DEVELOPING A PROTOCOL FOR THE SUSTAINABLE CULTURE OF MICROALGAE FOR MANGROVE OYSTER (*Crassostrea rhizophorae*) UNDER HATCHERY CONDITIONS IN JAMAICA.

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ABSTRACT

Algae produced for feed can be extremely beneficial to all growth phases of oyster development. The biophysiological makeup of algae offers key nutrients and minerals to bivalves that aid internal specialization, shell growth and the overall health of oysters. Culturing oysters is meant to supply food readily and at any period needed especially within and enclosed environment. Jamaica has over the last 41 years had a small-scale operation of growing mangrove oysters *Crassostrea rhizophorae* in the Bowden Bay area of Saint Thomas. Through the World Bank Project, plans are being put in place for the construction of a hatchery allowing Jamaica, to expand its production in mangrove oysters. Understanding different types of algal production methods and those that may be best suited for production, is the focus of this paper. Literature was examined; a site visit to SMEL to intern at their facility to produce alga , was conducted in Blainville-sur-Mer, Normandy France and a questionnaire developed to gain knowledge on existing protocols and how they can be tailored to the needs of Jamaica. Presenting this information in an easy to follow protocol, dealing specifically with indoor algal culture for Juvenile (D shaped larvae) to spat and some emphasis on broodstock in a simple guide, is the basis on which this paper was written. It is intended to meet the needs of Jamaica when the hatchery is built. Efforts to address the nutritional needs for Mangrove oysters must be made in order to: reduce mortality, increase production and ensure the provision of good quality and quantity of algae. This will determine the success of the Hatchery.

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LIST OF ABBREVIATIONS

CR-Crassostrea Rhizophorae

CV-Crassostrea Virginica

CG-Crassostrea Gigas

CGI-Crassostrea Gasar

CM-Conway Medium

TES-Trace element solution

SSS- Sodium Silicate Solution

1. INTRODUCTION

Jamaica's aquaculture development began in 1946 with the introduction of the Mozambique tilapia *Oreochromis mossambicus* from St Lucia as a means of providing locals with an alternative source of protein (Aiken, Morris, 2002). Tilapia was reintroduced to Jamaica in 1976 and since then has been the major fish cultivated on the island. Reduction of fishing efforts along the coast was a major priority of the government. Dwindling fish stocks were a major concern, and the need to diversify led to an examination of the potential for the cultivation of other species on the island. As a result, two marine organisms of interest were identified: The Irish moss or sea moss (*Gracilaria terete)*, generally referred to as G.T. and the mangrove oyster *Crassostrea rhizophorae* (Lovatelli & Sarkis, 2010). This was done through a project between the Jamaican Government and the International Development Research Centre of Canada resulting in the establishment of Oyster Culture Jamaica in Bowden Bay, Saint Thomas in 1977. Its main objective was to conduct biological and production research of mangrove oyster within the area with the intention to commercialise the species in the future. A total of 11 sites were surveyed to assess the best site for establishing oyster production (Figure 1). Bowden Bay was pinpointed as the ideal location to facilitate oyster production.

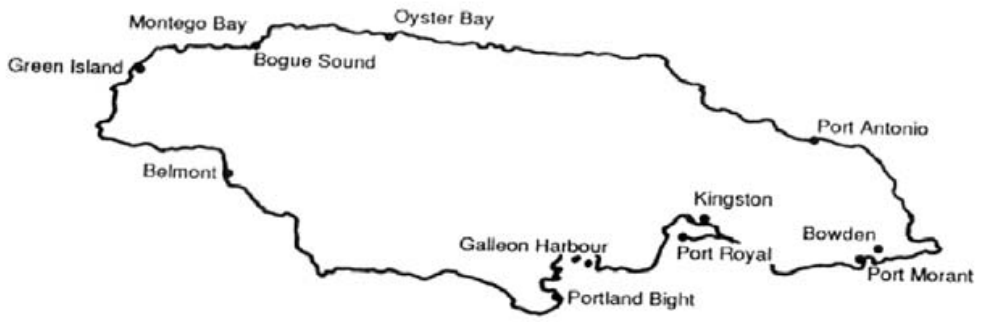

Figure 1 Picture of Surveyed sites for establishing Oyster culture production in Jamaica (Lovatelli & Sarkis, 2010)

Oyster Culture Jamaica was established only as a project and had been functioning as such from 1977. To date, the facility operates a basic culturing method relying on wild caught spat settling on cultches, that are then thinned out and placed on racks to grow out until ready for harvest. This process depends on seasonal availability of spawning oysters. In July of 2011, the idea of commercial culture was examined through a FAO regional technical workshop assessing the feasibility of a regional shellfish hatchery for the wider Caribbean area. One of the issues identified was that technical challenges include the absence of a reliable supply of spat. In Jamaica, mangrove oysters have two distinct spatfall periods (June to August and November to February, this is normally after the rainy months that happen in March and October.) Hence, the production of oysters is centred on these two periods and consequently, the supply of oysters and its associated products will be seasonal. To ensure a continuous and reliable supply of spat, Jamaica must invest in the development of an oyster hatchery (DeHaan, 2011).

2. PROJECT RATIONALE

Oyster culture Jamaica was established in 1977 and operates today as the Bowden Bay Research Facility. What began as a project and demonstration facility has managed to continue its operations. However, the facility uses the same basic techniques from its introduction, that is, collecting spat from the wild separating them on cultches and growing it out in designated spaces on racks in the Bowden Bay Harbour (Figure 2).

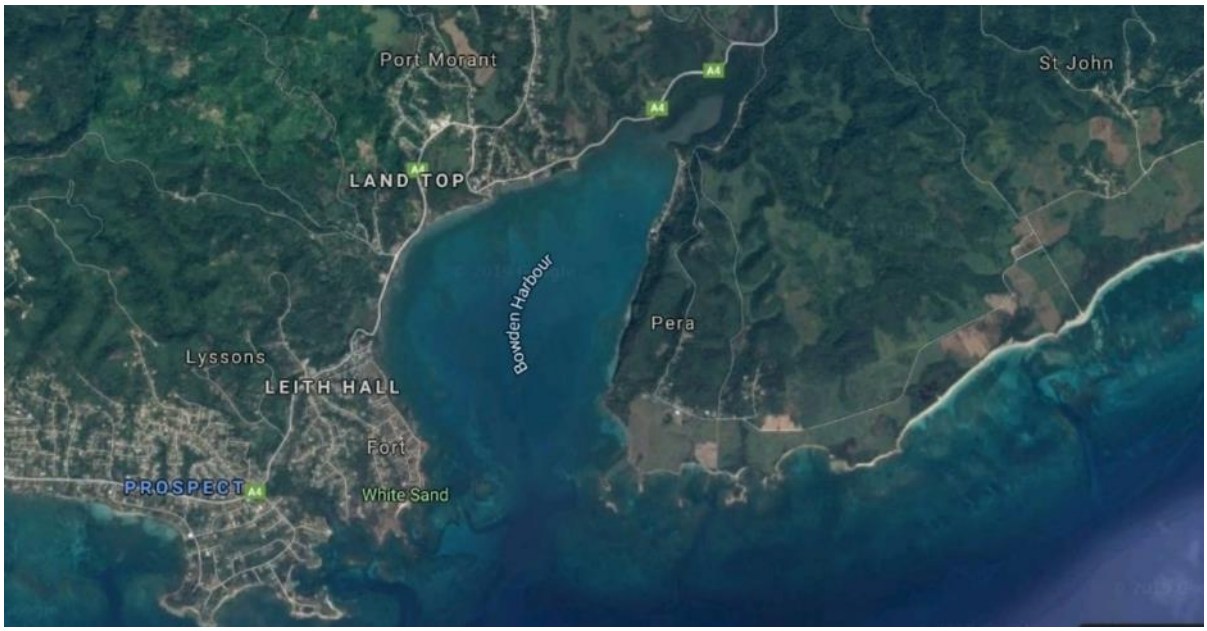

Figure 2 Aerial view of, Bowden Harbour, St Thomas Jamaica where the Bowden Bay oysters are grown. Google maps 2019

2.2 Economical Potential/Opportunities

The tourist sector has been Jamaica's primary and fastest growing industry for foreign exchange earnings. In 2018, Jamaica saw a total of 4.31 million tourists arrivals which generated approximately US$ 3.3 billion in earnings for the country (McIntosh, 2019). Importation of oyster products has steadily increased over the last nine years (Table 1).

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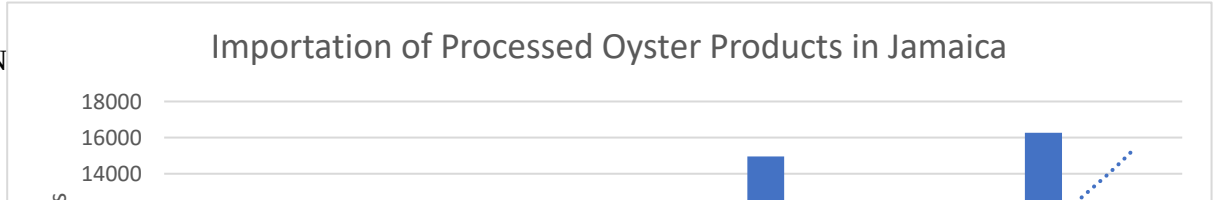

Figure 3. Demand trend for oyster products in Jamaica from 2009-2018. STATIN report 2018.

While there have been some fluctuations in the quantities of oysters imported, there exists a demand for oysters. Particularly from 2014, the quantity of oysters imported increased from approximately 6,000 kg to over 16,000 kg. This suggests that there exists a possible market for Jamaican oyster producers within the sector. Local interest, though undocumented, exists for the consumption of oysters. So much so that in 2013 at the annual Denbigh Agricultural show the concept and execution of an oyster bar to promote fresh oyster on the half shell and oyster punch consumption was established by the then Fisheries Division. This has been extremely popular with patrons with an estimated 300-350 persons visiting the booth over a three-day period. Initially they started with 108 kg of fresh oysters and 300 bottles of oyster punch per year and have averaged that amount up to the most recent staging in August 2019. Inquiries into the products have always been made, however the division has not always been able to fulfil the requests.

