SPRAYING DETERRENT STIMULI AS A TREATMENT METHOD FOR THE PREVENTION OF BIOFOULING CAUSED BY THE MUDWORM *POLYDORA LIGNI* WEBSTER ON NEW JERSEY OYSTER FARMS

by

EDWARD "NED" GAINE

A thesis submitted to the

Graduate School-Camden

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of Master of Science

Graduate Program in Biology

Written under the direction of

William Saidel Ph.D.

And approved by

____

William Saidel, Ph.D.

____

Daniel Shain, Ph.D.

____

Alejandro Vagelli, Ph.D.

Camden, New Jersey May 2012

ABSTRACT OF THE THESIS

Spraying Deterrent Stimuli as a Treatment Method for the Prevention of Biofouling Caused by the Mudworm *Polydora ligni* Webster on New Jersey Oyster Farms.

By Edward “Ned” Gaine

Thesis Director: William Saidel Ph.D.

Shellfish aquaculture, like all aquaculture, struggle with the recurring nuisance and pest of bio-fouling. Rack-and-bag oyster farms along the Delaware Bay shoreline of New Jersey fight a particular bio-fouling caused by the mudworm, *Polydora ligni*. Although methods of contending with the mud excreted by this worm on aquaculture equipment have been tried and are utilized, little work on preventative or proactive treatments has been conducted.

This proposed study addresses this deficiency by attempting to find deterrent stimuli for the *Polydora* Mudworm applied by means of a spray. The proposed study will conduct trials of various solutions in a laboratory setting leading to field testing of the successful lab trials.

By focusing on the natural thresholds for deterrence as applied by spray, the groundwork is laid for further exploration in environmental friendly pesticides for field use in aquaculture while still saving the farmer money in labor and crop losses.

TABLE OF CONTENTS

PAGE

ABSTRACT ii

TABLES OF CONTENTS iv

LIST OF ILLUSTRATIONS vi

1 INTRODUCTION 1

1.1 Description of Problem 4

1.2 Literature Review and Prior Work 5

1.3 Approach and Benefit 7

2 PROJECT DESIGN 9

2.1 Deterrent Solutions 10

2.2 Preliminary Laboratory Study 12

2.3 Field Trial 14

2.4 Field Applications 16

3 EVALUATION METHODS 18

3.1 Preliminary Laboratory Data 18

3.2 Field Trial Data 18

3.2.1 ImageJ 19

3.2.2 BioFouling Score BF_{score} 21

3.2.3 Nomenclature 22

3.2.4 Database 23

3.2.4.1 Fieldnames 23

3.2.4.2 Weekly Scoring Sheet 24

3.3 Experimental Design 26

3.3.1 Experiment 1: Stocking Density 27

3.3.2 Treatment Experiments 28

4.0 DISCUSSION 31

APPENDIX 36

BUDGET 36

Spreadsheet 42

Terms for Lease and Labor 43

Terms for Laboratory Technician 44

BIBLIOGRAPHY 45

ILLUSTRATIONS 48

LIST OF ILLUSTRATIONS

Illustration	Title	Page
A	Aerial View of Delaware Bay Grow-out Site	48
B	Map of New Jersey Showing Location of Grow-out Site	49
C	Rack-and-Bag Oyster Farm	50
D	Overhead View of One Bag Space on Rack with Hooks	50
E	View of Rack with Hooks and Straps, Oyster Bag Removed	51
F	Layout of Rack on Oyster Farm Showing Path for Quad and Worker Access	52
G	Overhead View of Oyster Bags with Varying Degree of Biofouling Caused by the Mudworm Polydora Ligni	52
H	Close Up View of Biofouling Coverage on Oyster Bags	53
I	Sample of Heavy Coverage	54
J	Sample of Medium Coverage	54
K	Sample of Light Coverage	54
L	Another Example of Heavy Coverage	55
M	Long View of Biofouling on Oyster Bag Rack	56
N	Randomized Bag Location Field Layout	57
O	Petri Dish Set-up with Plexiglas and Quadrants	58
P	Laboratory Recording Chart	59
Q	Color Coding Master List	60
R	Original Photographic Record and Duplicates	61
S	Enhanced Subsamples	62

T Original Photorecord and Enhanced Subsamples 63

U Blue Histogram of Subsample 3 64

V Adjusted Subsample 2 65

W Enhanced Subsample 1 Compared to Theshold Adjusted Subsample 2 66

X Illustration of Brightness Saturation 67

Y Comparision of Enhanced Subsample 1,Theshold Adjusted Subsample 2 & Illustrated Brightness Saturation Subsample 3 .68

Z Adjusted Subsample 2 69

AA Histogram of Adjusted Subsample 2 70

BB Comparision of Original Photorecord to Adjusted Subsample 71

CC Database File Structure 72

DD Weekly Score Sheet 73

EE Sample Weekly Score Sheet & Database 74

1.0 INTRODUCTION

The aquacultured oyster industry in Southern New Jersey has been growing and developing for several years from its infancy in 1992 (Littlewood) to as recently as the Winter of 2011, when the State of New Jersey opened over one thousand acres for lease with the specific aim of attracting new lease holders into the oyster farming industry. New lease holders are likely to mimic current oyster aquaculture practices in the area and experience the same problems faced by the current oyster farmers. One of these problems is recurring and very costly in the aquaculture industry - biofouling.

A 2011 survey of the United States shellfish aquaculture community suggested biofouling and its control accounts for an average of 14.7% of total operating costs for farmers, exceeding 21 million dollars (Adams et al., 2011). The specific type of bio-fouling facing New Jersey oyster aquaculturists is caused by the mudworm *Polydora ligni* (Webster) and its associated mud structure. This biofouling is so extreme that it threatens the quality and survival of the product, consequentially threatening the industry's overall profitability.

Current practices of oyster aquaculture along the Delaware Bayshore almost exclusively utilize a rack-and-bag methodology of oyster culture found in the intertidal zone of the Cape May peninsula (NJ Aquaculture Advisory Council, 2011) (Figure A, B, & C). Oysters are grown from seedling (less than one half inch) to market size or larger (greater than three inches) in enclosed plastic mesh bags of various sizes. The mesh bags are affixed to a welded rebar rack using bungee cord and steel hooks (Figures D & E). The rack prevents the bags from moving in the strong intertidal currents and high wave action. Oysters contained in these structures are kept three to four inches off the bottom of the substrate of the intertidal area. Racks are oriented end to end in long rows of approximately one hundred feet (Figure C). Two rows of racks are spaced three feet apart from each other to form a pair of rows. This pair of rows is separated from other pairs by six feet to allow a 4x4 All-Terrain Vehicle passage through the farm (Figure F). Oysters are maintained and cultivated in this rack–and-bag system for one to three years from seedling to market size.

Polydora ligni (Webster) commonly known as Polydora Mudworm, is a common invertebrate found throughout the coastal area including Eastern Oyster (*Crassostrea virginica*) habitats. *Poldora* forms a mud burrow structure which causes significant expense to the oyster fishing industry and oyster aquaculture operations (Loosanoff and Engle, 1943; Nel et al., 1996; Handley and Bergquist, 1997; Willemsen, 2005; Nell, 2007,). Since most seed is purchased from a hatchery, young oysters have an initial value which increases as market size is reached.

