RELATIONSHIP BETWEEN PLANKTON POPULATION AND THE SURVIVAL OF EPIDEMIC *VIBRIO CHOLERAE* IN BANGLADESH

A Dissertation Submitted to the University of Dhaka

for the partial fulfillment for the award of the degree of

Doctor of Philosophy (Ph. D.) in Biological Sciences

By

Nahid Sultana

Registration No. 191

Session: 2012-2013

Department of Zoology
University of Dhaka
Dhaka-1000
Bangladesh

June, 2021

Dedicated

To my

Beloved parents;

Caring better half Mr. Muntasir Mahbub

and

Loving kids Manha and Mayaz

Certificate

This is to certify that the work contained in the thesis entitled "Relationship between Plankton Population and the Survival of Epidemic *Vibrio cholerae* in Bangladesh", submitted by Nahid Sultana (Registration No. 191) for the award of the degree of Doctor of Philosophy (Ph.D.) to the University of Dhaka, Bangladesh is a record of genuine research work research works carried out by her under my direct supervision and guidance. I considered that the thesis has reached the standards and fulfilling the requirements of the rules and regulations relating to the nature of the degree. The contents embodied in the thesis have not been submitted for the award of any other degree or diploma in this or any other University.

Dr. Munirul Alam
Joint-Supervisor
Senior Scientist
Infectious Disease Division
ICDDR'B

M. Niamul Naser, Ph.D.
Supervisor
Professor
Department of Zoology
University of Dhaka
Bangladesh

Declaration

I do hereby declare that the work presented in this thesis entitled 'Relationship between Plankton Population and the Survival of Epidemic *Vibrio cholerae* in Bangladesh' was performed by me under supervision of Professor M. Niamul Naser, Ph. D, Department of Zoology, University of Dhaka, Bangladesh and Joint-supervision of Dr. Munirul Alam, Senior Scientist, ICDDR'B for the degree of Doctor of Philosophy. This thesis or any part of the thesis has not been presented before for any degree or any other form to any university. I also declare that the sources of information or data used from the laboratory of University of Dhaka and ICDDR'B has been properly acknowledged.

June, 2021

Nahid Sultana
Ph. D. Research Fellow
Department of Zoology
University of Dhaka
Dhaka-1000, Bangladesh.

Acknowledgements

At first my heartfelt gratefulness to the Almighty for giving me the strength and opportunity to perform the research work in the fine frame. I would like to express my sincere gratitude to my supervisor Professor M. Niamul Naser Ph. D, Department of Zoology, University of Dhaka for his guidance, suggestions and valuable time during the study period. His cooperation and immense knowledge help me a lot to perform the research.

I would like to extend my thanks and gratitude to Dr. Munirul Alam, Senior Scientist, ICDDR'B for giving me the chance to do my microbial part in his laboratory Infectious Disease Division and also his kind support, motivation and guidance during the study.

My earnest thanks to Dr. Marzia Sultana, Assistant Scientist, ICDDR'B for her immense cooperation and support during my research work. I would like to give thanks to all the researchers of Infectious Disease Division i.e., Dr. Monira, Ms. Fatema, Mr. Zillur, Mr. Arif and the staffs Mr. Mostofa and Mr. Rahman for their assistance and appreciation.

I am also grateful to Mr. Abul Hossain, Mr. Abdur Rahim, Mr. Sohrab uddin, Mr. Md. Shahjalal of Department of Zoology, University of Dhaka for their support and assistance during the four year study.

I am indebted to my family members especially my father and mother who always inspired me a lot to do the research. My younger brother is also praised for giving mental support throughout the study period. I am also grateful to my father-in-law, mother-in-law and sister-in-law for their cooperation and inspiration during the critical moment of my study period.

My sincere gratitude and thanks to my better-half Mr. Muntasir Mahbub and my loving kids Manha and Mayaz for their patience and cooperation when I was passing through very tough time of thesis writing.

My colleagues Mr. Rakibul Hasan, Senior Scientific Officer; Mrs. Mahmuda Begum, Scientific Officer and Mrs. Lailatul Ferdousi, Scientific Officer are also praised to be with me when I was busy with the research. Special thanks to Mr. Abdullah Al Helal, Research fellow of BCSIR for his cordial support during sampling. I am grateful to Mr. Shahriar Bashar, Senior Scientific Officer for his cordial support in some analytical part. Special thanks to my senior colleague Dr. Parvin Noor, Chief Scientific Officer and former Director of BCSIR for her continuous support and cooperation. I would also like to thank all of my friends who supported me in writing and incented me to strive towards my goal.

