THERMAL SHOCK SPAWNING OF PHILIPPINE CUPPED OYSTER *Crassostrea iredalie*, Faustino 1932

A Thesis

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Major in Aquaculture

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APPROVAL SHEET

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DEDICATION

This masterpiece is dedicated to:

God Almighty, the source of courage, strength,

enlightenment, hope and everything;

My very supportive husband, Leonilo;

My Loving son, Jed Danniel;

My Loving daughter, Ma. Kristina;

My motivator, Nanay Memang;

And to my brothers, sisters and relatives.

Bing

ABSTRACT

Induced spawning of Philippines cupped oyster *Crassostrea iredalie*, through thermal shock was investigated. Five (5)temperatures was tested namely; 40°C, 30°C, 27°C, 20°C, and 15°C. A preconditioning protocol was established. Oyster's length ranging from 9.6 cm to 12.6 cm spawned effectively when exposed to higher temperatures compared to lower temperatures. Thermal shock spawning was effective at 30°C compared to 27°C treatments which spawned constantly over 5 runs. 40°C was lethal to the experimental animal. No success in stimulating spawning in cold temperatures at 20°C and 15°C was observed. Spawning rates at 1,338,034 cells/650ml/female in 30°C, and 825,735 cells/650ml/female in 27°C respectively, did differ significantly at (P>0.05). Immersal periods were shorter in 30°C at 1.82 hours compared to 27°C at 2.05 hours which differ statistically at (P>0.05). Fertilized eggs produced from 30°C were better in terms of survival or egg density per hour from fertilized egg to trochophore stage compared to that produced at ambient temperature 27°C.

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Chapter 1

THE PROBLEM AND ITS SETTING

Introduction

Philippine slipper cupped oyster locally known as "talabang tsinelas" is a high value species in the Philippines, produced from both wild stocks and aquaculture. FAO's aquaculture yearbook of fisheries statistics reports a range of yearly production from around 325 metric tons in 1995 to 225 metric tons in 1999 in the Philippines. In the same period, the yearly aquaculture production ranges from around 11,874 metric tons in 1995 to 13,698 metric tons in 1999 FAO, 2010).

Some 192 small-scaled fisherfolks are engaged in the culture of *C. iredalie,* involving an area of 101, 416m^2 in Malaysia (Anonymous, 1996). Although the spat supply of this species is only confined to east coast of Peninsular Malaysia, culture operation has been very successful on the west coast through spat transplantation programs. Culturist on the west coast are those solely dependent on this spat supply from the east coast. However, spat collection (peak season is being April to June and October to December) on the east coast are hindered by monsoons which often result to total mortality of spat (salinity reaching 0ppt) during the November to January period when the spat supply was disrupted for farms on the west coast. The culturist would then resort to importing oyster spat from western Thailand (Devakie and Ali, 2000).

Shortage of quality spats is a perennial problem in the Philippines oyster industry. The need of hatchery-produced spats for farming is indeed badly needed. To date, the use of hatchery-produced spat in conjunction with selective breeding strategies has only been developed at an experimental scale and not yet by any private company in the country. It is not like the tiger shrimp industry where everything is already in place, so one know what problems to expect and the proper adjustments to make. In the oyster hatchery, one cannot say which technique is effective and which is not. One simply look for solutions each time he/she encounters problems (Surtida et.al. 2001).

Induced spawning of oyster by thermal shock is a common hatchery technique by increasing temperature or decreasing to trigger spawning of a sexually ripe matured *C. iredalie* (Devakie and Ali, 2000). In the wild oysters spawning is triggered when the water temperature rises in the spring. Sperm fertilize eggs in the water column. Fertilized eggs develop and progress through a series of free-swimming larval stages over a period of 14 to 20 days, depending on water temperature (Wallace et. al., 2008).

These stages are referred to as the trochophore, veliger and pediveliger. The trochophore larvae feed on very small algae as they move through the water column. Trochophore larvae quickly develop into more motile veliger larvae. Toward the end of the larval cycle, pediveligers develop a foot that helps them

find a suitable hard substrate on which to attach (set) and transform into small oysters (Wallace et. al., 2008).

Thermal manipulation is more successful than that of desiccation (Tew Chew, et. al, 2016). Strip spawning is also a process of induced spawning of oysters, where eggs are washed through a 80 µm nitex mesh to separate the debris, collected on a 30 µm nitex mesh and subsequently pooled. Sperm then is mixed with the mature eggs (Petton et al. 2013). The increase in temperature affected the morphology and growth of oyster larvae. There was a significant difference noted between the larval size reared in higher temperature (34°C) and the controlled (27°C) and lower temperature (20°C) (Tew Chew, et. al, 2016).

Oyster hatchery is deemed necessary to provide sterile diploid and haploid spat. Hatcheries are essential tool for securing spat availability to the industry, and for the dissemination of genetic stability and improvement. There are several reasons why hatcheries exist. These include the need to restock wild fisheries which have been depleted, to satisfy the demand by culturists and shellfish farmers for a consistent, high quality source of seed and to produce organisms that are not normally available (ICESCIEM, 2004).

This study will contribute to the development and field testing of a hatchery technique via thermal shocked technology for oysters. Hence, this study attempted to address the issue on hatchery produced oyster spats free from any

contaminants, harmful algal bloom (HAB), and provide oyster farmers sustainable supply of high quality single oyster spats for grow-out.

Statement of the Problem

The study aims to enhance the spawning and density rate oyster, C. *iredalie*, using thermal shock. Specifically, it seeks to answer the following questions:

1. What is the morphometric characteristic of the shell?
2. What pre-preparation process requirement for spawning and conditioning of shell?
3. What is the spawning rate of oysters through thermal shock at different temperature levels such as;
   - 3.1 15 ^{0}C;
   - 3.2 20 ^{0}C;
   - 3.3 30 ^{0}C; and
   - 3.4 40 ^{0}C.
4. What is the density per hour of fertilized egg to trocophore stage produced at different temperature levels?

Hypotheses

There is no significant difference in spawning rate and survival rate of oysters through thermal shock at different temperature levels.

Theoretical Framework

Oysters on natural reefs are stimulated to spawn when the water temperature rises in the spring. The release of sperm and eggs into the water further stimulates other oysters to spawn (Wallace, et. al., 2008). Variations in water temperature are the most likely stimuli to cause spawning and so water is heated to a matching temperature before the water exchange takes place (O. Connor et al., 2008).

Thermal shock was achieved by alternating exposure of broodstock to ambient and elevated (+5-6°C) water temperatures until spawning commence (Gervis and Sims, 1992; Southgate, 2008). On this study, ambient water temperature (27°C) as control was tested and raised to 30°C and 40°C, then from ambient decreased to 20°C and 15°C, to evaluate and determine optimum spawning rate among temperatures.

Conceptual Framework

The study was conducted at SSU-COFMAS Multi-Species Hatchery Mercedes Catbalogan City. The focus of the study was on the effects of temperature on the induced spawning of oyster's C. *iredalie*, by thermal shock under hatchery conditions.

Five temperature ranges were tested namely; 20° C, 25° C, 27° C, 30° C, and 40° C, to determine the optimum temperature in which oysters effectively and

efficiently spawn with emphasis to the spawning rate and density per hour of fertilized egg to trocophore produced. Constant monitoring and documentation of spawning from fertilized egg to trocophore was observed.

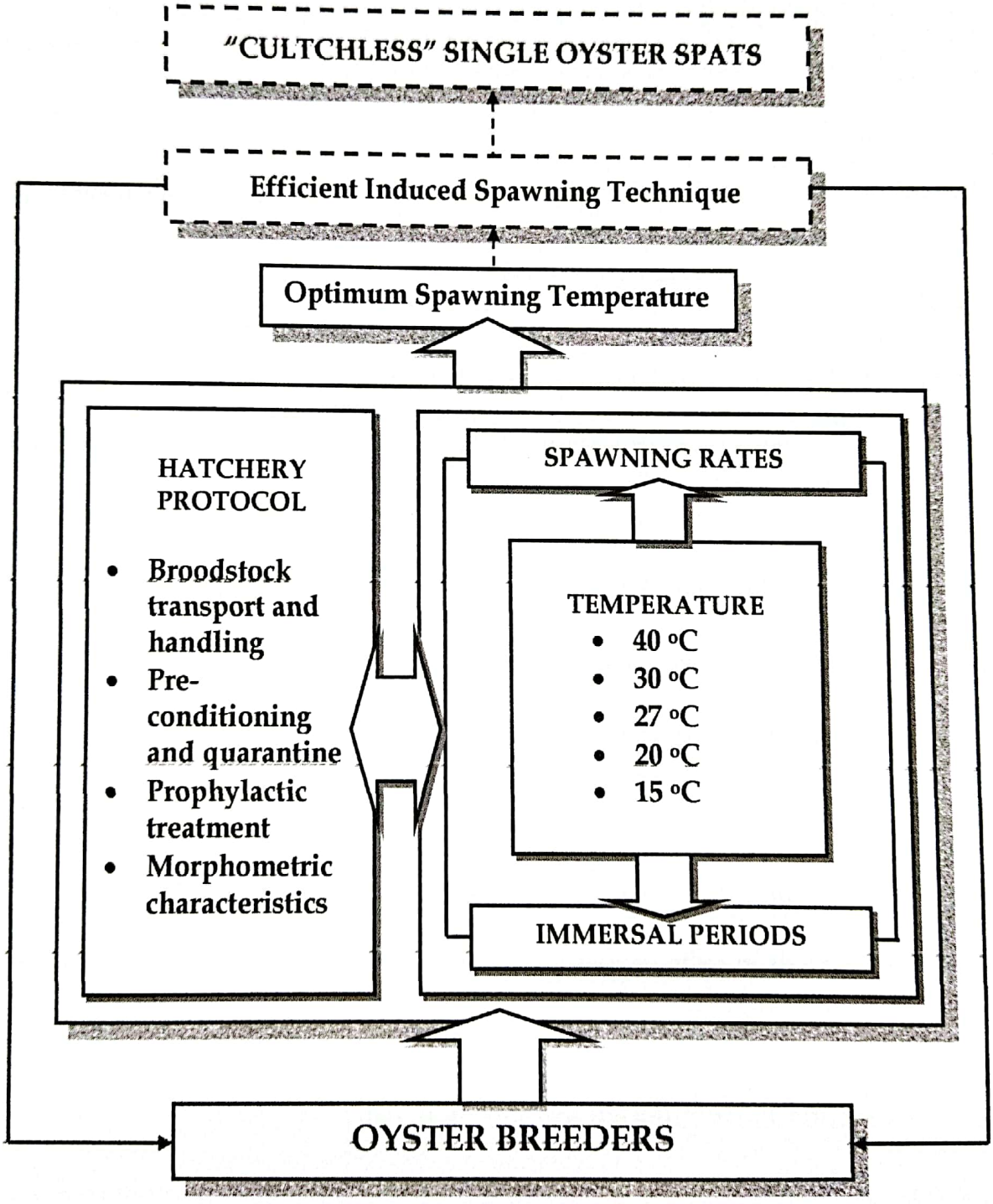

Figure 1. The Conceptual Framework of the Study

The results were subjected to statistical analysis and draw conclusions to recommend optimum conditions in respect to temperature on the spawning by thermal shock of oyster's C. *iredalie* in hatchery conditions. Furthermore, a protocol on thermal shock spawning of oysters was also partially formulated from the study.

Significance of the Study

Although oyster spawns throughout the year yet it cannot supply the demand of fish farmers in a sustainable manner. Furthermore, wild stocks are not readily available to our fish farmers even during off season. Thus, the results of this study was sought to address this issues by determining the feasibility of hatchery production of oyster spats by thermal shock to supply our farmers with readily available contaminant free, high quality oysters spats for farming. Moreover, the results of this research endeavour will directly or in one way benefit to the following stakeholder:

Investors/businessmen. The results of this study would enhance new business opportunities to businessmen and investors alike in the production and export of high value "cultchless" oysters.

Hatchery operators. Also, the results of the study would provide hatchery operators with an optimized hatchery protocol for the production of oyster spats using thermal shock technology.

Oyster farmers. The results of this investigation would directly benefit oyster farmers and provide them sustainable, contaminant free, high quality 'cultchless' single oysters for grow-out.

Researchers. Also, the study provide researchers first hand data that would serve as ready reference for future researches on the induced spawning of oyster through thermal shock.

Scope and Delimitation of the Study

This study focused on determining the spawning rate of oyster through thermal shock and limited to the effects of using low and high temperature levels such as 15°C, 20°C, 30°C, and 40°C respectively, increased or decreased from the control 27°C ambient water temperature. Oyster breeders used for the study was collected in Brgy. Bahay Tarangnan, Samar.

Pre-conditioning and conditioning preparations were standardized and followed in every thermal shock treatment. Thermal shock treatments were done in aquarias holding 30 liters of filtered seawater at every temperature treatment, egg collection was done after every 5 hours observation regardless of early spawning seizure. Egg counting was done using sedgewick and rafter counter in each treatment.

Definition of Terms

To promote understanding of the terms used in this research, these terms are defined conceptually and operationally.

Diploid or "Diploidy" (from the Greek word diplous, meaning 'double') is a term used to denote the existence of two sets of chromosomes within a cell or an organism (Wutz, 2014). In this study, it refers to male gamete.

Haploid. It is the condition of a cell having a one set of chromosomes (Hartwell et al., 2011). As used in the study, it refers to female gamete.

Induced spawning. Laying or spawning of eggs by artificial stimulation, e.g. changes in the light regime, fertility cycle, salinity or temperature, osmotic shock, UV irradiation (Fishbase, 2018). In this study, is a state of thermal shock spawning with varying temperatures such as 15°C, 20°C, 27°C, 30°C, 40°C.

Multi-species hatchery. A fish hatchery is a place for artificial breeding, hatching, and rearing through the early life stages of animals - finfish and shellfish in particular (Crespi, and Coche, 2008). Hatcheries produce larval and juvenile fish, shellfish, and crustaceans, primarily to support the aquaculture industry where they are transferred to on-growing systems, such as fish farms, to reach harvest size (FAO, 2010). In this study, it refers to hatchery with different purposes as to breeding, disposal and experimental.

Prophylactic treatment. A method of treating water and exposing cultured species at a certain extent or dosage so as to prevent or control of

parasitic, fungal, and bacterial diseases. As used in the study, it refers to the application of buffered formalin solution in UV treated filtered water at 4ppm for 10 minutes before quarantine so as to insure removal of disease causing bacterial pathogens.