A consistent production of oysters would be needed to facilitate greater demand, a task only a hatchery would be able to provide and that is dependent on the supply of algae available for consumption by oysters at the different stages of development from D shape to broodstock. The result would be enough spat for grow out and harvest. Bowden Bay however has remained operational and has within the last two years been able to begin to branch out into external spat provision. A small community called Green Island in the western part of Jamaica has been able to form a community group and establish a grow out operation. Bowden Bay has been the sole provider of spat to them.

At the different stages of development, quality and quantity of microalgae matter. Diet is an important component in the production of bivalves. For oysters in the wild, a diverse variety of phytoplankton is available for consumption. Within a hatchery environment, it is important to provide conditions as close to the natural environment as possible, with a variety of phytoplankton to ensure proper growth and development at every phase of the oysters' life. Most Crassostrea species consume a similar diet and depending on location, tropical or temperate, the phytoplankton needed will be able to be cultured. The Caribbean's climate is warm, thus cultivating phytoplankton that are found within our environs will assist in the successful production of condition tolerant species. We must also understand the species biology, the methods of cultivation, the periods for feeding and stages at which each type is to be fed. Feeding time frame must also be examined as this operation can be quite expensive if oysters are to be kept until ready for harvest in a hatchery environment. The practice of feeding from D larvae phase to pedivegular stage is implemented to reduce costs associated with this kind of production.

Throughout the entire system spawning to spat settlement whether being done by the hatchery itself or by a grow out farmer, be it pre-settlement pedivegular or post-settlement spat, a thorough understanding of the nutritional needs of mangrove oysters is necessary since it is the key to healthy Broodstock, healthy and functional eggs and healthy and thriving mangrove oyster larvae. Oysters consume various unicellular organisms in the wild therefore hatcheries must provide a diverse diet for Mangrove Oysters at different stages of development. Most importantly, conditions in each environment is different for temperate and tropical zones however, technologies can be adapted and tailored for the Jamaican situation.

3. GOALS AND OBJECTIVES

3.1 Goal

Develop a protocol for the culture of micro algae with attention given to the qualities, quantities of species at the different stages of oyster development. Egg to spat stage with some attention being given to the maintenance of broodstock oysters.

3.2 Objectives

1. Assess algal culture for oyster production with comparable oyster producing countries to see which methodologies can be adapted to the Jamaican context.
2. Documenting ideal methods to produce and calculate the required quantities of micro algae for feeding.

4. RESEARCH METHODOLOGY

An extensive review of the literature on *Crassostrea rhizophorae* was done to gain basic knowledge on its lifecycle and feeding habits in its natural environment. Knowledge on other Crassostrea species was used to fill in the gaps on reproduction, feeding and life cycle phases to gain the fundamental background on their environment, how it contributes to their food consumption habits and what needs to be provided when oysters are bred in a hatchery facility. In addition to this, a site visit to the research facilities of SMEL (Synergie Mer et Littoral) in Normandy, France was organised. The intention was to participate in a one-week internship (January 27 to 31) dedicated to production methodologies associated with the culture of Micro algae. This was associated with a questionnaire survey to ascertain specific information vital to understanding feeding requirements of oysters at different stages of development. Detailed analysis of material should enable the process to be replicated in Jamaica specifically tailored to our needs. France has a temperate climate with distinctive differences in temperature and climate. Conditions like this influence how they culture algae. Jamaica's environment experiences very little fluctuations in seasons and water temperature which are the ideal conditions to produce micro algae.

5. LITERATURE REVIEW

5.1 Oyster culture in Jamaica.

Bowden Bay location within a harbour (see figure 1) provides protection from active waves and currents. Over the last 43 years because of its affiliation with the subdivision Aquaculture Branch (which is a part of the entity formerly the Fisheries Division now named the National Fisheries Authority) as its caretaker. It remains the chief demonstration facility of mariculture practices and species diversification. It is the sole production facility of cultured oyster *Crassostrea rhizophorae* on the island. Much research has been done on varying species of Oysters. Where there is lacking information on the *Crassostrea rhizophorae,* other species closest to the mangrove oyster will be observed, e.g. *Crassostrea gigas, and Crassostrea virginica,* respectively. The information on method of production especially for Jamaica, physiological and biological make up is important for how this affects the growth and development of the species as well as emphasising the need to produce algae once hatchery production operations are adapted.

5. 2 Current method of Production

Production of oysters in Jamaica includes the collection of spat on 8*8 cm diameter tire cultches with 2.5 cm irrigation hose spacers in between that have been set as a medium for spat to settle on in the wild. After settlement is observed, the cultches are hung on a bamboo frame called a Hanson rack, at this stage oysters are exposed to air fortnightly between four to six hours for six weeks or until they have attained a size of 2.5 mm (figure 4). They are then transferred to grow out and harvested within another 20 weeks (five to six months) (Lovatelli & Sarkis, 2010).

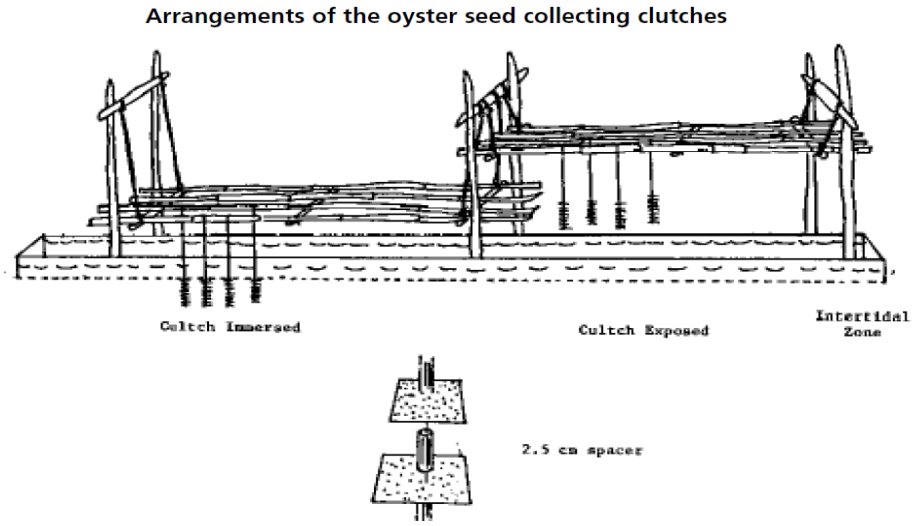

Figure 4. Diagram displaying Hanson Rack and Cultches. (Lovatelli & Sarkis, 2010)

Figure 5. Crassostrea rhizophorae out at sea on Hanson racks in Bowden Bay, Saint Thomas.

Oyster spat collection is highly dependent on the active rainy season, May and October to create ideal environmental conditions for oysters to spawn, eggs to develop and spat to settle. Changes in environmental activities, and the destruction of habitat due to global warming and anthropogenic activities disrupt oyster spawning cycles, reducing spat availability and ultimately reduces oyster production. The establishment of a controlled environment enables mangrove oyster spawning and grow out cycles to continue uninterrupted and more frequently thus increasing overall oyster harvesting quantities, mitigation is key to achieving stability.

5.3 Ecology: Mangrove Oyster (*Crassostrea rhizophorae* CR) other Crassostrea species

The world's mangrove resources cover approximately 100,000-200,00 km of tropical estuarine zones where mangrove trees can be found within Brackish water (waters that constitute a mixture of marine and fresh water.) (Lapègue et al., 2002). These areas are suitable aquaculture sites due to their frequent exposure with the rising and sinking of the tides.

5.3.1 Biology

The Mangrove oyster is a euryhaline bivalve molluscs that belongs to the Ostreidae family (Christo, Absher, & Boehs, 2010). Its shell is comprised of Calcium carbonate ($CaCO_3$,) approximately 96% and which in its mineral phase is called calcite) and varying miniscule trace elements (Christo et al., 2010). Distinct phenotypical description of the species is not easily found. However, based on physical analysis of oyster specimen at Bowden Bay Jamaica a general synopsis of the oyster can be described as having a deep cupping (figure 6 and 7) on one side containing the flesh area while the opposite side is slightly flat. The shape of the oysters also depends on the settlement material used in collecting spat, in the case of the *Crassostrea rhizophorae*, the underside of the oyster (the cupped area) is flat due to its settlement on recycled car tires used as cultches.

Figure 6. Underside (cupped portion) view of Crassostrea rhizophorae demonstrating its flat nature due to settlement on a rubber cultch.

Figure 7 External view of the top area of the flat area of the Crassostrea rhizophorae.

The *Crassostrea rhizophorae* (CR) can average in size up to 10cm (Menzel & Nascimento, 2019). Its internal anatomy comprises of the basic components such as the abductor muscle, responsible for the closing and opening of the shell, mouth, anus, mantle digestive gland, heart and other areas (Figure 8) These organs are responsible for the normal function of the oyster to carry out basic life processes: Ingestion, metabolism etc.