A very special gratitude goes to Bangabandhu Fellowship on Science, Information and Communication Technology for providing the funding which helps me a lot to do the research work.

Finally, I would like to express my special thanks and sincere gratitude to my working place BCSIR (Bangladesh Council of Scientific and Industrial Research) for the laboratory facilities required in my research work and also for giving me the opportunity to complete the research.

Author

ABSTRACT

In spite of the considerable number of critical work that has been conducted on the diarrhoea causing *Vibrio cholarae* bacterium, the ecological role of it's habitat, survival and association with plankton in Bangladesh still little known. The study was conducted at twelve ponds, one canal and one river ecosystems from two geographical locations i.e., Mathbaria and Chhatak, of Bangladesh between the year 2013 and 2014 to assess the role of selected climatic and limnological parameters on the *Vibrio cholarae* and plankton population. Sampling was done on weekly basis during the outbreak season of cholera, while fortnightly in non-infectious period. From the coastal seven ponds of Mathbaria 86 species of zooplankton was recorded, of which 27 species of protozoa, 43 species of rotifera, 8 species of copepod and 8 species of cladocera. Freshwater ponds and river of Chhatak exhibited in total of 100 species of zooplankton of which 14 species belonged to the phylum protozoa, 58 species of rotifera, 9 species of copepod and 19 species of cladocera. In Mathbaria two peak seasons of cholera existed, summer (March-May) and autumn (September-November) where site-2 (pond), site-8 (local canal) and site-11 (pond) were recognized as suspected *V. cholerae* contaminated ponds due to the isolation of toxigenic *V. cholerae* O1 from these water bodies. The total zooplankton, specially crustacean plankton was dominantly recorded during peak season of cholera in all water bodies. In Mathbaria, crustacean planktonic nauplii were recorded in highest number in both peak infection seasons. In non-contaminated ponds ponds (sites-5, 7 and 9) protozoa, rotifer and nauplii were dominant in the peak season of cholera. In Chhatak, peak season of cholera infection was occurred only once in autumn (September-November). Three sampling sites in Chhatak as site-1 (pond), site-10 (Surma River) and site-12 (pond) were suspected as *V. cholerae* contaminated. During the study in Mathbaria and Chhatak seasonal species of copepod *Cyclops sp.*, *Diaptomus sp.* and cladocera *Diaphanosoma sp.*were dominant plankton in two areas. Hydroclimatological factors like total rainfall in Mathbaria started to increase during summer peak (April) and then decrease at the end of autumn peak (November). On the other hand, in Chhatak total rainfall was highest during peak season (September-October) of cholera in the 2013. It was aided by highest air temperature during the peak seasons of cholera in Mathbaria and Chhatak. In pond ecosystem, micronutrients like nitrogen and phosphorus was found to be highest to improve primary

productivity during peak *V. cholerae* season. Laboratory based microcosm study on copepods from three water sources inoculated with the pure culture of toxigenic *Vibrio cholerae* O1 revealed that the count of bacteria was increased with the increased production of nauplii. Direct Fluorescent Antibody (DFA) and Scanning Electron Microscopy (SEM) images exhibits positive association of *Vibrio cholerae* O1 with extracted crab and shrimp chitin. Considering the biomass, the amount of minimum nauplii biomass was found to be 94.3 g per cubic meter dry weight during peak season in the water samples. From this study, it is evident that the hydroclimatic factors in association with the limnological parameters is creating a favorable condition for the emergence and survibility of *V. cholerae* bacteria in the. The water temperature, crustacean zooplankton abundance with certain biomass under favourable nutrients state in the contaminated water bodies help in ensuring the maximum environmental condition for *Vibrio cholera*e survibility. In spite of long geographical distances of coastal Mathbaria water bodies are more vulnerable to contamination and disease spread than freshwater Chhatak waters. However, the environmental challenges being overcome by the bacterium during the infection season.

