UNIVERSITY OF CAPE COAST

PROMOTING OYSTER CULTURE IN GHANA: STRATEGIES FOR OPTIMISING SEED COLLECTION AND GROWTH OF *CRASSOSTREA TULIPA* (LAMARCK,

1819) IN COASTAL WATER BODIES

ERNEST OBENG CHUKU

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BY

ERNEST OBENG CHUKU

Thesis submitted to the Department of Fisheries and Aquatic Sciences of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of a Master of Philosophy (M.Phil.) degree in Aquaculture

MAY 2019

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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:	ERNEST OBENG CHUKU	

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.

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ABSTRACT

The study explored simple strategies for optimising seed collection and growth of the mangrove oyster Crassostrea tulipa to support its large-scale farming in Ghana. Coconut shell, nylon mesh, recycled oyster shell, PVC slat and ceramic tile were assessed at different depths (top, middle and bottom) and at three different stations each in the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary. Ceramic tile, PVC and oyster shell were more efficient spat collectors than coconut shell. Spat settled more profusely on under-horizontal surfaces of collectors compared with the upper surfaces. Spatfall was highest at Narkwa Lagoon, recording 1.3, 2.5, and 9.8 times spatfall in the Densu, Benya and Whin systems respectively. Spatfall was highest in the dry season in Densu Delta and Narkwa Lagoon. In Benya Lagoon it was highest in the wet season but showed no seasonal pattern in Whin Estuary. Spatfall, spat sizes and growth of cultured C. tulipa were significantly greater on middle and bottom collectors than on top collectors. Dissolved oxygen (DO) and salinity were the significant predictors of spatfall whereas those for growth were DO and turbidity. C. tulipa spat stocked at 0.14 spat cm⁻² i.e. 1 spat per 7 cm² produced 'market-size' oysters in six months. There was a significant difference in the growth of C. tulipa among the different water bodies with fastest absolute growth rate recorded in Narkwa Lagoon (AGR = 0.33 mm day^{-1}). Fistubalanus pallidus was the most ubiquitous and *Ficopomatus* sp. was the most deleterious fouling organisms associated with the C. tulipa spat collection and culture. Further studies on genetic variability, nutrient loads and others are recommended in order to fully explain and consolidate the findings of this study.

KEY WORDS

Crassostrea tulipa

Spatfall

Cultch

Spat collector

Shell growth

Fouling

LIST OF ACRONYMS

AGR	Absolute growth rate
CSIR	Council for Scientific and Industrial Research
CS	Coconut Shell
СТ	Ceramic Tile
DFAS	Department of Fisheries and Aquatic Sciences
FAO	Food and Agriculture Organisation
FC	Fisheries Commission
MOFWRNAM	Ministry of Fisheries, Water Resources and National
	Assembly Matters (Republic of the Gambia)
MoFAD	Ministry of Fisheries and Aquaculture Development
	(Republic of Ghana)
OS	Oyster Shell
PVC	Polyvinylchloride
SDF	Skills Development Fund
SDGs	Sustainable Development Goals
SE	Standard Error
SGS	School of Graduate Studies
SH	Shell height

SL	Shell length
SR	Survival rate
ST	Station
TiLV	Tilapia Lake Virus
UAV	Unmanned Aerial Vehicle
UCC	University of Cape Coast
UN	United Nations
YEA	Youth Employment Agency

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DEDICATION

To my parents,

Thomas Gyasi Chuku and Monica Darkwa

TABLE OF CONTENTS

Content	Page
DECLARATION	ii
ABSTRACT	iii
KEY WORDS	iv
LIST OF ACRONYMS	v
ACKNOWLEDGEMENTS	vii
DEDICATION	viii
TABLE OF CONTENTS	ix
LIST OF FIGURES	XV
LIST OF TABLES	XX
CHAPTER ONE: INTRODUCTION	1
1.1 Background to the study	1
1.2 Statement of the Problem	5
1.3 Purpose of the Study	8
1.4 Research Objectives	8
1.5 Significance of the Study	10
1.6 Delimitations of the Study	11
1.7 Limitations of the Study	12
1.8 Definition of Terms	13

	1.9 Organisation of the Study	14
	1.10 Chapter Summary	14
(CHAPTER TWO: LITERATURE REVIEW	15
	2.1 Aquaculture Production and Development in Ghana	15
	2.1.1 Threats to Ghana's aquaculture	17
	2.1.2 Ghana's near-zero marine/coastal aquaculture (mariculture) sector	18
	2.2 Global Outlook on the Fisheries and Culture of Oysters	19
	2.3 The Oyster Fishery in Ghana	21
	2.3.1 Exploitation and consumption	21
	2.3.2 Culture prospects of the West African mangrove oyster	22
	2.4 Bivalve Molluscs	23
	2.4.1 Biology and ecology of bivalves: Oysters in focus	24
	2.4.2 The Mangrove Oysters	25
	2.4.3 Distribution of mangrove oysters	26
	2.4.4 Crassostrea tulipa (Lamarck, 1819)	27
	2.5 Obtaining Oyster Spat (Seed) for Aquaculture	28
	2.5.1 Wild seed	29
	2.5.2 Hatchery seed	29
	2.6 Materials for Spat Collection (Cultches)	29
	2.6.1 Selection of oyster spat collectors	29
	2.6.2 Types of oyster spat collector materials and associated techniques	30

2.7 Influence of Collector Orientation on Spat Setting Success	38
2.8 Chapter Summary	40
CHAPTER THREE: MATERIALS AND METHODS	41
3.1 Study Sites	41
3.1.1 Densu Delta	42
3.1.2 Narkwa Lagoon	44
3.1.3 Benya Lagoon	45
3.1.4 Whin Estuary	46
3.2 The Community Entry Process	47
3.3 Preparing the Collectors	47
3.3.1 Collating available spat collectors	47
3.3.2 Construction of experimental collectors	48
3.4 Experimental Design and Setup	51
3.5 Field Sampling and Data Collection	53
3.5.1 Estimating spat abundance on collectors	53
3.5.2 Size distribution of one-month old spat	54
3.5.3 Monitoring growth of cultured C. tulipa	55
3.5.4 Experiment on spat setting on upper- and under-horizontal	
surfaces of collectors	55
3.5.5 Hydrographic parameters	57
3.5.6 Identifying and estimating fouling organisms	57

3.6 Mapping Bathymetry, Oyster Presence and Spat Availability in the	
Water Bodies	57
3.7 Data Analysis	58
3.7.1 Estimation of spatfall	59
3.8 Chapter Summary	61
CHAPTER FOUR: RESULTS	62
4.1 Settlement of C. tulipa Spat on Experimental Collectors	62
4.2 Settlement of C. tulipa Spat on Upper and Under-Horizontal	
Surfaces of Collectors	64
4.3 Monthly Variations in Spatfall at Experimental Stations	67
4.4 Spatfall at different depths from the water surface	70
4.5 Size Distribution of One-Month Old C. tulipa Spat	73
4.6 Growth of Cultured C. tulipa	76
4.6.1 Comparing growth of <i>C. tulipa</i> in the different water bodies	76
4.6.2 Comparing growth of C. tulipa at different depths	80
4.6.3 Comparing growth of <i>C. tulipa</i> on the different collectors	80
4.7 Fouling and Associated Epibiotic Organisms	82
4.8 Prevailing Hydrographic Conditions	86
4.8.1 Temperature	86
4.8.2 Dissolved oxygen (DO)	87
4.8.3 Salinity	87

4.8.	.4 Turbidity	89
4.8.	.5 pH	89
4.8.	6 Relationship between hydrographic conditions, spatfall and spat	
grov	wth	89
4.9	Mapping Depth Profile, Spatfall and Presence of Adult C. tulipa in	
the	Coastal Water Bodies	91
CHAI	PTER FIVE: DISCUSSION	97
5.1	Efficiency of Collectors and Orientation for Optimising Collection	
of C	Crassostrea tulipa Spat From the Wild	97
5.2	Spatial Abundance of <i>C. tulipa</i> Spat in Some Coastal Water bodies	
in C	Ghana	102
5.3	Temporal Abundance of C. tulipa Spat in Some Coastal Water	
bod	lies in Ghana	104
5.4	Size Distribution of C. tulipa Spat	107
5.5	Growth Performance of C. tulipa Cultured on Different Cultches in	
Sele	ected Coastal Water Bodies	108
5.6	Potential Impact of Fouling and Epibiotic Organisms Associated	
with	h Spat Collection and Culture of C. tulipa	110
CHAI	PTER SIX: SUMMARY, CONCLUSIONS AND	
RECO	OMMENDATIONS	114
6.1	Summary	114
6.2	Conclusions	114

6.3 Recommendations	116
REFERENCES	118
APPENDICES	140

LIST OF FIGURES

Figure	Page
2.1: Aquaculture Production in Ghana from 1990 to 2016 Data set	
extracted from (FAO, 2018a).	16
2.2: Molluscan phylogenetic tree from transcriptome and genome data	
for all major lineages, highlighting bivalvia in rectangle. Adapted	
and modified from (Gosling, 2015). Monoplacophora insert after	
(Saleuddin & Mukai, 2017).	24
3.1: Maps of the study sites (A) Densu Estuary (B) Narkwa Lagoon	
(C) Benya Lagoon (D) Whin Estuary	43
3.2: The oyster bed at the Densu Delta (A). An oyster picker harvesting	
oysters by hand into canoe at high tide (B), and women and childre	n
are mainly involved in harvesting oysters from the oyster bed at low	N
tide (C).	44
3.3: Women and children harvesting oysters from the Narkwa Lagoon by	r
bending to reach the bottom of the water and collecting them by ha	nd
into pans (A). Oyster pickers getting oyster-filled pans ready at the	
bank of the lagoon for transportation (B).	45
3.4: Oyster spat collector materials tested for effectiveness (a) coconut	
shell, (b) fine mesh nylon net (mosquito net), (c) oyster shell	
(recycled), (d) PVC slats and (e) ceramic tiles.	48
3.5: Installation of racks and setting of collectors: (a) Stakes of experime	ntal
racks being mounted at low tide; (b) diving is required at stations w	vith

deep waters (ST2 of Densu Estuary), and (c) collectors being fitted	
to racks.	51
3.6: An illustration of the cross sectional view of the experimental setup	
at each station	52
3.7: Crassostrea tulipa spat showing shell height (SH) measurement.	
Illustration was done by researcher.	54
3.8: An illustration of (A) face down 0° and (B) face up 180° orientation	
and collector surfaces in the horizontal position	56
4.1: Mean (±S.E.) spatfall on experimental spat collectors in each of the	
four water bodies. Arabic alphabets show ANOVA comparison	
between collectors within the same water body only. Boldface	
Roman numerals show ANOVA comparison between water bodies	
for each type of collector. Means with different alphabets/numerals	
are significantly different (One-way ANOVA: $P < 0.05$)	
[see Appendix B2].	63
4.2: Percent mean number of spat on upper- (\square) and under- (\blacksquare)	
horizontal surfaces of experimental collectors (CS = Coconut shell;	
OS = Oyster shell; CT = Ceramic tile). * Significant difference	
between \square and \blacksquare ($P < 0.05$)	65
4.3: Mean (\pm S.E.) spat settlement on upper- (\square) and under- (\blacksquare)	
horizontal surfaces of experimental collectors (all 4 collectors	

pooled) in two positional orientations ("Face down" and "Face up") in Densu Estuary and Narkwa Lagoon.

xvi

- 4.4: Mean (±S.E.) monthly spatfall (spat m⁻²) at stations (ST) in the Densu Estuary, Narkwa Lagoon, Benya Lagoon, and Whin Estuary pooled from all collectors and different depths.
- 4.5: Mean (±S.E.) spatfall of *Crassostrea tulipa* at different depths (Top, Mid [middle] and Bot [bottom]) in water column at stations 1, 2 and 3 in the selected coastal water bodies.
 72
- 4.6: Pooled size (shell height) frequency distribution of one month old *Crassostrea tulipa* spat harvested monthly over 12 months (November 2017 October 2018) from the Densu Delta (D), Narkwa Lagoon (N), Benya Lagoon (B) and Whin Estuary (W). *Maximum observed size class; low frequency.
- 4.7: Mean (± S.E.) monthly shell heights of *Crassostrea tulipa* spat collected at the different stations (ST1, 2 and 3) of the four water bodies.
- 4.8: Mean (\pm S.E.) shell heights of *Crassostrea tulipa* spat cultured for 6 months at different depths (Top, Mid = middle, Bot = bottom) and in the different water bodies. Groups that do not share an alphabet are significantly different (ANOVA, Tukey's test: *P* < 0.05; see Appendix B10).
- 4.9: Monthly mean (± SE) shell heights of *Crassostrea tulipa* cultured on the different collectors over 9 months at the Densu Delta, Narkwa Lagoon and Whin Estuary (see ANOVA results in Appendix B10).

81

78

4.10: Biofoulers and mud associated with <i>Crassostrea tulipa</i> spat	
collection and culture: Serpulid worms (A) Ficopomatus spp. and	
(B) Spirobis sp, (C) Fistubalanus pallidus, (D) mud,	
(E) Chaetomorpha antennina, (F) Chondria bernardii, and	
(G) small-sized crab (<15 mm). Photographs were captured during	
field sampling for this study.	83

- 4.11: Biofoulers and mud associated with *Crassostrea tulipa* spat collection and culture: (H) *Armases* sp, (I) *Sesarma* sp., (J) *Littorina angulifera*, (K) sea anemones, (L) *Brachidontes* sp.,
 (M) *Membranipora arborescens*, and (N) ascidians. Photographs were captured during field sampling for this study.
- 4.12: Monthly mean (± SE) values for hydrographic parameters in the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary from November 2017 to October 2018.
- 4.13: Map showing depth profile at low tide (June 2018), oyster presence and availability of *Crassostrea tulipa* spat in the estuarine section of Densu Delta.93
- 4.14: Map showing depth profile at low tide (June 2018), oyster presenceand availability of *Crassostrea tulipa* spat in the Narkwa Lagoon.94
- 4.15: Map showing depth profile at low tide (June 2018), oyster presenceand availability of *Crassostrea tulipa* spat in the Benya Lagoon.95
- 4.16: Map showing depth profile at low tide (June 2018), oyster presenceand availability of *Crassostrea tulipa* spat in the Whin Estuary.96

5.1: Typical occurrence of spat on upper- (left) and under- (right)

horizontal surfaces of the same collector (ceramic tile). Photograph captured by researcher during field sampling. 101

LIST OF TABLES

Table	Page
2.1- Oyster Spat Collectors Used by Previous Workers	39
4.1 - Spat Settlement m ⁻² (mean \pm S.E., minimum, maximum) in the Dry	
and Wet Seasons in the Different Water Bodies	71
$4.2 - Mean (\pm SE)$, Minimum and Maximum Shell Heights of One-Mont	ħ
Old Crassosrea tulipa Spat at Different Depths in Water	75
4.3 – Overall and Monthly Absolute Growth Rates (AGR) of Crassostree	a
tulipa Cultured in Three Water Bodies	79
4.4 – Monthly Occurrence of Fouling and Epibiotic Organisms Associate	ed
with Crassostrea tulipa Spat Collection and Culture at the Differen	nt
Stations in the Four Water Bodies	85
4.5 – Coefficients and Contributions of the Linear Regression Model for	
Spatfall and Growth (Shell Height) (Factors) and the Measured	
Hydrographic Parameters (Predictors)	90

XX

CHAPTER ONE

INTRODUCTION

Fish and fisheries resources are important elements of good nutrition and health providing 60 % of Ghana's animal protein (Yankson, 2004; MoFAD, 2015). Unfortunately, the marine small pelagic stocks which form the greatest part of fish consumed are near collapse in Ghana (Lazar et al., 2018), and this calls for strengthening aquaculture as a means of bridging the widening fish demand gap. At present, tilapia culture constitutes about 95 % of total aquaculture production in Ghana (FAO, 2018a). Further focus of Ghana's aquaculture must therefore shift to diversifying the culture species to create a resilient aquaculture (fish production) system against catastrophes that characterize the culture of single species. The mangrove oyster, Crassostrea tulipa Lamarck 1819, provides a good opportunity for diversifying Ghana's aquaculture sector; it is abundant in many coastal ecosystems and serves as food for many and source of income for women in communities along the coast of Ghana. However, its fishery remains unregulated and its large-scale culture has not been attempted due to the paucity of relevant scientific information. This study, therefore, sought to provide relevant scientific data to support mass production and conservation of the species in Ghana.

1.1 Background to the study

As the global population keeps increasing, the ability to provide and equitably distribute good nutrition to all across the globe has become a major issue. Important among nutritional requirements is protein, which is essential for an

active and healthy life. The FAO in 2014, reported the role of fish and other aquatic animals in global nutrition and food security as providing low-cost animal protein, healthy fats and other micronutrients to people. Currently, a large number of people, estimated at about 56.6 million (FAO, 2016a) are involved primarily in the fisheries and aquaculture sector globally. In Ghana, as indicated earlier, the majority depend on fish and fish products for food; hence, fish serves as a primary source of animal protein with a significant proportion coming from the capture fishery (FAO, 2018b).

Ghana's coastal zone is noted to house about twenty percent of the country's 25 million population (Ghana Statistical Service, 2013) whose livelihood depends largely on fish and fisheries resources. However, fish catches continue to decline due to increasing fishing effort leading to high exploitation rates. Global climate change has also been identified as an additional stressor (Allison et al., 2009; Badjeck, Mendo, Wolff & Lange, 2009; Merino et al., 2012). The majority of fisheries resources are also "open access", and not properly regulated. This has led to a worrying situation of overcapacity, which is a major reason for much of the overfishing being recorded presently in some important fish species in Ghana, for example, the *Sardinella* spp (Lazar et al., 2017).

Whilst capture fisheries are declining, aquaculture is on the ascendency (FAO, 2018b). Aquaculture demonstrates the potential of producing enough fish to compensate for the shortfalls arising from wild capture fisheries. In fact, for the first time more fish was produced globally for human consumption from aquaculture (50.4%) than capture fisheries in the year 2014 (FAO, 2016a) and 53% production for same in 2016 (FAO, 2018b). Likewise, over the last few

years, aquaculture in Ghana has been reported to experience a continuous significant increase in production from 27,450 tonnes in 2012, to 32,513 tonnes in 2013, and further to 38,545 tonnes in 2014 (FAO, 2016b). Production in 2015 saw a further increase of about 10% from that of the previous year, yielding up to 44,610 tonnes (FAO, 2016b). This production represents about 11% of total fish produced in-country by marine and inland capture fisheries and aquaculture, and 4.5% of fish currently consumed nationally. Nonetheless, a perpetual 600,000 tonnes deficit in demand and supply of fish is compensated for by imports annually.

The promising outlook of Ghana's aquaculture sector and the huge potential for growth places the country in a strategic advantage to realign policy directions in order to harness maximum benefit from the sector. However, there are some challenges that may impede the rapid expansion of the enterprise. Low survival rates from disease and infections, limited culture species and technology, insufficient seed production, a polarized distribution of the culture operations, coupled with inadequate skilled personnel to deal with the challenges, hinder optimum yield through aquaculture at affordable costs for many people. The aquaculture enterprise in Ghana has been dominated by the culture of the Nile tilapia (*Oreochromis niloticus*), with some other species such as the African sharptooth catfish (*Clarias gariepinus*), the African bonytongue (*Heterotis niloticus*) and other tilapia species produced on a small scale.

In spite of the immense contribution aquaculture is making towards food security and national development in Ghana, its distribution across the country can only be described as being "landlocked" since it is concentrated inland on

rivers, streams and lakes especially on the Volta Lake, and dugout ponds but not popular along the stretch of Ghana's coastal zone. The enterprise is highly profitable and could be a source of livelihood and income for coastal dwellers. It could impact positively on sustainable exploitation of marine fisheries resources by reducing fishing pressure and overcapacity.

Aquaculture in Ghana, therefore, needs to be sustainably promoted by making the practice popular in the coastal areas *vis-à-vis* diversification of cultured species to include coastal species of economic value and culture potential. This will offer coastal dwellers the opportunity for a supplementary, if not an alternative to their seeming sole livelihood of fishing from the sea.

It is noteworthy in this regard, that there are several other food fish species in the coastal areas of Ghana including mullets (Blay, 1995; Dankwa, Blay & Yankson, 2005), shad (Blay & Eyeson, 1982), clams (Adjei-Boateng, Agbo, Agbeko, Obirikorang & Amisah, 2012; Obirikorang, Madkour, Amisah & Otchere, 2013), and cockles (Yankson, 2004). These are not cultured for use as food but collected from the wild largely for local consumption. The West African Mangrove Oyster (*Crassostrea tulipa*) is one of such species. It is an important bivalve seafood for Ghanaians (Obodai, Nyarko & Amponsah, 2010); the meat, like that of other bivalves, is of high nutritional value thus rich in proteins (Salaskar & Nayak, 2011) and other food nutrients for example vitamins, minerals and fatty acids (especially Ω -3 fatty acids), which are recommended for consumption among pregnant and lactating women (Reames, 2012). Oyster shells also have a wide array of uses in the building industry, agriculture and medicine (Yankson, 2004), making them economically very

important. That notwithstanding, only a few coastal communities endowed with natural populations of oysters are involved in active harvesting and trade in it.

Over the past years, quite significant exploitation of the species has been identified on a small scale primarily in three coastal communities namely, Elmina and Ekumfi Narkwa in the Central Region, and Tsokome in the Greater Accra Region. In addition to the three, the Whin Estuary has been noted to have an appreciable oyster harvesting activity due to the presence of oysters both on the roots of mangrove vegetation fringing the borders and a few on the sandy-mud bottom of the estuary. Women and children form the majority of those usually involved in harvesting oysters from either mangrove roots or oyster beds in shallow coastal intertidal areas, especially during low tides. Harvesting is usually done by handpicking when exposed at low tides. An improvised foot protection gear, usually old clothing such as trouser trunks are tied to the feet for protection during under-water harvesting at high water levels. Methods being used for collecting *C. tulipa* in Ghana at present may be laborious but exploitation remains unsustainable, as the fishery is open and unregulated by any specific legislation.

