged and tracked for determination of individual growth rates, especially for periods where very little growth is anticipated.

*Conclusions*

In conclusion, oysters grown in baskets arranged in-line were larger than those grown in baskets arranged cross-line, but their shells were not as deeply cupped. In addition, in-line oysters accumulated much more fouling than cross-line oysters. This difference would cause a grower to incur additional costs for cleaning oysters prior to sending them to market. The increase in fouling observed on in-line oysters may be a result of them being positioned lower in the water column. Before eliminating an in-line arrangement as a production option, it would be worth raising the baskets to the same growing position as the cross-line baskets and quantifying fouling.

There were very little, and no significant, differences between placing nine or six baskets cross-line in a bay. This is encouraging because it allows for a 33% increase in production, with no significant decline in product quality.

Oysters stocked at 75 per basket were smaller (in whole wet weight and shell height) than oysters stocked at 90 and/or 105 per basket. They were more cupped and had a higher condition index, however; both of which are important attributes of oysters destined for the premium half shell market.

## Chapter 3. Effects of basket arrangement and stocking density in an adjustable long-line system on polydorid infestation and shell strength

### Introduction

Along the northern Gulf of Mexico (GOM) coast, mud worms, mainly *Polydora websteri*, are present year-round and can be found in oysters cultured on-bottom (Loosanoff and Engle, 1943; Hopkins 1958) as well as off-bottom. Mud worms gain access to oysters as larvae when they settle on the growing margin of the bill (Haigler, 1969; Zottoli and Carriker, 1974). Once settled they construct a U-shaped burrow in the oyster shell that has two openings to the outside. These mud-lined burrows, often referred to as mud blisters, can be deep and may have a large terminal chamber. Worms use the oyster for protection and do not to have any direct impact on the tissue of the animal (Mohammed and Murad, 1972; Ghode and Kripa, 2001). The presence of a mud worm blister in an oyster, however, can reduce the internal cavity volume, disrupt feeding currents, be physically irritating, and can divert energy used for such things as somatic growth towards shell repair (O'Sullivan, 1996; Dunphy et al., 2005). An oyster is capable of confining the worm and repairing the blister by secreting a new layer of shell (Lunz, 1941).

Mud worms are a cosmetic nuisance known to infest many bivalve shellfish species (Haigler, 1969; Wargo and Ford, 1993; Lafferty and Kuris, 1996; Read, 2010) and they directly impact the marketability of oysters destined for the premium half-shell market (Littlewood et al., 1992; O'Sullivan, 1996; Handley and Bergquist, 1997). In addition to being unsightly, mud blisters caused by *P. websteri,* can be produce a foul odor if burst and the oyster's shell may become weak where burrows are located, causing the shell to break during packing, transportation (Korringa, 1951; O'Sullivan, 1996) or shucking. Some studies suggest, when environmental conditions are favorable, infestation with *P. websteri* does not impact growth and meat quality

(Loosanoff and Engle, 1943; Medcof, 1946; Mohammed and Murad, 1972; Bishop and Hooper, 2005). Others found that oysters infested with *P. websteri* had abnormal condition indices, were smaller, and that mortality increased with mud worm abundance (Owen, 1957; Wargo and Ford, 1993; Rheault and Rice, 1996).

To maximize profit, a proactive approach to preventing mud worms is preferable to a reactive approach that involves use of labor intensive eradication methods. Littlewood et al. (1992) suggested that in rack and bag culture infestation of *C. virginica* with *P. websteri* can be avoided by ensuring the oysters receive more than 40% aerial exposure. Ghode and Kripa (2001) suggested harvesting oysters as early as possible, preferably within the first year, to avoid a high degree of infestation. Use of the adjustable long-line system in the northern Gulf of Mexico can make use of both of these suggestions. The ALS design allows for easy aerial exposure of the baskets and oysters. Warm waters in the GOM allow oysters to grow quickly, potentially allowing farmers to harvest within one year of planting.

Shell strength of bivalve shellfish has been investigated in an ecological context (Kent, 1981; Bergman et al., 1982); however, shell strength is also important to the marketability of bivalve shellfish as it relates to breakage during transport and shucking. Bergman et al. (1982) found that *P. websteri* significantly weakened the upper valve of scallop, *Placopecten magellanicus*, shells. Due to the wide variety of factors contributing to the structure of bivalve shells, measurements of shell strength tend to be highly variable (Boulding and LaBarbera, 1986; Beadman et al., 2003; Grefsrud and Strand, 2006). Morphological aspects of the shell, which can be modified by culture techniques, can be important determinants of shell strength (Boulding, 1984; Lowell, 1986; Beadman et al., 2003; Grefsrud and Strand, 2006).

Mud Worm Eradication Using a Freshwater Soak

Several techniques to eradicate mud worms have been tested (Ghode and Kripa, 2001; Dunphy et al., 2005; Brown, 2012). Typically these treatments involve immersing oysters in a bath containing a liquid that will irritate the worms but, without prolonged exposure, not cause harm to the oysters. Solutions tested have included: hypersaline, hyposaline, hot water (70oC), formalin, and chlorine (MacKenzie and Shearer, 1959; Nel et al., 1996; Ghode and Kripa, 2001; Dunphy et al., 2005). A period of air exposure following the dip may be more effective at eradicating mud worms (Bishop and Hooper, 2005; Brown, 2012). Based on availability and cost-effectiveness, we chose a freshwater (FW) soak to encourage mud worms to evacuate their burrows.

The goal of this study was to determine how, when using an adjustable long-line system, different culture methods such as stocking density and basket arrangement impact the amount of *P. websteri* infestation observed in oysters, *Crassostrea virginica*. We quantified mud worm infestation by recording the number of active burrows, percentage of the interior surface of shell infested, and by counting the number of worms that evacuated each oyster during a three hour FW soak. These response variables were chosen because directly affect the marketability of premium Gulf oysters. We also sought to quantify relationships between mud worm infestation and shell strength as well as shell shape and shell strength.

## Materials and methods

### *Experimental Design*

This experiment was conducted in conjunction with a study on the effects of stocking density and basket arrangement on oyster production and product quality when using an adjustable long-line system for oyster grow-out (see Chapter 2). The space between riser posts in an adjustable long-line system was referred to as a ‘bay’. The experiment was replicated on the level of bay with 3 or 4 replicates of each of nine treatments (Table 3.1). Each treatment was randomly assigned to one of 34 bays in two runs of ALS gear at the Auburn University Oyster Research and Demonstration Farm in Portersville Bay, Alabama.

Table 3.1.
Experimental design to test for effects of basket arrangement and stocking density when using an adjustable long-line system for oyster grow-out.

| **Basket arrangement** | **Number of baskets/bay** | **Basket stocking density (oysters/basket)** | **Number of replicates** |
|---|---|---|---|
| Cross-line | 9 | 105 | 4 |
| Cross-line | 9 | 90 | 4 |
| Cross-line | 9 | 75 | 4 |
| Cross-line | 6 | 105 | 4 |
| Cross-line | 6 | 90 | 4 |
| Cross-line | 6 | 75 | 3 |
| In-line | 6 | 105 | 4 |
| In-line | 6 | 90 | 3 |
| In-line | 6 | 75 | 4 |

On September 10, 2012, three baskets were randomly selected from each of nine stocking density (3 levels) and basket arrangement (3 levels) treatments. With the exception of the 6 in-line basket arrangement stocked at 90 oysters per basket (6IL 90) treatment, each basket sampled was selected from a different bay (n=3). In the 6IL 90 treatment, two baskets were selected from the same bay, representing n=2 for this treatment. We transported the baskets to the Auburn University Shellfish Laboratory, where baskets were emptied, live and dead oysters were counted and five live oysters from each basket were haphazardly selected for soaking in freshwater.