CONTENTS

	Page no.
Acknowledgements	**i-ii**
Abstract	**iii-iv**
Contents	**v-viii**
List of Tables	**ix-x**
List of Figures	**xi-xiii**
List of Plates	**xiv**
List of Pictures	**xiv**
Chapter 1: Introduction	**1**
1.1 Genaral Introduction	**1**
1.2 Objectives	**6**
Chapter 2: Review of Literatures	**7**
2.1. History of Cholera	**7**
2.2. Cholera and it's ecosystem	**8**
2.3. Environmental persistence of *Vibrio cholerae*	**9**
2.4 Long term outbreak of cholera being observed	**10**
2.5 Conditions of the *Vibrio cholerae* contaminated ponds	**11**
2.6 Zooplankton in different aquatic habitats	**12**
2.7 Zooplankton and their association with *Vibrio cholerae*	**15**
2.8 Environmental influences on *Vibrio cholerae* association with plankton	**16**
2.9 *Vibrio cholerae* and it's relationships with chitin	**18**
2.10 Laboratory based microcosms of *Vibrio cholerae* and nutrients	**19**
2.11 Viability of *Vibrio cholerae* in different ecological habitats	**20**
2.12 Predicting the assessibility of chitin in water: Biomass of plankton	**21**
Chapter 3: Materials and Methods	**22**
3.1 Study Locations	**22**
3.1.1 Mathbaria	**22**
3.1.2 Chhatak	**25**
3.2 Weather Parameters	**28**
3.3 Water Quality Parameters	**28**

3.4 Crab's gut microbes analysis **28**

3.5 Zooplankton sampling and identification **28**
3.5.1 Zooplankton species composition (%) **29**
3.5.2 Relative abundance (%) **29**
3.5.3 Species diversity indices **29**

3.6 Microbiological Analysis **31**
3.6.1 Sampling for biological analysis **31**
3.6.2 Analysis for *Vibrio cholerae* **31**
3.6.3 Direct fluorescent antibody assay (DFA) **32**

3.7 Crab and shrimp shell for chitin extraction **32**

3.8 Fresh crab sample for microbial analysis **33**

3.9 Micro-ecosystem study (Microcosm) of chitin for the attachment of *Vibrio cholerae* **34**
3.9.1 Collection of strain **34**
3.9.2 Water sources and preparation **35**
3.9.3 Preparation of different micro-ecosystem with crab and shrimp based chitin **35**
3.9.4 Preparation of inoculums **35**
3.9.5 Two microcosms supplemented with three chitin flakes from three sources **36**
3.9.6 Processing of samples **36**
3.9.7 Counting procedure for *Vibrio cholerae* O1 **37**
3.9.8 Simple staining **37**
3.9.9 DFA **37**
3.9.10 DNA isolation **37**
3.9.11 M-PCR **38**

3.10 Micro-ecosystem study (microcosm) of copepods in different ecological habitats **38**
3.10.1 Preparation of microcosms of copepoda **38**
3.10.2 Inoculation of *Vibrio cholerae* **38**
3.10.3 Plate Count of *Vibrio cholerae* O1 **39**
3.10.4 Multiplex polymerase chain reaction (mPCR) **39**

3.11 Statistical analysis **39**

Chapter 4: Results and Observations **40**

4.1 Biological assessment of *Vibrio cholerae* affected ponds **40**
4.1.1 Biological assessment of *Vibrio cholerae* affected ponds in Mathbaria **40**
4.1.1.1 Zooplankton composition of different *Vibrio cholerae* affected ponds **40**
4.1.1.2 Zooplankton at Mathbaria ponds: A qualitative approach **51**
4.1.1.3 Species composition of zooplankton in Mathbaria ponds **53**
4.1.1.4 Distribution of Zooplankton in seven Mathbaria ponds **56**
4.1.1.5 Frequency of occurrence of zooplankton in Mathbaria **61**
4.1.1.6 Seasonal abundance of zooplankton species at Mathbaria ponds **64**

