UNIVERSITY OF CAPE COAST

ASSESSMENT OF EMBRYO CHARACTERISTICS, HATCHABILITY AND SURVIVAL OF CUTTLEFISH (*Sepia hierredda*) IN RECIRCULATING CULTURE SYSTEMS

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BY

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Thesis submitted to the Department of Fisheries and Aquatic Sciences, School of Biological Sciences of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of a Master of Philosophy degree in Fisheries Science

DECEMBER 2022

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature …… Date……..…

Name: ……..……..

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature……. Date…….

Name: ……..……

Co-Supervisor's Signature ……..…… Date…….

Name: ……..……

ABSTRACT

Globally, fish stocks are declining and the case is not different in Ghana, where small pelagic and some demersal stocks have also declined. It is therefore expedient to explore the culture potential of some economically valuable marine species for commercial production to augment fisheries production and improve coastal livelihoods within the context of promoting Ghana's blue economy. Cuttlefish is the most exploited cephalopod in the Ghanaian fisheries and the country is renowned as one of West Africa's top producers and exporters of cuttlefish. In this study, the embryo characteristics, hatching success, and survival of cuttlefish were assessed in recirculating aquaculture systems. The research involved the collection of fertilized eggs from near-shore waters of two coastal communities and culturing them in recirculating aquaculture systems (systems A and B) with three replicates each. System A contained eggs collected from cuttlefish landings at Nungua in the Greater Acca Region whereas system B contained eggs collected from Elmina in the Central region of Ghana. The embryos from Elmina showed significant growth evidenced by an increase in size during the period of incubation ($p<0.05$) whereas the embryos obtained from Nungua experienced little growth. The mean weight recorded for the embryos in system B was 0.956 ± 0.449 g whereas, in system A, embryos had a mean weight of 0.4776 ± 0.0603 g. The eggs hatched between 12 and 14 days after incubation. Eggs collected from Elmina had a hatching rate of 70% whereas those from Nungua had a lower rate of 16%. The survival of the species under culture was very low for eggs collected from Nungua and Elmina, with heavy mortalities (100%) occurring within 14 days after hatching.

KEYWORDS

Recirculating Aquaculture systems

Embryos

Hatchability

Physico-chemical parameters

Survival

Growth

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DEDICATION

To my supervisors: Dr. Isaac Okyere and Dr. Kwadwo Kesse Mireku, all of the Department of Fisheries and Aquatic Sciences, University of Cape Coast

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CHAPTER ONE

INTRODUCTION

This chapter is about the general introduction of the current work; background to the study, statement of the problem, justification, objectives, significance, delimitations and the limitations of the study.

Cephalopods are fast-growing, marine species that are very sensitive to environmental changes (Pierce, *et al*., 2008) and hence affecting their production and catch. They constitute a very important fishery resource in many coastal countries. Globally, cephalopod production has maintained relatively high steady growth for the past 20 years. However, recent catches have declined to about 3.6 million tonnes in 2018, from their peak catches of 4.9 million tonnes in 2014 (FAO, 2020). An increase in global demand for fish and seafood calls for alternative methods of mass production of this food resource to prevent excessive pressure on the capture fisheries. This work is focused on providing basic experimental knowledge of cuttlefish culture in the laboratory as an effort to explore the culture potential to augment declining stocks.

Background of the Study

The trade in cephalopods is a multi-billion-dollar industry worldwide that comprises wild capture production and aquaculture production of several highly commercially valuable species. It fully or partially supports the income and survival of tens of thousands of families around the globe (Ospina-Alvarez, de Juan Mohan, Pita, Ainsworth, Matos, Pita, & Villasante, 2021).

Approximately 2.5% of all seafood is made up of cephalopods. Landings have risen relatively by 416% from 1961, peaking at an all-time high

of almost 4.9 million tonnes in 2014 before declining to about 3 million tonnes in 2019 (Ospina-Alvarez, de Juan, Pita, Ainsworth, Matos, Pita, & Villasante, 2022). The biggest volume of production during the past 20 years has been centered in East Asia and South America (especially Peru and Argentina, including the Malvinas/Falkland Islands), which have held this leading position since the 1980s (Ospina-Alvarez, *et al*., 2022). The top 10 countries leading in cephalopod production (in decreasing order) in the world are China, Japan, Taiwan, South Korea, Vietnam, India, Spain, Italy, Morocco, and the USA (FAO, 2020).

Cephalopods are one of the most heavily exploited resources in Ghanaian fisheries. About 15-20% of all industrial landings are made up of cephalopods, the majority of which are cuttlefish. Ghana is renowned in West Africa as one of the top producers and exporters of cuttlefish to Asian and European markets (Sakyi-Djan, Fynn, & Lazar, 2022).

The meat of cuttlefish is made up of about 14.5% proteins; whereas essential minerals and fatty acids are also relatively high. The fatty acid profile analysis shows that it contains 71.67% saturated fatty acids, 17.87% polyunsaturated fatty acids, and 10.38% monounsaturated fatty acids. Carbohydrates and lipids constituted less than 1% of the total body weight and certain trace elements like Zn, Mn, B, Al, and Sr in appreciable amounts (Kavipriya & Ravitchandirane, 2021). Its viscera are used for aquafeed and some other natural products (Le Bihan, 2006). Its ink is used for food processing, whereas the cuttlebone, which is made of calcium carbonate (99 % aragonite) is used for medicinal and pharmacological purposes (Rocha, *et al*., 2005; Kim, Kim, Sung, You, & Lee, 2012). Cuttlefish (*Sepia officinalis*) has

been a popular model for basic scientific investigations because it has well developed nervous system and sensory organs (Gupta, *et al.*, 2018; Billard, Schnell, Clayton, & Jozet-Alves, 2020; Bowers, *et al*., 2020).

Cuttlefish belong to the family Sepiidae and the genus Sepia. The common cuttlefish *Sepia officinalis* L. (1758) previously comprised *Sepia officinalis officinalis* and *Sepia officinalis hierredda* subspecies. These subspecies are morphologically identical but phylogenetic analysis presents them as distinct species belonging to the same Genus (Guerra *et al*., 2001). Therefore *S. officinalis officinalis* is currently known as *Sepia officinalis* whereas *S. officinalis hierredda* is known as *Sepia hierredda.* Typically, *S. officinalis* is found in the Eastern Atlantic and Mediterranean waters. whereas, *S. hierredda* is endemic to the eastern central Atlantic. In Ghana, *S. hierredda* has a wide distribution, however, it is more abundant on the east coast of the country and found at 15 m to 87 m depth (Sakyi-Djan, Aggrey-Fynn, & Najih, 2022).

Cuttlefish has characteristics that justify it as an aquaculture candidate. These include large eggs that are easy to transport and maintain, tolerance to stocking at high densities, and a short generation time (Sykes, Domingues, Correia, & Andrade, 2006). With regards to feeding, cuttlefish require food with high protein content, and it is selective with feed. In culture, their live feed requirement for hatchlings as well as the inability of juveniles and adult stages to feed on pelleted feed, remain major bottlenecks in the culture of this species (Anil, 2003). This calls for increased knowledge of the biology and culture of cuttlefish.

Statement of the Problem

A recent global increased demand for seafood and fish products for food and non-food purposes has raised the exploitation of marine resources around the world. However, there is a global decline in capture fisheries production (FAO, 2020), and the situation is no different in Ghana. Ghana's small pelagic and some demersal stocks have dwindled and need immediate management interventions to remedy the situation. However, the FAO has documented the increasing production of aquaculture to augment capture fisheries production. This calls for an urgent exploration of marine resources of aquaculture potential in the context of promoting blue livelihoods for Ghana's blue economy.

Cephalopods are highly economically valuable resources that are patronized both locally and internationally. Cuttlefish is the most exploited cephalopod in Ghana and is exploited heavily on the eastern coast of the country (Sakyi-Djan *et al*., 2022). Cuttlefish have a short generation time, are tolerant to crowding in captivity, and are highly fecund. This makes it a good candidate for culture (Sykes, 2006).

However, there is sparse scientific documentation of the biology of *Sepia hierredda*, which is endemic to the Eastern Central to Southern Atlantic Ocean (Healey, *et al*., 2017), both in captivity and in the wild, as compared to other species of the same genus, like *S. pharaonis* and *S. officinalis*, which abounds in the Asian, European and Mediterranean coastal waters. The latter species have been studied extensively in experimental cultures (Palmegiano, & d'Apote, 1983; Sykes, 2006; Wu *et al*., 2021). and are due to enter the next phase of exploration, which is commercialization. Few studies conducted in

the country have identified *Sepia hierredda*'s distribution along the coast of Ghana, its breeding season, breeding grounds, and the collection and transportation of the species' embryos under optimum conditions (Okyere, Somers, Hatzipetro, Sakyi-Djan, Takyi, & Bossman, 2017; Sakyi-Djan, *et al*., 2022).

Justification

Fish consumption has risen over the years (FAO, 2020), thereby increasing the exploitation rate of fish and other seafood. Undoubtedly, the surging human population growth requires measures to ensure an adequate, constant food supply for it. However, natural resources need not be overexploited but harnessed sustainably. It is therefore imperative for the scientific community to design solution-oriented research to address such issues of global concern, as food security.

The study of the cuttlefish (*Sepia hierredda)* in captivity will provide scientific knowledge of their biology and behavior under controlled conditions so that their aquaculture potential can be sustainably harnessed. It is in this light that this study seeks to assess embryo characteristics, hatching success, and survival in culture.

This would be one of very few documented scientific knowledge on the aquaculture of *Sepia hierredda*. The completion of this work will set the stage for extensive research in other aspects of aquaculture of the species like its nutritional requirement, optimum growth, and maximum production in captivity.

Research Objectives

Main objective

This research seeks to assess the characteristics of embryos, hatching success, and the survival of *Sepia hierredda* in recirculating aquaculture systems.

Specific objectives

1 To assess embryo characteristics and the hatchability of eggs under optimized culture conditions.

2 To determine the survival rate of cuttlefish in recirculating aquaculture systems.

3 To determine water quality conditions for optimizing the survival and growth of cuttlefish.

Significance of Study

This work will contribute to the United Nations agenda 2030 sustainable development goals 2 and 14. These are; Goal 2- End hunger, achieve food security and improved nutrition and promote sustainable agriculture, and Goal 14- Conserve and sustainably use the oceans, seas, and marine resources for sustainable development (Desa, 2016). This work will also contribute to Goals 1 and 2 of the African Blue Economy Strategy as it will lead to the conservation and sustainable fisheries of the species. Also, wealth creation will be achieved as the commercial production of this species will create jobs and reduce poverty levels, especially in coastal communities. Mass food production through aquaculture technologies will have a great boost for the local economy as the country will have access to adequate protein nourishment and generate revenue from the exports of the highly

sought-after seafood. The aquaculture of cuttlefish will create jobs, thereby improving the livelihoods of the locals. It will also be a point of attraction for investment in the aquaculture of other valuable marine organisms. The nation's ability to generate income will rise with the commercial production of cuttlefish for export.

Delimitation of the study

The scope of this study comprises the collection of cuttlefish eggs from fishermen, the transportation of the eggs to the culture facility, and the characteristics, survival, and growth of the embryos studied under captivity. Cuttlefish are broadly distributed along the coast of Ghana; however, eggs were collected from Elmina in the Central region and Nungua in the Greater Accra region, based on the availability of eggs in these areas. The specific location in the waters where the eggs were collected is not taken into account in this work. Data used for analysis were collected from embryos which were maintained in recirculating aquaculture systems for twenty-six days.

Limitations of the Study

The breeding of cuttlefish is observed to be seasonal in Ghanaian waters. Therefore, the commencement of the research depended on the availability of eggs in the waters. The fertilization of cuttlefish eggs is external, and hence one could not guarantee that the eggs obtained from the wild were all fertilized - this might have affected the hatching rate of the eggs incubated in the culture systems. The low stocking density of eggs and hatchlings in the culture systems might have influenced the spread of the data analysed. However, low stocking density is necessary to maintain low nitrates and nitrite concentrations in the culture system. Also, the current method of

identification of microorganisms involves the use of modern molecular techniques like polymerase chain reaction and DNA sequencing which were not done in this study. An online identification portal was used to identify microbes, based on the similarity of biochemical test results in the online database.

CHAPTER TWO

REVIEW OF LITERATURE

This chapter reviews literature from blogs and articles published in books and journals relevant to the research topic. The subjects covered include the global distribution and trade of cuttlefish, their biology, and their growth and survival in captivity.

Distribution and Habitat

Cuttlefish are commonly found in coastal waters on muddy and sandy bottoms covered with seaweed and phanerogams (Luna, Rocha, & Perales-Raya, 2021). They can also be found down to a depth of about 200 m (Luna *et al*., 2021) and are present in both warm and temperate waters (Boyle & Boletzky, 1996). Reid, Jereb and Roper (2005) have also documented the occurrence of the common cuttlefish, *S. officinalis* on and near the seafloor in habitats with a sandy, muddy, or rocky substrate, with or without algal or seagrass beds (Nixon & Mangold, 1998).