Polydora causes both mortality and undesirable cultured oysters, both of which decrease profitability of the farm (Nel et al., 1996; Willemsen, 2005). Current practices for controlling the *Polydora* infestation in New Jersey aquaculture is to use one or more gas powered water pumps to wash the mud off the oyster bags with local seawater at high pressure and high flow rates. This routine task is limited to the low water condition of the daily tidal cycle. Depending on the size of a farm, it can take a month to wash every oyster bag free of mud. During high infestation periods mud structures can grow and encompass a grow-out bag within a few days after a washing (Figure G, H, I, J, K, L, & M). In this situation, a large farm can encounter the problem of reoccurring soiling between washes, resulting in oysters spending several days to weeks encased in mud structures. Therefore in many farms, at least one pump and one farm worker is dedicated to the washing of oyster bags during the entire of the infestation period.

Mortality is one concern during the infestation. However since any loss affects the overall profitability of the farm; however, mud burrows and structures formed by the mudworm can also have detrimental effects to the overall quality of the product produced. Mudblisters, yellowing of shell, decreased growth rate, and overall misshapenness are common effects of mud infested growout of the oyster crop (Nell, 2007).

In agriculture or commercial fishing, bio-fouling and pest control often leads to utilization of pesticides. In agriculture these pesticides are often delivered as either a powder/granules or sprays (Osteen and Szmedra, 1989). Currently aquaculture and commercial fishing apply pesticides in the form of coatings or dips.

1.1 Description of Problem

The use of pest deterrent has not been reported in any of the oyster farming areas of New Jersey. With the current expansion of available grounds, aquaculture practices along the New Jersey Bayshore are likely to follow current agriculture practices for pest management which include; manual pest removal/picking, cohabitation of pest predators, and the usage of pesticide.

Oyster farms along the Delaware Bay of the Cape May peninsula range in size. Large commercial scale operations typically have several thousand oyster bags.

The proposed study seeks to identify the responses of *Polydora* Mudworm to chemical stimuli delivered by a sprayer in both laboratory and field trials. By identifying which stimuli deter these pests, further work can be done to ensure the health and marketability of the crop using an inexpensive and proactive treatment procedure.

1.2 Literature Review and Prior Work

In 2005, Willemsen posed a statement that is not only true of European aquaculture but also of worldwide aquaculture. “Biofouling is a complex and recurring problem in all sectors of the European aquaculture industry. Given the low cost margins, current priorities of the industry and operating environments it is vital that low cost, practical and easily applicable methods are found and introduced to control biofouling” (Willemsen, 2005).

Among world-wide shellfish aquaculturists biofouling associated with mudworms of the *Polydora* genus are a major concern (Stauber and Nelson, 1940; Loosanoff and Engle, 1943; Skeel, 1997; Nell, 2007). *Polydora* Mudworms cause mortality, growth inhibition, processing and packaging complications and a decrease in the value of the crop (Nel et al., 1996; Handley and Bergquist, 1997; Willemsen, 2005).

Little remediation work aside from Carver et al. (2003) has been done with spraying. Typical methodology for combating the *Polydora* include mechanical cleaning using brushes, scraping, water pump washing (Hodson et al., 1997; Handley and Bergquist, 1997; Willemsen, 2005). Another method is the practice of an immersion dip (Loosanoff, 1961; Arakawa, 1980; Willemsen, 2005). This method is usually land based but can be conducted on a boat. Immersion dip is the method of submerging cultured oyster and gear in a container of water of extreme temperature, salinity or soluble chemicals. The use of immersion dips is employed by oyster farmers who use the farming techniques of floating bags or bottom cages ((Loosanoff, 1960; Nel et al., 1996, and Arakawa, 1980). These enclosures can be handled easily and immersed in a dip on a boat or on shore. The use of dips for rack-and-bag farmers is often avoided because of transportation expenses.

It has been suggested that the number of individual oyster stocked in one oyster bag may also affect the infestation of the oyster bag (Smith, 1984 in Handley and Bergquist, 1997). Current oyster farming practices range in stocking density. However it is assumed that lower density yields better results for fouling, oyster growth and overall quality of the crop.

Recent work by the Natural Products Utilization Research Unit of the US Department of Agriculture (Dayan et al., 2009) identified several natural products for use as a pesticide. Since aquaculture is still a young industry in the United States, the old practices of synthetic pesticides have not become entrenched in current practices on oysters, an edible aquatic filterer (NRCC, 2011). By utilizing natural products from the onset, oyster aquaculture may prove to be a model for incorporation of more natural techniques in farming specifically aquatic farming and aquaculture.

1.3 Approach and Benefit

The goal of the proposed study is to find an effective means of an intertidal, dispatchable, and preventative treatment for the mudworm infestation. The study is designed to discover if localized stimuli can prevent or reduce the mudworm's associated mud structure coverage thereby reducing the expenses incurred by the rack-and-bag oyster farmers of Cape May.

Concentrating on broad ranging categories of stimuli applied by spraying, specific treatments can lead to the development of a practical management of the recurring infestation. These broad categories of stimuli include: salinity, variation in pH (Acidity/Alkalinity), the recognized "organic" irritant capsaicin, and lastly an antifouling coating used in current commercial crabbing gear. Aside from the capsaicin oil and antifouling coatings these deterrent stimuli have been tried at other locations as an immersion dip methodology and have been effective in reducing the *Polydora* infestation (Loosanoff, 1961; Arakawa, 1980; Nel et al., 1996; Willemsen 2005). No such recorded trial has been conducted in New Jersey.

As stated previously, the current practices of oyster aquaculture along the Delaware Bay coastline of Cape May is to use a high-flow, pump-washing procedure to address the *Polydora* issue. The pump-washing method is labor intensive and not proactive.

While the use of an immersion dip may be considered proactive, the immersion dip technique for a rack-and-bag farmer would involve the transportation of bags to a shore based operation or the use of a barge/boat. Quantities of bags to be treated would be determined by how many bags could be removed from the rack system and brought to the immersion dip site during the course of low tide. Of consideration here is the dipped bags must then be returned, possibly during the next low tide. The numbers of bags treated are likely to be similar to that of the pump-washing regiment or possibly less. When culturing a crop intertidally in rack-and-bags, the transportation of bags to a land based system can prove costly, timely and ultimately labor intensive.

A practice that is dispatchable to the intertidal flats could reduce both time and labor costs. Moreover if the practice decreases or eliminates the frequency of the daily washing routine both profitability and the overall quality of the product can improve.

2.0 PROJECT DESIGN

A preliminary laboratory study will determine which, if any, of six solutions has a deterring effect on the mudworm *Polydora* when applied by spray. If the lab results show a deterrent impact, field trials of the solution will be tested.

Based on the laboratory results the field trials will be conducted on a state leased oyster farm, adjacent to current industry farms along the Delaware Bayshore of the Cape May peninsula. A simulated New Jersey oyster farm will be set up containing 27 racks and 162 bags.

During a six-week period, two farm hands/technicians will attend to the sample bags using established and experimental bio-fouling control methodology (Refer to 2.4 Field Application). The 162 sample bags are equally divided into two divisions: 81 pump-washed with a high pressure and high-flow water spray or 81 without the pump-washing procedure. The 81 sample bags of each division are further equally divided into three stocking density types: 27 low-density, 27 medium-density and 27 high-density. Three bags of each stocking density will be subjected to nine treatments: Fresh Water Salinity Spray, High Salinity Spray, Saturated Salinity Spray, Acid pH Spray, Alkaline pH Spray, Organic Irritant Pepper Spray, Placebo Spray, Antifouling Dip, and a Control group receiving no treatment.