4.1.1.7 Zooplankton community structures in Mathbaria **74**
4.1.2 Biological assesssment of *Vibrio cholerae* affected ponds in Chhatak **77**
4.1.2.1 Zooplankton composition of different *Vibrio cholerae* affected ponds **77**
4.1.2.2 Zooplankton at Chhatak ponds: A qualitative approach **86**
4.1.2.3 Species composition of zooplankton in Chhatak ponds **91**
4.1.2.4 Distribution of zooplankton in seven Chhatak ponds **94**
4.1.2.5 Frequency of occurrence of zooplankton in Chhatak **99**
4.1.2.6 Seasonal abundance of zooplankton species at Chhatak ponds **105**
4.1.2.7 Zooplankton community structures in Chhatak **117**
4.2 Climatic factors and it's relationships with ponds limnological dynamics **120**
4.2.1 Limnological dynamics of ponds in Mathbaria **120**
4.2.1.1 Interrelationsips of air and water temperature with rainfall **120**
4.2.1.2 Total rainfall and pH at Mathbaria ponds **122**
4.2.1.3 Total rainfall and it's relation with salinity **124**
4.2.2 Limnological dynamics of ponds in Chhatak **126**
4.2.2.1 Interrelationsips of air and water temperature with rainfall **126**
4.2.2.2 Total rainfall and pH at Chhatak ponds **128**
4.2.2.3 Total rainfall and it's relation with salinity **130**
4.3 Estimating zooplankton species of *Vibrio cholerae* affected ponds under two geographical conditions (Mathbaria and Chhatak) **132**
4.4 Climatic influx on changing environmental condition of pond **136**
4.4.1 Climatic influx on changing environmental condition of Mathbaria **136**
4.4.1.1 Hydroclimatic influence on the plankton count in Mathbaria ponds **136**
4.4.1.2 Interrelation between plankton group and major climatic factors in seven ponds of Mathbaria **144**
4.4.2 Climatic influx on changing environmental condition of Chhatak **147**
4.4.2.1 Hydroclimatic influence on the plankton count in seven ponds and river of Chhatak **147**
4.4.2.2 Interrelation between plankton groups and climatic factors in the studied ponds and river of Chhatak **155**
4.5 *Ex-situ* Experiments of *Vibrio cholerae* growth with zooplankton and chitin extraction **158**
4.5.1 Association of *Vibrio cholerae* with planktonic chitin **158**
4.5.1.1 Growth of *Vibrio cholerae* in Mathbaria water micro-ecosystem (microcosm) **158**
4.5.1.2 Growth of *Vibrio cholerae* in Paikgachha water micro-ecosystem (microcosm) **161**
4.5.1.3 Growth of *Vibrio cholerae* in Lake water micro-ecosystem (microcosm) **162**
4.5.2 Association of *Vibrio cholerae* with crustacean chitin in micro-ecosystem study **163**
4.5.2.1 Growth of *Vibrio cholerae* in microcosms of crab and shrimp chitin **163**
4.5.2.2 Physical observations of microcosms **164**
4.5.2.3 *Vibrio cholerae* in chitin supplemented micro-ecosystems: A DFA image study **167**
4.5.2.4 Association of *Vibrio cholerae* with different chitin structures in micro-ecosystems: A Scanning Electron Microscope (SEM) image study **172**

4.6 Bacterial colony growth in some crab samples **177**
4.7 Availability of nutrients in three *Vibrio cholerae* inhabiting ponds of Mathbaria **177**
4.8 Relationships with nauplii biomass in ponds during infection periods **179**