The distribution and habitat of *Sepia officinalis* stretch across the eastern Atlantic and Mediterranean Seas (Belcari, 1999). It stretches from the Shetland Islands and southern Norway in the eastern North Atlantic, through the Mediterranean Sea (including the Aegean Sea, Sea of Marmara, and Levantine Sea), and into northwest Africa, with the southern boundary roughly coinciding with the border between Mauritania and Senegal (Reid *et al*., 2005). However, they have also been noted to occur in a very diverse variety of waterways, from the North Sea and Baltic Sea to those near South Africa (Grati, *et al*., 2018). According to the FAO (2017), *Sepia* species are widely distributed from Morocco to Namibia, with varied abundances depending on

environmental factors, population dynamics, and habitat. Seasonal movements take place from offshore wintering habitats to coastal spawning and nursery sites. The geographic distribution of many cephalopod species in this region is unclear, uncertain, or unknown due to the lack of both biodiversity and resource-intensive studies (Krakstad, *et al*., 2011; Krakstad, *et al*., 2012). Although some surveys of the West African coast have been conducted on the distribution of cuttlefish, the overall records are scattered and incomplete (Luna *et al*., 2021). In Ghana, *S. hierredda* has a wide distribution however, it is more abundant on the east coast of the country and is found at 15 m to 87 m depth (Sakyi-Djan, *et al*., 2022).

Currently known distribution of *Sepia officinalis*: Eastern Atlantic and the Mediterranean: from the Shetlands and southern Norway (stray in the Baltic Sea), south to the Mediterranean Sea to northwestern Africa (Figure 1).

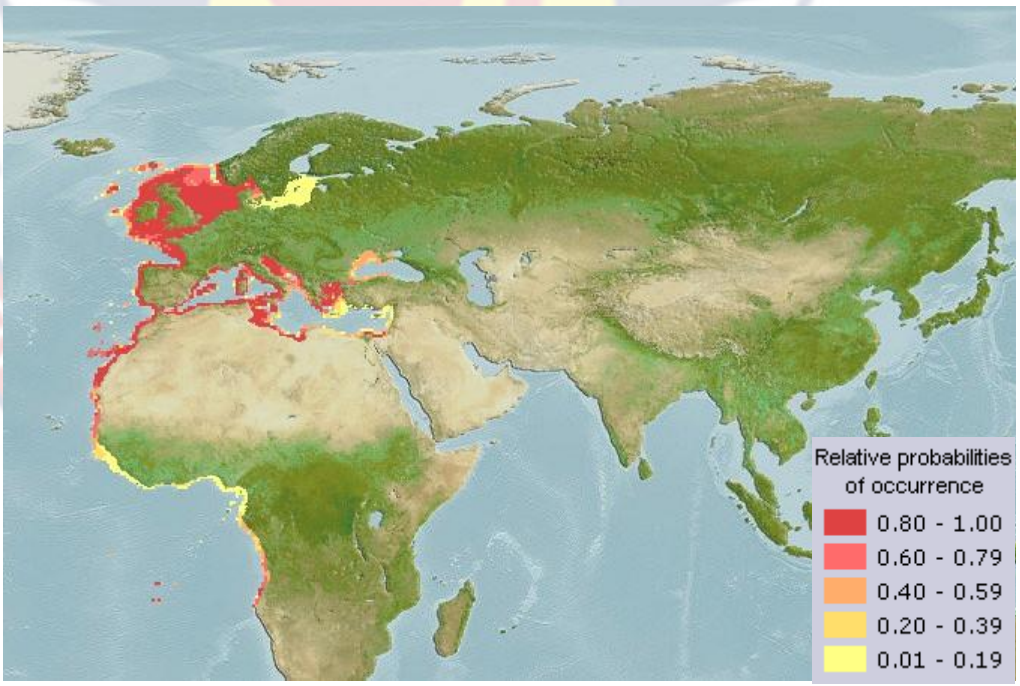

Figure 1: Computer Generated Native Distribution Map for *Sepia officinalis* (common cuttlefish), with modelled year 2050 native range map based on IPCC RCP8.5 emissions scenario (AquaMaps, 2019).

Biology and Life Cycle of Cuttlefish

Adult characteristics

The mantle of an adult cuttlefish is oval and the length measures up to 450 mm. The maximum mantle length (ML) is 300 mm for males and 250 mm for females (Mangold-Wirz, 1963; Nesis, 1987). The species has 8 arms and two tentacles. Each tentacular club has five or six suckers in each transverse row, one of which is relatively larger. The swimming keel does not reach proximally beyond the club's base as shown in Figure 2. After the 5–7 proximal sucker rows, there are 4–5 to 8–9 (medial) horizontal rows of decreased suckers on the left arm IV of males. The cuttlebone has parallel sides and a rounded posterior. The anterior striae are inverted U-shaped or shallow M-shaped. Cuttlefish are light brown but can change their color for camouflaging. There are white dots on the head, with black pigment around the eye orbits. They have a broad, longitudinal brownish band that runs medially across arms I–III, extending onto the head. During the breeding season, the dorsal mantle displays a prominent transverse zebra-stripe pattern. Their fins have a short white strip along the outer margin and little white dots that grow larger as they approach the mantle and fin junction. Mature males have white and black zebra bands and white spots on their arms IV.

The sexual maturity of common cuttlefish can occur in various sizes. Mature males with an ML of 6 to 8 cm have been spotted in the Mediterranean Sea. Males with a maximum length of 10 cm ML, on the other hand, may still be immature. Females find themselves in a similar situation. For females, the length at first maturity is about 13 cm ML.

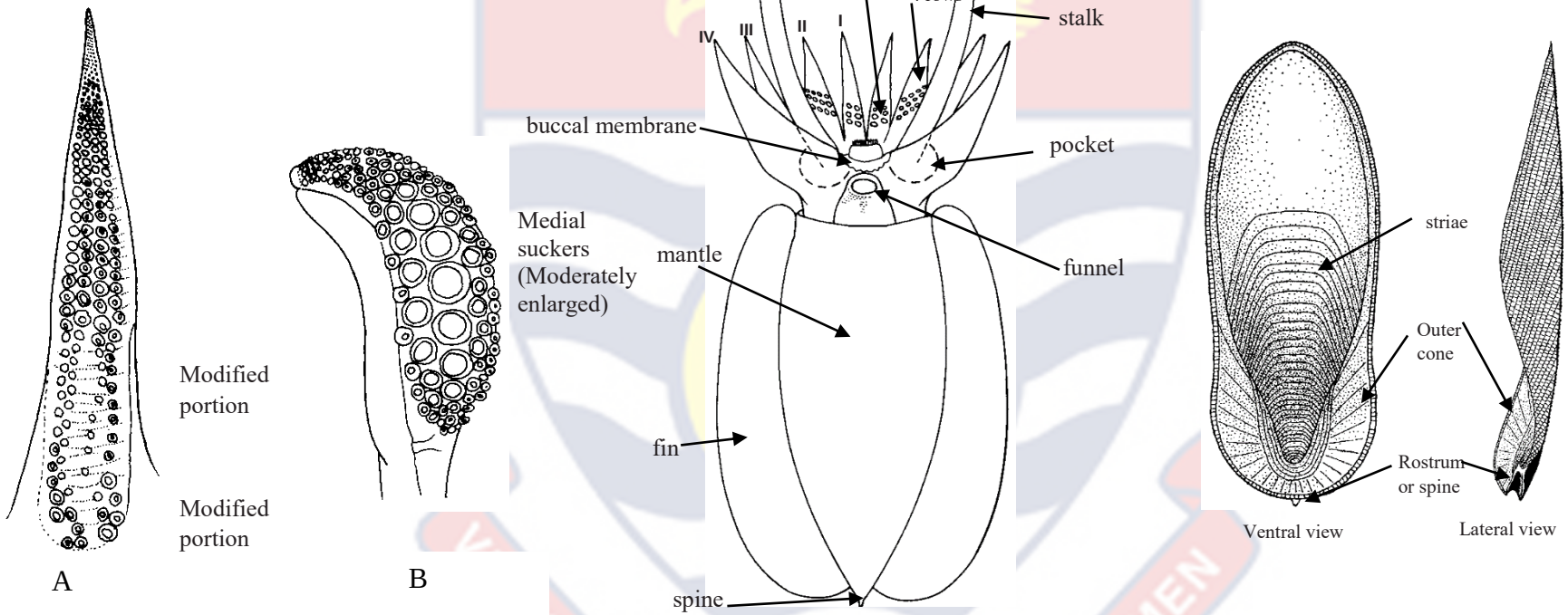

Figure 2: Diagram of the basic features of cuttlefish
A = left arm IV in male (hectocotylized); B = tentacular club; C = ventral view of cuttlefish; D = cuttlebone
(Roper et al., 1984; Guerra 1992; Reid et al., 2005)
A = left arm IV in male (hectocotylized); B = tentacular club; C = ventral view of cuttlefish; D = cuttlebone
(Roper et al., 1984; Guerra 1992; Reid et al., 2005)

Reproduction

The breeding season varies over the geographical distribution range, with spring to summer being the major breeding season; however, winter spawning has also been observed in the Mediterranean Sea and North Western Spain (Guerra, 2006). In their work, Önsoy and Salman (2005) also pointed out that the reproduction period of *S. officinalis* spans the whole year, as there were mature animals every month throughout the year. On the other hand, very little is known about *Sepia hierredda*'s reproductive behavior. However, observations of fishermen's nets indicate that the spawning period may span from September to March, with a peak around January, since the female cuttlefishes use the fishing nets as a spawning substrate. Outside the period between September and March, fishermen hardly find cuttlefish eggs attached to their nets when they go fishing.

The spawning strategy is categorized as an intermittent terminal spawner (Rocha, Guerra, & González, 2001), in which the spawning pattern is characterized by group-synchronous ovulation during only one reproductive cycle. According to laboratory observations, however, the reproductive strategy could be either intermittent spawning with periods of inactivity as long as one month, or continuous chronic spawning over many weeks (Boletzky, 1987). The number of oocytes being ovulated in this regard has been reported to be as many as 3000 eggs under culture conditions (Minton, Walsh, Lee, & Forsythe, 2001), but most findings report less than 1000 eggs for wild populations. For example, 99–543 eggs have been observed in Tunisian waters (Ezzedine-Najai, 1985), and 130–839 eggs in the Aegean Sea (Laptikhovsky *et al*., 2003). Hence, the wild populations seem to have lesser

viable oocytes being released (Boletzky, 1987). The egg size of *Sepia officinalis* varies from 6 x 5 to 9 x7 mm, and larger females produce larger eggs (Boletzky, 1983; Man-gold-Wirz, 1963).

S. officinalis has a lifespan of about two years. Given that the spawning season is followed by mass adult mortality, marine environmental conditions exert a strong effect on recruitment and spatial distribution (Boletzky & Boyle, 1983). Moreover, since egg masses are attached in clusters to seagrass, tube worms, drowned trees, ropes, and traps (Guerra, 2006), recruitment strongly depends on the availability of spawning substrates (Grati *et al*., 2018). The choice of the spawning substrate has been reported to depend on the female's preferences, which seem to favour tubular surfaces less than 10 mm in diameter (Boletzky, 1988). Since cuttlefish eggs take from 20 to 50 days to hatch (Domingues, Sykes, & Andrade, 2002), ropes and traps are often completely covered with eggs throughout the spawning season (Blanc, & Dagunzan, 1998). It is also common to find eggs attached to the fishing nets of artisanal fishermen in Ghana.

Culture of *Sepia* spp.

The Koreans and Japanese are considered to be the pioneers in cephalopod culture (of the *Sepiidae* family). Some first successful experimental cultures of the European cuttlefish were done by Richard (1971), Pascual (1978), and Boletzky (1979), keeping the species in culture for one or more consecutive generations. Explorative research on cuttlefish as an aquaculture candidate became intense in the 1980s (Sykes, 2006). Experimental cultures of the species in coastal lagoons have also been done (Palmegiano, & d'Apote, 1983). Some contributions on feeding and digestion

were made by Boucaud-Camou *et al*. (1985), Nixon (1985), and Guerra and Castro (1988); on fecundity by Boletzky (1987); and alternative prey items by DeRusha *et al*. (1989). Laboratory culture maintenance of cephalopods has been reviewed by Boletzy and Hanlon (1983) while addressing its biology and ecology. Social and sexual behaviour in cuttlefish captivity has also been previously studied (Adamo & Hanlon, 1996; Boal, 1996, 1997; Boal & Marsh, 1998; Hanlon *et al*., 1999). Valuable knowledge has also been acquired concerning crowding, and spawning methodologies when the species are in captivity (Minton, Walsh, Lee, & Forsythe, 2001; Guo, Zhang, Näslund, Wang, & Zhang, 2022).