As an additional protection against experimental error, each sample bag being exposed to any of the nine treatments, in each of the three density types, and each of the two pump-washing classifications has two identical sample bags which receive the same procedures and will be randomly placed (Figure N). Randomization of the sample oyster bag layout is provided by a computer program (Urbaniak and Plous, 2011).

Data for the final report will be provided by the analysis of the photographic records using *ImageJ* (Rasband, W.S. http://rsbweb.nih.gov/ij/) software. Statistical tests of the data will show if significant differences have been reached using treatments. The final report will indicate which treatments were successful in deterring the mudworm surface coverage of mud.

2.1 Deterrent Solutions

Salinity - To study the effects of salinity, chemical solutions will be made of three different salinity concentrations. The first solution is 0 ppt salinity and will use tap water directly. The second and third solutions will be made using a commercially available aquarium sea salt mixture. One solution will be made to 35 ppt at 24℃ (stronger than ambient local seawater) and another will be made to saline saturation at 24℃, ~260 ppt. Salinities will be verified by use of a refractometer.

pH - To study the effects of pH, an acidic solution will be compared with a basic solution. The acidic solution will be made with commercially available vinegar, 5% acetic acid and local baywater. Enough vinegar will be added to bring the local baywater to a pH of 5.0 as measured by a pH meter. The basic solution will be made by diluting commercially available baking soda (sodium bicarbonate) with local baywater. Baking soda will be added to bring the local baywater to a pH of 8.5 as measured by a pH meter.

Pepper - The solution for the capsaicin pepper spray will be made from a dilution of concentrated capsaicin oil with local baywater. The initial dilution will be 10% oil to water. However, the field trial solution will be modified after lab trials to use the least amount of oil required to deter the worms.

Placebo - A placebo solution will be used to ensure that the pressurized spray process alone does not affect the mudworm infestation. The solution will be composed of local seawater from the grow-out area and kept in the same storage vessels as other treatments.

Antifouling Bag Dip - One experimental test involves no spray solution use at all. Rather, this test involves application of an antifouling dip to be applied to unstocked oyster bags prior to field grow-out. All bags in this group will be dipped, suspended and dried for 2 days.

2.2 Preliminary Laboratory Study

Before field trials of different chemical sprays begin, it is prudent to ascertain that the solutions do, in fact, elicit a response from pest species of the genus, *Polydora*.

Laboratory trials will expose the targeted species to pressurized sprayed stimuli and movement will be observed and recorded. A repeatable response or vector movement away from stimuli or mortality of the targeted species will deem stimuli tests verified before initiating the field trials of the solution.

The laboratory testing apparatus is set up as a standard petri dish, filled with a gelatinous solution of agar made with local seawater. The petri dish is maintained at room temperature and positioned over a sheet of graph paper to aid in observation (Figure O). A single worm *Polydora ligni* is placed in the centralized local of a petri dish. A Plexiglas covering is placed over one-half of the petri dish, covering two quadrants. Solutions of the stimuli are uniformly sprayed from above the petri dish. The Plexiglas covering prevents the spray from contaminating two quadrants of the petri dish.

A photo record will be taken every fifteen seconds, for the duration of a two minute experiment. Directional movement (degrees), distance from the original position and quadrant of a worm will be analyzed from the photo record. Survival or mortality will be tabulated at the end of the two minute trials (Figure P).

The chemical stimuli to be tested are concentrations of salinity, differing pH levels and exposure to organic irritant capsaicin. Each solution test will be repeated over three trials.

Typical salinity at natural sites is 20 ppt – 24 ppt (Nguyen, 2003). Based on previous experiments and the literature regarding immersion dips (Arakawa, 1980; Nel et al. 1996), the mudworm will be exposed to different concentrations of salt or brine; no salinity 0 ppm, high salinity 35 ppt and saturated salinity solutions ~260 ppt.

Typical pH at the site and the local Delaware Bay waters is 7.8. The Polydora mudworm will be exposed to lower (5.0) and higher (8.5) pH levels. The pH stimuli solutions levels are based on previous investigations in the use of acid and alkaline immersion dips (Arakawa, 1980; Carver et al. 2003).

Most likely, the mudworm has never been exposed to the organic irritant capsaicin in nature. Therefore, concentrations of this irritant will be applied so the minimal diluted suspension of capsaicin oil to water that elicits a response is to be determined for field use. Capsaicin oil is both hydrophobic and not natural in the marine environment. Diluted suspension will be made and notes recorded to determine the minimal diluted suspension necessary to elicit a response. Initially 10% oil to water will be the upper limit. If such a diluted suspension elicits a repeatable response, that concentration will be diluted in 1% intervals; if not, the solution will be concentrated by 10% intervals until a 100% oil concentration is reached. This diluted suspension will be used for the photo analysis experiment to be conducted in the laboratory and field trials.

2.3 Field Trial

Once effective solutions and concentrations are determined in the laboratory, the solutions concentration will be used on a model commercial scale oyster farm.

Deterrent stimuli will be tested for efficacy in reducing the mudworm mud infestation on the surface of the oyster grow-out bags controlling for other factors including: stocking density, and the current practice of pump-washing. Concurrent with the spray treatment trials, the methodology antifouling dip coatings use by the fishing and crabbing industry will be incorporated in the field design and monitored in the same manner as the spray solutions.

As mentioned previously (Page 6), the number of individual oyster stocked in one oyster bag may influence the infestation of the oyster bag (Smith, 1984 in Handley and Bergquist, 1997). For this possibility, all tests will be conducted against three stocking densities of a light, medium and heavy stocking of oysters per bag. Stocking density of 100, 250, and 400 individual oysters per bag will be regarded as low, medium and high respectively.

To determine if any treatment is sufficiently effective to replace the current practice of high-pressure, pump washing, each treatment will be tested on both sample bags undergo pump washing and as sample bags that will not be pump-washed.

Orientation of adjacent sample bags will be randomized with respect to stimuli application (Figure N). To ensure the proper stimuli treatment is administered to the correct test bag, individual bag will be labeled using a color coded vinyl card affixed to the exterior. These vinyl cards will identify each sample bag and the unpumped test division from the pumped test division. The pumped test division will be marked with a card shaped in a triangle; and unpumped test division with a rectangular card. Coding for the vinyl cards is provided in Figure Q. For each experimental sample bag, two other experimental sample bags which will receive the same treatments. In total three sample bags will receive identical treatments and maintenance routines.

2.4 Field Application

The tests are designed so that each spray solution is tested on sample bags that are tried with both standard high pressure pump-washing methodology and those that are not pump-washed.

A photo record of each test bag will be made before any weekly washing or application of treatment is begun. Following the photo record, the washing procedure on one half of the population of sample bags will proceed. Using a gas powered pump, all visible mud will be washed off the oyster bags. All sample bags within the pumping division will have the top/upper surface washed first. After the topside of each oyster bag is washed free of mud, the bag hooks will be unfastened from the sample bag and each bag will be flipped over horizontally, the topside of oyster bag will be washed again (previous underside). Sample bags will then be returned to the original position and refastened to rack with the bag hooks. For those bags that are not being pump-washed, treatments will be applied directly to sample bags on the rebar rack, weekly. Bag clips will not be undone nor will the bag be flipped in any way.

