

**INVESTIGATION ON PARASITES OF WILD DAY OCTOPUS (*Octopus cyanea*)
IN SELECTED INDIAN OCEAN FISHING SITES IN TANGA, TANZANIA**

PENDO LASKA MSONGOLE

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE MASTER DEGREE OF PARASITOLOGY OF
SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

ABSTRACT

This is the first study done in Tanzania concerning with parasites of octopus, aimed to investigate parasites in *Octopus cyanea* along the Indian Ocean of Tanga region in Tanzania. Information from fishermen was required so as to determine their awareness on parasitic infections in octopus. Twenty five per cent (25.3%) agreed on the presence of parasites in octopus skin and muscles but these were not observed during laboratory investigation. Gamogony and sporogony of *Aggregata sp* (Apicomplexa: Aggregatidae) were observed during histopathological examination of the digestive tracts of octopuses collected along Tanga coastal area. *Octopus cyanea* was infected with coccidian parasite, *Aggregata spp* with a prevalence 41.1% (23 of 56 hosts examined) and organs like liver and gills were found be infected. Oocysts were sub-spherical in the mucosa wall of the intestine and caecum measuring 263 - 279 μm . Sporocysts were smooth-surfaced, dark-staining, spherical, typically 10–15 μm wide, and contained 9–22 banana-shaped sporozoites with a size of 2 -6 μm long and 0.2- 0.3 μm wide. The coccidian infection in octopuses accompanied with replacement of the infected cells with sporocysts. Parasite infection of *Aggregata spp* was not significantly correlated with the weight of octopus, study site or sex of the host. Molecular analysis was used to confirm the parasite species in which 68% of samples were positive when PCR products visualized in 1.5% agarose gel. Molecular characterization revealed that the coccidian was the *Aggregata octopiana* after sequencing its 18S rRNA gene using designed *Aggregata* primers. Alignments revealed that the coccidian from *O. cyanea* resembled for 89% with *Aggregata octopiana* isolated from common Octopus in Spain. Despite of the presence of *Aggregata octopiana* in *O. cyanea*, the parasite does not cause any effects to consumers unless the octopus is infected with other epizootiological agent.

DECLARATION

I, Pendo Laska Msongole, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Pendo Laska Msongole
(MSc. Candidate)

Date

The above declaration is confirmed by;

Prof. E. N. Kimbita
(Supervisor)

Date

COPYRIGHT

No part of this thesis may be reproduced, stored in any retrieval system, or transmitted in any form or by any means without the prior written permission of the author or the Sokoine University Agriculture in that behalf.

ACKNOWLEDGEMENTS

The present dissertation represents the end of a long journey and one more step in my career. There are a few people to whom I would like to give special acknowledgement. First of all, I thank God for giving me an opportunity of pursuing the Master degree of Parasitology which was dream of my life.

Then, I would like to begin by expressing my deepest gratitude to my supervisor, Professor E. N. Kimbita (Sokoine University of Agriculture) and Professor R. S. Silayo (Sokoine University of Agriculture) for their advice and guidance throughout the research process and for introducing me to the wonderful world of parasites. My sincere gratitude to academic staffs in the Department of Microbiology, Parasitology and Biotechnology (Sokoine University of Agriculture) especially Dr. J. S. Nzalawahe for advice and support during my research. I also have special word to Professor Mwita J. Chacha and Dr. B. Nyundo (University of Dar es Salaam), for their advice on how I could reach my research goals and come out with good findings.

Special words for my employer and sponsor, the Permanent Secretary (Ministry Livestock and Fisheries) for giving me an opportunity to be sponsored by the SWIO Fish project operating under fisheries sector in Coastal Regions. Thanks so much, I believe their financial support on my study will help in developing the fisheries sector to the benefit of government of Tanzania.

I would also like to direct my sincere thanks to all laboratory technicians for their technical support during sample analysis. I do appreciate their time and effort they used to assist me until I managed to finish up my laboratory work.

A special word of gratitude is due to my husband Constantine, M. Lukanyima, parents, family and relatives especially my uncle Mr. Emmanuel Msongole, for their encouragement, advice and unconditional supports before and since I started my postgraduate studies. I would like also to thank my friends and staffs in Laboratories of parasitology, pathology and molecular biology for their effort and support.

Most sincere thanks to the Kwale, Sahare, Kigombe and Mkinga local governments, fishermen and fisheries Officers for allowing me to collect information in their villages and much for their cooperation during data collection and support they given about octopus fisheries and parasites.

DEDICATION

This master's dissertation is dedicated to my parents and my husband for advising, encouraging and supporting me during master degree application and study time. Also I dedicate it to SWIO Fish project for their financial support.

TABLE OF CONTENTS

| | |
|--|-------------|
| ABSTRACT | ii |
| DECLARATION | iii |
| COPYRIGHT | iv |
| ACKNOWLEDGEMENTS | v |
| DEDICATION | vii |
| TABLE OF CONTENTS | viii |
| LIST OF TABLES | xi |
| LIST OF FIGURES | xii |
| LIST OF ABBREVIATIONS | xiv |
| CHAPTER ONE | 1 |
| 1.0 INTRODUCTION | 1 |
| 1.1 Background..... | 1 |
| 1.2 Problem Statement and Significance of the Study..... | 4 |
| 1.3 Objectives | 5 |
| 1.3.1 Overall objective | 5 |
| 1.3.2 Specific objectives..... | 5 |
| CHAPTER TWO | 7 |
| 2.0 LITERATURE REVIEW | 7 |
| 2.1 Octopus Catch and Overexploitation | 8 |
| 2.2 Parasites in Octopus | 9 |
| 2.3 Protozoa..... | 9 |
| 2.4 Dicyemid Mesozoans | 10 |
| 2.5 Copepods and Isopods (Crustacean) | 11 |
| 2.6 Parasite Identification..... | 12 |

| | |
|--|-----------|
| 2.7 Parasite Biodiversity in Marine Environment | 13 |
| CHAPTER THREE | 15 |
| 3.0 MATERIAL AND METHODS | 15 |
| 3.1 Location and Duration..... | 15 |
| 3.2 Sampling Strategy and Sample collection | 16 |
| 3.3 Questionnaire Administration..... | 17 |
| 3.4 Parasite Examination..... | 18 |
| 3.4.1 Gross examination | 18 |
| 3.4.2 Laboratory work- examination for ectoparasites..... | 18 |
| 3.4.3 Examination of intestinal parasites (Endo-parasites) | 19 |
| 3.5 Tissue Parasites | 19 |
| 3.6 Tissue Processing and Histopathology..... | 19 |
| 3.7 Molecular Identification | 20 |
| 3.7.1 Isolation and purification of parasite | 20 |
| 3.7.2 DNA extraction | 20 |
| 3.7.3 DNA amplification | 21 |
| 3.7.4 DNA cloning and sequencing | 21 |
| 3.7.5 Phylogenetic analysis..... | 22 |
| 3.8 Data Analysis..... | 22 |
| 3.8.1 Questionnaire data..... | 22 |
| 3.8.2 Parasite infection | 23 |
| CHAPTER FOUR..... | 24 |
| 4.0 RESULTS..... | 24 |
| 4.1 Questionnaire Administration (Field survey) | 24 |
| 4.1.1 Profile of the interviewed fishermen and octopus dealers | 24 |
| 4.1.2 Perception of fishermen on parasite existence in octopus | 25 |

| | | |
|-------|---|-----------|
| 4.1.3 | Response on type of pathogen (parasite) occurring in octopus..... | 26 |
| 4.1.4 | Fishermen’s perceptions on octopus disease in general | 27 |
| 4.2 | Laboratory Analysis of Parasite Infestation | 27 |
| 4.2.1 | Gross examination | 28 |
| 4.2.2 | Fresh smear examination | 30 |
| 4.2.3 | Fresh squash examination | 31 |
| 4.2.4 | Prevalence of parasite infection | 32 |
| 4.3 | Histopathology Examination | 34 |
| 4.4 | Sporocysts Counting | 37 |
| 4.5 | Analysis Results..... | 37 |
| 4.5.1 | Prevalence of infection by individuals | 37 |
| 4.5.2 | Infestation between sites | 38 |
| 4.5.3 | Infection between male and female hosts | 39 |
| 4.6 | Molecular Analysis | 40 |
| | CHAPTER FIVE | 44 |
| | 5.0 DISCUSSION | 44 |
| | CHAPTER SIX | 52 |
| | 6.0 CONCLUSION AND RECOMMENDATIONS | 52 |
| 6.1 | Conclusions..... | 52 |
| 6.2 | Recommendations | 53 |
| | REFERENCES | 54 |

LIST OF TABLES

| | | |
|----------|---|----|
| Table 1: | General features of the respondents who participated in the survey. | 24 |
| Table 2: | Fishermen opinions on parasites in octopus expressed as percentage of respondents..... | 25 |
| Table 3: | Fishermen responses on kind of cause of disease harboured by octopus along the coastline of Tanga | 27 |
| Table 4: | Shows the number of octopus examined for parasites, prevalence and number of octopus infected with parasites..... | 32 |
| Table 5: | Details on octopuses examined, state of infection, body weight and location of octopus collection..... | 33 |
| Table 6: | Results for molecular analysis showing the positive and negative samples for the PCR reaction for 1F and 2R primers | 42 |
| Table 7: | GenBank reference sequences of <i>Aggregata spp.</i> used in the construction of phylogenetic trees..... | 43 |

LIST OF FIGURES

| | | |
|------------|---|----|
| Figure 1: | Maps showing the study sites..... | 16 |
| Figure 2: | Respondent’s opinions on existence of parasites as the question answered by different age group | 26 |
| Figure 3: | A macroscopic view of dissected <i>Octopus cyanea</i> showing heavy infection with Coccidian parasite in the intestine, white cysts (arrow)..... | 29 |
| Figure 4: | Photograph showing macroscopic view of an octopus intestine with a heavily infected condition. Note number of white cysts (arrows) infecting the intestine and caecum of the octopus..... | 29 |
| Figure 5: | (A &B) Light microscopic examination of the fresh smear of intestinal contents. | 30 |
| Figure 6: | Fresh squash preparation of the octopus caecum infected with coccidian parasites..... | 31 |
| Figure 7: | A fresh squash preparation of the intestine showing sporocysts with sporozoites inside (arrows) | 32 |
| Figure 8: | Macrogamete with spongy cytoplasm in the microvilli of the intestine of <i>Octopus cyanea</i> | 34 |
| Figure 9: | (A and B) H & E section showing the mucosal cells of the intestine replaced with large Oocysts containing number of sporocysts (X10). An arrow shows Oocyst within the intestinal villi | 35 |
| Figure 10: | (A and B) H&E stain section LM examination of the octopus gill infected oocysts of the coccidian parasite..... | 36 |
| Figure 11: | Detachment of the epithelial cell seen (arrow). | 36 |
| Figure 12: | Relationship between number of sporocysts in an individual host and the weight of the infected hosts..... | 37 |

| | | |
|------------|--|----|
| Figure 13: | Prevalence of parasite infestation in octopus among the sites where octopuses were collected (1- Kigombe, 2-Kwale, 3-Pangani, 4- Sahare) | 39 |
| Figure 14: | Variation on coccidian infection in male and female <i>Octopus cyanea</i> | 40 |
| Figure 15: | Photograph showing PCR amplification of <i>Aggregata</i> species. | 41 |

LIST OF ABBREVIATIONS

| | |
|----------------|---|
| bp | base pair |
| F | Female |
| FAO | Food and Agriculture Organization of United Nations |
| HS | Host sex |
| I | Infection |
| K | Kigombe |
| KW | Kwale |
| M | Male |
| NBF | Neutral Buffered Formalin |
| O | Octopus |
| OK | Octopus collected from Kigombe |
| OKW | Octopus collected from Kwale |
| OP | Octopus collected from Pangani |
| OS | Octopus collected from Sahare |
| P | Pangani |
| PAST programme | Paleontological Statistical Software |
| S | Sahare |
| SWIOFish | South West Indian Ocean Fisheries Project |
| TAFIRI | Tanzania Fisheries Research Institute |

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Octopus fishing activity conducted along the Indian Ocean coast of Tanzania plays an important role as a source of protein and income that may improve fishermen's livelihood (Jiddawi and Ohman, 2002). Octopus has been processed and sold in the local market, while some is being exported to Kenya, Middle East and Spain (Guard, 2009). Local coastal communities of Tanzania mainly deal with artisanal fishing for White-spotted octopus (*Octopus chromatus*), common octopus, (*Octopus vulgaris*) and Day Octopus (*Octopus cyaneus*) as their source of employment and income generation (Mshana and Sekadende, 2014, Guard 2009, Guard and Mgya, 2002) and protein source (Bultel *et al.*, 1950; Gestal *et al.*, 2007 and Estevez *et al.*, 1996). But the most commonly found and exploited species in the coastal area of Tanzania is the Day octopus (*Octopus cyanea*) (Guard and Mgya, 2002).

Octopus belongs to Kingdom Animalia, Phylum Mollusca, Class Cephalopoda, Order Octopoda, Family Octopodidae (Emery *et al.*, 2016); the genus *Octopus* consists of more than 100 species (Ignatius and Srinivasan, 2006). These are marine Mollusk (von Boletzky and Villanueva, 2014), preferring benthic and reef environment. They are distributed worldwide and some being reared as mariculture candidates especially *Octopus vulgaris* in countries like Spain (Mladineo and Jozić, 2005, Estefanell *et al.*, 2011 and Gestal *et al.*, 2002). Day Octopuses (*O. cyanea*) are mostly found in East Africa coast and Madagascar to south-eastern Asia and Hawaii (Benbow *et al.*, 2014; Guard and Mgya, 2002).

In fact, *O. cyanea* the major octopus species in Tanzania coasts has short life span (12 to 15 months), highly fecund and fast growing like other cephalopods. It is named as Day octopus because of its activeness during the day and inhabits in tropical and intertidal reefs of Indo-West Pacific from Hawaii to East Africa (Jade *et al.*, 2012). *Octopus cyanea* is a predatory cephalopod foraging and feed on other invertebrates such as crustaceans (Mshana and Sekadende, 2014). Female lays hundreds of thousands of eggs compared to other cephalopods (von Boletzky and Villanueva, 2014). The size of male and female *O. cyanea* differs at sexual maturity; female may reach 0.6kg to 5kg at spawning while male may reach 300g at sexual maturity (Van Heukelem, 1983). In this fact cannibalism can happen to the small size male octopus during mating whereby large female forage even to a male (Hanlon and Forsythe, 2008).

Their life spans usually ends after their reproduction and spawning process ends, whereby male dies few weeks after mating, while the female reach senescence stage or die soon after eggs hatched (Pascual *et al.*, 2010). Spawning is usually determined with the age of the female while on the other hand male stop growing when the suckers at the edge of the web enlarge (Heukelem, 1973). High number of mature octopuses usually occurs in June every year (Guard and Mgaya, 2002). Parental care is only done by female whereby eggs are protected by a female and it starves until hatching (Guard, 2009). *Octopus cyanea* spawns throughout the year and the male can mate with several female before it dies (Heukelem, 1973).

Octopus fishing activities have been increasing in recent years due to demand and price increase in local and international market (Sauer *et al.*, 2011). Growth of the global market leads to heavy fishing of *O. cyanea* in Tanzania and East Africa in general (Guard and Mgaya, 2002). It has been reported that unsustainable fishing of Octopuses and other

invertebrates is due to increase of global demands and markets (Eriksson *et al.*, 2012). The global markets needs of octopus are higher compared to the catch source which leads to illegal and unreported fishing.

Despite of Octopus's importance in the coast of East Africa, there is no enough information concerning with their threats like diseases, host-parasite relationship, life cycle and ecology. Most of the studies on Octopus diseases and parasites infections have been conducted in European countries. Coccidiosis is among of the most important disease reported in octopus leading into malabsorption syndrome (Pascual *et al.*, 2010). The disease caused by the protozoa which are intracellular parasite affects both wild and cultured octopus through food-web relationship (Gestal *et al.*, 1999, Vidal and Haimovici, 1999). The enteric infection do not directly cause the octopus death but it expose the octopus into risk a risk of being affected by other disease causing agents (Pascual *et al.*, 2007).

Apart from protozoa, also octopus has been parasitized by Isopods, Digenea, nematodes, cestodes and copepods (Pascual *et al.*, 2007). Dicyemida, Spirurid nematodes and *Cystidicola sp.* has been reported to cause infection in the stomach of octopus (Gonzalez *et al.*, 2003; Pascual *et al.*, 1996). Ectoparasites like copepods (*Octopicola spp* and *Pennella spp*) cause heavy pathological condition in gills. Parasite and cephalopods relationship is common in all oceans, all known cephalopod species has parasite which associate with (Pascual *et al.*, 2007).

The fact is that, despite of the increased octopus landing in East Africa in recent years, the number of researchers involved in this field is still low and even the number of published papers concerning with parasites or octopus diseases in Africa is limited in general.