Physicochemical parameters during captivity

Temperature is an important factor in the regulation of the incubation period of eggs and of growth after hatching (Koueta, 2003). The embryonic development of cuttlefish takes a longer time at lower temperatures than at higher temperatures (Palmegiano, & d'Apote, 1983). This conditions the eggs and adds a final phase of embryonic "perfection" (Boletzky, 2004) to the developing embryos. On the contrary, higher temperatures lead to normal or premature hatching of eggs. A further means (other than low temperature) to minimize premature hatching is keeping the eggs under continuous light. However, a sudden change from dark to light should be avoided (Boletzky, 2004) as continuous light comparable in intensity to full day light at the sea surface is tolerated by many inshore cephalopods but may cause a delay in sexual maturation (Boletzky, 1974). And so, transparent aquarium walls should always be made opaque by any method available when artificial light is used in a culture system. Ambient temperature is a potential factor responsible

for differences in cuttlebone growth mechanisms and survival in *S. esculenta* larvae (Lei, Zhang, Liu, & Chen, 2012). Martínez, Bettencourt, Guerra, and Moltschaniwskyj (2000) pointed out the effect of temperature on survival and cuttlebone growth under conditions of different food levels. Based on our results on survival, the warmer water environment provides better living conditions for the growth of *S. pharaonis* juveniles (Jiang, Peng, Han, & Jiang, 2020). In simple, small-scale experiments run at temperatures below 20°C, stagnant seawater that is frequently changed and/or oxygenated by very gentle air bubbling can be used for early planktonic juveniles (Boletzky, 1974) or very young cuttlefish (Ré & Narciso, 1994).

A continuous seawater supply (fresh or recycled seawater) should renew the whole water mass of a culture tank at least once in 5 to 10 hours, depending on the activity level and density of the cultured animals and their prey. The true water quality can only be assessed by measuring the concentrations of oxygen and dissolved products of metabolism from the organisms present in the aquarium. Thus, standard culture conditions are generally defined by oxygen concentrations close to saturation, low concentrations of nitrogenous waste (ammonia and nitrite concentrations <0.1 mg/l, nitrate concentration <20 mg/l), with a pH close to 8 (7.8-8.3) (Boletzky & Hanlon, 1983). Regarding salinity, cuttlefish has a limited adaption range, making it extremely sensitive to salinity fluctuations, and this restricts its breeding methods (Wu *et al*., 2021).

Despite biological filtering, artificial or natural seawater that is recycled in a closed system may lose some constituents (e. g. trace elements, especially when “foam domes” are used to eliminate organic waste), or it

accumulates noxious elements or pathogenic microbes that may thrive in recycled seawater. Minimal changes in water chemistry may have far-reaching effects. A lack of strontium was found to cause malformations in statoliths and cuttlebones (Hanlon *et al*., 1989). A potential source of infestation or infection is sandy substrates, especially fine sand, and mud/sand mixtures. To prevent fouling, the tank bottom should be cleaned regularly (Boletzky, 2004a). Certain infections and skin diseases of unknown origin may occur despite all the care taken with a tank system. Some of these can be brought under control using antibiotics (Hanlon *et al*., 1984; Forsythe *et al*., 1990).

Food and Feeding habits

Cuttlefish are recognized for their opportunistic feeding behavior, with crustaceans and fishes being the most common prey (Castro & Guerra, 1990; Piczon du Sel, Blanc, & Daguzan, 2000; Alves, Cristo, Sendão, & Borges, 2006; Guerra, 2006). The nutritive value of live prey or food preparations depends above all on the quality of the available protein and lipid composition (Moltschaniwskyj & Jackson, 2000; Navarro & Villanueva, 2000). Natural diets enriched with polyunsaturated fatty acids were found to induce fast growth in newly-hatched cuttlefish (Koueta, Boucaud-Camou, & Noel, 2002). Mysids have been identified as important prey for juvenile cuttlefish specimens (Le Mao, 1985), while Domingues *et al*. (2001) found that cultured hatchlings easily grabbed mysids but had greater difficulty obtaining small grass shrimp.

Mysids stimulate greater growth during the first week of life on cultivated cuttlefish, according to Domingues *et al*. (2004), while larger prey like shrimps become more effective afterward. Darmaillacq, Chichery, Poirier,

& Dickel (2004) also discovered that very young cuttlefish preferred to feed on prey items that they are already familiar with, whereas older cuttlefish prefer novel prey items. The use of exclusively deep-frozen crustaceans as food does not provide cephalopods with sufficient amounts of copper for their hemocyanin synthesis (Le Pabic *et al*., 2015). Yet, hatchlings fed with frozen grass shrimps showed high survival and growth performance (Sykes, Gonçalves, & Andrade, 2013a). The use of pelleted feed showed poor survival rates and even caused cannibalism among the cuttlefish in culture (Castro, 1991; Castro & Lee 1994; Castro, DiMarco, DeRusha, & Lee, 1993).

Recirculating Aquaculture Systems

The rate at which the climate is changing globally, poses significant risks to global fish production, both in the wild and in captivity (Ahmed & Diana, 2016). To meet these issues, adaptation solutions must be developed and implemented, and "Recirculating Aquaculture Systems (RAS)" is a viable option. Recirculating aquaculture systems are indoor, fish-rearing facilities where tanks are used to house fish in a controlled setting, and purification of water is done via filtration. RAS were originally designed to produce freshwater and warm-water fish species (e.g., channel catfish, striped bass, and tilapia), but they can now be adapted and adopted to produce marine and brackish water and cold-water species (Helfrich & Libey, 1991). Recirculating aquaculture system is a type of intensive farming that produces a large number of fish, yet with the use of a comparatively little amount of water. This aquaculture system can come in a variety of sizes, such as small, medium, and large (Helfrich & Libey, 1991). A quantity of 400 - 500 tons of fish per year on average, can be generated from a large-scale RAS (Murray & Watson,

2014). RAS are frequently referred to as "hyper" or "super" intensive farming due to higher yield potential (O'Shea *et al*., 2019).

When recirculating aquaculture systems are used, fish can be raised in an indoor facility (built on land) with controlled environmental conditions. With RAS, the processes involved in production do not have direct contact with the natural environment, and this is a good measure to take in minimizing environmental pollution that results from fish and seafood production.

In the mid-1970s, the concept of commercially producing fish in recirculating aquaculture systems was initially proposed in Denmark. RAS was further developed in Australia, North America, Europe, and China during the 2000s (De Ionno, Wines, Jones, & Collins, 2006; Chen *et al*., 2015; Goldman, 2016).

RAS and its associated problems

RAS has the potential to produce large quantities of fish, yet it currently contributes just a small portion of worldwide aquaculture production (Waite, *et al*., 2014). This is attributed to a number of technological, social, and economic barriers. RAS are more often used in industrialized countries (like Europe, Canada, Australia, and the United States) than in less-developed ones. RAS employ sophisticated machinery like water filtration and sterilizing compartments, water-temperature regulators, automated control systems, and some advanced sensing and monitoring systems (O'Shea *et al*., 2019).

An annual cost of production with the use of RAS can be between US$2250 and US$ 8800, with each ton of fish generated (Waite *et al*., 2014). This is significantly higher than traditional aquaculture made in ponds with an annual cost of production of around US$2000 with each ton of fish produced. Recirculating aquaculture systems are expensive engineering techniques to

install and operate, with a large initial expenditure (Murray & Watson, 2014). Simple designs may assist in reducing capital and operational expenses but restrict overall productivity and stocking density. One of the major disadvantages of RAS is its high energy consumption, which drives up operating expenses. By adopting a microaerophilic assimilation reactor, the consumption of energy in RAS may be reduced. Technological improvements to reduce energy consumption in recirculating aquaculture systems (up to 75 percent) can help it become a more sustainable option for aquaculture, both economically and environmentally (Yogev & Gross, 2019). Hence, recirculating aquaculture systems with reduced energy consumption will be very instrumental to the growth of the aquaculture industry in developing countries like Ghana.

RAS and climate change adaptation

Climate change has a significant impact on the hydrological cycle, causing a change in rainfall patterns and intensity leading to increased flooding and drought occurrences (Trenberth, 2011). Increased rainfall intensity puts inland freshwater aquaculture at risk, as floods can result in structural damage, stock losses, and fish escape (Ahmed & Diana, 2016). Vietnam's striped catfish culture have recently been significantly impacted by some flood occurrence in the Mekong Delta (Nguyen *et al*., 2014). Coastal aquaculture can be harmed by increased rainfall intensity and variability. Coastal flooding is expected to quadruple by 2050, according to projections (Vitousek *et al*., 2017). By 2050, global average temperatures are predicted to rise by 1.5 degrees Celsius (IPCC, 2018). A small temperature rise in water can have a significant impact on the health, development, and production of

fish. (De Silva & Soto, 2009). Temperature rise can force cultured fish species to experience unfavorable environmental conditions. By 2050, 86 % of the ocean will become warmer and more acidic, globally (Henson *et al*., 2017). Ocean acidification can have an impact on seafood output, and a drop in water pH has been linked to a slew of detrimental effects on shellfish productivity (Clements & Chopin, 2017). By 2050, the level of the sea may rise to between 0.15 and 0.38 m. (Sweet, *et al*., 2017). Doney *et al*. (2012) reported that other parts of marine ecosystems are already being impacted by climate change.

However, RAS work in a controlled indoor environment and are thus regarded as a feasible climate change adaptation solution. The impacts of climate change as evidenced in rainfall variability, floods, droughts, global warming, and sea level rise have only a minor effect on RAS. RAS, which may work from sub-polar and temperate zones to tropical, desert, and dry locations (Dalsgaard *et al*., 2013) is unconcerned with global warming. Cyclones, storms, tsunamis, and typhoons may have little or no impact on RAS operations that are well-structured and well-located.

Studies that have been conducted on cephalopods in closed-recirculating aquaculture systems are rather few (Casalini *et al*., 2020; Kuo & Chiao, 2020; Oliveira *et al*., 2020) but in the advent of climate change and the global decline of fish stocks, technologies involved culturing cephalopods and other culturable seafood is set to improve and become widely explored.

CHAPTER THREE

MATERIALS AND METHODS

This chapter gives a detailed description of all the experiments performed in this study. It begins with the description of the experiment site. It further describes the culture systems used, experimental procedures, data collection and data analysis.

Experiment Site

The experiments were conducted at West Africa Aquatics, Greda Estates, Teshie-Nungua, Accra. West Africa Aquatics deals in the collection and exportation of tropical marine ornamentals and other fishery products. These wild-caught species are maintained in recirculating aquaculture systems of various sizes as shown in Figure 3, depending on the fish species and size before they are shipped to clients abroad.

Description of the Recirculating Aquaculture Systems

Two recirculating aquaculture systems were used for the study. These culture systems were by courtesy granted by West Africa Aquatics. As illustrated in Figure 4, each recirculating aquaculture system was composed of three glass tanks, each serving as a replicate, and a sump (also made of glass) for the filtration of the recirculating seawater. A net was fixed into the first compartment of the sump to sieve out food debris and other particles from the returning seawater from the glass tanks. Water from the first compartment cascaded into the second compartment, which was fitted with a protein skimmer to skim out nitrogenous waste from the water. The water from compartment 2 flowed into compartment 3 where a pack of coral rocks aided in biological filtration and regulation of water pH. The water pump in the fourth compartment then sent the water back into the glass tanks. The circulation of the seawater was facilitated by connecting pipe work.

Figure 3: Fish holding facility at West Africa Aquatics (Courtesy of Kudiabor Kofi Ntsunyo, 2022)

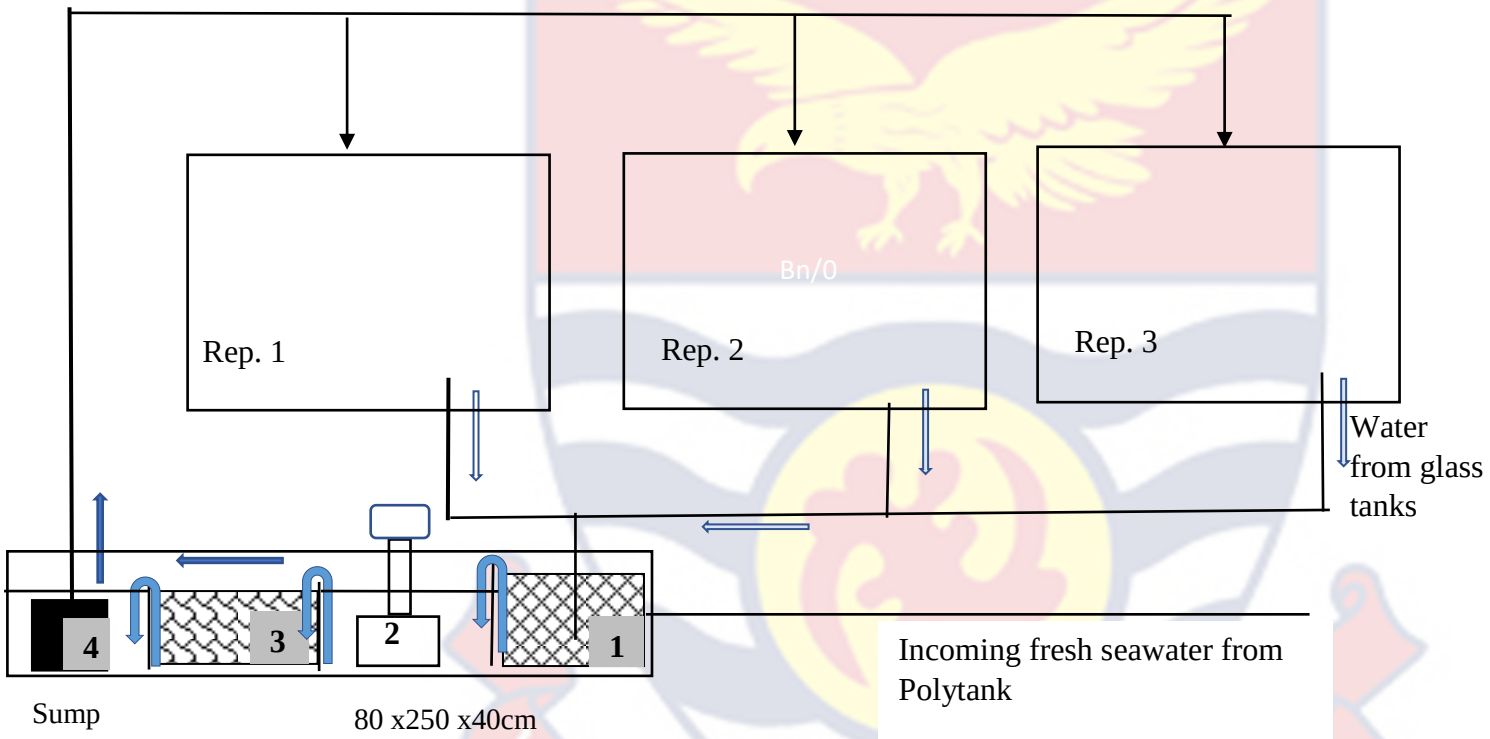

Figure 4: Design of the recirculating aquaculture system for Cuttlefish (*Sepia hierredda*) culture