Spawning rate. Conditioned oyster breeders are then placed in calibrated aquaria at 30 liters filtered seawater for thermal shock treatment. Due to the constraint of sex determination in oyster, five (5) oyster breeders are randomly selected for each treatment so as to allow favourable visibility on the aquaria for initial release of gametes during spawning. The number of broodstock used depends on the task hand and the size ad condition of the oysters to be spawned. While it is possible to have all the oysters to be spawned, it is wise to allow a generous margin of error (Connor et.al., 2008) . In this study, it refers to the number of eggs in which an aquatic organism releases or deposits in water.

Thermal shock. Is a process of alternating exposure of broodstock to ambient and elevated (+5-6oC) water temperatures until spawning commences (Gervis and Sims, 1992; Southgate, 2008). In this study, it refers to the different treatment used with corresponding temperature levels such as 15°C, 20°C, 27°C, 30°C, 40°C.

Trocophore. Free swimming larvae with hair-like cilia and it uses for swimming (Harvey, 1996). As used in the study, it refers to the stage size of a larvae survive within 5 hours which is the critical stage.

Chapter 2

REVIEW OF RELATED LITERATURE AND STUDIES

This chapter reviews various related literature and studies which are useful to the present study. The reviewed literature and studies provide relevant information and serve as guide in the formulation of the study particularly on the problem statement and variables used. This section presents and discusses ideas of authors of books, journals and others that were relevant to the research study.

Related Literature

For centuries, oysters have been a tantalizing delicacy. It has been documented that oysters were eaten in vast numbers by prehistoric man on the shorelines of Scandinavia more than 5000 years BC (Larsen et al., 1957). Oysters originated from Japan, where it has been cultured for hundreds of years (FAO, 2010). It is now the most widely farmed and commercially important oyster in the world, as it is very easy to grow, environmentally tolerant, and easily spread from one area to another (FAO, 2010).

Oyster hatcheries provide juvenile oysters for commercial production, restoration projects and research. (Wallace, et. al., 2008). Hatcheries are an essential tool for securing spat availability to the industry, and for the

dissemination of genetic stability and improvement. There are several reasons why hatcheries exist. These include the need to restock wild fisheries which have been depleted, to satisfy the demand by culturists and shellfish farmers for a consistent, high quality source of seed and to produce organisms that are not normally available (ICESCIEM, 2004).

An oyster hatchery simply creates a controlled environment for the early portions of the oyster life cycle (Wallace, et. al., 2008). Therefore, producers must understand oyster biology. Oysters occur naturally in dense aggregations, often called reefs or beds. Oysters thrive in estuarine waters with salinities of about 10 to 25 ppt, though they can tolerate lower and higher salinities. Oysters on natural reefs are stimulated to spawn when the water temperature rises in the spring. The release of sperm and eggs into the water further stimulates other oysters to spawn. This results in a mass release of reproductive products (Wallace, et. al., 2008).

Spawning oysters is the first step in the production of spat. As a rule, ten average females produce about 200 million eggs. Under good conditions 200 million eggs can result in 100 million or more early-stage larvae, which require 2,600 gallons (10,000 L) of treated water. Natural mortality and the need to thin out the larvae to proper densities should leave about 25 million eyed larvae ready for setting. Approximately 10 million spat can be expected from the 25 million eyed larvae (Wallace, et. al., 2008).

The early life stage is the most sensitive stage in the life cycle of a bivalve and its tolerance towards various environmental conditions increases as they develop into a benthic juvenile (Bayne et al. 1976; Chapporo et al. 2008). Once fertilized, the larval oysters or clams are held in tanks, fed several times daily, and develop over the course of about two weeks. During this time, they go through several developmental stages, even growing a small foot, which they use to detect proper substrate. When the larvae are ready, they will undergo a true metamorphosis, drastically changing their body shape into the form that you eventually recognize as an oyster or clam (NOAA, 2017).

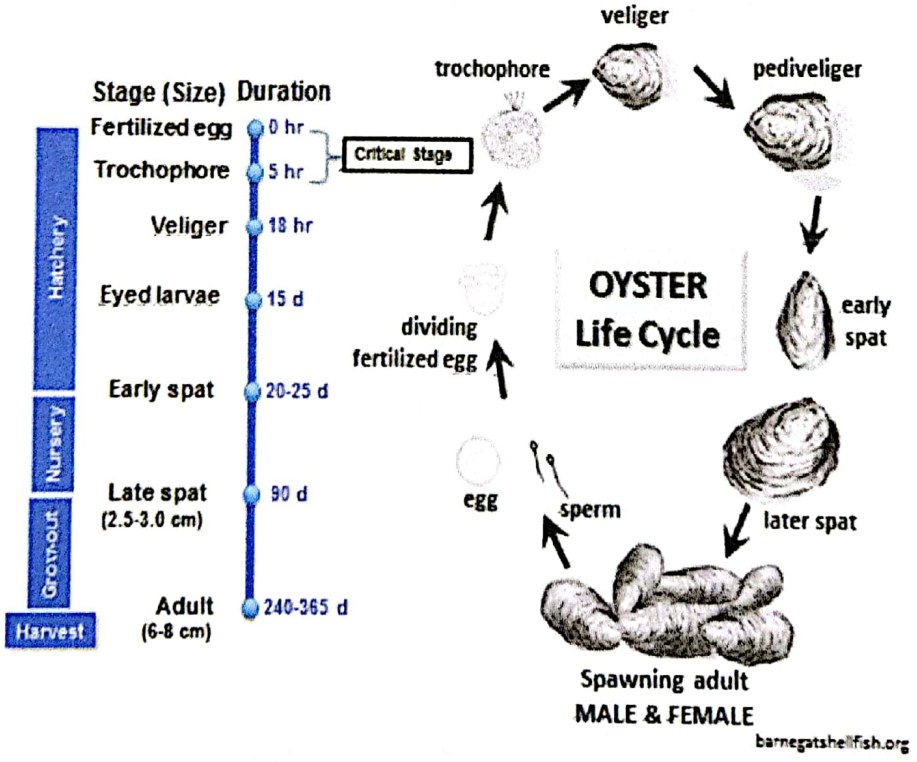

Figure 2. Life Cycle of Oyster

Oysters of the genus *Crassostrea* in general are considered to be euryhalineorganisms (Quayle and Newkirk, 1989). They have the ability to adapt well to temperature fluctuations (Angell, 1986.). Although temperature changes within the tropical or subtropicalzone may appear to have much less influence on the biology of bivalves, controlled laboratory experiments are still required to determine the effects of temperature,including effects with other parameters such as salinity and temperature (Devakie and Ali, 2017).

Researchers have studied the effects of temperature and salinity on the development and growth patterns of bivalve species in the wild and in the laboratory e.g., (Paul, 1980; Tettelbach and Rhodes, 1981); (Kalyanasundram and Ramamoorthi, 1986). Studies in temperate countries have been conducted in the laboratory to examine the combined effects of salinity and temperature on the larval settlement of *C. Gigas* (Lund, 1971;Henderson, 1983).; the effects of salinity on settlement of *C. Íirginica* larvae (Hidu andHaskin, 1971).; and the effects of temperature on settlement of *C. gigas* larvae settlement (Cooper and Shaw, 1984; Scholz et al., 1985).

Related Studies

Thermal or induced spawning techniques are the mainstay for spawning oysters. Oysters are placed in spawning tanks. Spawning is triggered by thermal shock, attained by progressively increasing the seawater temperature from 15 to 20-25 "C. *P. maximus* release male or female gametes indiscriminately (true hermaphrodites); however, initial spawns are generally males. Gametes are not released all at once but over a series of emissions (white for the spermatozoa, orange-coloured for theocytes) (Robert and Gerard, 1999).

Broodstock were removed from the conditioning system and cleaned using providone-iodine antiseptic solution and then left out of water in a cool dry area (16-20° C) for 24 hours before spawning induction (O. Connor, et. al. 2008). Spawning is typically undertaken in the spring when water temperatures rise above 77 °F (25 °C) in southern waters (K. Wallace et.al. 2008). From a study conducted by Tew et. al. in 2016, stated that the highest survival rate occurred when the larvae were reared in 20°C and 27°C. Larvae reared at 34°C exhibited reduced survival but increase in the growth rate. The growth rate in larvae reared in high temperature (34°C) was significantly higher compared to larvae reared in 20°C and 27°C.

Furthermore, temperature is a vital factor that influences the larval development, survival, and spawning of most of the marine invertebrate (Eversole 2001; Barber & Blake 2006). Thus, small fluctuation of the seawater

temperature will depress the rate of the survival and development of bivalve larvae (Loosanoff 1965; Fritz 2001; Cragg 2006).

From the Google Patent Database, publication number US4834024A, entitled Inducing Polyploidy in Oyster authored by Standish K. Allen, Jonathan A. Chaiton, Sandra L. Downing. A method of inducing polyploidy in oysters through the use of hydrostatic pressure is disclosed. The method includes separating oysters from one another such that male oysters are separated from female oysters, inducing the oysters to spawn, controlling the temperature of eggs from the oysters, fertilizing the eggs with sperm to form zygotes and then subsequently applying hydrostatic pressure to the zygotes to induce polyploidy.

In the case of Pacific oysters, in particular *Crassostrea gigas*, limiting the reproductive potential can be especially significant. In general, quality oysters are characterized by high levels of glycogen, which add texture and flavor to the meat. During the breeding season, the market quality of the oyster deteriorates because glycogen is diverted into gamete production instead of being stored. Triploid, and therefore neutered oysters will have reduced potential for maturing. Consequently, there exists a need in the art for a method of inducing polyploidy in oysters on a consistent and costs effective basis. The present invention fullfills this need, and further provides other related advantages. Utilizing this general method, approximately 30% triploidy may be obtained. When the eggs are maintained at approximately 25° C. and pressures of

approximately 6000 to 10,000 psi are employed, the pressure is applied approximately 15 minutes after fertilization for duration of 15 minutes. Triploid oysters may be obtained via the use of hydrostatic pressure at other temperatures if the timing of pressurization coincides with the formation of polar bodies. Although it is possible to induce polyploidy using pressures greater than 6000 to 10,000 psi, it is preferable to remain within this range due to the higher mortality rates exhibited when higher pressures are used (Allen *et.al*, 2006).

The present invention relates to the production of polyploid animals in general, and more specifically, to a method of inducing polyploids in oysters through the use of hydrostatic pressure. Triploids can have practical benefits in aquaculture. These benefits arise from the reproductive sterility that a triploid animal is expected to display. By circumventing the otherwise normal energy expenditure associated with maturation, ripening and spawning of gametes, triploids have been shown to surpass their diploid counterparts in growth and survival (Stanely, J. *et al.*, 1984).

Chapter 3

METHODOLOGY

This chapter discusses the method and procedure employed by the researcher in gathering pertinent data as well as necessary preparations of materials and equipment used in testing the hypothesis in the conduct of the study. This includes the research design, instrumentation, validation of the instruments, sampling procedure, data gathering procedure and statistical treatment of data.

Research Design

The research design was experimental considering that 5 temperature ranges were tested for spawning oysters using thermal shock in identifying the most efficient temperature for spawning and survival rates of fertilized oyster eggs to trocophore stage.

This study aimed to determine the induced spawning of oyster *Crasostrea iredalie* by thermal shock at different temperatures namely; 40^{0}C, 30^{0}C, 27^{0}C, 20^{0}C, and 15^{0}C. Hence, hatchery operation was employed using experimental laboratory. Five temperatures as treatments was tested and was categorized as high; 30^{0}C(T-1), 40^{0}C(T-2), while in low temperature; 15^{0}C(T-3),20^{0}C(T-4) and 27^{0}C(control), both high and low temperatures were ran five times using thermal

shock. Size ranges and individual weight, were also recorded and categorized into large and medium sizes.

Eggs collected during the immersal periods, morphometric data, and parameters within the aquaria from each treatment were tabulated accordingly using descriptive statistics. Egg density counts were presented in table and figure form. To assess performance from each temperature treatments, single factor ANOVA was employed and further analyzed by correlation coefficient analysis.

Instrumentation

To gather pertinent data, several instruments were used in the laboratory setup, techniques in the conditioning and induced spawning protocols including documentation listed below:

Glass aquaria. A glass aquaria measuring 3'x1.5'x1.5'ft. with a capacity of 100L was used. Oyster breeders were strategically placed inside with 30 liter volume of filtered sea water ready for thermal shock process.

Multi-parameter interphase. Oceanographic equipment used to monitor biological parameters in the aquaria specifically temperature, salinity, dissolved oxygen, and acidity level during the conduct of the thermal shock process. Probes specific to a parameter were positioned inside the aquaria connected to an interphase to monitor parametric ranges during the thermal shock process.

UV sterilizer. Ultraviolet water sterilizer was used to treat or disinfect marine water used for induced spawning to insure no living organisms e.g. plankton and other plankton like organisms are present on the marine water to be used.

Water heaters. Portable water heaters were used in increasing marine water to be poured in the aquaria to increase water temperature at the desired temperature to be tested. 3 liter treated seawater are poured to the heaters then turned on until water is 86-90°C then poured to the aquaria until the desired water temperature is reached.

Vernier caliper. Used in gathering morphometric data of oyster breeders to be subjected in thermal shock induced spawning procedure. Before prophylactic treatment and conditioning, length measurements were taken. Morphometric measurements include total shell length, width, and thickness in centimeters.

Analytical balance. An instrument used in determining total weight, meat weight, and shell weight, of oysters to be subjected in thermal shock induced spawning procedure. Oyster breeders (shell on) are simply placed on the scale to measure total weight or wet weight in grams.

Inverted microscope. Used in the density and metamorphosis monitoring of fertilized eggs produced by thermal shock. This instrument was used after each

thermal shock treatment and after every egg collection, 1ml samples from a 650ml fertilized egg concentrate is collected by a pipet and placed on the sedgewick rafter for counting.

Sedgwick rafter. Sedgewick Rafter counting chambers were intended for counting of particles and microorganisms in water or other transparent liquids. The cell of 50 x 20 x 1 mm (= 1 cm^3) is ruled in a 1 mm grid subdividing 1 ml in 1000 µl and used for counting egg collected from each induced spawning treatment. 1ml samples from a 650ml fertilized egg concentrate were collected by a pipet and placed on the sedgewick rafter for counting.

Graduated cylinder. Instrument used to measure the total quantity or volume of eggs produced from each induced spawning treatment. Prior to thermal shock, graduated cylinder was used to measure volume of hot/cold saltwater by adding and pouring water reaching the desired water temperature prior to thermal shock.