As oysters were selected from baskets they were placed in labeled 1-L plastic beakers. Each beaker was labeled according to the basket number from which the oyster came, and lettered A through E to differentiate between the five individuals from that basket. Oysters were exposed to air for approximately 3-4 hours prior to the FW soak, due to transport and basket processing time. Once all oysters had been collected in their respective beakers, the beaker was filled with FW from the Dauphin Island Water

Authority municipal water supply. Dunphy et al. (2005) determined that soaking Chilean oysters, *Tiostrea chilensis*, for 180 minutes in FW was as effective at eradicating mud worms, *Boccardia acus*, as a 300 minute soak. Based on this result, we soaked our oysters in FW for 180 ± 10 minutes. Beakers were kept in a shaded area for the duration of the soak. Water temperature ranged from 25.7°C at the beginning of the treatment to 24.4°C at the end.

After 180 minutes immersed in FW, each oyster was removed from its beaker, the FW was decanted, and all solids remaining in the bottom, including worms, were placed in labeled pre-weighed aluminum boats. Each oyster was then returned to its respective beaker and the beaker was placed in a 45 cm deep rectangular recovery tank filled with ambient one micron filtered aerated seawater (16.3 PSU) from the Gulf of Mexico. Oysters remained in the static seawater recovery tank for 24 hours to allow us to determine if the FW soak caused mortality of oysters. Following recovery, each oyster was examined to determine if it was dead or alive, labeled with a tag corresponding to its beaker tag, bagged, and placed in a Frigidaire® Gallery™ drop freezer (Frigidaire, Augusta, GA) at -16°C for at least one week.

Infestation by *P. websteri* was quantified by the number of individual worms that evacuated each oyster during the freshwater soak. Weigh boats, containing solids, including worms, decanted from each beaker, were refrigerated for 16-18 hours to allow solids to settle. Worms were then visually sorted and counted, by the naked eye, from the other contents of the boat.

*Evaluation of Oyster Condition*

On November 2, 2012, oysters were processed to determine condition index and shell metrics, and the interior of each valve was photographed to determine the extent of *P. websteri* damage. Fouling, shell metrics and condition index were determined according to the method described in Chapter 2.  After shells were air dried, each valve was photographed using an Olympus Stylus Tough 6020 (Olympus Imaging America Inc., Center Valley, PA) on a macro setting.  A standard field of view was maintained by positioning the camera 10 cm above the shell.  Each shell was then sealed in an air tight, labeled ZipLoc™ bag.

Each valve was then analyzed to determine the total area visibly impacted by *P. websteri* infestation using iSolution Lite image processing, measurement, and analysis software (IMT i-Solution Inc., Rochester, NY).  One valve at a time, images were loaded into the software and calibrated according to the shell measurements taken during shell metrics processing.  The perimeter of each valve was outlined by hand, yielding a measurement of the total area of the valve.  Each mud worm blister or burrow was then delineated by hand giving us the area of each visibly impacted area (Fig. 3.1).

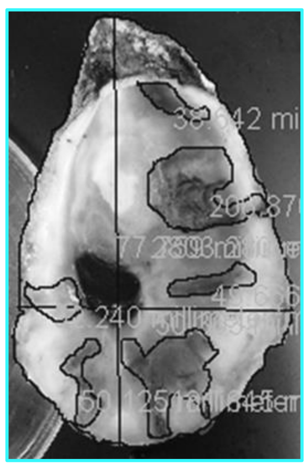

Fig. 3.1.  Cupped valve of oyster following iSolution Lite photo analysis.  Note that slightly discolored areas and chalky deposits were not considered as mud worm damage.

The total area visibly impacted by *P. websteri* was calculated by summing the area of each delineated polygon.  The percent area visibly impacted by *P. websteri* on each valve was calculated using the following formula:

% visibly impacted = (total area visibly impacted by mud worm / total valve area)*100

Areas of mild discoloration were not included in this analysis because it was unknown if this discoloration was caused by mud worm (Galtsoff, 1964). We delineated only those areas that were blisters, active burrows, or burrows in some stage of repair. Active burrows were defined as those that were continuous from the terminus of the burrow to the edge of the bill of the oyster and uniform in color (e.g. not in any stage of repair). The number of active burrows on each valve were counted to allow comparison with the number of worms evacuated during the FW soak.

*Shell Strength Testing*

Maximum load capacity of the cupped valve of each oyster was determined using a 50 kN capacity GeoComp LoadTrac II load frame (Geocomp Corporation, Acton, MA) equipped with a 2200 N (500 lb.) capacity load cell (Fig. 3.2). The cupped valve of the shell (the left valve) was placed, internal side down, on a fixed, smooth 10 cm diameter stainless steel platen. A 12 mm diameter stainless steel rod attached to the load cell was forced onto the highest point of the cupped valve at a strain-controlled rate of 2.5 mm/s until failure. Failure was deemed to have occurred when a peak load was reached and a subsequent dramatic decrease in load was observed. The maximum load was electronically displayed by the machine and subsequently recorded into a Microsoft Excel® spreadsheet.

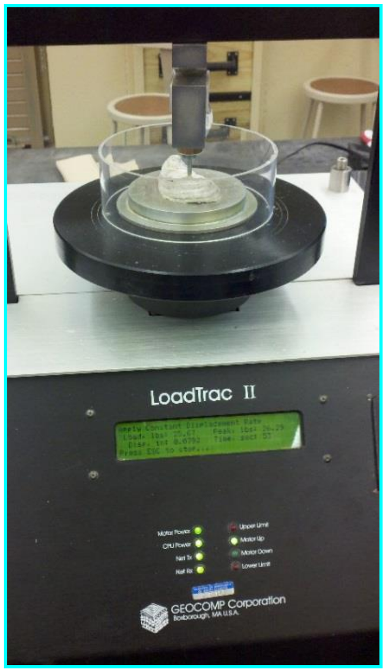


Fig. 3.2. Equipment configuration for testing shell strength.

*Data Analysis*

There were nine treatments in this two factor (3 x 3) study replicated on the level of bay with a total of 34 bays occupied. Data were analyzed by basket arrangement (n = 3, 2 df), stocking density (n = 3, 2 df), and any interaction between the two (n = 9, 4 df) for all of the response variables. Response variables included: percentage of each valve visibly impacted by *P. websteri*, number of active burrows in each valve, number of worms that evacuated the host during a FW soak, and maximum load capacity of each oyster's cupped valve. The following hypotheses, and their interactions, were tested for each response variable:

$H_o$: $\mu_{CL} = \mu_{IL}$; where CL = cross-line basket arrangement and IL = in-line basket arrangement

$H_a$: $\mu_{CL} \neq \mu_{IL}$

$H_o$: $\mu_{75} = \mu_{90} = \mu_{105}$; where 75, 90, and 105 refer to basket stocking densities

$H_a$: $\mu_{75} \neq \mu_{90} \neq \mu_{105}$

Systat® 12 software (Systat Software Inc., Chicago, IL) was used to analyze the data, using a two factor analysis of variance (ANOVA). Shapiro-Wilk test of normality and Levene's test for homogeneity of variance were used to verify the assumptions of normality and homogeneity of variance, respectively. Data were considered normally distributed and variances homogenous at $p>0.05$. No data transformations were necessary for any of the response variables to meet the ANOVA assumptions. Since no interactions were detected single factors were pooled across treatments (Appendix 1). For example, basket arrangement was pooled across densities and vice versa. All tests were performed with $\alpha=0.05$ and means were considered significantly different if $p<0.05$. Post-hoc pairwise comparison using Tukey's Honestly Significantly Difference Test ($p<0.05$) was used to further investigate differences identified by the ANOVA.