(1= nylon net; 2 = protein skimmer; 3 = a pack of coral rocks; 4 = a water pump) *Rep. 2*

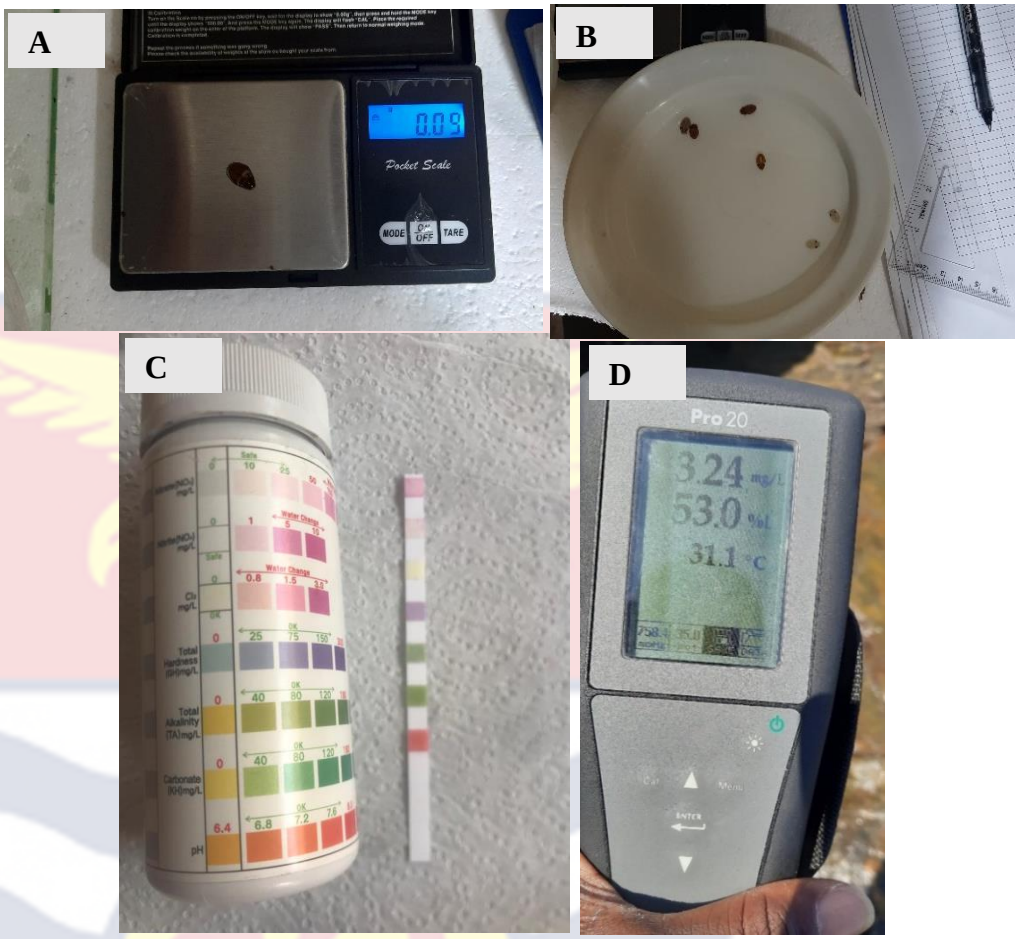

Figure 6: Apparatus used for the data collection on the hatchlings: electronic balance (A), small plastic bowl, (B) multiparametric test strip (C), and dissolved oxygen meter (D).

Data collection on physicochemical parameters in culture

The eggs from Nungua were incubated in culture system A and those from Elmina were incubated in system B. Eggs were macroscopic with a mean diameter of 1.59 cm and 1.77 cm for eggs from Nungua and Elmina respectively, and also a mean weight of 0.54 g and 0.69 g for eggs from Nungua and Elmina respectively. The salinity, dissolved oxygen, and temperature were recorded twice daily; at 0700 GMT and 1900 GMT. Salinity was recorded with a refractometer (Module REF113 ATC). A drop of seawater was placed on the main prism of the refractometer and the reading was recorded through the eyepiece. Salinity was maintained between 33 and

35 ppt in the culture systems. This was done by the addition of freshwater when the salinity increases as a result of evaporation from the systems. Dissolved oxygen and temperature were measured with a DO meter probe (Xylem YSI Pro 20) probe. The probe of the DO meter was lowered into the culture system and the most stable readings for both parameters on the LED screen of the DO meter were recorded. The pH, nitrates, and nitrites levels were determined using a test strip every 2 days. The test strip was dipped in the water for about 10 seconds. The readings were recorded within 60 seconds after removing it from the water. The bands on the test strip representing various parameters were compared against a colour chart on the wall of the container in which the test strips were packaged (Figure 6C). The corresponding values of the colour change were recorded.

Data collection on eggs and hatchlings

The longest axis of the eggs (in cm) and their weights (in g) were recorded daily during the period of incubation. After hatching, the number of hatchlings, their size, and their weight (in g) were also recorded daily. The longest axis (LA) of the eggs, the mantle length (ML), and the total length (TL) of the hatchlings were measured by the use of a ruler and a pair of dividers. The pair of dividers was opened so that each of the ends touched each end of the eggs and then the distance between the two ends of the dividers was measured on a ruler. This measurement was recorded as the length for the longest axis (Figure 6BI). The mantle length and the total length of the hatchlings, as illustrated in Figure 7, were measured using the same procedure. The eggs and hatchlings were weighed (to 0.01 g) with a digital balance of capacity, 500 g. During data collection, the eggs and hatchlings

were removed from a culture system with a fish net and placed in a small bowl containing seawater from that system. After the lengths (ML, TL, LA) have been taken, they were weighed after blotting off excess water on their body. However, it was ensured that the organisms were not completely dry. Also, to avoid stressing the organisms in the process of handling them, data collection on them was done in the evenings after 1900GMT. Immediately after data collection, they were placed back into the culture system.

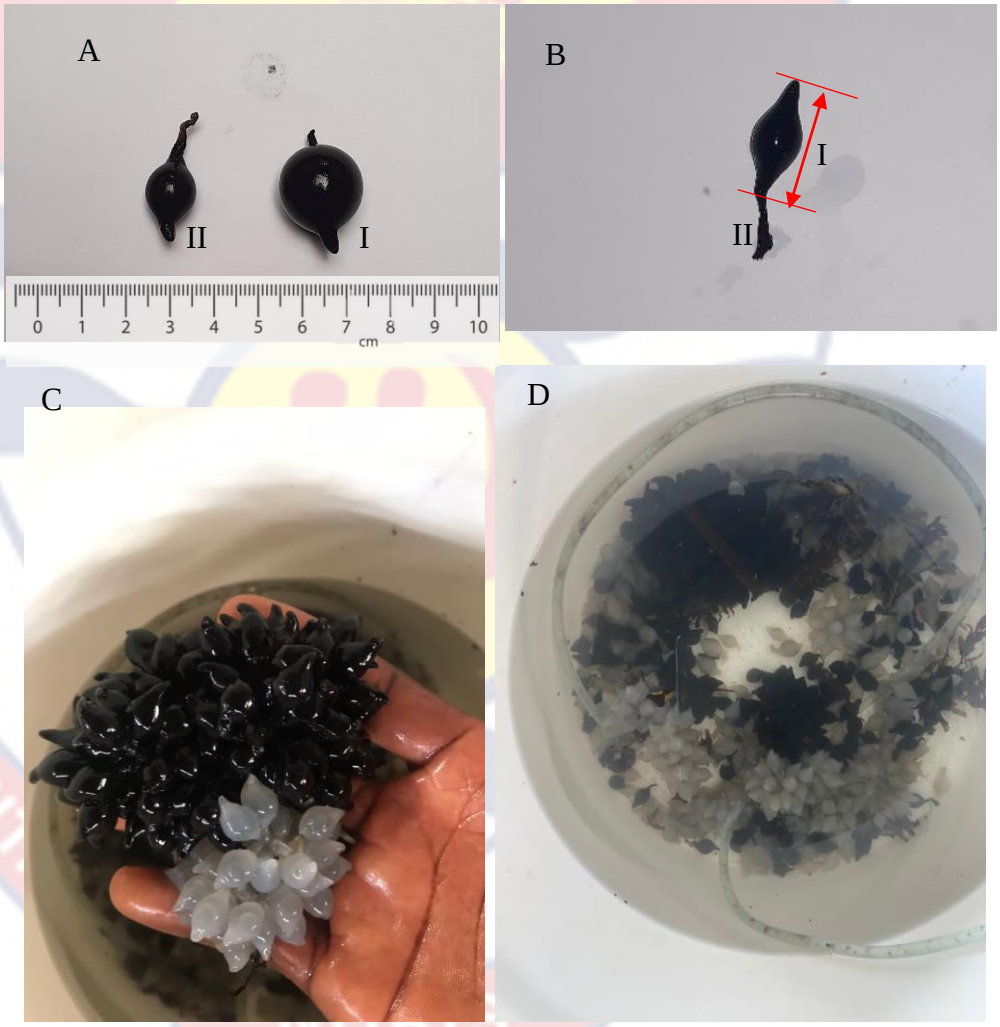

Figure 7: Difference in the sizes of eggs collected from Nungua (AI) and Elmina (AII). The measurement of the axis of the eggs (BI) and the point of attachment (BII) to the bunch of eggs. Pigmentation in eggs from Nungua (C) and the process of acclimatizing them to the culture systems (D).

The illustrations in Figure 8 show the dorsal mantle length and the total length of the cuttlefish hatchlings.

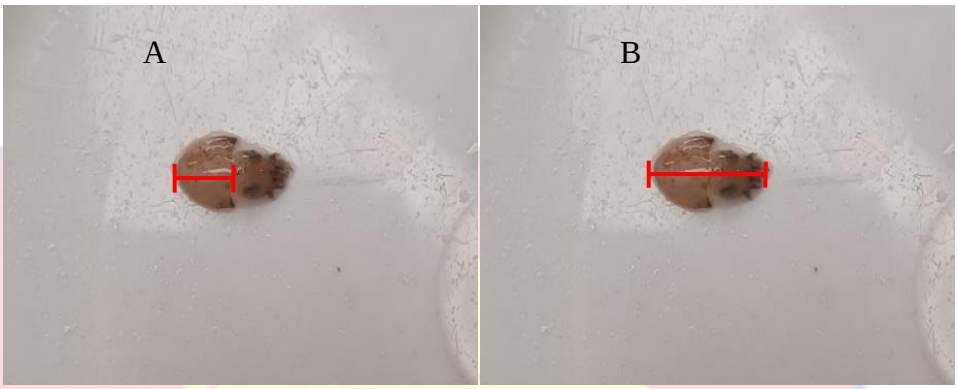

Figure 8: Illustrations showing the mantle length, ML (A) and the total length, TL (B) measurements (in cm) of the hatchlings.

Maintaining Hatchlings in Recirculating Aquaculture Systems

The eggs of the cuttlefish started hatching after 12 days of incubation. The hatchlings were kept in perforated plastic containers placed in the glass tanks to prevent them from passing through the outlet into the sump. During the experiment, empty egg shells were removed when they floated on the surface of the water. Water conditions during incubation were maintained throughout the experiment

Feeding of Hatchlings

Hatchlings were fed *ad libitum* with live brine shrimp (*Artemia salina*) and their mean weights and lengths (ML and TL) were recorded daily. The hatchlings were fed on the brine shrimp from the first day of hatching until their death. The brine shrimp cysts were placed in jars containing seawater and placed on a flat surface with a bright light installed to shine on the jars. Very high illumination was necessary to induce the hatching of the cysts. Rubber tubes connected to an aerator were placed in the jars to ensure a constant bubbling of the mixture. The set-up was left for 18-24 hours, within which

they hatched. They were then used to feed the hatchlings *ad libitum*. The brine shrimp used (Golden Lake Artemia) was obtained from Planted Aquarium Concepts, LLC USA Tempe, AZ.