Seawater ice cubes. Before thermal shock process, seawater ice cubes was used to decrease the ambient water temperature by reaching the desired temperature. Salt water ice cubes are melted in 5 gallon filtered seawater concentrate and poured to the aquaria until the desired water temperature were reached.

The data gathered were tabulated, classified, organized, and presented in statistical form. The data processing tool used was MSOffice Excel and Statistica software. Statistical methods employed were the following: frequency count, percentage, arithmetic mean, standard deviation, weighted mean, single factor ANOVA, and correlation coefficient analysis.

Validation of Instruments

All activities in the conduct of the study followed standard *in vitro* operating procedures including all instruments and equipment's to be calibrated following instructions and manuals of the specific instrument that was used.

Glass aquaria. Glass aquaria was calibrated and labelled upright on its side by a masking tape and permanent marker. Each grid measured 5 liters of water up to 100 liters capacity.

Multi-parameter interphase. Oceanographic equipment (Science Cube KDS E-A900-05-3048A) multi parameter interface connected to a laptop with corresponding probes was used to monitor salinity (ppt; salinity probe KDS-1055), dissolve oxygen (O^2ml/L; KDS-1043), temperature (^oC; temperature probe KDS-1013), pH (KDS-1048), all probes was calibrated using calibration procedures and ammonium electrode standard solution at 100ppm prescribed standard to the

equipment before use. All parameters were measured before and after the induced spawning procedure using thermal shock.

UV sterilizer. The water sterilizer was installed in a horizontal direction. From the reservoir tank marine water passes through a filtration tank system into the UV sterilizer and distributed to the aquaria's.

Water heaters. Five heaters with a capacity of 3 liters each, was used to increase water temperature of seawater up to 88^{0}C, the calibration process was done 3 times to insure accuracy. Temperature treatments and volume of hot water needed to increase ambient water temperature is shown on the table below:

Table 1. Required Volume of Hot Sea Water to Increase Water Temperature at 88^{0}C.

Treatment (^{0}C)	Required Volume of Hot Water at 88^{0}C (liters)
40	15
30	3.5

Table 1 shows the required volume of hot sea water to increase water temperature at 88^{0}C. In 40^{0}C, the required volume of hot water is 15 liters and 3.5 liters in 30^{0}C.

Vernier caliper. As used to gather morphometric data from mussel. Measuring process is shown below (Figure 3) to compute for total cavity volume thus, condition index.

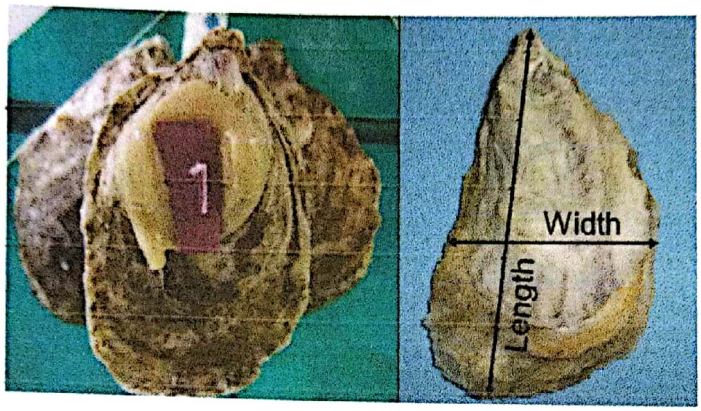

Figure 3. Standard Measurements of Oyster *C. iredalie*

Analytical balance. Meat, shell, and whole weights of oyster breeders were determined using analytical balance (0.1g) before lengthwise data is taken. Mortalities and or cracked specimens were discarded so as to insure homogeneity of samples.

Inverted microscope. The inverted microscope was used in conjunction with sedgewick rafter and handy tally counter for counting eggs collected at every treatment. To insure accuracy of counting the microscope were always set to 4X magnification at low light intensity, counting is done after an hour the eggs are collected.

Sedgwick rafter. A distinct sedgewick rafter was used on all samples for counting fertilized eggs to insure homogeneity of results. Counting was done by taking 1ml sample from a 650ml fertilized egg concentration in a graduated cylinder.

Graduated cylinder. A 1000ml of hot/cold saltwater is added gradually until the desired temperature is reached.

Saltwater ice cubes. Filtered sea water was made into ice cubes and was used to decrease water temperature in the aquaria before thermal shock. Saltwater ice cubes were weighed and melted in basins with filtered sea water and then poured to the aquaria until the desired temperature reached, the calibration process was done 3 times to insure accuracy. Temperature treatments and volume of cold saltwater at 3°C needed to decrease ambient water temperature as shown on the table below.

Table 2. Required Weight of Ice to Decrease Water Temperature at 3°C.

Treatment (°C)	Required Volume of Cold Water at 3°C (Liters)
20	8.5
15	13

Table 2 shows the required weight of ice to decrease water temperature at 3°C. For 20°C, the required volume of cold water is 8.5 liters and 13 in 15°C .

Data Gathering Procedures

In data gathering procedure, the researcher used the standard *in vitro* operating procedures using aquaria systems in thermal differential were observed.

Raw materials (oyster breeders) were collected from Brgy. Bahay Tarangnan, Samar. Three sacks or 15 hanging cultivation lines containing 20 oyster breeders in average per line were harvested from raft method, to maximize number of samples and allow selection in terms of length and weight in relation to fecundity. Cultivation lines were placed in sacks and transported via land to the hatchery immediately upon harvest to minimize stress.

Upon arrival, oyster breeders were removed from their clutches individually and were cleaned from barnacles, algae, or other fouling organisms using hard bristle brush removing any soft sponges, sea grass, mud, bryozoans, and etc. then rinsed in a basin with tap water.

Morphometric data (wet weight, length, width, thickness) were gathered using analytical balance (0.1g) and vernier caliper by standard methods.

Oyster breeders were subjected to prophylactic treatment using buffered formalin solution at 4ppm for 10 minutes before quarantine. Quarantine was

done by (4 hours depuration) and pre-conditioning so that felts and parasites and other bio-fouler organisms were removed.

Oyster broodstock samples were conditioned in strong aerated tanks or aquarium prior to induce spawning to stimulate recovery from stress of pre-conditioning. While conditioned oyster breeders were placed in calibrated aquaria at 30ltrs filtered sea water for thermal shock treatment. Five (5), oysters breeders are randomly selected for each treatment so as to allow favourable visibility on aquaria for observation during spawning. Size ranges was also random in selecting broodstock and were treated for thermal shock, so as to allow diversity in terms of length of oysters samples.

From the ambient water temperature, the broodstock were subjective to high (40^0C and 30^0C) temperatures for treatment 1 and 2, respectively. Then reduced temperature (low) for treatment 3 (25^0C) and treatment 4, (20^0C) and the control is at 27^0C.

Water temperature in the aquaria were closely monitored by the interphase connected to laptop, and was maintained in each treatment by constantly adding hot/cold saltwater when fluctuations are visible. As oyster breeders begin to spawn adding hot/cold saltwater is seized to prevent cease of spawning activity.

The immersion periods for each treatment was also observed and recorded. Spawned larvae was syphoned and collected using 20 μm sieves then put in

beaker (1000ml cap.) in 650ml volume of filtered seawater then counted using microscope at 4x magnification with a Sedgwick-Rafter and tally counter. The counting of eggs spawned was done hourly for every treatment right after eggs were collected at 5 hours observation.

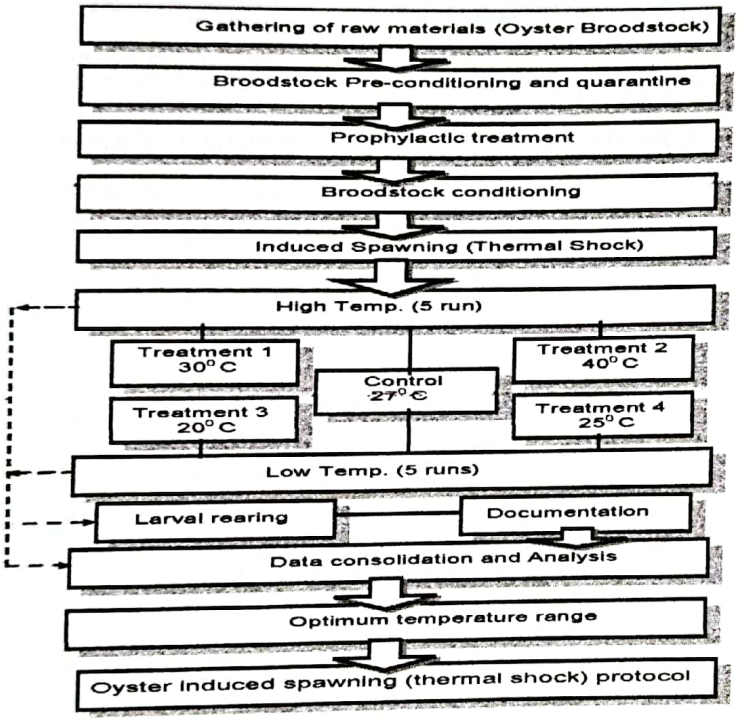

Figure 4. Workflow on Data Gathering Process

Statistical Treatment of Data

Raw data was transformed to arcsine values prior to analysis to normalize the data, based on Snedecor and Cochran 1989. Raw data from triploid gametes were subjected to test of normality Kolmogoroff-Smirnoff test.

Single-factor ANOVA was used to determine if interactions occurred between temperature and spawning rate effects, and in between immersal period and temperature ranges (high and low) effects. Larval sets from different treatments were assessed by using one-way ANOVA, while comparisons of their means were conducted using Tuckey's honesty significant differences (HSD) method.

Moreover, data from each induced spawning procedure was pooled by considering all size batches together to evaluate the overall growth rate for each treatment. All analyses were performed using Statistica Version 10 Software and MSOffice Excel program.

Chapter 4

PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA

This chapter presents the data based on hatchery *in vitro* experiment with the corresponding analysis and interpretation. Included in this chapter were the morphometric characteristics of oyster breeders in relation to its spawning behavior through thermal shock and what pre-preparations and conditioning that needs to efficiently spawn at the optimum temperature by thermal shock induced spawning. Further, this chapter reveals the survival rate of fertilized eggs to trocophore stages of oysters produced through induced spawning.

Morphometric Characteristics

A total of 120 experimental animal of matured oyster breeders were treated by induced spawning. Out of 120 conditioned and subjected to induced thermal shock 40 oyster samples spawned with length range of oyster spawned are reflected on the table below: Spawning oysters is the first step in the production of spat. As a rule, ten average females produce about 200 million eggs (Wallace, 2008). Under good conditions 200 million eggs can result in 100 million or more early-stage larvae, which require 2,600 gallons (10,000 L) of treated water. Natural mortality and the need to thin out the larvae to proper densities should leave about 25 million eyed larvae ready for setting. Approximately 10 million spat can be expected from the 25 million eyed larvae (Connors et.al., 2012).

Table 3. Length Ranges of Oyster Spawned Via Thermal Shock

Size range (cm)	Total Samples Treated (ind.)	Total Samples Spawned (ind.)
7 to 9	25	3
9.1 to 12	49	23
12.1 to 15	46	14
Total	120	40

Table 3 shows the total number of oyster subjected to thermal shock spawning. Out of 120 only 40 individuals responded to thermal shock or 33% of the total number reacted to different temperature gradient. On the other hand, 77% of the total number of experimental animals was not yielded to spawn using either increased temperature or decreased temperature. Spawning is typically undertaken in the spring when water temperatures rise above 77°F (25°C) in southern waters (Connors et al., 2012).

The maximum shell length observed to spawned was 15.60cm and the smallest was 7.60cm, mean length was 10.91cm. As shown on the table, 3, 33.33% of the total population spawned from a total 120 oysters treated.

Table 4. Class Sizes of Spawned Oysters

Class sizes	Oysters spawned(ind.)
7.5 - 8.5	2
8.6 - 9.5	4
9.6 – 10.5	5
10.6 – 11.5	8
11.6 – 12.5	9
12.6 – 13.5	12
13.6 – 14.5	0
14.6 – 15.5	0
15.6 – 16.5	0

As shown in Table 4, class size between 12.6 cm to 13.5 cm shell length that responded to thermal shock followed by 11.6cm to 12.5cm shell length, while bigger shell length did not yield to spawn. In either case, oysters larger than 3 inches (76 mm) were selected, although males were smaller, and then a sample was examined for reproductive readiness. Experienced hatchery personnel can judge the state of "ripeness" by removing the right or upper shell and noting the development of the gonads (Wallace, 2008).

Pre-condition

The pre-conditioning phase of the protocol starts when the oyster breeders arrived in the hatchery. Oyster breeders were still attached to their cultches to minimize stress and mortalities at transport. They were immediately cleaned and dislodged from their substrate individually. Barnacles, algae, and other fouling

organisms such as soft sponges, seagrass, mud, bryozoans, etc. are gently scrubbed using hard bristle brush then rinsed in a basin with tap water before morphometric data is taken. The range of temperatures chosen was based on the water temperature monitored from the oyster setting tanks in the hatchery (Devakie, *et.al*, 2012) were used at five temperatures 24^{0}C, 27^{0}C, 30^{0}C, 33^{0}C, and 36^{0}C.

Prophylactic treatment. Oyster breeders were subjected to prophylactic treatment using buffered formalin solution in UV treated filtered water at 4ppm for 10 minutes before quarantine so as to insure removal of disease causing bacterial pathogens.

Quarantine. By obtaining broodstock from coastal locations, the hatchery is vulnerable to translocation of diseases and pathogens into the hatchery (O. Connor et al., 2008). Quarantine was done by four (4) hours depuration so that felts and parasites and other bio-fouling organisms was removed. Broodstock were placed in 2 ton capacity tanks in a recirculating flow through water system for 4 four hours without feeding.

Broodstock conditioning. Broodstock samples are conditioned in strong aerated tanks at 20 ind./m^2 stocking density for a day feed with *chlorella spp.* at 450 cells/ml/1ltr tank water volume prior to induce spawning to stimulate recovery from stress of pre-conditioning. Water change during conditioning was

not permitted to prevent premature spawning due to the sudden fluctuations in water temperature. For variations in water temperature were the most likely stimuli to cause spawning (O. Connor et al., 2008).