1.2 Statement of the Problem

Harvesting of oysters from some lagoons and estuaries in Ghana's coastal areas along the Gulf of Guinea is often challenged with fluctuating yields attributed mainly to the different seasons, the wet and dry seasons. During the wet season, oyster fishers have reported low yield whereas periods after the rains have seen increased yields. The trend is however highly speculative at present and this

impedes exploitation to the full potential of the species for subsistence and its potential large-scale aquaculture.

For successful aquaculture of the mangrove oyster, sustainability measures, which may require investment commitments into conservation and rearing of the species, need be considered. Irrespective of the approach, there will be a heavy reliance on spat from the wild and from hatcheries or laboratories. According to Kennedy & Roberts (2006), adequate data on the seasonal history of spatfall and patterns of settlement are important as the presence and distribution of planktonic oyster larvae at various sites around coastal ecosystems serve as a precursor to studies of spatfall patterns. However, the availability of such relevant data is currently limiting for *C. tulipa*. Hence, the apparent absence of any practical attempt to culture the species on a large scale in Ghana, in addition to technological limitations. Yankson (1990) reared *Crassostrea tulipa* from fertilized eggs through to settled spat in laboratory cultures and Obodai (2010) reported of the embryonic development by *in vitro* fertilisation, yet the information generated has been insufficient for the desired transformation.

Without the necessary hatchery and laboratory facilities for producing oyster spat for culturing purposes, as is the case in Ghana, the obvious option is to rely on natural supplies of seed from the wild. It is for this reason that marine bivalves are widely cultured using the extensive system, otherwise referred to as open-water culture. Further, there is established evidence of myriad factors that influence the setting of spat in the natural ecosystem in various parts of the world. Food supply to larvae of the European oyster (*Ostrea edulis* L.) and the

Pacific oyster (*Crassostrea gigas* Thunberg) prior to and during spatfall directly affected setting success (Laing, 1995). Climate variation on juvenile abundance (Kimmel & Newell, 2007), structure, texture of the substrate type and seasons (Monteforte, Kappelman & Lopez, 1995), reproductive activity of the oysters (O'Connor & Lawler, 2004) are examples of such factors. In view of this, Mann (1983) prescribes that for successful extensive aquaculture of marine bivalves, proper methods for obtaining seed are required. Initial tests are essential to provide information about the spatial and temporal distribution of the seed to be collected. In addition, the depths at which optimum spatfall occurs, and the materials to use as collectors in order to enhance spat settlement (Cendejas, Carvallo & Juarez, 1985) are essential information gaps that first need to be addressed. These sets of information may form a solid basis for informed management decisions and the development of appropriate technology for large-scale oyster production.

Although some attempts were made, dating nearly two decades ago (Obodai, Yankson & Blay, 1991) or even close to half a century (Yankson, 1974) to provide information on the seasonality of *C. tulipa* spatfall and spat collectors, there still remains critical questions unanswered as to where are the best places and times of the year for spat collection, as well as the type of material to be used for optimising spat collection. In addition, considering the time elapsed, available information may be obsolete with emerging global climate trends and impacts. Thus, there is the need for reassessment to furnish current information to support the potential aquaculture and local oyster fishery.

1.3 Purpose of the Study

C. tulipa trade is a major livelihood option for women, who usually ply the trade with their children in coastal communities in Ghana. The study, therefore, sought to document strategies for optimizing the availability of *C. tulipa* spat within four important coastal aquatic systems namely the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary. This is an attempt to promote oyster culture in the coastal areas of Ghana, by providing relevant scientific data on the most efficient collectors, spatfall regimes (spatial and temporal), growth of cultured oysters and associated fouling organisms. The outcome of the study is expected to be beneficial to prospective oyster farmers, specifically in identifying which periods of the year to invest resources for seed collection for culturing. The study is also expected to inform policy formulation for the management of oyster fisheries in Ghana. It is anticipated that the outcome will motivate private investors to venture into the commercial production of the species.

1.4 Research Objectives

This study, therefore, sought to identify strategies for optimising spat collection and growth of *Crassostrea tulipa* for its mass production in coastal water bodies in Ghana. It focuses on two lagoons, one delta and one estuary to identify efficient materials to be used as spat collectors, locations and periods within which to collect oyster spat in large quantities for use by prospective *C. tulipa* farmers. The specific objectives were to:

- i. Determine the effectiveness of collectors prepared from locally available materials for collecting *C. tulipa* spat,
- ii. Assess the influence of collector orientation on *C. tulipa* spatfall,
- iii. Estimate the spatial and temporal distribution and abundance of *C*. *tulipa spat*,
- iv. Determine the growth of *C. tulipa* spat cultured on selected collector materials and at different depths,
- v. Assess some physical and chemical hydrographic conditions of the coastal water bodies and their possible effects on *C. tulipa* spatfall regimes and growth,
- vi. Identify fouling organisms and determine their seasonal and spatial occurrences, and
- vii. Map out suitable oyster spat collection areas and bathymetry of the selected water bodies.

The following hypotheses were therefore tested based on the set objectives:

- 1. H₀ The type of collector material does not influence *C. tulipa* spatfall
 - H₁ The type of collector material influences C. tulipa spatfall
- 2. H₀ There is no difference in *C. tulipa* spat settlement on the upper and under-horizontal surfaces of spat collectors

- H₁ There is a difference in *C. tulipa* spat settlement on the upper and underhorizontal surfaces of spat collectors
- 3. H₀ There are no spatial (among and within water bodies) and temporal variations in *C. tulipa* spatfall
 - H₁ There are spatial (among and within water bodies) and temporal variations in *C. tulipa* spatfall
- H₀ Growth of *C. tulipa* spat does not vary on the different collectors and at different depths in water column
 - H₁ Growth of *C. tulipa* spat varies on the different collectors and at different depths in water column
- 5. H₀ Sizes of *C. tulipa* spat harvested monthly do not vary seasonally
 - H₁ Sizes of *C. tulipa* spat harvested monthly vary seasonally
- 6. H₀ Hydrographic conditions do not influence *C. tulipa* spatfall and growth
 - H₁ Hydrographic conditions influence C. tulipa spatfall and growth
- 7. H₀ There are no seasonal and spatial variations in the abundance of fouling organisms
 - H₁ There are seasonal and spatial variations in the abundance of fouling organisms

1.5 Significance of the Study

Eradicating poverty and hunger is of global concern, underscored by the United Nations (UN) Sustainable Development Goals (SDGs). Within the context of the developing nations e.g. Ghana, the significance of this study is deeply

rooted in the values of sustainable development and social and economic empowerment, especially of women and children. The study further encapsulates the potential to enhance the nutrition and health of coastal inhabitants, who are generally poor and malnourished.

Due to its importance to national development, the study has attracted the attention of Ghana's Ministry of Fisheries and Aquaculture Development (MoFAD), visiting one of the field experimental sites, Narkwa, during a tour to assess viable sites for marine/coastal aquaculture in Ghana. The findings of the study will be useful to MoFAD and for the training of traditional oyster collectors within the communities where the study was conducted and the lessons transferred to other coastal communities with wild, exploitable oyster populations.

1.6 Delimitations of the Study

In Ghana, oysters do not thrive only in the four coastal ecosystems considered under this study. However, these systems were selected for two reasons: first to fit in the regions of interest of the funds provided for the research; and secondly because initial survey revealed these four to be relatively more important due to evidence of thriving oyster fishery in the respective communities.

Although the literature presents a vivid account on several materials for collecting oyster spat, it was practically impossible to assess all of them, thus, the study was restricted to five collectors initially. These were chosen based on which materials were readily available and easily obtainable for mass production in Ghana. However, the study ended with four out of the initial five

collectors as one of the collector materials, the fine mesh netting, was excluded after three months of no spat harvest. The possibility could have been to change the technique for installing the net. However, for the purpose of comparison among collectors, it was inappropriate to alter the technique, hence the fine mesh netting was eliminated, with the conclusion that it is not suitable per the experimental design.

Sampling for physicochemical parameters of the water was done in triplicates within the water column at the respective stations without considering vertical stratification because the selected ecosystems are generally shallow. With some stations completely exposed at low tide, it was assumed that there would be negligible differences in hydrographic conditions from top to bottom.

1.7 Limitations of the Study

In Narkwa Lagoon, heavy rains destroyed and drifted the experimental rack at Station 2 in June 2018, probably into the sea, as it was never retrieved. It is noteworthy that although a security person was employed to keep watch of the setups, it was beyond his ability to salvage the situation, amidst the heavy flooding and rapid rush of water. Theft ended the culture experiment after six months of study, also in the Narkwa Lagoon. Destruction of experimental setups at Densu Delta prevented successful spat collection at Station 3. A similar event at the Beyna Lagoon prevented culture experiment in the lagoon. Although these incidences are worth mentioning as uncontrollable limiting factors to the study, it is important to note that their influence is only on the scale of the research without any influence on the scientific inferences made from the study. They only underscore the complex nature of such experiments conducted in open aquatic systems where natural harsh conditions and disasters are bound to happen.

1.8 Definition of Terms

Aquaculture – rearing of aquatic animals and plants, for food and other economic purposes

Bathymetry – measurements of depth in aquatic systems

Cultch – any material, in the natural environment or deliberately deployed and used as substrates for settlement by oysters

Culture – maintain under conditions suitable for growth

Epibiotic – living on the surface of plants or living animals usually parasitically

Fouling organism – undesired organism living and/or growing on culture facilities

Geotaxis – the motion of a motile organism or cell in response to the force of gravity

Phototaxis – the bodily movement of a motile organism in response to light, either towards the source of light (positive phototaxis) or away from it (negative phototaxis)

Spat – newly settled oyster larva

Spatfall (settlement/setting) – attachment of the larvae of bivalves (such as oysters or mussels) to a substrate

Xenomorphism – having the shape (e.g. of oysters) determined by contours of the substrate

1.9 Organisation of the Study

There are six chapters in this thesis. Chapter 1 introduces the whole concept of the study, giving a background and stating the problem, purpose, the objectives and significance of the study.

Chapter 2 reviews literature relevant to the study into detail. In-depth review of the literature on oyster spat collectors used around the world is presented. In Chapter 3, the methods employed for the research are described, using diagrams where necessary. Study areas are well-described and statistical tools and applications used to analyse the data collected are explained.

Chapters 4 and 5 present results and discussion, respectively. Results obtained from the research are presented in graphs, maps, charts and tables with brief descriptions in Chapter 4. Detailed analysis of results and inferences are drawn in Chapter 5, in the form of a discussion structured under the main themes of the study. Conclusions and recommendations are given in Chapter 6. Other sections presented in this thesis include a list of references and appendices.

1.10 Chapter Summary

The underlining rational for the study has been presented in this chapter, providing justification as well as benefits to be derived by coastal communities from large-scale oyster culture. The objectives have been outlined and terms have been defined to guide readers.

CHAPTER TWO LITERATURE REVIEW

This chapter reviews the literature relevant to the study. Issues pertaining to Ghana's aquaculture and its status along the coast, biology and ecology of oysters, and an overview of the global status of oyster fisheries are examined. An in-depth review of available materials used for collecting oyster spat around the world is presented.

2.1 Aquaculture Production and Development in Ghana

Aquaculture in Ghana is largely practised in freshwater systems. In the past, aquaculture was practised mainly as subsistence small-scale extensive farming in earthen ponds. Presently there are intensive practices such as commercial cage farming on the Volta Lake in addition to large ponds and raceways adopted by some farms recently. Species reared include the African bonytongue (*Heterotis niloticus*), Nile tilapia (*Oreochromis niloticus*) and the North African catfish (*Clarias gariepinus*). Culture of the giant tiger prawn has also commenced. For many years, the monosex culture of Nile tilapia (*Oreochromis niloticus*) has been the dominant culture practice in Ghana. Tilapia fingerlings are produced in concrete tanks and hapas, whilst grow-out is practised in cages and ponds.

State institutions, both government and research – Ministry of Fisheries and Aquaculture Development (MoFAD), Fisheries Commission (FC), Council for Scientific and Industrial Research (CSIR) – have contributed immensely to the
advancement of aquaculture through a number of interventions. Research and adoption of cage technology around the mid-2000s and the development of the local strain of Nile tilapia (Akosombo Strain) are cited as the turning point of the aquaculture industry in Ghana (S. Agyarkwa, personal communication, October 18, 2016). The development of the Akosombo strain of the Nile tilapia has been supported by the WorldFish and the Food and Agriculture Organisation (FAO) of the United Nations, through a selective breeding programme at the Water Research Institute's Aquaculture Research and Development Centre of CSIR. These interventions may have encouraged private sector investment into the large-scale production of tilapia. As a result, a tremendous fourteen times growth has been recorded in Ghana's total aquaculture production (Figure 2.1) in a decade from an annual production of about 3,820 metric tonnes in 2007 to an estimated volume of 52,480 metric tonnes in 2016 (FAO, 2018a).



Figure 2.1: Aquaculture Production in Ghana from 1990 to 2016 Data set extracted from (FAO, 2018a).

It is noted over the same period that almost all of Ghana's cultured aquatic resources focuses on tilapia production. This is evident in the aquaculture production data having the contribution of Nile tilapia averaging 95 % from 2007 to 2016. The aquaculture sector continues to receive significant attention from the Government of Ghana with recent interventions promoting aquaculture entrepreneurship through training with funding from the Skills Development Fund (SDF) and the Youth Employment Agency (YEA).

2.1.1 Threats to Ghana's aquaculture

In spite of the gains made in the aquaculture sector of Ghana, there exist a number of constraints limiting the desired growth of the sector. These include lack of access to start-up funds, high cost of fish/aqua feed, lack of technology for highly intensive aquaculture, inadequate extension services, and limited number of skilled professionals to address the challenges posed by the changing global climatic conditions to aquaculture in the country. Due to this, issues pertaining to the causes of mass mortalities on fish farms, as reported by Graphic Online (2018), are not fully understood.

It is noteworthy that the overdependence of Ghana's aquaculture sector on the production of *O. niloticus* is the single most dangerous threat to the sector especially in the wake of the deadly Tilapia Lake Virus (TiLV), which has been confirmed by FAO to be present in some countries in Asia, Africa and Latin America. In their Special Alert release on 26th May 2017 (FAO, 2017), the significance of threat posed by the virus to tilapia farming at the global level is emphasised. This is further deepened by the ban on the importation of "all

ornamental fishes and tilapia species (live and dead) including gametes-eggs and milt into the country effective from July 1, to December 31, 2018," issued in a press release by the Ministry of Fisheries and Aquaculture Development (MoFAD) on 18th June, 2018.

2.1.2 Ghana's near-zero marine/coastal aquaculture (mariculture) sector

As mentioned earlier in the introductory section (Section 1.1), the distribution of aquaculture in Ghana is best to be described as being "landlocked" since it is concentrated heavily inland on rivers, streams and lakes especially on the Volta Lake, and dugout ponds. Until recently, aquaculture in Ghana's coastal zone was literally non-existent. At present, there is only one known production site for Giant tiger prawn (marine species) in the Volta Region. According to the FAO (2018), only 10 mt of the shellfish species was produced out of the estimated 52,480 metric tonnes of fish produced by culture in 2016. This represents 0.02 % of total aquaculture production in Ghana in 2016.

Although in Ghana, there are important shellfish species mainly, bivalves (e.g. clams, cockles and oysters), that have the potential of being reared on commercial scale, no such industry has yet been realised. Culture attempts have been made but only at the level of research. Yankson (1990) and Obodai, Yankson & Blay (2007) successfully performed artificial fertilization of the oyster *C. tulipa* in laboratory cultures. Yankson (1990) further reared the oyster larvae to settle as spat while the latter researchers provided a detailed description of the embryonic development of the fertilised eggs.

Many shellfish species are found in Ghana's marine and coastal waters. Cephalopods (cuttlefish, octopus and squid), bivalves (oysters, clams, mussels and cockles), and crustaceans (shrimps, prawns, lobsters, and crayfish) are examples. Popular among bivalve species of economic potential in Ghana which have attracted the attention of researchers are the Volta clam, *Galatea paradoxa* (Amisah, Adjei-Boateng, Obirikorang & Quagrainie, 2009; Obirikorang, Adjei-Boateng & Amisah, 2009; Adjei-Boateng et al., 2012; Adjei-Boateng & Wilson, 2012; Obirikorang et al., 2013), the West African bloody cockle, *Anadara senilis* (Yankson, 1982), the mangrove oyster *Crassostrea tulipa* (Plahar & Obodai, 1989; Obodai et al., 1991; Yankson, 1996; Asare, 2017), the brown mussel, *Perna perna* (Krampah, Yankson & Blay, 2016) and the freshwater oyster, *Etheria elliptica* (Ampofo-Yeboah & Owusu-Frimpong, 2014).

2.2 Global Outlook on the Fisheries and Culture of Oysters

Oysters have been fished from coastal intertidal zones in many parts of the world mainly for food (Yankson, 2004; Satia, 2011; Lucas & Southgate, 2012; Adite, Abou, Sossoukpê & Fiogbé, 2013; Asare, 2017) and pearls found in some species (Southgate & Lucas, 2008). Dried oyster, clam and mussel products have been commonly recommended and consumed in Asia over many centuries (Wen et al., 2018).

Eagling, Ashton, and Eagle (2015) give an account of fishing of *Ostrea edulis* in Scotland. There are nine oyster-harvesting communities within the Tanbi Wetlands National Park in the Republic of the Gambia. The collection, processing and marketing of mangrove oysters (*Crassostrea gasar/tulipa*)

constitute economic activities by predominantly women operating in individual family units within their respective communities (MOFWRNAM, 2012). Rice (2011) reports of experimental wild spat collection in the Gambia, and the periodic and sequential appearance of associated fouling organisms, that could potentially serve as competitors for space and/or available food.

Before the development of cultured pearl oyster farming in Japan coupled with the discovery of oil in the 1900's, pearls from the oysters Pinctada spp. were a valuable trade commodity and earner of foreign exchange for such states in the Gulf region as the Kingdom of Bahrain (G. Chemali, personal communication, June 23, 2018), and Omar and Qatar (Carter, 2005). In Qatar, oysters were fished intensely for pearls from the late 1700s up until the early 1900s (Smyth, Al-Maslamani, Chatting & Giraldes, 2016). High rates of exploitation of pearl ovsters may have contributed to the decline of the pearl industry. A recent study in Qatar reports of collapsed oyster banks that were once an extremely productive economic resource, due to intense overfishing, extreme environmental conditions and anthropogenic impacts (Eagling et al., 2015). Concerns about the over-exploitation of the oyster beds could be traced back to 1770, when a pearl trader, Justamond described the beds as overfished and exhausted (Carter, 2005). In the same vein, the intensity of the fishing was estimated by Captain Durand of the East India Trading Company in 1878 at over 4000 pearling boats, working the oyster banks of the Gulf (Carter, 2005).

However, the absence of regulation and ownership are believed to have promoted irresponsible fishing (Eagling et al., 2015) as fishers appear to have unlimited fishing rights and rationally, fishermen will fish as much and as

quickly as they can until doing so is no longer profitable. For sustained shellfish production, it has become more important to venture into farming. The practice does not only produce more food for local communities but also helps in the conservation of oyster populations in oyster beds, mangrove areas and other favourable habitats. All of the cultivated molluscs, apart from a small group of gastropods (haliotids or abalones), are bivalves and include the mussels, scallops, oysters, clams and cockles (Spencer, 2002). Obtaining larvae, either from the wild or produced in the hatchery is a crucial stage in the rearing of oysters (Yankson, 1990; Friedman, Bell & Tiroba, 1998a; Southgate, Beer & Ngaluafe, 2016; Legat, Puchnick-Legat, Gomes, Sühnel & Melo, 2017).

Different species of oysters have been farmed at various levels and production scales, using varying methods in many of these countries. Velasco and Barros (2010) performed suspended culture experiments of the Atlantic pearl oyster, *Pinctada imbricata*, in the Colombian Caribbean using pearl nets and pocket nets as holding facilities. The Pacific oyster, *Crassostrea gigas*, which is well known throughout the world because of its ability to adapt to a wide range of environmental conditions, was introduced for cultivation into France on a massive scale in the 1970s (Cognie, Haure & Barillé, 2006).

2.3 The Oyster Fishery in Ghana

2.3.1 Exploitation and consumption

A number of coastal communities are involved in the harvesting of wild oysters on an appreciable scale from lagoons and estuaries. The oysters are harvested from the stilt roots of mangroves and sandy-mud substratum or rocks at the

bottom of these water bodies during low tides. Women and children form the majority of the oyster harvesters. Asare (2017) reports oyster fishing and trading as the second major economic activity within the Narkwa community, where oysters could be available all year round but with low catches from April to August in 2016.

Although there is no organised oyster culture in Ghana, there are about 27 markets where oysters are sold as well as 54 waterbodies found to be suitable for oyster culture (Yankson, 2004). Presently, there are some efforts being made by a group of oyster fishers to rear the species in estuarine areas of the Densu River Delta, near Tsokomey, Accra. There are no traditional preservation methods to extend the shelf life of oyster meat (Asare, 2017) implying all quantities harvested are readily consumed by processing into kebab, cooking in stew or frying (Yankson, 2004; Asare, 2017). Although oyster harvesting in Ghana is virtually on a small scale, it remains unsustainable, as the fishery is open and unregulated by any specific legislation.