Octopus diseases and parasites are not yet considered as an important issue in Indian Ocean even though parasite infects even cephalopods as reported by other researchers in other continents (Vidal and Haimovici, 1999). Parasitic infection (like coccidiosis) has been reported in octopus of the Mediterranean water, Atlantic and Pacific Ocean which lied much in common octopus (Gestal *et al.*, 2007). The *Aggregata* coccidian has been reported to infect the digestive tract of more than 13 species of octopus worldwide (Estevez *et al.*, 1996).

This study aimed to investigate the possibility of octopus species selected, *O. cyanea* hosting parasites, investigating the endoparasites and the ectoparasites along the coastal area of Tanga.

1.2 Problem Statement and Significance of the Study

Problem statement

Parasitic disease aetiology information in wild cephalopod population especially octopus in Indian Ocean is limited. Parasite infections have been reported to threaten growth rate in both wild and reared octopus worldwide. Many researchers reported parasites threatening only few wild and reared octopuses such as *O. vulgaris*, *O. maya* and some other cephalopods in European water. *O. cyanea* has been harbouring agents of diseases including parasites without being noticed that it lowers the quality of octopus product when it comes to international market. Limited studies have been done on the parasites of day octopus, *O. cyanea* or any other cephalopod in the Indian Ocean, coastal of Tanzania.

Significance of the study

This study aimed to identify parasites in *O. cyanea* which has emerged as an important fisheries product in Tanzania for exports and contributing to national income.

Investigation on parasites in *O. cyanea* was needed in order to improve the quality of product for export and for local consumption and deliver some information about octopus parasites in Indian Ocean water. Identifying parasites in these Octopuses will enable fisheries sector to design a new fisheries regulations which will guide the octopus processing industries to avoid the parasites in products.

The study on wild octopus parasites will also contribute new knowledge that will assist in planning ways for monitoring and managing diseases of reared octopus and other cephalopods in large scale aquaculture in coming years. In addition, this study will open a field and call for the Tanzania Fisheries Research Institute to conduct more research along the Indian Ocean coast of Tanzania concerning with octopus parasitic infections, octopus aquaculture development and catch statistic, in order to promote octopus fisheries, increase octopus value and amount of catch for the benefits of the fishing communities and national income development.

1.3 Objectives

1.3.1 Overall objective

- i. To investigate the parasites infecting *Octopus cyanea* along the Coast of Tanga, Tanzania.

1.3.2 Specific objectives

- i. To assess local people`s knowledge, attitudes and practices with respect to octopus diseases and parasites in Tanga coastal area.
- ii. To determine prevalence of endoparasites and ectoparasites infecting the *Octopus cyanea* along the coast of Tanga.

- iii. Molecular characterization of gastrointestinal coccidian parasite, *Aggregata sp* in *Octopus cyanea*.

CHAPTER TWO

2.0 LITERATURE REVIEW

Octopus cyanea is a diurnal, medium sized and major motile predator, which feed on other small marine organisms such as fish, worms, shrimps and crabs (Neto *et al.*, 2014). Octopuses catch their prey by mimic mechanism whereby they cope with the environment so that they are not recognized by their prey (Boyle and Rodhouse, 2005). Octopuses also defend themselves from enemies by crypsis and camouflage mechanisms.

Octopuses have been reported to have short life cycle and single spawn (Emery *et al.*, 2016). A female octopus lays thousands of eggs depending on species for instance *O. vulgaris* 500 000 while *O. cyanea* 700 000 (Gibson *et al.*, 2008). Eggs hatching depend on the environmental condition in terms of temperature and embryos develop faster in higher temperature. In *O. cyanea* for example embryo development takes about 20 to 36 days and female die within 10 days of the last hatch (Emery *et al.*, 2016).

Octopuses are a good source of proteins, vitamins and long-chain polyunsaturated fatty acids (Mshana and Sekadende, 2014; Neto *et al.*, 2014). Tanzanian coastal communities consume octopuses and other cephalopods as source of protein (Jiddawi and Ohman, 2002). Octopus has been consumed much by men because of local beliefs that, it has aphrodisiac effect (Mshana and Sekadende, 2014). Also octopuses are mostly preferred seafood in Asian countries, Spain and Japan (Castellanos-Martinez *et al.*, 2014).

Traditionally, coastal communities regarded octopus fishing as the source of income for women and children (Westerman and Benbow, 2013). Octopuses are collected by walking on lower intertidal reef flat or by snorkelling along the reef edge (Guard and Mgaya,

2002). Recently, men became involved due to increasing demand in countries like Spain, Japan, Argentina and Middle East (Rocliffe and Harris, 2016). The artisanal fishery of octopus is now a major fishing practices in the local coastal fishing communities of Tanzania and East Africa in general (Msuya, 2013).

2.1 Octopus Catch and Overexploitation

Octopus contributes about 12% of the world cephalopod catch though has been the most desirable fisheries products than other fish (Guard and Mgya, 2002). Octopus catch in Tanzania has increased by 160% from 1990 to 2012 and being a good octopus exporter in the year of 2012 with total of 47.3% per year compared to Kenya, Mozambique, and Madagascar as reported by Rocliffe and Harris (2016). Octopus reproduction has been threatened by bad fishing practices methods and overfishing (due to increase of market demand and tourism) along the Indian Ocean coast of Tanzania and other Western Indian Ocean coast countries, which in turn threaten the market of octopus. Illegal fishing of using dynamites, trawling and beach seine (the non- selective fishing gear), are destructive fishing techniques which disturb breeding site of octopuses (Guard, 2009, Jiddawi and Ohman, 2002).

Tanga coastal area has been reported to experience overfishing and having small fishing area which contain reefs where octopus seems to flourish (Guard, 2009, Jiddawi and Ohman, 2002). Small reefs site in Tanga coast has been contributing to unsustainable fishing. Tanga and Mtwara are the coastal areas reported with high catch but involved in octopus overfishing and with fishing undersized octopus (Guard, 2009). Overfishing includes even the small under sized octopus (<500g) which under Tanzania fisheries regulation are restricted. In addition, overfishing destroys nests of other marine fish without excluding other cephalopods. Octopus demand has been increasing in recent years

compared to finfish demand (Vidal *et al.*, 2014). Overexploitation is then the result of increase of market and demand of the cephalopod species especially octopus and cuttlefish (FAO, 2004). It has been reported that Spain, Japan and Italy are the major importers of octopus in Europe and Asia (Vidal *et al.*, 2014); they import tons of octopus from different countries including Tanzania and other East African countries (Kiwale, 2003). Octopus and other cephalopods species has been reported by fishermen to decline in the Coastal of Indian Ocean of Tanzania as the result of overfishing which is influenced by increase of octopus demand and market (Katikiro, 2014; TAFIRI, 2012).

2.2 Parasites in Octopus

Wild cephalopods usually host parasites like protozoan, dicyemids, crustaceans (copepods and isopods) and metazoans (Pascual *et al.*, 1996). All these parasites can be found in skin, gill, digestive tract, digestive gland (liver) and kidney (Sykes and Gestal, 2014; Gestal *et al.*, 1999). There is no history of parasite infection in *Octopus cyanea*, even though previous report described that all cephalopods harbours some parasites. The mostly reported parasites in cephalopods include protozoa, dicyemids and crustaceans (Pascual *et al.*, 1996).

2.3 Protozoa

The coccidian infections have been documented as big problem in both wild and farmed octopus (Gestal *et al.*, 2007). The coccidian, *Aggregata spp* (Protozoa: Apicomplexa) has been reported to be a main epizootiological agent in wild and reared squids and octopus species in European water (Mayo-Hernandez *et al.*, 2013, Gestal *et al.*, 2000). The protozoa cause major parasitic infection in reared common octopus in Europe (Pascual *et al.*, 2006), leading to malabsorption, growth retardation and expose both wild and common octopus to other stressors.

Life cycle *Aggregata sp.*

Aggregata sp are intracellular coccidian parasites which infects the gastrointestinal cells of octopuses and other cephalopods. Like some other coccidian parasites, *Aggregata sp* involve two hosts to complete their life cycle (Castellanos-Martínez and Gestal, 2013; Gestal *et al.*, 2007). The parasite life cycle complete with definitive host, cephalopods including octopuses. Intermediate host crustacean is where sexual (gamogony and sporogony) and asexual (merogony) stage happens in the digestive tracts of their hosts (Tedesco *et al.*, 2017; Gestal *et al.*, 2005). Octopuses become infected once they feed on the infected intermediate host (crustacean) such as crabs, shrimps (Betancor *et al.*, 2013).

The parasitic stage of the coccidian occurs as gamogony and sporogony in the digestive tract of octopus as the definitive host (Gestal *et al.*, 2002). A predatory octopus feed by means of foraging the intermediate host especially crabs and prawns (Betancor *et al.*, 2013). The intermediate hosts become infected when they feed on plant material, decaying organic matter, microorganisms, small shellfish and worms and other food contaminated with faeces of the infected octopus (Lima *et al.*, 2014).

The caecum has been reported to be the most infected portion of the digestive tract in octopus with the protozoan parasites. Coccidian infections can also spread to other tissues and muscles of octopus (Estevez *et al.*, 1996). They are very specific in terms of host species, so each *Aggregata sp* has its own specific host according to Gestal *et al.* (1999). Also heavy infection has been noted in reared cephalopods where the dose of parasites becomes heavier than in wild environment.

2.4 Dicyemid Mesozoans

The Dicyemids usually infects the renal appendages or kidney of cephalopods including octopuses, squids and cuttlefish (Sykes and Gestal, 2014) where they destroy the brush

border of their host's renal appendages. The parasites are widely distributed in almost all geographical locations without excluding the Indian Ocean (Castellanos-Martinez *et al.*, 2011). Up to this point about 122 dicyemids species have been described by different authors from different geographical localities (Castellanos-Martinez *et al.*, 2016). Furuya, *et al.* (1992) reported that, most of the *Dicyema spp* are usually found in large sized shallow water cephalopods that the size of cephalopods tends to be proportional to the number of parasites.

Four *Dicyema spp* have been reported in cephalopods, where two *Dicyema madrasensis* and *Dicyema octopusi* infects octopus of East Coast of India (Kalavati *et al.*, 1984) and nine species of dicyemids from different octopus species from Mexico (Castellanos-Martinez *et al.*, 2016). The parasites have been reported on the other hand to be beneficial to the host, since it enhance of ammonia excretion in cephalopods (Finn *et al.*, 2005). The parasite feed on their host's urine all the time when they are in their host renal sacs.

2.5 Copepods and Isopods (Crustacean)

Post-embryonic stage of copepods (genus *Pennella*) has been reported to cause heavy infections in octopus and other cephalopods gills and mantle cavity (Cavaleiro and Santos, 2014; Castellanos-Martinez and Gestal, 2013; Pascual *et al.*, 2007; Pascual *et al.*, 1996). Sometimes these ecto-parasites can be attached on the body surface of cephalopods like in the arms of octopuses and squids (De Baets *et al.*, 2015). Copepods impair the wellbeing of cephalopods by affecting respiratory activities of the gill. Heavy infestation of copepods in gills has been reported to affect the conditions of cephalopods at cellular, tissue, molecular and individual level (Pascual *et al.*, 2005). Isopods have been reported as the ecto-parasites and sometime endo-parasites on cephalopods mantle, body surface and

tissue. Isopods can invade the host tissue and cause damage of tissues (Pascual *et al.*, 2002).

2.6 Parasite Identification

Identification of *Aggregata* species has been based on differences in morphological features such as size and shape of sporogonial stages and host specificity (Castellanos-Martínez and Gestal, 2013). *Aggregata* oocysts is characterized by having a large number of sporocysts containing three to eight sporozoites compared to other coccidian oocysts (Gestal *et al.*, 2004). Gestal *et al.* (2000) used sporocysts number during *Aggregata sp* identification. Fresh squash examination of a caecum under a light microscope shows the sporocysts of coccidian as done by Gestal *et al.* (2000) and Gestal *et al.* (2004).

Molecular diagnostic techniques have been developed as a means for diagnosis, identification and characterizing *Aggregata sp* in octopus and other cephalopod (Castellanos-Martínez *et al.*, 2014). Molecular characterization has been done for confirmation of *A. octopiana* and *A. eberthi* of the common octopus by Castellanos-Martínez *et al.* (2014) by sequencing the parasite small subunit 18S rRNA gene of both using Polymerase Chain Reaction technique. *Aggregata sagittata* has been reported in cuttlefish (*Todarodes sagittatus*) of North-East Atlantic Ocean, *A. dobelli* in *Octopus dofleini* and *A. millerorum* in *Octopus bimaculoides* of North-East Pacific Ocean (Gestal *et al.*, 2000). *Aggregata kudo* has been also reported in cuttlefish, *Sepia elliptica* of North West Indian Ocean (Castellanos-Martínez *et al.*, 2014; Silas, 1985).

Other parasites like helminthes and arthropods have not been yet more discussed compared to protozoa, the coccidian. Only few of them described by Castellanos-Martínez, and Gestal (2013) and Pascual *et al.* (1996) in Common octopus, *Octopus*

vulgaris and other cephalopods using different diagnostic techniques. Apart from *A. octopiana* they also reported the third stage larvae of anisakis (nematode) parasite and ectoparasite copepods, in gills of octopus. The helminthes has been reported to use octopus and other cephalopods as their paratenic, secondary or third host to reach their definitive hosts (Castellanos-Martínez and Gestal, 2013).

2.7 Parasite Biodiversity in Marine Environment

Marine water comprises large number of the aquatic organisms due to its long term ecosystem stability compared to fresh water environment (Palm, 2011). The availability and large number of fish species and invertebrates including cephalopods in marine water relates with parasites biodiversity. Parasite species richness has been described in different ways depending on the author's opinions. Bio geographical modelling and host population has been reported to influence parasite diversity on the environment (Luque *et al.*, 2004). In fact, host-parasite relationship in marine environment play an important role as a driver for ecological function and structure (Gomez and Nichols, 2013). Palm *et al.* (1999) described about 191 metazoan parasites in marine organisms.

The susceptibility of marine fish and invertebrates to parasites like protozoa has increased due to factors like anthropogenic stressors (Palm, 2011; Wood *et al.*, 2010). All global oceans are now experiencing pollutants deposition due to increase of human population along the coastal areas (Palm, 2011), where some pollutants alter the immune system of marine organisms hence becomes more susceptible to parasites (Sures, 2004). For example, pollutants like diesel oil have been reported to cause fluctuations of some immunological parameters like haemoglobin and hematocrits in invertebrates like prawns (Carballeira *et al.*, 2012).

On the other hand, we can say human activities such as introduction of waste materials in the ocean have been contributing to changing the parasite diversity in the aquatic environment, as the marine habitats experience increase of parasite population (Wood *et al.*, 2010). Some waste material contains some parasites hence increasing parasite number in the sea. Industrial and aquaculture activities can also affect the nearshore ecosystems through wastewater from ponds hence increase number bacteria and other parasites (Carballeira *et al.*, 2012).

Ecological and behavioural features of octopuses and other cephalopods life cycles have been reported to be the main reason for their vulnerability to parasites (González and Sánchez, 2002). Octopus's life cycles are particularly the same if compared to the already discussed octopuses from European water. In that matter, the octopus from Indian Ocean water could experience the same vulnerability to parasite as octopuses of European water. González and Sánchez (2002) suggested that host external factors cannot be the important determinants for the octopus parasite diseases risk; this was the reason why this research was decided to be done in octopuses like *Octopus cyanea* of the Coast of Indian Ocean in Tanga to investigate the presence of parasites in them.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Location and Duration

Octopuses were purchased from the fishermen in four landing sites located in four districts of Tanga Region from December 2017 to January 2018. The study site were Kwale, Sahare, Kigombe and Pangani villages located in Tanga Region, (05°04', 39°06' E), which represents four District, Mkinga, Tanga city, Muheza and Pangani. The Tanga Region has only four coastal districts which were selected as the study area of this research. The four coastal districts are involved in fishing activities including octopus fishing.

Study design:

The study design used in the study was cross sectional study design, Purposive sampling, 4 landing sites, with a Sample size, n calculated as

$$\text{Sample size, } n = Z\alpha^2 \times p (1-q)^2 / d^2 \text{ (Charan and Kantharia, 2013).....(1)}$$

Where; $Z\alpha$ (from the table) at Type I error of $p=0.05 = 1.96$, d = effect size.