Spawning rate

Conditioned oyster breeders were placed in calibrated aquaria at 30ltrs filtered sea water for thermal shock treatment. Due to the constraints of sex determination in oysters, five (5) oyster breeders were randomly selected for each treatment so as to allow favorable visibility on the aquaria for the initial release of gametes during spawning. The number of broodstock used depends on the task at hand and the size and condition of the oysters to be spawned. While it is possible to have all the oysters to be spawned, it is wise to allow a generous margin of error (Connor et al., 2008).

Oyster spat are predominantly male. The sex ratio after the breeding season is influenced by environmental conditions and physiological stress. Oysters that settle in unfavorable environments or experience physical injury do not tend to develop as females. Coping with environmental or physiological stress may limit the amount of energy that can be invested in female gonad development (Lorio and Malone, 1994).

A significant amount of eggs and sperm can be produced by just a few oysters, but not every oyster may spawn and it is a good practice to have the eggs and sperm from several oysters. Therefore, 20 to 30 large oysters are thoroughly cleaned and scrubbed, then placed in a shallow, black tank containing 4 to 6 inches (101 to 153 mm) of filtered (Wallace, 2008).

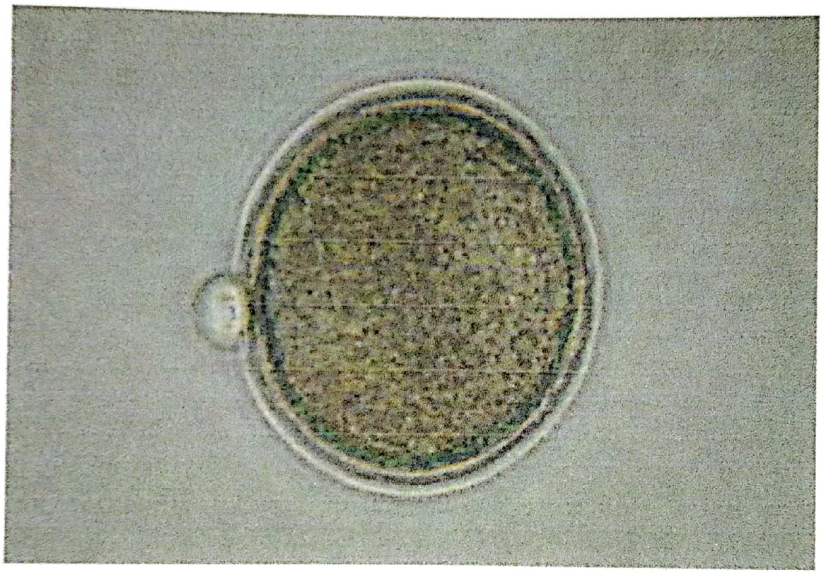

Figure 5. Fertilized Egg with First Polar Body (after 15-20 minutes).

The critical phase of the process was that, as soon as hot/cold water is introduced to the aquaria observations must be constant and consistent for sex determination of oyster breeders. When oysters begin releasing gametes, the whitish sperm and eggs can be easily seen against the black background of the tank. Males release a near constant stream of sperm and females release eggs during periodic shell closures (Wallace, 2008). Fecundity (number of eggs

released) in oysters is high, with the female capable of producing in excess of 20 million egg per spawning, while a male produces many times this number of sperm. (Connor, 2008).

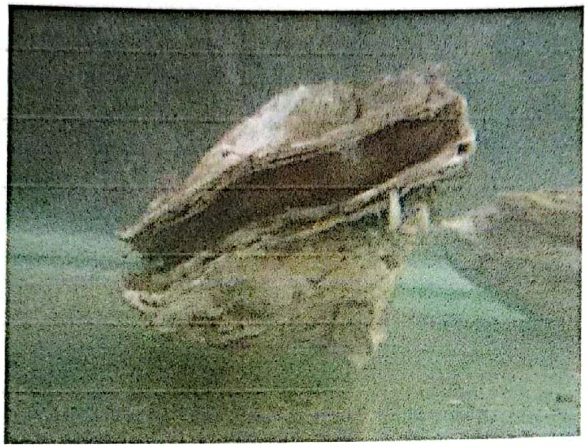

Figure 6. Male Oyster while spawning

Figure 7. Female Oyster while spawning

Figure 6 and 7, shows the spawning activities and behavior of male and female oysters. Female was first spawned and more gametes were released many

times and the body moves upon spawning. Then followed by male, less gametes were released.

Table below presents the average collected eggs from 5 treatments at 5 thermal shock runs. It can be viewed that spawning only occurred in treatment 2 and 3, with an average of 2.67:1.50 male to female ratio in treatment 2, and 2.50:1.25 in treatment 3 with an average spawning period of 1.51 and 1.92 hours respectively. Water temperatures above 35^{0}C may limit the extent of spawning. Spawning does not depend directly on tidal cycles except in shallow bays where the water may warm up rapidly during low tide and stimulate spawning (Lorio and Malone, 1994). Within 45 minutes of spawning all the eggs can be sieved on a 50-µm screen to remove debris (Wallace, 2008). In the study 5 hours of observation was done after removal of spawned gametes.

Table 5. Average Eggs Collected from 5 Treatments in 5 Runs

Treatment		Temp. (^{0}C)	Male (ind.)	Female (ind.)	Average Spawning Period (hours)	Average Eggs Collected (cells/650ml)
High	1	40	0	0	0	0
	2	30	2.67	1.50	1.51	1,973,786
Control	3	27	2.50	1.25	1.92	1,039,311
Low	4	20	0	0	0	0
	5	15	0	0	0	0
Averages			**1.03**	**0.55**	**0.69**	**602,619.40**

The ambient water temperature (27°C), and high temperature stimulated spawning at 30°C and differ statistically at ($P>0.5$). Therefore, the results were consistent in contrasts to the study of (Wallace et al., 2008) that spawning is typically undertaken in the spring when water temperatures rise above 77°F (25°C) in southern waters. Sexually mature oysters are placed on the spawning table in seawater that is the same temperature as that from which they are being held for conditioning, commonly 24°C, 35% salinity and filtered to 1µm (Connor, 2008).

At 40°C treatments probed lethal consequences to oyster breeders after 5 hours immersion period. In the process of increasing the temperature 5°C per 10 minutes, it was observed that upon reaching 37°C oyster breeders close their shells and response to the warm water was seized there was no spawning observed thereafter. Cheney et al. (2000) demonstrated that oysters can survive repeated exposure to high temperatures over 40°C.

Mean spawning rates per female from five induced spawning treatments at 5 runs are reflected on table 7. It can be seen that oyster breeders spawned much constantly in higher temperatures (37°C) which spawned at every run compared to lower temperatures which has no success of inducing spawning. The control at 27°C, induced spawning only 3 times in five runs which is attributed to the reproductive condition or the reproductive readiness of the oyster breeder used in

thermal shock process. As explained by (O. Connor et al., 2008), that period of conditioning depends on the reproductive condition of the oyster when they enter the hatchery.

Table 6. Average Spawning Rate from 5 Treatments

Treatment		Temp (°C)	Spawning (cells/650ml/female) @ 5 runs					Ave
			1st	2nd	3rd	4th	5th	
High	1	40	0	0	0	0	0	0
	2	30	1,445,600	1,326,547	1,265,354	1,476,435	1,176,236	1,338,034
Control	3	27	0	1,050,400	1,254,305	0	998,234	825,735
Low	4	20	0	0	0	0	0	0
	5	15	0	0	0	0	0	0

Egg densities and immersal periods, from two successful thermal shock treatments namely (27°C and 30°C), were then transformed to arcsin values to normalize the data through arcsin transformation and analyzed using analysis of variance single factor.

Table 7. Immersal Periods from 5 Treatments at 5 Runs

Treatment		Temp (°C)	Spawning (hours) @ 5 runs					Ave
			1st	2nd	3rd	4th	5th	
High	1	40	0	0	0	0	0	0
	2	30	2.3	1.47	1.32	2.1	1.89	1.82
Control	3	27	0	3	2.51	0	2.7	2.05
Low	4	20	0	0	0	0	0	0
	5	15	0	0	0	0	0	0

Statistical analysis reveals that there is significant difference among egg densities produced at different temperatures and in immersal periods at ($P>0.05$) level of significance.

Table 8. Analysis of Variance Output

Source of Variation	SS	df	MS	F	P-value	F crit
Egg Density (27°C, 30°C)	2.0481E+11	4.0000E+00	5.1202E+10	1.2065E-01	9.7349E-01	2.8661E+00
Total	8.6926E+12	24				
Immersal periods (27°C, 30°C)	1.1258	4.0000	0.2815	0.2110	0.9293	2.8661
Total	27.807736	24				

Density from fertilized egg to trocophore

After 5 hours of observation all the fertilized eggs was sieved on a 20µm plankton net and concentrated in a 1000ml beaker at 650ml concentrated volume and counted. Average density counts per hour are seen on figure below:

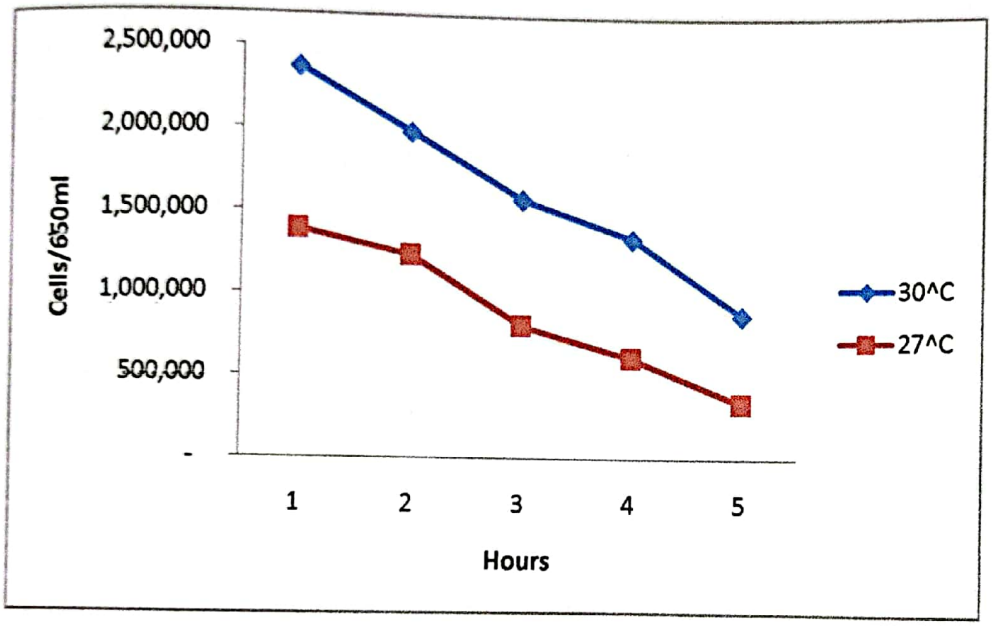

Figure 8. Density of Fertilized Egg Per Hour

The result shows that the density rate is declining from cells over time. Larvae are ready to set when they have a well-developed eye spot and are 290 µm or more in length. Larvae that are ready to set are usually selected by sieving them through a 180-µm screen (254-µm diagonal opening). Larvae that pass through are restocked. The retained larvae are sieved again on a 210-µm screen (296-µm diagonal opening). Those that pass through are also restocked to a

separate tank. The retained larvae (larger than 296 µm) are pooled and counted before being transferred to setting tanks. This procedure is repeated every day until the desired number of eyed larvae is obtained or the number of eyed larvae dwindles to the point that it is no longer effective to continue (Wallace et al., 2008).

Chapter 5

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the summary of findings with the corresponding conclusions based on the findings of the study and the recommendations based on the conclusions drawn from the findings of the study.

Summary of Findings

The following are the salient findings of the study:

1. It was observed that oyster's length ranging from 9.6cm to 12.6cm spawned effectively when exposed to higher temperatures compared to lower temperatures. Maximum shell length was at 15.60cm, smallest was smallest was 7.60cm. From a total 120 oysters treated 33.33% of the total population spawned.
2. Pre-conditioning process in thermal shock spawning of oysters includes the cleaning and removal of any attached fouling organisms to the oyster shells, before prophylactic treatment to insure removal of disease causing bacterial pathogens. Quarantine is also performed by 4 hours depuration to effectively remove other remaining felts and parasites. Lastly, broodstock conditioning is essential to stimulate recovery of oyster broodstock from the pre conditioning process.

3. Induced spawning was effective at 30°C compared to 27°C treatments, which spawned constantly over 5 runs. 40°C was lethal to the experimental animal with no response to the thermal shock process.
4. There was no success in stimulating spawning in cold temperatures 20°C and 15°C.
5. Spawning rates was at 1,338,034 cells/650ml/female in 30°C, and 825,735 cells/650ml/female in 27°C respectively, and did differ significantly at (*P>0.05*).
6. Immersal periods were also shorter in 30°C at 1.82 hours compared to 27°C at 2.05 hours which differ statistically at (*P>0.05*).
7. Fertilized eggs produced from 30°C was better in terms of survival or egg density per hour from fertilized egg to trocophore stage compared to that produced at ambient temperature 27°C which showed inferior performance.

Conclusions

From the aforecited summary of findings, the following conclusions were drawn:

1. Oysters breeders ranging 9.6 to 12.6cm in length are most likely to stimulate spawning by induced spawning through thermal shock.

2. The pre-conditioning protocol used was effective with minimal mortalities observed. This stage is critical to avoid translocation of pathogens, and bacteria to the hatchery and minimize spread of diseases thus mortalities.
3. The optimal temperature to use in induce spawning of oyster through thermal shock is at 30°C which produced significantly higher spawning rates, lesser immersal periods, and higher survival rates of fertilized egg to trocophore development stage.
4. Survival rate or density of eggs per hour from fertilized egg to trocophore was superior in 30°C.

Recommendations

From the conclusions drawn based on the findings of the study, the researcher strongly recommends the following:

1. It is highly recommended to utilize oyster breeders ranging from 9.6 to 12.6cm in length. Results from the study reveals that oysters within this sizes ranges are most likely to spawn through thermal shock induced spawning process.
2. The pre-conditioning protocol is effective but it is recommended to further enhance or refine feeding regimes on broodstock conditioning and test other prophylactic treatment processes.

3. It is recommended to use the optimum temperature at 30°C, in induced spawning of oysters through thermal shock process. Results from the study reveals that this temperature stimulates spawning effectively and efficiently, for it induces higher spawning rates in lesser immersal periods. Further, it produces high quality eggs, which performs relatively higher survival rates in larval development from fertilized egg to trocophore stages.