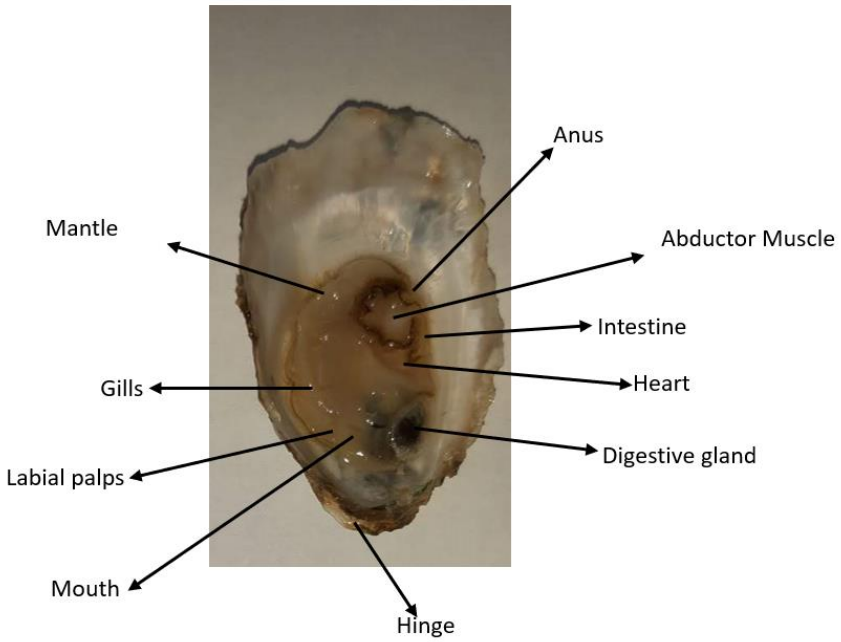

Figure 8 Detailed diagram of the internal anatomy of Crassostrea rhizophorae

Suitable conditions for the survival of Oysters during different phases is dependent on a range of water parameters. Ideal water salinity levels of 15 to 25 ppt, oxygen concentrations of 2-5 mg/l, a temperature range between 22-28^{o}C especially in estuary environments allows for the best survival of CR (Neto et al., 2013). Tropical and temperate waters (except arctic waters) are the preferred areas of habitation. Within different regions, they are located on the coast of the country at either the intertidal or shallow subtidal water level. Typical group behaviour will find oysters in clusters either on the aerial prop roots of the mangrove (rhizophorae) or on sedimentary or shelly bottoms (Bayne, 2017; Lapègue et al., 2002). CR is found all over the Caribbean, Atlantic, South America region.

5.3.2 Reproduction and development in Crassostrea species.

Crassostrea reproduction processes are similar and can be likened to varying *Crassostrea* species/subspecies. Where information is lacking on *CR*, *Crassostrea gasar* (CGI) will be used to demonstrate the phases of reproduction in the species. As a tropical area some similarities exist in the processes needed for reproduction to take place. CG is a tropical species found in Brazil and West Africa (Hannah, Brown, & Milner, 2007) which have climates similar to those in the Caribbean. Of the two countries, Brazil is closest to the Caribbean and like the Caribbean water temperatures are within a consistent range between 28^{o}C-30^{o}C (Paixão, Ferreira, Nunes, Fonseca-Sizo, & Rocha, 2013) making it suitable for the commercial culture of Crassostrea species of oysters.

Crassostrea species are single sex containing organisms (dioecious) that release sperm and eggs externally, this action is normally triggered via water parameter factors especially that of salinity which fluctuates during the rainy and dry season. Within the estuary environment an increase in organic

matter via the inflow of water runoff containing nutrients and the decrease in salinity allows for a higher concentration of phytoplankton bloom. The abundant and diversified phytoplankton is consumed by the oyster providing the energy needed for gametogenesis (development of gametes) and once mature, release of said gametes result thus, diffusion of reproductive material and the existence of free floating fertilized zygotes (Paixão et al., 2013) compared to those in temperate climate where gametogenesis (egg maturation) will occur when temperatures begin to fluctuate. For *Crassostrea gigas* that process happens at a temperature above 10°C with the actual range between 17°C-18°C (Ubertini et al., 2017) while mature *Crassostrea virginica* oysters reproduce at temps above 16°C. In C gigas, earlier gamete production can occur at temps of 24°C (Ramos, Gomes, Magalhães, Santos, & Melo, 2014). This factor is different for each species depending on their geographical location and climate conditions which will determine their period of sexual development which involves accumulation of reserves, gametogenesis maturation, and spawning (Ramos et al., 2014). Jamaica's rainy season takes place in two phases, From April to May and September to October. The collection of spat at Bowden Bay happens November to February, and April to June which also coincides with a similar dry season and dry rainy season transition.

Crassostrea spawning mechanism is universal and functions by first releasing their eggs externally and thus fertilisation will begin once the sperm meets egg (Wallace, Waters, & Rikard, 2008). Fusion of these two haploid gametes produces a zygote with a polar body (circular bubble in egg). This indicates that fertilisation has occurred. At this point cell division begins immediately until it has become a larva. The larva then goes through a series of free-swimming stages the first of which is the trochophore stage, followed by D umbo shaped larvae, eye larvae, veliger, pediveliger, plantigrade (early spat), Spat, Juvenile, Adult (Fakhrina et al., 2018; Wallace et al., 2008). Figure 9 describes the process of the oyster lifecycle in detail.

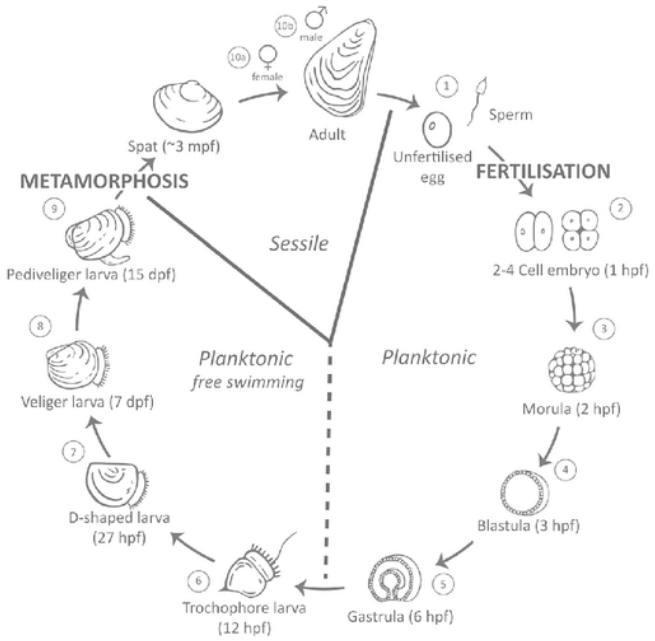

Figure 9. Description of the lifecycle of the Crassostrea gigas in detail along with time frame for each development period. (Vogeler, Bean, Lyons, & Galloway, 2016) In the Caribbean, the period between spat and adult normally takes place within three to four months

Crassostrea species begin development within the first hour of fertilisation, by cell division. At the 12th hour they are classified as Trochophores. Organogenesis and shell development are initiated, and the larvae are free swimming depending on circular ciliary bands for movement. Following the trochophore phase, they develop into D shaped larvae. Vital organs and nervous system have begun to further develop, while new organs such as the velum and foot begin to develop (Vogeler, Bean, Lyons, & Galloway, 2016). Veliger and Pediveliger phases mark the development of further expansion of the oyster shell growth along with the presence of an eye spot that is sensitive to light and is also a prominent feature. This is followed by the full development of the foot and eyespot which signals its preparation to settle on substrate. During the metamorphic phase, Crassostrea species internal organs will change, i.e. a loss of velum occurs, and gills take its place, while the foot would have either disappeared partially or completely. This organ would secrete a cement like substance and settlement would have taken place making them spat (miniature oysters that contain fully developed replicas of adult oyster internal organs and external features) (Baker & Mann, 1994; Fakhrina M N et al., 2018; Wallace et al., 2008). The importance of producing phytoplankton within a hatchery environment allows for the proper growth and development of oyster in the first few phases of their life.

5.3.3. Feeding mechanism in Oyster

The uptake of food particles by bivalves can be categorised in two ways; (a.) netting- using mucous nets or strings whether they are internal (within the mantel cavity) or external, or (b.) ciliate action, a process where molluscs rely on cilia (finger like structures) to retrieve food (Ward & Shumway, 2004). Oysters can be classified as ciliate action organisms. Food is captured through filtration. At the earlier stage of development, oysters at the D larval phase feed using their cilia and velum. Researchers explain that in *Crassostrea angulate* (CA), for example, seawater is ingested using the finger like protrusion (cilia) which can be found orally and within the velum at which point micro algae are retained and water is filtered out. Further filtration of algae occurs when water has reached the velum (developing digestive tract of oysters during the D larvae stage) (Qiu, Liu, Zheng, Zhang, & Qi, 2015). In adults, water is taken into their body when the shell slightly opens, and the cilia starts a beating action on the gills which causes the movement of the cilia through the mantle, begin circulating the water particles which exist on the other side. Cilia transport the filtered food to the mouth of the oyster, after ingestion, it is moved to the stomach (gut) then passed on for processing and absorption in the digestive diverticular (Ganapathi Naik. M, 2015). Understanding feeding processes is important to aid in providing the right microalgae in a hatchery environment; consideration must be given to the size, shape and quantity of microalgae made available to the oyster for consumption.

5.4 Importance of a Hatchery.

Jamaica is currently embarking on a campaign to encourage the local consumption of products using the tag line: "*Grow what we eat, eat what we grow."* to spearhead programmes that encourage food security for the island. The aim of these programmes is to encourage growth in cultivation and further processing of more local products making them available for consumption and export. The Blue economy (products from the earth's water bodies) has not been extensively explored for standardisation in the island and making use of its potential sustainably falls under this category. The government organisation, The National Fisheries Authority, (NFA) formally the Fisheries Division has been mandated to better utilise sustainably, the resources from the blue economy efficiently and effectively, and involves the exploration of underutilised species such as oysters (establishing a Hatchery for their production) sea urchin production, Sea cucumber, Glass eel fisheries while improving and further regulating activities associated with FAD and long line, artisanal and Industrial pelagic fishing and Live Lobster production. Climate change is a constant threat that is changing the way our world operates. Adapting new technologies to mitigate against this issue is crucial to the

survival of the aquaculture industry. Part of the protocol to ensure Caribbean readiness is to become actively involved in changing the operation of our activities.

5.4.1. Essential Hatchery Operations-Algal Production

Oyster hatcheries provide juvenile oysters for commercial production, restoration projects and research. (Wallace et al., 2008). It is meant to provide the ideal environment, good water quality parameters (Temp, DO, ph., Total Dissolved solids, nutrient availability), adequate food sources that facilitate bivalve spawning and egg development, D shaped larvae nutrition and spat production. Not only oysters, but other bivalves such as clams, mussels, sea urchins, etc., can be cultured under these conditions. Hatchery operations normally consist of a Broodstock/nursery area for maintaining spat and Broodstock and a separate algal culture facility for feeding of oysters at each stage. (Sarkis & Lovatelli, 2007). Some layouts may be in the order of Broodstock maintenance and spawning, larval culture, setting and cultch preparation and algal culture (Breese & Malouf, 1975) (Crisp, Yule, & White, 1985). No matter the order, their primary purpose is to maintain (feed and fatten oyster Broodstock to ensure healthy eggs are fertilised) and induce spawn intended Broodstock, care for hatched larvae after spawning has occurred, and prepare mediums intended to allow spat to settle before they are sent out to sea for further grow out until harvest.