Microbial Assessment of Water Samples

The microbial quality of the water used to culture the cuttlefish was assessed to identify whether pathogenic bacteria that may be detrimental to the health of the cuttlefish were present. Water samples obtained from Nungua beach– the usual point of collecting seawater– were transferred into sterile plastic bottles and kept at 4 ^{o}C for 48 hours, after which they were transported in an ice chest containing ice packs to the Molecular Biology laboratory of the University of Cape Coast. In the laboratory, the water samples were immediately transferred into the refrigerator at 4 ^{o}C. Nutrient agar and nutrient broth were prepared to culture bacteria present in the water samples.

Water samples used in the assessment of microbial quality were collected from Nungua beach since it was the place where collected seawater was used for the culture of the cuttlefish. Since water from Elmina beach was not used in the culture systems, they were not sampled for microbial analysis.

Media preparation

Nutrient agar was prepared by mixing 7.0 g of dehydrated nutrient agar in 250 ml of distilled water and the resultant mixture was microwaved until the agar melted into a clear liquid. The nutrient broth was prepared by dissolving 2.6 g of dehydrated nutrient broth in 200 ml of distilled water. About 5 ml of it was dispensed into test tubes. The test tubes were plugged with cotton and autoclaved at 121 °C, and 101.325kpa.

Serial dilution of samples

Ten-fold serial dilutions were made for the water samples by pipetting 1.0 ml of the 10 ml stock solutions into a test tube containing 9.0 ml of sterile distilled water. From the new 10 ml stock, 1.0 ml was pipetted into a second test tube containing 9.0 ml of sterile distilled water. This procedure was repeated for a third and a fourth test tube. The resultant was the preparation of 0.1M, 0.01M, 0.001M, and 0.0001M concentrations of the stock solution.

Isolation of and culturing of bacteria

The Pour Plate Method of Isolation and culturing of bacteria was used. For obligate and anaerobic bacteria, the pour plate method is a typical plating technique (Ben-David & Davidson, 2014). By serial dilutions, this method is utilized to isolate microbial colonies. The 0.01M, 0.001M, and 0.0001M concentrations of the stock solution were used as inoculum to isolate bacteria. For each concentration, replicates were done whereas, sterile distilled water was used as inoculum for the control setup. The Petri dishes were labelled with their respective sample names, dilution factors, and the date of inoculation. An inoculum of 1.0 ml, from each sample, was pipetted into their respective Petri dishes. The media was poured into the Petri dishes and gently swirled to mix with the inoculum in the Petri dishes. They were subsequently incubated in an inverted position in an incubator at a temperature of 30°C. Bacterial growth was observed after 24 hours. The bacteria were subsequently subcultured to obtain axenic cultures. Eight axenic cultures were obtained. These were used for microscopy and biochemical test.

Microscopy of bacterial cultures

An inoculation needle was used to transfer a very small piece of the axenic cultures on nutrient agar onto a clean glass slide. The bacterial cells were placed on the slide, stained with bromophenol blue, and covered with a cover slip. The slide was then viewed under the microscope under oil immersion. The bacterial cells were identified as bacilli, cocci, or streptococci.

Biochemical tests

Bacteria were subcultured into 7.0 ml of nutrient broth at 25 °C in 15.0 ml tubes until the broth became turbid between 24 to 32 hours. The bacterial cultures in these test tubes were used as the inoculum for the biochemical tests. Some of the biochemical tests conducted included; the Voges-Proskauer (VP) test (McDevitt, 2009), the Citrate test (Difc, 1998; MacFaddin, 2000; MacWilliams, 2009), the Indole test (MacWilliams, 2009), the Catalase test, Triple Sugar Iron (TSI) test (Lehman, 2005), Urease test (Brink, 2010), and carbohydrate fermentation tests using Phenol red nutrient broth base (PRNBB) (Kali, Srirangaraj, & Charles, 2015). The compounds used in this test were lactose, glucose, xylose, arabinose, maltose, and glycerol. All these were prepared according to standard protocol.

Statistical Analysis

The morphometrics of eggs and hatchling collected were organized in Microsoft Excel sheets and various means were calculated with Microsoft Excel. Percentages of hatching and mortality were also calculated using Microsoft Excel. A test of significance on group means was conducted using student's t-test on Minitab version 18. Graphical presentations were made using Microsoft Excel (MS Office 2019).

Bacteria isolates were identified based on the analysed results obtained from their culture appearance, microscopy and biochemical tests using an online microbial identification database (https://www.microrao.com/identify.htm).

CHAPTER FOUR

RESULTS

The findings of the study are reported in this chapter. A brief description that explains the trend of the outcomes is provided with each result.

Physicochemical Parameters Recorded During the Incubation of Embryos

The salinity in system A increased from 27.5 ppt to 32 ppt within the first four days of incubation after which it stabilized and increased steadily for the rest of the culture period. However, in system B, the salinity decreased from 30 ppt to 28 ppt in the first three days of incubation but increased to 32 ppt by the sixth day and remained fairly constant for the rest of the culture period as seen in Figure 9.

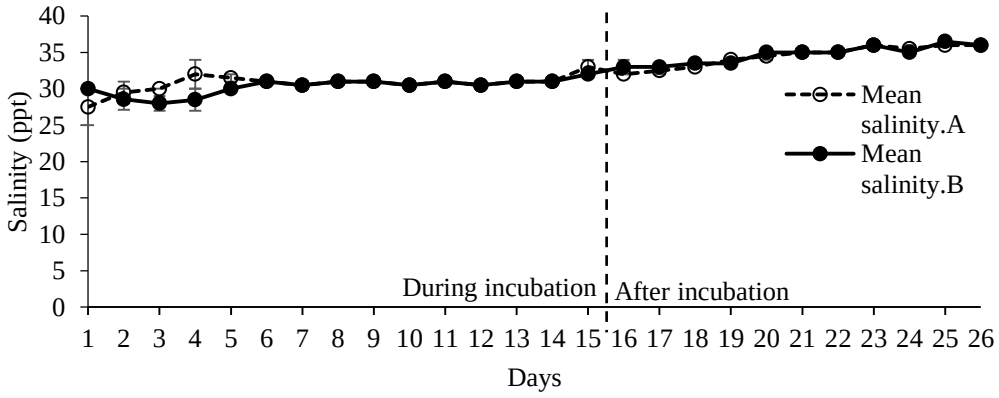

Figure 9: Trend of salinity in systems A and B before and after incubation

There was an increase in the dissolved oxygen levels in the first three days of incubation, from 1.43 mg/l to 4.43 mg/l in system A after which it declined gradually to 1.77 mg/l during the incubation period (Figure 10). However, it increased to a peak of 2.59 mg/l on day 15 of the culture period

and declined steadily for the rest of the culture period. A similar trend was observed in system B. The DO increased from 2.70 mg/l to 4.27 mg/l on the fourth day of incubation but declined for the rest of the incubation period. A slight increase was observed within the first four days after hatching but declined thereafter for the rest of the culture period.

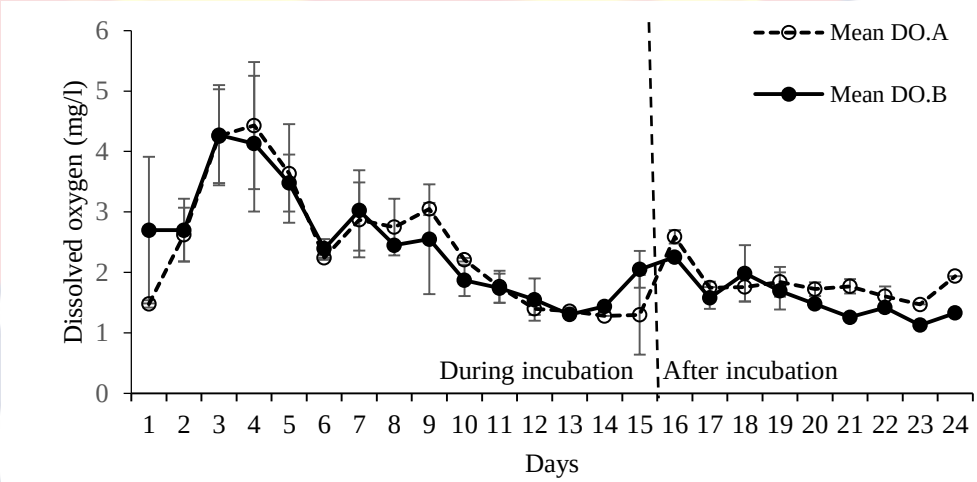

Figure 10: Trend of dissolved oxygen levels in systems A and B before and after incubation

From the beginning of the incubation period, the temperature decreased from 28.35 ^{o}C to 26.55 ^{o}C and in system A. It increased gradually to 28.15 ^{o}C by the thirteenth day of the culture period but decreased sharply to 26 ^{o}C. Thereafter, it increased to 27.85 ^{o}C after which some fluctuations were observed toward the end of the culture period. In system B, a decline in temperature was observed in the first eight days of incubation after which a subsequent gradual increase in temperature occurred between the eighth day and the eleventh day of incubation. The temperature remained constant briefly, after which it fluctuated for the rest of the culture period as seen in Figure 11.

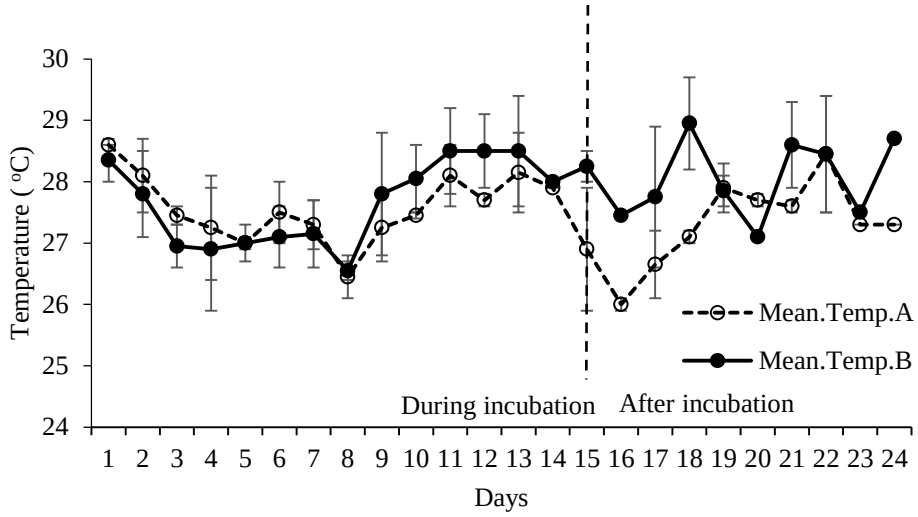

Figure 11: Mean temperature recorded each day of the culture period

As seen in Figure 12, the pH of the two systems was recorded once every other day. In system A, the pH remained constant at around 9.2 during the incubation period but decreased to 8.4 on day 13. The pH further decreased to 7.6 and remained around that level for the rest of the culture period. The pH was considerably stable between 9.2 and 9.46 in the first four days of incubation in system B but reduced to 8.0 on day 9 of incubation and further declined to 7.6 for the rest of the culture period.

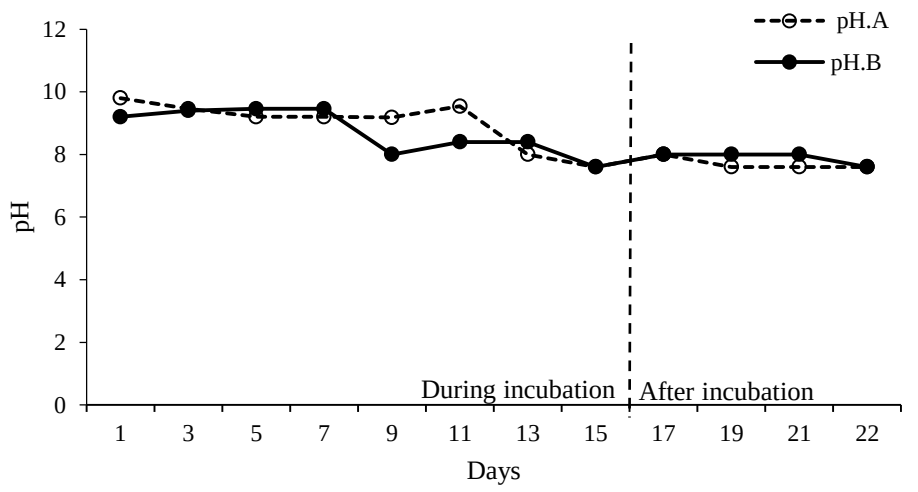

Figure 12: pH of systems A and B recorded over the culture period

Characteristics of Eggs during Embryonic Development in the Culture Systems

Cuttlefish eggs obtained from Elmina (Figure 7AI) were generally bigger than those from Nungua (Figure 7AII). The weight of eggs from Nungua ranged from 0.43 g to 0.72 g and the length of their longest axis ranged from 1.1 cm to 1.9 cm whereas eggs from Elmina weighed between 0.56 g and their longest axis measured from 1.5 cm to 1.9 cm. The eggs were oval and tapered at both ends. One end of the egg was blunt while the other end possessed a threadlike projection which served as the point of attachment to the bunch of eggs collected from the wild (Figure 7BII). The eggs obtained from Nungua were a mixture of all-black and all-whitish eggs (Figure 7C). Upon arrival at the culture facility, the eggs were acclimatized to the culture water conditions by dropping the water in the system onto them through a narrow rubber tube as shown in Figure 7D.