4. It is also recommended to further refine the technology through further investigations and utilize other induced spawning techniques such as application of regents e.g. serotonin, etc. and other parametric manipulations to stimulate spawning.

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APPENDICES

APPENDIX A

Table 9. First Run, Egg Density and Immersal Periods at Different Thermal Shock Treatments

Treatment		Temperature (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40			*	*	*	*	*			
	2	30	3	2	1	1	1	*	*	2.3	2,891,200	1,445,600
Control	3	27			*	*	*	*	*			
Low	4	20			*	*	*	*	*			
	5	15			*	*	*	*	*			

Table 10. Second Run, Egg Density and Immersal Periods at Different Thermal Shock Treatments

Treatment		Temperature (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40			*	*	*	*	*			
	2	30	4	1	*	1	1	*	*	1.47	1,326,547	1,326,547
Control	3	27	3	2	*	*	1	1	1	3	1,050,400	525,200
Low	4	20			*	*	*	*	*			
	5	15			*	*	*	*	*			

Table 11. Third Run, Egg Density and Immersal Periods at Different Thermal Shock Treatments

Treatment		Temperature (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40			*	*	*	*	*			
	2	30	2	3	1	1	*	*	*	1.32	3,796,062	1,265,354
Control	3	27	3	2	*	1	1	1	*	2.51	2,108,610	1,054,305
Low	4	20			*	*	*	*	*			
	5	15			*	*	*	*	*			

Table 12. Fourth Run, Egg Density and Immersal Periods at Different Thermal Shock Treatments

Treatment		Temperature (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40			*	*	*	*	*			
	2	30	4	1	1	1	1	*	*	2.1	1,476,435	1,476,435
Control	3	27			*	*	*	*	*			
Low	4	20			*	*	*	*	*			
	5	15			*	*	*	*	*			

Table 13. Fifth Run, Egg Density and Immersal Periods at Different Thermal Shock Treatments

Treatment		Temperature (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40			*	*	*	*	*			
	2	30	3	2	1	1	*	*	*	1.89	2,352,472	1,176,236
Control	3	27	4	1	*	1	1	1	*	2.17	998,234	998,234
Low	4	20			*	*	*	*	*			
	5	15			*	*	*	*	*			

Table 14. Mean Egg Density and Immersal Periods at Different Temperatures from 5 Thermal Shock Runs.

Treatment		Temp (Degrees Celsius)	Male (ind.)	Female (ind.)	Spawning (hours) @ 5 hours observation					Spawning period (hours)	Eggs collected (cells/650ml)	Eggs collected (cells/female)
					1	2	3	4	5			
High	1	40	0.00	0.00	0	0	0	0	0	0.00	0.00	0.00
	2	30	2.67	1.50	4	5	3	0	0	1.51	1,973,786	1,115,029
Control	3	27	2.50	1.25	0	2	3	3	1	1.92	1,039,311	644,435
Low	4	20	0.00	0.00	0	0	0	0	0	0.00	0.00	0.00
	5	15	0.00	0.00	0	0	0	0	0	0.00	0.00	0.00
Averages			1.03	0.55	0.80	1.40	1.20	0.60	0.20	0.69	602,619.40	351,893

APPENDIX B

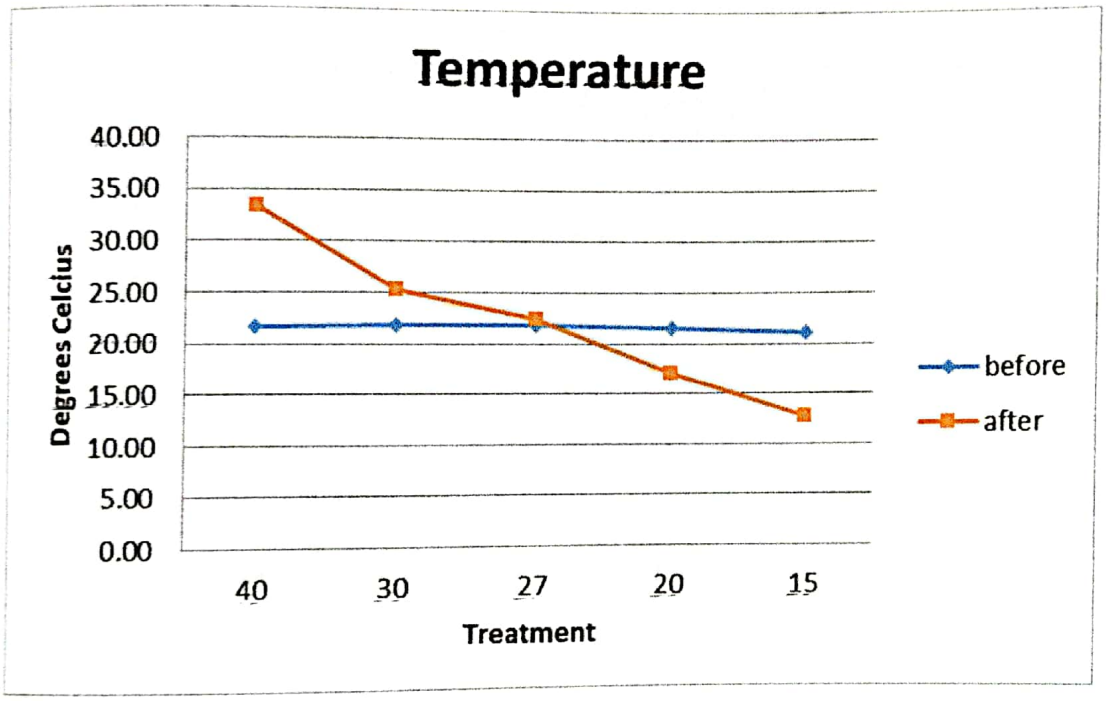


Figure 9. **Mean Temperature Ranges Before and After Effects from 5 runs Thermal Shock Treatment at Different Temperatures.**

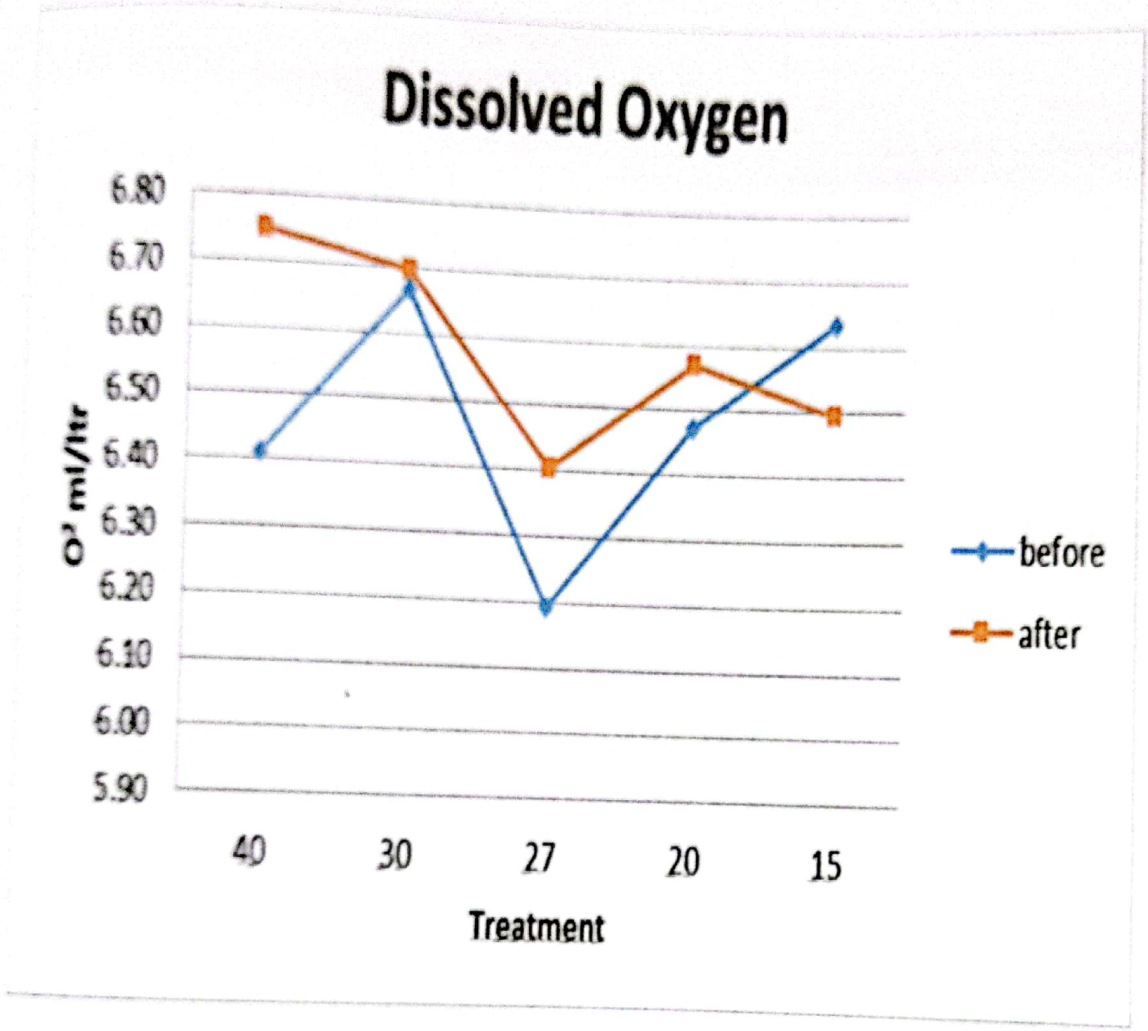


Figure 10. **Mean Dissolved Oxygen Rranges Before and After Effects from 5 Runs Thermal Shock Treatment at Different Temperatures**

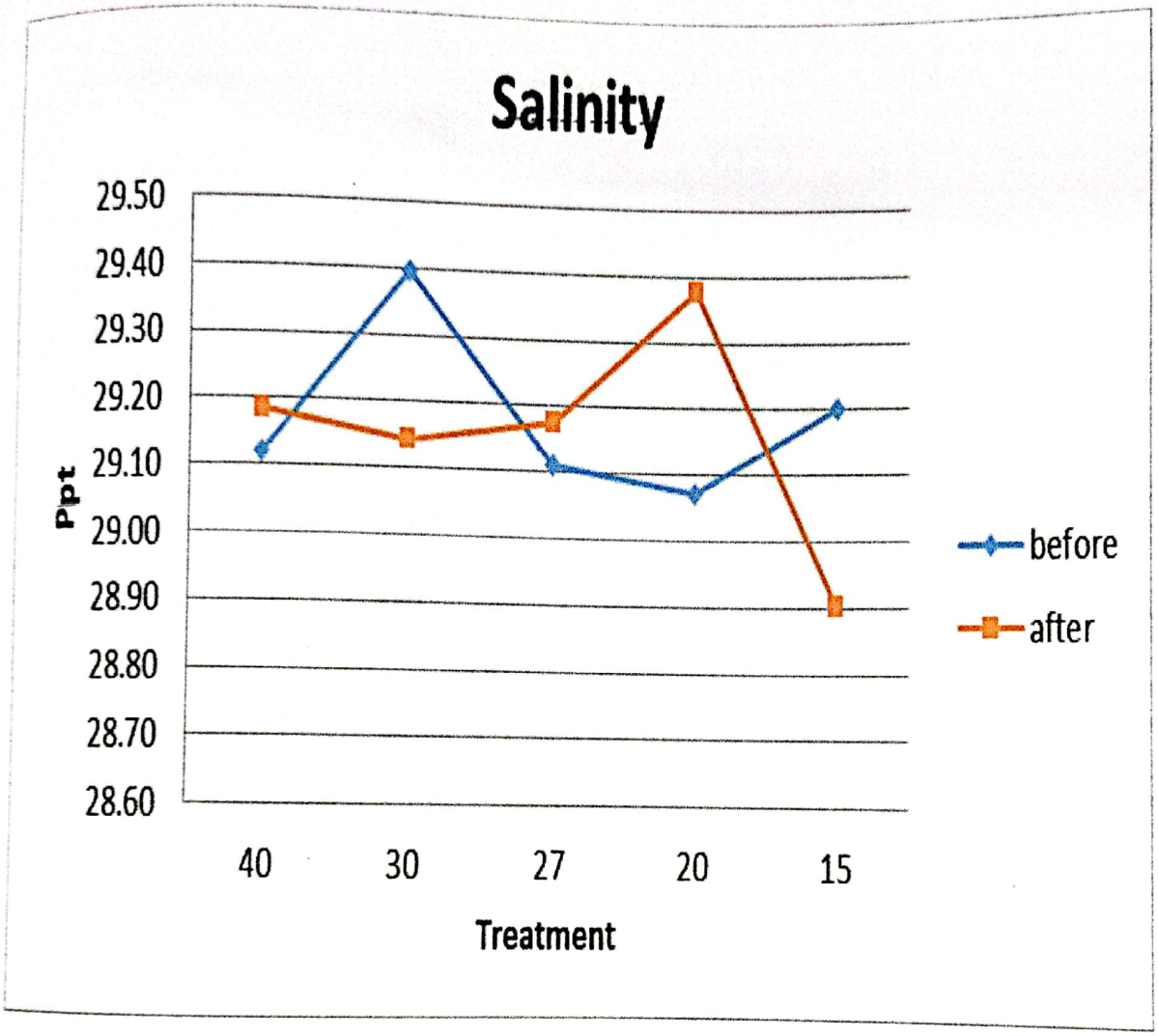


Figure 11. Mean Salinity Ranges Before and After Effects from 5 Runs Thermal Shock Treatment at Different Temperatures.