Chapter 5: Discussions **183**

Chapter 6: Summary and Conclusion **191**

Chapter 7: Recommendations **197**

References **198**

Annexure **216**

LIST OF TABLES

Table	Title of Table	Page no.
Table-1	Comparison of water parameters in Mathbaria and Paikgachha	**35**
Table-2	Quantitative analysis of Zooplankton at Mathbaria pond (Site-2) in 2013 and 2014	**44**
Table-3	Quantitative analysis of Zooplankton at Mathbaria pond (Site-5) in 2013 and 2014	**45**
Table-4	Quantitative analysis of Zooplankton at Mathbaria pond (Site-7) in 2013 and 2014	**46**
Table-5	Quantitative analysis of Zooplankton at Mathbaria pond (Site-8) in 2013 and 2014	**47**
Table-6	Quantitative analysis of Zooplankton at Mathbaria pond (Site-9) in 2013 and 2014	**48**
Table-7	Quantitative analysis of Zooplankton at Mathbaria pond (Site-10) in 2013 and 2014	**49**
Table-8	Quantitative analysis of Zooplankton at Mathbaria pond (Site-11) in 2013 and 2014	**50**
Table-9	Zooplankton species identified from Mathbaria ponds	**51**
Table-10	Diversity of Zooplankton at seven study sites during January 2013-December 2013	**57**
Table-11	Diversity of Zooplankton at seven study sites during January 2014-December 2014	**59**
Table-12	Frequency of Occurrence of particular zooplankton species in Mathbaria on a four degree scale; Absolute Constant Species (AS)- >75%, Constant Species (S)- 51-75%, Absolute Species (A)- 26-50% and Accidental Species (P)- < 25%	**62**
Table-13	Relative abundance of Protozoa at different sites of Mathbaria according to four seasons during two years of study	**64**
Table-14	Relative abundance of Rotifera at different sites of Mathbaria according to four seasons during two years of study	**67**
Table-15	Relative abundance of Copepoda at different sites of Mathbaria according to four seasons during two years of study	**71**
Table-16	Relative abundance of Cladocera at different sites of Mathbaria according to four seasons during two years of study	**73**
Table-17	Diversity Indices of Zooplankton in Summer	**75**
Table-18	Diversity Indices of Zooplankton in Rainy Season	**75**
Table-19	Diversity Indices of Zooplankton in Autumn	**75**
Table-20	Diversity Indices of Zooplankton in Winter	**75**
Table-21	Quantitative analysis of zooplankton at Chhatak pond (Site-1) in 2013 and 2014	**79**
Table-22	Quantitative analysis of zooplankton at Chhatak pond (Site-2) in 2013 and 2014	**80**
Table-23	Quantitative analysis of zooplankton at Chhatak pond (Site-4) in 2013 and 2014	**81**
Table-24	Quantitative analysis of zooplankton at Chhatak pond (Site-9) in 2013 and 2014	**82**
Table-25	Quantitative analysis of zooplankton at Chhatak pond (Site-10) in 2013 and 2014	**83**
Table-26	Quantitative analysis of zooplankton at Chhatak pond (Site-11) in 2013 and 2014	**84**
Table-27	Quantitative analysis of zooplankton at Chhatak pond (Site-12) in 2013 and 2014	**85**
Table-28	Zooplankton species identified from Chhatak ponds	**86**
Table-29	Diversity of zooplankton at seven study sites during January 2013-December 2013	**94**
Table-30	Diversity of zooplankton at seven study sites during January 2014-December 2014	**97**
Table-31	Frequency of Occurrence of particular zooplankton species in Chhatak on a four degree scale; Absolute Constant Species (AS)- >75%, Constant Species (S)- 51-75%, Absolute Species (A)- 26-50% and Accidental Species (P)- < 25%	**101**

Table-32	Relative abundance of Protozoa at different sites of Chhatak according to four seasons during two years of study	**105**
Table-33	Relative abundance of Rotifera at different sites of Chhatak according to four seasons during two years of study	**108**
Table-34	Relative abundance of Copepoda at different sites of Chhatak according to four seasons during two years of study	**114**
Table-35	Relative abundance of Cladocera at different sites of Chhatak according to four seasons during two years of study	**115**
Table-36	Diversity indices of zooplankton in Summer	**119**
Table-37	Diversity indices of zooplankton in Rainy season	**119**
Table-38	Diversity indices of zooplankton in Autumn	**119**
Table-39	Diversity indices of zooplankton in Winter	**119**
Table-40	A comparison of the plankton production in Mathbaria and Chhatak (n=161)	**132**
Table-41	Testing the equality of plankton production in two study areas i.e., Mathbaria and Chhatak	**133**
Table-42	Comparison of plankton production in different month of the year	**134**
Table-43	Comparison of plankton groups between ponds	**135**
Table-44	Correlation among zooplankton groups and some hydroclimatic factors that can influence the abundance of plankton in seven domestic ponds of Mathbaria	**145**
Table-45	Correlation among zooplankton groups and some hydroclimatic factors that can influence the abundance of plankton in seven domestic ponds of Chhatak	**156**
Table-46	Amount of micronutrients analyzed of some infected ponds in Mathbaria during the peak season of cholera	**178**