The mean length of the longest axis (diameter) of the eggs in system A remained almost unchanged during the incubation period (Figure 13). In system B, however, there was a conspicuous increase in the length of their longest axis after the tenth day of the incubation period.

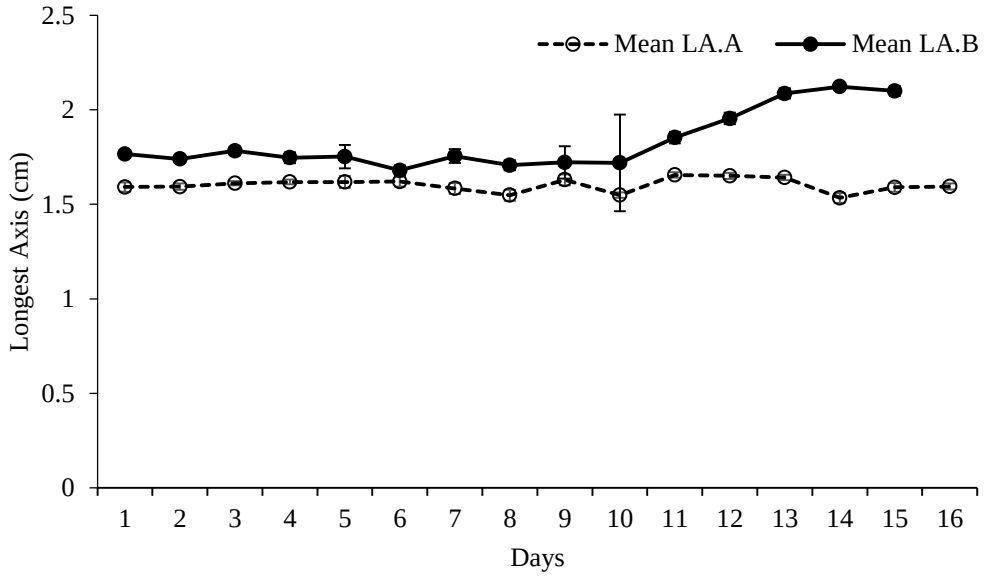

Figure 13: Mean diameter of embryos on each day of incubating embryos LA. A = Longest axis of the embryos in system A; LA. B = Longest diameter of the embryos in system B.

The weight of the eggs in system A was considerably constant for the entire period of incubation. On the contrary, the weight of eggs in system B exhibited an upward trend towards a peak of 1.54 g on the fourteenth day of incubation (Figure 14).

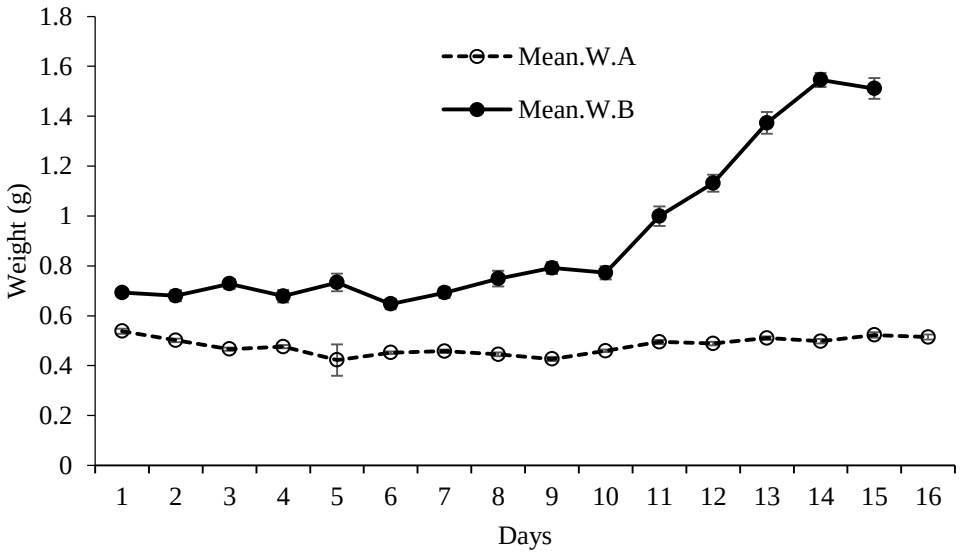

Figure 14: Mean weight of embryos on each day of incubation

Hatching and Mortality rates in Systems A and B

In system A, the percentage of hatching increased gradually from the first day (0.5%) to a maximum on the fourth day (10%) as seen in Figure 15. Thereafter, there was a sharp decline on the fifth day and this reduced further to zero on the seventh day. In system B, the percentage of hatching increased sharply from the first day (2%) to a maximum of 16% on the fourth day after which a decline to 3.3% was observed on the fifth day. There was no hatching on the sixth day, however, there was a gradual increase in hatching after the sixth day and a subsequent decline until hatching ceased on the eleventh day.

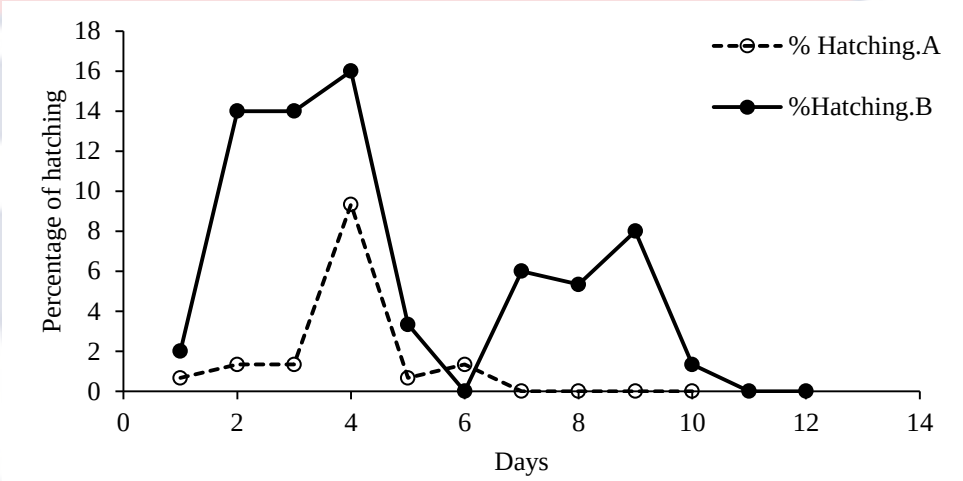

Figure 15: Percentage of hatching by day in culture systems A and B.

As seen in Figure 16, no mortalities were observed in system B on the first day but mortalities increased gradually and peaked at 23.3% on the sixth day, declined to 4% on the seventh day, and increased to 13.3% and declined gradually until the twelfth day. In system B, the highest mortality (10%) was observed on day 4. There were no deaths on the following day but the rest of the hatchlings died on the sixth and seventh days. The trends of the hatching rate and mortalities in system A (Figure 15 and Figure 16) are very similar since the hatchlings in this system died the same day after hatching.

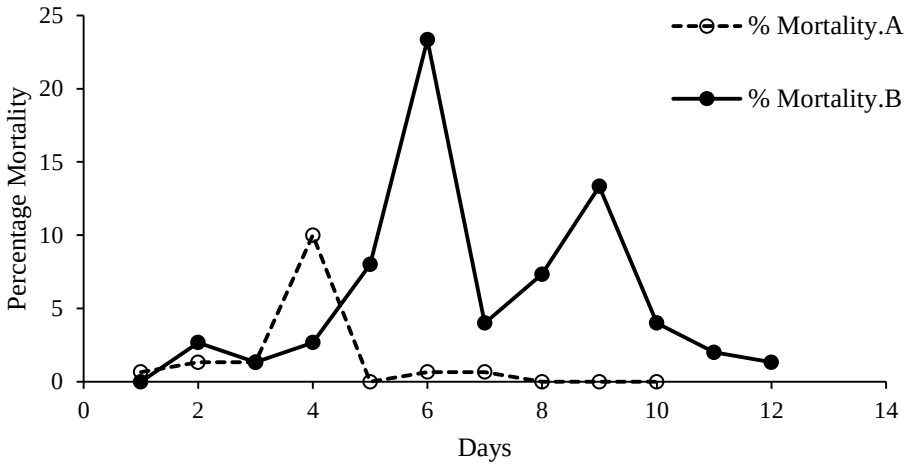

Figure 16: Percentage mortality by day in culture systems A and B

The cumulative results from the three hatching experiments have been presented in Table 1. The eggs from Elmina, in system B, had a cumulative hatching rate of 70% whereas eggs from Nungua, in system A had a low hatching rate of 16%.

Table 1: *Summary of Hatching Success and Mortality of Cuttlefish Embryos in Systems A and B*

	System A	System B
Number of eggs	450	450
Number of eggs hatched	72	315
Number of unhatched eggs	378	135
Hatching rate (%)	16	70
% Mortalities after 8 days	100	100

The growth of Hatchlings in the Systems

The morphometrics of the hatchlings have been graphically presented in Figures 17-19. During data collection, only hatched and living organisms present in the systems were measured, implying that more or fewer hatchlings were available to be measured on some days, which might have influenced the means of the data sets obtained. Due to this inconsistency, some data points

were not included in the graphical presentation of the results. Since hatchlings in system A died the same day after hatching, a trend could not be established with the data obtained. Due to insufficient data from system A, only data from system B have been represented in this section.

The mantle lengths of hatchlings in system B increased slightly from day 4 to day 8 after hatching. However, an overall trend could not be established because, on some days, hatchlings died before data collection (Figure 17).

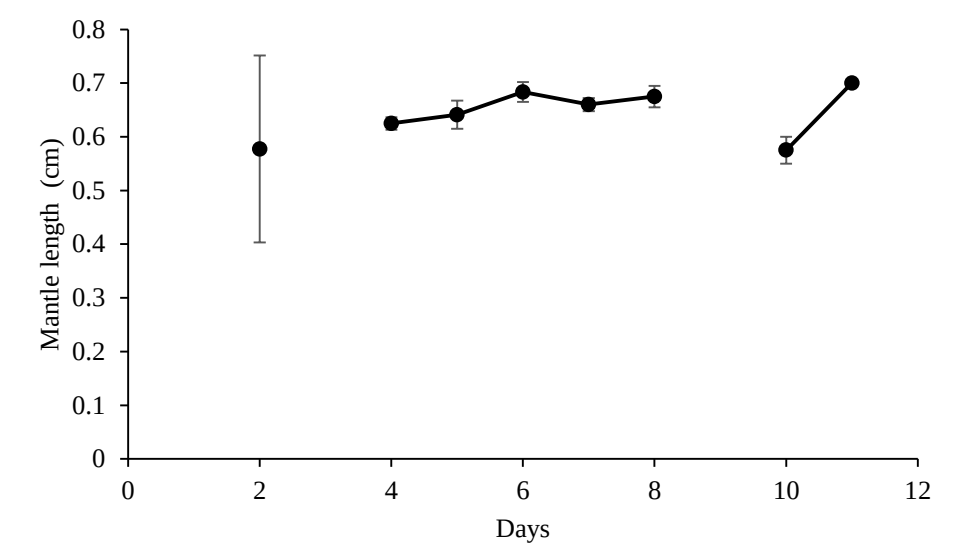

Figure 17: Mean mantle lengths (ML) of hatchlings in system B

There was a marginal but steady increase in the total length of the hatchlings in system B from 0.09 cm on the second day to 1.09 cm on the sixth day, and from 1.07 cm on the eighth day to 1.10 cm on the tenth day (Figure 18).

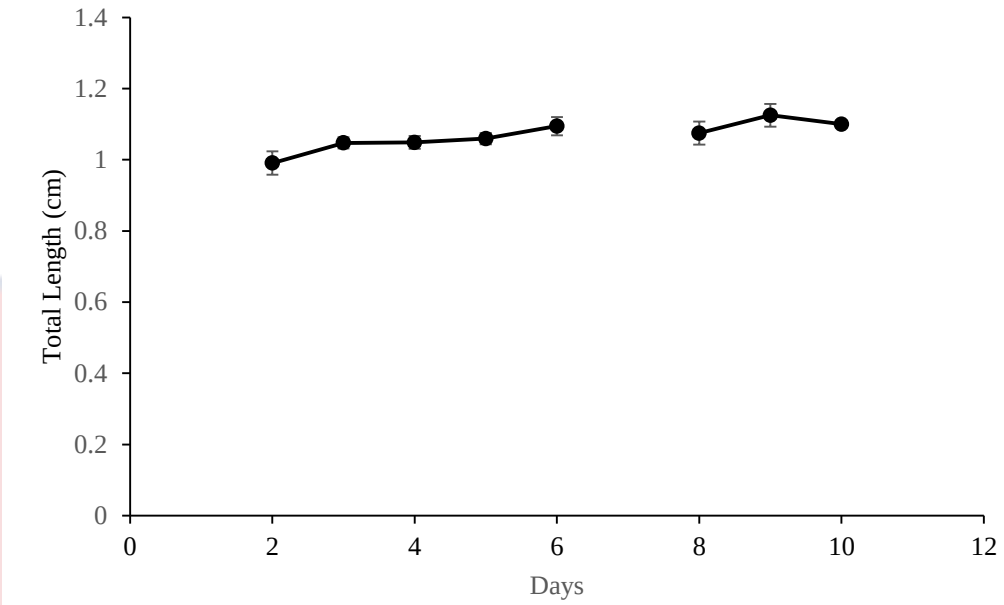

Figure 18: Mean total lengths (TL) of hatchlings in systems B

The dorsal mantle length of the hatchling increased in the course of the culture. From day 4 to day 6, a gradual increase in the mantle length was observed as presented in Figure 19.