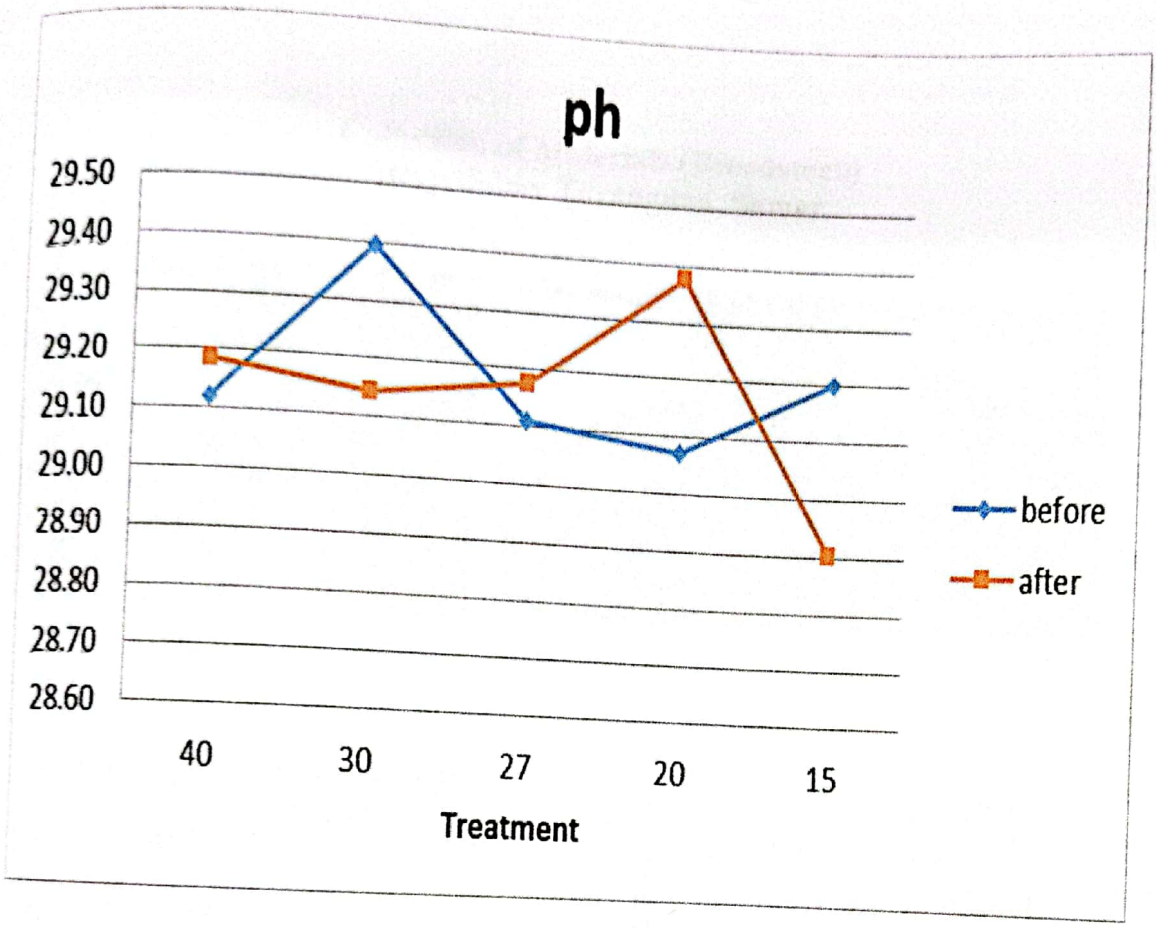
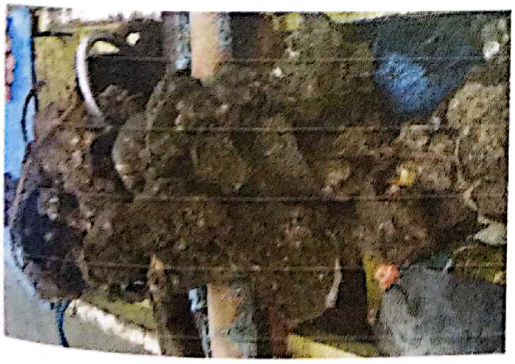
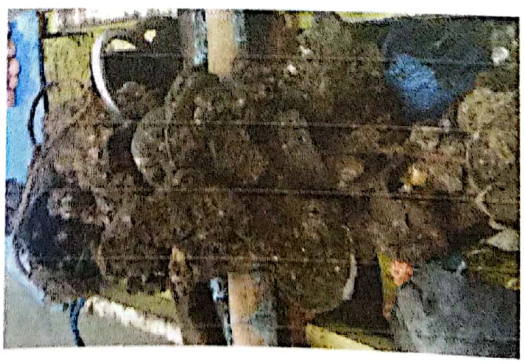


Figure 12. **Mean pH Ranges Before and After Effects from 5 Runs Thermal Shock Treatment at Different Temperatures**

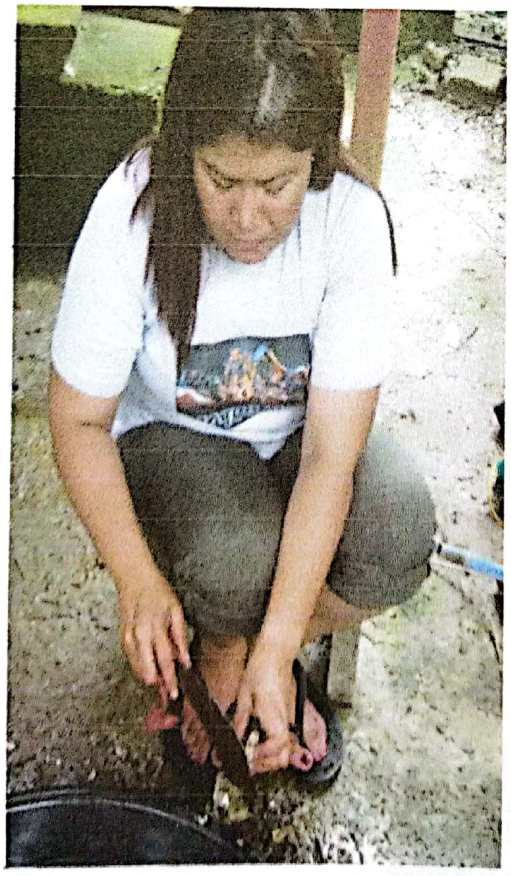
APPENDIX C

Collection of Materials (Broodstock)
Brgy Bahay Tarangnan, Samar

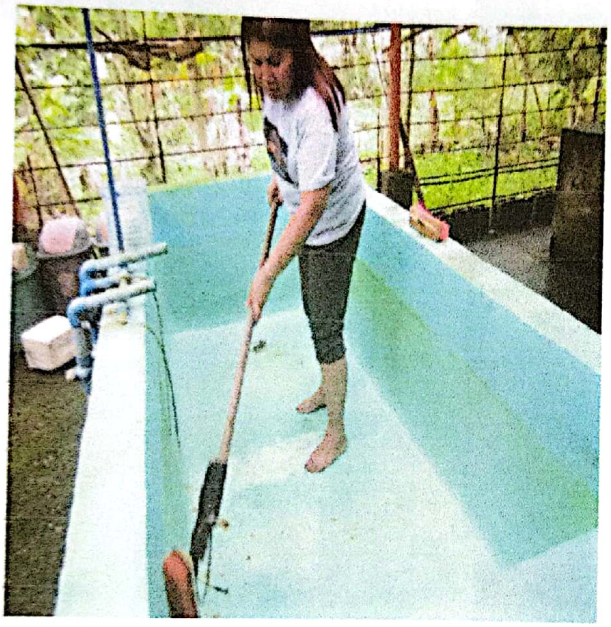


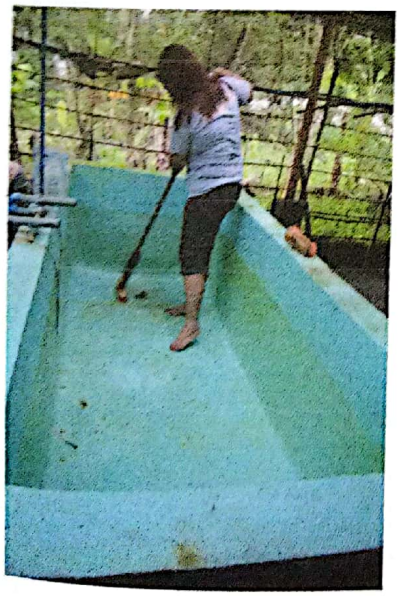


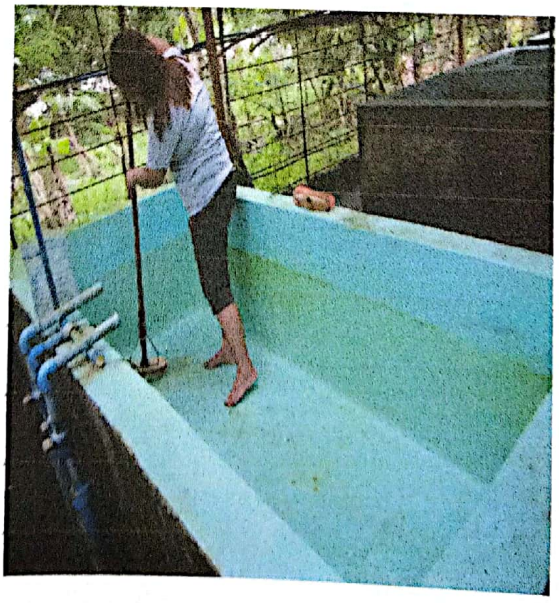
Cleaning of Broodstock
College of Fisheries and marine Sciences Multi- Hatchery
Mercedes, Catbalogan City



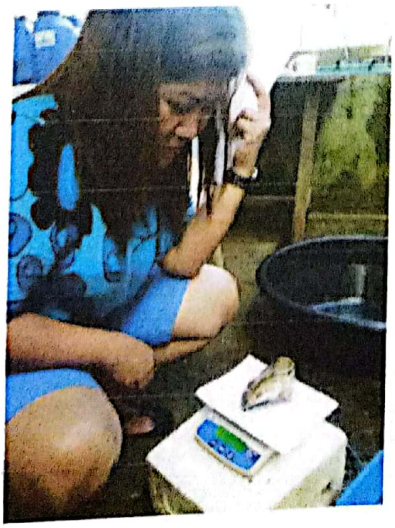
Cleaning of Hatchery Conditioning Tank
College of Fisheries and marine Sciences Multi- Hatchery
Mercedes, Catbalogan City



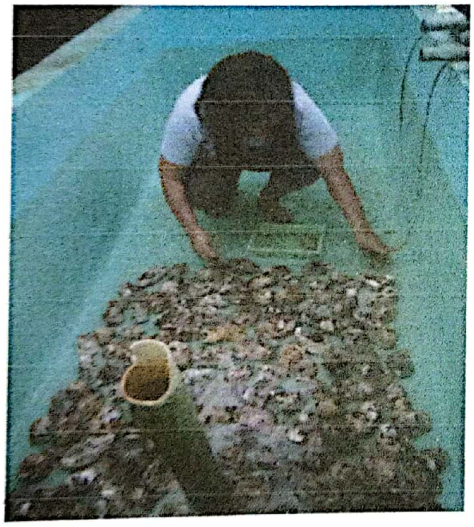


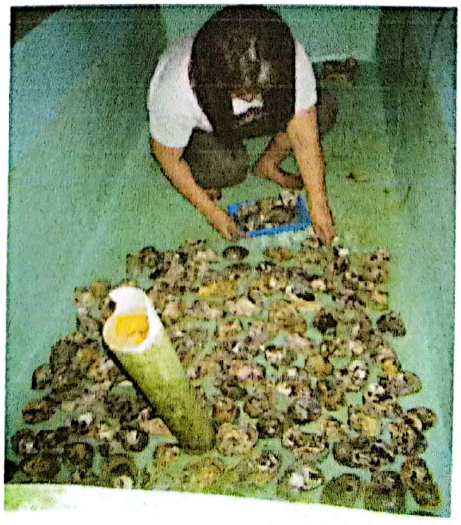


Morphometric of Broodstock
Measuring the Thickness, Width, Length, and Weight

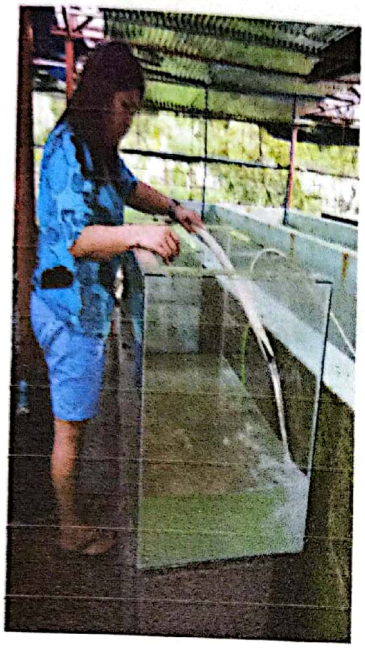


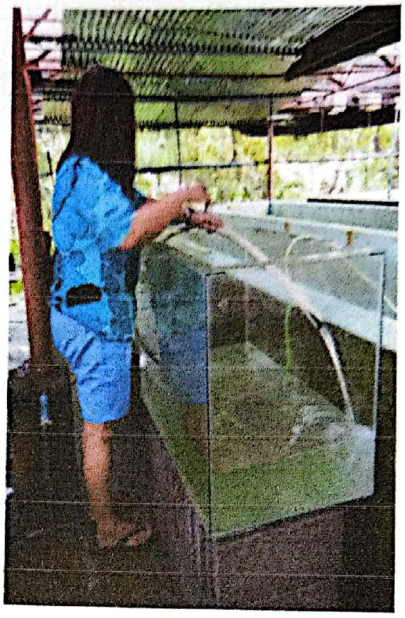
Preparation for Prophylactic Treatment of Broodstock at Conditioning Tank





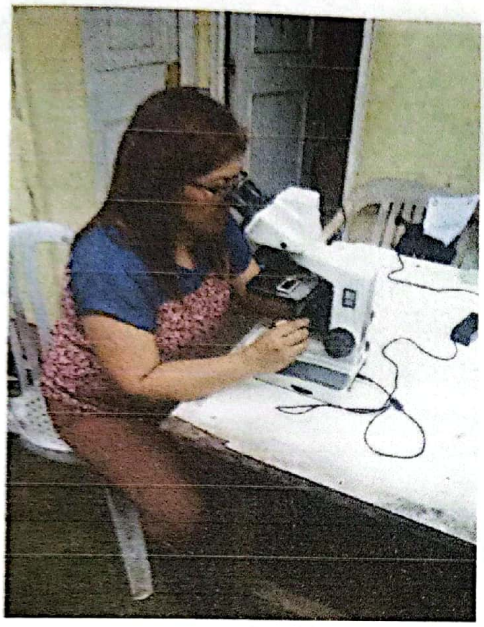
Filling of 30 Liters UV Treated Seawater in 5 Aquaria's in Preparation for Thermal Shock Spawning (T1, T2, T3, T4, T5 with Aerator)