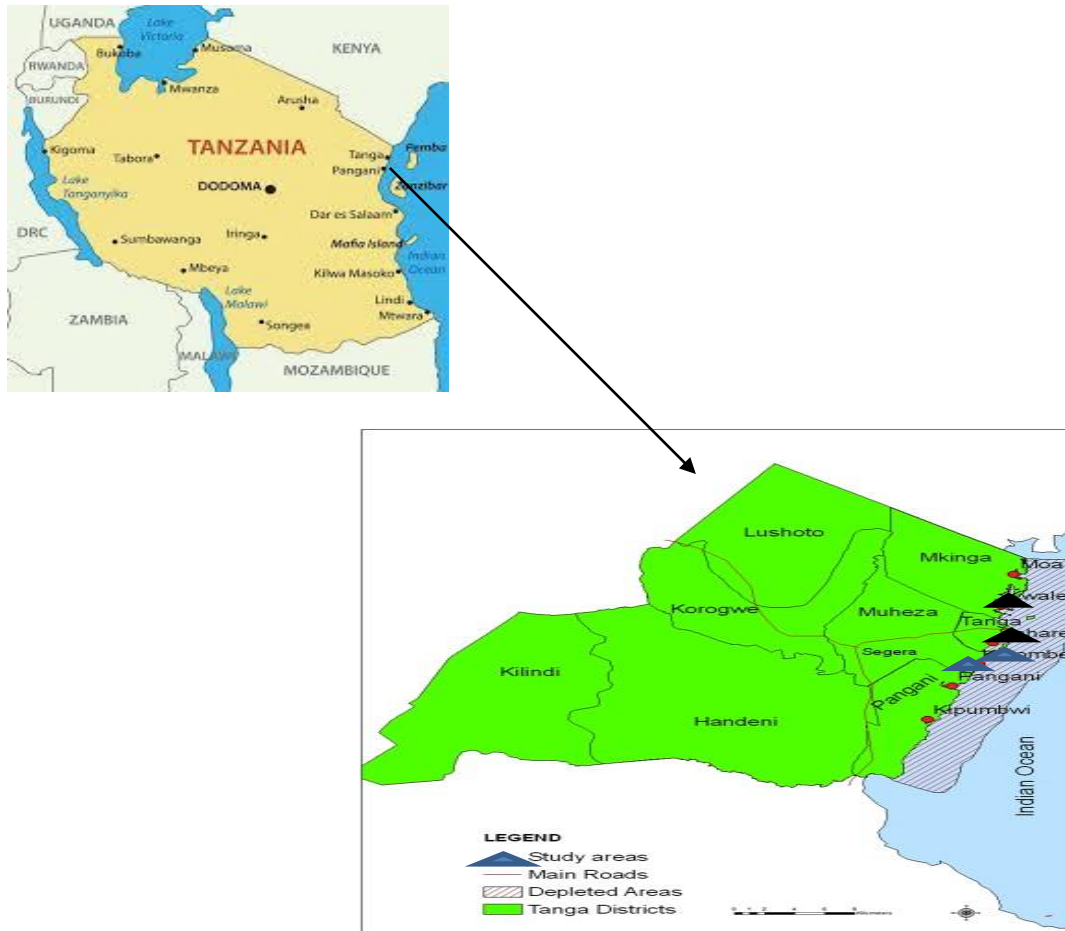


Figure 1: Maps showing the study sites

3.2 Sampling Strategy and Sample collection

Samples were collected in four districts which are actively involved in octopus artisanal fishing. Total of 56 octopus (about 12 octopuses from each site), *Octopus cyanea* with average weight of 400.51g from four districts (one coastal village per district), were collected purposively. Octopuses were caught by artisanal gear used by local fishermen. Fresh small piece kidney, liver and caecum from each octopus were taken after mantle dissection and fixed in 10% buffered formalin for histological purpose. Octopuses were then frozen for preservation then transported in a sterile cool box to the laboratory and thawed prior to necropsy at Sokoine University of Agriculture, Morogoro for parasite examination and identification.

3.3 Questionnaire Administration

A total of 156 fishermen were selected randomly at four villages Kwale, Sahare, Kigombe and Pangani for questionnaire. Thirty nine (39) fisherman from each selected site were required to answer prepared questions concerning with octopus parasites, fishing activities, habits of octopus consumption, type of octopus captured and processing activities. Sample selection was based on the age groups of the respondents as the inclusion criteria during sampling, where the age of fishermen was above 18 years old.

Although octopus fishing activities in these areas is seasonal, it was possible to visit fishermen and get some information concerning with octopus fishing and parasites. The reason of choosing these sites were to gain information on fishermen's perceptions on the presence of parasites on octopuses, which octopuses species they preferred, ways of octopus processing for consumptions and how they overcome any side effect caused by octopus consumption in human.

The structured questionnaire was given to respondent in their corresponding landing sites mainly near the coast when they had returned from fishing activities or during repairs and mending of their gear where it was easy to meet with them. Questions were in Swahili. For the fishermen who were not able to read and answer the questions on their own, each question was read to them to get some information about fishing activities of octopus and octopus parasites or diseases. The structured questions were both open and closed ended, the respondents were free to discuss during interview.

The questionnaires were about species of octopus, the presence of parasites (both ectoparasites and endoparasites), effects of the undercooked octopus to consumers and awareness of people on octopus parasite. The collected data were grouped according to the

group age of the respondents and sex. Few of the respondents were female at an age group of 30-53, they were mostly octopus dealers. During the survey, the questions were asked even about some infectious agents such as bacteria and fungus. Respondents were also required to give their opinions on effects of any parasites of octopuses to human being. Some questions were asked on the measures they took to prevent parasites, bacterial or fungal effects in octopus to human.

3.4 Parasite Examination

3.4.1 Gross examination

Macroscopic examination of parasites on the body surface and intestine for each octopus was done in the field after mantle dissection. The entire octopus mantle and body surface was observed for the ectoparasites and endoparasites. The octopuses were inspected well so as to observe each parasite. The mantle was cut with scissors to observe endoparasites which could be seen by naked eyes in the internal organs.

3.4.2 Laboratory work- examination for ectoparasites

Scrapings from the skin and gills of the octopus specimen were smeared on clean glass slides, covered with cover slides and examined under light microscopes for ectoparasites. Each sample was examined independently for parasites according to the protocol outlined previously by Obiekizie and Ekanem (1995). Skin scrapings and wet mounts from skin and gills were examined for abundance and distribution of larval stage of worms and other ectoparasites like leaches. Gills were taken into a petri dish with tap water and placed under dissecting microscope for larval worm examination.

3.4.3 Examination of intestinal parasites (Endo-parasites)

In the laboratory, octopuses were weighed using a weighing balance and their weight were recorded in grams (g). Octopuses were then dissected to get intestinal contents, caecum, liver, kidney and faecal sample. The intestinal contents of the weighed octopus were taken into a beaker, mixed with concentrated salt solution, filtered, poured into a test tube then covered with cover slip for floatation process to recover the parasites oocysts. After 10 minutes a cover slip was removed for microscopic examination. The intestinal contents were also examined for adult worms.

3.5 Tissue Parasites

In each dissected octopus, kidney, caecum and liver were isolated. Small pieces of liver, kidney and caecum were smeared on glass microscope slides, which were immediately fixed in methanol and some pieces stored in 70% ethanol. The smears were then stained in Giemsa solution (Bruno *et al.*, 2006), for coccidian oocyst and sporocysts examination. Squashing was the major means of detecting parasites from the octopus tissue. Squash preparations of fixed tissues from the intestine and caecum were examined by light microscopy and the size of the parasites were measured by using a calibrated ocular micrometer and are expressed in micrometers (μm).

3.6 Tissue Processing and Histopathology

Heavily parasitized tissue of gills, kidney, liver and caecum of some samples were fixed in 10% buffered formalin and then trimmed in processing cassettes. The trimmed kidney, gills, liver and caecum tissues in processing cassettes were fixed in 10% NBF for 48 hours, then processed routinely and embedded in paraffin wax using standard paraffin procedure. The standard paraffin process (tissue processing) implemented in this work moves specimens through a series of steps so the soft tissue is supported in a medium that

allows sectioning. The standard steps starts with fixation that preserves the tissue. This was followed by dehydration through graded ethanol (70%, 90% and absolute), clearing in chloroform and finally infiltration of the tissue with molten paraffin wax. It was followed by embedding that allows the orientation of the specimen in a block that can be easily sectioned and also makes it easy to handle and to store. Then sectioning was done using a rotary microtome (Baird and Tatlock) to produce 4 μm thin sections that were placed on paraffin section mounting bath (Electrothermal) microscope slides. Sections (4 μm) were stained with H & E following standard procedures (Culling *et al.*, 1985). These sections were dried overnight in paraffin oven (Electrolux) at 50°C.

3.7 Molecular Identification

3.7.1 Isolation and purification of parasite

The infected digestive tract of *Octopus cyanea* were dissected and homogenized in 10 ml of distilled water 1% Tween20 using motor and paste. Tissue homogenates were filtered twice with nylon meshes of 100 μm and 41 μm , respectively, to remove tissue fragments, and then concentrated NaCl was added to make the sporocysts and oocyst float. The mixture was then centrifuged at 1000 x g for 5 min in a centrifuge KUBOTA S100. The sporocyst were purified by density gradient centrifugation method according to Gestal *et al.* (1999) and preserved in 70% ethanol for DNA extraction.

3.7.2 DNA extraction

Genomic DNA was extracted from *Aggregata* parasite sporocysts isolated from the digestive tract of *Octopus cyanea* using ZYMO Research DNA extraction kit following the manufacturer procedures. Sporocysts were suspended in 500 μl of extraction buffer (NaCl 100mM, EDTA 25mM pH 8, SDS 0.5%) and opened by sonication on ice (5 cycles, 40W, 50 s) to release sporozoites. After Proteinase K (Sigma) digestion (1mg ml⁻¹) at

37°C overnight, the DNA was purified following alcohol extraction method, as described by Sambrook *et al.* (1989). DNA was precipitated with ethanol and sodium acetate overnight at -20 °C. The precipitated pellet was resuspended in 50 µl of Tris-EDTA (TE) buffer.

3.7.3 DNA amplification

The small subunit 18S rRNA gene with the size of 970 bp of extracted coccidian was amplified by PCR using conserved primers designed for *Aggregata spp.* (Kopečná *et al.*, 2006) and derived from GenBank sequences: (*Aggregata* 1-F: 5'-ATGATGAAACTGCGAAGAGC-3'; *Aggregata* 2-R: 5'-CGACGGTATCTGATCGTCTT-3'); PCR reactions were performed in a total volume of 25 µl containing 1 µl of 10mM dNTP mix, 0.25 µl Taq DNA polymerase (Roche), 2.5 µl Taq10x buffer, 1 µl 2.5 mM MgCl₂, 1 µl of each primer and 1 µl of template DNA at 100 ng µl⁻¹. The temperature profile for primers 1-2 included an initial denaturation at 94°C for 10 min; 35 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 10 min. For primers 3-4, we used an annealing temperature of 55°C. PCR products were separated on 1.5% agarose gels, stained with Gel Red including a 100-bp ladder size standard (Invitrogen) and visualized using UV transilluminator.

3.7.4 DNA cloning and sequencing

PCR products were cloned using TOPO® Cloning Kit (Invitrogen) according to the protocol supplied by the manufacturers and transformed in TOP 10 F' competent bacteria *Escherichia coli* (Invitrogen). Screening of clones carrying 18S rRNA-coding region fragments was performed by PCR adding the positive colony directly to the PCR mixture reaction using the corresponding *Aggregata* primers. Positive clones were purified by Microcon-centrifugal filter YM-50 (Millipore).

The purified PCR products were bi-directionally sequenced using BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) following manufacturers procedures, using the 15 positive sequences from PCR. DNA sequencing was performed on an ABI 3500XL DNA Analyser (Applied Biosystems), which uses a 24-capillary at Mbeya Zonal Referral Hospital. The sequenced fragment from clones was then assembled and arranged together using Genius Software into consensus sequences which were then entered in a Genebank for alignment with other sequences of *Aggregata spp.*

3.7.5 Phylogenetic analysis

The aligned sequence products were used to construct a phylogenetic tree using sequences of other Apicomplexa taxa available in Genebank so that to determine the species of the *Aggregata* isolated from *O. cyanea*. The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis version 7.0 (MEGA-7 software) (Kumar *et al.*, 2016).

3.8 Data Analysis

3.8.1 Questionnaire data

Data from survey were collected, entered and stored into the spread sheet using Microsoft Excel version 12 of 2007 and analysed using a Statistical Package for Social Sciences (SPSS) version 16.0. Qualitative descriptive analysis was used to describe the awareness of respondents on octopus parasites. Chi-square test was done to test the goodness for the observed frequencies such as the number of respondents on parasite existence yield statistical difference with age group of fishermen at a p-value 0.05.

3.8.2 Parasite infection

To describe parasite infection in *Octopus cyanea*, the prevalence of parasite infection was calculated according to Mitchell *et al.* (2000).

$$\text{Prevalence} = \frac{\text{number of individuals of hosts infected with parasites}}{\text{Number of hosts examined}} \times 100 \dots \dots \dots (2)$$

Data for parasite infection were collected and stored in Microsoft Excel version 12 of 2007. For normally distributed data One way ANOVA (performed using PAST programme) used to compare the means differences of parasite infection between sites. Two sample t-tests was performed to determine if there was statistical significance difference of parasite infection between host's sex (male and female). Linear regression analysis was carried out to determine if there is significant relationship of rate of infections between individuals (r applied to determine the degree of association between parasite intensity and host-related factor such as octopus weight). Quantitative analysis performed for statistical significant of ectoparasites and site of infection. A P- value of ≤ 0.05 was considered statistically significant. Parasite population between sites of sample collection were analysed using prevalence (P, %) of infection as a parameter according to Mitchell *et al.* (2000). DNA sequence data analysis was done using the Predictive Analysis Software PASW Statistics 19.0 statistical program. The results were presented as percentage.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire Administration (Field survey)

4.1.1 Profile of the interviewed fishermen and octopus dealers

A total of 156 fishermen from four study sites were interviewed. Most of the respondents were men (94.87%) and the rest were women (5.12%). The respondents mean age was 40.07 years and majority of them were in age ranging 31-53years. According to their response on filling the questionnaire, it was observed that only 32% were able to read and answer the questions educated and able to read the questionnaire (25% primary education, 7% secondary education) while 68% were not educated and unable to read the questionnaire, that was one of the challenges on getting information from them. Majority of the fishermen along the Tanga coastline were found to have poor knowledge of parasites in general, meaning they did not know the meaning of parasites. 80% of the fishermen responded that they fished only one species of octopus which is *Octopus cyanea* even though they don't know the name of the octopus they fish. Most fishermen identified octopus by local name "pweza".

Table 1: General features of the respondents who participated in the survey.

| Characteristic | Sampling site N (% of respondents) | | | | Total |
|------------------|------------------------------------|-----------|-----------|-----------|------------|
| | Sahare | Kigombe | Pangani | Kwale | |
| Age group | | | | | |
| Young (18-30) | 7(17.1) | 11(26.8) | 16(35.6) | 10(34.5) | 44(28.2) |
| Middle (31-53) | 23(56.1) | 20(48.8) | 24(53.3) | 16(55.2) | 83(53.2) |
| Old (>54) | 11(26.8) | 10(24.4) | 5(11.1) | 3(10.3) | 29(18.6) |
| Sex | | | | | |
| Male | 39(95.1) | 41(100) | 41(91.1) | 27(93.1) | 148(94.9) |
| Female | 2(4.9) | - | 4(8.9) | 2(6.9) | 8(5.12) |
| | 41 | 41 | 45 | 29 | 156 |

4.1.2 Perception of fishermen on parasite existence in octopus

The results from fishermen and octopus dealer's response were grouped according to the respondent age and sex. Age and sex was used as one of the criteria to get information about octopus parasites along the coast of Tanga Region. According to the data obtained from respondents, it seems the issue of parasites in octopus is not well known especially among older fishermen as shown in table 2 below followed by young fishermen with age 18-30.

Table 2: Fishermen opinions on parasites in octopus (n=156) expressed as percentage of respondents

| Characteristic | Parasite status (% of respondents) | | | Total |
|------------------|------------------------------------|-----------|------------|------------|
| | Present | Absent | Don't know | |
| Age group | | | | |
| Young (18-30) | 2(4.54) | 23(52.45) | 19 (43) | 44 |
| Middle (31-53) | 5(6.02) | 42(50.6) | 36(43.4) | 83 |
| Old (>54) | 8(2.8) | 10(34.5) | 11(37.9) | 29 |
| Sex | | | | |
| Male | 13(86.7) | 69(92) | 66(100) | 148 |
| Female | 2(13.3) | 6 (8) | 0(0) | 8 |
| | 15 | 75 | 66 | 156 |

Fishermen in the middle age group had some experiences on octopus parasite compared to young one and some elders had given some good information on octopus parasites than young age groups. During survey we observed that, long time octopus fishing and processing by the fishermen gave them an understanding of some abnormal conditions in octopus including presence of diseases. Elders were experienced in fishing and it was expected that they could give some good information about parasite infections and diseases in octopuses.