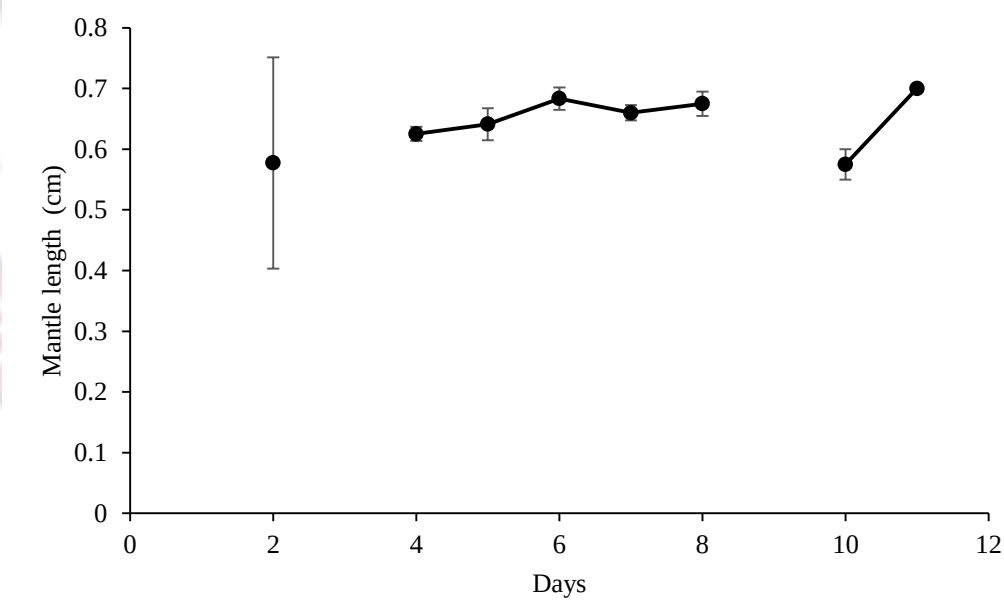

Figure 19: Trend of the dorsal mantle length of hatchlings in system B

There was an increase in the mean weight of hatchlings in system B. Hatchlings increased in weight from day 3 and peaked at 0.115 g on day 6. On the other hand, little growth was observed from day 8 to day 11 (Figure 20).

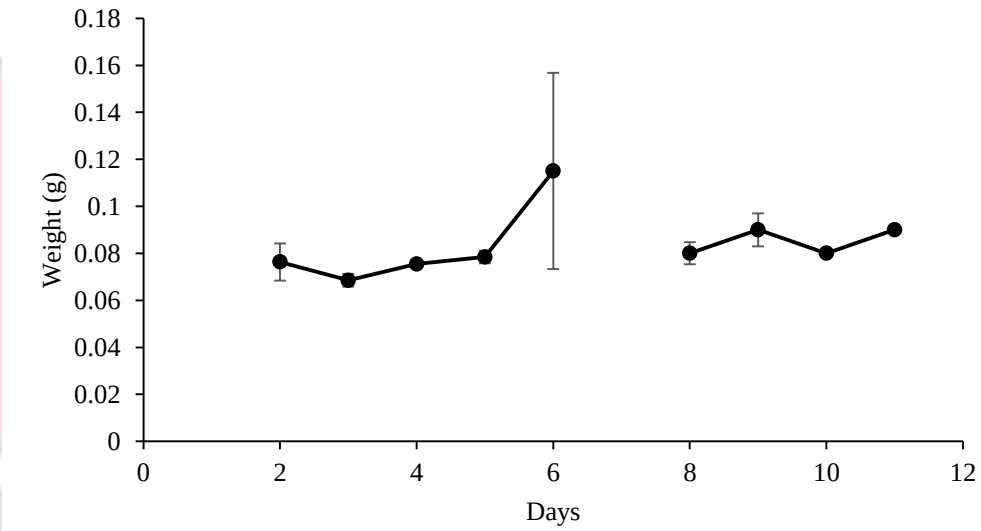

Figure 20: Mean weight of hatchlings in system B

Some photos of hatchlings on different days after hatching have been presented in Figure 21. Little growth was observed during the time they were maintained in the culture system. Figure 21A shows a day-old hatchling, and Figure 21B shows a 5-day-old hatchling. Hatchlings that survived long enough, became lethargic on day 9 (Figure 21C) and died by day 11.

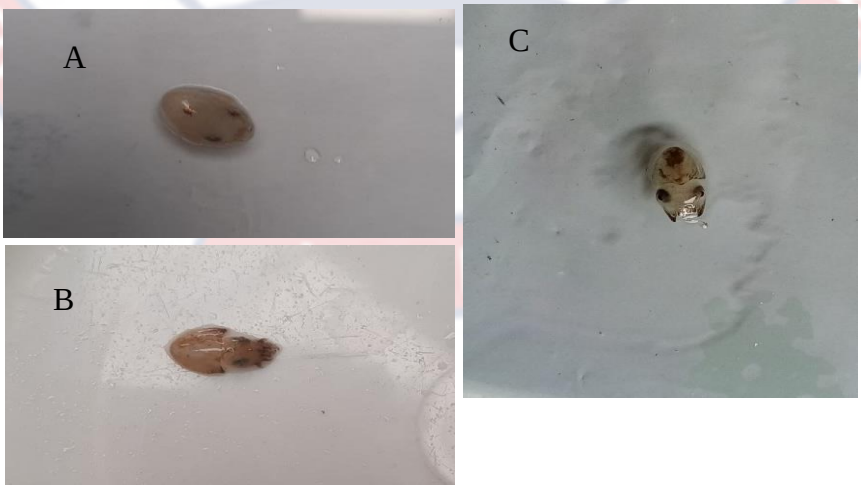

Figure 21: Hatchlings at day1 (A), day 2 (B), and day 9 (C) after hatching

The culture characteristics comprising the form, margin, surface appearance, colour, and size of colonies are outlined in Table 2. Also, the shape of the cells after observation under the light microscope has been stated.

Table 2: Bacterial Culture Characteristics and Shape under the Microscope

Isolate	Form	Margin	Surf. Appearance	Color	Size	Microscopy
1	Filamentous	Irregular	Smooth	Opaque	Large	Cocci
2	Punctiform	lobate	Smooth	peach	small	Streptococci
3	Irregular	Irregular	Dull	opaque	Large	cocci
5	Spindle	entire	Dull	opaque	medium	bacillus
6	Punctiform	undulate	Glistening	opaque	medium	bacillus
10	Irregular	Irregular	spreading/dull	transparent	Large	Cocci
12	Irregular	lobate	Smooth	Opaque	Large	Streptococci
15	Irregular	undulate	Rough	Opaque	Large	Streptococci

The test results of some biochemical tests conducted for the bacterial cultures have been tabulated in Table 3. These tests were done to identify the type of bacteria present based on their ability to utilize or produce certain compounds for their metabolism under specific culture conditions.

Table 3: Some Biochemical Tests Conducted with the Bacterial Cultures

Isolate	**Catalase**	**Oxidase**	**Slant**	**Butt**	**Gas**	**H_2S**	**Urease**	**Citrate**	**Indole**	**Gram+/-**
1	+	-	red	Yellow	-	-	-	-	-	+
2	+	-	red	Yellow	-	-	-	-	-	+
3	+	-	red	Yellow	-	-	-	-	-	+
4	+	-	red	Yellow	-	-	-	-	-	+
5	+	-	red	Yellow	-	-	-	+	-	+
6	+	-	red	Yellow	-	-	-	+	-	+
7	-	-	red	Yellow	-	-	-	-	-	+
8	-	-	red	Yellow	-	+	-	-	-	+

Definition of symbols; (+) = reactive; (-) = non-reactive

Table 3 continued

Isolate	L-Arabinose	VP	Maltose	Lactose	Glycerol	Glucose	D-Xylose
1	-	-	-	-	+	+	+
2	+	-	-	-	-	+	+
3	-	+	-	-	+	+	+
4	-	+	-	-	+	+	-
5	-	-	+	+	+	+	+
6	-	+	-	-	-	+	-
7	-	-	+	-	+	+	-
8	-	-	+	-	+	+	-

Definition of symbols; (+) = reactive; (-) = non-reactive

The bacteria identified in each isolate have been outlined in Table 4 along with a brief description of the species.

Table 4: Identified Bacteria in the Seawater used in the Culture Systems

Isolate	Bacteria	Description
1	*Staphylococcus haemolyticus*	It is a component of human skin flora, and the places with the highest concentrations of it are often the axillae, perineum, and inguinal regions. Primates and domestic animals are also colonized by *S. haemolyticus*.
2	*Micrococcus roseus*	Found in soil, freshwater and marine environment.
3	*streptococcus faecalis*	Normally in the gastrointestinal tracts of humans but can withstand harsh environmental conditions
4	*Staphylococcus capitis*	It is a typical component of the flora on the scalp, face, neck, scrotum, and ears of humans.
5	*Staphylococcus saprophyticus*	It is a normal flora in the human, pig, and cattle gastrointestinal tract, urethra, perineum, and cervix.
6	*Micrococcus halobius*	Warm-blooded animals and humans are the main reservoirs. It is found in terrestrial and aquatic habitats.
7	*Aerococcus viridans*	It is a marine bacterium causing fatal disease in lobsters.
8	*Streptococcus pneumoniae*	It is a gram-positive bacterium responsible for causing community-acquired pneumonia.

DISCUSSION

This chapter discusses the implications of the results obtained. Comparisons between the results of the current study and relevant previous studies are also made to ascertain the conformity of this work to previous knowledge or otherwise.

Embryo Characteristics

In this study, eggs of *Sepia hierredda* were obtained from Elmina and Nungua. The eggs collected were typically black, and oval and had gelatinous but firm shells. All the eggs obtained from Elmina were black but those from Nungua were made up of a mixture of white (translucent) and black eggs. There was no change in coloration during the incubation period contrary to what was reported by Okyere *et al*. (2017). Black eggs remained black throughout the incubation period, likewise the whitish eggs.

During the incubation period, the increase in length of eggs in Systems B in comparison with those in System A was significant (APPENDIX A). Some of the eggs in system B measured 3.0 cm from end to end a few days before hatching and this agrees with the findings of Okyere *et al*. (2017), who reported a height (longest axis) of 2.5 cm to 3.0 cm, a few days before hatching. The weight of eggs collected from Nungua ranged from 1.1 g to 1.9 g and those from Elmina ranged from 1.5 g to 1.9 g on the day of collection, and this is within the range of 0.1 g to 2.5 g reported by Sykes *et al*. (2006). There was also a significant increase in the weight of the eggs in system B over the incubation period ($p < 0.05$). This indicates the presence of metabolic activities in the eggs during incubation. In contrast, the weight of eggs in system A remained almost constant, and this may signal less or the absence of

metabolic activity in the eggs, which could account for the low hatching rate observed in that system. The shells of the eggs in both systems became more turgid and softer during incubation. The eggs were observed to be floating more easily a few days before their hatching. Upon hatching, some eggs shells floated while others remained at the bottom of the tanks. Most hatched eggs looked entire but with a little opening on one side from which the hatchling came out.

Water Quality Parameters and Hatchability

Cuttlefish which are exclusively marine organisms require a narrow range of salinity, pH, and ammonia concentrations to survive and thrive. Very low salinity has been observed to cause significant pathological alterations in the gills of cuttlefish hatchlings (Wu *et al.*, 2021). The pH was kept in an acceptable range of 7.8-8.3. The mean nitrite and nitrate concentrations in System B were significantly lower than in System B (APENDIX B). In system B, nitrites and nitrates concentrations were within an acceptable range of 0.1 mg/l and <20 mg/l respectively as suggested by Boletzky and Hanlon (1983) whereas, in system A, these concentrations were significantly higher.

Cuttlefish eggs are known to be easily transported and maintained and have a high hatching rate (Jones, Ridgway and Richardson, 2009; Sykes *et al.*, 2006). The eggs are abandoned, attached to the substrates upon which they were spawned from there they hatch into miniature adults. Therefore, eggs did not need much maintenance while in the culture system. A constant movement of the seawater in the recirculating aquaculture systems was sufficient to allow hatching. It is reported that cuttlefish embryos can hatch within salinities of 25-33 ppt, with the highest hatching rate occurring at 31 and 33 ppt

(Palmegiano, & d'Apote, 1983). This is not so different from what was observed in this study (34.00 ± 1.39 ppt and 33.70 ± 1.55 ppt for Systems A and B respectively), with system B having the highest hatching rate.