2.3.2 Culture prospects of the West African mangrove oyster

The suitability of the mangrove oyster for aquaculture within the coastal marine environment has been investigated by some Ghanaian researchers (Obodai, 1990; Yankson, 1990; Obodai et al., 1991; Yankson, 1996; Asare, 2017). That notwithstanding, it is clear that a lot more investigation needs to be conducted to provide further information to form the basis for financial investment into the mariculture of the species. Information on its general biology regarding feeding ecology, filtration rates (Sutton, Yankson & Wubah, 2012), breeding and reproduction (Obodai et al., 1991; Yankson, 1996) and nutritional value

(Yankson et al., 1989) within Ghana's coastal ecosystems are available. Larvae produced with cryopreserved spermatozoa have been successfully reared (Yankson & Moyse, 1991) providing hope for possible year-round hatchery production of spat to support a viable large scale oyster culture in this country.

Trials of bottom and suspended culture methods and stocking densities have been successfully conducted by Obodai (2000). Studies by Otchere (2003) and Essumang (2010) showed levels of heavy metals in oysters from many lagoons in Ghana to be within acceptable limits. In addition, the meat could be a year round rich and cheap source of calcium, iron and phosphorus (Yankson et al., 1989; Obodai et al., 1991).

2.4 Bivalve Molluscs

One of the most important, diverse and largest groups of organisms in the animal kingdom is the phylum Mollusca, having at least 50,000 species described and about 200,000 living species, largely marine (Gosling, 2015). Cladistics organises molluscs into eight classes (Figure 2.2), mostly based on analysis of morphological characters in extant and fossil taxa (Gosling, 2015). Species of the phylum Mollusca and belonging to the class Bivalvia are known as bivalve molluscs or bivalves for short. Bivalves are the second largest class within the Mollusca. These organisms are typically soft-bodied invertebrates and have two separate valves of the shell secreted by two mantle lobes held together by a hinge at the dorsal end (Gosling, 2015).

2.4.1 Biology and ecology of bivalves: Oysters in focus

Bivalves generally have a shell, which is in two parts, the left and right valves, secreted by the outer fold of the mantle edge and held together by an adductor muscle. The mantle also has a muscular inner fold that largely controls the flow



Figure 2.2: Molluscan phylogenetic tree from transcriptome and genome data for all major lineages, highlighting bivalvia in rectangle. Adapted and modified from (Gosling, 2015). Monoplacophora insert after (Saleuddin & Mukai, 2017).

of water entering and leaving the mantle cavity generated by the cilia on the gills (Spencer, 2002). The shell grows in circumference by the addition of material from the edge of the mantle and grows in thickness by deposition from the general mantle surface. Calcium for shell growth is obtained from the diet or taken up from seawater (Gosling, 2015). Although there is a general notion that the concentric markings can be used to determine the age of oysters, this

method may not be accurate. Kraeuter, Ford & Cummings (2007) suggested the method could not be used and proposed sectioning of the hinge plate for an accurate estimate. Respiration and feeding by bivalves are achieved by syphoning large volumes of seawater, filtered through the gills (Leavitt, 2010). Lateral cilia are set along the sides of the gill filaments, which are responsible for drawing water into the mantle cavity and passing it through the ostia, and then upwards to the exhalant chamber and onwards to the exhalant opening (Gosling, 2015).

In the genus *Crassostrea*, the sexes are separate (Quayle & Newkirk, 1989). The female gonad or ovary produces eggs (ova). The male gonad or testis produces spermatozoa. In both cases, the gonadal products (spermatozoa and eggs i.e. gametes) are discharged externally into the open water, where fertilization takes place. The genital pores, through which the gametes are discharged, exit into the exhalent chamber above the gills (Spencer, 2002).

2.4.2 The Mangrove Oysters

Mangrove oysters have drawn a great deal of attention due to the growing biological and commercial interest as well as the wide distribution of the species. Three species of mangrove oysters, *Crassostrea rhizophorae*, *C. brasiliana*, and *C. gasar* (see Yankson [1988] for comments on nomenclature), have been recorded along the Atlantic shores of Africa and South America as important for aquaculture (Ajana, 1980; Lapegue, Boutet, Leitao, Heurtebise & Cia, 2002).

2.4.3 Distribution of mangrove oysters

C. rhizophorae attaches in the intertidal zone, either to mangrove (*Rhizophora mangle*) roots or to rocks in the intertidal zone whilst *C. brasiliana* would normally attach to rocks in the subtidal zone. In Nigeria, *C. gasar* (cf. *tulipa*) favours the subtidal zone, although it may occur a little above the level of low tide in the dry season (Ajana, 1980), also on mangrove roots, but occasionally on rocks.

In their work investigating the trans-Atlantic distribution of mangrove oysters, Lapegue et al. (2002) sampled 15 populations from South America and the western coast of Africa. DNA and karyological analysis identified two haplotypes. One haplotype, representing C. gasar (cf. tulipa), was observed in both locations, whereas the other, representing C. rhizophorae, was only observed in South America, specifically, in Brazil. In addition to the two species, C. brasiliana was already known to be present along the coasts of South America. Shipping between South America and the west coast of Africa may have transferred C. gasar (cf. tulipa) from one coast to the other (Lapegue et al., 2002). Natural transport may also be responsible for the present geographic range of C. gasar (cf. tulipa). Larval dispersal might have been possible, even over such a long distance (at least 3000 km), because the larval stage of most *Crassostrea* species lasts about 3 weeks. The predominant surface circulation patterns in this part of the Atlantic Ocean could promote C. gasar (cf. tulipa) transport from Africa to America (Lapegue et al., 2002). However, Amaral & Simone (2014) dispute the presence of C. gasar (cf. tulipa) in Brazil, an assertion that raises further uncertainty about the distribution of the species.

2.4.4 Crassostrea tulipa (Lamarck, 1819)

Like other oyster species, *C. tulipa* is a filter feeder, hence relies on natural primary productivity of phytoplankton within their habitat for nutrition (Adite, Sonon & Gbedjissi, 2013). This biological characteristic reduces the cost of production of the species in terms of feed cost because there is virtually no investment cost in this regard; as supplementary feeding is not required. Maximum average filtration rates are achievable in culture tanks at 100% and 80% seawater, that is, approximately 34‰ and 27‰ respectively (Sutton et al., 2012).

Good nutrition and favourable environmental conditions stimulate reproduction in mature oysters. Gametes of both sexes are released into the open water column where fertilisation occurs randomly when male and female gametes meet by chance. A male-female sex ratio approximated at 1:1 is reported for the species (Yankson, 1996). For culture purposes, the addition of glycine to the cryoprotective diluent was found to enhance the overall viability of the spermatozoa of *C. tulipa* without influence on larval vigour. In addition, 15-day reared larvae from cryopreserved spermatozoa did not exhibit latent freezeinduced injury (Yankson & Moyse, 1991). This could be useful in hatchery rearing of *C. tulipa*. According to Obodai et al. (1991), *C. tulipa* bred continuously in the Benya lagoon while the reproductive activity was seasonal in the River Pra estuary where the non-breeding season coincided with the period of low transparency and salinity. However, there was an apparent lack of direct relationship between individual hydrographic factors and the breeding of oysters in the lagoon (Obodai et al., 1991).

Mean growth of 5.4 cm shell height was reported for *C. tulipa* after 12 months of culture in Benin (Adite et al., 2013a). The species is naturally well adapted to the rigorous environmental conditions of coastal intertidal areas, surviving wide temperature ranges 20-30 °C (Ajana, 1980), 24 - 31.5 °C and 27- 36 °C (Obodai et al., 1991). Both lagoon and estuarine oysters maintained moderate to high condition for most of the year (Yankson et al., 1989). In relation to interactions with the environment, *C. tulipa* could be used as a biomarker of bacterial and Polycyclic Aromatic Hydrocarbon (PAH) contamination of the mangrove ecosystem (Ebong, Udoinyang, Njoku & Eyen, 2010). Adite et al., (2013b) also refer to the species as an indicator of environmental quality due to its ability to accumulate pollutants.

2.5 Obtaining Oyster Spat (Seed) for Aquaculture

Larval development of oysters continues until a size of about 300 microns, where a distinctive foot is formed and it is ready to settle on a suitable substrate (Doiron, 2008). A juvenile oyster newly attached to a substrate is called a spat. Juvenile oysters of size ranging from 0.8 mm (Urban, 2000) through 1.2 mm (Urban, 2000; Taylor, Southgate & Rose, 1998b) to 12.5 mm (Yankson, 1990) or more may be considered as spat. Aquaculture is heavily dependent on the availability and regular supply of viable seed, thus culturing oysters either requires seed from the natural environment or artificially produced in a hatchery or laboratory.

2.5.1 Wild seed

Oyster spat could be collected from the wild by providing favourable substrate/collector prior to spawning and removal after desired quantities have set. According to Mann (1984), collecting oyster spat from the wild does not require the culturist to have technical knowledge of breeding and fertilisation but requires knowledge on suitable materials, periods and localities of abundance and techniques for maximising harvest.

2.5.2 Hatchery seed

In the hatchery, larval oysters may be reared and provided suitable substrates for spat to set on them. Oyster seed production in hatchery, laboratory and nursery systems relies on the production of microalgae, which may be unpredictable (Tanyaros & Chuseingjaw, 2016) because of the level of specific controls required, for the nourishment of larvae. Specialised techniques and capital are required for hatchery setup and successful rearing and collection of spat (Mann, 1984).

2.6 Materials for Spat Collection (Cultches)

2.6.1 Selection of oyster spat collectors

There are many ways to collect oyster seed from the wild and in hatcheries. The choice of material is dependent on the oyster aquaculturist's own personal preference in the collection, and on the conditions in the area. Any substrate available or provided for settling larva is a cultch (Gosling, 2015; Quayle & Newkirk, 1989). In the wild, oysters at the spat stage set on available hard stable substrates within their habitat. For rearing purposes, different artificial cultches

could be deployed into areas where spat abound for a period spanning about two weeks to one and half months (Kvingedal, Evans, Taylor, Knauer & Jerry, 2008; Laing & Spencer, 2006; Taylor, Southgate & Rose, 1998a; Velasco & Barros, 2010) or up to six months (Friedman, Bell & Tiroba, 1998b) to harvest spat. In some instances, the collector/cultch is covered in a thin layer of a mixture of cement, lime and sand to assist in setting and subsequent removal of spat from collectors.

Whereas information on biology and ecology with respect to oyster aquaculture could remain as current information for a long time, the rearing techniques, equipment, and operations may change after some time (Doiron, 2008). Likewise, materials and techniques for harvesting oyster spat are expected to vary with time and across geographical locations based on what works most efficiently. Ideally, in addition to being a suitable substrate for collecting spat, the materials utilised as spat collectors should be cheap, durable and locally available (Vakily, 1989). The following section describes various materials used to collect oyster spat in different parts of the world.

2.6.2 Types of oyster spat collector materials and associated techniques

This presentation highlights some merits and demerits of different oyster spat collectors/materials but it is important to begin by introducing a common useful technique that is usually employed to maximise spat setting.

<u>Cement coating technique</u>: Dipping collectors in fine cement slurry is a common practice in modern oyster aquaculture. This is done a few weeks before deploying collectors into the wild or into hatchery facilities where spat

will be collected. The technique is applicable to most if not all, the different kinds of collectors reviewed below. Doiron (2008) and Leavitt (2018) suggest that in using cement for spat collection, the mixture should constitute equal volumes of cement, slaked lime and moderately fine sand with enough water added to obtain a thin slurry of about 2 mm. A series of activities are employed to cure the cemented spat collector materials; they are kept out of the wind and sun, sprayed with water for three or four days after they are limed. This, according to Leavitt (2018), is essential as it prevents the mixture from hardening too fast to avoid the formation of cracks, and being subsequently worn away by waves and currents when deployed. After curing, the mixture is allowed to harden and washed by the rain in the open for a minimum of two weeks before they are used to collect spat. Various collector materials are now discussed below:

Chinese hat

The Asian conical hat or the popular rice hat, also known as the Chinese hat and coolie hat (in the UK) has become an essential tool in the shellfish aquaculture industry. The hat, according to the Merriam Webster Online Dictionary (2019), "is a conical-shaped, usually straw, hat worn especially to protect the head from the heat of the sun". In what is a rather modernised aquaculture-use of the Chinese hat, as applied in shellfish aquaculture for spat collection, a number of the hats are stacked with spaces between them and then smeared or dipped in fine cement mortar. Since its introduction in one of Canada's Maritime Provinces - New Brunswick - in the 1970s the Chinese hat has proven to be an effective oyster spat collector (Doiron, 2008).

Stone-bridge concrete slabs

Pillay & Kutty (2005) describe a method for collecting spat using structures made from concrete or cement/mortar. The structure is often moulded in the form of a V and placed in a series of inverted Vs looking like stone-bridges. This method of collecting spat on cement slabs has been employed in culturing oysters (*Crassostrea rivularis*) in muddy areas in China and New Zealand (Pillay & Kutty, 2005).

Plastic mesh/shademesh

Plastic mesh nettings are used in different forms and shapes for harvesting oyster spat. Different names are given depending on the form and/or technique of its application. Plastic mesh could be of monofilament nylon material (Taylor et al., 1998a). Urban (2000) estimated the abundance of spat by deploying collectors comprised of "onion bags" of plastic mesh 80 × 30 cm², mesh size 0.8 cm. Black plastic shade-mesh (Friedman et al., 1998a) or shade-cloth (Southgate & Lucas, 2008) have also been successfully used to collect oyster spat. The perforated, multi-stranded structure of shade-mesh provides a greater number of sites for the spat to settle (Friedman et al., 1998a). Plastic mesh bags, of larger mesh sizes, are used in combination with shell-cultch in certain areas for spat collection (Pillay & Kutty, 2005). It may be called "flower-type" collector, where a strip of shade-mesh is folded in concertina-style (Gervis and Sims, 1992: In Pillay & Kutty, 2005), or the "accordion-style" collector, where it is threaded in accordion-fashion (Haws and Ellis, 2000: In Pillay & Kutty, 2005).

Plastic (polyethylene) sheet

Plastic polyethylene sheeting is also used (Nayar, Rajapandian, Gandhi & Gopinathan, 1980; Friedman et al., 1998a). Plastic sheet could be deployed as folded broad sheets of plastics or sliced into strips and bound or held with a rope, which is held in water column fastened to a horizontally placed frame or long line. Although plastic sheet is capable of harvesting spat and are relatively cheap, their relatively large, smooth surfaces appear to be fouled quickly and extensively than mesh material (Friedman et al., 1998a). Using this technology however, spat are easily removed from the polyethylene sheet and reared separately.

Polyvinyl chloride (PVC) materials

Tubes of PVC material are the most utilised collectors in recent times for largescale oyster spat collection (Gosling, 2015). PVC pipes may be cut into curved slats (Taylor et al., 1998a) or maintained as cylindrical pipes (Tanyaros & Chuseingjaw, 2016) and used as spat collector units. Matt surfaced PVC sheets (Helm & Bourne, 2004) have been used as settlement substrate for oyster spat, where collectors are placed on the base of larval culture tanks. According to the Washington Sea Grant (2002), elevating longlines in rows above the bottom with PVC pipe stakes is a successful commercial spat harvesting method. PVC pipes may also be combined with other materials (Velasco & Barros, 2010), serving as support poles (Rybovich, 2014) or spacers (De Silva, 1998) during the harvest of spat. A rather modern method of collecting oyster seed involves using coupelles and modified PVC drainpipes. Coupelles are used on largescale oyster culture farms because they are designed to have a large surface area available for spat to settle and it is easy to clean and maintain, making it suitable for industrial use (Godeep Shellfish Aqua, 2019).

Polypropylene/nylon rope

Ropes of polypropylene or nylon material are very useful in setting cultches for spat collection in the wild. Although their use is mostly accessory, often as preferred fasteners and holders (Mann, 1983; Pillay & Kutty, 2005), they are capable of supporting substantial spat setting (Taylor et al., 1998a). In addition, polypropylene ropes experience lesser biofouling compared to many other spat collector materials (Pillay & Kutty, 2005).

Recycled oyster (bivalve) shells

Traditionally, on-bottom oyster seed collection includes the spreading of empty oyster shells on the sediment of the coastal intertidal zone. Empty shells are often gathered in piles within communities where there is some exploitation, thus readily available for recycled use as collectors. In Japan and many other countries, the most common collector used consists of scallops or oyster shells strung on ropes or wires (Pillay & Kutty, 2005). Such strings of shells are suspended from rafts, long lines or specially constructed bamboo frames. Finely crushed shell (microcultch) is commonly used in hatcheries as substrates for spat setting (Dégremont, Ernande, Bédier & Boudry, 2007; Walton et al., 2013).

Oyster sticks/stakes

Another traditional method of oyster seed collection is the use of sticks in the wild and sometimes in hatcheries. This has been a mainstay in Australian and Japanese oyster farming for a long time (Pillay & Kutty, 2005). Although still

widely used, such traditional collection methods expose oysters to predators in the wild, thus replaced with rather advanced options for oyster seed collection. The earliest is probably the stake or stick culture system, where bamboo, wooden or cement stakes or sticks are driven into the bottom or set out horizontally on racks to catch spat (Pillay & Kutty, 2005). The stake or stick system is particularly useful in intertidal areas with soft mud bottoms. Since spat of mangrove oysters attach themselves in nature to mangrove roots, Nikolić, Bosch & Alfonso (1976) developed a method for harvesting *C. tulipa* spat using branches of the red mangrove hanged from the horizontal frames of wooden racks. The cement coating technique described earlier could be applied to sticks with reinforced concrete for stability (Pillay & Kutty, 2005). Stakes may also be of plastic or fibreglass materials, which are often used if the spat are to be removed from the collectors for growing in trays or other containers (Pillay & Kutty, 2005).

Ceramic tiles

Tiles are also suitable materials in oyster seed production (Galtsoff, 1964; Giese & Pearse, 1979; Miller & Hall, 1995; Quayle & Newkirk, 1989). Tiles used for harvesting oyster spat may come in different forms such as wall tiles (Yankson, 2004) or roofing tiles (Ajana, 1980; Quayle & Newkirk, 1989). According to Pillay & Kutty (2005), collectors used in some European countries for the European flat oyster and the Portuguese oyster generally, consist of semi-cylindrical ceramic roof tiles about 10 - 12cm in diameter and 30cm long. The tiles may be coated with lime to facilitate the subsequent removal of the spat.

Egg crates

Egg crates have been tested and found to be successful at harvesting spat from the wild. They were successful to a very satisfactory extent, especially on a hard bottom, but tended to have a lowered efficiency on muddy, silt, or soft bottom. The disadvantage is that the crate, lying in the oyster ground, impedes free flow of water over the surfaces and the individual cells contain relatively still water and result in their filling with silt (Hopkins, 1937). Bonnot (1940) successfully used egg crates dipped in a mixture of cement and sand, in collecting oyster spat.

Stacked paper/cardboard "government collector"

To avert the disadvantages of the regular egg-crate, a special modification called "government collector" was developed (Hopkins, 1937). This collector is made of a paper unit of two rows with seven cells each. The cells are of equal height and width but are twice as long as those of the egg-crate are. The advantage over the egg-crate is that the shape of this collector causes it to lie on its side, standing somewhat upright, presenting several horizontal surfaces within the water column with free flow of water (Hopkins, 1937). The "government collector" is suitable for shallow water.

Plywood

The plywood collector is often constructed into units similar to that of the cardboard "government collector" (Bonnot, 1940). The surfaces of the assembled units are separated by equidistance creating square chambers for settlement of spat. A current of water flowing through these narrow spaces

between the rough, uneven cemented surfaces will roll and eddy (Bonnot, 1940). Consequently, any swimming spat that may be present will be tumbled and whirled and thus be more likely to meet both the upper and lower surfaces. Plywood may be dipped in mortar cement and allowed to dry to form a thin concrete layer (Kamara, 1982) before use. The difficulty with the use of this collector is that the removal of the cement with the attached oysters is quite a slow and costly operation.

Coconut shells

Shells of already consumed coconut are recommended for use as spat collectors mainly because they are cheap (Quayle & Newkirk, 1989). Haws (2002) described the construction of coconut cultches for collecting oyster spat. A hole is created at the end of each coconut half, and then using a piece of 14 mm polypropylene rope, a knot is tied at one end, then threaded on a coconut husk (Haws, 2002). Another knot is tied about 20 cm above this and another coconut half is added and repeated until 5 or 6 coconut halves are on the collector and then hung on the main line (Haws, 2002). According to Quayle & Newkirk (1989) a less durable and disintegrating cultch such as coconut shell is ideal for bottom culture.

Asbestos

Asbestos is cited for use as spat collector (Kamara, 1982; Quayle & Newkirk, 1989; Taylor et al., 1998a; Pillay & Kutty, 2005). However, Quayle & Newkirk (1989) recommend its use in strips of about 10 cm x 10 cm cut from sheets about 10 mm thick for experimental purposes. Asbestos could be combined

with other methods, for example, asbestos strips held in trays (Pillay & Kutty, 2005). Corrugated asbestos roofing cut to shell length may also be used in collecting spat (Kamara, 1982).

Rubber

Collectors consisting of rubber material are considered suitable for the settlement of oyster spat, hence employed in collecting spat (Galtsoff, 1964; Quayle & Newkirk, 1989). Rubber boots and tires are all capable of supporting spat setting (Galtsoff, 1964).

Ancillary spat collectors

Other methods of oyster spat collection may consist of one or more of the materials reviewed, thus considered as methods instead of materials in themselves. The list of materials may be inexhaustible as oyster spat are found on rocks, gravel, wood, stems and leaves of marsh grass, and on a great variety of miscellaneous objects such as tin cans, that have found their way to the bottom or deliberately used as spat collectors (Galtsoff, 1964). The collectors reviewed are summarised in Table 2.1.