A trailer is to be deployed to test site containing all equipment needed for stimuli application and evaluation protocols. Sprayers and chemical storage vessels contained on the trailer are color coded to match the color of vinyl cards attached to the sample bags and ensure the proper stimuli is applied to the correct sample bag.

The treatment process begins by the spray application of a fresh water solution (Treatment 1) on the first of three bags in the pump division. After the initial bag, the other two bags in the pump division are sprayed. The spray application of the fresh water solution is repeated on the three bags in the not pumped division. Once application of Treatment 1 (Fresh Water) is completed, the same process is repeated for all the remaining spray treatments. All application of solutions will be sprayed uniformly and evenly as possible.

The field trials will be run for five weeks during mid-summer, at average to peak mud worm infestation. To ensure that the pressurized sprayer used in administering stimuli has no effect on the mudworm infestation, a placebo spray will be conducted using the same water as the conventional high pressure washing pump, Delaware Bay water.

3.0 EVALUATION METHODS

3.1 Preliminary Laboratory Study

The purpose of the laboratory study is to determine the efficacy of spraying already known deterrent stimuli on the *Polydora* mudworm. The methodology, described in the previous section, will provide quantitative and descriptive data

Data from laboratory tests (mortality and directional movement) will be evaluated such that repeatable data of either directional movement of the species away from the stimulus or death is a successful trial; whereas, survival and no movement or movement towards the stimuli-affected quadrants is a failed trial.

3.2 Field Trial Data

A photo record of the mudworm infestation will be conducted weekly on each sample bag in the field trials. Each sample oyster bag will be photographed from approximately the same distance. All sample bags are of uniform shape, size and mesh size. Photo records will be recorded for each sample bag each week before the weekly washing routine is begun.

A comprehensive photo collection consisting of 162 photos per week, 972 photos total will be compiled and formatted for use in an analytical image processing program.

3.2.1 *ImageJ*

Following the field data collection (photo record) the analysis of the treatments uses *ImageJ*, an image processing and analysis program (Rasband, W.S. http://rsbweb.nih.gov/ij/), which allows: (1) Selection of same size bag area; (2) Adjustment of specific color thresholds; (3) Equalization of histograms for increased contrast; and (4) Counting of the colored pixels in each same size photographic image. *ImageJ* produces the values used to calculate the scores of the surface coverage of the mud infestation on each bag.

Using these scores, the effectiveness in reducing surface mud/biofouling (Figures J, K & L) caused by the mudworm (*Polydora ligni*) on sample bags exposed to various treatments, stocking densities, pump utilization will be statistically tested.

A section of each sample bag photographic record, 2750 ±2 pixels high by 1350 ±2 pixels wide, is cropped and duplicated three times, resulting in an original photograph and three, identically-cropped subsample images (Figure R).

Two of the three subsamples (subsample 1, subsample 2) will be adjusted by enhancing the contrast level by 70%. The third subsample (subsample 3) will be adjusted by enhancing the contrast level by 70% while equalizing the histogram (Figure S). These enhancements increase the difference and intensity of the colors of the photographic records. This procedure is used to enhance the mud on the surface of the samples bags, taking a normally brown mud on black mesh bags and enhancing the mud to a hue of yellow, while keeping the mesh bag black. The results of this procedure are an original photographic record and three enhanced subsamples (Figure T).

Subsample 1 will serve as an enhanced cropped original, subsample 2 will serve as the sample to be evaluated, and subsample 3 will be used to determine a threshold criteria.

Using subsample 3, a histogram is compiled of the blue colors only (Figure U). The *ImageJ* histogram provides the mean value for blue pixels in the subsample. This value will be used to determine the threshold value for evaluation of the mud coverage on subsample 2.

Subsample 2 is adjusted by color threshold using the mean blue value from subsample 3 as the minimal threshold value on the blue-yellow spectrum (Figure V). Since the *ImageJ* program uses only whole numbers in determining threshold, the mean value is rounded up to the next integer. Subsample 2 image is rendered into black and white with those values exceeding threshold appearing red (Figure W). These red values represent the surface mud on the photographic record.

A visual comparison of subsamples 1 & 2 will reveal if any false positives have occurred. If false positives are found, adjustments can be made by editing subsample 2.

A more common error occurs as the result of the brightness saturation of the original photographic record. This saturation occurs due to the field conditions of camera flash or sun positioning. This error is shown on subsample 2 as white space surrounded by red pixels. These white spaces can be illustrated by evaluating subsample 3 for brightness and saturation maxima (Figure X). Adjustments to subsample 2 can be made by comparison with subsample 1 and subsample 3 (Figure Y, Figure Z).

To compose a score of surface mud coverage (biofouling) a histogram of subsample 2 is compiled (Figure AA). The *ImageJ* histogram provides the total pixel count and the total red count represented by the count of value 85 (Figure BB).

3.2.2 Biofouling Score

The biofouling score (BF) will be calculated as the ratio of the count of total red pixels (R_{pixel}) to the total pixels (Tot_{Pixel}).

$$BF = R_{pixel} / Tot_{Pixel}$$

Where, BF = biofouling score
R_{pixel} = Red pixels
Tot_{Pixel}= Total pixels

3.2.3 Nomenclature

The nine experimental treatments are represented as:

$$T_{1\ldots9}$$

Where, T = treatment,
1 ...9 = nine treatment procedures.

BF scores for each of the treatments are represented as:

$$BF_{1\ldots9}$$

The experimental set-up is divided into two groups: Those which are pumped to 0% mud coverage weekly and those that are not. This division (pumped and not-pumped) allows investigation into effectiveness of deterrence of infestation on a weekly level as well as a broader 5 week scale.

These divisions are represented as:

$$T^{p}, \quad T^{np}$$

Where, p = pumped,
np = not pumped.

Within these divisions are the nine treatments and the stocking density groups. The three stocking densities are 100, 250 and 400 oysters per bag and are represented respectively:

$$^{100}T,\ ^{250}T,\ ^{400}T$$

3.2.4 Database

For conducting the statistical experiments, proper management of the database and the records contained within, is essential. When completely populated from a weekly scoring sheet, the database will contain 972 individual records gathered from each of the 162 sample bags photographed weekly. The *ImageJ* program will provide pixel counts which, when computed, will result in BF scores.

3.2.4.1 Fieldnames

The first field, Record #, (See Figure CC) is a number from 0001 to 972 where each record is a distinct image. Field # 2 is the classification of the Pump v. No Pumping Division where P = the sample bags receiving weekly pumping process and NP = the sample bags not receiving the pumping process. Field # 3 is the classification of Stocking Density where Low = Stocking Density of 100 oysters per sample bag; Medium = Stocking Density of 250 oysters per bag; and, High = Stocking Density of 400 oysters per bag. Field #4 is the classification of Treatments where T_1 = fresh water spray; T_2 = high salinity water spray; T_3 = saturated salinity water spray; T_4 = acidic solution spray, T_5 = alkaline solution spray; T_6 = pepper solution spray; T_7 = antifouling bag dip treatment; T_8 = Placebo (spray with ambient sea water); and, T_9 = receive no treatment (control group). Field #5, Treatment

Sample, identifies the three sample bags (A,B,C) receiving the same treatment but randomly placed (Figure N) at the experimental farm to control for experimental error. Field #6, Sample Bag #, is the assigned number of that sample bag from 001 to 162 (See Figure Q). Field #7 Week identifies the week (0 – 5). Field #8is the photograph number.