Linear regression was used to determine if there was a relationship between *P. websteri* infestation and the maximum load capacity, or shell strength, of the cupped (left) valve of the oyster. Three variables, each requiring transformation to meet assumptions of normality, were used to quantify *P. websteri* infestation: total number of worms evacuated from each oyster during the FW soak, number of active burrows in the cupped valve of each oyster, and percentage of each cupped valve visibly impacted by mud worms. Square root transformation was used on count data and an arc sin transformation was used on percentage data (Underwood, 1997). Maximum load capacity data were log transformed due to the high values and high variability measured during the experiment (Underwood, 1997). Following transformation, all variables met the assumption of normality ($p>0.05$) when using Kolmogorov-Smirnov Test. Each variable was plotted in relation to the dependent variable, shell strength, and we considered the independent variable to be a good predictor of shell strength at $p<0.05$.

We also used linear regression to determine relationships between shell metrics and shell strength. We sought to determine if shell height, shell length, shell width, cup ratio, fan ratio, and dry shell weight were good predictors of shell strength as measured by maximum load capacity on the cupped valve. Again, we used the Kolmogorov-Smirnov Test to ensure the assumption of normality was satisfied ($p>0.05$). Log transformed maximum load capacity data were plotted versus each shell descriptor and we considered the independent variable to be a significant predictor of shell strength at $p<0.05$. When significant relationships were detected, we constructed a correlation plot of the variables to determine the strength of the relationship, as indicated by the $r^2$ value.

## Results

In the analyses of the tests of basket arrangement, stocking density and their interaction, no significant interactions were observed. Each single factor (basket arrangement, stocking density), therefore, is discussed separately below.

### *Basket Arrangement*

Basket arrangement had no significant effect or on the total number of active burrows present on the interior shell surface of each oyster (ANOVA, p=0.055) nor on the number of worms that evacuated an oyster during the three hour FW soak (ANOVA, p=0.898) (Fig. 3.3).

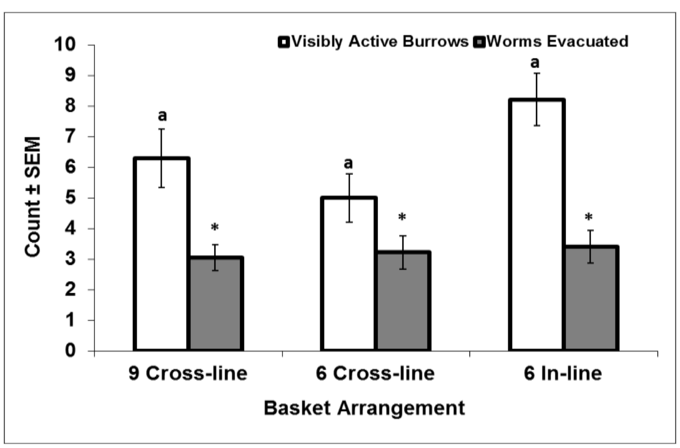


Fig. 3.3. Number of active burrows and evacuated worms (mean count ± SEM, n ≥ 2) recorded on oysters grown in baskets arranged in a cross-line or in-line configuration in an adjustable long-line system. No statistically significant differences were observed for either response variable.

Basket arrangement had no effect on the percentage of the interior of the cupped valve impacted by *P. websteri*. On average, 13.7±1.45% of the cupped valve's interior surface was visually impacted by mud worms. There was a significant effect of basket arrangement upon the visual impact of mud worms on the interior surface of the flat valve of oysters (ANOVA, p=0.002); oysters grown in baskets arranged in-line were more visually impacted by *P. websteri* than oysters grown in baskets arranged in either cross-line configuration (Tukey, p≤0.047) (Fig. 3.4). On average, 7.2±0.9%, 5.2±0.8%, and 10.3±0.9% of the flat valve of oysters grown in bays with nine baskets cross-line, six baskets cross-line, and six baskets in-line was visibly impacted by mud worms, respectively.

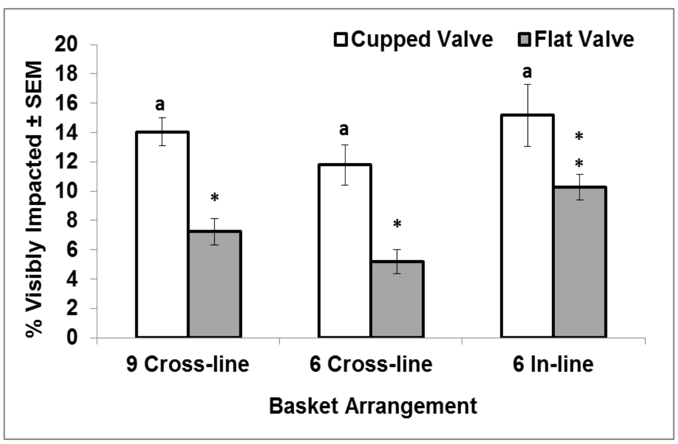


Fig. 3.4. Percentage of the interior of each valve (mean ± SEM, n ≥ 2) cosmetically damaged by *P. websteri* when grown in baskets arranged in-line and cross-line in an ALS. No statistically significant differences in the cupped valve. Stacked astericks (*) indicate signifcant differences in the flat valve series.

Basket arrangement did not significantly impact the maximum load capacity of the cupped valves of oysters (ANOVA, p=0.405). Despite lack of a statistically significant effect, possibly due to the high variance, there was a trend indicating that the shells of oysters grown in baskets arranged in-line were stronger than those grown in baskets arranged cross-line (Fig. 3.5). On average, fracture occurred when 260.4±12.7

lbs. of load was applied. Two cupped valves exceeded the 500 lb. capacity of the load cell. These values were treated as 500 lbs. in analysis of the data.

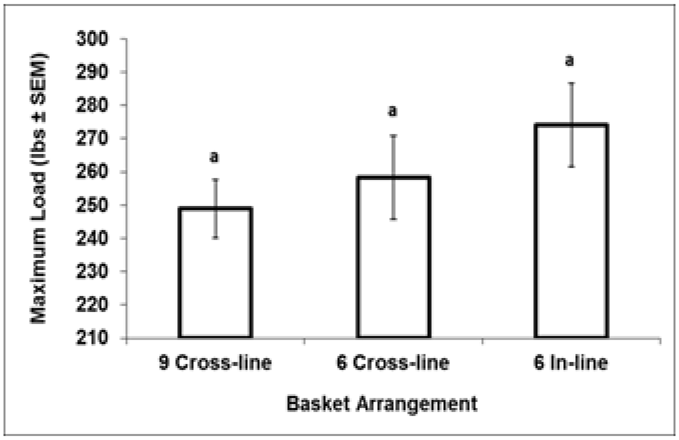


Fig. 3.5. Shell strength of the cupped valve (mean ± SEM, n ≥ 2) of oysters grown in baskets arranged in-line and cross-line in an adjustable long-line system.