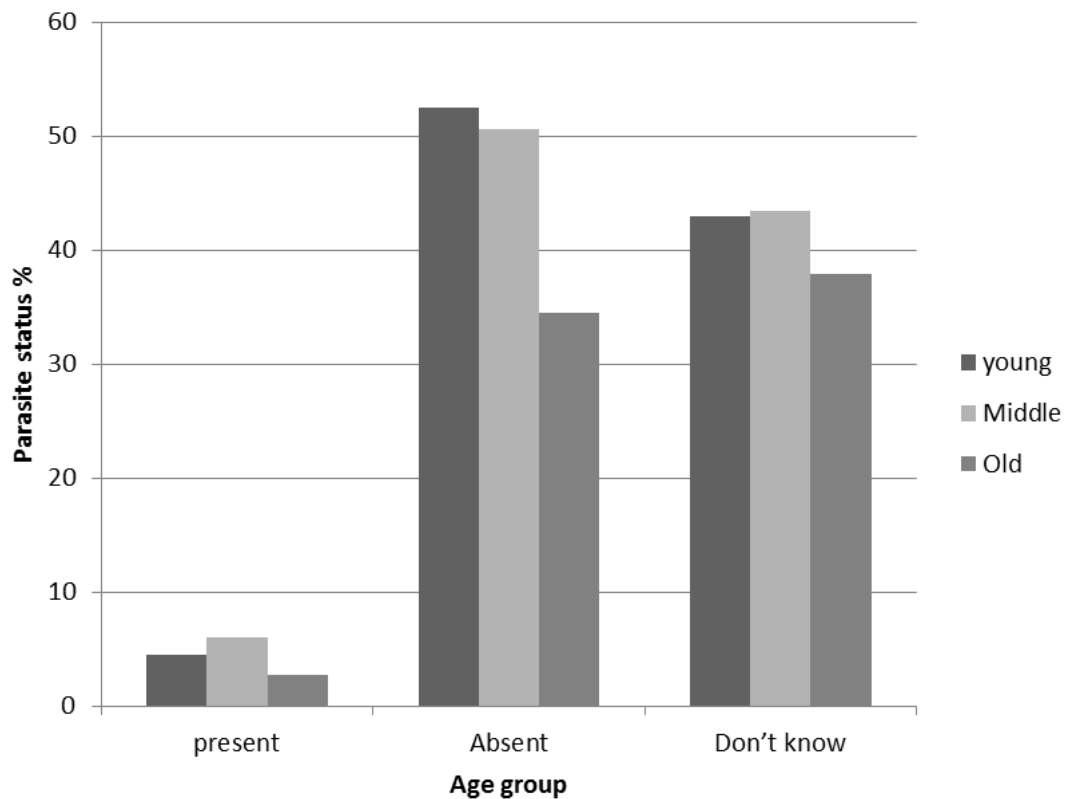


Figure 2: Respondent's opinions on existence of parasites as the question answered by different age group

In Figure 2, respondents were asked if octopus they catch harbours or there is any observable parasite. Majority of respondents 97/156 didn't know or thought octopus does not harbour parasites; while a small minority agreed to be aware of parasites on octopus.

4.1.3 Response on type of pathogen (parasite) occurring in octopus

Majority of fishermen in each age group had no idea on the kind of parasite infecting octopus. It was difficult to get information from them concerning this question. Fishermen were asked to describe the appearance of any parasites they saw in octopus during fishing and processing. The respondents were also required to answer if the parasites have any effects to consumers.

Table 3: Fishermen responses on kind of cause of disease harboured by octopus along the coastline of Tanga

| Age group | Cause of disease (n=156) | | | | Total |
|---------------|--------------------------|----------|----------|------------|------------|
| | Worms | Bacteria | Fungus | Don't know | |
| Young (18-30) | 6 | 0 | 0 | 41 | 47 |
| Middle(31-53) | 5 | 1 | 3 | 71 | 80 |
| Elders (>54) | 3 | 1 | 1 | 24 | 29 |
| Total | 14 | 2 | 4 | 136 | 156 |

4.1.4 Fishermen's perceptions on octopus disease in general

Fishermen and octopus dealers were asked to mention and describe any disease in octopuses they have ever seen. The responses to the question were recorded based on the age group of the respondents. The respondent age group was statistically significance at 0.05 levels ($X^2 = 2.72$, $p = 0.033$) to octopus disease status.

4.2 Laboratory Analysis of Parasite Infestation

Among of fifty six (56) *Octopus cyanea* collected from four study sites examined for parasites, only 41% (23) of octopuses were infested with parasitic protozoa, the coccidian *Aggregata spp*, all of which were found in the octopus digestive gland (Liver), kidney and intestine. Table 4 shows octopus samples examined and the number infested at each selected study site. Cysts were found on the octopus body, digestive tract especially the cecum.

96% of the infected octopuses weighed above 500g, while 89.3% octopus below this weight were found uninfested by any parasites. The bigger the size of octopus the higher was the probability of having parasite especially gastrointestinal protozoan increased. Octopuses with large weight had many cysts observed in the intestine than octopuses with small body weight.

External lesions were observed in some octopuses while some of them had been injured with their arms being chopped. The lesions could be a result of ectoparasite invasion having attached, fed and detached leaving some wounds on their host's body. Fishermen have blamed the predator eel fish to be the main reason of causing lesions and cutting the arms of octopuses.

Histopathological evaluation of octopus intestine, liver, kidney and gills shown substitution of tissues with developed oocysts with sporocysts and gametes in some tissues. The gametes were mostly observed in the intestinal mucosa especially the caecum, while no gametes were found in the gills, liver and kidney. Figure 8, 9, 10 and 11 represents the tissues in the intestinal mucosa substituted by large number of oocysts. Histological sections of the kidney and liver shown little infections with protozoa compared to that of digestive tracts especially a caecum. The mucosa of many infected octopuses was found to have lesions caused by invasion of the protozoa oocysts which replaced the host mucosal cells.

4.2.1 Gross examination

White cysts containing *Aggregata octopiana* were commonly found by gross observation in the wall of digestive tracts of some octopuses sampled from Kwale, Pangani, Kigombe and Sahare (Figure 3 and 4).

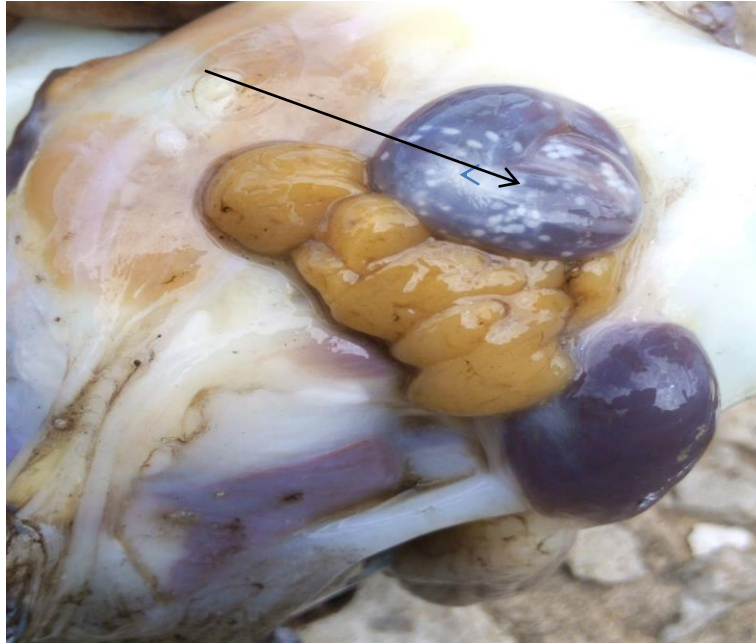


Figure 3: A macroscopic view of dissected *Octopus cyanea* showing heavy infection with Coccidian parasite in the intestine, white cysts (arrow)



Figure 4: Photograph showing macroscopic view of an octopus intestine with a heavily infected condition. Note number of white cysts (arrows) infecting the intestine and caecum of the octopus

The cysts were clearly observed with naked eyes in the field soon after opening the mantle. Most of the cysts were seen in the intestines of the *Octopus cyanea* especially in the cecum. In figure 3 and 4 cysts were seen as white spots in the wall of the intestine and sometime they were observed in the digestive gland of heavily infected octopus. Apart from the observable white cysts, there was no any other parasite observed in the surface of the mantle or intestine. No ectoparasites found during examination of parasites in field and laboratory tissue parasite examination. Fresh smear of eyes, skin and gills showed no worms whether in larval stage or adult stage were found in octopuses collected from all selected study sites.

4.2.2 Fresh smear examination

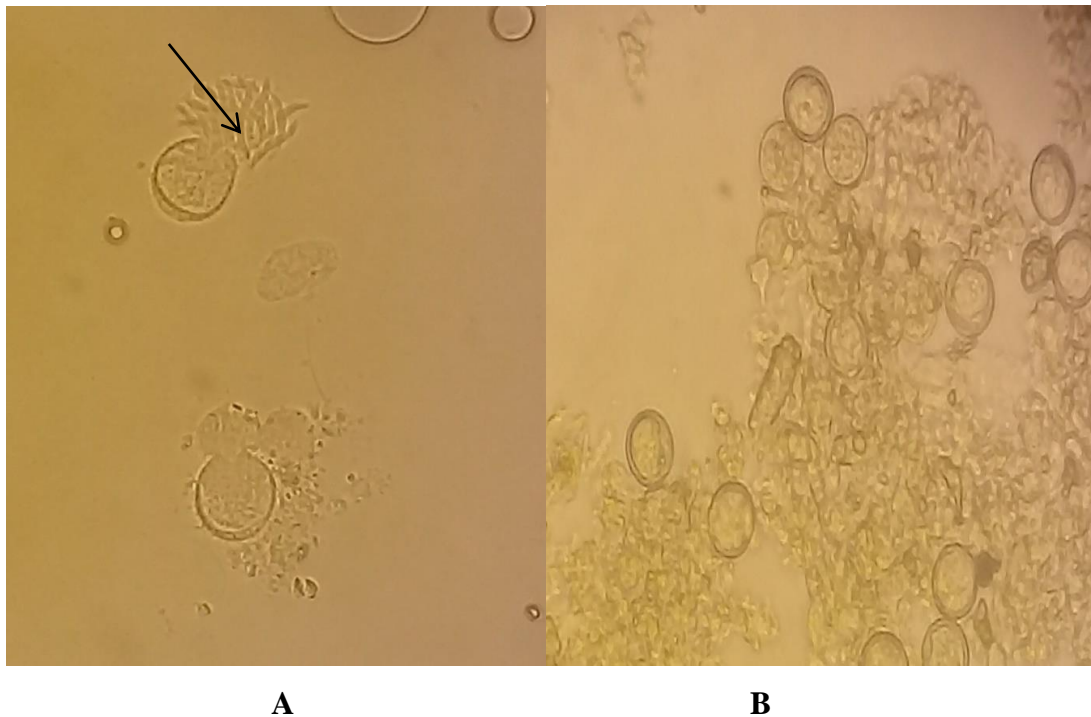


Figure 5: (A &B) Light microscopic examination of the fresh smear of intestinal contents.

A- showing the sporocyst ruptured to release the banana-shaped sporozoites. **B** - Several sporocysts with sporozoites inside

In fresh smear preparation, it was found some of the sporocysts had already ruptured as shown in Figure 5(A) with the sporozoites being easily seen with light microscope with different magnifications. The sporocysts were ovoidal and smooth containing large number of sporozoites.

4.2.3 Fresh squash examination

The results for Giemsa stain squash preparation of the intestine and caecum showed the spherical in shape sporocysts, the size ranging 10-15 μ m containing sporozoites (Figure 6 and 7). The Light Microscope observation of fresh tissue squash showed that intestine and kidney to be infected with parasites while the liver was less infected.

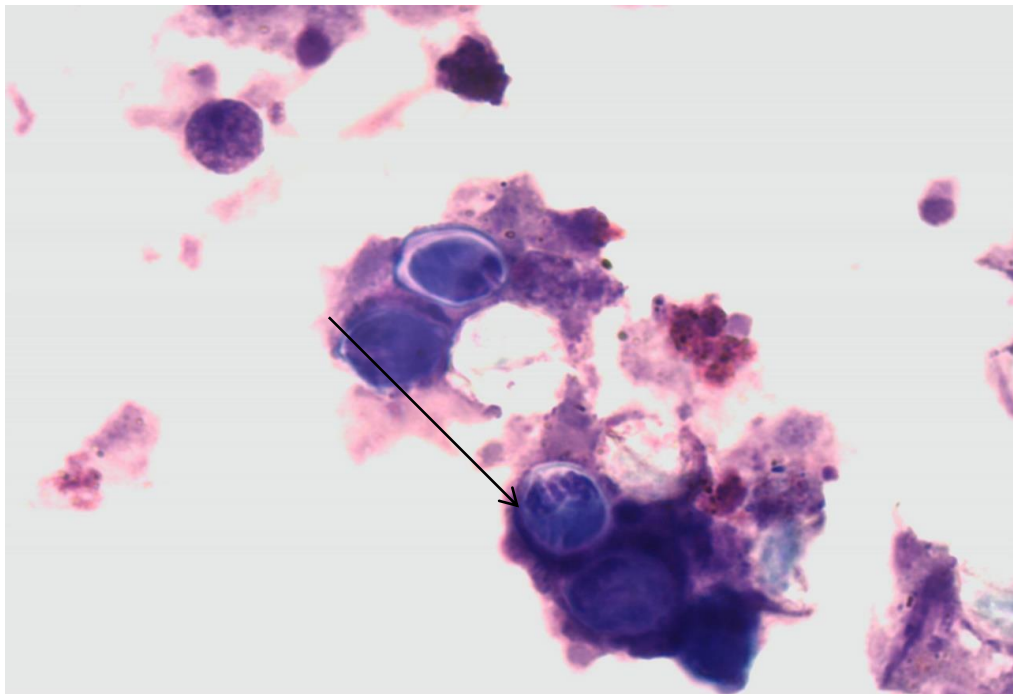


Figure 6: Fresh squash preparation of the octopus caecum infected with coccidian parasites. The figure show the sporocysts with sporozoites (arrow)

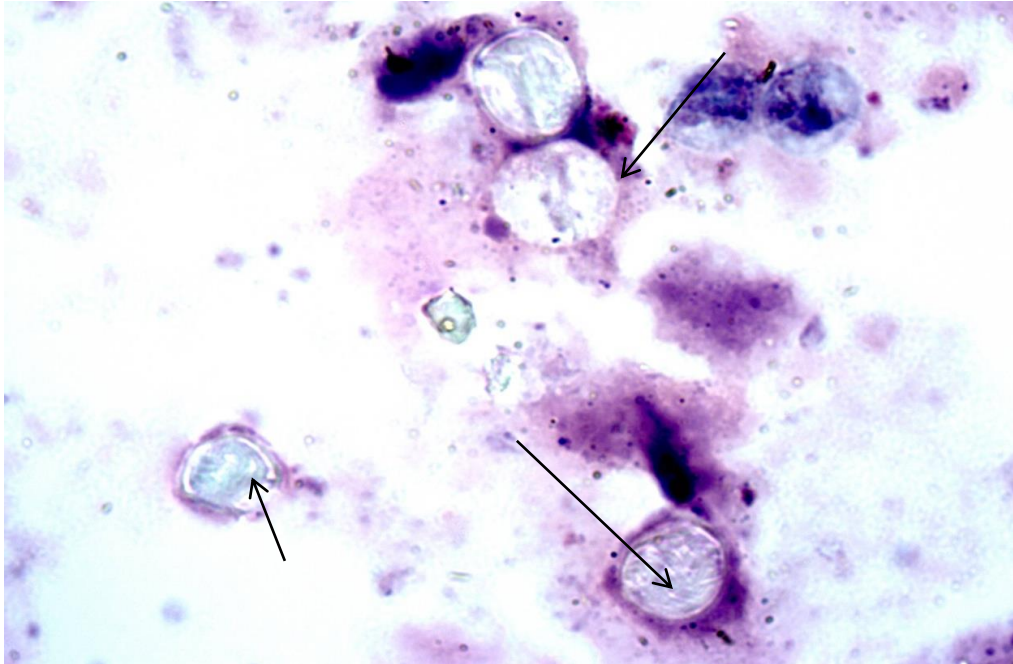


Figure 7: A fresh squash preparation of the intestine showing sporocysts with sporozoites inside (arrows)

4.2.4 Prevalence of parasite infection

Parasite infection was predicted by observing on the prevalence of parasites in all the four sites where octopuses were collected. The prevalence of parasites was calculated after light microscope examination for the parasite using smear, squash preparation and histopathology examination. The results were grouped depending on the number of host examined with respect to the site where sample were collected. All prevalence rates were tabulated as shown in the Table 4 below.