2.7 Influence of Collector Orientation on Spat Setting Success

It appears that the shape (Taylor et al., 1998a) and orientation (Hopkins, 1937; Taylor et al., 1998a) of materials deployed for spat collection have great influence on the number of spat harvested. Hopkins (1937) concludes that *Ostrea lurida* settles most at the under-horizontal surface of collectors. Similarly, Schaefer (1937) and Cole & Knight-Jones (1939) observed

Collector	Material	Source
Asbestos	Asbestos	Kamara (1982); Quayle & Newkirk (1989); Taylor et al. (1998); Pillay & Kutty (2005)
Ceramic tiles	Ceramic tiles	Yankson (1974); Giese & Pearse (1979); Ajana (1980); Quayle & Newkirk (1989); Miller & Hall (1995); Pillay & Kutty (2005)
Chinese hat	Straw	Doiron (2008)
Coconut shells	Coconut shells	Quayle & Newkirk (1989); Obodai & Yankson (2000); Haws (2002)
Coupelle collector	Industrial grade plastic	Leavitt (2018); Godeep Shellfish Aqua (2019)
Egg crates	Plywood/Cardboard	Hopkins (1937); Bonnot (2011)
Government collector	Paper/Cardboard	Hopkins (1937); Bonnot (2011)
Nylon rope	Polypropylene	Mann (1983); Taylor et al. (1998); Pillay & Kutty (2005)
Oyster sticks/stakes	Stick	Nikolić, Bosch & Alfonso (1976); Pillay & Kutty (2005)
Plastic mesh/ shademesh	Soft plastic	Friedman et al. (1998); Taylor et al. (1998); Urban (2000); Southgate & Lucas, (2008)
Plastic sheet	Polyethylene	Nayar, Rajapandian, Gandhi & Gopinathan (1980); Friedman et al. (1998)
Plywood/wood	Plywood/wood	Hopkins (1937); Bonnot (1940); Yankson (1974); Kamara (1982); (Obodai, 1990)
Polyvinyl chloride (PVC)	Polyvinyl chloride (PVC)	De Silva (1998); Taylor et al. (1998); Velasco & Barros (2010); Helm & Bourne (2004); Rybovich (2014); Gosling (2015); Tanyaros & Chuseingjaw (2016)
Recycled shells	Bivalve shells	Yankson (1974); Pillay & Kutty (2005); Dégremont, Ernande, Bédier & Boudry (2007); Walton et al. (2013)
Rubber	Rubber	Quayle & Newkirk (1989)
Stone-bridge	Concrete	Pillay & Kutty (2005)

Table 2.1- Oyster Spat Collectors Used by Previous Workers

significantly higher numbers of *Osterea edulis* and *Crassostrea gigas* respectively, on the under-horizontal surface of collectors.

2.8 Chapter Summary

Literature on Ghana's aquaculture and its status along the coast, biology and ecology of oysters, and an overview of the global status of oyster fisheries have been reviewed in this Chapter. Examples of available materials used for collecting oyster spat around the world have also been summarised.

40

CHAPTER THREE

MATERIALS AND METHODS

This Chapter gives an account of the materials and methods used to execute the research. The study locations are described in detail. Where necessary, illustrations are provided to explain the methods adopted for the study. Statistical analytical tools and software employed to make inferences are also indicated.

3.1 Study Sites

Experiments were set up in four coastal water bodies (two lagoons, a delta and an estuary) along the coast of Ghana to test the effectiveness of selected materials/collectors for oyster spat settlement. These locations spanned a distance of about 185 km from the Densu Delta in Greater Accra Region, through Narkwa and Benya Lagoons in the Central Region, to the Whin Estuary in the Western Region. An initial survey was conducted and these sites were selected based on the existence of thriving populations of *Crassostrea tulipa* and reports of communities deriving livelihood from the species (see Yankson, 1990; Obodai & Yankson, 2002; Asare, 2017; Janha, Ashcroft & Mensah, 2017). Narkwa and Tsokomey are the most vibrant *C. tulipa* fishing communities among the four, where the oyster is an important food and source of income for the people. The site maps in Figure 3.1 depict current states, from base maps captured with an Unmanned Aerial Vehicle (UAV) – DJI Phantom 3 Professional in 2018.

3.1.1 Densu Delta

The larger Densu River Delta wetland is a Ramsar site and covers a vast lowlying land mass encompassing the concession of a salt manufacturing company. Excluding the salt company, it covers an area of about 8.2 km². It is located within the perimeter $0^{\circ}16'43''$ W, $5^{\circ}34'07''$ N and $0^{\circ}20'02''$ W, $5^{\circ}30'21''$ N – west of Dansoman, south of Mallam, McCarthy Hill and Aplaku, and east of Bortianor. The wetland is heavily influenced by freshwater from the Densu River, which is dammed upstream (the Weija Dam) and divides into small tributaries downstream, and into the sea. The amount of freshwater in the delta is controlled by the spilling and closing of the dam during and after the wet season respectively.

The study however focused on the southern part of the delta within the estuarine region (Figure 3.1 A) where there is a thriving oyster population. Aside serving as feeding and nesting grounds for migratory birds, crabs and fish, the Densu Delta, with its sandy mud bottom, provides suitable habitat for a significant population of *C. tulipa*. Oyster fishing is a major livelihood for women, supported by their children (Figure 3.2), in the Tetegu and Tsokomey communities. Here, the oyster bed is sometimes exposed at low tide. Common fishing methods within the delta are akaja/atidza fishing (i.e. open water culture, using tree branches), basket fishing, cast-net, set-net and hand fishing.



Figure 3.1: Maps of the study sites (A) Densu Estuary (B) Narkwa Lagoon (C) Benya Lagoon (D) Whin Estuary



Figure 3.2: The oyster bed at the Densu Delta (A). An oyster picker harvesting oysters by hand into canoe at high tide (B), and women and children are mainly involved in harvesting oysters from the oyster bed at low tide (C).

3.1.2 Narkwa Lagoon

The brackish ecosystem found at Narkwa in the Ekumfi District orients parallel to the Gulf of Guinea with a somewhat centrally placed mouth, which is usually open to the sea (Figure 3.1 B). Its specific location is within the perimeter 0°56′22″ W, 5°12′17″ N and 0°54′41″ W, 5°12′32″ N. Occasional closure of the mouth by a sand bar occurs once in several years (observed during the course of this study) causing floods within the low-lying settlement. The sand bar gets broken by the force of water from the riverine source and rains after a few months (E. Mensah, Personal communication, May 18, 2018) or mechanically opened with a bulldozer to prevent flooding. Conservatively, the system covers an area of about 0.6 km². The occupations of the community members are fishing and farming. Fishing with bottle, hook-and-line, and

shellfish (oysters and cockles) collection (Figure 3.3) are common in the lagoon. Sand is also mined from the sand bar for building and construction purposes. A nearby salt mining company draws saltwater from the lagoon for the extraction of salt.





Figure 3.3: Women and children harvesting oysters from the Narkwa Lagoon by bending to reach the bottom of the water and collecting them by hand into pans (A). Oyster pickers getting oyster-filled pans ready at the bank of the lagoon for transportation (B).

3.1.3 Benya Lagoon

Benya Lagoon (Figure 3.1 C) is a man-made open lagoon which maintains contact with the Gulf of Guinea throughout the year (Obodai et al., 1991). It meanders almost 90° northwards along its length within the perimeter 1°20'50" W, 5°04'59" N and 1°21'26" W, 5°05'18" N. Dykes have been constructed at the mouth of the lagoon to maintain a permanent opening. Irrespective of this,

continuous sand accretion renders the mouth shallow, sometimes exposed at low tide, preventing canoes from navigating into or out of the inland landing quay and boatyard close to the mouth. There is no notable freshwater source feeding into the lagoon.

The lagoonal area at present is approximately 0.13 km² along a length of about 2 km, from the mouth to the head region marked by a heap of refuse excluding the mangrove marshes and saltpans. The lagoon is bordered on the east by a densely populated urban community, Elmina, and to the immediate west by mangrove vegetation, beyond which lie large saltpans. Two major livelihood activities are predominant along the lagoon i.e. artisanal fishing trade (including boat-building) and piggery. Although *C. tulipa* abounds on roots of the mangrove fringing the lagoon, there is no active fishing of the species. Rather, children are periodically seen collecting the cockle, *Anadara senilis*, from the muddy bottom. Dumping of household refuse and open defecation into the lagoon are sanitary issues of great concern.

3.1.4 Whin Estuary

The Whin Estuary located between 1°46′47″ W, 4°52′52″ N and 1°46′04″ W, 4°52′30″ N, lies on the eastern fringes of New Amanful, a suburb of the Takoradi Township in the Ahanta West District (Figure 3.1 D). The estuary is oblique to the Gulf of Guinea, relative to the orientation of the prevailing shoreline at its narrow mouth, which is about 90 m wide, as opposed to the middle region where it stretches over 350 m wide. It is largely a shallow system with some deep portions. Members of the New Amanful community depend on the Whin Estuary for their livelihoods, mainly fishing and oyster harvesting. A

fish farm is located on the eastern bank of the head of the estuary. Freshwater constantly flows into the estuary from the Whin River that passes through a dense highland forest some 9 km northwest. The Takoradi Airport is located about 1.5 km north of the estuary. Two main effluent channels are directly connected to the estuary, a smaller one running from the New Amanful community, and a relatively bigger one runs from an industrial-residential area (Adakope and Kokompe) northeast of the estuary.

3.2 The Community Entry Process

The design of the research required that racks be constructed and mounted at various sections of the selected water bodies. As these coastal aquatic ecosystems are traditionally considered sacred and not accessible on certain days and at some periods in the year, it was important that proper steps were put in place to avert any possible conflict with traditional leaders. In view of this, a community entry technique was employed to acquaint with the norms as well as perform traditional rights in all research sites. Visits were paid to the communities in late August and early September 2017. An important arrangement during this process was to seek permission from the traditional authorities and engage with key persons who acted as security persons and kept watch over the experimental setups in each community throughout the study.

3.3 Preparing the Collectors

3.3.1 Collating available spat collectors

Information was gathered from desktop research, which involved review of publications including books, peer-reviewed journal articles and manuals in the

field of marine bivalve culture. The literature search was followed by a field survey for information on natural substrates for settlement of *C. tulipa* spat in the selected water bodies by on-site inspection during the community entry. The review has been presented as part of Chapter 2 and outcomes summarised in the results section (Chapter 4) of this thesis.

3.3.2 Construction of experimental collectors

Five collectors were initially selected for assessment (Figure 3.4). These were the coconut shell, fine mesh nylon net (2 mm mosquito net), oyster shell (recycled), PVC and ceramic tile. Four-inch (10.16 cm) diameter PVC pipes were cut into smaller cylinders of height 10 cm and each cylinder cut into three equal pieces along its circumference, producing curved slats of area 106 cm².



Figure 3.4: Oyster spat collector materials tested for effectiveness (a) coconut shell, (b) fine mesh nylon net (mosquito net), (c) oyster shell (recycled), (d) PVC slats and (e) ceramic tiles.

Fifty by fifty cm ceramic tiles were sliced to sizes 10×10 cm², likewise the fine mesh nylon net. Coconut and oyster shells were collected, cleaned and dried before using for the study. For each material 240 collectors were prepared, making 1300, to be used throughout the period of the experiment.

Measuring surface area for coconut and oyster shells

Since PVC, tile, and mesh were cut into definite sizes, their surface areas were determined by the length and breadth $(L \times B)$. On the other hand, considering the irregular and varied sizes of coconut and oyster shells, the surface area of each cultch (see Appendix A1) was determined following Nelson Education Ltd. (2009), for subsequent use in computing spatfall on each collector. To achieve this, the shape outline of each cultch was drawn on a 2 cm square-grid paper with 0.2 cm minor grids. All major and minor grids within the outline were counted, multiplied with their respective areas (4 cm² and 0.04 cm²) and summed up to obtain the area of one side of each collector, not leaving out half grids. For each collector and for all collector materials, the surface area obtained was multiplied by two because of their double sides. Precaution was taken to wrap grid paper around the convex surface of both oyster (when left valve was used) and coconut shells before drawing outlines, to obtain the right surface area values.

Creating holes in collectors

Holes were drilled in the middle portion of all materials except the net, where sizeable holes were created by manoeuvring an object through the central mesh hole. The holes were created to facilitate stringing cultches on polypropylene ropes. A hand-held electric drilling machine (SBY Impact Drill no. 2600r/min) fitted with a drill bit was used for the coconut and oyster shells. For PVC pipes, cutting and drilling were done mechanically at the University of Cape Coast College of Agriculture and Natural Sciences Workshop, using a similar-sized drill bit. To make holes in the ceramic tiles, an expert tiler was engaged. The technique used was to cross-mark the back of the tiles with the tile cutter, creating a deep cross wedge on one side and penetrating the opposite side as a small hole or in some instances, a small plus-shape hole. These holes were further widened manually with a wall nail during stringing if they were too small for ropes to penetrate.

Stringing collectors

Collectors were strung on 4 mm nylon ropes following Chuku & Osei (2017) to minimise wastage of materials. For each string, three cultches of the same material were fastened equidistant from each other. Cultches were held horizontally as this position is confirmed to harvest maximum spat (see Hopkins, 1937). To obtain the right rope length, average high water depth at each station was measured (Appendix A2). Additional 1.2 m length was included to cater for the about 0.2 m rope length anticipated to be lost through the tying of six knots, two to hold each collector, and allow for a 0.5 m rope each at both ends for fastening to rack frames (see Chuku & Osei, 2017). Stringing of collectors was done in the same style and direction. Each string was marked to identify collectors with their surface areas.

3.4 Experimental Design and Setup

Racks measuring approximately $1 \times 1 \text{ m}^2 (L \times B)$ were installed in October 2017 as holding facilities for all experiments (Figure 3.5). Three racks with all the collectors were mounted in each of the four waterbodies one rack at each of three stations (ST1, ST2 and ST3) for spatfall and collector assessment experiments. An additional rack was mounted at ST2 of each water body for the growth experiment. The height of each rack was adjusted by the position of the upper frames to the level of observed high tide mark at each station.

For each collector material, a cultch was made of 3 units which were hung vertically on the racks, one each at the top, middle and bottom water column (see Figure 3.6). Collectors at the top were placed ≈ 0.1 m below water surface at high water level, bottom ≈ 0.1 m off the bottom, and middle collectors placed



Figure 3.5: Installation of racks and setting of collectors: (a) Stakes of experimental racks being mounted at low tide; (b) diving is required at stations with deep waters (ST2 of Densu Estuary), and (c) collectors being fitted to racks.
equidistant from the two at each station. Collectors were hung in one direction to ensure uniformity. In all, 720 collectors were deployed i.e. $540 = [(3 \times 3 \text{ of collector}) \times (5 \text{ materials}) \times (3 \text{ racks}) \times 4 \text{ water bodies}]$ for spat collection, + 180 for the growth experiment. An extra set of 540 collectors were prepared for monthly replacements in the spat collection experiment.



X = Replicate cultches

Figure 3.6: An illustration of the cross sectional view of the experimental setup at each station

3.5 Field Sampling and Data Collection

Monthly sampling was conducted for twelve months spanning the dry and wet seasons, from November 2017 to October 2018 in all four water bodies. The experimental setup was mounted in the third quarter hence sampling was done within the same period in subsequent months. At each sampling, all collectors in the field were removed and replaced with a new set. Spat-laden collectors removed were carefully arranged, bound and marked according to stations to prevent mixing and transported in a sac to the Fisheries and Coastal Research Laboratory at UCC for counting and measurement. Oyster growth parameters as well as some hydrographic parameters were measured in the field.

When laboratory analysis was completed, each collector was cleaned before returning to the field the following month for replacement. First, spat and other attached organisms were scraped from the surfaces of the collectors. Then collectors were washed with scrubbing brush and freshwater to clear sand/mud and algae and sundried for at least three days before the next sampling.

3.5.1 Estimating spat abundance on collectors

In the laboratory, collectors were examined for settled spat. For each collector, all spat were counted and recorded separately for the upper and underhorizontal sides. When spat were too many to count, a subsampling method was employed, dividing the settlement area into four parts and counting spat from one portion, to extrapolate the whole. A study lamp was used to provide a higher localised luminescence relative to laboratory ambience for increased visibility. In order to identify very small spat (<1 mm), the lamp was used in conjunction with a hand lens.

3.5.2 Size distribution of one-month old spat

After counting, the shell heights (SH) of spat (Figure 3.7) were measured to the nearest 0.5 mm with the aid of the adapted lamp-and-lens technique. Shell height (SH) of *C. tulipa* spat was measured as the vertical distance from the tip of the umbo to the base of the posterior margin. A metre rule was used for direct measurements. Where it was difficult to measure directly, a pair of dividers were used and transferred onto the metre rule to read values. For each collector, 30 spat were measured. All spat were measured when less than 30 occurred on the collector. Anterior and posterior determinations followed Spencer (2002).



Figure 3.7: Crassostrea tulipa spat showing shell height (SH) measurement. Illustration was done by researcher.

3.5.3 Monitoring growth of cultured C. tulipa

Spat were collected on the different collectors at the top, middle and bottom over three months (October 2017 to January 2018), allowing crowding (averagely 26 spat per cultch), after which monthly shell measurements began from the third to the ninth month. No further settlement was allowed after the third month. Shell height (SH) measurement (see Figure 3.7) was used for the growth studies as it provides a robust dimension for comparison across populations (see Peterson, 1958; Eisma, 1965; Ivell, 1979 as cited by Yankson, 2000). SH was measured to the nearest 0.5 mm with a metre rule in conjunction with a pair of dividers.

3.5.4 Experiment on spat setting on upper- and under-horizontal surfaces of collectors

Another spatfall experiment was set up in February 2018 to assess spat setting preference on the underside and upper side of collectors from the first three months of sampling. Because the two sides of each collector material were different either in texture or in shape, it was appropriate to test the influence of the surfaces on spat settlement. In this confirmation experiment, collectors were strung in the opposite direction to the regular spatfall experiment as shown in Figure 3.8 and collectors removed in March 2018. Spat were counted, measured and a 0° (face down) versus 180° (face up) comparison done statistically.



Figure 3.8: An illustration of (A) face down 0° and (B) face up 180° orientation and collector surfaces in the horizontal position

3.5.5 Hydrographic parameters

Some physico-chemical parameters of water namely, temperature, salinity, dissolved oxygen (DO), and turbidity were measured *in situ* at all stations during each sampling, mostly at low tide whereas pH was measured in the laboratory. Temperature (°C) and DO (mg L⁻¹) were measured using a dual parameter water quality instrument (YSI Environmental EcoSence[®] DO200A). Salinity (ppt) was measured with the E-LINE Refractometer 44-8080 and turbidity (NTU) with the OAKTON[®] Turbidimeter T-100. All water quality readings were triplicated. Triplicate water samples were transported on ice and allowed to thaw to room temperature in the lab for the determination of pH with the OAKTON[®] pH 700 by dipping the probe into the water sample.

3.5.6 Identifying and estimating fouling organisms

In the field and during the laboratory work, collectors were inspected for any unwanted materials and organisms that were attached to the cultches or on the settled spat. These organisms were identified to the lowest taxonomic level possible. Each fouling organism was estimated by percentage coverage on the collectors, on a scale of 0 - 4 and indicated with the plus "+" sign (0; 1 = +; 2 = ++; 3 = +++; 4 = ++++), where; 0 = no fouling on collector, 1 = 1 - 25 %, 2 = 26 - 50 %, 3 = 51 - 75 % and 4 = 76 - 100 % coverage on collector.

3.6 Mapping Bathymetry, Oyster Presence and Spat Availability in the Water Bodies

Aerial images of water bodies were captured with UAV – DJI Phantom 3 Professional and image classification and digitisation was done with the

Pix4Dmapper[®] software. To obtain the depth profile of the water bodies, a depth sounder (3-126-D15; 0.6-800 m) was used to measure depth along grids that were created remotely with ArcGIS software. The sizes of grid areas varied based on the sizes of the systems under study. Within each grid area, depth was measured and specific location re-marked with a GPS device (Garmin GPSMap64 Handheld Navigator). Bathymetry data and corresponding coordinates were entered into Microsoft Excel spreadsheet and exported to the ArcMap 10.3 software in Comma-separated Values (.csv).

The data was then converted to shapefiles and interpolated using Inverse Distance Weighting (IDW) tool to create depth profiles. Areas having oyster populations within the water bodies were identified and marked, as well as traditional knowledge on oyster harvesting locations. In mangrove swamps, the extent of mangrove vegetation with oysters on roots was measured by horizontal distance landwards.

3.7 Data Analysis

Two-sample t-test and Analysis of Variance (ANOVA; One-Way, Two-Way, Multi-Way using Minitab 17 Statistical Software) were used to deduce statistical differences between sample groups at 95% confidence level (α = 0.05). Tukey's Pairwise and Simultaneous Comparisons were used to identify specific differences among means. Where comparison against best/highest means was required (e.g. in determining most suitable collector), the Honestly (Highest) Significant Unit (Hsu) Multiple Comparison with Best (MCB) was applied.

When ANOVA analysis included stations, "Station" was nested into "Water body" considering that each station was characteristically different among water bodies, and Densu Delta had two stations whilst the others had three each. Homogeneity was confirmed with Bartlett's test and the appropriate data transformations applied to satisfy assumptions before ANOVA, where necessary.

Interval plots, bar charts, growth curves, histograms as well as tables and photographs were used to present data graphically. Unless otherwise stated, standard error (\pm SE) values and bars were used for means in values and charts respectively.

Change in shell height was considered as primary growth parameter; hence absolute growth rate (AGR) was determined for cultured *C. tulipa* using the formula:

$$AGR = \frac{Final SH - Initial SH}{Number of days} \dots \dots \dots \dots \dots (Equation 1)$$

3.7.1 Estimation of spatfall

An equation was derived for computing spatfall on collectors considering the number of replicates and varied surface areas of some of the collectors (Eqn. 4). To calculate spatfall on each collector, the total count of spat was divided by surface area (Eqn. 2). Note that for flat materials, total surface area includes both (2) sides.