5.5 Microalgal culture

Microalgae as defined by Phycologists are chlorophyll containing organisms with a plant body that does not differentiate into roots, stem or leaves (Amos Richmond, 2004). They are usually microscopic in nature and require specialised equipment such as a microscope for viewing. They can be classified as unicellular (single individual), colonial (grouped) or filamentous (thread like) (Amos Richmond, 2004). Together in a confined space, they present a green hue or tinge in water. They can be found in both fresh and marine environments, however for the feeding of bivalves that live in a marine environment, a marine alga is the corresponding food for the Mangrove oyster *Crassostrea rhizophorae*.

When cultivating these species, there are several factors to consider: (1) physical factors such as, light, water temp, ph and other abiotic factors, (2) microalgal species and biochemical presentation (nutrient factors), (3) algal growth phases for harvesting algae at its optimum state, (4) steps in producing a microalgal starter culture, (5) the methods of culture (e.g., batch, continuous, semi continuous, indoor vs outdoor), (6) calculating ideal algal quantities for feeding, (7) algal species combination recommended for the different phases of bivalve development (FAO, 2014). All of which will contribute to the successful and continuous production of microalgae especially in a hatchery environment in Jamaica.

Different species require different types of algae; however, geographical location determines the type of algae present and is most likely the type of algae being consumed by oysters in the wild. Under hatchery conditions, oysters that are cultivated are not fed according to the content of the surrounding sea but are rather fed a general species of algae that is used throughout the industry. A basic profile of algal species fed to oysters are in Table 1 of the listing of species of algae cultured for oyster production. The process of culture, the nutritional make up and feeding densities of the micro algal species listed is part of the work to be examined by this paper to better understand how to culture, what types and how much algae should be fed based on stocking densities in both the broodstock, juveniles and spat phases of oyster culture under hatchery conditions.

Table 1. Specific Algal species cultured for oyster production under hatchery conditions worldwide.

Oyster Species Cultured	Common Flagelate Algae Cultured	Common Diatoms Cultured	Source
Ostrea edulis L., *Crassostrea gigas* *Mytilus edulis L.* *Crassostrea Gigas* *Crassostrea Rhizophorae*	*Hochrysis galbana*	*Chaetoceros calcitrans*	(Sarkis and Lovatelli, 2007) (FAO, 2014) (Crisp et al., 1985;) (Gerdes, 1983)
	Rhodomonas baltica	*Chaetoceros gracilis*	
	Tetraselmis suecica	*Thalassiosira pseudonana*	
	Dunaliella tertiolecta	*Skeletonema costatum*	
	Isochrysis galbana	*Chaetoceros muelleri*	
	Isochrysis		
	Pavlova lutherii	*Phaeodactylum tricornutum*	
	Nannochloris occulata		
	Tetraselmis chuii		
	Chaetoceros calcitrans		
	Phaeodactylum tricornutum		

5.5.1 Abiotic factors for Microalgae.

Microalgal production is common in hatcheries across the world. Producing these organisms requires specific conditions to enable successful culture. At its core, microalgae make their own food via photosynthesis which involves the uptake of nutrients from the water and the use of light and carbon dioxide. Within a hatchery setting providing these parameters along with nutrients such as nitrates, phosphates, essential trace elements to treated seawater ensures that the production of algae is continuous (FAO, 2014a). The specification for the parameters mentioned above in algal culture should be in the following range (Table 2).

Table 2 Water parameters ranges for Micro algal production in a Hatchery setting.

Parameter	Range	Optimal	Source
Temperature (°C)	16-27	18-24	(Brown & Blackburn, 2013)
Salinity (g.l)	12-40	20-24	
Light intensity (lux) (depends on volume and density)	1,000-10,000	2,500- 5,000	
Photoperiod (light:dark, hours)	6:8 (max)	24:0 (min)	
pH	7-9	8.2-8.7	

5.5.2. Micro algal species and biochemical composition

Understanding types of algae and its nutritive requirements is key to supplying bivalve larvae, juvenile to broodstock with a balanced diet. All nutrients do not exist in a single algae and a diverse mixture of microalgae ensures nutritive components such as protein, essential amino acids, essential fatty acids, carbohydrates, vitamins, minerals and trace elements (Knauer & Southgate, 1999) are readily available for absorption throughout their juvenile stages and as adults, for the development of gonads to produce offspring and maintenance of everyday functions. The most common classes of microalgae cultured and species belonging to those classes are listed in the table. The stages at which the mollusks can be fed the varying species particularly for oysters is also noted in table 3.

Table 3. Common microalgae cultured in aquaculture which normally feed oyster species. Definition of abbreviations include BPL-Bivalve Post Larvae, BB- Bivalve broodstock, BL- Bivalve Larvae, MZ Marine Zooplankton (Brown & Blackburn, 2013).

Micro algae species Used in Aquaculture of Bivalves				
Class	Genus	Example of Micro algae	Category of use	Source
Parsinophytes	Tetraselmis	T. suiceia, T. chuii		(Brown & Blackburn 2013)
	Pyraminamonas viginica		BP, BL	
	Micromonas pursila		BL MZ	
Cryptophytes	Rhodomonas	R. lens	BL BP MZ	
		R. salina		
Chlorophytes	Dunaliella tertiolecta			
	Chlorella	C.minimutissima	BP BB MZ	
		C. virginica	MZ	

Table 4 (cont.) Common microalgae cultured in aquaculture which normally feed oyster species. Definition of abbreviations include BPL-Bivalve Post Larvae, BB- Bivalve broodstock, BL- Bivalve Larvae, MZ Marine Zooplankton (Brown & Blackburn, 2013).

Micro algae species Used in Aquaculture of Bivalves				
Class	Genus	Example of Micro algae	Category of use	Source
Diatoms	Skeletoma	S.costatum	BL BB BP	(Brown & Blackburn 2013)
		S.pseudocostatum		
	Thalassirosira	T. pseudonana		
		T. oceanica		
	Chaetoceros	C. calcitrans		
		C. muelleri		
Premnesiophytes	Isocrysis	I. galbana		
		I. spp		
	Pavlova	P. lutheri		
		P.salina		
		P.pinguis		

Carbohydrates, vitamins and minerals are the basic components found within algae. For carbohydrates they become available to the oyster readily as hydrolysable polysaccharides. They allow bivalves to utilise protein and lipids for biosynthesis. Proteins, the precursors to amino acid, aid in tissue biosynthesis through the supply of nitrogen and essential amino acids (EAAs). Fatty acids in algae are numerous. However, the most important of these are DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid) and ARA (arachidonic acid). The overall purpose of these fatty acids especially in bivalves contributes to egg viability and spat survival after fertilisation. DHA and EPA contribute to membrane fluidity (viscosity of lipid bilayer of cell membrane) of bivalves. The role of vitamins and minerals is not fully understood but the essential minerals calcium, normally absorbed from the seawater, is most useful and is suggested to contribute to shell growth of bivalves. Vitamins are linked to the survival of larvae especially at the pedivegular stage (Brown & Blackburn, 2013; Knauer & Southgate, 1999) by providing a mixture of micro algae to cover the basic nutritional needs of the CR.

5.5.3. Algae growth phases

Depending on the methods used to produce microalgae a clear and concise understanding of algal phases and the ideal times of harvest ensures that algae provided along each phase of the oyster's development retains the maximum nutrient components. The growth phases of algae can be separated into four segments (Table 5):

Table 4 Growth phases of algae (FAO, 2014a)

Induction Phase (Lag stage) **1** Very little growth occurs here. It may long if culture is transfer of Algae from plate to liquid culture or short of liquid is inoculated with exponential growth algae. There is little increase in cell density at this phase due to the algal cells' physiological adaptation of cell metabolism to the process	**Exponential Phase (Growth stage)** **2** There is an increase in cell density, this may be due to algal species exposure to elements such as: light intensity and temperature.
Stationary Phase **3** At this phase, there is a balance between the limiting factor and growth rate.	**Death Phase (Crash Phase)** **4** Deterioration in water quality and a depletion in nutrient results in a reduction in cell density and an eventual crash in the algal culture.

Ideally, culture should be continuously maintained at the stationary phase (3). Once beyond this threshold there is a likelihood to produce toxic metabolites, composition deficit and non-digestibility (FAO, 2014a).

5.6 Starter culture methodologies

Starter culture are normally referred to as axenic, i.e. pure strain cultures free of other types of algae and can be classified as the building blocks that will allow a hatchery to produce large volumes of specific species of algae whenever it is needed. By means of inoculating the designated container of clean sea water. The process of culture begins in the following order: stock culture, starter culture, inter mediate scale culture. The first of which is kept without aeration and CO_2 in a climate controlled environment that has low temperature (FAO, 2014a)

5.6.1. Methods of algal culture

There are two main modes by which algae can be produced: Indoor, which is normally intensive and requires artificial light on the outside of the vessel housing the microalgae or Outdoor culture which is more exposed to the elements and relies on natural lighting and takes place in large tanks or ponds (FAO, 2014a). Common species produced include: *Monochrysis lutheri* and *Isochrysis Galbana*, *Thalassiosira pseudonana* (Breese & Malouf, 1975; Fakhrina et al., 2018). There are three main algal culture methodologies incorporated to ensure quality is maintained. Table 6 gives a brief explanation of each method.