Table 4: Shows the number of octopus examined for parasites, prevalence and number of octopus infected with parasites

| SITE | No. examined | No. infected | Prevalence % |
|--------------|--------------|--------------|--------------|
| Kwale | 14 | 5 | 35.7 |
| Kigombe | 15 | 8 | 53.3 |
| Sahare | 13 | 3 | 23.1 |
| Pangani | 14 | 7 | 43.8 |
| Total | 56 | 23 | 41 |

Table 5: Details on octopuses examined, state of infection, body weight and location of octopus collection

| Host No. | BW (g) | Locality | HS | I | No. sporocyst/g | Site of infection |
|----------|--------|----------|----|---|-----------------------|-------------------------|
| O 3 | 387 | KW | F | + | 2 x 10 ³ | caecum |
| O 5 | 586 | KW | F | + | 1.2 x 10 ³ | Digestive tract |
| O 1 | 962 | S | M | + | 3 x 10 ³ | Digestive tract |
| O 4 | 1532 | KW | F | + | 8 x 10 ² | Digestive tract |
| O 5 | 1540 | K | F | - | | |
| O 6 | 442 | K | M | - | | |
| O 8 | 261 | P | M | - | | |
| O 7 | 253.5 | KW | M | - | | |
| O 9 | 567 | P | F | + | 5 x 10 ² | Caecum |
| O 10 | 262 | P | M | - | | |
| O 2 | 317 | P | F | - | | |
| O 1 | 217.6 | K | F | - | | |
| O 5 | 255.4 | P | F | - | | |
| O 1 | 250 | P | M | - | | |
| O 7 | 261 | P | M | - | | |
| O 2 | 263 | S | M | - | | |
| O 9 | 567 | KW | M | - | | |
| O 3 | 341.8 | P | M | + | 6 x 10 ³ | Caecum |
| O 4 | 512 | K | M | - | | |
| O 6 | 442 | KW | F | - | | |
| O 11 | 511.4 | P | M | + | 8 x 10 ² | Digestive tract |
| O 6 | 347 | S | F | - | | |
| O 3 | 712 | K | F | + | 1.6 x 10 ³ | Caecum |
| O 7 | 422 | K | F | + | 6 x 10 ² | Digestive tract |
| O 1 | 387 | KW | M | - | | |
| O 10 | 262 | KW | M | - | | |
| O 4 | 267 | S | F | - | | |
| O 3 | 253 | S | M | - | | |
| O 2 | 337 | K | F | + | 8 x 10 ³ | Digestive tract |
| O 6 | 250.5 | P | F | + | 3 x 10 ³ | Digestive tract |
| O 10 | 312.8 | S | M | - | | |
| O 9 | 251.4 | K | F | - | | |
| O 7 | 560 | S | M | - | | |
| O 12 | 712.6 | K | M | + | 5 x 10 ³ | Kidney, Digestive tract |
| O 11 | 461 | S | F | - | | |
| O 13 | 502.3 | P | M | + | 1 x 10 ⁴ | Digestive tract |
| O 14 | 281 | KW | F | - | | |
| O 15 | 637 | P | F | + | 4 x 10 ³ | Digestive tract |
| O 16 | 511.9 | P | F | - | | |
| O 4 | 252 | P | F | - | | |
| O 12 | 251.8 | P | M | - | | |
| O 10 | 524 | K | M | + | 6 x 10 ³ | Digestive tract |
| O 9 | 378.2 | S | F | - | | |
| O 12 | 428 | KW | M | + | 1.2 x 10 ³ | Digestive tract, kidney |
| O 14 | 390.3 | K | F | + | 8 x 10 ² | Digestive tract |
| O 11 | 442 | KW | F | - | | |
| O 12 | 571 | S | F | + | 1 x 10 ³ | Digestive tract |
| O 15 | 419 | K | F | + | 8 x 10 ² | Digestive tract |
| O 5 | 267.6 | S | M | - | | |
| O 13 | 704.7 | KW | M | - | | |
| O 11 | 672 | K | F | + | 2 x 10 ³ | Digestive tract |
| O 13 | 254.8 | S | M | - | | |
| O 5 | 1540 | KW | M | + | 3.4 x 10 ³ | Caecum |
| O 14 | 734.2 | P | M | + | 2.8 x 10 ³ | Digestive tract |
| O 13 | 302 | K | M | - | | |
| O 2 | 586 | KW | M | - | | |

Abbreviations: M, male host; F, female host; O, host numbers; KW, Kwale; P, Pangani; S, Sahare; K, Kigombe; BW, Octopuses body weight; HS, host sex; I, Infection

4.3 Histopathology Examination

Histopathological study shown that each organ of the infected octopus had at least with number of huge oocysts containing many sporozoites and gametes. The gamogony stage was observed in some organs especially the intestine and the caecum where the microgametes and macrogametes were seen as shown in figure 8 (observe arrows). The parasites were mostly found in sub mucosa and muscularis layer. The detachment of epithelial cells was observed especially during sporogonic stage of *Aggregata sp* (figure 11). Some nuclear displacements were observed in gills and mucosal cell due to the presence of large number of sporocysts and developing macrogametes (figure 10). However there was no much tissue destruction in gills and kidney compared to intestine and caecum.

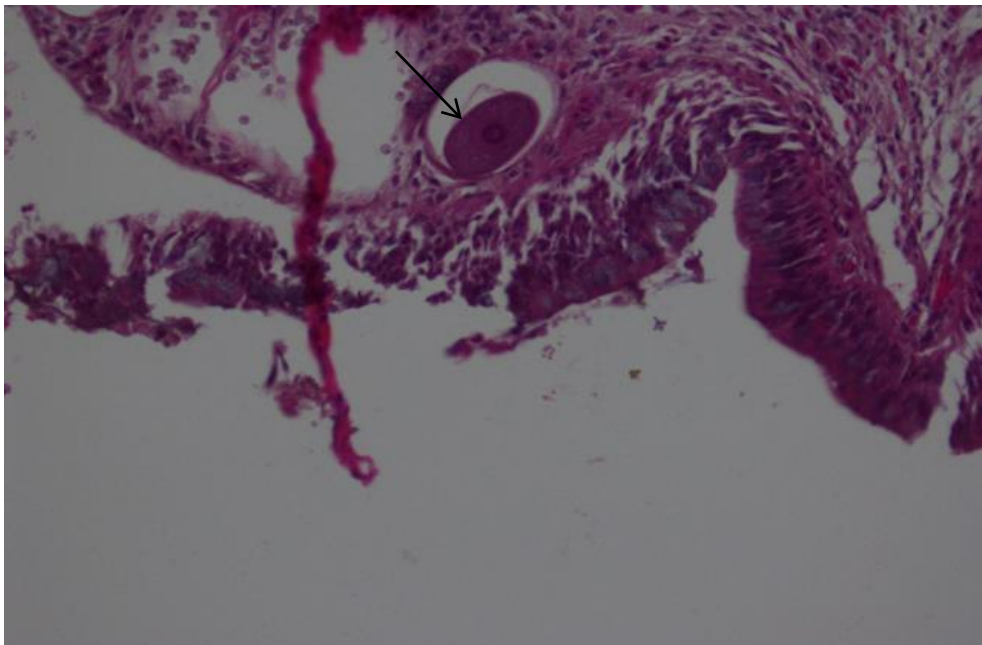


Figure 8: Macrogamete with spongy cytoplasm in the microvilli of the intestine of

Octopus cyanea

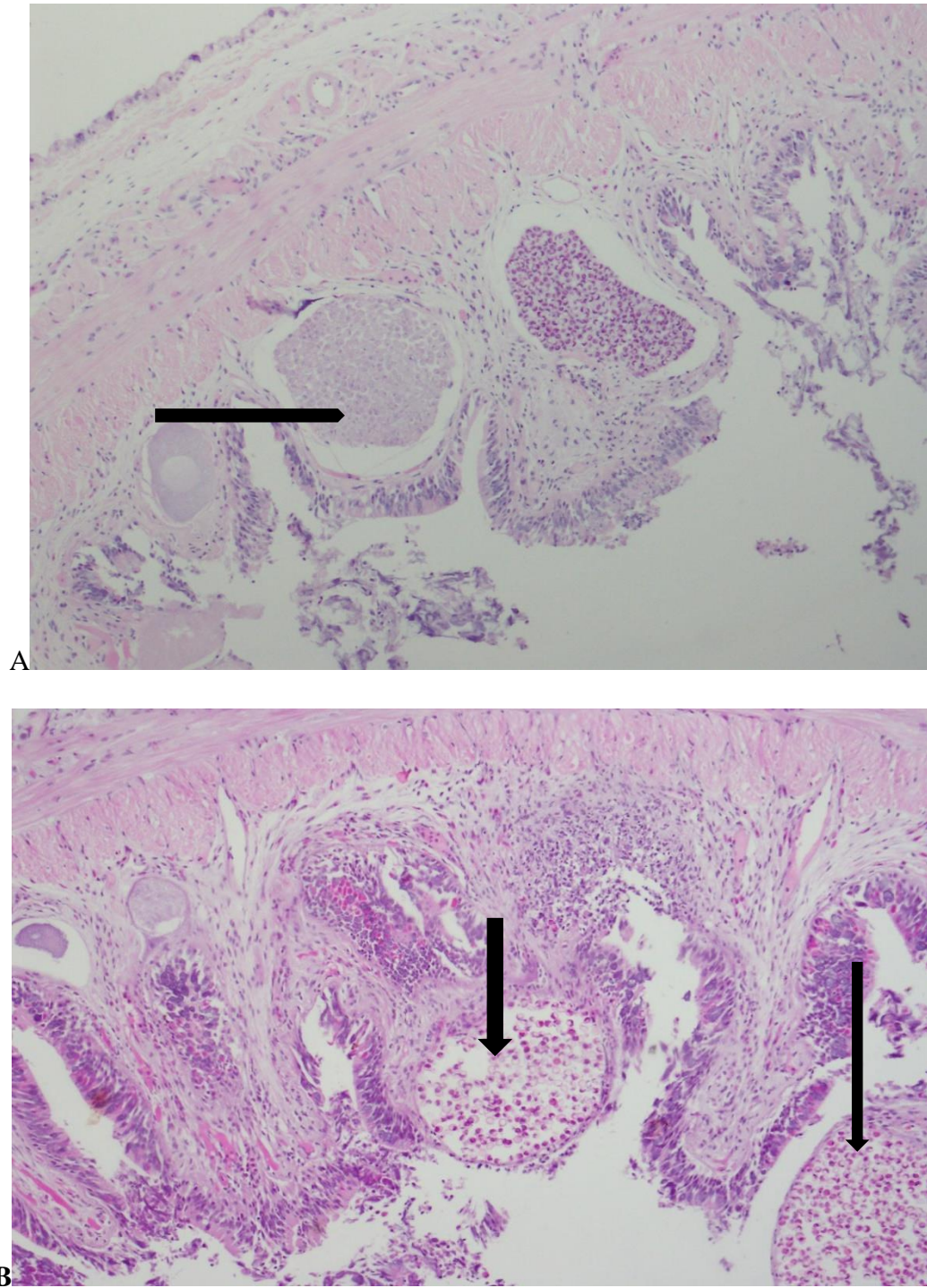


Figure 9: (A and B) H & E section showing the mucosal cells of the intestine replaced with large Oocysts containing number of sporocysts (X10). An arrow shows Oocyst within the intestinal villi

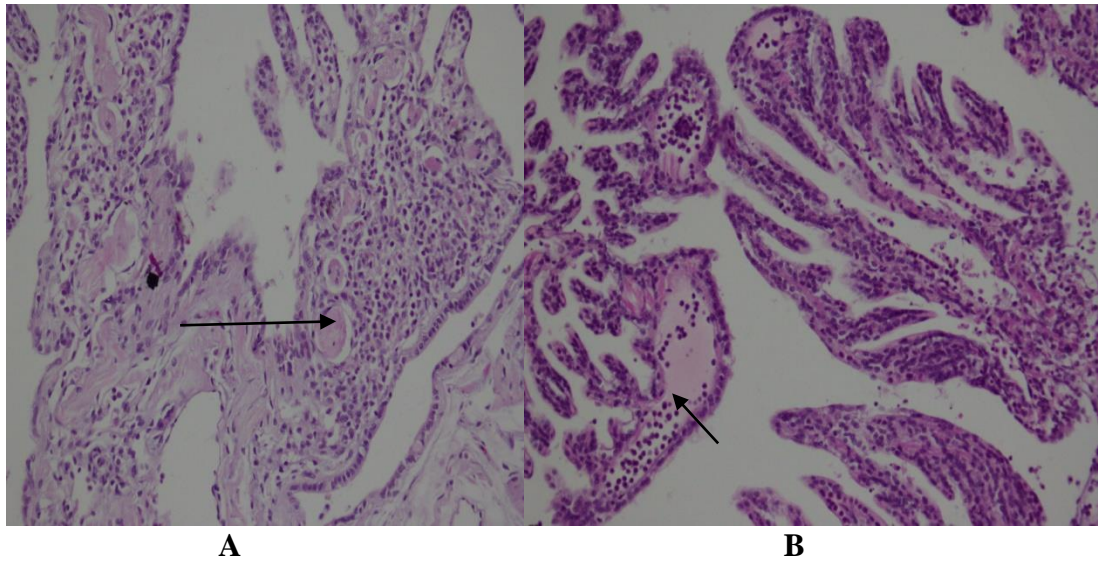


Figure 10: (A and B) H&E stain section LM examination of the octopus gill infected oocysts of the coccidian parasite

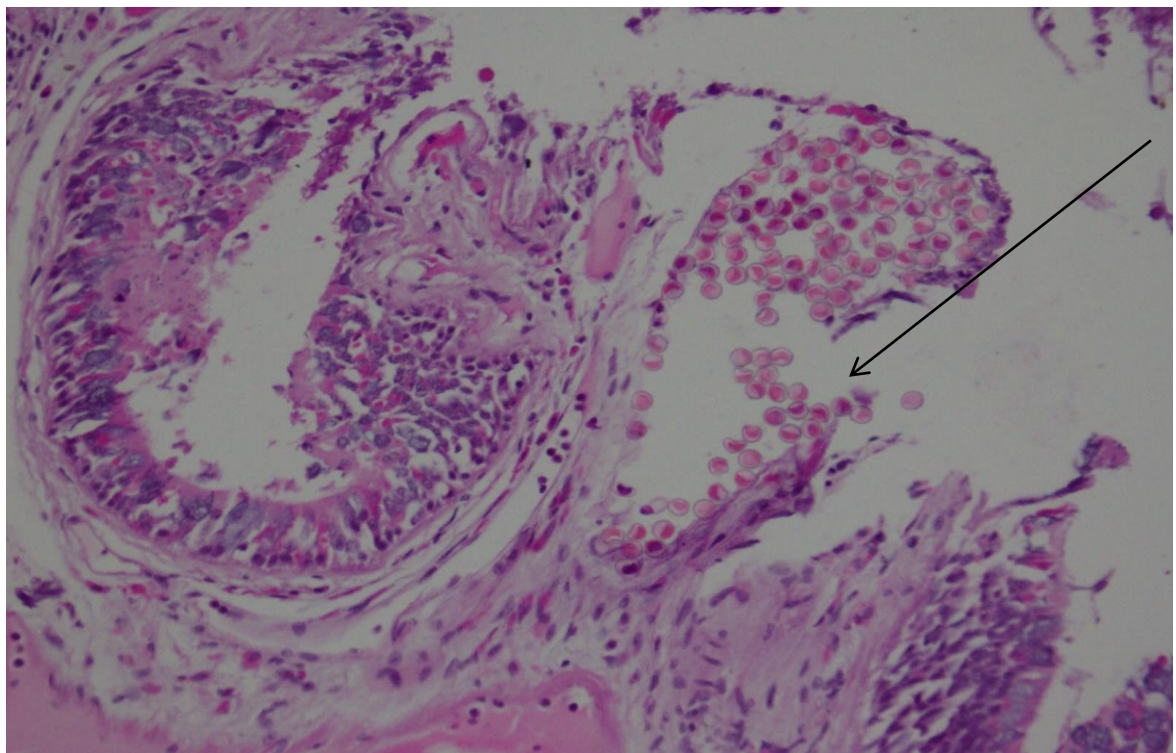


Figure 11: Detachment of the epithelial cell seen (arrow).

4.4 Sporocysts Counting

During sporocysts isolation, large numbers of sporocysts were observed under the light microscope. With the aid of light microscope, number of sporocysts was recorded using Neubers chamber as shown in table 5. Also during sporocysts counting, some had already released the sporozoites (Figure 5A).

4.5 Analysis Results

4.5.1 Prevalence of infection by individuals

The comparison was carried out to see whether there is any association between the density of infections and the weight of individual octopus. The data were normally distributed, and a linear regression analysis was performed.