Where Sf = Spatfall, Ns = Number of spat on collector material, Ac = Total surface area of collector material, i = 1, 2, 3 ... n^{th} replicate collector.

Mean spatfall is a summation of spatfall for all replicate collectors, divided by number of replicates (Eqn. 4).

Where n = number of replicates

Eqn. 3 is further simplified to Eqn. 4 by introducing the sigma equation and inserting Eqn. 2:

$$= \left[\sum_{i=1}^{n} \left(\frac{\mathrm{Ns}_{i}}{\mathrm{Ac}_{i}}\right)\right]/\mathrm{n}$$

$$=\frac{1}{n} \cdot \sum_{i=1}^{n} (Sf_i)$$

Mean spatfall obtained from the derived equation (per cm²) was extrapolated to the number of spat per m² by the expression \overline{X} Sf × 10000.

3.8 Chapter Summary

The materials and methods used to execute the research, as well as the study locations have been described in detail in this Chapter. Statistical analytical tools and software employed to make inferences have also been indicated.

CHAPTER FOUR

RESULTS

Information reviewed from the literature on oyster spat collectors, data collected from various field experiments and observations have been organised and presented in this chapter. The chapter describes outputs from data analysis, including descriptive and inferential statistics. In addition, the data and observations have been organised into comprehensible tables, charts, diagrams and photographs and presented as appropriate. Unless otherwise stated, standard error (\pm SE) values and bars were indicated for means in tables and charts respectively. Statistical tests of differences were done using 95 percent confidence interval, thus significant differences were inferred at *P* < 0.05. Significant differences have been denoted by regular/superscript alphabets in graphs and tables to facilitate comprehension.

4.1 Settlement of C. tulipa Spat on Experimental Collectors

Figure 4.1 presents the performance of the different oyster spat collectors assessed in each water body. Spat settlement was significantly different on the different spat collectors (see Appendix B1). In the Densu Delta, however, there was no significant difference in spat settlement on oyster shell, PVC and ceramic tile (P > 0.05), all of which were significantly higher than that of coconut shell (P < 0.05). There was a significant difference in spat settlement on the difference in spat settlement difference in spat settlement difference in spat settlement on the difference spat settlement difference in the Narkwa Lagoon (P = 0.004). Further, Tukey pairwise comparisons showed that oyster shell (3165 ± 191 spat m⁻²) and ceramic tile (3451 ± 254 spat m⁻²) had significantly higher spatfall than coconut



Figure 4.1: Mean (±S.E.) spatfall on experimental spat collectors in each of the four water bodies. Arabic alphabets show ANOVA comparison between collectors within the same water body only. Boldface Roman numerals show ANOVA comparison between water bodies for each type of collector. Means with different alphabets/numerals are significantly different (One-way ANOVA: P < 0.05) [see Appendix B2].

shell (2089 ± 168 spat m⁻²), with PVC (3112 ± 246 spat m⁻²) having spatfall not significantly different from the others (P > 0.05).

In the Benya Lagoon, ceramic tile had the highest number of settled spat (1769 \pm 140 spat m⁻²) whilst coconut shell had the least (725.8 \pm 73.1 spat m⁻²). Again, spatfall was significantly minimal on coconut shell in the Whin Estuary, compared to those of oyster shell, PVC and ceramic tile, which did not differ significantly from each other (*P* > 0.05). Tukey simultaneous comparisons are presented in Appendix B2.

There was a significant difference in spat settlement among the four water bodies studied (P < 0.05). Densu Delta averaged 2373 ± 129 spat m⁻² month⁻¹, Narkwa Lagoon had the heaviest spatfall averaging 3069 ± 129 spat m⁻² month⁻¹ ¹ whilst Benya Lagoon and Whin Estuary had 1288.3 ± 58.0 spat m⁻² month⁻¹ and 312.4 ± 26.9 spat m⁻² month⁻¹ respectively. For each collector, spatfall was not significantly different in the Densu Delta and Narkwa Lagoon but had significantly more spat than Benya and Whin Estuaries, Whin being the least (illustrated by I, II and III in Figure 4.1).

4.2 Settlement of *C. tulipa* Spat on Upper and Under-Horizontal Surfaces of Collectors

There was consistently higher spat settlement on the under-horizontal compared to upper-horizontal surfaces (P < 0.05) of collectors deployed in each water body throughout the study as shown in Figure 4.2 (see Appendix B3). Figure 4.2 and Figure 4.3 summarise spatfall on the different surfaces of each collector material and orientations respectively (details in Appendix B4). These were



Figure 4.2: Percent mean number of spat on upper- (\square) and under- (\square) horizontal surfaces of experimental collectors (CS = Coconut shell; OS = Oyster shell; CT = Ceramic tile). * Significant difference between \square and \square (P < 0.05)



Figure 4.3: Mean (\pm S.E.) spat settlement on upper- (\square) and under- (\square) horizontal surfaces of experimental collectors (all 4 collectors pooled) in two positional orientations ("Face down" and "Face up") in Densu Estuary and Narkwa Lagoon.

observed with collectors placed in a "Face down"/0° orientation; hence, experiments were set up to test spat settlement when collectors were oriented in the opposite "Face up"/180° direction, as illustrated in Chapter 3 (see Fig. 3.8). Figure 4.3 shows higher spat settlement on the under-horizontal surface, recurring in both Densu Delta and Narkwa Lagoon when collectors were faced upwards (P < 0.05) (see Appendix B5 for details of comparisons). The underhorizontal surfaces collected twice or more, the number of spat settling on the upper surfaces. Factorial analysis (Appendix B6) however revealed that depending on the collector deployed, orientation may/not matter with respect to total spat yield; e.g. for PVC slats, orientation did not influence total number of spat harvested ("Face down" = 983 ± 203 m⁻², "Face up" = 975 ± 141 m⁻²) whereas for the other collectors, a 0° orientation i.e. "Face down" had significantly more spat (see Appendix B6).

4.3 Monthly Variations in Spatfall at Experimental Stations

Spatfall showed significant monthly variations in all the four water bodies (P < 0.05) (Appendix B7). In the Densu Delta spatfall fluctuated from November 2017 to June 2018 and reduced to extremely low or no settlement from July to October 2018 (Figure 4.4). Highest spatfall was recorded in May 2018 at both Stations (Station 1 = $6867 \pm 655 \text{ m}^{-2}$; Station 2 = $8968 \pm 1284 \text{ m}^{-2}$).

Progressively decreasing fluctuations in spatfall were observed at all Stations in the Narkwa Lagoon from November 2017 to May 2018 and remained very low afterwards (Figure 4.4). During this period of low spatfall, Stations 1 and 3 maintained spatfall below 850 m⁻² whereas there was no spatfall at Station 2 in June, September and October 2018. Both Stations 1 and 3 had their respective



Figure 4.4: Mean (\pm S.E.) monthly spatfall (spat m⁻²) at stations (ST) in the Densu Estuary, Narkwa Lagoon, Benya Lagoon, and Whin Estuary pooled from all collectors and different depths.

spatfall peaks, $6031 \pm 1006 \text{ m}^{-2}$ and $9455 \pm 1357 \text{ m}^{-2}$, in December 2017 whilst Station 2 had highest mean spatfall of $10705 \pm 918 \text{ m}^{-2}$ in February 2018.

At least there was some spatfall in each month throughout the study period in the Benya Lagoon. However, spatfall was low from November 2017 to April 2018 and from August to October 2018. Least spatfall in the Benya Lagoon was recorded at all stations in March 2018 (Station $1 = 41.0 \pm 10.4 \text{ m}^{-2}$; Station $2 = 106.7 \pm 19.1 \text{ m}^{-2}$; Station $3 = 73.2 \pm 17.1 \text{ m}^{-2}$). Hikes in spat densities were observed from May to July 2018 in the Benya Lagoon, with Stations 2 (8700 \pm 699 m⁻²) and 3 (4371 \pm 379 m⁻²) recording maximum spatfall in May 2018 (Figure 4.4). On the other hand, Station 1 had the highest spatfall in July (3706 \pm 343 m⁻²).

Spatfall in the Whin Estuary was relatively low with two small peaks, one in January and the other in May 2018 (Figure 4.4). For January, Station 1 had a peak of 465.7 \pm 84.3 spat m⁻², Station 2 had 703 \pm 200 spat m⁻² whereas Station 3 had 1394 \pm 333 spat m⁻². The peaks in May were more pronounced across all stations (Station 1 = 2108 \pm 311 spat m⁻²; Station 2 = 2265 \pm 421 spat m⁻²; Station 3 = 1641 \pm 390 spat m⁻²). There was no spatfall in September and October 2018 at all three stations.

The months were categorised into dry and wet seasons by calculating rainfall anomalies using rainfall data (Appendix C1) obtained from the online global climate database (Tutiempo.net, 2019). Consecutive months of average total rainfall lower than the annual (12 months study period) mean were assigned to the dry season category, and vice versa. Hence, dry season was the period from November 2017 to April 2018, whereas the wet season was from May 2018 to October 2018. Spatfall for the two seasons differed significantly with the dry season recording 2359.6 \pm 80.6 spat m⁻² month⁻¹ and the wet season, 1050.4 \pm 49.6 spat m⁻² month⁻¹ pooled from all water bodies studied (*P* = 0.000). There were however some discrepancies with respect to specific water bodies (Table 4.1). Spat settlement in the Densu Delta was 3058 \pm 171 spat m⁻² month⁻¹ in the dry season, and 1689 \pm 188 spat m⁻² month⁻¹ in the wet season.

Spatfall was 5724 \pm 209 and 413.7 \pm 25.4 m⁻² month⁻¹ in the Narkwa Lagoon for dry and wet seasons respectively. In the Benya Lagoon, the situation was reversed as spat settled at 658.1 \pm 25.3 m⁻² month⁻¹ in the dry season and 1919 \pm 108 m⁻² month⁻¹ in the wet season. Whin Estuary also had 231.6 \pm 26.5 spat m⁻² month⁻¹ in the dry season, and 393.2 \pm 46.6 spat m⁻² month⁻¹ in the wet season.

4.4 Spatfall at different depths from the water surface

Spatfall varied depending on depth at which collectors were placed, as shown in Figure 4.5 (see ANOVA results in Appendices B7 and B8). In the Densu Delta, the bottom (≈ 0.10 m off bottom) and middle collectors had similar spatfall of 2986 ± 257 m⁻² month⁻¹ and 2905 ± 249 m⁻² month⁻¹ respectively (*P* > 0.05) whereas the top (≈ 0.10 m below high water level) collectors had significantly lower spatfall of 1228 ± 125 m⁻² month⁻¹ (*P* < 0.05). Similarly, in the Narkwa lagoon, spatfall increased significantly from top (1993 ± 189 m⁻² month⁻¹) through middle (3023 ± 203 m⁻² month⁻¹) to bottom (4190 ± 258 m⁻² month⁻¹) (*P* < 0.05).

Watan bada		Dry season (Nov	v-17 - Apr-18)	Wet season (May-18 - Oct-18)			
water body		Spat $m^{-2}(\pm SE)$	Month-Yr	Spat $m^{-2}(\pm SE)$	Month-Yr		
Densu Estuary	Mean	3058 ± 171		1689 ± 188			
	Minimum	826 ± 104	Nov-17	0 ± 0.00	Jul, Sep, Oct - 18		
	Maximum	5255 ± 374	Feb-18	7918 ± 727	May-18		
Narkwa Lagoon	Mean	5724 ± 209		413.7 ± 25.4			
C	Minimum	1932 ± 159	Mar-18	160.9 ± 28.4	Sep-18		
	Maximum	8245 ± 508	Feb-18	792.3 ± 75.4	May-18		
Benya Lagoon	Mean	658.1 ± 25.3		1919 ± 108			
	Minimum	73.65 ± 9.51	Mar-18	133.6 ± 14.7	Sep-18		
	Maximum	1030.8 ± 73.6	Apr-18	5393 ± 367	May-18		
Whin Estuary	Mean	231.6 ± 26.5		393.2 ± 46.6			
5	Minimum	31.62 ± 6.09	Nov-17	0 ± 0.00	Sep, Oct - 18		
	Maximum	854 ± 137	Jan-18	2005 ± 217	May-18		
All water bodies	Mean	2359.6 ± 80.6		1050.4 ± 49.6			
(pooled)	Minimum	760.6 ± 61.6	Mar-18	80.31 ± 9.47	Sep-18		
	Maximum	3404 ± 233	Feb-18	3673 ± 222	May-18		

Table 4.1 - Spat Settlement m^{-2} (mean \pm S.E., minimum, maximum) in the Dry and Wet Seasons in the Different Water Bodies



Figure 4.5: Mean (\pm S.E.) spatfall of *Crassostrea tulipa* at different depths (Top, Mid [middle] and Bot [bottom]) in water column at stations 1, 2 and 3 in the selected coastal water bodies.

Highest spatfall in Benya Lagoon was $1586 \pm 117 \text{ m}^{-2} \text{ month}^{-1}$ on middle collectors although not significantly different from that recorded on bottom collectors $(1375.1 \pm 97.9 \text{ m}^{-2} \text{ month}^{-1})$ (P > 0.05). Again, collectors at the top had significantly lower spatfall (904.2 \pm 80.1 m⁻² month⁻¹). Like Narkwa Lagoon, spatfall differed significantly on top ($12.35 \pm 5.14 \text{ m}^{-2} \text{ month}^{-1}$), middle ($224.9 \pm 34.5 \text{ m}^{-2} \text{ month}^{-1}$) and bottom ($699.9 \pm 68.6 \text{ m}^{-2} \text{ month}^{-1}$) collectors in the Whin Estuary.

In the Narkwa lagoon, whereas Station 1 had extremely low spatfall at the top $(190.8 \pm 34.8 \text{ m}^{-2} \text{ month}^{-1})$ compared to middle $(2481 \pm 259 \text{ m}^{-2} \text{ month}^{-1})$ and bottom $(4090 \pm 405 \text{ m}^{-2} \text{ month}^{-1})$, Stations 2 and 3 had similar spatfall levels at all depths. There was no spatfall on top collectors at Stations 2 and 3 of the Whin Estuary.

4.5 Size Distribution of One-Month Old C. tulipa Spat

On the average, spat harvested in one month were observed to be of significantly different sizes relative to the water bodies (Appendix B9). Figure 4.6 shows the size frequency distribution of spat harvested from all four water bodies studied. Mean shell heights of spat were 4.32 ± 0.05 mm, 7.5 ± 0.07 mm, 2.69 ± 0.02 mm and 4.65 ± 0.08 mm for Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary respectively (Table 4.2). Highest size range (SH = 0.5 - 45.0 mm) was recorded in the Narkwa Lagoon, whilst Benya Lagoon had a relatively narrow size range (SH = 0.5 - 22.0 mm). It could be observed in Figure 4.6 that for all water bodies, a significant majority of spat had sizes 10.0 mm and below.



Figure 4.6: Pooled size (shell height) frequency distribution of one month old *Crassostrea tulipa* spat harvested monthly over 12 months (November 2017 - October 2018) from the Densu Delta (D), Narkwa Lagoon (N), Benya Lagoon (B) and Whin Estuary (W). *Maximum observed size class; low frequency.

Water body	Shell height (mm)	Тор	Middle	Bottom	Overall	
Densu Delta	Mean (± SE)	1.89 ± 0.04^{c}	4.84 ± 0.08^{b}	$5.53\pm0.08^{\rm a}$	4.32 ± 0.05	
	Minimum	0.5	0.5	0.5	0.5	
	Maximum	11.0	44.0	22.0	44.0	
Narkwa Lagoon	Mean (± SE)	$6.23\pm0.13^{\text{ c}}$	$7.50\pm0.13^{\text{ b}}$	8.28 ± 0.11 a	7.5 ± 0.07	
	Minimum	0.5	0.5	0.5	0.5	
	Maximum	39.0	40.0	45.0	45.0	
Benya Lagoon	Mean (± SE)	$1.5\pm0.02^{\:c}$	$2.65\pm0.03^{\text{ b}}$	$3.65\pm0.04~^a$	2.69 ± 0.02	
	Minimum	0.5	0.5	0.5	0.5	
	Maximum	9.0	22.0	14.0	22.0	
Whin Estuary	Mean (± SE)	$2.66\pm0.37^{\text{ b}}$	$3.16\pm0.10^{\text{ b}}$	5.47 ± 0.11 $^{\rm a}$	4.65 ± 0.08	
	Minimum	0.5	0.5	0.5	0.5	
	Maximum	10.0	20.0	35.0	35.0	

Table 4.2 – Mean (± SE), Minimum and Maximum Shell Heights of One-Month Old Crassosrea tulipa Spat at Different Depths in Water

^{a, b, c} Mean values that do not share a superscript are significantly different (One-Way ANOVA; Tukey's test; P < 0.05)

A positively skewed unimodal size distribution, with modal size class, 1.1 - 2.0 mm, was observed for *C. tuipa* spat in each water body. Skewness was however most pronounced for the Whin Estuary (skewness = 2.06) whereas the opposite was true for Narkwa Lagoon (skewness = 1.29). Further analysis (Table 4.2) revealed size variations in spat collected at different depths.

In addition, sizes observed in the water bodies differed strongly in the Narkwa Lagoon and Whin Estuary temporally, with largest sizes in March-April 2018 and reducing considerably afterwards (Figure 4.7) (see also Appendix B9). At the Narkwa Lagoon, spat at Station 1 were relatively larger than those at Stations 2 and 3 from June to October 2018. The converse was true for the preceding January to April.

4.6 Growth of Cultured C. tulipa

4.6.1 Comparing growth of C. tulipa in the different water bodies

Sizes of *C. tulipa* at six months showed varying growth (P < 0.05) among the water bodies indicated with 'A' and 'B' in Figure 4.8. Spat cultured in the Narkwa Lagoon (denoted 'A' in Figure 4.8) exhibited fastest growth (Absolute Growth Rate [AGR] = 0.33 mm d⁻¹), attaining shell height (SH) of 59.86 ± 0.64 mm in six months. Growth was however similar (P = 0.051) in the Densu Delta and Whin Estuary at six months (denoted 'B' in Figure 4.8) although relatively bigger sizes were obtained in the Densu Delta. Mean SH recorded in the two water bodies were 47.16 ± 0.72 mm and 44.02 ± 1.47 mm with growth rates of 0.26 mm d⁻¹ and 0.24 mm d⁻¹ respectively in six months. Monthly shell heights and growth rates are presented in Table 4.3.



Figure 4.7: Mean (\pm S.E.) monthly shell heights of *Crassostrea tulipa* spat collected at the different stations (ST1, 2 and 3) of the four water bodies.



Figure 4.8: Mean (±S.E.) shell heights of *Crassostrea tulipa* spat cultured for 6 months at different depths (Top, Mid = middle, Bot = bottom) and in the different water bodies. Groups that do not share an alphabet are significantly different (ANOVA, Tukey's test: P < 0.05; see Appendix B10).

	Densu Es	stuary	Narkwa I	Lagoon	Whin Estuary			
		AGR		AGR		AGR		
Month-Yr.	Mean SH \pm SE	(mm day ⁻¹)	Mean SH \pm SE	(mm day ⁻¹)	Mean SH \pm SE	(mm day ⁻¹)		
Oct-2017	0.00 ± 0.00	0	0.00 ± 0.00	0	0.00 ± 0.00	0		
Nov-17	** **	0.22	** **	0.30	** **	0.12		
Dec-17	** **	0.22	** **	0.30	** **	0.12		
Jan-2018	19.89 ± 0.64	0.22	26.87 ± 0.41	0.30	10.94 ± 0.95	0.12		
Feb-18	29.96 ± 0.64	0.34	41.21 ± 0.54	0.48	21.60 ± 1.19	0.36		
Mar-18	38.14 ± 0.68	0.27	55.02 ± 0.59	0.46	34.84 ± 2.18	0.44		
Apr-18	47.16 ± 0.72	0.30	59.86 ± 0.64	0.16	43.90 ± 2.16	0.30		
May-18	$50.10 \pm 0.7 $	0.10		-	44.12 ± 2.03	0.01		
Jun-18	50.43 ± 0.65	0.01		-	$46.49 \pm 1.89 $	0.08		
Jul-18	52.70 ± 0.69	0.08		-	46.81 ± 2.01	0.01		
AGR at end of April (6 months)		0.26 ^b		0.33 ^a		0.24 ^b		
Overall AGR (9 months)		0.20 ^c				0.17 ^d		

Table 4.3 – Overall and Monthly Absolute Growth Rates (AGR) of Crassostrea tulipa Cultured in Three Water Bodies

** No measurements taken

^{a, b, c, d} Values that do not share superscript are significantly different from each other (Tukey's test; P < 0.05)

After the sixth month, growth monitoring was terminated at the Narkwa Lagoon due to the stealing of the experimental setup. The final sizes (SH) of *C. tulipa* in this experiment after nine months in the Densu Delta and Whin Estuary were $52.70 \pm 0.69 \text{ mm}$ (AGR = 0.20 mm d⁻¹) and $46.81 \pm 2.01 \text{ mm}$ (AGR = 0.17 mm d⁻¹) respectively. The difference in growth was significant (*P* = 0.001).

4.6.2 Comparing growth of C. tulipa at different depths

Figure 4.8 further depicts growth of *C. tulipa* on top, middle and bottom collectors in the water bodies after 6 months. Growth was significantly different at all depths in the Densu Delta (P < 0.05). In the Narkwa Lagoon, growth was low on the top collectors, whereas the middle and bottom collectors had similar (P = 0.715) but significantly higher (P < 0.05) growth than the top collectors.