Table 5. Description of the methods of algal culture practised to produce marine algae, continued on page 20 (Sorgeloos, Center, Ghent, & Ghent, 1996)

Methods of Algal Culture

Method	Description	Advantage	Disadvantage	Source
Batch Culture	This method uses fertilised seawater that has been inoculated with a culture of algae, this is left to grow until ready for harvest when algal has reached near maximum to maximum density.	Simple, Flexible, allows specie change remedies defects in culture system rapidly.	Must be maintained for a specific time period Harvest quality less predictable Contamination prevention must be practised in inoculation phase. Labour intensive harvest. (containers must be cleaned, sterilized and refilled.	(Sorgeloos, Center, Ghent, & Ghent, 1996)

Method	Description	Advantage	Disadvantage	Source
Continuous Culture (Turbiostat/Chemo stat)	Method whereby continuous supply of fertilized sea water is being pumped into a growth chamber while there is an overflow of excess algae culture.	Produces a more predictable quality algae. Technologicall y adaptable and therefore less labour intensive. Relativly reliable algal culture system.	Bags can be large and cause self-shading, resulting in low algae densities Overall cost can be expensive. Restricted to indoors due to the need for high illumination requierments	(Sorgeloos et al., 1996)
	T.stat-Dilution of algal culture with medium to keep algal concentration at a preset level (automatic)	Gives optimal yield especially for Dinoflagelate		
	C.stat-Introduction of fresh medium via steady flow-This influences growth rate and not cell density.	Can be reasonably affordable		
Semi continuous	Periodic harvest of algae immediately topped up to original volume and supplemented with nutrients for prolonged use of the batch.	Flexible method can be used indoor or outdoor Yields more culture than the batch method.	Unpredictable duration. Susceptible to contamination especially outdoor culture. Build-up of metabolites can make the culture useless.	

6. FINDINGS

SMEL algal culture station

An internship at SMEL, an algal culture station, located in Blainville-sur-Mer in Normandy France was an important part of this research. SMEL produces algae using simple techniques that are cost effective and efficient. As the leading facility in bivalves research to aid farmers in the production of bivalves, particularly cupped oysters, of the specie *Crassostrea Gigas*. They have over thirty years of experience in this activity. As facilitators of technological transfer, their influence reaches as far as Africa and now the Caribbean. Algal production technology transfer was done via a visit to the facility which encompassed: lectures, practical experience in production methodologies and interviews via a questionnaire (appendix 8) collecting resident expertise to pinpoint specific information on algae production and their techniques through the different phases of oyster development. Information gathered will assist Jamaica in the way forward in algae production specific to its needs under hatchery conditions.

SMEL employs the batch culture method, i.e. producing unicellular algae using a single vile, to establish a culture in a sterilized beaker with nutrients. This stored in a temperature-controlled room at temperatures of 18^{o}C, with a salinity of up to 32 ppm. *Isochrysis Galbana, Tetraselmis suecica and Chaetoceros calcitrans* are the three main species produced. These are obtained from the local algal bank in the form of either a petri dish or borosilicate vile. Examples of the three species were observed under a microscope and photographed (see appendix 2). The batch culture method for algae production is applied to all species they produce. This way they can maintain the stock at the exponential growth phase by consistently harvesting a portion of the algae, refilling with treated sea water, and adding nitrogen-phosphate/nitrogen-silicate-phosphate solution for Flagellates and Diatoms. Simple equipment is used for the process (see appendix 3) making it cost effective.

Algal production

Production of algae begins with a three-phase system. Stock culture is the building block for creating the other three stages: Starter, immediate and large-scale culture. The former is viable for up to a month. Constant observation and monitoring are needed to ensure it is healthy enough to be used for the three culture phases. Secondly, assessing the state of microalgae is part of the monitoring process. By observing algae under the microscope and noting the features and activity and using the spectrophotometer to read concentration of the desired microalgae, will assist in maintaining quality of the algae during its lifecycle at any of the phases of culture present in the lab. Establishing a culture relies on the use of clean seawater, sanitized holding containers and providing essential nutrients for the growth of the algae being produced; this comes in the form of producing nutrient rich solutions or culture mediums.

Algal Culture for Broodstock.

Broodstock feeding requirements are slightly different at this stage. As full-grown oysters, they can handle all three species of algae being produced by SMEL. There is no specific algal strain combination, more important is the diversification of algae available for the oysters to consume. Feeding of broodstock normally takes place once a day, Algae ration is calculated by assessing a sample of no less than 10-12 adult oysters for flesh quality and dry weight. Flesh water content can be removed with a compact dehydrator. Oyster can be fed anywhere between 2-4 % of their dry weight (see appendix 5). Algae for feeding should be chosen and divided in two to reflect the percentage at

which the oysters are being fed. The dry weight of each oyster species is also needed to complete the calculation (FAO, 2014a). A detailed break down and example of how to calculate algal proportions is seen in (appendix 5). A spectrophotometer is used daily to access algal concentration and based on a formula calculation of feeding quantity for the day is done. Algal quality is not compromised due to the precision of feeding through calculating feed quantity. Because oysters a great filter feeder, and the quantity being consumed is measured there is no waste produced except for excrement which is removed via a flow through system.

Algal Culture for Larvae phase.

Oysters at the vegular and pedivegular phase, are small and need big enough observation of the species under a microscope after spawning can help to track the progress of development. Upon notice that they have reached D shape, then feeding can begin. The combination of algae matters. It is important to feed algae with a Diatom and a Flagellate to ensure they are receiving a diversity in the food being provided. A single algae species cannot provide all the nutrients needed to satisfy their requirement. At this stage of development, it is ideal to give Isochrysis and Chaetoceros due to their small size and their easy intake by the D shaped larvae, as well as their nutritive component. They are then fed starting with small quantities. Feed demand fluctuates as the larvae grow; however, their growth increases their filtration rate and thus the demand for more food. CR filtration rate is not known and could be explored in further studies. Larvae are fed everyday once a day. Food distribution is done by hand, an amount is measured and given to satisfy their needs (see appendix 5). Water quality is maintained by water exchange. Maintenance of water beings 24 hours after spawning has taken place and exchanged up to three times per week (FAO, 2014)

Algal culture for Spat.

Oyster at the spat stage are miniature versions of adult oysters. Here they can consume the three species produced at SMEL without complication. Like the larvae, demand for food will fluctuate, increasing in quantity due to the growth of the spat. Settlement normally occurs when they have reached the pediveliger stage and have gills and a foot appendage present, which can be observed under a microscope (Fakhrina M N et al., 2018). SMEL inspect their larvae every two days to observe the development taking place. Feed distribution can be done in two ways: Feeding according to container capacity. (knowing the volume of the container) or feeding according to increase in growth of the oyster larvae. It is recommended that the former be used for ease of execution (FAO, 2014a) This methodology can also prove useful to Jamaica and calculations can be tweaked according to the quantity of larvae it produces.

Culture Medium Production

Different mediums are tailored to meet the needs of the algae being produced. Species cultured at SMEL use Conway medium (CM) along with treated seawater as the base for their mediums. CM which is made up of a Trace element solution TES and additional elements is easy to prepare and contains the ideal nutritive make up. Diatoms require a sodium silicate solution (SSS) along with the Conway medium, to provide the algae with the building blocks needed to construct its silicate wall. Flagellates do not need it due to their lack of a silicate wall. Starter cultures need a medium that will enable growth and viability for up to a month. Erdschreiber culture maintenance medium, Guillard's F/2 media or HESAW media can be used (FAO, 2014a). However, CM is used by SMEL the preparation for which can be seen in (appendix 4). The use of Conway medium is also recommended for use in Jamaica for ease of medium production.

Conway medium is made up of two formulations: A trace element solution and added nutrients. The former should be prepared to enable the production of (CM), the preparation for which can be found in (Appendix 4). The formulation can be adjusted to make as little or as much medium as desired. While stock cultures are not aerated and starter cultures are given enriched CO_2 (FAO, 2014a). SMEL does not use this methodology, their location to the sea allows for the constant supply of UV treated filtered sea water to combat cross contamination in their operations. This water contains some form of $C0_2$. which can be expensive in compressed form. The exclusion of it in SMEL's culture process lessens the complication of the process. Jamaica's proximity and access to sea water that can be treated and filtered, can prove beneficial for micro algal culture system setup. UV rays and filtration reduces the potential for any disease-causing agent hence the need to produce these nutrient rich solutions to replace the depleted microalgae.

7. DISCUSSION

Hatchery culture has provided a solution for high oyster demand. Through the culture of microalgae, bivalves can obtain essential nutrients for complete growth and development. Healthy and robust gonads produce viable eggs for reproduction and contributes to survival of the offspring. Hence the importance of food availability. Though the main guide for algal culture can be found in the FAO book: The Hatchery Culture of Bivalves: a practical manual. The experience from SMEL demonstrated methods that Jamaica can adapt to suit its needs at different phases from broodstock, Juvenile (vegular and pedivegular) stage and spat.

Recommended Method for Algal culture in Jamaica.

Batch culture production of microalgae is practiced because of the cost effectiveness of this method; which constitutes to approximately 40% of bivalve production costs in a hatchery (FAO, 2014a). It would be ideal for Jamaica to adapt this methodology, as it can be done in a small facility, with minimal but essential equipment necessary for clean culture production (see appendix 8) and a possibility to expand based on future demand from the Hatchery. Jamaica's climate can facilitate the growth of algae; however, a temperature control room will be ideal to allow maximum control of the environment and aid in slowing down the growth of algae. This would be done to allow algae to develop at different stages avoiding any crash and extending its shelf life to facilitate the establishment of other cultures. Counting of algae daily can be time consuming and strenuous on hatchery technicians. It cannot be stressed how important a spectrophotometer, microscope and desktop are to the efficiency of performing the daily duty of observing the state of algae at different quantities of culture. Likewise, an orbital lab shaker allows gentle agitation of stock culture to extend shelf life. Outdoor culture could prove useful in Jamaica especially if demand for alga is high and cannot be fulfilled by largescale culture. A closed bag culture system within a greenhouse away from direct sunlight supported by an outside frame, could also prove cost effective. Polyurethane is affordable and frames once constructed and cared for, can be used indefinitely, and repaired repeatedly. Alterations would have to be made to accommodate aeration to keep algae agitated and water oxygenated. A closed system also minimises contamination from outdoor elements. The drawback to this is the need for single use plastic. Alternatives that can be sanitized should be considered.