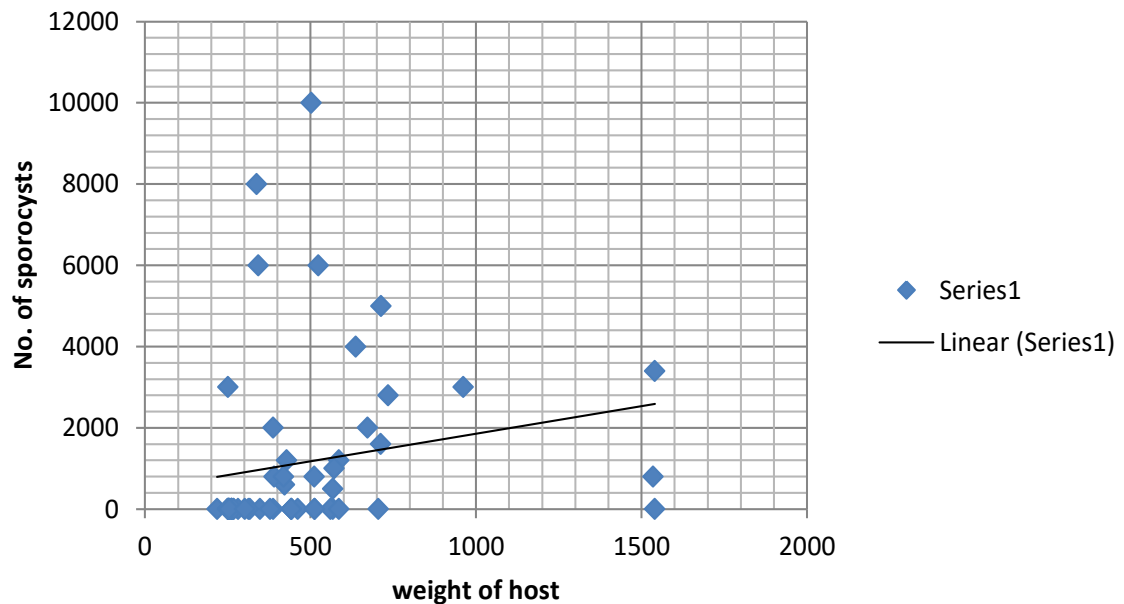


Figure 12: Relationship between number of sporocysts in an individual host and the weight of the infected hosts

The correlation between weight of an individual octopus and number of sporocysts showed no statistically significant at 0.05 level ($r = 0.18927$, $p = 0.16238$). The plot of number of sporocysts and weight of hosts showed weak positive correlation. Significant

difference was not found in the linear regression patterns that means the weight of the host doesn't determine the number of sporocysts the host could have. The number of sporocysts depends on the infective dose of parasites which the host ingested from the intermediate host and not its weight.

Regression equation: $y = 7.157 - 2288.5x$,(3)

$r = 0.18927$

$r^2 = 0.035825$ (i.e., only 3.5825% of variation in density of infection can be explained by the weight of the host).

$t = 1.4165$, $p = 0.16238$ (not significant).

4.5.2 Infestation between sites

On the normally distributed data One way ANOVA was performed to compare the means of sporocysts between study sites. The means of infections were not significantly different among sites at 0.05 level ($F = 1.63$, $p = 0.1936$). A Tukey's Honest Significant Difference test indicated that parasite infestation in samples from Kigombe were higher than those from other sites followed by Pangani and the rest two sites infections were not significantly different. Since there was no statistical significance between parasite infestations in octopuses among sites, the post-hoc testing was not required in this case.

| | Sum of sqrs | df | Mean square | F | p-value |
|-----------------|-------------|----|-------------|------|---------|
| Between groups: | 2.21581E07 | 3 | 7.38602E06 | 1.63 | 0.1936 |
| Within groups: | 2.35562E08 | 52 | 4.53003E06 | | |
| Total: | 2.5772E08 | 55 | | | |

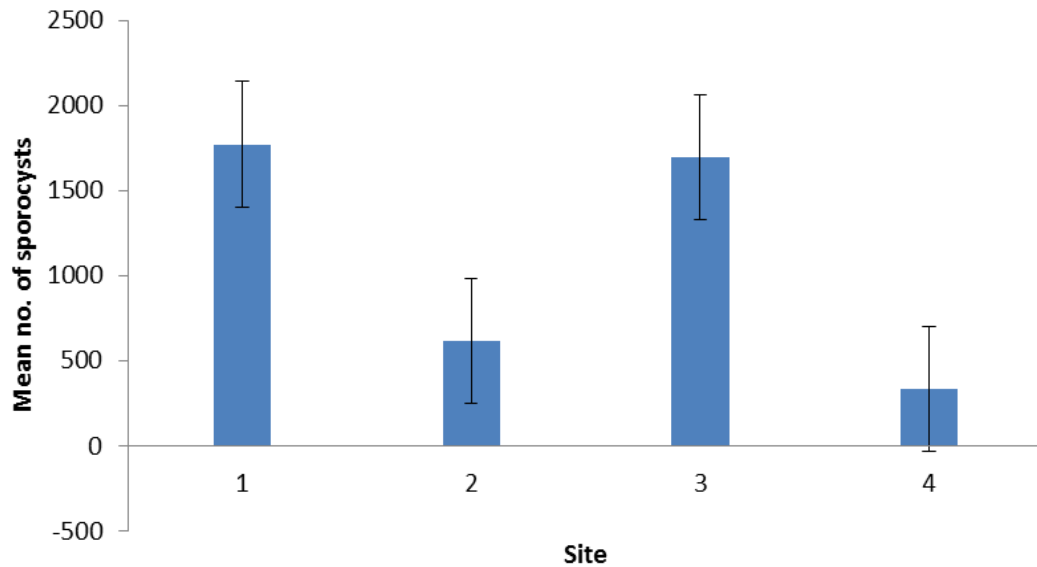


Figure 13: Prevalence of parasite infestation in octopus among the sites where octopuses were collected (1- Kigombe, 2-Kwale, 3-Pangani, 4- Sahare)

Prevalence of parasite infection was positively and significantly correlated among sites ($r = 0.98$, $p = 0.008$, $df = 3$).

4.5.3 Infection between male and female hosts

The results showed that the infection was higher in male octopus than in female octopus as indicated in the bar graph figure 14 as the mean number of sporocysts for female and male were 974.0741 and 1317.241 respectively. The comparison of means between two sexes of hosts was performed using two sample t-tests and there was no statistical significant difference in parasites infection between the two sexes.

| | |
|---------------------|--------------------|
| FEMALE | MALE |
| N: 27 | N: 29 |
| Mean: 974.07 | Mean: 1317.2 |
| 95%: (284.9 1663.2) | 95%: (360.44 2274) |
| Var.: 3.0351E06 | Var.: 6.3272E06 |

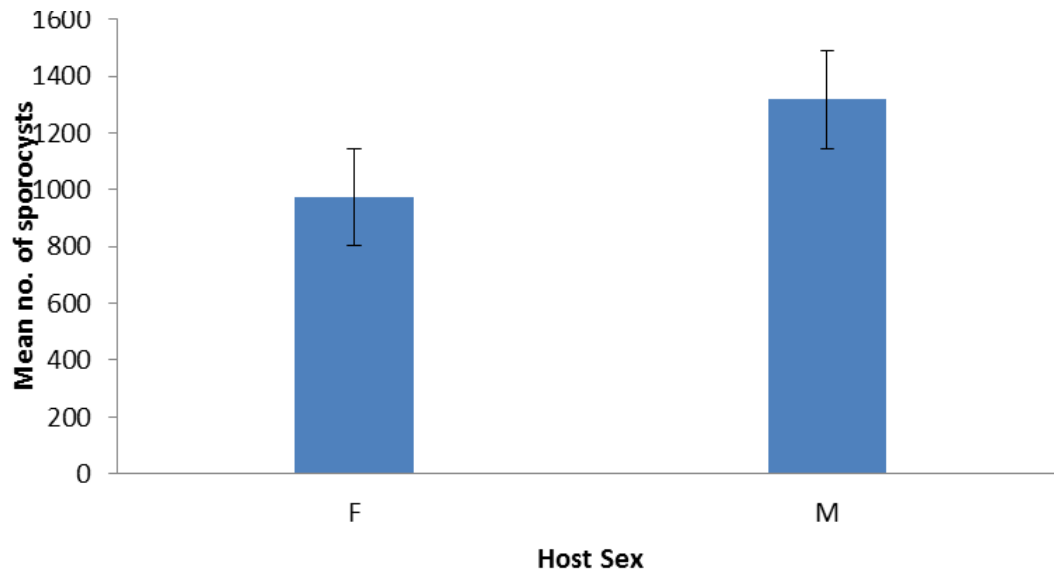


Figure 14: Variation on coccidian infection in male and female *Octopus cyanea*

4.6 Molecular Analysis

A total of 25 samples of coccidian parasites isolated from the intestine of *Octopus cyanea* were run in TAKARA PCR Thermo cycler machine using a pair of primers to confirm the presence of *Aggregata* species in the isolated intestinal parasites. Seventeen samples were positive for the 1F-2R primers and eight of them were negative. In figure 20, are representative samples which show positive results in first pair of primer visible in 1.5% agarose gel.

Aggregata sp. DNA was found to be present in pooled intestinal sample collected from 23 infected *Octopus cyanea* of Indian Ocean. In proportion, 68% of DNA samples performed (amplified) in a PCR were positive and the remaining 32% were negative. The positive PCR products were cloned for sequencing in order to confirm the *Aggregata spp* found in *Octopus cyanea* of the Indian Ocean coast of Tanga region.

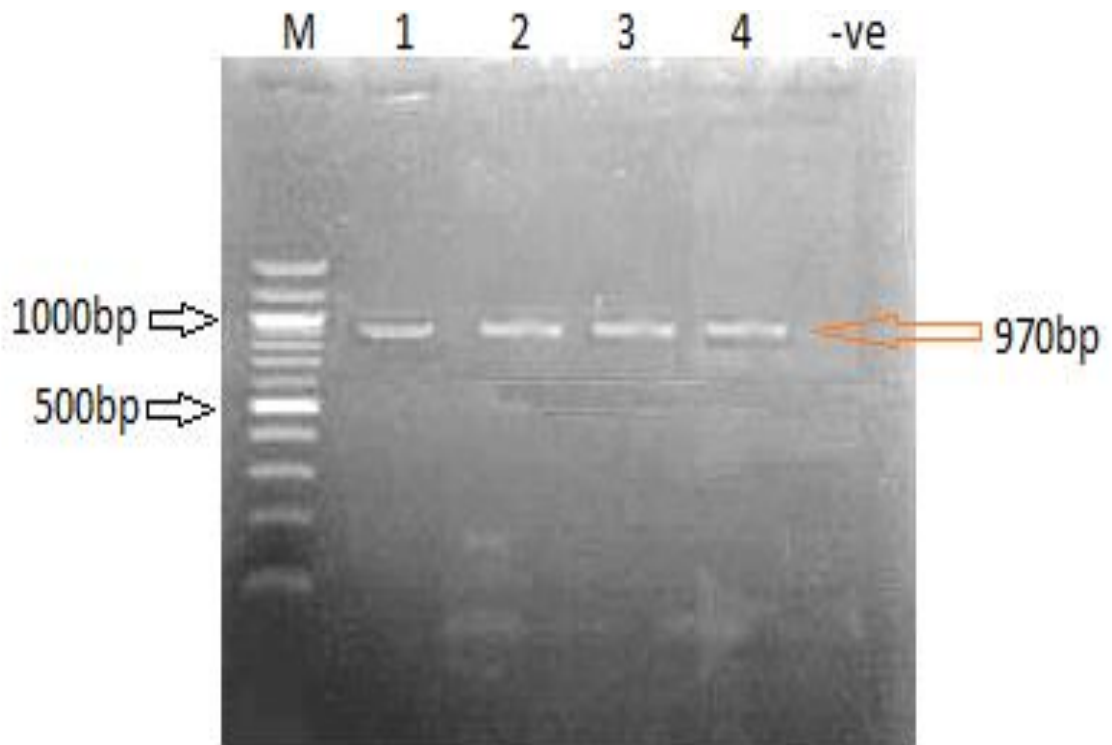


Figure 15: Photograph showing PCR amplification of *Aggregata* species. Using universal primers, *Aggregata* primers 1F and 2R were used to amplify an 18S rRNA gene. The positive product 970bp is visible in 1.5% agarose gel. M is 100bp Marker and the gel was stained with Gel Red

Seventeen of the 25 samples tested reacted positively with the designed *Aggregata* primer pair as seen in table 6. The remaining eight samples had no identifiable PCR amplicons with the designed primers. For the 17 positive samples, two of them were weak and they were not selected for DNA sequencing analysis.

Table 6: Results for molecular analysis showing the positive and negative samples for the PCR reaction for 1F and 2R primers

| S/N | Specimen name | Specimen no. | DNA Results |
|-----|---------------|--------------|-------------|
| 1 | OP | 3 | + |
| 2 | OK | 12 | - |
| 3 | OKW | 3 | - |
| 4 | OP | 9 | - |
| 5 | OS | 3 | + |
| 6 | OS | 1 | + |
| 7 | OP | 14 | - |
| 8 | OK | 10 | - |
| 9 | OKW | 5 | + |
| 10 | OK | 3 | + |
| 11 | OK | 15 | + |
| 12 | OK | 7 | + |
| 13 | OKW | 5 | + |
| 14 | OS | 12 | + |
| 15 | OK | 11 | + |
| 16 | OP | 13 | + |
| 17 | OK | 14 | - |
| 18 | OS | 8 | + |
| 19 | OK | 12 | + |
| 20 | OP | 6 | + |
| 21 | OS | 7 | + |
| 22 | OKW | 4 | + |
| 23 | OP | 15 | + |
| 24 | OP | 11 | - |
| 25 | OK | 2 | - |

Abbreviations: OKW: octopus from Kwale, OP: Octopus from Pangani, OS: octopus from Sahare, OK: octopus from Kigombe. (+) - DNA present, (-) - DNA absent

Sequencing identification of coccidian parasite

Histology and microscopic examination aided on phylogenetic identification of *Aggregata spp.* Cloning and sequencing were done for the 15 strongly positive DNA samples in order to confirm the parasite species. The sequenced samples were edited and assembled in Genius Software to form a consensus sequences aligned in a GenBank in order to determine the resembling sequence for species confirmation. The alignment showed that all the sequences were identical to *Aggregata octopiana* isolate H1 18S ribosomal RNA gene for 89% with Accession number KC188342 followed by *A. eberthi* gene isolate RV2 18S ribosomal RNA for 84% with Accession number KC188343.

Table 7: GenBank reference sequences of *Aggregata spp.* used in the construction of phylogenetic trees

| Field Sequence | Name | Isolate | Accession No. | % Identity | Country | Host | Year |
|----------------|---------------------|---------|---------------|------------|----------------|----------------------|------|
| S1 | <i>A. octopiana</i> | RV1 | KC188342.1 | 86 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 83 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 82 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| | <i>A. eberthi</i> | | DQ096838.1 | 81 | Czech Republic | <i>S.officinalis</i> | 2006 |
| S2 | <i>A. octopiana</i> | RV1 | KC188342.1 | 86 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 84 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 82 | | <i>O. vulgaris</i> | 2006 |
| | <i>C. ubiquitum</i> | JFM10 | AB697056.1 | 77 | Japan | <i>A. speciosus</i> | 2013 |
| S3 | <i>A. octopiana</i> | RV1 | KC188342.1 | 86 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 84 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 82 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| S4 | <i>A. octopiana</i> | H1 | LC186909.1 | 89 | Spain | <i>O. vulgaris</i> | 2017 |
| | <i>A. octopiana</i> | RV1 | KC188342.1 | 85 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 83 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 82 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| | <i>A. eberthi</i> | | DQ096838.1 | 81 | Czech Republic | <i>S.officinalis</i> | 2006 |
| S5 | <i>A. octopiana</i> | RV1 | KC188342.1 | 87 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 84 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 83 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| | <i>A. eberthi</i> | | DQ096838.1 | 82 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| S6 | <i>A. octopiana</i> | RV1 | KC188342.1 | 86 | Spain | <i>O. vulgaris</i> | 2013 |
| | <i>A. eberthi</i> | RV2 | KC188343.1 | 83 | Spain | <i>S.officinalis</i> | 2013 |
| | <i>A. octopiana</i> | | DQ096837.1 | 82 | Czech Republic | <i>O. vulgaris</i> | 2006 |
| | <i>A. eberthi</i> | | DQ096838.1 | 81 | Czech Republic | <i>S.officinalis</i> | 2006 |

Abbreviations; O- octopus; S- Sepia; A- Apodemus; A- aggregata; C- cryptosporidium. S- Consensus sequences

Table 7 above shows the summary of alignment of the consensus sequences during BLAST analysis and the percentage of identity with the *Aggregata octopiana* sequences from a GenBank. The results revealed that the compared sequences from coccidian parasite form *Octopus cyanea* collected along the Indian Ocean coast of Tanga was the *Aggregata octopiana* as the percentages of identity showing in the above table.