There was not enough spat settlement on the top collectors in this experiment in the Whin Estuary. Nonetheless, the middle (SH = 35.00 ± 1.90 mm) and bottom (SH = 57.31 ± 2.26 mm) collectors showed significantly different growths (*P* = 0.00).

4.6.3 Comparing growth of C. tulipa on the different collectors

Figure 4.9 shows a generally similar growth pattern of *C. tulipa* on all the collectors in the various water bodies. In the Densu Delta, however, significantly higher growth was recorded on the ceramic tile than the other collectors in January 2018 (SH = 23.63 ± 1.25 mm) and February 2018 (SH = 35.15 ± 1.19 mm). Final shell heights in July (nine months culture period) showed a significant difference only between the oyster shell and ceramic tile cultches. The cultured oysters had significantly lower growth on the oyster shell



Figure 4.9: Monthly mean $(\pm$ SE) shell heights of *Crassostrea tulipa* cultured on the different collectors over 9 months at the Densu Delta, Narkwa Lagoon and Whin Estuary (see ANOVA results in Appendix B10).

collectors (49.09 \pm 1.03 mm) than the ceramic tiles (55.17 \pm 1.37 mm) (*P* < 0.05). In the Narkwa Lagoon, growth on ceramic tile and oyster shell were not significantly different (*P* = 0.837) but higher than those of PVC and Coconut shell (*P* < 0.05) in six months. PVC and Coconut shell had similar growth (*P* = 0.999).

The ceramic tile had the highest growth (SH = 53.47 ± 3.15 mm) in the Whin Estuary. PVC (SH = 46.54 ± 3.63 mm) and Oyster shell (SH = 44.21 ± 3.42 mm) had similar sizes of oysters after nine months culture (*P* > 0.05). Coconut shells at the bottom were disintegrated by fouling organisms by the sixth month (April 2018).

4.7 Fouling and Associated Epibiotic Organisms

Photographs of foulers of the experimental clutches are shown in Figure 4.10 and 4.11. The serpulid worm *Ficopomatus* sp. was the most dominant and persistent fouling organism through the 12 month period at Stations 2 and 3 of the Whin Estuary (Table 4.4), predominantly on bottom collectors. The species was observed in appreciable numbers in the Densu Delta and appeared in the Narkwa Lagoon only in April 2018 (see Table 4.4). The other serpulid *Spirobis* sp., appeared on collectors in the Benya Lagoon in the last month of sampling (October 2018).

The barnacles (*Fistubalanus pallidus*) occurred in high densities in the Narkwa Lagoon, reaching extreme levels at Station 2, in August 2018. The species was present in all the water bodies studied. Also growing on collectors were some green (*Chaetomorpha antennina*) and brown (*Chondria bernardii*) algae; the

latter being present solely at Station 1 of the Whin Estuary. *C. antennina* was present in all four water bodies.



Figure 4.10: Biofoulers and mud associated with *Crassostrea tulipa* spat collection and culture: Serpulid worms (A) *Ficopomatus* spp. and (B) *Spirobis* sp, (C) *Fistubalanus pallidus*, (D) mud, (E) *Chaetomorpha antennina*, (F) *Chondria bernardii*, and (G) small-sized crab (<15 mm). Photographs were captured during field sampling for this study.



Figure 4.11: Biofoulers and mud associated with *Crassostrea tulipa* spat collection and culture: (H) *Armases* sp, (I) *Sesarma* sp., (J) *Littorina angulifera*, (K) sea anemones, (L) *Brachidontes* sp., (M) *Membranipora arborescens*, and (N) ascidians. Photographs were captured during field sampling for this study.

Month-Yr	Densu Delta		Narkwa Lagoon		on	Benya Lagoon			Whin Estuary		
	ST 1	ST2	ST 1	ST2	ST 3	ST 1	ST2	ST 3	ST 1	ST2	ST 3
Nov-2017	CE+	C+	0	CE++	CE+	0	0	0	0	A++	A++
Dec-17	0	0	0	CE++	CE++	D++	D+	E+	CE+	A++	A++
Jan-2018	ACEL+	C+	0	CDM++	C++	D+	D+	D+	CEF+	A+	AEM+
Feb-18	ACEL+	C+	0	C+	CM+	0	0	0	CDEF++	AM+	A+
Mar-18	ACEL+	AC+	0	EKN+++	C+	E+	E+	E+	ACEM+	ACJM++	AC++
Apr-18	ACE+	AC+	0	AC+	A++	D+	D+	D+	CE+	ACJ+	A+
May-18	AC+	AC+	0	0	0	0	0	0	CE+	A++	A+
Jun-18	AC++	ACE+	D++	0	0	0	0	0	ACE+	AJ+	A+
Jul-18	AGE+	AE+	0	C++	C+	0	D+	0	E+	A+	A+++
Aug-18	CE+	A+	C+	C++++	C++	CE++	C+	C+	C+	ACI+	A+
Sep-18	0	ACE+	C+	C+++	C++	0	C+	C+	EC+	ACI+	A+
Oct-18	0	0	0	CG+	C+	0	B+	0	H+	IJ+	AI+

Table 4.4 – Monthly Occurrence of Fouling and Epibiotic Organisms Associated with Crassostrea tulipa Spat Collection and Culture at the Different Stations in the Four Water Bodies

+ Degree of fouling (all foulers) for the month: '0'none, + low, ++ moderate, +++ heavy, ++++ extreme

(A) *Ficopomatus* sp., (B) *Spirobis* sp, (C) *Fistubalanus pallidus*, (D) mud, (E) *Chaetomorpha antennina*, (F) *Chondria bernardii*, (G) small-sized crabs (<15 mm), (H) *Armases* sp, (I) *Sesarma* sp., (J) *Littorina angulifera*, (K) sea anemones, (L) *Brachidontes* sp., (M) *Membranipora arborescens*, and (N) ascidians.

Three species of crabs were found associated with the collectors; *Armases* sp. (Figure 4.11 H), *Sesarma* sp. (Figure 4.11 I) and unidentified small-sized (<15 mm including walking legs) crab (see Figure 4.10 G). The unidentified small crab was seen in July and October 2018 at Densu and Narkwa respectively (Table 4.4). *Littorina angulifera*, an estuarine snail was commonly found on collectors at the Whin Estuary and occasionally in the Densu Delta, in small numbers. The mussel *Brachidontes* sp. mostly occurred in the Densu Delta and the Narkwa Lagoon. In the Benya Lagoon, collectors were mostly fouled with mud.

The bryozoan *Membranipora arborescens* was seen at both Narkwa and Whin in January and February 2018 but only at Whin Estuary in March 2018. Sea anemones (Figure 4.11 K) and ascidians (Figure 4.11 N) were encountered at the Narkwa Lagoon.

4.8 Prevailing Hydrographic Conditions

Values for hydrographic parameters were pooled to obtain monthly means for each parameter in each of the water bodies studied. Nonetheless, there were few cases of significant differences among some stations. Figure 4.12 presents annual trends in hydrographic conditions.

4.8.1 Temperature

Annual mean temperatures were 28.5 ± 0.2 °C, 29.1 ± 0.27 °C, 28.0 ± 0.2 °C and 29.1 ± 0.2 °C for the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary respectively. Temperatures were similar among the Densu, Narkwa and Whin systems (P > 0.05). However, the mean temperature in the Benya Lagoon was significantly lower than the Narkwa and Whin systems (P < 0.05). The general trend in monthly variations depicts highest temperatures from February to April 2018 and minimum temperatures in July and August 2018 (Figure 4.12).

4.8.2 Dissolved oxygen (DO)

In general, mean concentrations of DO were higher and followed a similar pattern in the Densu Delta, Narkwa Lagoon and Whin Estuary than the Benya Lagoon throughout the study (Figure 4.12). Annual mean DO concentrations in these water bodies were $3.0 \pm 0.3 \text{ mgL}^{-1}$, $3.6 \pm 0.3 \text{ mgL}^{-1}$ and $2.9 \pm 0.3 \text{ mgL}^{-1}$. On the other hand, relatively low DO levels were observed in the Benya Lagoon ($1.1 \pm 0.1 \text{ mgL}^{-1}$). Dissolved oxygen fluctuated, with highest concentrations recorded in February and April 2018 and minimal concentrations recorded from June to October 2018 (Figure 4.12).

4.8.3 Salinity

For the period of study (November 2017 – October 2018), salinity averaged 17.4 ± 1.6 ppt, 20.0 ± 0.9 ppt, 28.7 ± 0.7 ppt and 14.7 ± 0.9 ppt in the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary respectively. Salinity was generally high in the water bodies from January to April 2018 and reduced thereafter, showing no clear pattern after April 2018 (Figure 4.12). In the Benya Lagoon, however, higher salinities were recorded after April 2018 with the highest (39. 8 ± 0.5 ppt) in August 2018.


Figure 4.12: Monthly mean $(\pm$ SE) values for hydrographic parameters in the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary from November 2017 to October 2018.

4.8.4 Turbidity

Annual mean turbidity values were 12.2 ± 0.6 NTU, 19.8 ± 2.0 NTU, 12.9 ± 0.3 NTU and 24.8 ± 1.9 NTU in the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary respectively. It is seen in Figure 4.12 the Narkwa Lagoon and Whin Estuary had wider fluctuations in turbidity with peaks in December 2017, May and June 2018 (see Figure 4.12). On the other hand, Densu Delta and Benya Lagoon had relatively low fluctuations in turbidity. In the Narkwa and Whin systems, high turbidities were observed in December 2017 and from May to September 2018.

4.8.5 pH

Annual mean pH in the water bodies (Densu = 8.0 ± 0.1 , Narkwa = 7.8 ± 0.1 , Benya = 7.6 ± 0.0 and Whin = 7.9 ± 0.1) indicate slight alkalinity in the coastal water bodies studied. Densu Estuary had the highest pH of 9.6 ± 0.4 in November 2017 whereas the least pH (6.6 ± 0.1) for the study period was recorded at Narkwa Lagoon in July 2018 (Figure 4.12).

4.8.6 Relationship between hydrographic conditions, spatfall and spat growth

The hydrographic parameters were analysed as predictors of spat availability and growth for the study period using multiple linear regression. The results indicated that DO and Salinity were the most significant (P = 0.00) predictors of spatfall in the four water bodies studied; explaining 18 % and 9 % of data variations respectively. Hydrographic factors accounted for 31.36 % of variation in the data for spatfall (Table 4.5). For growth, 24.36 % of data

Parameters (Pre	dictors)				
Term	Coef	SE Coef	95% CI	T-Value	P-Value
Spatfall					
Constant	-0.550	5.290	(-11.03, 9.92)	-0.100	0.917
Temperature	-1.840	3.030	(-7.83, 4.16)	-0.610	0.545
DO	1.789	0.452	(0.895, 2.683)	3.960	0.000
Salinity	0.997	0.251	(0.500, 1.494)	3.970	0.000
Turbidity	0.331	0.406	(-0.472, 1.134)	0.820	0.416
рН	3.430	4.010	(-4.50, 11.36)	0.860	0.394
	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	5	57.240	11.448	11.510	0.000
	R ² 31.36%				
Growth (Shell Height)					
Constant	-0.570	1.090	(-2.73, 1.59)	-0.520	0.603
Temperature	1.153	0.607	(-0.050, 2.357)	1.900	0.060
DO	0.260	0.092	(0.0779, 0.4420)	2.830	0.006
Salinity	-0.099	0.070	(-0.2370, 0.0395)	-1.420	0.160
Turbidity	-0.223	0.093	(-0.4078, -0.0378)	-2.390	0.019
рН	-0.198	0.811	(-1.805, 1.409)	-0.240	0.807
	DF	Adj SS	Adj MS	F-Value	P-Value

1.287

5 R² 24.36%

Regression

0.257

Table 4.5 – Coefficients and Contributions of the Linear Regression Model for Spatfall and Growth (Shell Height) (Factors) and the Measured Hydrographic Parameters (Predictors)

7.020

0.000

variation was explained by hydrographic conditions. DO and turbidity were the significant (P < 0.05) predictors of growth within the ecosystems studied (Table 4.5). Although, temperature explained, to the largest extent i.e. 11.89 %, the variation in shell height-hydrography data, its *P*-value was not significant.

4.9 Mapping Depth Profile, Spatfall and Presence of Adult *C. tulipa* in the Coastal Water Bodies

Depth profiles and presence of adult oyster populations in the water bodies as well as spatfall data at stations are presented as maps to guide site selection for spat collection and oyster culture (Figure 4.13 - Figure 4.16). Low tide depths of the water bodies ranged from 0 m to 2 m. Average depths of the water bodies were 0.4 ± 0.01 m (Densu Delta), 0.5 ± 0.03 m (Narkwa Lagoon), 0.7 ± 0.02 m (Benya Lagoon) and 0.5 ± 0.03 m (Whin Estuary). The water bodies had maximum depths of 2 m, 1.5 m, 1.4 m, and 1.3 m respectively.

Adult *C. tulipa* were most abundant on sandy-mud bottom of the Densu Delta and Narkwa Lagoon. Although there were oysters at the deep north-eastern part of the Densu Delta, spat collection and culture setups appeared to interfere with the active fishing activities of fishers, hence marked as an insecure zone (see X in Figure 4.13). The central portions in the Narkwa Lagoon had sandy bottom, thus the absence of adult oysters in this area. The depth range of adult oysters was 0.2-2 m and 0.5-1.5 m for Densu Delta and Narkwa Lagoon respectively. In the Benya Lagoon and Whin Estuary, there was highest concentration of the adult oysters on stilt roots of red mangroves fringing their banks. The bottom substrate in Benya Lagoon was predominantly muddy and not suitable for

oysters to inhabit. The bottom substrate of Whin Estuary was mainly sandy, and did not have a significant presence of adult *C. tulipa*. However, there was a rocky area of about 0.4 m depth between the middle (Station 2) and head (Station 3) regions of the estuary (see Figure 4.16), referred to by the inhabitants of Amanful in local parlance as "Adante-bu" to wit "oyster-hub", where large-sized oysters are collected. Additionally, there was settlement of spat on collectors at Station 1 where there were no adult oysters.



Figure 4.13: Map showing depth profile at low tide (June 2018), oyster presence and availability of *Crassostrea tulipa* spat in the estuarine section of Densu Delta.



Figure 4.14: Map showing depth profile at low tide (June 2018), oyster presence and availability of *Crassostrea tulipa* spat in the Narkwa Lagoon.



Figure 4.15: Map showing depth profile at low tide (June 2018), oyster presence and availability of *Crassostrea tulipa* spat in the Benya Lagoon.



Figure 4.16: Map showing depth profile at low tide (June 2018), oyster presence and availability of Crassostrea tulipa spat in the Whin Estuary.

CHAPTER FIVE

DISCUSSION

The results presented in Chapter 4 are discussed in this chapter. The discussion covers the efficiency of the different types of collectors and their orientation for optimising collection of *Crassostrea tulipa* spat from the wild. Spatial (horizontal and vertical) and temporal abundance of *C. tulipa* spat in the selected coastal water bodies as well as the pooled size distribution of spat collected monthly are elaborated with reference to relevant literature. Growth of *C. tulipa* cultured in the different water bodies is also discussed. The potential impact of fouling by epibiotic organisms on spat collection and culture of *C. tulipa* is also examined.

5.1 Efficiency of Collectors and Orientation for Optimising Collection of *Crassostrea tulipa* Spat From the Wild

Apart from the nylon mesh, which was eliminated after three months of not attracting spat, the other spat collectors proved to support the settlement of *C*. *tulipa* spat in natural ecosystems. Instability of the nylon net in the water column may have contributed to unsuccessful spat settlement on the nylon mesh collector. Perhaps, a finer mesh-size material adapted to a more stable setup in the water column may produce positive results. Previous studies have demonstrated the use of wall tiles (Yankson, 1974), oyster shells (Yankson, 1974; Obodai, 1990) and coconut shells (Obodai, 1990; Obodai, 1997; Asare, 2017), for collecting *C. tulipa* spat in Ghana. PVC was used to collect spat of other species of oysters; *Crassostrea gigas* in Wales (Laing & Earl, 1998),

Crassostrea virginica in Georgia (Manley, Power & Walker, 2008) and *Pinctada maxima* in Indonesia (Taylor, Rose & Southgate, 1997; Taylor et al., 1998a,b). Ruwa & Polk (1994) successfully collected *Crassostrea cucullata* spat on coconut shells in Kenya.

In the present study, ceramic tiles, oyster shells (recycled) and PVC slats showed similar potential at collecting *C. tulipa* spat, whilst coconut shell collectors had the least number of spat in all the water bodies. In the Narkwa Lagoon and Whin Estuary, however, coconut shells appeared to have the potential to collect as many spat as recycled oyster shells. Therefore, in the two water bodies both collectors could be deployed for *C. tulipa* spat collection in the absence of ceramic tiles and PVC slats. There was further indication that spat settlement on collectors could be a random activity, underscored by the large standard errors observed for mean spatfall. Nonetheless, the results showed that ceramic tiles and PVC slats were clearly more efficient *C. tulipa* spat collectors than coconut shells.

The differences in efficiency among some of the spat collectors used in this study may be due to a number of factors. Taylor et al. (1998a) identified surface contour to promote settlement of the pearl oyster *Pinctada maxima*. However, this phenomenon did not apply to *C. tulipa* in this study, as it would be expected that relatively contoured collectors (coconut and oyster shells) would have been most effective. Instead, the hard nature of materials such as ceramic tiles, oyster shells and PVC, with somewhat resistance to water absorption, appeared to have provided a more stable substratum for the attachment of *C. tulipa* spat. On the other hand, the water absorption capacity of coconut shells (Rao, Swaroop,

Rao & Bharath, 2015) and the tendency to disintegrate in water may have rendered a relatively softer substrate, not as attractive as the other collectors for cementing by *C. tulipa* during attachment.

A comparative advantage of ceramic tiles could be their weight (each ceramic tile weighed 200 g; the other collectors weighed < 50 g each) and probably relatively less perturbed by water currents, providing more stability for settlement of C. tulipa spat. However, it is extremely difficult to detach whole/live spat from ceramic tiles and oyster shells as experienced during monthly cleaning of collectors prior to deployment. In addition, tile collectors are brittle and break easily when they fall. This may increase losses and affect production cost. The utilisation of ceramic tiles as collectors on a practical C. *tulipa* farm would require intensive labour for cleaning collectors and great care to prevent losses. In contrast, coconut shells required moderate effort whilst PVC required minimal time and effort to detach almost all spat undamaged, corroborating the 95-100 % spat removal success reported by Wedler (1980) for PVC. This is probably the reason PVC is the most widely used in recent times for oyster culture (Gosling, 2015). Further, preparation and construction of ceramic tiles and PVC collectors require skilled labour whilst coconut and oyster shells can be prepared by the culturist with little skill.

The culturist, therefore, will have to decide on the type of collector based on available resources, expertise and purpose. *C. tulipa* spat to be collected and detached for onward culturing in different grow-out facilities may be best done using PVC slats. Those meant for rearing on collectors would be ideal on ceramic tiles and oyster shells. The biodegradable coconut shells would be ideal

for bottom culture (Quayle & Newkirk, 1989) and useful in oyster restoration programmes. In addition, growth and survival of spat may be critical in the choice of collectors by culturists beyond settlement.

Experimental racks in this study were fitted with vertical series of horizontally strung collectors. This was guided by conclusions on horizontally placed collectors yielding the greatest number of spat by several workers in the past (see Hopkins, 1937; Schaefer, 1937; Cole & Knight-Jones, 1939; Taylor et al., 1998a). However, observations of spat settlement on upper and lower surfaces of horizontally placed collectors are inconclusive. The present study demonstrated the abundance of C. tulipa spat on under-horizontal surfaces of all collectors in every month. Hopkins (1937) and Schaefer (1937) experimented effects of angles of collector surface to the horizontal on the intensity of setting for Ostrea lurida and C. gigas spat respectively and found a more intense settlement on the under surface similar to the findings in the present study. Earlier work by Cole and Knight-Jones (1939) however, showed a marked tendency for the larvae of Ostrea edulis to attach in daylight. In contrast, Shaw, Arnold and Stallworthy (1970) concluded that setting activity in mature larvae of C. virginica was encouraged by dark and partially inhibited by light. Ajana (1979) also recorded best concentration of C. gasar (=tulipa) spat on shaded collectors. These observations support the profuse settlement of C. tulipa spat on under-horizontal surfaces of collectors observed in the present study. This demonstrates a possible escape from light or simply a quest for shaded areas (i.e. negative phototaxis) by the pediveliger. It could be assumed that undersides of the collectors used in this study received relatively lesser

illumination and therefore attracted more spat than the upper surfaces (see Figure 5.1).

The description of the morphological and anatomical structure of the larvae of *C. virginica* (Galtsoff, 1964) and *O. edulis* (Cole & Knight-Jones, 1939; Cranfield, 1974) suggests larval movement prior to setting could most likely account for the observation for *C. tulipa* in this study. The free-swimming veliger possesses a foot for attachment near the velum (which is an outgrowth of the prototroch of the previous trochophore larva) with cilia for swimming forward and upward with foot and velum uppermost (Galtsoff, 1964). Since larval formation is identical for *Ostrea* and *Crassostrea* (Galtsoff, 1964), the *C. tulipa* larvae, like other oyster larvae, swimming upside down with the foot uppermost, presents the best chance of attaching to undersides of horizontally suspended substrates even under turbulent natural conditions. Upward swimming of competent larvae of *C. virginica* was found to persist in highly turbulent flow by Wheeler et al. (2013). In addition, Baker (1997) provides



Figure 5.1: Typical occurrence of spat on upper- (left) and under- (right) horizontal surfaces of the same collector (ceramic tile). Photograph captured by researcher during field sampling.