The final two fields are computer generated once the red pixel (R_{pixel}) value and total pixel value (TOT_{pixel}) are entered on the Weekly Scoring Sheet (see below). Field #9, BF score (refer to Page 21 for formula), is a decimal value. Field #10, Mean Treatment Sample BF Score, is the average of the three treatment samples bags (A, B and C identified in Field 5) for the same Pumping Division, Stocking Density, and Treatment.

3.2.4.2 Weekly Scoring Sheet

The database is populated from items and calculations entered on the Weekly Scoring Sheet and the stored data of Figure N, Randomized Bag Location Field Layout. In order to differentiate fields on the database from those on the Weekly Scoring Sheets, the fields on the database have no superscript and use numerical values while the fields on the Weekly Scoring Sheet use letters and the superscript format $\text{Field}^{\text{WSS}}$.

A template worksheet will be generated for each of the 162 Sample Bags (See Figure DD). There are seven fields on the Weekly

Score Sheet. $Field^{WSS}$ A is the Sample Bag #. Once the Sample Bag # ($Field^{WSS}$ A) is entered, the database program will retrieve from Figure N's stored data all the information associated with that sample bag (Pump Division, Stocking Density, Treatment, and Treatment Sample). $Field^{WSS}$ B is the Week #, where Week is a value between 0-5. $Field^{WSS}$ C is the Photograph identifying #.

The experimenter then enters the R_{pixel} values ($Field^{WSS}$ D) and TOT_{pixel} value ($Field^{WSS}$ E) for the photographic record of that week. The program will compute and enter the BF_{score} for that image in $Field^{WSS}$ F and assigns a Record # ($Field^{WSS}$ G).

After completing entries for each record on the Weekly Score Sheet, the database program imports the values from Figure N to the database for Fields 2, 3, 4, & 5 and transfers the values from $Fields^{WSS}$ A, B, C, F and H into the corresponding database Fields 6, 7, 8, 9, and 1. Until all three treatment sample bags needed to calculate the mean are entered on the Weekly Score Sheet, the program will return an error message for Field 10, Mean BF_{score} for Treatment Samples. Figure EE shows how the Weekly Score Sheet and the Database will appear after trial data of two weeks is entered for Sample Bag 001.

3.3 Experimental Design

The unique field project design allows for consideration of multiple variables without the need for replication of field trials. The field project design has the division of weekly pumping vs. no pumping to explore the potential of replacement or supplementation to the common practice of oyster farms. Each experiment is conducted on both divisions indicated by the notation H^{p} Pump or H^{np} No Pump.

The full experimental design will be dependent on results of an initial test of the impact of stocking density. The basic sample size for each treatment experiment will either be n= 3 or n= 9. Hence, if stocking density in Experiment 1 has no effect (H_o not rejected), then all subsequent stocking densities classification can be collapsed. (${}^{100}T + {}^{250}T + {}^{400}T \rightarrow T$) thereby consolidate 3 stocking density classifications of 3 samples for each treatment (${}^{100}T_{1-9}, {}^{250}T_{1-9}, {}^{400}T_{1-9}$) into 9 samples for each treatment(T_{1-9}). If however stocking density is found to have an effect (H_o rejected) then each treatment experiment will need to be replicated for stocking density levels. For the purposes of this report, H_o is assumed to be rejected therefor n = 15 which represent the BF scores of 3 sample bags measured for 5 weeks.

For Experiment 1 and the treatment experiments with H_o rejected, the decision rule for rejection of the null hypothesis at the 95% confidence level is to reject H_o if the T-Value is > -2.048 or T-Value < +2.048 with DF = 28. For other treatment experiments when H_o is not rejected the n = 90 and the rule for rejection of the null hypothesis at the 95% confidence level is to reject H_o if the T-Value is > -1.6623 or T-Value < +1.6623 with DF = 88.

Since the purpose of the proposed study is to identify deterrent stimuli controlling for stocking density, pumping practice and application methods, the salient statistic chosen to determine significance is the measure of difference between the mean using the t ratio.

3.3.1 Experiment 1: Stocking Density

To evaluate if the stocking density has an effect on the mudworm infestation a statistical experiment will be conducted to compare the mean differences of the BF scores of the three stocking densities (${}^{100}T, {}^{250}T, {}^{400}T$).

Null hypothesis: Stocking density does not affect the surface mud coverage. The mean difference of the BF scores of all stocking density samples (${}^{100}T - {}^{250}T - {}^{400}T$) will fall within a magnitude which can be reasonably explained by sampling variation.

$$\boxed{H_o^p\colon \bar{X}^{400}BF_9^p = \bar{X}^{250}BF_9^p = \bar{X}^{100}BF_9^p \qquad H_o^{np}\colon \bar{X}^{400}BF_9^{np} = \bar{X}^{250}BF_9^{np}}$$

3.3.2 Treatment Experiments

Experiment 2 - To evaluate if the spraying procedure alone has an effect on the mudworm infestation, a statistical experiment will be conducted to compare the mean differences of the control BF scores (T_9) with those of the placebo spray of local baywater (T_8) within each pumping division.

Null hypothesis: The spraying procedure alone does not have an effect to the surface mud coverage. The mean difference of the BF scores between the placebo spray and the control group $(T_8 - T_9)$ will fall within a magnitude which can be reasonably explained by sampling variation.

$$\boxed{H_0^p\text{: } \bar{X}\, BF_8^p = \bar{X}\, BF_9^p \qquad H_0^{np}\text{: } \bar{X}\, BF_8^{np} = \bar{X}\, BF_9^{np}}$$

Experiment 3 - To evaluate the effectiveness of the antifouling bag dip as a deterrent to mudworm infestation a statistical experiment will be conducted to compare the mean difference of the BF scores of the bag dip treatment (T_7) to the control group (T_9) within each pumping division.

Null hypothesis: The antifouling dip does not affect the mudworm infestation. The mean difference between the BF scores of sample bags with antifouling dip applied and control group

$(T_7 - T_9)$ will fall within a magnitude which can be reasonably explained by sampling variation.

$$H_0^p\colon \bar{X}\,BF_7^p = \bar{X}\,BF_9^p \qquad H_0^{np}\colon \bar{X}\,BF_7^{np} = \bar{X}\,BF_9^{np}$$

Experiment 4 - To evaluate the effectiveness of pH spray solutions of acid or alkaline baywater deterrence to the mudworm infestation a statistical experiment will be conducted to compare if the mean difference of the BF score of either the acid (T_4) or the alkaline (T_5) solutions to the control group (T_9) within each pumping division.

Null hypothesis: Sprayed pH solutions does not affect the surface mud coverage. The mean difference between the BF scores for either treatment and the control group $(T_{4,5} - T_9)$ will fall within a magnitude which can be reasonably explained by sampling variation.

$$H_0^p\colon \bar{X}\,BF_4^p = \bar{X}\,BF_9^p \qquad H_0^{np}\colon \bar{X}\,BF_4^{np} = \bar{X}\,BF_9^{np}$$

$$H_0^p\colon \bar{X}\,BF_5^p = \bar{X}\,BF_9^p \qquad H_0^{np}\colon \bar{X}\,BF_5^{np} = \bar{X}\,BF_9^{np}$$

Experiment 5 - To evaluate effectiveness of the capsaicin pepper spray as a deterrent to the mudworm infestation a statistical experiment will be conducted to compare the mean difference of the BF scores of the capsaicin treatment (T_6) to the control group (T_9) within each division.