Medium preparation and Sanitation Practices.

Mediums used to produce marine algae include Erdschreiber culture maintenance medium, HESAW media, Guillard's F/2 media, Conway medium, are standard mediums used for culture, however. Erdschreiber, HESAW, Guillard's F/2 media can prove difficult to produce and may require additional ingredients. Conway medium is versatile, easy to prepare. And can be used up to Intermediate culture. However, for large scale culture it is best to use Potassium Nitrate phosphate solution to enable this form of culture as Conway medium is too expensive for large scale culture. Sodium metasilicate pentahydrate can be added to facilitate diatom culture. It should be eliminated for flagellates due to their lack of a silicate wall (see appendix 4). Sanitation practices are standard and important to minimise cross contamination, it is therefore important to practice good hygiene and put elements in place for this to be practiced. A hand wash and hand sanitation station, the use of 70% alcohol and the use of an autoclave and autoclavable borosilicate glass vials and plastic caps (which are reusable and will reduce environmental waste) is ideal for this practice. Importantly, using separate hoses for each bin should be a day to day practice, the use of alcohol and distilled water as a means of sanitization for utensils reduces cross contamination. Because of the consistent use of distilled water, a distilled water machine should be purchased and readily maintained for the use in almost all aspects of algal culture.

There is much research to be done in understanding the behavior of algae in Jamaican waters and our mangrove oysters thus we must bear in mind the recommendations in chapter 8.

8. RECOMMENDATIONS AND CONCLUSION

Jamaica's tropical climate possesses little challenge to the cultivation of algae. There are a few things to consider when establishing an algal operation. Gaps in the production of microalgae in Jamaica include knowledge of the aquatic microbiome, especially those surrounding potential areas for oyster cultivation. Analysis of the type of algae present in Jamaican waters, i.e. observing algae unique to our Caribbean territories that can adapt to our conditions readily. Research is needed to:

1. Understand marine microbiome of Jamaica. Isolation of oyster favored algae. If we can capture enough of our indigenous algae and practice methods of culture on them then we can produce large volumes of algae suitable for our environment. The experiment does not have to be expensive and only requires the settlement of sea water left alone for two to three days and identifying and isolating the desired algae under a microscope. Algae isolation can be done through two means. Competition isolation - Removing as much of the desired algae as possible and setting up an environment to see which algae will grow faster than the other. Or pure Isolation - Larger quantities of the desired algae are removed and incubated to allow optimal growth of the species intended for use.

2. Secondly, understanding the best method of cultivation for algae and the specific types of algae that mangrove oysters tend to prefer, by comparing growth of CR groups fed different diets is a basis for which research can be conducted.

3. Understanding the behavior of Jamaican Mangrove Oyster, is another aspect that should be explored for research. The focus could be placed on filtration rate, natural feeding versus lab cultured algae feeding and how it influences the growth and development of the oyster are gaps to be filled only through research.

4. An experimentation of the use of different mediums as suggested in FAO's The Hatchery culture of Bivalves: A practical guide, could be undertaken to see how effective their use is for our conditions.

Considerations for algal culture that are of great importance include:

- Access to clean seawater is imperative to the culturing needs. Contaminated water gives rise to unwanted algae that may compete for food and nutrients being provided for the algae of choice. They also become an avenue for disease development. Having sanitation methods like UV lighting alongside filtration for water coming in and out of the facility.

- Data collection must be the focus of algal production. A comprehensive data collection and storage protocol allows for long-term accurate analysis of methodologies employed, state of water quality and performance of oysters. It gives an idea of the way forward when designing programs for research. As well as making simple improvement of current and future systems

- The production of algae outdoors is something that Jamaica can consider. As a much cheaper alternative to indoor culture. This can either be an open or closed system. It is recommended that a closed system be used to culture algae in larger quantities. This will eliminate the need for large vessels such as vats and artificial light. The drawback of this method is the risk of contamination, low light penetration due to excess debris and quick failure of culture (FAO, 2014b). Improving methods of outdoor culture for Jamaican purposes can be researched.

In order to allow Bowen Bay to be the premier institution for bivalve production we must employ new and emerging technologies that facilitate strategic management of resources for algal culture and oyster production. Attention must be concentrated on having a state-of-the-art lab. One that can facilitate observation analysis and production of algae in convenient environment. This should in future be able to supply the market with oyster and oyster products boosting the Jamaican economy and eventually becoming the ideal place in the Caribbean for which research can take place on marine and freshwater microbiome.

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11. APPENDICES

APPENDIX 1. MICROALGAL PRODUCTION AT SMEL

SMEL's Microalgal production room and algal culture phases

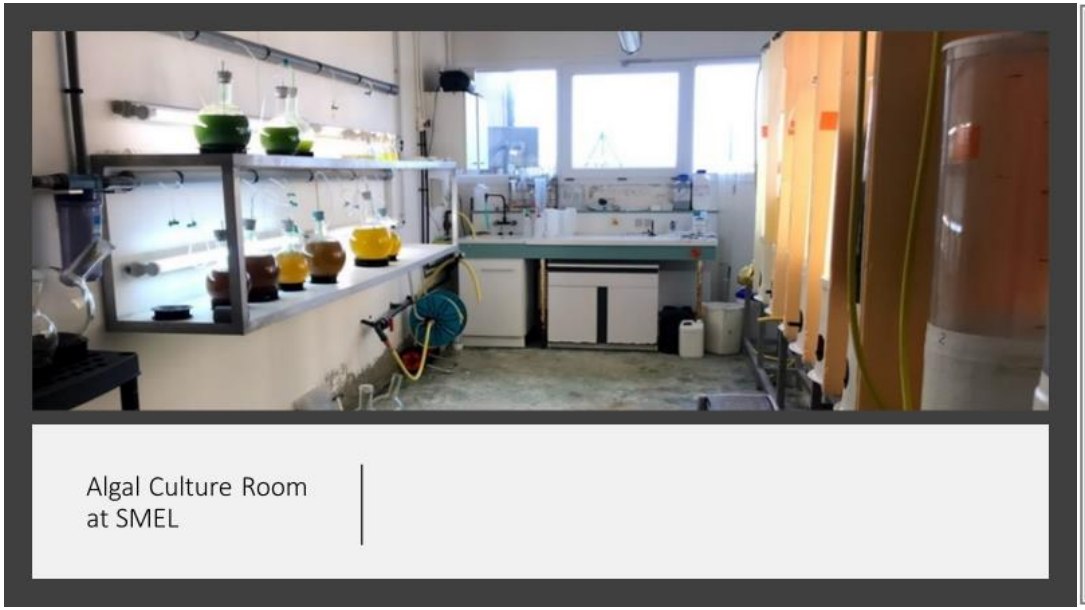


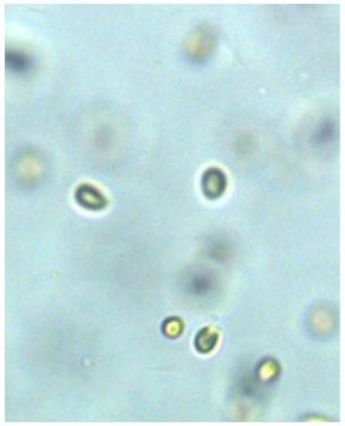
Figure 10 Algal production room at SMEL in Blainville-sur-Mer, Normandy France

Figure 11 Example of the Stock culture used to produce the three-phase process of algal culture.

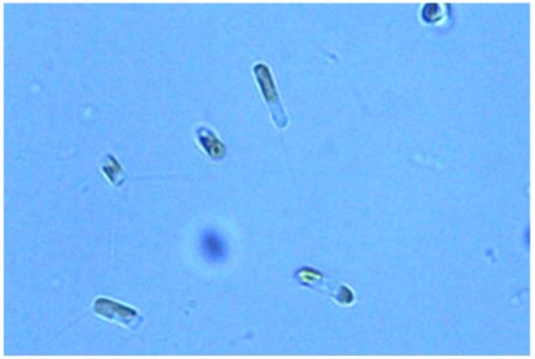
APPENDIX 2 MICRO ALGAE SPECIES PRODUCED AT SMEL.

Figure 12 Examples of Micro algae in medium and cell under microscope.

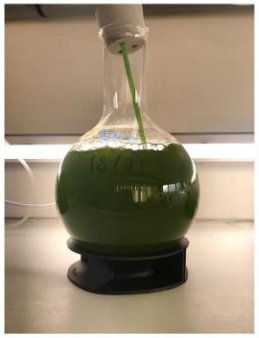
Isochrysis Galbana

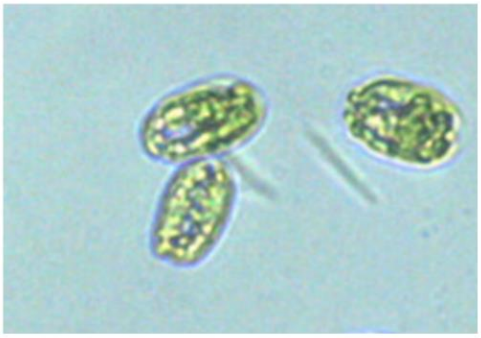


Chaetoceros calcitrans



Tetraselmis suecica





APPENDIX 3 PROCESSES FOR STARTING MICRO ALGAL CULTURES.

Table 6.Methodologies for producing starter culture algae.

Starter Culture of Micro algae

You will need:

- Stock culture
- Sanitized 2L/4L Borosilicate round bottom flask and flask stand
- Pipette
- Air tubing and rubber stopper
- Nitrogen phosphate solution
- Silicate solution (For Diatoms only)
- Bunsen Burner
- Cotton stop.

1. In a sanitized area fill 2L/6L flask with clean sea water. (Fill to 75% capacity)
2. Flame Bunsen burner.
3. Add Conway medium solution to flask (1ml/l) with a sanitized pipette
4. Add Silicate Medium if starting a diatom culture (1ml/L) with a sanitized pipette.
5. Remove capping from stock culture tilt flask and flame the mouth of beaker.
6. Flame another clean pipette allow cooling, then insert in stock culture flask and remove 25 ml of culture.
7. Add pipetted culture to prepared flask (s).
8. Flame the mouth of stock beaker cover immediately and set aside.
9. Cover 2L/6L flask with a rubber stop place in area with light.
10. Insert a sanitized aeration tube and allow culture to grow over the next 7-14 days
11. For Diatoms growth can occur between 3-5 days.