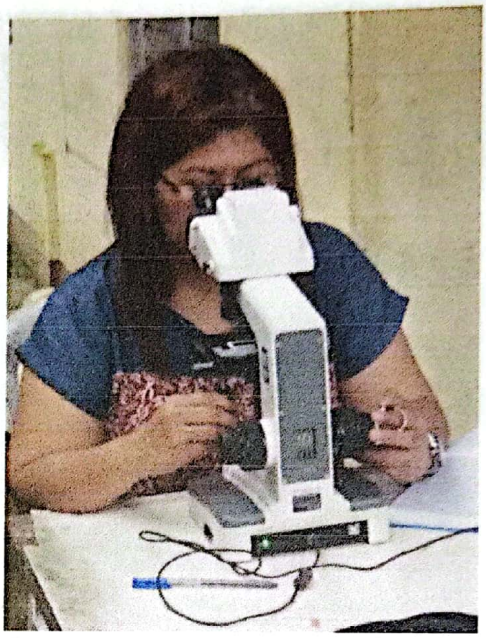


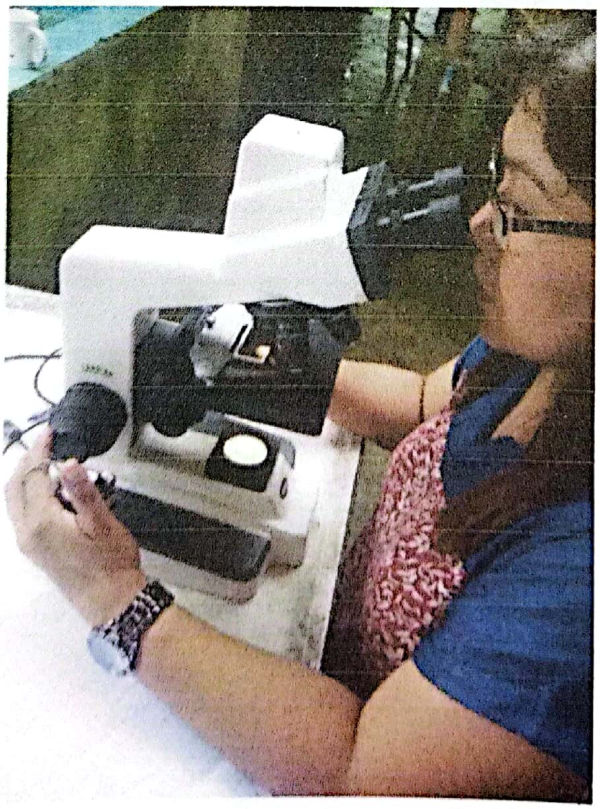


Aquarium Set-up at Different Temperature Levels (T1- 30°C, T2- 40°C, T3- 15°C, T4- 20°C, T5- 27°C) at COFMAS Multi-Species Hatchery

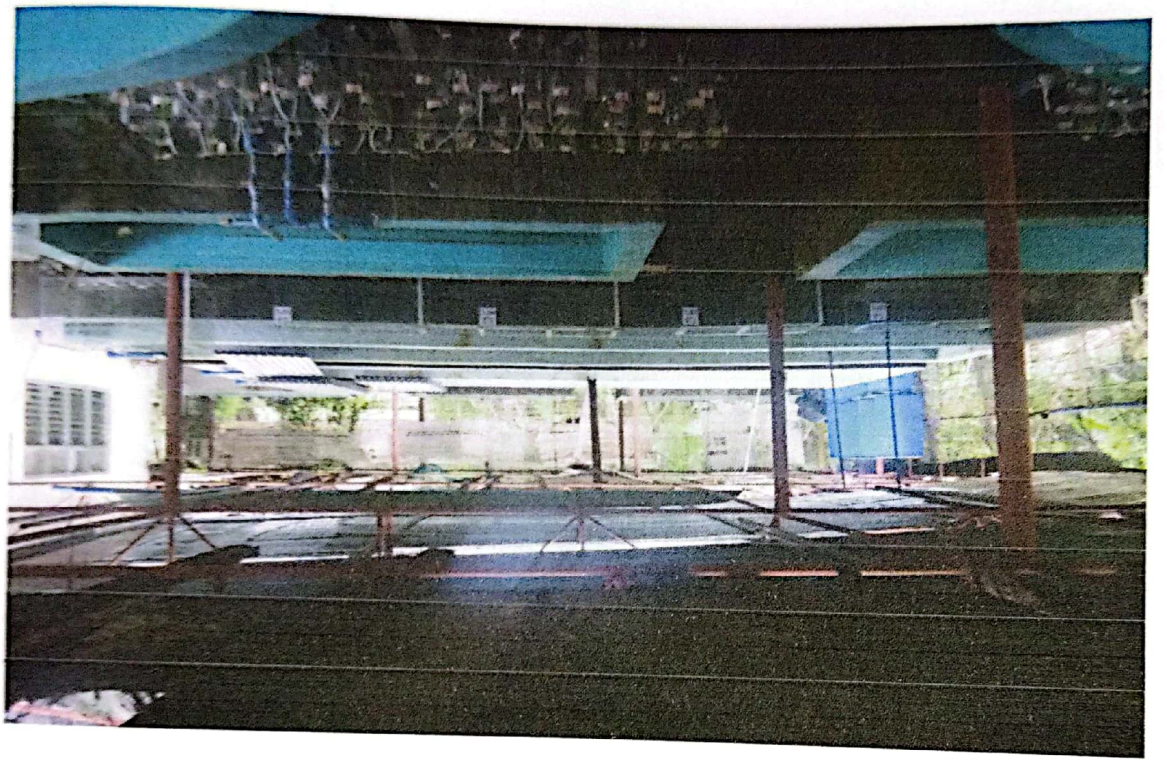
Microscopic Activity
Counting of Trocophore (Spawned Oyster)

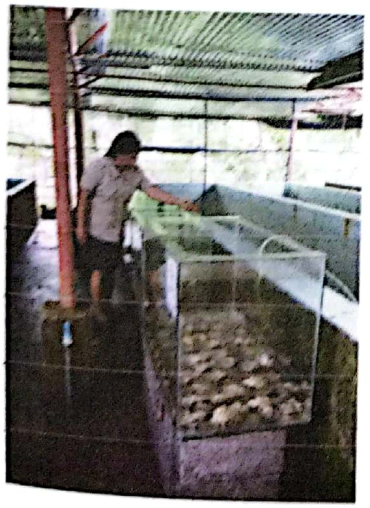


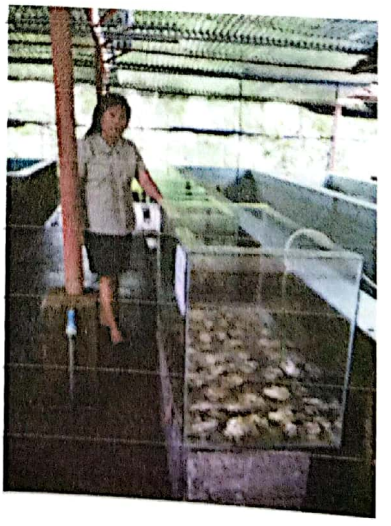




College of Fisheries and Marine Sciences Multi-Species Hatchery
Mercedes Catbalogan City
Site for Experimental Laboratory







APPENDIX D

SUPPORTING DOCUMENTS

ON THESIS WRITING

nt's Copy

SAMAR STATE UNIVERSITY
COLLEGE OF FISHERIES AND MARINE SCIENCES

JAS-ANZ
Certificate No.: AJA17.1013

SSU-UNREG-FR-001
01-Aug-2017 Rev: 1

ENROLLMENT FORM

[ ] New Student
[ ] Old Student
[ ] Quitter

t No.

Name: **MARIETTA B. ALBINA**

2nd Sem SY 2017-2018 Nov. 20, 2017

Master in Fisheries Technology (MFT)

Y	TIME	ROOM	SUBJECT CODE	Unit	INSTRUCTOR	SIGNATURE
			THESIS WRITING	6		

d by:

JANE C. MENDIOLA
Registrar III

Verified Correct:

LOLITO O. AMPARADO, Ph.D.
Campus Director

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the University President

JAS-ANZ
AJA
Certificate No.: AJA17.1013

1st ENDORSEMENT
January 30, 2018

Respectfully returned to **Ms. Marietta B. Albina**, Faculty Researcher, this University, the herein letter of request for approval concerning on her enrolment for thesis writing *(Master in Fisheries Technology)* in this University.

Supporting on the professional and personal development of the faculty, the undersigned interposes no objection on the request of **Ms. Albina** provided that her responsibilities as faculty researcher are not adversely affected.

Please be guided accordingly.

MARILYN D. CARDOSO, Ph.D.
University President

Copy furnished:

Dr. Ronald L. Orale
VP for Planning, Research and Extension Services

SAMAR STATE UNIVERSITY
OPRES - UDC
RELEASED
BY:
DATE: 1/31/18 TIME: 9:10 AM

JAS-ANZ
Acc. No.: MZ7009039M
Certificate No.: AJA17.1013

January 29, 2018

SAMAR STATE UNIVERSITY
OPRES - UDC
RECEIVED
BY:
DATE: 1/29/18 TIME: 2:15 PM

DR. MARILYN D. CARDOSO
University President
This University
Catbalogan City, Samar

Madam:

I have the honor to request permission to enroll on thesis writing in Master in Fisheries Technology (MFT) in Samar State University Mercedes Campus, Catbalogan City, Samar for the Second Semester, School Year 2017-2018, under the College of Graduate Program.

My study will not hinder, in any manner, my function as a faculty researcher at the Office of the Vice President for Planning, Research and Extension Services for I will do my best not to use my official time and function in the Office. I promise to diligently and successfully do my duties and responsibilities to the best of my ability and for the best service and benefit of the clienteles of the University.

I hope for your favorable consideration.

Very truly yours,

MARIETTA B. ALBINA
Faculty Researcher

NOTED:

RONALD L. ORALE, Ph.D.
VP for Planning, Research & Extension Services

APPROVED:

MARILYN D. CARDOSO, Ph. D.
University President

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Dean I College of Graduate Studies

Certificate No.: AJA17.1013

FORM CGS 17

ASSIGNMENT OF THESIS/DISSERTATION ADVISER

PROF. RENATO C. DIOCTON
Faculty
Mercedes Campus
This University

January 22, 2018

Dear Prof. Diocton:

I am glad to inform you that you were designated as thesis adviser/consultant of MARIETTA B. ALBINA candidate/s for the degree of **Master in Fisheries Technology major in Aquaculture** who propose(s) to write a thesis entitled: THERMAL SHOCK SPAWNING OF PHILIPPINE CUPPED OYSTER *Crassotrea iredalie* as approved by the panel of evaluators last January 17, 2018.

I am looking forward for your favorable response on this matter.

Thank you very much for your continued support to our student.

Very truly yours,

FELISA E. GOMBA, Ph. D.
Acting Dean, College of Graduate Studies/
Vice-President for Academic Affairs

CONFORME:

PROF. RENATO C. DIOCTON, MSci
Adviser

01-22-2018
Date

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Vice-President for Planning, Research and Extension

SAMAR STATE UNIVERSITY
OVPRE - UDC
RECEIVED
BY: cem
DATE: 02/01/18 TIME: 8:50 AM

AJA ISO 9001:2008 Acc. No.: MZ7000919PM
Certificate No.: AJA17.1013

January 31, 2018

DR. RONALD L. ORALE
VP for Planning, Research and Extension Services
This University

Dear Sir:

I am writing this letter to formally request permission to conduct my thesis works in between the spare time of my office hours, in preparation for my final defense this upcoming February 26, 2018 as a course requirement of my chosen master's degree in Fisheries Technology at Samar State University, College of Graduate Studies.

In accordance with this, I would like to inform you that due to the sensitive nature of my thesis entitled, "Thermal Shock Spawning of Philippine Cupped Oyster *Crassostrea eridalie*" critical data gathering, persistent collection of samples/results and consistency of measured parameters are all subject of concern which requires an ample amount of time to fully commit with those demands. These activities are undertaken at COFMAS Hatchery Experimental Site, Mercedes Catbalogan City.

I am hoping for your consideration regarding this endeavor of mine thus supporting my profession and personal development. Rest assured, I will be responsible and still be cooperative with any activities and programs in the office, the research center I belong and the University wishes from me to do so. On this regard, I am hoping for a positive feedback regarding this effort.

Thank you and more power.

Sincerely,

MARIETTA B. ALBINA
Faculty Researcher - CESTI

Approved by:

RONALD L. ORALE, Ph.D.
VP-Planning, Research & Extension Services

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Dean I College of Graduate Studies

Certificate No.: AJA17.1013

FORM CGS 17

January 22, 2018

TO: **PANEL OF EXAMINERS/EVALUATORS**

DR. RONALD L. ORALE - **MEMBER**
DR. RICARDO T. SEVERO - **MEMBER**
DR. EMILIO H. CEBU - **MEMBER**

1/26/2018

FOR : **MARIETTA B. ALBINA**
COURSE : **MFT Major in Aquaculture**

It gives me pleasure to appoint you to the Panel of Examiners/Evaluators of the aforementioned candidate. The oral defense will take place at CGS Dean's Office on **JANUARY** 27, **2018 (** Saturday **) at** 1:30 **o'clock in the** afternoon.

Thank you.

FELISA E. GOMBA, Ph. D.
Acting Dean, College of Graduate Studies

Republic of the Philippines
SAMAR STATE UNIVERSITY
COLLEGE OF GRADUATE STUDIES-
ASSISTANCE FOR RESEARCH CENTER (CGS-ARC)
Catbalogan City
Telephone Numbers: (055)-543-8394/(055)-251-2139
Website: www.ssu.edu.ph

APPLICATION FOR ORAL DEFENSE

Name of Applicant: MARIETTA B. ALBINA
Course and Major: MFT
Type of Defense Applied for:

- [✓] Proposal Defense
- [ ] Final Defense

Research Category:

- [ ] Masteral Level
- [ ] Doctorate Level

Schedule of Defense: Date: 01-23-2018 Time: _____

Fees to be paid:

Particulars	Proposal Defense		Final Defense		Finalization/Final Draft
	Masteral	Ph. D.	Masteral	Ph. D.	
Adviser	3,200.00	4,000.00	3,200.00	4,000.00	2,000.00 – Ph. D. 1,600.00 - Masteral
Chair	1,000.00	1,300.00	1,200.00	1,500.00	-
Member	600.00 x 3	800.00	800.00	1,000.00	
Adm Cost	500.00	500.00	500.00	500.00	
Recorder	300.00	300.00	300.00	300.00	-

Members of the Examination Committee:

Name	Designation	Name	Designation
Prof. RENATO BIOCTON	Adviser	Dr. EMILIO CEBU	Member
Dr FELISA E. GOMBA	Chair		Member
Dr RONALD L. OTGALE	Member	Ms NATHALIE ALAGA	Secretary/Recorder
Dr RICARDO T. SEVERO	Member		

Total Fees: ₱6,800.