*Stocking Density*

Stocking density did not have a significant effect on the number of burrows present on the interior surface of the oyster (ANOVA, p=0.102) or on the number of oysters evacuated during a three hour FW soak (ANOVA, p=0.292) (Fig. 3.6).

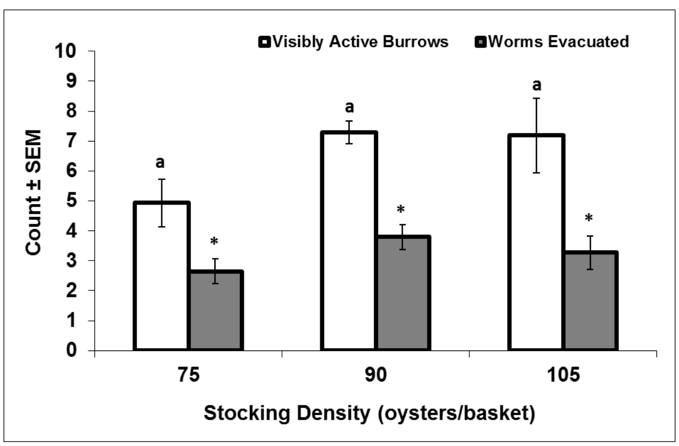


Fig. 3.6. Number of active burrows and worms (mean count ± SEM, n ≥ 2) recorded on oysters grown at various densities in BST-brand ALS baskets. No statistically significant differences.

Stocking density had no effect on the proportion of the interior of the cupped or flat valves damaged by *P. websteri* (ANOVA, p≥0.102) (Fig. 3.7). On average, 13.6±1.44% of the interior surface of the cupped valve was marred by mud worm damage. Only 7.5±2.55% of the interior surface of the flat valve had visible damage. As expected, infestation of the cupped valve, as measured by percent of the interior surface of the valve with visible mud worm damage, was positively correlated with infestation of the corresponding flat valve ($p \leq 0.001$, $r^2 = 0.1021$).

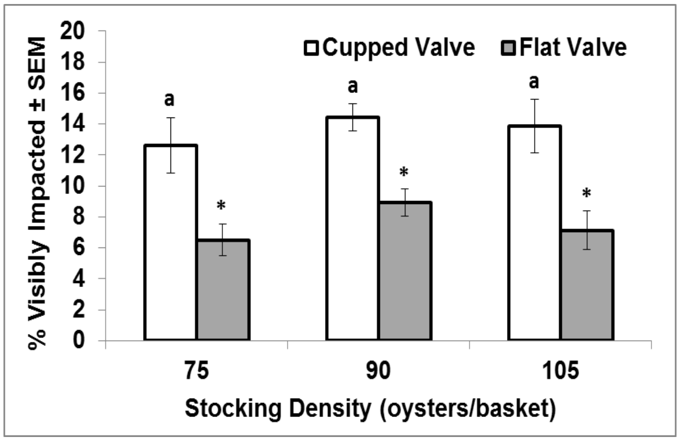


Fig. 3.7. Percentage of the interior of each valve (mean ± SEM, n ≥ 2) cosmetically damaged by *P. websteri* when oysters are grown at various densities in BST-brand ALS baskets. No statistically significant differences were observed.

Stocking density had no effect on the maximum load capacity of the cupped valve of oysters (ANOVA, p=0.838) (Fig. 3.8). Across all densities, oysters held on average 260.1±4.85 lbs. prior to fracture.

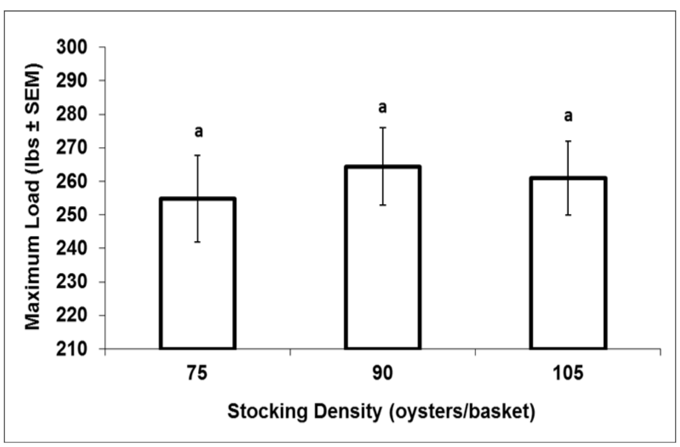


Fig. 3.8. Shell strength of the cupped valve (mean ± SEM, n ≥ 2) of oysters grown at various densities in BST-brand ALS baskets. No statistically significant differences.

*Predictors of Mud Worm Infestation*

Interestingly, there was no relationship between the number of worms evacuated during the FW soak and the number of burrows visible on the inside of the oyster ($p=0.762$, $r^2 < 0.001$). For every worm evacuated following a FW soak, there were, on average, 2.93±3.08 active burrows inside the oyster. During the FW soak, 9% of the oysters had no worms evacuate, but these same oysters had, on average, 5.67±5.84 active burrows on the interior surface of the shell.

The amount of bio-fouling accumulated on the exterior of the oyster was a poor indicator of both mud worm activity and mud worm damage. There was no significant relationship between the amount of bio-fouling accumulated on the oyster and number of active burrows present on the shell's interior surface ($p=0.505$, $r^2 = 0.0097$). Similarly, the number of worms evacuated during the FW soak was not correlated with the amount of bio-fouling accumulated on the oyster ($p=0.251$, $r^2 = 0.0079$).

*Predictors of Shell Strength*

Due to the lack of a relationship between shell strength and stocking density or basket arrangement, we examined the possibility that *P. websteri* infestation was dictating the load capacity of the oysters. We found no evidence to suggest that mud worm infestation was the main variable dictating the maximum load capacity of the cupped valves of these oysters (Figs. 3.9-3.11). Neither the percentage of the cupped valve marred by mud worm damage (Fig. 3.9) nor the number of active burrows present on the cupped valve (Fig. 3.10) were able to predict the load capacity of the shell ($p = 0.288$ and 0.519, respectively). Similarly (Fig. 3.11), shell strength could not be predicted by the number of worms evacuated during the FW soak ($p = 0.850$).

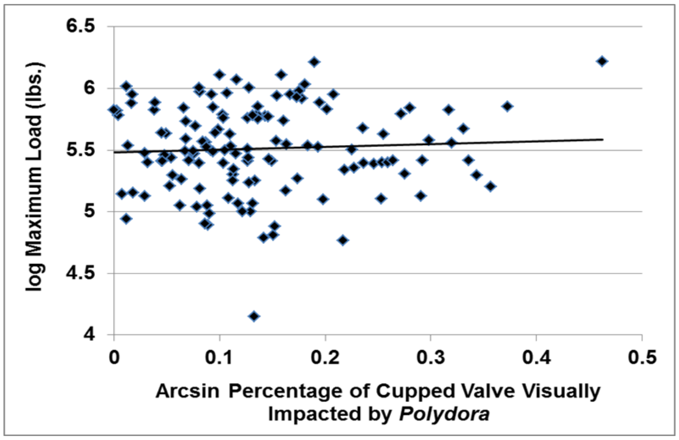


Fig. 3.9. Simple linear regression of the relationship between shell strength and the percentage interior surface of the cupped valve visibly damaged by *P. websteri* (n = 133).