Null hypothesis: The capsaicin pepper spray does not affect the fouling of the sample bags. The mean difference of the BF scores of the capsaicin treated sample bags and the control group $(T_7 - T_9)$ will fall within a magnitude which can be reasonably explained by sampling variation.

$$H_0^p\colon \bar{X}\, BF_6^p = \bar{X}\, BF_9^p \qquad H_0^{np}\colon \bar{X}\, BF_6^{np} = \bar{X}\, BF_9^{np}$$

Experiment 6 - To evaluate the effectiveness of the various salinity solutions deterrence to the mudworm infestation a statistical experiment will be conducted to compare the mean difference of the BF scores of each of the three salinities {Fresh (T_1), high (T_2), and saturated (T_3)} with that of the control group (T_9) within each pumping division.

Null hypothesis: The individual salinity spray treatments do not have an effect on the surface mud coverage. The mean difference of the BF scores of each of the three salinity groups and that of the control group $(T_{1,2,3} - T_9)$ will fall within a magnitude which can be reasonably explained by sampling variation.

$$H_0^p\colon \bar{X}\, BF_1^p = \bar{X}\, BF_9^p \qquad H_0^{np}\colon \bar{X}\, BF_1^{np} = \bar{X}\, BF_9^{np}$$

$$H_0^p\colon \bar{X}\, BF_2^p = \bar{X}\, BF_9^p \qquad H_0^{np}\colon \bar{X}\, BF_2^{np} = \bar{X}\, BF_9^{np}$$

$$H_0^p\colon \bar{X}\, BF_3^p = \bar{X}\, BF_9^p \qquad H_0^{np}\colon \bar{X}\, BF_3^{np} = \bar{X}\, BF_9^{np}$$

4.0 DISCUSSION

The overall goal of this proposed study is to discover if treatments, applied by spraying, can be a significant deterrent to surface mud accumulation caused by the mudworm *Polydora ligni*. An understanding of the success or failure of these treatments, conducted on a commercial field site, will aid in directing future work in preventative measures against this and other recurring issues for oyster farmers.

Additional experimentation variables were incorporated into the design to ascertain the impact of treatments with other practices currently used by commercial oyster farmers. These variables include stocking density (Experiment 1), pump-washing process and antifouling dip.

Experiment 1 (stocking density) is designed to explore if stocking density does affect the mudworm infestation and the effectiveness of treatments. Assessment of this variable will aid oyster farmers in their future husbandry techniques with respect to the number of oysters in each bag.

Since little work has been conducted on the spraying of treatments on oyster bags, Experiment 2 (placebo spray) is designed to discover if the spraying process itself affected the surface mud accumulation. This experimental control is used to ensure that the chemical treatments are the effector and not the spray alone.

Experiment 3 (antifouling dip), is designed to evaluate if current antifouling methods employed by the similar industry of commercial crabbing might be effective for incorporation by the oyster industry. If this treatment is effective a future study should be conducted to compare the results of this treatment with those of future treatments applied as a spray (See 4.0 Discussion).

Experiments 4-7 (stimuli treatments) are designed to test the specific treatments for effectiveness. The treatments themselves were selected to reflect basic biological stimuli which the mudworm *Polydora ligni* may be susceptible. Success or failure of these treatments at the commercial level will aid future research and procedures which the farmer or scientist may utilize as preventative treatments. (See 4.0 Discussion) The pump-washed vs not pump-washed division was intended to explore if: (a) deterrent stimuli spraying could replace the current, labor-intensive practice of weekly pump washing; and, (2) if it would be advantageous to incorporate spraying into the pump-washing routine.

Regardless of the success or failure of the experiments, important knowledge will be gained regarding solutions and treatment processes in both the laboratory and the field phases of the proposed study.

If a solution failed the preliminary trials for stimuli response in the laboratory, that solution is not to be field tested. While the solution is rejected, it should be considered that only the concentration of the solution failed – it may still be an effective deterrent at a different concentration. This is particularly the case in evaluation of pH and salinity experiments.

In the field, success or failure could be application specific. The solution may not be the determining factor; rather, the solution as a field spray may be the determining factor since the solution had shown to be effective in the laboratory trials. Therefore, the field application as a spray is ineffective in showing a response. This situation can occur because of the inability of the spray to reach all parts of the oyster bag or oysters as compared to the immersion dip methodology where full coverage is ensured.

Using the data collected in the proposed study, future studies could explore baseline data of surface mud growth over time exploring possible correlation to weekly or seasonal conditions and possibly lunar cycles. This avenue of research might investigate the mudworm infestation relationship to local water conditions and generic trend forecasting using data from the nearest realtime water condition monitor at the Brandywine Shoal Light.

Another expansion of this proposed study could test which, if any, is the most effective among the treatments. No additional field work would be required to pursue inquiry along this avenue of research.

During the time frame of the proposed study mudworm infestation may not be at peak. Therefore, future work could be conducted using effective deterrent stimuli to explore if prevention prior to the mudworm growing season or before going into winter months could be successful in reducing the infestation throughout the whole growing season.

Due to the business nature of aquaculture a cost benefit analysis should be required part of continued inquiry. Data collected from the evaluation section, combined with the Project Budget (See Appendix) could be used to determine the economic feasibility of any new treatment process. Projections to full-scale farms and full-season applications compared to labor savings and crop yield will indicate the true potential.

Antifouling dip methods used by the related crabbing industry may prove to be an effective deterrent. If found to deter, the evaluation techniques discussed in this paper should be utilized over a longer period to evaluate the lifespan of effectiveness of the single application. Experimentation on the process may disclose that when evaluated in combination with a cost benefit analysis a single application procedure may prove to be a costly initial procedure that eliminates or greatly reduces labor cost over the long term.

The photographic image analysis process (*ImageJ*) described in this proposed study could have applications in other aspects of oyster farming such as floating or submerged cages. Another potential application includes the evaluation of young oyster native collection (spatfall). *ImageJ* could prove to be a valuable aid to gauge spatfall rates on collection gear and the oyster crop. Since the removal of spatfall on oyster crop can be another timely and costly endeavor to the oyster farmer, *ImageJ* analysis of effective stimuli found in the proposed study could be utilized by the industry to provide relief to the costly and labor intensive practice of manually scraping oysters clean.

Finally it should be noted, this proposed study about deterrent treatment applied by a spray to control biofouling caused by the mudworm *Polydora ligni* further expands the applicable knowledge of preventative methods to the oyster farmer and scientist alike. Upon completion of the study, the industry will gain valuable insight to direct future research in the aim of developing a cost effective deterrence to a recurring pest species. The study demonstrates the value of the applied research of Rutgers University – Camden addressing Southern New Jersey industry needs.

APPENDIX

Project Budget

Equipment and personnel are the key components of exploring and field testing the basic deterrent stimuli. This proposed study requires a total budget less than $ 10,000.00 for a three month period. This proposed budget assumes one funding agency. However, if needed, it can be modified to include several overlapping state and federal funding agencies.

Item # 1, 2 Personnel:

An experienced and well-trained lab technician will be needed to conduct the "lab" proportion of the proposed study. It is estimated one person for two, eight-hour days at $ 25 hour, for a total of $400. This is a contracted position.