MARIETTA B. ALBINA
(Printed Name & Signature of Applicant)

Amount Paid: ₱6,800.00
OR No: 3121384
Date: 18 JAN 2018

PAID

Noted:

FELISA E. GOMBA, Ph. D.
VPAA/Acting Dean, College of Graduate Studies

ERWIN T. ABAYAN
Collecting Officer

(To be accomplished in 2 copies: 1 – applicant's file; 1 – SSU-CGS file)

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Dean I College of Graduate Studies

Certificate No.: AJA17.1013

FORM CGS 17

ASSIGNMENT OF THESIS/DISSERTATION ADVISER

PROF. RENATO C. DIOCTON
Faculty
Mercedes Campus
This University

February 21, 2018

Dear Prof. Diocton:

I am glad to inform you that you were designated as thesis adviser/consultant of MARIETTA B. ALBINA candidate/s for the degree of **Master in Fisheries Technology major in Aquaculture** who proposed and passed the pre-oral defense entitled: THERMAL SHOCK SPAWNING OF PHILIPPINE CUPPED OYSTER *Crassotrea iredalie* as approved by the panel of evaluators last January 27, 2018.

I am looking forward for your favorable response on this matter.

Thank you very much for your continued support to our student.

Very truly yours,

FELISA E. GOMBA, Ph. D.
Acting Dean, College of Graduate Studies/
Vice-President for Academic Affairs

CONFORME:

PROF. RENATO C. DIOCTON, MSci
Adviser

02-21-2018
Date

Date of Final Oral Defense:
February 26, 2018
9:00 AM

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Dean I College of Graduate Studies

Certificate No.: AJA17.1013

FORM CGS 17

February 21, 2018

TO: **PANEL OF EXAMINERS/EVALUATORS**

DR.RONALD L. ORALE	-	MEMBER
DR.EMELIO H. CEBU EMILIO	-	MEMBER
DR. RICARDO T. SEVERO, JR.	-	MEMBER
PROF. RAUL B. CELMAR	-	MEMBER

FOR : **MARIETTA B. ALBINA**
COURSE : **MFT - Aquaculture**

It gives me pleasure to appoint you to the Panel of Examiners/Evaluators of the aforementioned candidate. The final oral defense will take place at CGS Dean's Office on **FEBRUARY 26, 2018 at 9:00 o'clock in the morning.**

Thank you.

FELISA E. GOMBA, Ph. D.
Acting Dean, College of Graduate Studies

CGS-ARC Form 1

Republic of the Philippines
SAMAR STATE UNIVERSITY
COLLEGE OF GRADUATE STUDIES-
ASSISTANCE FOR RESEARCH CENTER (CGS-ARC)
Catbalogan City
Telephone Numbers: (055)-543-8394/(055)-251-2139
Website: www.ssu.edu.ph

APPLICATION FOR ORAL DEFENSE

Name of Applicant: MARIETTA B. ALBINA
Course and Major: MFT - Aquaculture
Type of Defense Applied for:

- [ ] Proposal Defense
- [x] Final Defense

Research Category:

- [x] Masteral Level
- [ ] Doctorate Level

Schedule of Defense: Date: 02-26-2018 Time: 9:00 AM

Fees to be paid:

Particulars	Proposal Defense		Final Defense		Finalization/Final Draft
	Masteral	Ph. D.	Masteral	Ph. D.	
Adviser	3,200.00	4,000.00	3,200.00	4,000.00	2,000.00 – Ph. D. 1,600.00 - Masteral
Chair	1,000.00	1,300.00	1,200.00	1,500.00	-
Member	600.00	800.00	800.00x4	1,000.00	-
Adm Cost	500.00	500.00	500.00	500.00	
Recorder	300.00	300.00	300.00	300.00	-

Members of the Examination Committee:

Name	Designation	Name	Designation
Prof. RENATO C. DOCTON	Adviser	Dr. RICARDO T. SEVERO, Jr.	Member
Dr. FELISA E. GOMBA	Chair	Prof. RAUL B. CELMAR	Member
Dr. RONALD L. ORALE	Member	Ms. VIANE C. VILLARIN	Secretary/Recorder
Dr. EMILIO H. CEBU	Member		

Total Fees:

₱ 10,000.-

(Printed Name & Signature of Applicant)

Amount Paid: PAID
OR No: FEB 15 2018
Date: 3123446

Noted:

FELISA E. GOMBA, Ph. D.
VPAA/Acting Dean, College of Graduate Studies
(To be accomplished in 2 copies: 1 – applicant's file; 1 – SSU-CGS file)

March 3, 2018
Date

The University Registrar
Samar State University
Catbalogan City

Madam:

I have the honor to apply for graduation from Samar State University, Catbalogan City, under the Master in Fisheries Technology (MFT) major in Aquaculture at the end of the Second semester, school year 2017 - 2018 /summer ____.

Signature of Student

==========

STUDENT PERSONAL INFORMATION

A. Personal Background

Name *(Please Print)*: ALBINA (Last Name) MARIETTA (First) BERGULA (Middle)

Maiden Name *(if Married)*: BERGULA Civil Status: Married Religion: RC
Date of Birth: April 05, 1970 Place of Birth: Catbalogan City, Samar
Home Address: P-2 Manlong Catbalogan City, Samar
Catbalogan Address: ____
Name of Father: Marcial Diaz Bergula Sr (living ____ deceased ✓) *(Please Check)*
Name of Mother: Maxima Pido Bacalando (living ✓ deceased ____) *(Please Check)*
Address of Parents: P-2 Manlong Catbalogan City, Samar

If sent to school other than parents:

Name of Guardian or Spouse: LEONILO CALISTE ALBINA
Address of Guardian or Spouse: P-2 Manlong Catbalogan City, Samar

OR# 3132063 P1,86...
3/8/18

B. Educational Background:

	Name of School	Address	Highest Grade Completed	School Year
Elementary	Cabu IV Central ES	Manlong	Graduate	1979 - 1980
Secondary	SRSF	Mercedes	Graduate	1984 - 1988
College	SPIFT VSU	Mercedes Tolosa, Leyte	Graduate Graduate	1988 - 1988 1990 - 1991

Degree/Title to be obtained: Master in Fisheries Technology 2017 - 2019
Major: Aquaculture Minor: Aquaculture
Expected date of Graduation:

C. Signature of Instructors in each subject you are presently enrolled implying his/her recommendation to you being a candidate for graduation at the end of the present school term:

Subjects	Signature	Recommendation
Thesis Writing		subject to submission of final approved copy of thesis
"Morphometric Characterization of Philippine Cupped Oyster Crassostrea virginalis"		

Date: March 2, 2019

Signature of Student

OR No.
Date Issued
Amount Paid :

Contact #: 09778268872

DISCLAIMER

CANDIDATES FOR DEGREE AND DIPLOMA

THE CANDIDATES WHOSE NAMES ARE LISTED DO NOT AUTOMATICALLY QUALIFY FOR GRADUATION DESPITE THEIR PRESENCE AT THE GRADUATION CEREMONIES AND THE DECLARATION OF CONFERMENT BY THE UNIVERSITY PRESIDENT OR HIS AUTHORIZED REPRESENTATIVE.

IT IS, THEREFORE, IMPERATIVE FOR THE CANDIDATES TO COMPLY WITH ALL THE ACADEMIC REQUIREMENTS FOR GRADUATION BEFORE THEY CAN BE CONSIDERED GRADUATE.

MARIETTA B. ALBINA
Student's Name and Signature
Date: March 2, 2018

Parents Name and Signature:

_____ _____
Mother *Father*
Date: _____ *Date:* _____

If sent to school other than parents:

_____
Guardian's Name and Signature

Date: _____

Republic of the Philippines
SAMAR STATE UNIVERSITY
Desktop Publishing Center

JAS-ANZ
AJA
Certificate No.: AJA17.1013

2018 YEARBOOK SUBSCRIPTION

Name of Subscriber (*Please print*)

ALGINA	MARIETTA	BERGULA
Last Name	**First Name**	**Middle Name**

Permanent Address: P-2 Maulong Catbalogan City, Samar

Date of Birth: April 08, 1970

Department/ College: CGS

Degree Pursued: MFT - Master in Fisheries Technology

Major/ Field of specialization (*If any*): Aquaculture

Dissertation/ Thesis Title (*For CGS graduates only*): "Thermal Shock Spawning of Philippine Cupped Oyster Crassostrea irridalis

Academic Honor/ s _____

Participation in Extra Curricular Activities: (*Please provide additional sheet if needed*):

_____ _____ _____ _____ _____ _____ _____ _____ _____

Ambition / Motto in life: "What we are is God's gift to us, what we become is our gift to God"

Mode of Payment: (✓) Full
( ) Partial

Photo taken: _____

O.R No.: 3132063

Date Issued: 3/8/18

Amount: 1,078.

I certify that the above foregoing data are true and correct.

MARIETTA B. ALGINA
Name & Signature of Subscriber

09758268872
Telephone / Cellphone No.

Note: Please return this form after paying the Graduation fees. Thank you!

O.R # 3132063 Date

Republic of the Philippines
SAMAR STATE UNIVERSITY
Office of the Dean| College of Graduate Studies

Certificate No.: AJA17.1013

Certification

To whom it may concern:

THIS IS TO CERTIFY that **MS. MARIETTA B. ALBINA** had the final oral defense of her thesis entitled **"THERMAL SHOCK SPAWNING OF PHILIPPINE CUPPED OYSTER *Crassostrea iredalie*, Faustino 1932"** on **February 26, 2018** with a rating of **PASSED.**

This certification is issued upon request of the interested party for whatever legal purpose it may serve.

Given this 8th day of March, 2018 in Catbalogan City, Samar.

FELISA E. GOMBA, Ph. D.
Acting Dean, College of Graduate Studies

NOT VALID
WITHOUT SEAL

SAMAR STATE UNIVERSITY
ODCGS - UPPER
RECEIVED
BY:
DATE: 3-8-18 TIME: 11:10 AM

Cert Fee OR # : 3126666
Doc Stamp OR # : 3126666
Date Paid : 3/8/2018

CURRICULUM VITAE

CURRICULUM VITAE

Name	:	Marietta Bergula-Albina
Date of Birth	:	April 5, 1970
Place of Birth	:	Catbalogan City, Samar
Home Address	:	Purok-2, Barangay Maulong, Catbalogan City, Samar
Civil Status	:	Married
Spouse	:	Leonilo C. Albina
Children	:	Jed Danniel B. Albina Ma. Kristina B. Albina
Parents	:	Marcial D. Bergula (Deceased) Maxima P. Bacalando
Present Position	:	Instructor I (Faculty Researcher)
Office/Agency	:	Samar State University
Office Address	:	Barangay Guindapunan, Arteche Blvd, Catbalogan City, Samar
Eligibility	:	NC 2 – Aquaculture NC 11 – Data Encoder

EDUCATIONAL ATTAINMENT

Graduate	:	**Samar State University - Mercedes Campus** Master in Fisheries Technology - Aquaculture March 2018
College	:	**Visayas State University - Tolosa Campus** Bachelor of Science in Fisheries - Aquaculture March 1991
	:	**Samar Regional Institute of Fisheries Technology** Diploma in Fisheries Technology - Aquaculture March 1988
Secondary	:	**Samar Regional School of Fisheries** Major in Aquaculture Catbalogan City. Samar March 1985
Elementary	:	Catbalogan IV Central Elem School Maulong Catbalogan City, Samar March 1980

: **General Clerical (Job Order)**
Samar State University – Mercedes Campus
Detailed - Accounting Section
Catbalogan City, Samar
1997 - 2002

Researcher : **Senior Research Assistant (Contractual)**
Joint Project SSUMC with DOST
Establishment of Red Tide Monitoring Center
in Eastern Visayas
Catbalogan City, Samar
1996 - 1997

Administrative : **General Clerical (Job Order)**
Samar State University – Mercedes Campus
Detailed - Accounting Section
Catbalogan City
1995 - 1996

Researcher : **Biologist - 3 (Contractual)**
BFAR Central Office, Research Div, Oceanography Sec
FSP Red Tide Monitoring Program
Aug 1992 - Aug 1995

IN SERVICE TRAININGS/SEMINARS/ WORKSHOP/CONFERENCE ATTENDED

Title	Date	Level
Seminar on Thesis and Dissertation Writing	January 13 & 20, 2018	Local
Training on Ecosystem Approach to Fisheries Management EAFM	November 12-15, 2017	National
Conference on CLCP, SAFE, CEST Programs and EVHRDC Call for Proposals of the 2017 Regional Science and Technology Week	September 20, 2017	Regional
Seminar on WHATs and HOWs of a Research Journal	September 15, 2017	Local
National Conference on Poverty and Sustainable Development	September 13-15. 2017	National
Writeshop on Disaster Risk Reduction (DRR) and Climate Change Adaptation (CCA)	September 6-7, 2017	Local
Workshop on Writing and Publishing in ISI Journals	July25-26, 2017	Local
Training on Managing Records with the Law in Mind	May 29-31, 2017	National
Basic Documentary Video Production Seminar Workshop	April 21-24, 2017	Local
CGS Strategic Planning Workshop	October 29, 2016	Local
Training on Team Development and Developing Customer Service Excellence for Frontliners	August 18, 2016	Local

Continued

Title	Date	Level
Seminar-Workshop on Records Management for Newbies	March 9-11, 2016	National
Training on ISO 9001:2008 Awareness Course (Quality Management System)	February 24, 2016	Local
In-House Seminar Workshop on SSU Academic Personnel	May 5 – 8, 2016	Local
Regional Assembly and Lecture Series on Research	August 29, 2015	Regional
Workshop on Target-Setting and Accomplishment	October 8, 2010	Local
Civil Registration for School Registrar and Media Practitioner	December 9, 2005	Local
In-House Seminar Workshop on Frontline Service Management	September 29, 2004	Local
Seminar on Civil Service Updates	September 9, 2004	Local
In-Service Training on CSC Updates and New College Policies	December 17, 2003	Local
Regional Seminar-Workshop on Higher Education Management Information System	March 3, 1996	Regional
Red Tide Monitoring and Data Management	March 25 – 29, 1996	National
Seminar on Red Tide	July 7 - 28, 1993	National

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