The eggs incubated in system B were collected from Elmina and transported for 10 hours to Accra, with no oxygen in the plastic bucket in which they were placed, yet a 70% hatching rate was observed. However, eggs obtained from Nungua beach in Accra and transported in 2.5 hours on ice showed a very low hatching rate (16%). This brings to the fore the possibility of the negative effects of transporting cuttlefish eggs on ice. This may have caused a temperature shock and lowered the hatchability of the eggs. Jones *et al.*, (2009) achieved a hatching rate of 82.8% after transporting cuttlefish eggs for 8.5 hours in seawater without aeration or ice. The total time for the transportation takes into account the time of collection of the eggs from Elmina waters into the boat of fishermen and the duration of transporting them by car to the culture facility at Teshie. There was heavy traffic on Accra-Cape Coast road, and Nungua to Greda Estates, Teshie on the days on which the eggs were transported.

Hatching of the embryos in both systems did not produce a normal distribution curve as expected but fluctuated over the period until it ceased on the eighth day in system A and the tenth day in system B as seen in Figure 15. There were also days when no hatching was recorded. Some authors have documented that cuttlefish incubation time is strongly dependent on temperature (Palmegiano & d'Apote, 1983). For instance, Mangold-Wirz (1963) reported that cuttlefish eggs can remain in incubation for up to 87 days (at 15 °C), 47 days (at 18.4 °C), and 31 days (at 21.4 °C) before hatching.

Also, Sykes *et al*. (2006) identified that the embryonic development of cuttlefish requires between 25 days at 25 °C to 30 days at 20 °C. In this study, the cuttlefish eggs were kept in the culture system for 27 days at 27.338± 0.763 °C and 28.015± 0.593 °C for systems A and B respectively but hatching ceased at day 21 in system A and at day 25 in system B.

Since the culture facility was not an enclosed, temperature-controlled area, intermittent rainfall reduced the atmospheric temperature and had a corresponding change in the culture systems. An attempt was made to reduce the water temperature in system A by dropping ice (seawater) into the sump but a constant temperature could not be achieved, as this process was inefficient. This made temperatures fluctuate in the system as seen in Figure 11. On the other hand, the temperature in system B was kept relatively the same as the surrounding temperature in the culture facility. This marked a smooth and gradual increase or decrease in temperature due to the change in temperature of the surroundings. However, the temperature in system A (mean 27.338 ± 0.763 °C), differed slightly from that in system B (27.823 ± 0.679 °C). Contrary to some previous observations that embryos go through a process of 'perfectioning' in embryo development at low temperatures during incubation (Boletzky, 2004), the embryos in system A had a very low hatching rate. The difference in water temperature in systems A and B was however not significant ($p > 0.05$) to be considered to have influenced the hatching of the eggs. Moreover, Domingues *et al*. (2002) have also demonstrated that the species tolerates a wide temperature range (10-32 ^{o}C), so the low hatching rate in system A cannot be attributed to a lower temperature in the system.

The importance of oxygen for the metabolism of any organism cannot be overlooked. Dissolved oxygen is an essential requirement in the life processes of aquatic organisms like fishes and cephalopods. As suggested by Sykes *et al*. (2006), the hatching tanks were adequately aerated to keep eggs moving to increase the hatching rate and decrease egg spoilage. However, optimum levels of dissolved oxygen could not be maintained. The dissolved oxygen levels recorded in both systems were extremely low (System A = 1.814 ±0.334 mg/l; System B = 1.629 ±0.364 mg/l) as compared to recorded values in the species' breeding sites at Mumford (6 .5 ±0.5 mg/l), Cape Coast (6.0±1.0 mg/l), and Elmina (6.2 ±0.5 mg/l) (Okyere *et al*., 2017). Since the levels of dissolved oxygen were low for both systems and not significantly different from each other ($p>0.05$), low hatching success in system A cannot be attributed to low oxygen content as well. Moreover, the DO meter may have had an inherent malfunction to have recorded such low DO levels, considering the level of aeration the systems received. To be certain of the functionality of the DO meter, it was sent to Nungua beach to measure the DO levels in the open seawater but readings were always below 4.0 mg/l at 4 different locations in the beach waters.

Since cuttlefish undergo external fertilization and the certainty of obtaining fertilized eggs from the wild is not guaranteed, it is not clear whether all the eggs acquired from Nungua were fertilized, giving a reason for an observed low hatching rate of those eggs. Moreover, the little growth in the eggs in system A as compared to those in system B (Figures 13 and 14) indicates less metabolic activity in the eggs and further strengthens the

possibility that not all the eggs might have been fertilized before collection from the wild, and thus some were not viable.

Survival and Growth

Survival and growth of hatchlings in captivity do not seem to be a major hindrance in the culture of cuttlefish based on previous studies (Minton *et al*., 2001; Domingues, Bettencourt & Guerra, (2006); Sykes, Domingues, & Andrade, 2006). Some of these authors have cultured *Sepia officinalis* to maturity and proceeded to assess its economic viability in commercial culture. However, previous studies in Ghana (Okyere *et al*., 2017) maintained hatchlings up to about fourteen days after hatching in non-circulating fish tanks. The current study maintained them in recirculating aquaculture systems yet there was 100% mortality by the twelfth day after hatching. This is unlike what Sykes, Pereira, Rodríguez, Lorenzo, and Andrade (2013b) reported in their studies where cuttlefish were maintained to the juvenile stage from eggs captured in the Ria Formosa lagoon (Faro, South Portugal). Eggs were maintained to the juvenile stage in a flow-through system of between 9-30ºC temperature, 25-38‰ salinity, nitrites below 0.1mg/l and nitrates below 80mg/l.

Feeding is one of the crucial factors determining both survival and development, and its importance is enhanced by temperature (Domingues, Bettencourt & Guerra 2006; Sykes *et al*., 2006). Most of the mortality in cuttlefish culture occurs at the hatchling stage (Sykes *et al*., 2006) and this may be caused by several factors such as the amount and quality of prey (Domingues, Kingston, Sykes & Andrade 2001a; Domingues, Sykes & Andrade 2001b; Domingues *et al*., 2004).

A key barrier to the successful large-scale culture of cephalopods is the lack of appropriate prey that may be used to successfully culture their early stages (Lee, 1995; Domingues *et al*., 2003, 2004). The hatchlings require live feed and since there is an abundance of zooplankton and small crustaceans in the open seawater, they feed and thrive on these. However, hatchlings have been fed a variety of prey species, such as amphipods, *Artemia* spp., and the mysid shrimp *Paramysis nouvelli* (Domingues *et al*. 2001), In this study, brine shrimp *Artemia salina* was used to feed the hatchlings due to the unavailability of zooplankton. Cuttlefish hatchlings were seen moving toward the feed given to them in the system but, it was not clear if they fed on the *Artemia*. However, the results on the growth of the hatchlings indicate a slight increase in the ML, TL, and weight for the 12 days they were maintained in culture. This suggests that hatchlings fed but not as voracious as they have been observed to do in previous studies (Grigoriou & Richardson, 2004). Also, some amphipods were obtained but these were bigger than the gape size of the hatchlings, so they could not feed on them.

Although aquaculture systems are meant to be a replication of the natural aquatic environment, it can be difficult to maintain suitable conditions during the entire culture period (Farto, Fichi, Gestal, Pascual & Nieto, 2019). For better industrial culture output, understanding the bacterial species involved in cephalopod culture and their ecological function is crucial (Farto, *et al*., 2019). Physico-chemical and geographical parameters such as water temperature, latitude, and salinity of seawater have a variable effect on the microscopic population density and a high level of microbial diversity (Harder & Yee, 2009; Farto *et al*., 2019). One of the biggest obstacles to successfully

producing cephalopods in aquaculture or maintaining them in adequate settings in captivity is the occurrence of diseases, many of which are bacterially induced (Prado-Álvarez & García-Fernández, 2019; Sykes & Gestal, 2014). The microbial quality of the water used in the culture system in this study was also assessed to determine if any pathogenic bacteria in the seawater might have been detrimental to the health of the embryos and hatchlings.

Most of the bacteria identified in the water sample were mainly normal flora on mammalian skin and gastrointestinal tract. This is relatable to the condition of the beach from which the seawater was obtained. Open defecation is a norm at Nungua beach and since the collection of seawater was done 5 m away from the shore at high tide, the water was probably contaminated with human fecal matter. Thus, the identified microbes are mainly contaminants from human excreta. Also, the area where the water was collected is a usual spot for swimmers. Among the bacteria identified in this study, *Aerococcus viridans* has been found to cause a lethal systemic disease known as gaffkemia in both the American lobster, *Homarus americanus*, and the European lobster, *Homarus gammarus* (Saxegaard & Hâstein,1978; Clark & Greenwood, 2011) but no relationship has been drawn to cephalopods in the scientific literature yet. *Micrococcus halobius*, which was also among the bacteria species identified in this study, is considered a probiotic strain present in the gut microflora of fishes and enhances their growth (Sivakumar, Janani, & ShreeRama, 2015).

CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The findings made and their implications are briefly discussed in this chapter. It also provides a summary of the entire study and some recommendations meant to advance current research and broaden understanding in the future.

Summary

Cuttlefish is an economically important seafood in Ghana. It has high nutritional value and is a delicacy among coastal dwellers. A lot of research has been conducted on cuttlefish in the past six decades. Various conditions have been modified to suit the laboratory culture of cuttlefish and varying successes have been achieved at different salinities, temperatures, and the use of different feeds as well. However, these studies have centered on *Sepia officinalis* and *Sepia pharaonis*. In this study, the embryo characteristics, hatching success, and survival of *Sepia hierredda*, which is endemic to the Eastern Central Atlantic waters have been assessed in recirculating aquaculture systems. The setup for the experiment included a sump comprised of a net to remove food debris, a skimmer to remove nitrogenous waste, and a pack of coral rocks to aid in biological filtration. The water parameters were maintained at optimum levels to ensure the hatching of the eggs. Some bacteria species were also identified in the seawater used to culture the cuttlefish. This work is among the very few that have considered the culture potential of *S. hierredda.*

Conclusions

Through this study, it has been established that maintenance of the eggs and embryonic development of *Sepia hierredda* in captivity is not problematic. Cuttlefish eggs increase in size and weight during incubation indicating metabolism and growth. The seawater used to culture the cuttlefish contained microbial communities that were mainly contaminants from human excreta indicating the polluted condition of seawater from Nungua beach. No pathological relation to the death of the cuttlefish has been drawn from the seawater used in their culture systems.

This study has also shown that hatching success is high for cuttlefish eggs in the salinity range of 34.19 ± 1.67 ppt, and temperature of 28.025 ± 0.622 ^{o}C. Eggs collected from the wild began to hatch after 12-14 days of incubation, however, hatching did not follow a normal distribution pattern.

Survival was extremely low as all hatchlings died within 14 days after hatching. The feeding of the hatchlings is very crucial in the first week of hatching, yet their requirement for live feed makes the culture of their early stages very challenging.

Recommendations

Based on this study, the following recommendations have been suggested:

1. The use of copepods and other zooplankton should be assessed to identify local live feed that can sustain *S. hierredda* in captivity.
2. In subsequent studies in a culture facility, equipment like an industrial sand filter and UV filters should be installed to aid in the purification of the seawater before use.

3. Studies on the viability of eggs of cuttlefish obtained along the coast of Ghana should be assessed.
4. Studies on human-induced mortalities of eggs and hatchlings should be assessed.

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APPENDICES

APPENDIX A

Results from 2-Sample t-test of the morphometrics of cuttlefish eggs in system B (95% CI)

Sample	N	Mean	StDev	T-Value	DF	P-Value
LD.A	675	1.607	0.150	-15.26	813	0.000
LD.B	596	1.828	0.325			
Weight.A	675	0.4776	0.0603	-25.77	610	0.000
Weight.B	593	0.956	0.449			

LA.A = Longest axis of eggs in system A;

W.A = Weight of eggs in system B.

APPENDIX B

Results from 2-Sample t-test of the physicochemical parameters recorded for the culture period (95% CI)

Sample	N	Mean	StDev	SE	T-Value	DF	P-Value
Sal.A	26	32.29	2.29	0.45	0.26	49	0.796
sal.B	26	32.12	2.46	0.48			
DO.A	24	2.220	0.894	0.18	0.29	45	0.772
DO.B	24	2.145	0.882	0.18			
Temp.A	24	27.462	0.616	0.13	-1.93	45	0.061
Temp.B	24	27.823	0.679	0.14			
pH.A	12	8.565	0.896	0.26	0.32	21	0.755
pH.B	12	8.460	0.724	0.21			
Nitrate.A	12	7.08	7.22	2.1	2.79	14	0.015
Nitrate B	12	0.83	2.89	0.83			
Nitrite.A	12	0.833	0.389	0.11	5.36	20	0.000
Nitrite.B	12	0.083	0.289	0.083			

A or B attached to the parameters denotes system A or B from which the parameter was recorded.