CHAPTER FIVE

5.0 DISCUSSION

Day octopus, the benthic species occurring in coral reefs is widely distributed in the Indo-Pacific regions from East Africa to Hawaii Islands (McKeown *et al.*, 2017). The octopus represents an important economic artisanal fishery product around the Indian Ocean coast of East Africa providing national income and source of proteins to East African countries (Guard and Mgya, 2002). *Octopus cyanea* artisanal catch comprises about 99% of octopus catch in the coast of Indian Ocean of Tanzania (Guard, 2009). The fishermen community depends on octopus fisheries as their source of protein and employment. During survey, it was observed young men to the elders and women dealt with artisanal octopus fishing as their source of income and food as reported by Jiddawi and Ohman (2002).

Even though Octopus fishing has been a major activity in coastal areas, fishermen community lacks the knowledge on octopus parasites and diseases. The reason would be due to the fact that, octopus parasites and other threats have not been given attention as other commercial animals (Pascual and Guerra, 2001). Knowledge on octopus-parasite relationship in wild population is still poor and it has not been given much attention since the parasites especially coccidian has not been reported to cause any harm to consumers (Bentacor *et al.*, 2013) and do not cause direct mortality to octopuses (Castellanos-Martínez *et al.*, 2013; Gestal *et al.*, 2007; Pascual and Guerra, 2001; Pascual *et al.*, 1996). For this reason, it is not possible for the fishermen communities and even the consumers to easily be able to recognize the parasitic infections in octopuses.

According to fishermen information and gross observation of octopus in the field, it has been observed that, octopuses are infected with some unknown agents that cause lesions on their body and arms. The lesion can be caused by biotic and abiotic factors occurring on the environment where octopuses live. Pascual *et al.* (2006) reported that injuries can be caused by parasites, bacterial or viral infections or sometimes with any substance that can cause injury to the animal in their natural environment. Fishermen proposed that injuries in octopuses were mainly caused by predators like eel fish and other carnivores that hunt on octopus. Considering the definition of parasite and predator, eel fish sucks on octopus and not killing, they could be termed as parasite not predator but more studies are needed to determine the association between eel fish and octopus.

The fishermen opinions of parasite existence in octopus were observed by categorizing them by their age groups. This was intended to get different opinions depending on the respondent's age and experience in octopus fishing. Age groups helped somehow on gaining some information concerning with parasite infection in octopus. Fishermen in the middle age group (31-53) were more knowledgeable on octopus parasite existence than other age groups. Most of the fishermen interviewed perceived that octopus has neither being infected by any parasite nor having any disease while some of them knew nothing about parasites supporting that the knowledge of parasite and disease in octopus is limited (Pascual and Guerra, 2001).

It was possible to get some information about the effects of octopus consumption among the fishing community. In the survey, some fishermen confirmed occurrence of allergies in some people after eating octopus resulting in rashes, stomach ache and vomiting. According to previous reported documents on octopus diseases and parasites, these allergies could be associated with some worms reported in octopuses such as nematodes,

Anisakis sp (Tsabouri *et al.*, 2012; Angelucci *et al.*, 2011). According to the experiences of fishermen community, they overcame the allergic reaction by different ways including proper octopus meat cooking or stopping the victim from eating octopus.

The reported results for histopathology study and fresh squash examinations of the liver, gills, kidney and intestine especially cecum of *O. cyanea* were likely similar to those reported by Betancor *et al.* (2013) and Licciardo *et al.* (2005) in the intestine of common octopus (*Octopus vulgaris*). Results from this study found no association between parasite load and weight of octopus although host size has been reported to be one of many factors contributing to parasite infection (Catalano *et al.*, 2014). The weight of octopus has an influence on the parasite pathology as described by Betancor *et al.* (2013). In fact, octopus with small body weight and size were observed no or slightly infected as described by Storero and Narvarte (2013). The larger in size and weight of octopus, the greater the possibility of harbouring parasites or being heavily infected with protozoan parasites as discussed by Storero and Narvarte (2013) octopuses' body weight.

In this study, no ectoparasites were found even though some findings reported existence of ectoparasites in octopus such as copepods *Pennella spp* and *Octopicola spp* located in gills of common octopus (Pascual *et al.*, 1996). During parasites examination in gills, skin eyes and arms of *O. cyanea* it was expected that, ectoparasites like copepods or any other crustacean like isopods could be identified. Nematode larva or Monogenean trematodes like *Diphyllbothrium sp* were also expected to be seen attached in gills as reported by Pascual *et al.* (1996) in common octopus. But the finding was different as the results revealed no any ectoparasite observed in *Octopus cyanea* captured along the coast of Tanga. Even though, ectoparasites were not found, but some of the octopuses were found

to have lesion on their skin as discussed earlier, these could be the result of parasite or any other mechanical damage occurred during octopus catch.

Most recent papers indicate that enteric coccidian infection in octopuses is the most important consequence to deal with than other parasitic organisms reported in cephalopods Gestal *et al.*, 1999) since they have been causing economic loss in reared cephalopods. It has been reported that the Coccidian parasite, *Aggregata sp* is a host-specific parasite in cephalopod (Hochberg, 1990) but being parasitizing all octopus species and are widely distributed in all geographical localities. In this study, almost all the infected octopuses examined were found to have an enteric coccidian infection. The reported coccidian infections in this study were the same as those reported in previous studies done in other octopuses and some squids in European water. The coccidian parasite reported here had the same morphological feature of having oocysts with large number of sporocysts as that reported by Gestal *et al.* (2000).

In comparison with other *Aggregata spp* like *Aggregata octopiana* and *Aggregata eberthi*, the *Aggregata sp* found in *O. cyanea* had sporocysts with more than 8 sporozoites as shown in figure 5. Poynton *et al.* (1992) reported *Aggregata dobelli* in *Octopus dofleini* from North East Pacific Ocean with sporocysts having more than 8 sporozoites as those observed in this study. *Aggregata octopiana* has sporocysts with 8 sporozoite and *A. sagittata* has sporocyst with 4-8 sporozoites with smooth cover (Gestal *et al.*, 2000). The sporocysts of *Aggregata sp* in octopuses from coastal area of Tanga region were found to have a cover resembling that of *A. octopiana* for 86% compared to other coccidian species but having 9-22 numbers of sporozoites.

The caecum was heavily infected with coccidian parasite compared to other extra-intestinal organ such as gills and kidney, since the caecum is the usual site of infection with coccidian as described by Gestal *et al.* (2002) and Estevez *et al.* (1996). Some

pathological effects of coccidian parasite noticed in histopathological study of the intestine, caecum and gills were the same as those described by Gestal *et al.* (2000). In heavily infected octopus, the mucosal and epithelial cells were replaced by coccidian parasites (Pascual *et al.*, 2007) as shown in figures. Betancor *et al.* (2013) reported pathological reactions of *Aggregata octopiana* in the caecum, intestine and gills of wild and reared octopus which are likely to those reported in infected *Octopus cyanea* in this study.

The replacement and destruction of mucosal and epithelial cells with sporocysts cause malabsorption syndrome and loss of intestinal and caecum epithelium (Baldascino *et al.*, 2017). Pathology caused by these coccidian parasites reported to weaken the host and increase the susceptibility to other pathogens (Pascual *et al.*, 2007). The host and parasite (*Aggregata sp*) has been reported to have influence on pathological state (Gestal *et al.*, 2002). In this study, it was noted that parasite pathology was not much high in wild Day octopus compared to the pathology reported by Betancor *et al.* (2013) in reared and wild common octopus. The pathology depend on the infective dose of *Aggregata sp* the host has ingested, in reared octopus there is intensive infection due to high infective dose of parasite octopuses ingest (Betancor *et al.*, 2013).

Apart from *Aggregata sp*, Dicyemid mesozoans also reported to be host specific and found in Northern Indian Ocean with no evidence in Eastern Indian Ocean (Castellanos-Martínez *et al.*, 2011; Furuya, 1999) as in this study none of the octopus examined were found to be infected with these mesozoans. Most of the described mesozoan parasites have been reported to be host specific (Castellanos-Martínez *et al.*, 2011) so may be it could be possible to find one in *Octopus cyanea* but only *Aggregata sp* was observed in octopus. It cannot be concluded that mesozoan parasites are completely absent in this specie of

octopus, more work has to be done in order to prove the reality of dicyemids condition along the Indian Ocean.

The existence of visible cysts in the wall of the digestive tract, oocysts having large number of sporocysts, the shape of oocysts and sporocysts used as an evidence and key taxonomic features of identifying gastrointestinal protozoan as *Aggregata sp* since they are only coccidian parasites infecting cephalopods including octopuses (Baldascino *et al.*, 2017; Pascual *et al.*, 2007). Pathology of parasite in the host intestine like mechanical damage of large portion of intestinal tissue had also useful for parasites identification in this case, since are the well-known common feature of any coccidian parasite infection in their host gastrointestinal tissue (Pascual *et al.*, 2007; Mladineo and Jozic, 2005).

This is the first report of parasite existence especially coccidian parasite infection in *O. cyanea* along the coast of Indian Ocean of Tanzania. Many studies have been reported *A. octopiana* infections in common octopus, *Octopus vulgaris* in European countries like Spain (Gestal *et al.*, 2005). Isolating *A. octopiana* in Day octopus, *O. cyanea* is a new finding. The task of this study was to determine parasites affecting *O. cyanea* but only one parasite *A. octopiana* was identified and was confirmed by molecular techniques. The body condition in *Octopus cyanea* found along the coast of East Africa could be the same as that in common octopus in European water.

The prevalence of coccidian parasites found in examined octopuses differed depending on the geographical location (site) where they were collected as shown in table 2. Geographical location has a great influence on distribution of parasite as reported by Catalano *et al.* (2014), Storero and Narvarte (2013) and Castellanos-Martínez *et al.* (2011). In this study, the location where octopus collected can be one of the factors which

lead into parasite abundance variation. Apart from geographical location, ecosystem structure of where the octopuses collected could also be not the same that's why the parasite abundance among host species was different. Gonzalez *et al.* (2003) documented that, ecosystem structure has something to do with parasite richness, so this could also be the reason for the variation of parasite prevalence among the octopuses collected from four study sites.

Molecular identification gave out the results showing that, the isolated coccidian parasite of *O. cyanea* was the *Aggregata octopiana* after sequencing the 18S rRNA gene of the parasite. Molecular identification was done to prove the existence of *Aggregata spp* in the present study. Morphological and molecular identification of the parasites done to compare the results with other researcher's findings described about octopus parasites. The results from this study confirmed the presence of coccidian parasites in *O. cyanea*, so more works has to be done for more descriptions about this *Aggregata spp* in the Indian coast of Tanzania and East Africa in general. Combining morphological and molecular identification revealed that the enteric coccidian parasite was the *Aggregata octopiana* which was also found in common octopus, *Octopus vulgaris* (Gestal *et al.*, 2002). This is not enough; more studies as suggested earlier should be done for the parasite of *Octopus cyanea* to prove the existence of coccidian parasite inhabit this species of cephalopod.

From confirmed 23 coccidian infected octopuses obtained from four study sites, 17 microscopically positive animals were confirmed by PCR. The PCR was the most specific technique of diagnosing the parasite than using the microscope which shown 23 animals were positive. The PCR results showed that four octopuses from Pangani, five from Sahare, three from Kwale and five from Kigombe were positive which were less compared to the positive number obtained from microscopic examination. In most disease cases,

PCR has been used and reported to be the most sensitive technique of disease confirmation than using the less sensitive microscopic method (Johnston *et al.*, 2006).

In addition, the absence of morphological and structural information of helminthes, crustacean and coccidian parasite affecting *Octopus cyanea* made it difficult to identify them. The presence of *Aggregata* in *Octopus cyanea* from Indian Ocean has never been discussed earlier or even any other parasites reported along the coast of Indian Ocean. There could be some confusion on identifying the parasite in *Octopus cyanea* due to the absence of detailed information on the parasite harbouring them. Further investigation is needed in our coastal area so as more information about octopus parasite is achieved.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The contribution that should be given to the fisheries sector from this study is that, octopuses along the Indian Ocean coast of Tanzania also harbours and is infected by parasites like other octopus species in other Oceans. About 41% of the collected octopuses were found to be infected with parasites, the coccidian parasite which has been reported in European water. The parasites are dangerous when it comes to octopus rearing aspect and cause mortality to the infected host once it is exposed to other biotic or abiotic stressors. The parasite does not cause any disease to consumers but it is advised octopus has to be well cooked to prevent any disease causing agents. Combining this study and other previous report on octopus parasites, the condition has to be taken as the serious problem so as to bring more awareness to East African countries on the existence of parasites in octopuses along the Indian Ocean.

The expectation is that, more works can be done on investigation of more parasite species found in all *Octopus spp* along the Indian Ocean coast especially in Tanzania where the octopus species is being exploited. *Aggregata octopiana* was found as the only parasite in the present study, more stains of coccidian or *Aggregata spp* from *O. cyanea* even in other cephalopod species are expected to be discovered in Indian Ocean as many species have been described in countries like Spain. This is the first time octopuses along the Tanzanian coastal have been investigated for parasites, literatures for references was difficult to assess but this challenge will be reduced if more researches will be conducted on octopus so as to simplify the future researchers work.

6.2 Recommendations

According to the findings from this study, it is recommended that the Government of Tanzania and Fisheries research institutes have to plan more researches on octopus parasites so as to increase the value of octopus in national and international markets. Instead of not only dealing with other aquatic organisms but also octopus wellbeing has to be considered in researches by investigating their diseases and parasites infections on their natural environment.

To this moment, there is no information on octopus parasites along the Indian Ocean coast of Tanzania. There have been some difficulties for identification of parasites in octopuses from the fishermen community levels because of limited information. More studies on octopus parasites and diseases are recommended to be conducted in order to obtain more information which will help on planning for diseases and parasites management in future. The fishermen and octopus processing factories owners are also recommended to be trained on the existence of parasites in octopuses so as they can improve in terms of hygiene during product processing.

REFERENCES

- Angelucci, G., Meloni, M., Merella, P., Sardu, F., Madeddu, S., Marrosu, R., Petza, F. and Salati, F. (2011). Prevalence of *Anisakis spp.* and *Hysterothylacium spp.* larvae in teleosts and cephalopods sampled from waters off Sardinia. *Journal of Food Protection* 74(10): 1769-1775.
- Baldascino, E., Di Cristina, G., Tedesco, P., Hobbs, C., Shaw, T., Ponte, G. and Andrews, P. L. (2017). The gastric ganglion of *Octopus vulgaris*: Preliminary characterization of gene-and putative neurochemical-complexity, and the effect of *Aggregata octopiana* digestive tract infection on gene expression. *Frontiers in Physiology* 8: 1001.
- Benbow, S., Humber, F., Oliver, T. A., Oleson, K. L. L., Raberinary, D., Nadon, M., Ratsimbazafy, H. and Harris, A. (2014). Lessons learnt from experimental temporary octopus fishing closures in south-west Madagascar: benefits of concurrent closures. *African Journal of Marine Science* 36(1): 31-37.
- Betancor, M. B., Estefanell, J., Socorro, J., Roo, J. and Caballero, M. J. (2013). First description of parasitization by *Aggregata octopiana* in common octopus, *Octopus vulgaris*, in Canary Islands. *Fish Pathology* 33(1): 13-20.
- Boyle, P. and Rodhouse, P. (2005). From egg to recruitment. *Cephalopods: Ecology and Fisheries*. Blackwell science, Oxford. 146-160pp.
- Bruno, D. W., Nowak, B. and Elliott, D. G. (2006). Guide to identification of fish protozoan and metazoan parasites in stained tissue sections. *Diseases of Aquatic Organisms* 70: 1- 36.

- Bultel, E., Doherty, B., Herman, A., Le Manach, F. and Zeller, D. (1950). An update of the reconstructed marine fisheries catches of Tanzania with taxonomic breakdown. *Fisheries catch Reconstructions in the Western Indian Ocean* 2010; 151-161.
- Carballeira, C., De Orte, M. R., Viana, I. G., DelValls, T. A. and Carballeira, A. (2012). Assessing the toxicity of chemical compounds associated with land-based marine fish farms: the sea urchin embryo bioassay with *Paracentrotus lividus* and *Arbacia lixula*. *Archives of Environmental Contamination and Toxicology* 63(2): 249-261.
- Castellanos-Martínez, S. and Gestal, C. (2013). Pathogens and immune response of cephalopods. *Journal of Experimental Marine Biology and Ecology* 447: 14–22.
- Castellanos-Martinez, S., Aguirre-Macedo, M. L. and Furuya, H. (2016). Two new species of dicyemid mesozoans (Dicyemida: Dicyemidae) from *Octopus maya* Voss and Solis-Ramirez (Octopodidae) off Yucatan, Mexico. *Systematic Parasitology* 93(6): 551-564.
- Castellanos-Martinez, S., Diz, A. P., Alvarez-Chaver, P. and Gestal, C. (2014). Proteomic characterization of the hemolymph of *Octopus vulgaris* infected by the protozoan parasite *Aggregata Octopiana*. *Proteomics* 105 (13): 151-163.
- Castellanos-Martinez, S., Gómez, M. C., Hochberg, F. G., Gestal, C. and Furuya, H. (2011). A new dicyemid from *Octopus hubbsorum* (Mollusca: Cephalopoda: Octopoda). *The Journal of Parasitology* 97(2): 265-269.