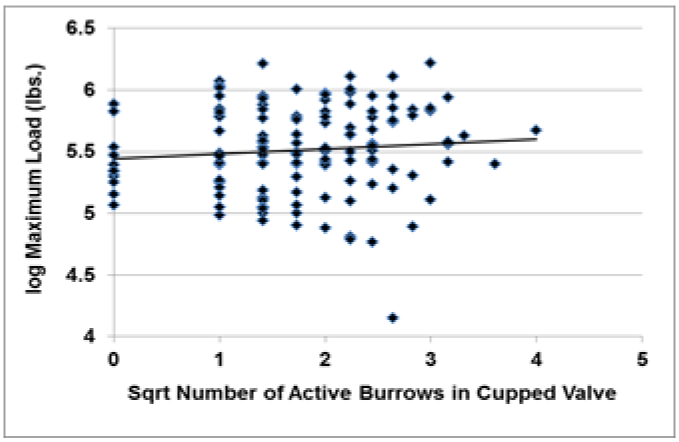


Fig. 3.10. Simple linear regression of the relationship between shell strength and the number of active *P. websteri* burrows present on the interior surface of the cupped valve (n = 133).

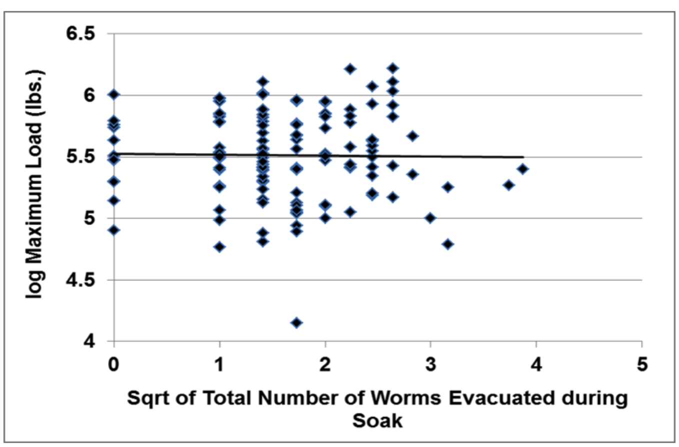


Fig. 3.11. Simple linear regression of the relationship between shell strength and the number of *P. websteri* individuals evacuated from the whole oyster during a three hour freshwater soak (n = 133).

We found shell morphology to be a better predictor of breaking strength than any of the mud worm response variables.  Shell height, shell width and dry shell weight were all significantly related to the maximum load capacity of the cupped valve of the oysters (p = 0.024, 0.000, and 0.000; respectively) (Figs. 3.12-3.14).  Shell strength was positively correlated with each of these variables, but most strongly with dry shell weight ($r^2 = 0.1576$).  Shell length, cup ratio, and fan ratio were not significantly related to the maximum load capacity of the cupped valve (p=0.059, 0.566, and 0.141; respectively).

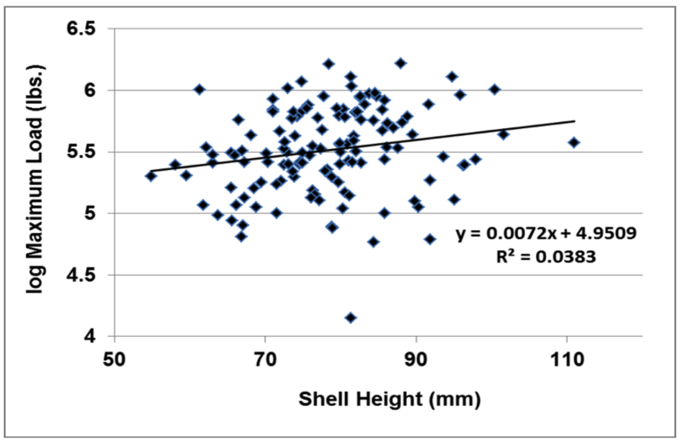


Fig. 3.12.  Simple linear regression of the relationship between shell strength and shell height (n = 133).

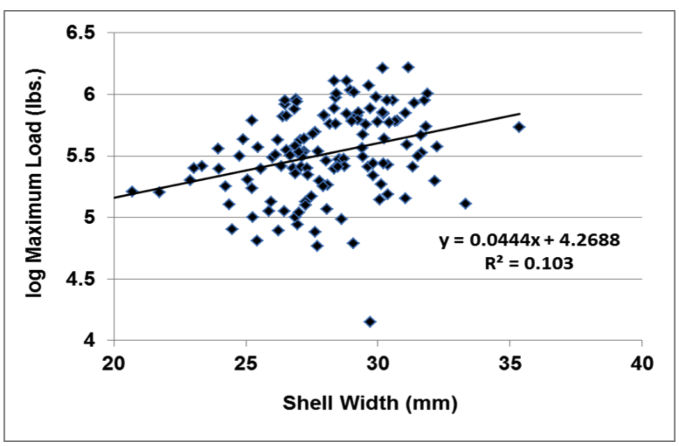


Fig. 3.13.  Simple linear regression of the relationship between shell strength and shell width (n = 133).

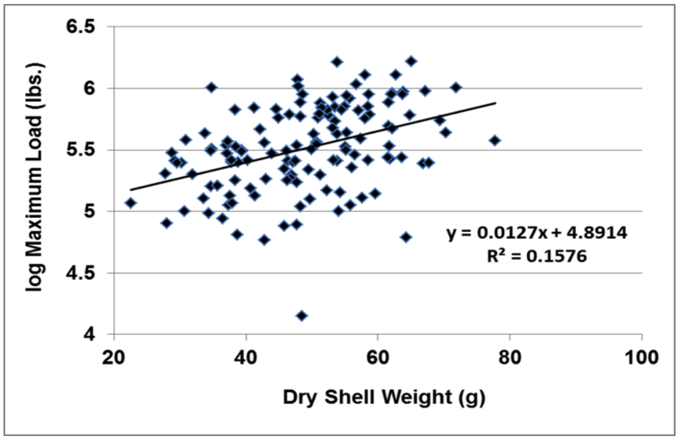

Fig. 3.14. Simple linear regression of the relationship between shell strength and dry shell weight (n = 133).

**Discussion**

When using an adjustable long-line system for grow out of oysters, *C. virginica*, the culture methods of basket arrangement and stocking density at the levels tested, with one minor exception, had no effect on the degree of *P. websteri* infestation. Previous studies indicated that oysters with more aerial exposure or grown higher off the substrate had a lower degree of mud worm infestation (Loosanoff and Engle, 1943; Littlewood et al., 1992). Although our oysters grown in baskets arranged in-line hung 20 cm lower in the water column than those grown in baskets arranged cross-line, we did not observe any difference in the number of worms on the oyster, the number of active burrows, or the amount of damage in the cupped valve of the oyster. The percentage of the flat valve of oysters grown in-line were more damaged by mud worms than the flat valve of oysters grown cross-line (Fig. 3.4). This may be because oysters grown in baskets arranged in-line grew more rapidly, and, therefore, may have had a thinner growing edge, than oysters grown in baskets arranged cross-line (see Chapter 2). The thin growing margin of the oyster is where mud worm larvae gain access to oysters (Haigler, 1969; Zottoli and Carriker, 1974; Bishop and Hooper, 2005).