LIST OF FIGURES

Figure	Title of Figure	Page no.
Figure-1	*Vibrio cholerae and* chitin binding at different hierarchical scales in the ecosystem, environment and human cell response (e.g. cell multiplication, chemotaxis, competence), biofilm formation, association with chitinous organisms, C and N cycling, and pathogenicity for humans (adopted from Carla *et al.*, 2008)	**4**
Figure-2	Pie-chart showing species composition in the Year-2013 and 2014 at site-2	**54**
Figure-3	Pie-chart showing species composition in the Year-2013 and 2014 at site-5	**54**
Figure-4	Pie-chart showing species composition in the Year-2013 and 2014 at site-7	**55**
Figure-5	Pie-chart showing species composition in the Year-2013 and 2014 at site-8	**55**
Figure-6	Pie-chart showing species composition in the Year-2013 and 2014 at site-9	**55**
Figure-7	Pie-chart showing species composition in the Year-2013 and 2014 at site-10	**56**
Figure-8	Pie-chart showing species composition in the Year-2013 and 2014 at site-11	**56**
Figure-9	Pie-chart showing species composition in the year 2013 and 2014 at site-1	**91**
Figure-10	Pie-chart showing species composition in the year 2013 and 2014 at site-2	**92**
Figure-11	Pie-chart showing species composition in the year 2013 and 2014 at site-4	**92**
Figure-12	Pie-chart showing species composition in the year 2013 and 2014 at site-9	**92**
Figure-13	Pie-chart showing species composition in the year 2013 and 2014 at site-10	**93**
Figure-14	Pie-chart showing species composition in the year 2013 and 2014 at site-11	**93**
Figure-15	Pie-chart showing species composition in the year 2013 and 2014 at site-12	**93**
Figure-16	Interrelationships among air and water temperature and total rainfall in Mathbaria ponds	**120-121**
Figure-17	pH of seven Mathbaria ponds and it's relationships with rainfall of that region	**122-123**
Figure-18	Salinity of seven Mathbaria ponds and it's relationships with rainfall of that region	**124-125**
Figure-19	Interrelationships of air and water temperature with rainfall in seven Chhatak ponds	**126-127**
Figure-20	pH of seven Chhatak ponds and it's relationships with rainfall of that region	**128-129**
Figure-21	Salinity of seven Chhatak ponds and it's relationships with rainfall of that region	**130-131**
Figure-22	Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-2	**137**
Figure-23	Impact of precipitation on the abundance of zooplankton at site-2	**137**
Figure-24	Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-5	**138**
Figure-25	Impact of precipitation on the abundance of zooplankton at site-5	**138**
Figure-26	Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-7	**139**
Figure-27	Impact of precipitation on the abundance of zooplankton at site-7	**139**
Figure-28	Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-8	**140**

Figure-29 Impact of precipitation on the abundance of zooplankton at site-8 **140**

Figure-30 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-9 **141**

Figure-31 Impact of precipitation on the abundance of zooplankton at site-9 **141**

Figure-32 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-10 **142**

Figure-33 Impact of precipitation on the abundance of zooplankton at site-10 **142**

Figure-34 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-11 **143**

Figure-35 Impact of precipitation on the abundance of zooplankton at site-11 **143**

Figure-36 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-1 **148**

Figure-37 Impact of precipitation or rainfall on the abundance of zooplankton at site-1 **148**

Figure-38 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-2 **149**

Figure-39 Impact of precipitation or rainfall on the abundance of zooplankton at site-2 **149**

Figure-40 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-4 **150**

Figure-41 Impact of precipitation or rainfall on the abundance of zooplankton at site-4 **150**

Figure-42 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-9 **151**

Figure-43 Impact of precipitation or rainfall on the abundance of zooplankton at site-9 **151**

Figure-44 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-10 **152**

Figure-45 Impact of precipitation or rainfall on the abundance of zooplankton at site-10 **152**

Figure-46 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-11 **153**

Figure-47 Impact of precipitation or rainfall on the abundance of zooplankton at site-11 **153**

Figure-48 Impact of maximum and minimum temperature as hydroclimatological factors on the abundance of zooplankton at site-12 **154**

Figure-49 Impact of precipitation or rainfall on the abundance of zooplankton at site-12 **154**

Figure-50 Growth of *V. cholerae* in Mathbaria water micrococsm (Without feed) **160**

Figure-51 Growth of *V. cholerae* in Mathbaria water micrococsm (With feed) **160**

Figure-52 Growth of *V. cholerae* in Paikgachha water micrococsm (Without feed) **161**

Figure-53 Growth of *V. cholerae* in Paikgachha water micrococsm (With feed) **161**