Table 7 Methodologies for producing intermediate algal culture.

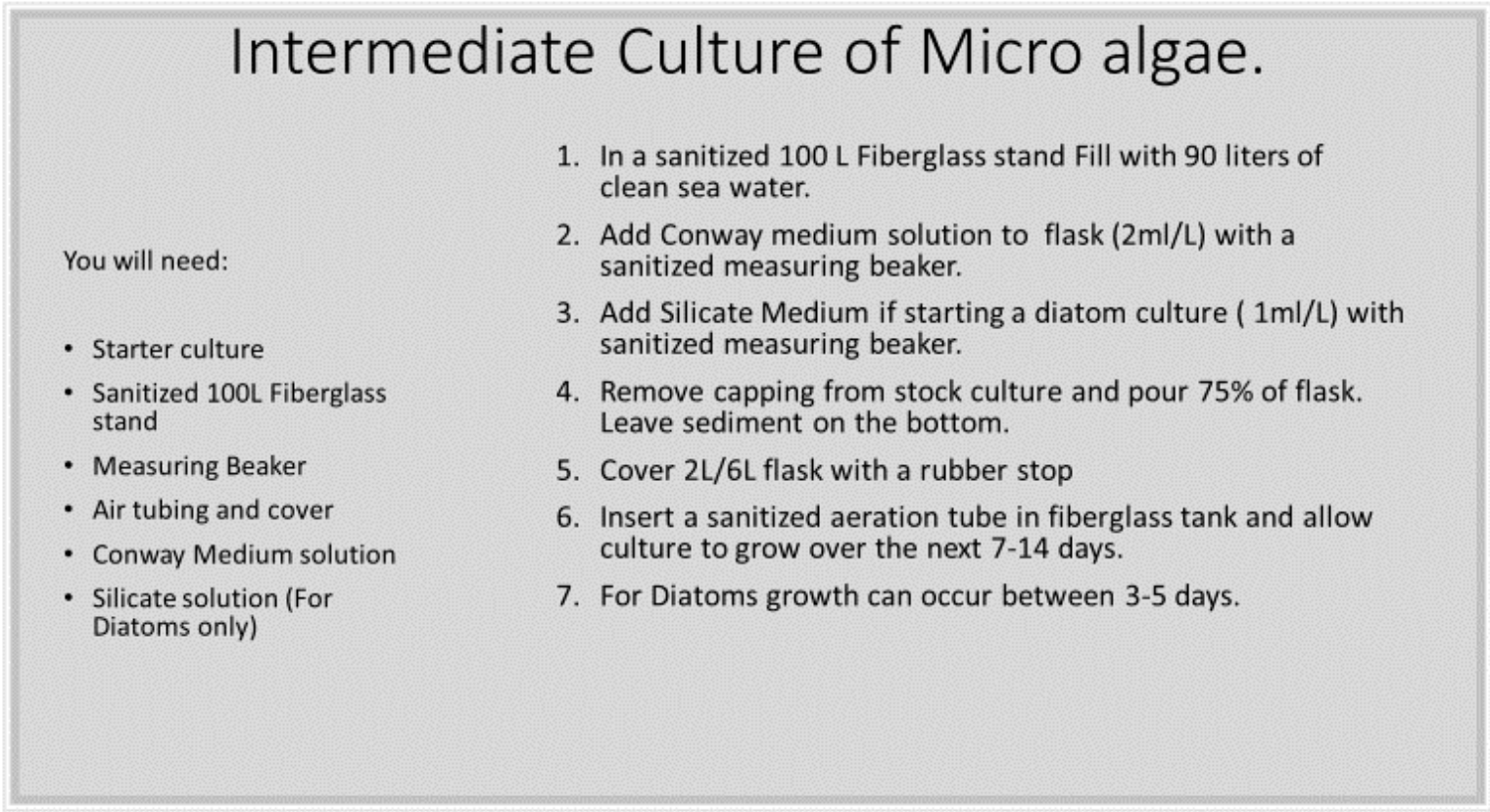
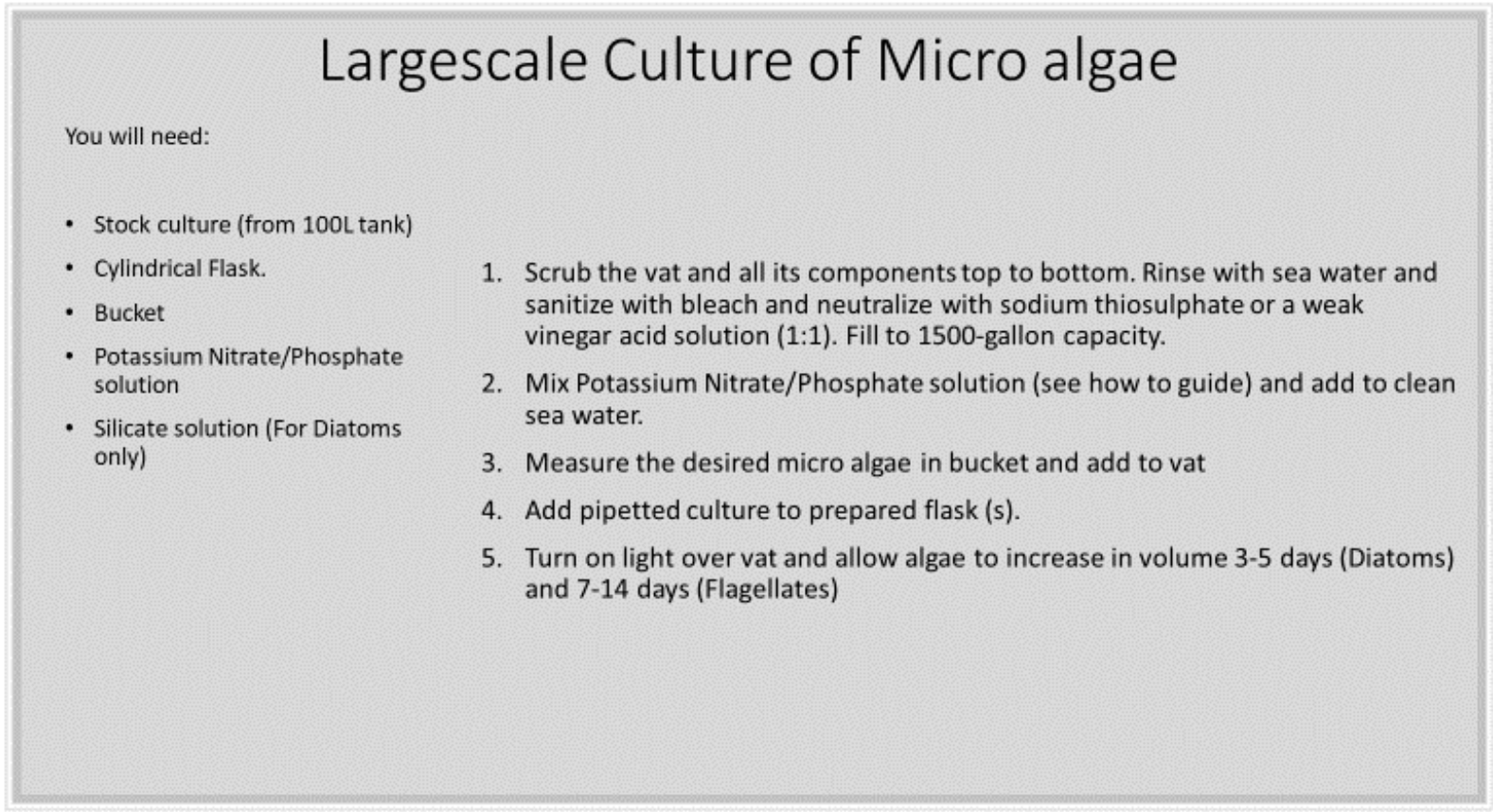


Table 8 Methodologies for producing largescale algal culture.



APPENDIX 4 CULTURE MEDIUM FORMULATIONS

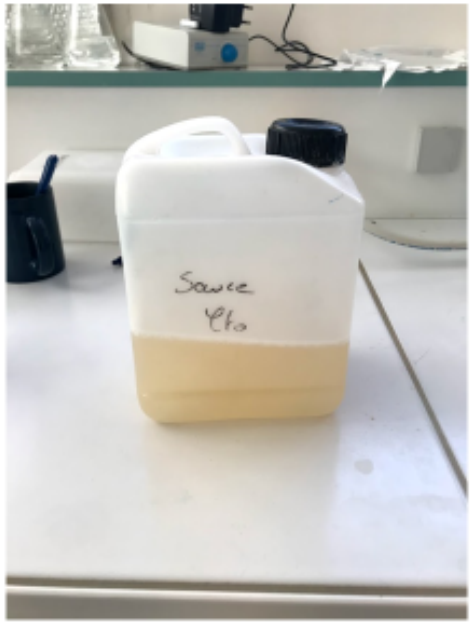
Table 9 Constitution Trace Element Solution (TES) normally used to produce CM.

Trace Element Solution Formulation(TES)

ELEMENT		QUANTITY
Iron Sulphate $FeSO_4$		15.5 g
Manganese sulphate monohydrate	$MnSO_4$	1.55 g
Zinc sulphate heptahydrate	$ZnSO_4$. $7H_2O$ or $H_{14}O_{11}SZn$	0.22g
Copper sulphate pentahydrate	$CuSO_4.5H_2O$	0.019g
Cobalt sulphate pentahydrate	$CoSO_4$	0.024
Sodium molybdate dihydrate	Na_2MoO_4	0.012
Distilled water	H_2O	1 L
Hydrogen chloride	HCL	Add until you obtain a lemon yellow

Table 10. Constitution of Conway medium Solution (CM) normally added to the culture vessel of Flagellates and Diatoms as a base for nutrients.