101

evidence of geotaxis, i.e. movement against gravity, as a stronger settlement cue than phototaxis and rugotaxis for *C. virginica*, stating pediveligers of both *Crassostrea* and *Ostrea* possess statocysts, which are thought to be geosensory.

The above notwithstanding, in the Narkwa Lagoon, which had the highest availability *C. tulipa* spat, upper (lamellate) sides of Oyster shell collectors collected similar spat as the under-horizontal sides in the peak spatfall period. This, although rare in this study, gives a clue of the potential of multiple attachment sites presented by the lamellate outer surface of oyster shells.

5.2 Spatial Abundance of *C. tulipa* Spat in Some Coastal Water bodies in Ghana

Spatfall was highest at Narkwa Lagoon, which was 1.3, 2.5, and 9.8 times those of Densu Delta, Benya Lagoon and Whin Estuary respectively. These water bodies are within 185 km stretch of the coastline of Ghana and approximately 62 km apart. Oysters in the Densu Delta and Narkwa Lagoon occurred on the bottom sandy-mud sediment whereas, in the Benya Lagoon and Whin Estuary, they were found attached either on the submerged main stem or on stilt roots of mangrove vegetation on the banks, with very limited occurrence on their respective muddy and sandy bottoms. It appears that bottom-sediment populations were more prolific than mangrove populations of *C. tulipa* in this study. This could be attributed to environmental differences, for example, the relatively high DO concentration in the Narkwa Lagoon could be a major contributor to the high spatfall observed in that system as DO was found to be one of two key factors influencing spatfall. Genetic variations in reproductive capacity of the different populations could also be a reason; but this would

suggest the likelihood of different sub-populations of *C. tulipa* along the coast of Ghana, which needs further investigation.

Further, within each water body, spat abundance varied in space i.e. at different stations and by depth also suggesting the effect of environmental differences. In the Densu Delta, Stations 1 and 2 had equally abundant *C. tulipa* spat whilst Station 3 could not support spat collection as a result of interference with other fishing activities.

Stations 2 and 3 of Narkwa Lagoon presented the most favourable sections for harvesting spat, yielding 3592 ± 252 spat m⁻² and 3360 ± 227 spat m⁻² respectively in a month. The relatively low spatfall at Station 1 could be due to the contrast in environmental conditions. Stations 2 and 3 were located at the eastern part of the lagoon and had similar depth (≈ 1 m at high water) compared to Station 1, which was very shallow (≈ 0.5 m at high water) hence exposed for longer periods, with muddy substratum and only a few adult oysters present. The hydrodynamics of the Narkwa lagoon is also such that Station 1 had a narrower salinity range (11 - 32 ppt) as recorded in this study, whereas Stations 2 and 3 had a wider salinity range (1.4 - 34 ppt). The combined effect of these could have made these stations more suitable for oysters to thrive.

In the Benya lagoon where hydrographic conditions were similar at all stations, the duration of submergence of collectors appears to have accounted for the differences in spat availability among stations. The present study revealed that the relatively deep portions (Station 2) where middle and bottom collectors were inundated at low tide had about twice as much spat as higher beds (Stations 1 and 3) where all collectors were exposed at low tide. The uniform

but low spat settlement observed at the Whin Estuary could be due to the similar hydrographic conditions at all the stations as well as the shallowness of that water body.

The general increase in abundance of *C. tulipa* spat with increasing depth in all the four water bodies observed in this study could be attributed to differences in exposure and hence food availability. In an experiment conducted by Laing (1995), setting success was affected by the amount, type and concentration of algal diet supplied to larvae of *O. edulis* and *C. gigas* before and during spatfall. The apparent interminable inundation of most bottom collectors, in such fixed culture systems as the one used in this study, could also lead to the faster formation of biofilms providing suitable conditioning of collector surfaces, attractive to larvae. Biofilms play a vital role in larval settlement in oysters by increasing settlement rate and metamorphosis (Tanyaros, 2011; Tanyaros & Chuseingjaw, 2016). Longer tidal exposure may have limited conditioning time of top collectors than their counterparts at the lower levels.

5.3 Temporal Abundance of *C. tulipa* Spat in Some Coastal Water bodies in Ghana

The results outlined in section 4.3 demonstrate seasonality in the availability of *C. tulipa* spat, with dry season (i.e. November 2017 to April 2018; total precipitation \approx 448 mm; average temperature \approx 28.2 °C) having more spat than the wet season (i.e. May to October 2018; total precipitation \approx 1390 mm; average temperature \approx 26.5 °C). This was the case for Densu Delta and Narkwa Lagoon. The reverse was true for Benya Lagoon whilst Whin Estuary showed no distinct seasonal variation. Seasonal variations in oyster spat have been

attributed to temperature (Flores-Vergara, Cordero-Esquivel, Cerón-Ortiz & Arredondo-Vega, 2004; Cognie et al., 2006; Saucedo, Ormart-Castro & Osuna-García, 2007) and other conditions of the aquatic environment (Hofmann, Powell, Klinck & Wilson, 1992; Dekshenieks, Hofmann, Klinck & Powell, 2000). The observed year-round availability of spat in the Narkwa and Benya Lagoons in this study is consistent with Obodai (2007) and suggests continuous spawning by *C. tulipa* in these water bodies.

The preponderance of dry season availability of C. tulipa spat was accentuated in the Narkwa Lagoon where there was a drastic and persistent decline right from the onset of rains until the end of the wet season. Therefore, major spat settlement occurred from November 2017 to April 2018 at Narkwa. The apparent absence of spat settlement at Densu from July to October 2018 is a probable effect of freshwater intrusion in the delta due to the opening of the Weija dam. Densu Delta is routinely flooded in the wet season with freshwater spilt from the Weija Dam located further upstream, forcing saltwater back to the sea and making the entire system almost fresh, causing mortalities in wild oysters. In addition, a reduction in activities for acquiring energy whilst concentrating on expending more energy to regulate cell volume and to prevent osmotic shock (Solan & Whiteley, 2016) as a survival strategy, may have compromised spawning. Further, fertilisation of successfully spawned gametes if any, and survival of zygotes would not be achieved in salinity < 10 ppt (Pechenik, Pearse & Qian, 2007; Allen & Pechenik, 2010; Fang, Peng, Yen, Yasin & Hwai, 2016). This underscores the significance of salinity on spatfall established by the results of the linear regression model in this study (see Table 4.5). When conditions were favourable, spat were available in the Densu Delta from November 2017 to June 2018, with major settlement at Station 2 in April-May 2018.

In Benya Lagoon, unlike the other water bodies, the wet season had increased salinity possibly caused by the washing of salt from the numerous saltpans located at the periphery into the lagoon system coupled with the daily tidal seawater inflow. In view of this, major spat settlement season for *C. tulipa* in this lagoon occurred from May to July 2018. Longest continuous peaks observed by Obodai (2007) in Benya Lagoon (April to June 1996) corroborate the findings of this study. Again, the effect of salinity appears to determine the seasonality in the availability of *C. tulipa* spat in the Benya Lagoon. The Whin Estuary, on the other hand, showed no distinct seasonal pattern in availability of *C. tulipa* spat but had two small peaks occurring separately in January and in May 2018. This pattern is not easy to explain, but it should be noted that spatfall in this water body was generally very low (see Section 4.4).

An important observation in this study was the slight natural revamping of *C*. *tulipa* spat in October 2018 in the Narkwa Lagoon. Stations 1 and 3 appeared to be regaining whereas Station 2, which hitherto yielded more spat, had none. The relocation of the mouth of the lagoon close to Station 2 after three months of closure of the original mouth resulted in the deposition of large volumes of sea sand into this section due to escalated rate of erosion on the banks of the new mouth of the lagoon. The impact of such changes in the geomorphology of coastal intertidal areas, brought about by artificial openings in the sand bar, on *C. tulipa* populations, has not been assessed. However, the observation in this study reveals potential negative consequences on the availability of spat

possibly from adult *C. tulipa* being buried under sand deposits and/or dying as a result. Potentially suitable sites for harvesting *C. tulipa* spat may therefore be endangered by the process of creating artificial openings in sand bars close to oyster beds. This needs further investigation.

5.4 Size Distribution of C. tulipa Spat

The marked differences in sizes of *C. tulipa* spat collected monthly from the four water bodies in this study (Densu = 4.3 ± 0.05 mm; Narkwa = 7.5 ± 0.07 mm; Benya = 2.7 ± 0.02 mm; Whin = 4.7 ± 0.08 mm) could be due to differences in availability of nutrients and other environmental conditions. Additionally, as mentioned earlier for spat abundance, genetic variations could also produce faster- or slower-growing sub-populations, which may be responsible for the size differences. Sizes of *C. tulipa* spat collected during one month in this study, increased with increasing depth in the four water bodies. This is in tandem with spat availability by depth, found in the present study.

Again, because bottom collectors were subjected to longer periods of submergence, settlement of *C. tulipa* could occur earlier on such collectors due to better conditioning, and settled spat would have the benefit of more nutrients and required aquatic conditions under longer inundation. Collet et al. (1999) found earlier settling larvae of *C. gigas*, to have greater post-metamorphic growth. Results of this study also show relatively larger sizes of *C. tulipa* spat in the dry season, increasing up to April when spat with greatest shell heights were observed especially in the Densu, Narkwa and Whin ecosystems. It appears that the wet season, and its accompanying environmental changes, reduced the growth potential of successfully settled *C. tulipa* spat. However,

Station 1 of Narkwa Lagoon with less freshwater inflow, and all the stations in Benya Lagoon (an open lagoon with minimal freshwater inflow) were not affected by this phenomenon. The outcome of the multiple regression analysis suggests that dissolved oxygen and turbidity may be the most critical conditions for growth of *C. tulipa* spat; perhaps through enhanced respiration, active metabolism and efficient filtration of food by the newly settled larvae.

5.5 Growth Performance of *C. tulipa* Cultured on Different Cultches in Selected Coastal Water Bodies

C. tulipa spat cultured at an average density of 0.14 spat cm⁻² (1 spat per 7 cm²) i.e. 30 spat per cultch in the present study, appeared to grow at rates comparable to relatively lower stocking densities from previous studies. For instance, in Asare (2017), shell heights of C. tulipa at stocking densities ranging from 1-8per cultch grew at a rate of 0.82 ± 0.14 cm per month (≈ 0.27 mm day⁻¹) over seven months in the Narkwa Lagoon whereas absolute growth rate of 0.33 mm day⁻¹ was recorded over six months in the same lagoon in the present study. Previous studies on the culture of C. tulipa (Obodai, 2000; Asare, 2017) showed a general insignificant difference in sizes obtained at varied stocking densities ranging from one to eight oysters per cultch. In addition, Urban (2000) and Manley et al. (2008) established density-independent growth in studies on the growth of *Pinctada margaritifera* and *Crassostrea. virginica* respectively. Further, sizes reached in six months in the present study are market-ready as the mean sizes are larger than the average market size of 4.08 cm reported by Asare (2017). There is, therefore, the potential to capitalise on high stocking densities to boost revenue using the same facility in C. tulipa farming.

However, further studies may be required to validate the comparative advantage of such high stocking over lower ones concurrently to offset possible effects of environmental differences.

The faster growth of *C. tulipa* cultured in the Narkwa Lagoon (AGR = 0.33 mm day⁻¹) compared with growth at Densu Delta (AGR = 0.26 mm day⁻¹) and Whin Estuary (AGR = 0.24 mm day⁻¹), suggests Narkwa Lagoon to be the most suitable among the three systems for farming the species. The factors influencing spat availability and size distribution (sections 5.2 - 5.4) are most likely responsible for the differences in growth performances of *C. tulipa* among the water bodies. Also, the suppressed growth of spat on top collectors in all the water bodies in this study could be due to the effect of tidal exposure and consequent reduced feeding time as mentioned earlier in section 5.4.

Superior growth performance of *C. tulipa* on ceramic tile collectors, relative to the others in this study, may have resulted from early growth advantage due to the phenomenon of xenomorphism in oysters described by Quayle & Newkirk (1989). According to the authors, oysters naturally take the shape of contours and space presented by cultches. Therefore, the flat surfaces of ceramic tiles may be advantageous in producing relatively greater shell heights in cultured *C. tulipa* especially as cultched specimens would navigate the contours of curved and/or undulating surfaces of the other collectors, losing vertical height in the process.

Growth pattern of *C. tulipa* in this study showed a rapid increase in shell height in the first six months (October 2017 to April 2018) beyond which growth rate retarded on all collectors. From growth simulation of mussels and oysters using

109

modelled growth curves (Kraeuter et al., 2007; Sarà et al., 2012), it would appear that the observed growth pattern in this study could be the general growth form for bivalves. If this presumption is right, considering the presence of larger wild oysters in the selected waterbodies than the sizes reached in this study, then a longer culture period may be required to ascertain the full growth pattern and potential of *C. tulipa* under culture in these water bodies.

5.6 Potential Impact of Fouling and Epibiotic Organisms Associated with Spat Collection and Culture of *C. tulipa*

Fouling organisms associated with spat collection and culture of C. tulipa in the Narkwa and Benya lagoons were documented by Obodai and Yankson (2000). For the Densu Delta and Whin Estuary, this study is the first record. Several macro-organisms (fauna and flora) and thick layers of sediment fouled the collectors deployed for collection and rearing of the oyster spat. The most biologically significant fouling organism was the barnacle Fistulobalanus pallidus due to its ubiquitous and relatively dense occurrence within the geographical range of the study. F. pallidus reached extreme abundance, covering completely all surfaces of the majority of collectors deployed for spat collection, at Station 2 of Narkwa lagoon in August-September 2018, when temperature, dissolved oxygen and salinity were generally lower than the annual average. In earlier similar work in the same lagoon (Obodai & Yankson, 2000) and another in the Gulf of Mannar (Alagarswami & Chellam, 1974), barnacles were the major fouling organisms and their peak occurrence coincided with the period observed in the present study. Aggravated fouling possibly caused by run-offs in the wet season, as seen in this study, was also

reported by Friedman and Bell (1999) and Friedman et al. (1998a) in the Solomon Islands. It could be considered a fortune that the extreme occurrence of *F. balanus* abundance peaks occur at a period that is unfavourable for spat collection, per the results of this study.

Furthermore, in the present study, the polychaete Ficopomatus sp. caused considerable damage to the shells of live oysters as well as coconut shell collectors in Whin Estuary particularly on bottom collectors, similar to observations by Alagarswami and Chellam (1974). The process of using calcium glands to construct tubes of crystalline calcium carbonate and mucopolysaccharide matrix by this serpulid polychaete (Ten Hove & Kupriyanova, 2009) disintegrated coconut shells, whilst gaping shell valves of cultured oysters appeared to be prevented from clamping, resulting in mortalities. However, significant quantities of this polychaete were limited to Densu Delta and Whin Estuary dominating fouling in the latter. Spirobis sp. is not likely to create problems for oyster culturists in these water bodies because of its one-time appearance in very low numbers, in the Benya Lagoon. Other than mechanical removal, adopting suspended culture systems such as the raft could minimise the harm caused by such benthic epifauna (Arakawa, 1990). Nonetheless, this option may be impracticable in shallow systems such as Whin estuary.

Filamentous green macroalgae (Chlorophyceae: *Chaetomorpha antennina*) were consistent on upper surfaces of spat collectors and were most abundant on top collectors in all water bodies in this study. Although spat were often found attached to surfaces with algal fouling [as in Obodai & Yankson (2000)], this,

according to Brand, Paul, and Hoogesteger (1980), has the potential to decrease numbers of spat near the surface. *Chaetomorpha* sp. was reported to be of no threat in experimental spat collection and culture of *C. tulipa* in the Narkwa and Benya Lagoons by Obodai and Yankson (2000). The alga *Chondria bernardii* was limited to Station 1 of the Whin Estuary, whose possible effects may be similar to its green alga counterpart in this study.

Crabs are known predators of farmed oysters (Brand et al., 1980; Friedman et al., 1998a; Urban, 2000). However, it is difficult to clearly report of direct predation by the few crabs observed, as there were no marked indications on shells of either spat or cultured *C. tulipa* in this study. On the other hand, small/juvenile crabs, abundant in the Densu Delta during the wet season, were found in opened valves of dead oysters, possibly scavenging on the carcass.

Biofilm and algal growth may have created a suitable grazing substratum for the mangrove snail *Littorina angulifera* (Kohlmeyer & Bebout, 1986; Gutierrez, 1988). Thus, the species could be rather beneficial for algal control, the veracity of which would require further research.

Whilst different species of bryozoa are reported to form majority of fouling communities in some studies (Brand et al., 1980; Guenther, Southgate & De Nys, 2006; Beer & Southgate, 2008; Rodriguez & Ibarra-Obando, 2008), the species in this study, *Membranipora arborescens*, only colonised small portions of spat collectors from January to February 2018 in the Narkwa Lagoon and February to March 2018 in the Whin Estuary. The species encrusted collector surfaces as well as on settled spat. Conversely, *C. tulipa* spat settled on the bryozoans as well, similar to observations by Brand et al. (1980).

In the present study, sea anemones and ascidians occurred exclusively and rarely, on collectors submerged for long periods for culture purposes in the dry season in the Narkwa Lagoon, with no appearance on those deployed monthly for spat collection. According to Rodriguez and Ibarra-Obando (2008), oysters and ascidians have similar modes of feeding (filter-feeding), likewise their selection of food resources. Hence, the authors pre-empt that because ascidians cover the valves they could have better access to the flow stream, and probably filter suspended food particles at the expense of host oysters. The mussel, *Brachidontes* sp., encountered on cultured *C. tulipa* in the Densu, Narkwa and Whin systems could be a similar competitor. Fortunately, it did not occur in significant numbers to pose problems.

In summary, there was recruitment of several sessile and vagile species, which may be present and/or deleterious to *C. tulipa* depending on the period of immersion and season, on collectors used in this study. Collectors deployed for culturing would require periodic removal of *F. pallidus* and *Ficopomatus* sp. especially in the Narkwa Lagoon and Whin Estuary respectively. Checking biofouling of such sessile epifauna is labour intensive and mechanical injury and detachment of oysters are common losses to expect in their removal. Nonetheless, fouling may not be a significant menace in spat collection over one month from the wild as extreme fouling of the ubiquitous fouler *F. pallidus* and high *C. tulipa* spatting season appear to be out of phase.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The main aim of the study was to identify strategies for optimising spat collection and growth of *Crassostrea tulipa* for its mass production in coastal water bodies in Ghana. The study provides useful information needed to support successful spat collection and culture of C. tulipa, in four waterbodies along the coast of Ghana. Data on the suitability of spat collectors and their positioning effects, spatio-temporal availability of spat, high stocking density culture performance on different collectors and at varying depths in water, and fouling organisms associated with C. tulipa spat collection and culture have been provided. Whereas some previous non-spatial data on spatfall were available for the Narkwa and Benya lagoons, this study elicited more details by considering spatial dynamics, by depth and localities within the water bodies. The present study further covered two other water bodies, Densu Delta and Whin estuary, which have no published data on spatfall, growth and fouling for C. tulipa. Information on the bathymetry of the four water bodies (shallow systems) are also mapped for the first time to serve as a reference in selecting potential sites for oyster aquaculture in the selected water bodies.

6.2 Conclusions

The following conclusions are made from the present study:

Ceramic tiles, PVC and recycled oyster shells were more suitable in collecting *C. tulipa* spat than coconut shell. Ceramic tiles were most preferred by *C. tulipa* larvae followed by PVC, oyster shells and coconut shells in that order. Two-millimetre mesh nets used in this study did not harvest any *C. tulipa* spat.

C. tulipa spat settled profusely on the undersides of horizontally placed collectors whilst total spat yield increased when concave and rough/lamellate surface contours were positioned downward in the water column.

There were both spatial and temporal variation in the distribution of *C. tulipa* spat in the water bodies studied. Narkwa lagoon had the highest spatfall and therefore the most important source of *C. tulipa* spat followed by Densu Delta, Benya Lagoon and Whin Estuary, in that order. For all the collectors used in this study, those placed in mid-water to bottom water column harvested more *C. tulipa* spat than those at the top in all the water bodies.

With the exception of Benya Lagoon, the dry season (November 2017 – April 2018) was largely a more appropriate period for optimal harvest of *C. tulipa* spat. High growth rates of *C. tulipa* were recorded in middle to bottom water column compared to growth on collectors near the surface, with the best growth performance recorded in the Narkwa lagoon followed by Densu Delta and Whin Estuary. High stocking density (0.14 spat cm⁻² i.e. 1 spat per 7 cm²) produced 'market-size' *C. tulipa* in six months.

Dissolved oxygen and salinity were the most significant predictors for spatfall of *C. tulipa* whereas growth of the spat was most significantly affected by dissolved oxygen and turbidity.

With the exception of *Ficopomatus* sp., which caused disintegration of coconut shells and penetrated the shells oyster spat, there was no marked evidence of deleterious effects of biofouling on growth of spat in this study. *Fistubalanus pallidus* was the ubiquitous fouling organism and its extreme occurrence was out of phase with peak spatfall season in the Narkwa Lagoon. However, considering the fact that most of the fouling organisms are likely to compete with *C. tulipa* for space and food, a prudent management practice would need to be adopted.