Two farm hands/ technicians will be required for the weekly duties required of the field trial portion of the proposed study. The hands are responsible for transportation of equipment to the experiment site, the washing and flipping of bags in the "pumping" group, the dipping procedure, the administration of chemical stimuli sprays, and the weekly recording of photographic data. Estimated cost of labor at a rate of $ 15/hr for 3 hours/day; twice a week service for six weeks brings a total of

$1,080. This can be included in a contract with farm lessor to provide farm hands and equipment.

Item # 3 Petri Dishes with Culture: Three dozen petri dishes with a mixed solution of standard agar and sea water: estimated $50.

Item #4 Worms Field collections from the field site by laboratory technician $0.

Item # 5 Plexiglas: A Plexiglas cover is needed to cover one half of each petri dish as the spray is applied. One, 12″ x 12″ sheet will be cut by the laboratory technician into nine, 4″ x 4″ squares. Thirty-six 4″ x 4″ squares are needed. Estimated 4: 12″ x 12″ sheets at $25 a sheet including shipping costs, $100.

Item # 6 Solution Spray Containers: A pressurized spray container is needed for each of the experimental solutions that allows for uniform and consistent spray distribution. Each chemical resistant tank with a wand is $50. Estimated cost for 7 with shipping included is $350.

Item # 7 Solutions and Storage: A total of eight fluids will be used in this project; six of which are solutions which need to be mixed and stored. Total combined costs $1,010.

<u>Vinegar</u> – 55 Gallon, industrial container is estimated at $275.

<u>Baking Soda</u> – 25 lbs is estimated to cost $25.

Sea Salt Mix – *Instant Ocean* Aquarium Natural Sea Salt Mix. One 5 gallon bucket provides enough to make to make both high and saturated solutions. Estimated cost $50.

Antifouling Dip – *Flexgard* Antifouling coating is required for a single application of dip throughout the course of the experiment. Estimated cost: $150 for 2 gallon pail.

Capsaicin Oil – The active ingredient in pepper spray, available as a concentrate: www.iamm.com/capsicum.htm is estimated at $200 per 1 gallon.

55-gallon drums – Are needed to store mixed solutions for repeated applications during the field trial. Estimated costs for six drums with removable covers is $510 ($85 x 6).

Item # 8 Refractometer – In order to verify the salinity levels in the mixture of three solutions, a high quality, portable refractometer with PPT optical readout is required: Hanna Instruments HI 96822 $200.

Item # 9 pH Meter – In order to verify one solution of local baywater being mixed to a pH level of 5.0 and another to 8.5, a high quality pH meter is required : Hanna Instruments pH 2700 Meter $597.

Item # 10 Camera – In order to document laboratory results and to provide data for the *ImageJ* photo analysis, a high quality, moisture resistant digital camera is required: Olympus TG610 14.0 Megapixel $280.

Field Equipment and Supplies

Item # 9 Oyster Oysters utilized in the experiment will be of uniform size ½" or larger. The oysters will not be available for commercial sale after the project. The estimated purchase price of ½ inch oyster is $15 per thousand. The experiment calls for 28,500 oysters, at a cost will be $427.50.

Item # 10 Bags New oyster grow-out bags 3/8" mesh. These bags are used as the experimental unit for field trials. The estimated cost for 162 bags at $5/bag is $810.

Item # 11 Pump and Hoses: One half of all groups will be exposed to water pump treatment. Pump -Honda BE-TP-3080HM, 286 GPM; $1,389.70. Hoses, one intake and one output Apache Water Pump 3" x 15 feet, two need at $85 costs $170.

Item # 12 Dipping Tub: A durable heavy weight tub is needed to submerge the oyster bags in the application of the antifouling dip. Estimated cost is $220.

I**tem # 13 Individual Field Sprayer, Hoses and Nozzles**

For maintenance of chemical standards, the stimuli to be administered to the crop should be stored in its own vessel and utilize an individual sprayer. Such a configuration ensures that each sample only receives a spray of solution composed of a single specific treatment. Each sprayer unit is equipped with an individual pump and DC power cables. Seven Sprayer units at $90 each totals $630.

Item # 14 Field DC Battery A single 12V battery is sufficient to power all the sprayer pumps individually for the duration of the experiment, while providing the pumps with power. One UPG Sealed Marine Battery — 12V, 110 Amps, Estimate cost $320.

Item # 15 Trailer A modified trailer of sufficient size to store and transport all chemical vessels with pumps and power source to testing site. Northern Industrial Watercraft Trailer 610-Lb. Capacity, Model# LCI-881PA Estimated cost $530.

Item # 16 Fuel: Gasoline for running pump and 4x4 ATV during the six week field test estimated cost $200.

Item # 17 Recording Supplies: Graph paper, wax pencils, pens, paper towels, other miscellaneous supplies for the lab experiment, and printing of some digital photographs has estimated cost of $50.

Item # 18 Other Office Expense: Copying, printing, postage and telephone reimbursement to field technicians for lab and field reports, estimated $50.

Item # 19 Travel: Assuming a two-hour, round trip with a two times a week frequency for six weeks would have an estimated gas charge for project supervisor of $100.

Item # 20 Leases and Service Contracts

To conduct the experiment in known area of recurring mudworm infestation, space needs to be obtained from an existing commercial oyster farm. A rental fee for usage of farm lease grounds, racks and hooks is estimated at $10 per rack for 27 racks equal $270. In addition, it is expected that a contract could be negotiated with the same owner of the oyster farm for part time services to be provided by farm hands with the use of a ATV. $50 a week for six weeks a total of $300.

For proposed terms of the lease and labor see Figure U. Estimated costs for rack/farm lease, $270; add Quad use, $300.

A contractual arrangement for a qualified lab technician (2 day costs detailed in item #1.) The proposed the terms for Laboratory Technician are described in Figure V. Legal review of lease and contracts is estimated at $150.

Project Budget Spreadsheet

Terms for Farm Lease and Labor

In order to conduct the field trials in a known area of recurring mudworm and simulate industry conditions, space needs to be obtained from an existing commercial oyster farm on a state leased oyster farm along the Delaware Bayshore of the Cape May peninsula. A reasonable estimate to lease space for 162 bags on 4 racks for a six-week period would be $270.

Since this project is for a short time and requires only part time field hands, it is possible that the farm lessor could assign his/her employees to this project and equip them with a quad. A reasonable labor estimate would be $ 15/hr for 3 hours a day. Calculating twice a week for six weeks would be $ 1200. If the lessor could provide a Quad ATV with the farmhands for an additional $ 300, the total lease, equipment rental and labor assignment would gross the lessor $ 1,770.

Field hands/ technicians duties:

- Apply antifouling dip to eighteen bags.
- Affix color coded vinyl cards to grow-out bags.
- Stock oyster grow-out bags with appropriate number of oyster.
- Deploy grow out bags to field site.
- Record photo data, twice per week.
- Wash one half experimental bags with bay water from provide pump, weekly.
- Transport and return spray equipment to and from field site.
- Conduct spray treatments once per week.
- Conduct final mortality counts.

Terms for Laboratory Technician

An experienced and well-trained lab technician will be needed for conducting the laboratory proportion of the proposed study. The individual should be knowledgeable of basic experimental procedures, preparation of experiments and agar plates, proficient in report generation.