CM Conway Medium Formulation



Element	Quantity
Trace Element Solution	80 ml
Potassium Nitrite	80 g
Dipotassium phosphate	16 g
EDTA	4 g
Vitamin B12	1ml
Vitamin B1	0.01 g
Distilled Water	1L
This makes One liter of CM solution	

Solution should be stirred with a magnetized mixer, placed in a sanitized bottle and stored in a cool place.

Table 11. Constitution and calculation of Sodium Silicate Solution (SSS) normally added to the medium for Diatom culture e.g. Chaetoceros calcitrans.

Sodium Silicate Solution Formulation(SSS)

- **This solution constitutes: Sodium metasilicate pentahydrate ($Na_2SiO_3.5H_20$) and Distilled water.**
- *To make 1 liter (1000 ml) of solution*
  - *(4.0 g Sodium metasilicate pentahydrate Silicate should be added to 100 ml distilled water (FAO, 2014)*
  - (1L)1000ml/100*4.0g= 40g
  - Amount can be adjusted depending on the volume being made.

Solution should be stirred with a magnetized mixer, placed in a sanitized bottle and stored in a cool place.

Table 12. Constitution and calculation of Potassium Nitrate/Phosphate Solution (PNPS) normally used for creating medium for Large culture (2000L) of Diatom culture e.g. Chaetoceros calcitrans.

Potassium Nitrate/Phosphate Solution (Used for large volume culture)

- **This solution constitutes: Potassium Nitrate (KNO_3) Sodium metasilicate pentahydrate ($Na_2SiO_3.5H_20$) and Distilled water.** *To make a mix for a volume of 100 L for Chaetoceros calcitrans.*
- *You will need:*

Potassium Nitrate- 0.0145g /l *100= 1.45g

Sodium metasilicate pentahydrate 0.017g /l*100= 1.70g (Eliminate if culturing Flagellate)

Phosphorous 0.0235 g/l*100= 2.35g

Dissolve into 1000ml of distilled water, then use in entire mix in 100L tank.

APPENDIX 5 FEED CALCULATIONS

Calculating feed for larvae/Spat

Calculating Algae Quantities in Larvae

Feed volume required= $\frac{required\ cell\ density * V}{Cell\ density\ of\ Harvested\ Algae\ \{cells\ /\mu\}}$

V represents- volume of larvae culture tank in L

E.g: Crassostrea rhizophorae larvae are being fed a diet of *Isochrysis galbana* and *Chaetoceros calcitrans* at a total rate of 125 cells per µl and 50 cells per µl respectively. The volume of the culture tank is 3000L. Cell density quantities for each specie is as follows:

Isochrysis galbana 36,000 cells µ/Chaetoceros calcitrans 24,000 cells µ.

CALCULATIONS for *I. galbana*: $\frac{125*3000}{36000}$ **= 10.41 L CALCULATIONS for *C.calcitrans*:** $\frac{50*3000}{24000}$ **= 6.25 L**

Total feed amount: .16 66L of *I. galbana* and *C. calcitrans*

Calculating Feed in Adults

- **Method calculation for feeding one oyster with one algae**
- $dry\ weight\ meat = \frac{\Sigma(dry\ weight\ of\ the\ flesh\ of\ 10\ oysters)}{10}$

- Choose which percentage of the dry weight you want to provide between 2 and 4 % (depend of the state of the oyster). Then choose the fraction of this percentage you want to give to your broodstock .
  - Exemple : I want to feed my broodstock at 4 % with Chaetoceros and Isochrysis half and half => I'll give 2 % of chaetoceros and 2 % of Isochrysis (because 2 % +2 % = 4 %)
  - Find the dry weight of the algae you want in the bibliography or calcutate it (for 1 million cells)

$$Ration\ per\ day = \frac{(percentage\ of\ dry\ weight\ meat\ \times dry\ weight\ meat\ (mg))/100}{dry\ weight\ of\ algae\ cells\ (mg)}$$

$$Volume\ of\ algae\ per\ day = \frac{Ration\ per\ day}{harvest\ density\ of\ the\ day}$$

For example : With Chaetoceros : $percentage\ of\ dry\ weight\ meat = 2$ (Choice made to feed algae)

$dry\ weight\ meat\ (mg) = 5000\ mg$ (calculation of 10 oyster dry flesh)

$$dry\ weight\ of\ chaetoceros\ cells\ (mg) = 0{,}007\ mg$$

$$Ration\ per\ day = \frac{(2 \times 5000)/100}{0{,}007}$$

$$Ration\ per\ day = 1\ 4286\ millions\ cells$$

$$harvest\ density\ of\ the\ day = 1{,}5\ millions\ cells\ per\ ml$$

$$Volume\ of\ chaetoceros\ per\ day = \frac{1\ 4286}{1{,}5}$$

$$Volume\ of\ chaetoceros\ per\ day = 9\ 524\ ml\ or\ 9{,}5\ L$$

For feed all the broodstock, you need to multiply $Volume\ of\ algae\ per\ day$ by the number of adult and to repeat this method for all the algae you want to use.

APPENDIX 6 LIST OF TOOLS AND EQUIPMENT.

List of Items needed for algal culture lab

Name	Category
	Glass and Plastic Items
Serological pipette and Pump (varying sizes)	
Borosilicate Measuring Flask (250-1L)	
Borosilicate Pipette tube (Varying sizes)	
Borosilicate Round Flask/stand (4L,6L)	
Petri dish	
Storage bottles (Glass and Plastic)	
Chemical squeeze Bottles (250 ml)	
Plastic storage containers (varying sizes)	
Graduated cylindrical flask (0.5, 500, 1000 ml)	
Funnel	
Borosilicate measuring Beaker (1, 250, 500, 1000 ml)	
Drying Oven (Tabletop)	
Transfer pipettes/Glass Pasteur Short and long	
Plastic Pipette Tips	
Mechanical Pipette	
Pipette stand	

Name	Category
Magnetic stirrer	Electronics, Equipment and Apparatus
Magnetic stir bar retriever	
Orbital shaker	
Spectrophotometer	
Desktop computer	
Microscope	
Chemical Fridge	
Freezer	
Biological Fridge	
Precision Scale	
Gram Scale	
Distilled water Machine	
Electronic Pipette	
Autoclave	
pH meter and electrode	
Non- porous shelf space	
White Board	
Hand soap Dispenser	
Hand Sanitizer Dispenser	

Name	Category
Iron Sulphate	Chemicals
Magnesium Sulphate	
Zinc sulphate heptahydrate	
Copper sulphate pentahydrate	
Cobalt sulphate pentahydrate	
Sodium molybdate dihydrate	
Copper Sulphate	
Potassium Nitrite	
Dipotassium phosphate	
EDTA	
Vitamin B12	
Vitamin B1	
Sodium metasilicate pentahydrate	
Phosphorous	
70% Ethanol	

Name	Category
Nitrile gloves	Disposables
Weighing paper and boats	
Paper Towel	
pH paper	
Aluminum foil	
Cotton wool	
Syringes and needles	
Marking tape and pens	
Haemocytometer	
Burners and flints	
Timers (including a stopwatch)	
Microscope slides/Slide covers	

APPENDIX 7 QUESTIONNAIRE

SMEL Visit Investigative Questionnaire

1. Which algal production technique is practiced exclusively? Batch or Continuous?
2. Do you practice any form of outdoor culture as a temperate country?
3. How has the culture methods been made cost effective to a hatchery?
4. What are the major strains of algae produced?
5. Are all methods of algae culture Continuous or Batch applicable to all species you culture?
6. Is isolation of the culture a normal practice?
7. How long are the algal strains isolated and maintained for production until replenishing the supply?
8. Does the species of Algae being produced influence the medium you choose to culture?
9. Can you explain the method of culture you use in detail?
10. How are algae assessed to ensure quality is maintained especially at the stationary phase?
11. At what phase is the introduction of CO_2 vital
12. Do all your algae being cultured Flagellates and Diatoms require CO_2
13. Are your algae cultured to mirror the biome of the French region or is it bought from a sterile facility?
14. How is water quality for algae controlled?
15. Is frequent analysis done to assure quality of algae provided
16. What methods of analysis are performed?
17. How do you compensate for short falls in nutrient load of algae?
18. What Macro and Micronutrients do you use to ensure algal production is successful?
19. Which specific materials can supply these macro and micronutrients?
20. How do you substitute for lack of nutrients component?
21. What are the ideal water parameter levels for algal production?

Relating to algal culture corresponding to Oyster phases and Broodstock.

1. What species of micro algae do you cultivate specifically for this phase?
2. What quantities are recommended for broodstock for

   a. Maintenance before reproduction
   b. Reproduction viability
3. What algal strain combinations do you feed your broodstock?
4. Do the dietary needs of the brood stock stay constant or does it change according to their physical needs, e.g. pre and post reproduction?
5. How often do you feed your broodstock?
6. How do you calculate the quantity needed by your broodstock?
7. Which method of distribution do you use to feed the broodstock?
8. Do you feed broodstock in schedules or is it done ad-lib?
9. What is the average amount of larvae that the broodstock will produce?
10. How long are the algae viable after feeding?

Larvae

1. How do track the progress of the different stages development to know when it is time to start feeding?
2. Which algal species are ideal for this phase of development?
3. Is feed demand constant or does it fluctuate?
4. How is feed quantity calculated to ensure adequacy at this phase
5. How often do you feed at this phase of development?
6. Is there a specific combination of algae fed at the Larvae stages?
7. What methods are used to distribute algae to the larvae?
8. How long are the algae viable after feeding?

Spat

1. Are there physical signs to indicate when spat is about to settle?
2. Does algal specie and quantity requirement change at this phase?
3. Which algal species combination are ideal for this phase of development?
4. Is feed demand constant or does it fluctuate?
5. How is feed quantity calculated at this phase of development?
6. How is mortality calculated at this phase?
7. How often do you feed the spat?
8. Is there a specific combination of algae fed at the spat stages?
9. What methods are used to distribute algae to the Spat?
10. How long are the algae viable after feeding?