Although stocking density had no effect on mud worm infestation or damage, we observed that, as with basket arrangement, the cupped valve was more damaged than the flat valve and that there were more active burrows in the oysters than there were number of mud worms that evacuated during the freshwater soak. A mud worm constructs a U-shaped burrow that has two openings to the outside (Haigler, 1969; Zottoli and Carriker, 1974). We counted individual burrows and did not observe any burrows that were U-shaped at their terminal end. If every individual burrow we counted was treated as one half of a U-shaped burrow, we would expect two active burrows for every worm evacuated during the treatment, assuming all worms present on the oyster evacuated during the treatment. Our data suggest that this may not be a fair assumption, because we observed, on average, 2.93±3.08 burrows for every worm evacuated. In addition, some oysters had no worms evacuate during the treatment, but had many active burrows inside the oyster. This suggests either: 1) some worms had evacuated their burrows prior to the FW soak; 2) all worms did not evacuate during the treatment and/or, 3) one worm can create more than two burrows, or more than one U-shaped burrow.

We found a greater proportion of the cupped valve was impacted by *P. websteri* than the flat valve. Using a similar image analysis method as we used but only examining the flat valve, Wargo and Ford (1993) found that one and two year old oysters had, respectively, 4 and 15% of the valve infested with *P. websteri*. Our oysters were 1.5 years old at harvest and had, on average, 7.6% of the flat valve visually impacted by *P. websteri*, suggesting similar levels of infestation. In addition, we hypothesize that the cupped valve may provide more protection for the mud worm or provide an advantage in relation to feeding currents. In addition, the cupped exterior of the cupped valve is rougher than the flat valve, which may make it easier for mud worms to gain access to the shell.

Basket arrangement, stocking density, and mud worm infestation appear not to be as important as shell morphology in predicting shell strength. Basket arrangement and stocking density had no significant effect on shell strength; however, there was a trend with respect to basket arrangement as might be expected given the effect basket arrangement can have upon shell morphology (Chapter 2). Oysters grown in baskets arranged in-line were able to withstand a higher load than oysters grown in baskets hung cross-line in either configuration (Fig. 3.5). As in previous studies, maximum load readings were highly variable, possibly due to the many factors that can affect shell strength (Boulding, 1984; Beadman et al., 2003; Grefsrud and Strand, 2006). Shell morphology, such as shell width and shell weight, appears to be the best predictor of shell strength when a load is applied to the apex of the cupped valve. Bergman et al. (2003) tested the effects of *P. websteri* on the strength of scallop shells by applying the load to the apex of the cupped valve when the valve was placed interior surface down. This method may be useful in ecological applications, because this is the surface where a predator would gain access to the animal's tissue. We are concerned, however, more with how shell strength affects the marketability of the oyster. In other words, does it break when transported or shucked? Applying the load at the apex of the cup may be applicable to strength during transportation, but in the future, we should consider an apparatus that will allow us to apply load in a way that mimics stress applied when shucking. This breakage likely depends on the method of shucking used, but is frequently observed across the flat valve and/or at the hinge.

*Conclusions*

Basket arrangement and stocking density when using the adjustable long-line system for grow-out of oysters have very little to no effect on mud worm, *P. websteri*, infestation. Basket arrangement had no effect on the percentage of the cupped valve visually impacted by mud worms or on the number of worms evacuated during a three hour FW soak. Basket arrangement did have an effect on the percentage of the flat valve visually impacted by mud worm, with the flat valve of in-line oysters being more infested than the flat valve of either cross-line treatment. Stocking density had no effect on the percentage of either the cupped or flat valve visually impacted by *P. websteri* or the number of worms evacuated during the FW soak.

Basket arrangement and stocking density had no significant effect on the maximum load capacity of the cupped valve of oysters. Shell characteristics, such as dry shell weight, shell height and width, which can be manipulated with culture techniques, were good predictors of shell strength. Although there were significant effects of basket arrangement and stocking density on shell morphology, the differences were not large enough to be reflected in shell strength testing. To be applicable to marketability or ‘shuck-ability’ of premium half shell oysters, future tests of shell strength may require an apparatus that better mimics stress applied during shucking.

**Chapter 4. Optimizing Production of Premium Half Shell Oysters When Using the Adjustable Long-line System in Alabama**

The purpose of this chapter is to present the results of our basket arrangement and stocking density experiments in a way that can be applied by oyster farmers and to make recommendations on how the results could be put to use on their farm in order to maximize product quality and, in turn, profit.

An off-bottom oyster farmer using the adjustable long-line system has two primary basket arrangement options; cross-line or in-line (Fig. 4.1). The cross-line arrangement will allow nine baskets in a bay, whereas the in-line arrangement allows only six baskets in a bay. Being able to put nine baskets cross-line could increase production 33% over an in-line arrangement in the same area and using the same infrastructure, but what effect does this have on product quality?

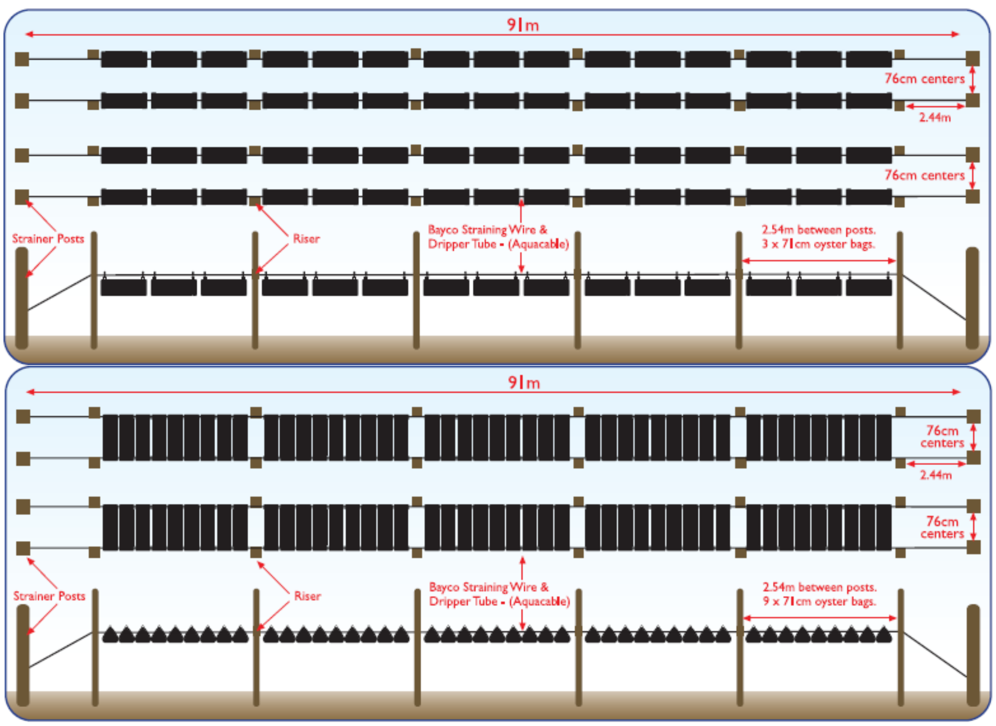


Fig. 4.1. Schematic of in-line (top) and cross-line (bottom) basket arrangements as they would be deployed in a commercial production setting. Note that cross-line allows a greater number of baskets per bay. (BST Oyster Supplies Pty Ltd. 2012).

Stocking density can also affect product quality and, in turn, profitability. In conjunction with our study on basket arrangement, we also tested three stocking densities; 75, 90 or 105 oysters per basket. These densities were chosen based on the 1/3 volume rule of stocking baskets and prior work that had shown that exceeding this rule can have negative effects on growth and fouling (Holliday et al., 1993; Honkoop and Bayne, 2002; Comeau et al., 2011). This caused the number of oysters present in a bay to differ between our nine baskets cross-line and six baskets in-line test, so we put six baskets cross-line in some bays to be sure that we had a fair test of in-line versus cross-line arrangements (Table 4.1). Our runs were 100 m long and had 34 bays each.