Figure-54 Growth of *V. cholerae* in Lake water micrococsm (Without feed) **162**

Figure-55 Growth of *V. cholerae* in Lake water micrococsm (With feed) **162**

Figure-56 Association of *V. cholerae* in two different microcosms supplemented with Raw Crab chitin and Raw Shrimp chitin **163**

Figure-57 Mathbaria nauplii data, showing the monthly weight (log) distributions and their relationships with two infection seasons and the ponds (solid line sites 2, 8 and 11 were the contaminated ponds) in 2013. The non-contaminated pond data were shown as in dotted line. March to May and September to November were the infected season of Mathbaria. The December sampling was missing due to strike in communication. The highlighted zone is the disease outbreak period of the sampling sites **179**

Figure-58 Mathbaria nauplii data, showing the monthly weight (log) distributions and their relationships with two infection seasons and the ponds (solid line sites 2, 8 and 11 were the contaminated ponds) in 2014. The non- contaminated pond data were shown as in dotted line. March to May and September to November were the infected season of Mathbaria. The highlighted zone is the disease outbreak period of the sampling sites **180**

Figure-59 Chhatak nauplii data, showing the monthly weight (log) distributions and their relationships with single infection season and the ponds (solid line sites 1, 10 and 12 were the contaminated ponds) in 2013. The non- contaminated pond data were shown as in dotted line. September to November were the infected season of Chhatak. December sampling was missing due to communication strike. The highlighted zone is the disease outbreak period of the sampling sites **181**

Figure-60 Chhatak nauplii data, showing the monthly weight (log) distributions and their relationships with single infection season and the ponds (solid line sites 1, 10 and 12 were the contaminated ponds) in 2014. The non- contaminated pond data were shown as in dotted line. September to November were the infected season of Chhatak. The highlighted zone is the disease outbreak period of the sampling sites **182**

LIST OF PLATES

Plate	Name of Plates	Page no.
Plate-1	Some protozoan plankton identified in Mathbaria and Chhatak	**89**
Plate-2	Some rotifers in Mathbaria and Chhatak	**89**
Plate-3	Copepoda and cladocera species recorded in Mathbaria and Chhatak	**90**
Plate-4	DFA images of copepods to view the attachment position of *V. cholerae* to their carrier	**159**
Plate-5	Physical observation of microcosms supplemented with chitin in Mathbaria water	**165**
Plate-6	Physical observation of microcosms supplemented with chitin in Paikgachha water	**166**
Plate-7	Epifluorescent micrographs of different stages of biofilm formation of *V. cholerae* in Mathbaria water microcosms supplemented with raw crab chitin	**168**
Plate-8	Epifluorescent micrographs of different stages of biofilm formation of *V. cholerae* in Mathbaria water microcosms supplemented with raw shrimp chitin	**169**
Plate-9	Epifluorescent micrographs of different stages of biofilm formation of *V. cholerae* in Paikgachha water microcosms supplemented with raw crab chitin	**170**
Plate-10	Epifluorescent micrographs of different stages of Biofilm formation of *V. cholerae* in Paikgachha water microcosms supplemented with raw shrimp chitin	**171**
Plate-11	Scanning Micoscopic Images of the attachment of *V. cholerae* with raw crab chitin in Mathbaria water microcosm at room temperature	**173**
Plate-12	Scanning Micoscopic Images of the attachment of *V. cholerae* with raw shrimp chitin in Mathbaria water microcosm at room temperature	**174**
Plate-13	Scanning Micoscopic Images of the attachment of *V. cholerae* with raw crab chitin in Paikgachha water microcosm at room temperature	**175**
Plate-14	Scanning Micoscopic Images of the attachment of *V. cholerae* with raw shrimp chitin in Paikgachha water microcosm at room temperature	**176**

LIST OF PICTURES

Map	Name of Map	Page no.
1	GPS Mapping of Mathbaria Upazila showing the location of studied pond	**23**
2	GPS Mapping of Chhatak Upazila showing the location of studied ponds	**26**

Chapter-1. Introduction

1.1 General Introduction

Cholera is an ancient epidemic disease which was pandemic about fifty years ago among the third world countries. On the contrary, it has been disappeared from the developed countries at that time. It is most frequently occurred by the microbial agent *Vibrio cholerae* through ingestion of water contaminated with fecal matters or vomitus of cholera patients. Cholera pathogen *Vibrio cholerae* is a life threatening and therefore important to understand the ecology and survival for extended periods of time in aquatic ecosystem (Xu *et al.*, 1982). According to the World Health Organization *V. cholerae* infects three to five million people each year, causing diarrhoea that can range from mild to very severe consequences.