This will be an independent contractor (IRS 1099). Two days expected work at $25 hour for approximately $400.

The assigned responsibilities will include:

Laboratory technician:

- Collect target species from field grow-out site.
- Make testing petri dishes from agar and local baywater.
- Delineate quadrants on testing petri dishes.
- Cut Plexiglas into squares for spray guards.
- Make stock solutions for both laboratory and field experiments.
- Conduct laboratory experiment and photo record.
- Fill out laboratory recording chart.
- Write up final report detailing results.

BIBLIOGRAPHY

Adams, C.M., Shumway, S.E., Whitlatch, R.B. and Getchis, T. 2011. Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. Journal of the World Aquaculture Society, 42. pp 242-252.

Arakawa, K.Y. 1980. Prevention and removal of fouling on cultured oysters. A handbook for growers. (Translated from Japanese by R. Gillmor) Mar. Sea Grant Tech Rep. No 56. pp 1-37.

Browdy, C.L. and J.A. Hargreaves (editors). 2009. Overcoming technical barriers to the sustainable development of comprehensive Marine Aquaculture in the United States. U.S. Department of Commerce, Silver Spring, MD USA. NOAA Technical Memo NMFS F/SPO-100. 114pp.

Carver, C. E., Chrisholm A. and A. L. Mallet. 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. Journal of Shellfish Research 22. pp. 621-631.

Dayan F.E., and Cantrell C.L. and S.O. Duke. 2009. Natural products in crop protection. Biorganic and Medicinal Chemistry. 17:12. pp. 4022-4034.

Handley S.J. and P.R. Bergquist. 1997. Spionid polychaete infestations of intertidal pacific oysters *Crassostrea gigas* (Thunberg), Mahurangi harbor, northern New Zealand. Aquaculture 153. pp. 191-205

Hartman, O. 1945. The marine annelids of North Carolina. Duke University Marine Station Bulletin. 2. pp. 1-54

Hodson S.L. T.E. Lewis and C.M. Burke. 1997. Biofouling of fish-cage netting: the efficacy problems of in situ cleaning. Aquaculture 152. pp. 77-90

Littlewood D.T.J., R.N. Wargo, J.N. Kraeuter and R.H. Watson. 1992. The influence of intertidal height on growth, mortality and *Haplosporidium nelsoni* infection in MSX mortality resistant Eastern Oysters, *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research, 11:1. pp. 59-64

Loosanoff V.L. and J.B. Engle. 1943. Polydora in oysters suspended in the water. Biological bulletin 85:1. pp. 69-78

Loosanoff Victor L. 1961. Recent Advances in the Control of Shellfish Predators and Competitors. Proc. Gulf Carib. Fish. Inst. 13th Annu. Sess. pp. 113-128

Nel R., P.S. Coetzee, and G. Van Niekerk. 1996. The evaluation of two treatments to reduce mud worm (*Polydora hoplura* Claparede) infestation in commercially reared oysters (*Crassostrea gigas* Thunberg). Aquaculture 141 pp. 31-39

Nell, John. 2007. Controlling mudworm in oysters… Primefact 590, New South Wales Department of Primary Industries www.dpi.nsw.gov.au/primefacts

Nelson, T. C. and Stauber, L. A. 1940. Observation of some common polychaetes on New Jersey oyster beds with special reference to *Polydora*. *Anatomical Record* 78. 102A–103A.

Nguyen, J. Delaware Bay Cape Shore 1999-2002 Water quality monitoring report. 2003. NJDEP Bureau of Marine Water Monitoring 50 pp.

NJ Aquaculture Advisory Council. 2011. Opportunities and potential for aquaculture in New Jersey. NJ Department of Agriculture. 14pp.

NRCC 2011 Bivalve aquaculture gear and biofouling control Code 400. Natural Resources Conservation Service, Conservation Practice Standard. pp 1-3

Orth R. J. 1971. Observations on the planktonic Larvae of *Polydora ligni* Webster (Polychaeta: Spionidae) in the York River, Virginia. Chesapeake Science 12:3 pp 121-124.

Osteen C.D. and P.I. Szmedra. 1989. Agricultural pesticide use trends and policy issues. USDA. Agricultural economic report No 622. pp 93

Rasband, W.S. *ImageJ*, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2008.

Skeel, M.E. 1977. Further investigations on mudworms in oysters. Aust. Fish. pp. 22-23.

Smith, I.R. Diseases important in the culture of the Sydney Rock Oyster. Rep. Brack. Wat. Fish Cult. Res. Stn., N.S.W. Dept. Agric., 10pp

Stauber, L. A. and T.C. Nelson. 1940. Some observations on *Polydora ligni* Webster, a polychaeta worm on the oyster beds of Delaware Bay, New Jersey. Proc. Natl. Shellfish Assoc., Pro. Conv. Add., Milford Lab. Dedication . Aug 1,1940

Urbaniak, G. C., & Plous, S. (2011). Research Randomizer (Version 3.0) [Computer software]. Retrieved on April 22, 2011, from http://www.randomizer.org/

Webster D. 2007. Oyster Production Methods *www.mdsg.umd.edu/images/uploads/.../2_**Production**%20Methods. p...*

Willemsen, P R. 2005. Biofouling in European Aquaculture: Is There an Easy Solution? European Aquaculture Society Special Publication No 35: pp. 82-87. (Available from: www.crabproject.com).

Figure A
Aerial View of Delaware Bay Grow-out Site

Figure B
Map of New Jersey Showing Location of Grow-out Site

Figure C
Rack-and-Bag Oyster Farm

Figure D
Overhead View of One Bag Space
on Rack with Hooks

Figure E
View of Rack with Hooks and Straps, Oyster Bag Removed

Figure F
Layout of Racks on Oyster Farm
Showing Path for Quad and Worker Acess

Figure G
Overhead View of Oyster Bags with Varying Degrees
of Biofouling Caused by the Mudworm *Polydora ligni*

Figure H

Close-Up View of Biofouling on Oyster Bag

Figure I

Sample of Heavy Coverage

Figure J

Sample of Medium Coverage

Figure K

Sample of Light Coverage

Figure L
Another Exaxmple of Heavy Coverage

Figure M
Long View of Biofouling on Oyster Bag Rack

Figure N
Randomized Bag Location Field Layout

Figure O
Petri Dish Set-Up with Plexiglas and Quadrants

Figure P
Laboratory Recording Chart

Figure Q
Color Coding Master List

FIGURE R
Original Photographic Record and Duplicates

FIGURE S
Enhanced Subsamples

FIGURE T
Original Photorecord and Enhanced Subsamples

FIGURE U
Blue Histogram of Subsample 3

FIGURE V
Threshold Adjusted Subsample 2

FIGURE W

Enhanced Subsample 1 Compared to Theshold Adjusted Subsample 2

FIGURE X
Illustration of Brightness Saturation Subsample 3

FIGURE Y
Comparision of Enhanced Subsample 1,
Theshold Adjusted Subsample 2
& Illustrated Brightness Saturation Subsample 3

FIGURE Z
Adjusted Subsample 2

FIGURE AA
Histogram of Adjusted Subsample 2

FIGURE BB
Comparision of Original Photorecord to Adjusted Subsample

FIGURE CC
Database File Structure

FIGURE DD
WEEKLY SCORE SHEET

Figure EE
SAMPLE WEEKLY SCORE SHEET AND DATABASE