Table 4.1.
Basket arrangements and stocking densities tested using an adjustable long-line system.

| Basket arrangement | Number of baskets/bay | Basket stocking density (oysters/basket) | Number of oysters/bay |
|---|---|---|---|
| Cross-line | 9 | 105 | 945 |
| Cross-line | 9 | 90 | 810 |
| Cross-line | 9 | 75 | 675 |
| Cross-line | 6 | 105 | 630 |
| Cross-line | 6 | 90 | 540 |
| Cross-line | 6 | 75 | 450 |
| In-line | 6 | 105 | 630 |
| In-line | 6 | 90 | 540 |
| In-line | 6 | 75 | 450 |

We found that nine baskets can be placed cross-line in a bay with very little difference in product quality compared to six baskets placed cross-line in a bay. Oysters grown in bays with nine baskets cross-line were similar in shell dimensions, cup, fan, whole weight, meat condition, tissue weight and shell weight compared to oysters grown in bays with six baskets cross-line. In addition, those oysters had similar growth rates and survival rates. The amount of fouling on the oysters at nine and six cross-line baskets per bay was also similar, with only slightly more fouling on the six cross-line oysters. This means that a run could produce roughly 27, 000 oysters, which may have only been producing 18,000 oysters with six baskets per bay (Table 4.2).

Table 4.2
Production and profit estimates based on choice of basket arrangement and stocking density when using the adjustable long-line system for oyster grow-out. Assumptions used for calculations included below.

| | 9 Baskets Cross-line | | | 6 Baskets Cross-line | | | 6 Baskets In-Line | | |
|---|---|---|---|---|---|---|---|---|---|
| | **75** | **90** | **105** | **75** | **90** | **105** | **75** | **90** | **105** |
| Number of Oysters/Bay | 675 | 810 | 945 | 450 | 540 | 630 | 450 | 540 | 630 |
| Number of Oysters/Run | 22,950 | 27,540 | 32,130 | 15,300 | 18,360 | 21,420 | 15,300 | 18,360 | 21,420 |
| Number of Baskets/Run | 306 | 306 | 306 | 204 | 204 | 204 | 204 | 204 | 204 |
| Number of Oysters to Market | 22,261 | 26,714 | 31,166 | 14,841 | 17,809 | 20,777 | 14,841 | 17,809 | 20,777 |
| Gross Revenue/Run | $11,131 | $13,357 | $15,583 | $7,421 | $8,905 | $10,389 | $7,421 | $8,905 | $10,389 |
| Cost of Goods Sold/Run | $5,120 | $6,144 | $7,168 | $3,413 | $4,096 | $4,779 | $3,413 | $4,096 | $4,779 |
| Net Income/Run Before Cleaning | $6,011 | $7,213 | $8,415 | $4,007 | $4,808 | $5,610 | $4,007 | $4,808 | $5,610 |
| Fouling Multiplier | 1 | 1 | 1 | 1.167 | 1.167 | 1.167 | 1.769 | 1.769 | 1.769 |
| Hours Req'd to Clean One Run of Oysters | 63.8 | 76.5 | 89.3 | 49.6 | 59.5 | 69.4 | 75.2 | 90.2 | 105.3 |
| Labor Cost to Clean One Run of Oysters | $510 | $612 | $714 | $397 | $476 | $555 | $601 | $722 | $842 |
| Net Income/Run After Cleaning | $5,501 | $6,601 | $7,701 | $3,610 | $4,332 | $5,054 | $3,406 | $4,087 | $4,768 |

| **Assumptions** | |
|---|---|
| Number of Bays in a Run | 34 |
| Market Price of an Oyster | $0.50 |
| Cost of Goods Sold per Oyster | $0.23 |
| Time to Clean One 9 Cross-line Oyster (sec.) | 10 |
| Labor Cost (per hour) | $8.00 |

When comparing oysters grown in bays with six baskets cross-line to oysters grown in bays with six baskets in-line, we found the in-line oysters on average were larger on all shell dimensions, were heavier in whole weight, shell weight, and tissue weight. The in-line oysters, however, were not as cupped or fanned as their cross-line counterparts. Whether oysters were grown in in-line or cross-line, baskets appeared to have no effect on meat condition or survival. In-line oysters grew at a faster rate than cross-line oysters, possibly due to competition with fouling organisms for food and/or space.

Despite a weekly routine of 18-24 hours of air drying, oysters grown in in-line baskets were much more fouled with oyster spat, barnacles, etc. than oysters grown in cross-line baskets (Fig. 4.2).

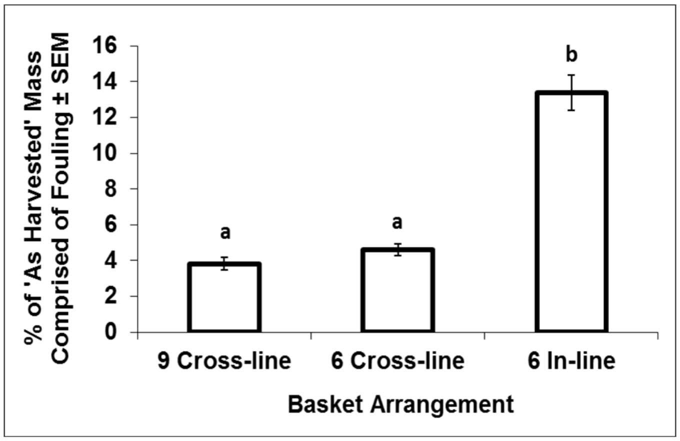


Fig. 4.2. Effect of basket arrangement on the amount of fouling accumulated on oysters at harvest time. Different letters indicate a statistically significant difference ($p<0.05$).

Our in-line baskets hung roughly 20 cm lower in the water column than our cross-line baskets, and this may have been the cause of the increased fouling, but we have not conducted follow-up studies as yet to confirm this. An in-line oyster on average had 77% more fouling on it than an oyster grown in a nine cross-line basket. More fouling means increased labor cost for cleaning oysters prior to sending them to market (Table 4.2) or a potentially less attractive product. For this reason, growing oysters in baskets arranged in-line is not recommended unless fouling can be decreased considerably from what we experienced, or a more aggressive fouling control program is implemented (which might, in turn, reduce growth).

Stocking density had a significant effect on shell height, meat condition, whole wet weight, and dry shell weight of oysters. Perhaps surprisingly, oysters stocked at 105 per basket grew faster and larger, but their shells were significantly less cupped and fanned than oysters stocked at 75 or 90 oysters per basket. Survival was also slightly lower in baskets with 105 oysters. Due to the significant benefits to meat condition and shell shape, we recommend stocking oysters at either 75 or 90 per basket. Due to the significant difference in cup and fan shape (Fig. 4.3) between oysters stocked at 75 and

90 per basket, it may be possible to have two grades of product, though it remains to be seen if consumers or buyers would distinguish between these differences.