- Catalano, S. R., Whittington, I. D., Donnellan, S. C. and Gillanders, B. M. (2014). Dicyemid fauna composition and infection patterns in relation to cephalopod host biology and ecology. *Folia Parasitologica* 61(4): 301.
- Cavaleiro, F.I. and Santos, M.J. (2014). Numerical and functional responses to the presence of a competitor—the case of *Aggregata* sp.(Apicomplexa: Aggregatidae) and *Octopicola superba* (Copepoda: Octopicolidae). *Parasitology* 141(2): 216-226.
- Charan, J. and Kantharia, N. D. (2013). How to calculate sample size in animal studies? *Journal of Pharmacology and Pharmacotherapeutics* 4(4): 303-306
- Culling, C. F. A., Allison, R. T. and Barr, W. T. (1985). *Cellular Pathology Techniques*. Butterworth and Co. London. pp. 3-163.
- De Baets, K., Keupp, H. and Klug, C. (2015). Parasites of ammonoids. In *Ammonoid Paleobiology: From anatomy to ecology*. Springer, Netherlands. pp. 837-875.
- Emery, T. J., Hartmann, K. and Gardner, C. (2016). Management issues and options for small scale holobenthic octopus fisheries. *Ocean and Coastal Management* 120: 180-188.
- Eriksson, H., De La Torre-castro, M. and Olsson, P. (2012). Mobility, expansion and management of a multi-species scuba diving fishery in East Africa. *PLOS One* 7(4): 35504-35559.

- Estefanell, J., Socorro, J., Tuya, F., Izquierdo, M. and Roo, J. (2011). Growth, protein retention and biochemical composition in *Octopus vulgaris* fed on different diets based on crustaceans and aquaculture by-products. *Journal of Aquaculture* 322: 91-98.
- Estevez, J., Pascual, S., Gestal, C., Soto, M., Rodriguez, H. and Arias, C. (1996). *Aggregata octopiana* (Apicomplexa:Aggregatidae) from *Octopus vulgaris* off NW Spain. *Disease of Aquatic Organisms* 27: 227-231.
- FAO, Food and Agriculture Organization of United Nations (2004). Fishery and Aquaculture Country profiles. Mauritius. In: *FAO Fisheries and Aquaculture Department*. Rome: Food and Agriculture Organization. [http://www.fao.org/fishery/countrysector/FI-CP_MU/en] site visited on 12/11/2018.
- Finn, J. K., Hochberg, F. G. and Norman, M. D. (2005). Phylum Dicyemida in Australian waters: First record and distribution across diverse cephalopod hosts. *Phuket Marine Biology Research Center Bulletin* 66(8): 96.
- Furuya, H. (2008). Three New Dicyemids from *Octopus sasakii* (Mollusca: Cephalopoda: Octopoda). *Journal of Parasitology* 94(5): 1071-1081.
- Furuya, H., Tsuneki, K. and Koshida, Y. (1992). Two new species of the genus *Dicyema* (Mesozoa) from octopuses of Japan with notes on *D. Misakiense* and *D. Acuticephalum*. *Zoological Science* 9: 423-437.
- Gestal, C., Belcari, P., Abollo, E. and Pascual, S. (1999). Parasites of cephalopods in the northern Tyrrhenian Sea (western Mediterranean): new host records and host specificity. *Science Marine*. 63(1): 39-43.

- Gestal, C., Guerra, A. Abollo, E. and Pascual, S. (2000). *Aggregata sagittata* sp. (Apicomplexa: Aggregatidae), a coccidian parasite from the European flying squid *Todarodes sagittatus* (Mollusca: Cephalopoda). *Systematic Parasitology* 47: 203–206.
- Gestal, C., Guerra, A., Pascual, S. and Azevedo, C. (2002). On the life cycle of *Aggregata eberthi* and observations on *Aggregata octopiana* (Apicomplexa: Aggregatidae) from Galicia (NE atlantic). *Protistology* 37: 427-435.
- Gestal, C. and Azevedo, C. (2005). Ultrastructure of *Goussia cruciata* (Apicomplexa: Coccidia) infecting the liver of horse mackerel, *Trachurus trachurus* (L.), from Ibero-Atlantic waters. *Journal of Fish Diseases* 28(3): 125-132.
- Gestal, C., Guerra, A. and Pascual, S. (2007). *Aggregata octopiana* (Protista: Apicomplexa) a dangerous pathogen during commercial *Octopus vulgaris* on-growing. *Journal of Marine Science* 64: 1743-1748.
- Gibson, R. N., Atkinson, R. J. A. and Gordon, J. D. M. (2008). Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology: Annual Review* 46:105-202.
- Gómez, A. and Nichols, E. (2013). Neglected wild life: parasitic biodiversity as a conservation target. *International Journal for Parasitology: Parasites and Wildlife* 2: 222-227.
- González, A. F., Pascual, S., Gestal, C., Abollo, E. and Guerra, A. (2003). What makes a cephalopod a suitable host for parasite? The case of Galician waters. *Fisheries Research* 60(1): 177-183.

- González, M. and Sánchez, P. (2002). Cephalopod assemblages caught by trawling along the Iberian Peninsula Mediterranean coast. *Scientia Marina* 66(S2): 199-208.
- Guard, M. (2009). Biology and fisheries status of octopus in the Western Indian Ocean and the Suitability for marine stewardship council certification. *United Nations Environment Programme (UNEP) and The Institute for Security Studies (ISS)*. pp. 1-21.
- Guard, M. and Mgaya, Y. D. (2002). The artisanal fishery for *Octopus cyanea* Gray in Tanzania. *AMBIO: A Journal of the Human Environment* 31(7): 528-536.
- Hanlon, R. T. and Forsythe, J. W. (2008). Sexual cannibalism by *Octopus cyanea* on a Pacific coral reef. *Marine and Freshwater Behaviour and Physiology* 41(1): 19-28.
- Heukelem, W. V. (1973). Growth and life-span of *Octopus cyanea* (Mollusca: Cephalopoda). *Journal of Zoology* 169(3): 299-315.
- Hochberg, F. G. (1990). Diseases of Mollusca: Cephalopoda. Diseases caused by protistans and metazoans. *Diseases of Mollusca: Cephalopoda. Diseases caused by protistans and metazoans*. Biologische Anstalt Helgoland, Hamburg-Germany. pp. 47-227.
- Ignatius, B. and Srinivasan, M. (2006). Embryonic Development in *Octopus aegina* Gray, 1849. *Current Science* 91: 1089-1092.

- Jiddawi, N. S. and Öhman, M. C. (2002). Marine fisheries in Tanzania. *Ambio: A Journal of the Human Environment* 31(7): 518-527.
- Johnston, S. P., Pieniazek, N. J., Xayavong, M. V., Slemenda, S. B., Wilkins, P. P. and da Silva, A. J. (2006). PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of Clinical Microbiology* 44(3): 1087-1089.
- Kalavati, C., Narasimhamurti, C. C. and Suseela, T. (1984). Four new species of mesozoan parasites (Mesozoa: Dicyemidae) from cephalopods of Bay of Bengal. *Proceedings: Animal Sciences* 93(7): 639-654.
- Katikiro, R. E. (2014). Perceptions on the shifting baseline among coastal fishers of Tanga, Northeast Tanzania. *Ocean and Coastal Management*. 91:23-31.
- Kiwale, M. J. (2003). The Tanzania fish export sector. Paper presented on Sector Diagnostic Report of Board of External Trade, Dar es Salaam, Tanzania. *Food Control* 16(1): 1-9.
- Kopečná, J., Jirků, M., Oborník, M., Tokarev, Y. S., Lukeš, J. and Modrý, D. (2006). Phylogenetic analysis of coccidian parasites from invertebrates: search for missing links. *Protistology* 157(2): 173-183.
- Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology Evolution* 33(7): 37.
- Licciardo, G., Garziano, A., Nocera, G., Gaglio, G., Marino, F. and De Vico, G. (2005). Contributo alla conoscenza dell'azione patogena di *Aggregata octopiana*

- (Apicomplexa: Aggregatidae) in *Octopus vulgaris* nel sud del Mar Tirreno. *Ittiopatologia* 2: 193-198.
- Lima, J. D. F., Garcia, J. D. S. and Silva, T. C. D. (2014). Natural diet and feeding habits of a freshwater prawn (*Macrobrachium carcinus*: Crustacea, Decapoda) in the estuary of the Amazon River. *Acta Amazonica* 44(2): 235-244.
- Luque, J.L., Mouillot, D. and Poulin, R. (2004). Parasite biodiversity and its determinants incoastal marine teleost fishes of Brazil. *Parasitology* 128: 671–682.
- Mayo-Hernandez, E., Barcala, E., Berriatua, E. and Munoz, P. (2013). *Aggregata* (Protozoa: Apicomplexa) infection in the common octopus, *Octopus vulgaris* from West Mediterranean Sea: The infection rates and possible effects of faunistic, environmental and ecological factors. *Journal of Sea Research* 83:195-201.
- McKeown, N. J., Taylor, A. L. and Shaw, P. W. (2017). Isolation and characterisation of polymorphic microsatellite loci for studies of the big blue octopus, *Octopus cyanea*. *Marine Biodiversity*. pp.1-3.
- Mitchell, A. J., Salmon, M. J., Huffman, D. G., Goodwin, A. E. and Brandt, T. M. (2000). Prevalence and Pathogenicity of a Heterophyid Trematode Infecting the Gills of an Endangered Fish, the Fountain Darter, in Two Central Texas Spring-Fed Rivers. *Journal of Aquatic Animal Health* 12(4): 283-289.
- Mollusca, Cephalopoda) in Spain: an updated perspective. *Aquaculture* 142(1-2): 1-10.

- Mshana, J. G. and Sekadende, B. (2014). Assessment of Heavy Metal Pollution in *Octopus cyanea* in the coastal water of Tanzania. *Journal of Health and Pollution* 4: 10-18.
- Msuya, F. E. (2013). Social and economic dimensions of carrageenan seaweed farming in the United Republic of Tanzania. *Social and economic dimensions of carrageenan seaweed farming, Fisheries Aquatic Technology Paper*. 580: 115-146.
- Neto, M. J. L., de Souza Nascimento, E., Maihara, V. A., Silva, P. S. C. and Landgraf, M. (2014). Evaluation of As, Se and Zn in octopus samples in different points of sales of the distribution chain in Brazil. *Journal of Radioanalytical and Nuclear Chemistry* 301(2): 573-579.
- Obiekezie, A. and Ekanem, D. (1995). Experimental infection of *Heterobranchus longifilis* (Teleostei, Clariidae) with *Trichodina maritinkae* (Ciliophora, Peritrichida). *Aquatic Living Resources* 8(4): 439-443.
- Palm, H. W. (2011). Fish parasites as biological indicators in a changing world: Can we monitor environmental impact and climate change? In; *Progress in Parasitology*. Springer, Berlin, Heidelberg. pp. 223-250.
- Palm, H. W., Klimpel, S. and Bucher, C. (1999). Checklist of metazoan fish parasites of German coastal waters. *Berichte aus dem Institut für Meereskunde an der Universität Kiel* 307: 1-148.
- Pascual, S. and Guerra, A. (2001). Vexing question on fisheries research: the study of cephalopods and their parasites. *Iberus* 19(2): 87-95.

- Pascual, S., Gestal, C., Estévez, J. M., Rodríguez, H., Soto, M., Abollo, E. and Arias, C. (1996). Parasites in commercially-exploited cephalopods (Mollusca, Cephalopoda) in Spain: an updated perspective. *Aquaculture* 142(1): 1-10.
- Pascual, S., González, A. and Guerra, A. (2007). Parasites and cephalopod fisheries uncertainty: towards a waterfall understanding. *Reviews in Fish Biology and Fisheries* 17(2-3): 139-144.
- Pascual, S., Gonzalez, A. F. and Guerra, A. (2005). The recruitment of gill-infesting copepods as a categorical predictor of size-at-age data in squid populations. *ICES Journal of Marine Science* 62(4): 629-633.
- Pascual, S., González, A. F. and Guerra, A. (2006). Unusual sites of *Aggregata octopiana* infection in octopus cultured in floating cages. *Aquaculture* 254: 21-23.
- Pascual, S., González, A. F. and Guerra, A. (2010). Coccidiosis during octopus senescence: preparing for parasite outbreak. *Fisheries Research* 106(2): 160-162.
- Pascual, S., Vega, M.A., José Rocha, F. and Guerra, A. (2002). First report of an endoparasitic epicaridean isopod infecting cephalopods. *Journal of Wildlife Diseases* 38(2): 473-477.
- Poynton, S. L., Reimschuessel, R. and Stoskopf, M. K. (1992). *Aggregata dobelli* n. sp. and *Aggregata millerorum* n. sp. (Apicomplexa: Aggregatidae) from two species of octopus (Mollusca: Octopodidae) from the eastern North Pacific Ocean. *The Journal of Protozoology* 39(1): 248-256.

- Rocliffe, S. and Harris, A. (2016). *Blue Ventures Report*. The status of octopus fisheries in the Western Indian Ocean. 1-40pp.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (No. Ed. 2). Cold spring harbor laboratory press. 1546pp.
- Sauer, W. H. H., Potts, W., Raberinary, D., Anderson, J. and Perrine, M. S. (2011). Assessment of current data for the octopus resource in Rodrigues, Western Indian Ocean. *African Journal of Marine Science* 33(1): 181-187.
- Silas, E. G. (1985). Cephalopod fisheries of India - An introduction to the subject with methodologies adopted for the study. In: E.G. Silas (Ed.) *Cephalopod bionomics, fisheries and resources of the EEZ of India*. Bull. Central Marine Fisheries Research. Institute 37: 1-4.
- Storero, L. P. and Narvarte, M. A. (2013). Coccidian infection may explain the differences in the life history of octopus host populations. *Journal of invertebrate pathology* 114(3): 222-225.
- Sures, B. (2004). Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* 20(4): 170-177.
- Sykes, A. V. and Gestal, C. (2014). Welfare and diseases under culture conditions. In; *Cephalopod Culture*. Springer, Dordrecht. pp. 97-112.
- TAFIRI, Tanzania Fisheries Research Institute (2012). Baseline Information on the status of Commercially important Fisheries and Exploitation Perceptions by

Artisanal Fisheries on the Mainland Coastal Waters. Tanzania Fisheries Research Institute. Dar es Salaam, Tanzania.

Tedesco, P., Gestal, C., Begić, K., Mladineo, I., Castellanos-Martinez, S., Catanese, G., Terlizzi, A. and Fiorito, G. (2017). Morphological and molecular characterization of *Aggregata* spp. Frenzel 1885 (Apicomplexa: Aggregatidae) in *Octopus vulgaris* Cuvier 1797 (Mollusca: Cephalopoda) from central Mediterranean. *Protistology* 168(5): 636 - 648.

Tsabouri, S., Triga, M., Makris, M., Kalogeromitros, D., Church, M. K. and Priftis, K. N. (2012). Fish and shellfish allergy in children: review of a persistent food allergy. *Pediatric Allergy and Immunology* 23(7): 608-615.

Van Heukelem, W. F. (1983). *Octopus Maya. Cephalopod Life Cycles*. Academic press, London 1: 311-323pp.

Vidal, E. A. and Haimovici, M. (1999). Digestive tract parasites in rhynchoteuthion squid paralarvae, particularly in *Illex argentinus* (Cephalopoda: Ommastrephidae). *Fishery Bulletin* 97: 402-405.

Vidal, E. A., Villanueva, R., Andrade, J. P., Gleadall, I. G., Iglesias, J., Koueta, N., Rosas, C., Segawa, S., Albertin, B. C., Caamal-Monsreal, C., Chimal, M. E., Edsinger-Gonzales, E., Franco-Santos, R. M., Gallardo, P., Grasse, B., Le Pabic, C., Pascual, C., Roubledakis, K. and Wood, J. (2014). Cephalopod culture: Current status of main biological models and research priorities. *Advances in Marine Biology* 67: 1–98.

- Von Boletzky, S. and Villanueva, R. (2014). Cephalopod biology. In; *Cephalopod Culture*. Springer Netherlands. pp. 3-16.
- Westerman, K. and Benbow, S. (2013). The role of women in community-based small-scale fisheries management: the case of the southern Madagascar octopus fishery. *Western Indian Ocean Journal of Marine Science* 12(2): 119-132.
- Wood, C. L., Lafferty, K. D. and Micheli, F. (2010). Fishing out marine parasites? Impacts of fishing on rates of parasitism in the ocean. *Ecology Letters* 13: 761 - 775.