6.3 Recommendations

In order to achieve optimum production (spat collection and culture) of *C*. *tulipa* under natural conditions in Ghana,

- Ceramic tiles, PVC slats and Oyster shells should be selected over coconut shells. However, for commercial scale, the PVC would be ideal, as its use would facilitate easy removal of spat for cultchless culture,
- 2. Collectors should be placed in mid-water or close to the bottom for optimum *C. tulipa* spat collection and growth, and
- Spat collection in Densu Delta should be done at Stations 1 and 2 and for Narkwa Lagoon at Stations 2 and 3. In both water bodies, this should be done from November to May

Pertinent questions remain unanswered, which could further explain observations in the present study, thus further studies are recommended to be undertaken to:

- 1. Assess the genetic variability of *C. tulipa* populations in coastal water bodies of Ghana as a precursor to comprehending the varied growth potentials observed in the different water bodies,
- Determine the nutrient load and critical constituents of the diet of *C*.
 tulipa in the different water bodies,
- 3. Elucidate the potential or otherwise of high stocking density for the intensification of *C. tulipa* aquaculture,
- 4. Confirm *C. tulipa* spatfall regime through extended studies on spatfall in various coastal water bodies,
- 5. Explore the suitability of other locally available materials for *C. tulipa* spat collection,
- 6. Assess the effect of artificial opening of lagoons on natural populations of oysters and availability of spat, and
- 7. Evaluate the transfer of spat among water bodies to form the basis for collecting and transplanting spat from high yielding water bodies.

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APPENDICES

APPENDIX A - SUPPORTING DATA

Table A1 – Mean (\pm S.E.) Surface Areas (cm²) of One Side of Coconut and Oyster Shells Used for Spat Collection in the Various Waterbodies

				S -	+ 1			C.	+ 2	
	uo			Se	t I			26	et 2	
	Stati	Water Column	Cocon	ut shell	Oyste	r shell	Cocon	ut shell	Oyste	er shell
		Тор	153.3	±3.1	118.3	±12.7	111.0	±3.1	80.0	±11.0
	1	Middle	153.3	±6.0	110.5	±9.9	106.0	±3.5	89.3	±8.7
		Bottom	154.2	±15.8	139.0	±15.8	125.7	±8.4	123.7	±13.2
ary		Тор	133.3	±18.1	109.0	±20.7	136.7	±2.3	119.7	±34.1
Estu	2	Middle	122.2	±12.0	119.0	±6.0	113.3	±0.9	86.0	±8.5
/hin		Bottom	153.3	±16.8	158.3	±17.6	134.7	±25.4	96.0	±6.0
*		Тор	165.0	±19.7	152.0	±29.6	109.0	±6.5	98.3	±23.2
	3	Middle	159.7	± 8.8	156.0	±28.0	134.0	±11.5	91.7	±1.2
	5	Bottom	136.7	±14.1	124.0	±7.1	144.0	±4.5	116.7	±8.7
		Тор	170.3	±12.9	86.0	±7.0	128.3	±15.9	116.7	±20.7
	1	Middle	151.0	±9.5	81.0	±8.7	119.0	±1.5	88.3	±5.2
		Bottom	121.3	±18.2	102.3	± 17.0	150.0	±9.0	114.7	±10.4
g001		Тор	122.3	±28.2	90.7	±20.8	120.0	±6.7	136.3	±38.5
a La	2	Middle	151.3	±13.1	104.0	± 17.4	127.7	±7.8	103.7	±16.1
eny		Bottom	144.7	±15.7	137.3	±37.6	148.3	±19.3	90.0	±13.5
Щ		Тор	147.7	±6.4	89.3	±10.2	114.7	±9.2	72.3	±2.3
	3	Middle	145.3	±5.8	98.3	±2.7	144.7	±28.6	75.0	±10.2
	-	Bottom	142.3	±9.3	199.7	±50.4	122.0	± 8.6	67.7	±6.8
		_								
		Тор	160.3	±24.7	88.0	±26.8	134.3	±19.4	96.3	±17.3
Ę	1	Middle	155.3	5.6	126.0	±15.0	126.7	±4.1	83.3	±11.6
1goo		Bottom	158.3	±20.4	114.3	±39.6	128.0	±24.1	107.3	±9.8
∕a Lá		Тор	195.3	±30.0	81.0	±19.9	105.7	±5.4	123.3	±7.9
arkw	2	Middle	146.3	±28.0	60.3	±22.6	119.7	±2.3	86.3	±19.3
ž		Bottom	147.7	±19.1	57.7	±1.2	94.3	±7.2	117.0	±11.3
	3	Тор	155.7	±12.4	91.0	±28.6	168.0	±25.8	98.0	±6.4
	5	Middle	171.7	± 42.4	79.3	±21.6	112.3	±6.1	126.7	±14.9

			Set 1			Se	et 2			
	Station	Water Column	Cocon	ut shell	Oyste	r shell	Cocon	ut shell	Oyste	er shell
		Bottom	164.7	±9.2	98.0	±32.1	133.7	±9.8	114.0	±14.0
stuary		Тор	125.7	±5.7	121.7	±25.6	134.3	±26.2	116.3	±21.9
ta Es	1	Middle	142.0	±10.3	168.0	±63.1	109.3	±7.9	99.3	±8.7
Dell		Bottom	163.0	±7.6	91.0	±10.1	116.7	±6.1	122.0	±12.6
iver		Тор	150.7	±15.6	94.0	± 18.8	131.3	±16.2	94.0	±11.0
su R	2	Middle	150.3	±7.3	67.3	±15.5	137.0	±19.5	103.3	±15.3
Den		Bottom	138.7	±4.8	56.7	±14.4	113.3	±3.7	129.3	±23.9

Surface Area of Tiles = 100 cm^2

Surface Area of PVC Slats:

PVC diameter = 4 inches = 10.16 cm

Circumference/perimeter = $2\pi r = 31.92$

Therefore, *Length* (*L*) = $31.92/3 = 10.64 \approx 10.6$ cm, and

Height (H) = 10 cm

Area (A) = $10.6 \text{ cm} \times 10 \text{ cm} = 106 \text{ cm}^2$

			D	epth (m)	
	Station	R 1	R2	R3	Average
Densu	1	0.52	0.76	0.58	0.62
	2	0.95	1.01	0.85	0.94
	3	1.82	1.76	1.51	1.70
Narkwa	1	0.26	0.35	0.38	0.33
	2	1.00	0.79	0.80	0.86
	3	0.69	0.79	0.73	0.74
Benya	1	0.73	0.69	0.71	0.71
	2	0.92	0.90	0.88	0.90
	3	1.24	1.04	0.96	1.08
Whin	1	0.98	0.88	1.00	0.95
	2	0.52	0.49	0.66	0.55
	3	0.46	0.43	0.41	0.43

Table A2 – Average Depths at Stations 1, 2 and 3 in the Water Bodies

APPENDIX B - ANOVA TABLES

Source of variation	df	Adj SS	Adj MS	F	Р
Collector	3	108	36	24.270	0.000
Water body	3	2129	710	477.150	0.000
Depth	2	717	358	240.870	0.000
Collector \times water body	9	10	1	0.740	0.675
Collector × depth	6	14	2	1.600	0.144
Station (water body)	7	98	14	9.450	0.000
Waterbody \times depth	6	147	24	16.410	0.000
Collector × station (water body)	21	21	1	0.660	0.878
Collector \times water body \times depth	18	14	1	0.530	0.943
Station \times depth (waterbody)	14	251	18	12.040	0.000
Collector \times station \times depth (waterbody)	42	19	0	0.310	1.000
Error	4620	6872	1		
Total	4751	10466			

Appendix B1 – Results of Three-Way ANOVA for Interaction between Collector, Water Body and Depth on Spatfall of C. tulipa

Appendix B2 – Tukey Simultaneous Tests for Differences of Means for C. tulipa Spatfall on Collector materials

Difference of Levels	Difference of Means	S.E. of Difference	Т	Adj. P
Densu estuary				
Oyster shell - Coconut shell	0.2511	0.0703	3.57	0.002
PVC - Coconut shell	0.1997	0.0698	2.86	0.022
Ceramic tile - Coconut shell	0.2568	0.0692	3.71	0.001
PVC - Oyster shell	-0.0514	0.069	-0.74	0.879
Ceramic tile - Oyster shell	0.0057	0.0684	0.08	1.000
Ceramic tile - PVC	0.0571	0.0679	0.84	0.835
Narkwa lagoon				
Oyster shell - Coconut shell	0.1202	0.0623	1.93	0.216
PVC - Coconut shell	0.1903	0.0628	3.03	0.013
Ceramic tile - Coconut shell	0.2001	0.0617	3.24	0.007
PVC - Oyster shell	0.0701	0.0626	1.12	0.677
Ceramic tile - Oyster shell	0.0799	0.0615	1.30	0.564
Ceramic tile - PVC	0.0098	0.062	0.16	0.999
Benya lagoon				
Oyster shell - Coconut shell	0.2807	0.05	5.61	0.000
PVC - Coconut shell	0.2974	0.0499	5.96	0.000
Ceramic tile - Coconut shell	0.3748	0.049	7.65	0.000
PVC - Oyster shell	0.0167	0.0496	0.34	0.987
Ceramic tile - Oyster shell	0.0941	0.0488	1.93	0.215
Ceramic tile - PVC	0.0774	0.0486	1.59	0.383
Whin estuary				
Oyster shell - Coconut shell	0.1731	0.0817	2.12	0.147
PVC - Coconut shell	0.2799	0.0826	3.39	0.004
Ceramic tile - Coconut shell	0.281	0.0798	3.52	0.002
PVC - Oyster shell	0.1068	0.0771	1.39	0.509
Ceramic tile - Oyster shell	0.1078	0.074	1.46	0.464
Ceramic tile - PVC	0.001	0.075	0.01	1.000

Table B3 – *Results of Four-Way ANOVA for Effects of Collector Surface* (*Upper and Under-Horizontal*) on Spat Settlement on Collectors through the 12 Months Duration of Study

Source of variation	df	Adj SS	Adj MS	F	Р
Collector surface	1	2502.6	2502.6	2572.2	0.000
Water body	3	3460	1153.4	1185.45	0.000
Month	11	5069.2	460.83	473.66	0.000
Collector	3	221.6	73.85	75.91	0.000
Collector surface \times water body	3	463.3	154.44	158.74	0.000
Collector surface \times month	11	98.5	8.95	9.2	0.000
Collector surface \times collector	3	166.3	55.43	56.98	0.000
Water body \times month	33	2913.7	88.29	90.75	0.000
Water body \times collector	9	32.3	3.58	3.68	0.000
Month \times collector	33	75.4	2.29	2.35	0.000
$Collector \ surface \times waterbody \times month$	33	324.6	9.84	10.11	0.000
Collector surface ×Waterbody × collector	9	41.3	4.59	4.72	0.000
$Collector \ surface \times month \times collector$	33	71.6	2.17	2.23	0.000
Water body \times month \times collector	99	154	1.56	1.6	0.000
Collector surface \times water body \times month \times collector	99	79.3	0.8	0.82	0.898
Error	9120	8873	0.97		
Total	9503	24209			

Source of variation	df	Adj SS	Adj MS	F	Р
Orientation	1	3.72	3.72	6.21	0.013
Collector	3	17.06	5.69	9.48	0.000
Collector Surface	1	37.36	37.36	62.30	0.000
Waterbody	1	68.69	68.69	114.55	0.000
Orientation × collector	3	6.62	2.21	3.68	0.013
Orientation \times collector surface	1	2.45	2.45	4.09	0.044
Orientation \times waterbody	1	10.17	10.17	16.95	0.000
Collector \times collector surface	3	5.51	1.84	3.07	0.029
Collector × waterbody	3	6.36	2.12	3.54	0.015
Collector surface \times waterbody	1	4.63	4.63	7.72	0.006
$Orientation \times collector \times collector \ surface$	3	4.80	1.60	2.67	0.048
$Orientation \times collector \times water \ body$	3	10.61	3.54	5.90	0.001
$Orientation \times collector \ surface \times waterbody$	1	0.00	0.00	0.00	0.951
Collector \times collector surface \times waterbody	3	1.25	0.42	0.69	0.556
Orientation \times collector \times collector surface \times water body	3	2.56	0.85	1.42	0.237
Error	256	153.51	0.60		
Total	287	335.297			

Appendix B4 – Results of Four-Way ANOVA for Effects of Orientation and Collector Surface on C. tulipa Spat Settlement on the Different Collectors in the Densu Estuary and Narkwa Lagoon

Appendix B5 – Tukey Simultaneous Tests for Differences of Means for C. tulipa
Spatfall on Upper and Under-Horizontal Surfaces of Collector materials and
for Orientation

Difference of Levels	Difference of Means	S.E. of Difference	Т	Adj. P
12 months of stud	у			
Densu estuary				
Upper - Under	-0.607	0.037	-16.49	0.000
Narkwa lagoon				
Upper - Under	-0.272	0.033	-8.38	0.000
Benya lagoon				
Upper - Under	-0.532	0.030	-17.69	0.000
Whin estuary				
Upper - Under	-0.255	0.049	-5.15	0.000
Collector material	s			
Coconut shell				
Upper - Under	-0.3349	0.0415	-8.07	0.000
Oyster shell				
Upper - Under	-0.2041	0.0345	-5.92	0.000
PVC				
Upper - Under	-0.432	0.039	-11.07	0.000
Ceramic tile				
Upper - Under	-0.4504	0.0388	-11.6	0.000
Confirmation in M	Iarch 2018			
Face down (0°)				
Densu estuary				
Upper - Under	-0.139	0.147	-0.95	0.349
Narkwa lagoon				
Upper - Under	-0.566	0.089	-6.39	0.000
Face up (180°)				
Densu estuary				
Upper - Under	-0.557	0.101	-5.49	0.000
Narkwa lagoon				
Upper - Under	-0.288	0.074	-3.91	0.000



Appendix B6: Factorial plot of interactions between collector material, surface and orientation on spat settlement



Source of variation	df	Adj SS	Adj MS	F	Р
Month	11	2905.7	264.2	817.18	0.000
Waterbody	3	2129.3	709.8	2195.78	0.000
Station (waterbody)	7	98.4	14.1	43.49	0.000
Depth	2	716.6	358.3	1108.46	0.000
Month \times waterbody	33	1636.5	49.6	153.41	0.000
Month \times station(Waterbody)	77	720.7	9.4	28.95	0.000
Month \times depth	22	70.1	3.2	9.86	0.000
Waterbody × depth	6	146.5	24.4	75.52	0.000
$Month \times waterbody \times depth$	66	351.6	5.3	16.48	0.000
Station \times depth (waterbody)	14	250.8	17.9	55.42	0.000
Month \times station \times depth (waterbody)	154	287.4	1.9	5.77	0.000
Error	4356	1408.1	0.3		
Total	4751	10466			

Table B7 – Results of a Four-Way Nested ANOVA for Effects of Spatial (Water Body, Station [Water Body] and Depth) and Temporal (Month) Factors on Settlement of C. tulipa Spat

Table B8 – Tukey Simultaneous Tests for Differences of Means for C. tulipa Spatfall at Depths (Near surface [top], middle [middle] and bottom [bottom])

Difference of Levels	Difference of Means	S.E. of Difference	Т	Adj. P
Densu estuary				
middle - bottom	0.0122	0.0566	0.22	0.975
top - bottom	-0.3354	0.0597	-5.62	0.000
top - middle	-0.3476	0.0599	-5.8	0.000
Narkwa lagoon				
middle - bottom	-0.1429	0.0509	-2.81	0.014
top - bottom	-0.2668	0.0555	-4.8	0.000
top - middle	-0.1239	0.056	-2.21	0.069
Benya lagoon				
middle - bottom	0.0546	0.0424	1.29	0.403
top - bottom	-0.1312	0.0442	-2.97	0.008
top - middle	-0.1858	0.0442	-4.21	0.000
Whin estuary				
middle - bottom	-0.2618	0.0565	-4.63	0.000
top - bottom	-0.559	0.117	-4.79	0.000

top - middle	-0.297	0.119	-2.49	0.034
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Table B9 – Tukey Simultaneous Tests for Differences of Means for Shell Heights of One-month Old C. tulipa, in the Different Water Bodies, on Different Collectors, and at Different Depths (Near surface [top], middle [middle] and bottom [bottom])

Difference of Levels	Difference of Means	S.E. of Difference	Т	Adj. P
Water body				
Narkwa Lagoo - Densu Estuary	0.195	0.006	30.16	0.000
Benya Lagoon - Densu Estuary	-0.173	0.007	-26.57	0.000
Whin Estuary - Densu Estuary	0.021	0.009	2.30	0.098
Benya Lagoon - Narkwa Lagoon	-0.368	0.006	-64.88	0.000
Whin Estuary - Narkwa Lagoon	-0.174	0.009	-20.41	0.000
Whin Estuary - Benya Lagoon	0.194	0.009	22.65	0.000
Depth				
middle - top	0.209	0.006	32.76	0.000
bottom - top	0.334	0.006	53.27	0.000
bottom - middle	0.125	0.006	22.55	0.000
Collector material				
Oyster shell - Coconut shell	0.059	0.007	7.91	0.000
PVC - Coconut shell	-0.015	0.007	-1.99	0.193
Tile - Coconut shell	0.044	0.007	6.07	0.000
PVC - Oyster shell	-0.074	0.007	-10.17	0.000
Tile - Oyster shell	-0.015	0.007	-2.12	0.147
Tile - PVC	0.058	0.007	8.36	0.000

Tukey Pairwise Comparisons (99% Confidence) for Monthly Shell Heights of C. tulipa Spat

Month	Ν	Mean	Grouping
Mar-18	1734	0.831	А
Apr-18	2782	0.816	А
Feb-18	2564	0.685	В
Oct-18	671	0.551	С
Aug-18	730	0.534	С
Jun-18	2401	0.497	C D
May-18	3113	0.479	D E
Jul-18	1678	0.442	E F

Nov-17	2456	0.424	F
Jan-18	2687	0.376	G
Sep-18	516	0.303	Н
Dec-17	2451	0.159	Ι

Means that do not share a letter are significantly different

Table B10 – Tukey Simultaneous Tests for Differences of Means for **Final Shell Heights (SH)** of Cultured C. tulipa between and within Water Bodies at Different Depths and on Different Collectors

Difference of Levels	Difference of Means	S.E. of Difference	Т	Adj. P
WATER BODIES				
APRIL (6 months)				
Narkwa Lagoon - Densu Estuary	12.70	1.08	11.77	0.000
Whin Estuary - Densu Estuary	-3.14	1.34	-2.34	0.051
Whin Estuary - Narkwa Lagoon	-15.84	1.31	-12.07	0.000
JULY (9 months)				
Whin Estuary - Densu Estuary	-5.89	1.70	-3.46	0.001
DEPTH				
Densu Estuary				
middle - top	10.50	1.65	6.37	0.000
bottom - top	5.98	1.63	3.67	0.001
bottom - middle	-4.52	1.51	-3.00	0.008
Narkwa Lagoon				
middle - top	7.93	1.50	5.29	0.000
bottom - top	6.75	1.53	4.42	0.000
bottom - middle	-1.18	1.51	-0.78	0.715
Whin Estuary				
bottom - middle	22.31	2.99	7.45	0.000
COLLECTORS				
Densu Estuary				
PVC - Ceramic tile	-1.51	1.86	-0.81	0.849
Coconut shell - Ceramic tile	-1.82	2.05	-0.89	0.812
Oyster shell - Ceramic tile	-6.08	1.80	-3.39	0.004
Coconut shell - PVC	-0.31	2.10	-0.15	0.999
Oyster shell - PVC	-4.57	1.84	-2.48	0.063

Oyster shell - Coconut shell	-4.26	2.04	-2.09	0.156
Narkwa Lagoon				
PVC - Ceramic tile	-7.65	1.75	-4.38	0.000
Coconut shell - Ceramic tile	-7.01	1.77	-3.96	0.000
Oyster shell - Ceramic tile	-1.43	1.71	-0.84	0.837
Coconut shell - PVC	0.64	1.80	0.35	0.985
Oyster shell - PVC	6.22	1.75	3.56	0.002
Oyster shell - Coconut shell	5.58	1.77	3.15	0.009
Whin Estuary				
PVC - Ceramic tile	-6.94	4.81	-1.44	0.478
Coconut shell - Ceramic tile	-24.22	8.76	-2.76	0.036
Oyster shell - Ceramic tile	-9.26	5.17	-1.79	0.286
Coconut shell - PVC	-17.29	8.56	-2.02	0.191
Oyster shell - PVC	-2.33	4.81	-0.48	0.962
Oyster shell - Coconut shell	14.96	8.76	1.71	0.328

APEENDIX C

Appendix C1 – Rainfall Data and Calculated Anomalies from November 2017 to October 2018 for Meteorological Stations within the Catchment of the Study

Station	Month	Rain (PP)	Rain (Anom)
Accra	Nov-17	52.58	2.08
	Dec-17	11.43	-39.07
	Jan-18	0.00	-50.50
	Feb-18	5.08	-45.42
	Mar-18	4.06	-46.44
	Apr-18	36.58	-13.92
	May-18	194.57	144.07
	Jun-18	91.45	40.95
	Jul-18	0.76	-49.74
	Aug-18	39.89	-10.61
	Sep-18	4.58	-45.92
	Oct-18	98.29	47.79
Saltpond	Nov-17	0.00	-50.50
	Dec-17	38.87	-11.63
	Jan-18	0.00	-50.50
	Feb-18	24.89	-25.61
	Mar-18	0.00	-50.50
	Apr-18	17.78	-32.72
	May-18	70.36	19.86
	Jun-18	48.52	-1.98
	Jul-18	4.83	-45.67
	Aug-18	198.12	147.62
	Sep-18	14.49	-36.01
	Oct-18	78.74	28.24
Takoradi	Nov-17	0.76	-49.74
	Dec-17	76.20	25.70
	Jan-18	0.00	-50.50
	Feb-18	69.59	19.09

	Mar-18	0.00	-50.50
	Apr-18	89.91	39.41
	May-18	218.95	168.45
	Jun-18	80.78	30.28
	Jul-18	24.63	-25.87
	Aug-18	28.20	-22.30
	Sep-18	54.86	4.36
	Oct-18	138.42	87.92
Average		50.50	0.00

Appendix C2: Photograph of researcher displaying C. tulipa cultured for six months in the Narkwa Lagoon