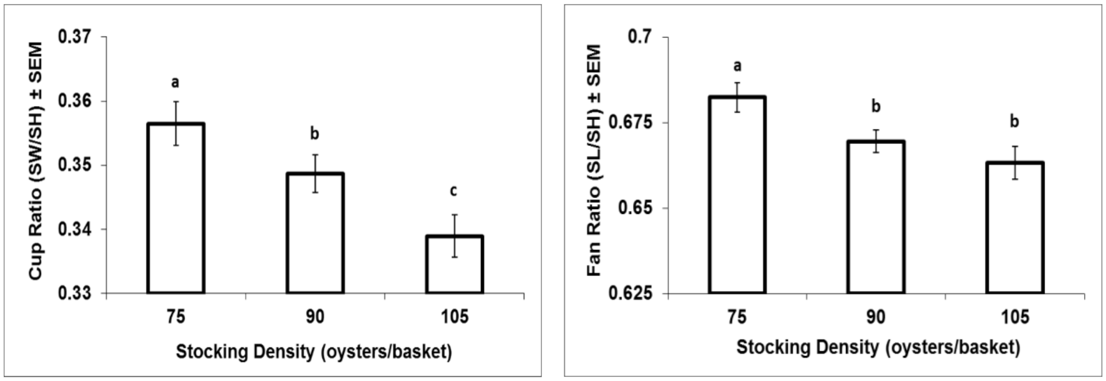


Fig. 4.3. Significant effect of stocking density on cup and fan ratio of oysters stocked at various densities in BST brand ALS baskets. Different letters indicate a statistically significant difference ($p<0.05$).

Optimum production can be defined as producing as many oysters with desirable attributes (i.e. good shell shape and meat condition) as possible within a given space or for a given cost. The results of our study suggest that optimum production in a given space when using an adjustable long-line system can be achieved by arranging nine baskets cross-line per bay and stocking each basket with 75 oysters (Table 4.2). This method should produce an oyster with a better cup, better meat condition, and with less fouling than growing oysters at a higher density or arranging baskets in-line. Our results suggest it may be possible to produce two grades of oysters by having a system with nine baskets cross-line and using stocking densities of either 75 or 90 oysters per basket. Although not considered in our estimates, this may increase profit by allowing you to market the 75 per basket oysters as a higher grade product.

*Effects of Basket Arrangement and Stocking Density on Mud Worm Infestation*

Presence of mud blisters caused by *Polydora websteri* can greatly impact a farmer's ability to sell his or her oysters. We looked at the effects of basket arrangement and stocking density on the amount of *Polydora* infestation in oysters grown using the adjustable long-line system.  We measured the percentage of the inside of the shell visually impacted by mud worm to an extent that may deter buyers (i.e. areas that were repaired were not counted).

We found that oysters grown in baskets arranged cross-line had a similar amount of mud worm damage as oysters grown in in-line baskets.  Similarly, whether oysters were stocked at 75, 90, or 105, oysters per basket had no effect on mud worm damage. We also tested the shell strength of the cupped valve of oysters using a 500 lb. load cell. We found no significant relationship between the amount of mud worm infestation and shell strength.

We soaked the oysters for three hours in freshwater and found that for every worm evacuated during the soak there could be up to three burrows inside the oyster. Surprisingly, we found that oysters could have no worms evacuate during the soak but could still have on average six burrows inside.  This suggests that the number of worms evacuated during a freshwater soak should not be used as an indicator of the amount of damage inside the shell.

Based on our results, and results of previous studies, we recommend that husbandry practices to reduce mud worm infestation in the first place be relied on rather than dips to eradicate them after damage has likely been done.

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Appendix 1

Summary of response variables (mean ± SEM) on harvest sampling data for each of nine basket arrangement and stocking density treatments.

| | 9 Cross-line | | | 6 Cross-line | | | 6 In-line | | |
|---|---|---|---|---|---|---|---|---|---|
| RESPONSE VARIABLE | **75** | **90** | **105** | **75** | **90** | **105** | **75** | **90** | **105** |
| ***Shell Dimensions (mm)*** | | | | | | | | | |
| Shell Height | 76.0±0.616 | 77.6±0.685 | 77.5±0.652 | 76.0±0.906 | 77.1±0.755 | 79.7±0.786 | 80.9±0.680 | 84.1±0.919 | 87.5±0.987 |
| Shell Length | 52.4±0.349 | 51.8±0.316 | 51.9±0.301 | 51.2±0.386 | 51.9±0.397 | 53.1±0.339 | 54.2±0.414 | 54.9±0.438 | 55.7±0.443 |
| Shell Width | 27.6±0.223 | 27.0±0.216 | 26.7±0.192 | 27.0±0.308 | 27.0±0.252 | 27.0±0.224 | 27.8±0.252 | 28.4±0.330 | 28.2±0.281 |
| ***Shell Shape*** | | | | | | | | | |
| Cup Ratio | 0.365±0.003 | 0.352±0.004 | 0.348±0.003 | 0.359±0.005 | 0.353±0.004 | 0.342±0.004 | 0.346±0.003 | 0.338±0.004 | 0.327±0.004 |
| Fan Ratio | 0.693±0.004 | 0.672±0.005 | 0.674±0.004 | 0.678±0.006 | 0.677±0.005 | 0.672±0.006 | 0.675±0.005 | 0.657±0.006 | 0.644±0.006 |
| ***Shell Quality*** | | | | | | | | | |
| Fouling Index | 4.07±0.239 | 4.28±0.264 | 3.12±0.195 | 4.24±0.455 | 4.06±0.301 | 5.37±0.418 | 11.5±0.700 | 13.1±0.778 | 15.4±0.991 |
| Dry Shell Weight (g) | 43.3±0.715 | 42.7±0.694 | 43.2±0.635 | 43.1±1.022 | 42.8±0.830 | 44.5±0.759 | 52.9±0.929 | 52.9±1.12 | 56.6±0.990 |
| Maximum Load Capacity (lbs.) | 256±28.9 | 256±17.9 | 251±20.4 | 266±24.79 | 248±19.7 | 261±27.6 | 244±21.9 | 321±20.8 | 271±21.3 |
| % cupped valve infested with mud worm | 15.1±2.56 | 13.7±2.25 | 14.1±2.24 | 10.4±1.66 | 14.4±2.39 | 10.6±1.42 | 14.7±2.94 | 13.4±2.73 | 16.9±2.20 |
| % flat valve infested with mud worm | 6.37±1.76 | 8.55±1.71 | 7.07±1.28 | 4.27±1.18 | 7.45±1.34 | 3.89±0.908 | 8.88±1.90 | 11.24±1.48 | 10.7±1.73 |
| # of visibly active burrows | 5.27±0.968 | 7.21±1.20 | 6.86±1.39 | 2.93±0.371 | 7.07±1.14 | 5.00±0.730 | 6.60±0.994 | 7.80±0.841 | 10.0±1.35 |
| # worms evacuated during FW soak | 2.93±0.556 | 4.14±0.553 | 2.07±0.539 | 1.67±0.287 | 3.93±0.892 | 4.07±0.959 | 3.33±0.754 | 3.13±0.568 | 3.73±0.714 |
| ***Meat Quality*** | | | | | | | | | |
| Dry Tissue Weight (g) | 1.26±0.024 | 1.22±0.020 | 1.23±0.023 | 1.25±0.034 | 1.24±0.027 | 1.28±0.026 | 1.37±0.029 | 1.39±0.033 | 1.45±0.036 |
| Condition Index | 6.13±0.065 | 5.87±0.052 | 5.88±0.061 | 6.07±0.080 | 5.88±0.073 | 5.84±0.061 | 6.04±0.088 | 5.78±0.089 | 5.65±0.081 |