The genus *Vibrio* belongs to the family Vibrionaceae and consists of 44 recognized species of which 12 species are related to human infections (Brenner *et al.*, 2005). The serotype *V. cholerae* O1 is the causative agent of pandemic cholera of the historical past. *V. cholerae* O139 strain (isolated from Bengal) was isolated from estuarine water to be another causative agent of cholera (Ramamurthy *et al.*, 1993). The epidemic causal strains of *V. cholerae* (O1 or O139 serogroups) produce cholera toxin (CTX) which is the major contributing factor for profuse diarrhoea (cholera gravis) with rice water like stools, dehydration and electrolyte imbalance. Cholera Toxin (CTX) encoded by ctx AB is responsible for the severe diarrhoeal symptoms elicited by *V. cholerae* (Kaper *et al.*, 1995).

V. cholerae O1 serogroup that produces CTX has long been responsible with epidemic and pandemic cholera in the region. Some isolates of *V. cholerae* O1 do not produce CTX and also do not possess the ctx genes encoding CTX (Kaper *et al.*, 1981). Environmental strains are usually CTX negative and are considered to be non-pathogenic (Levine *et al.*, 1982). However CTX negative *V. cholerae* O1 strains has been isolated from occasional cases of diarrhoea or extraintestinal infections (Morris *et al.*, 1984).

V. cholerae is naturally present in the environment and is autochthonous in riverine, coastal, and estuarine ecosystems. The organism residing in both human host and marine or estuarine environments, however estuarine environments supposed to be the best environmental condition for *V. cholerae* (Colwell and Spira 1992; Huq and Colwell 1996 and Faruque *et al.,* 1998). *V. cholerae* possesses very effective strategies for long-term survival in aquatic systems. The ability to survive nutrient deprivation, to enter a viable but non-cultural stage, and to attach to certain substrates may explain why the organism survives and resides in aquatic environment (Xu *et al*., 1982; Singleton *et al.,* 1982; Baker *et al*., 1983 and Colwell *et al*., 1985).

Colwell and associates (Colwell 1970; Colwell *et al.,* 1977; Kaper *et al.,* 1979; Colwell *et al.,* 1984 and Colwell *et al*., 1981) hypothesized that *V. cholerae* O1 (CT+) is an estuarine or brackish water bacterium, demonstrating characteristics primarily of environmental advantage but, possibly, also accidentally causing diarroheal disease in humans.

Surveys performed in non-endemic areas have shown that the majority of *V. cholerae* strains isolated are non-toxigenic (Faruque *et al.,* 2004; Haley *et al.,* 2012; Islam *et al.,* 2013) which suggests that associations with the human host is only one small aspect of the *V. cholerae* life cycle and is not necessary for environmental persistence.

Attachment of *V. cholerae* to various aquatic organisms has been well documented. The bacterium is strongly associated with plankton forming commensal and symbiotic relationships, mainly with copepods (Islam *et al*., 1989; Colwell and Huq, 1995 and Shukla *et al.,* 1995). The copepod exoskeleton has been shown to support large populations of vibrios, including the pathogenic species *V. cholerae* (Tamplin *et al*., 1977; Colwell *et al.,* 1981; Colwell *et al*., 1983 and Huq 1999). Adherence to the roots of water hyacinth, common duckweeds, other freshwater plants and certain blue and blue-green algae has also been shown (Spira *et al.,* 1981; Islam *et al*., 1989).

V. cholerae O-group serotype 1 from cholera patients produces chitinase, suggesting that pandemic strains may have an extra-human ecological niche associated with chitinous organisms (Dastidar and Narayanswami, 1968). This theory (Editorial, 1976; Nalin, 1976; Colwell *et al.,* 1977; Kaper *et al.,* 1979) is consistent with the ecological data on non-agglutinable *V. cholerae* in the Chesapeake Bay (Kaper *et al.,* 1979) and with the recent occurrenceof O-group 1 cholera serotypes linked to ingestion of crabs in Louisiana (Center for Disease Control, 1978) and the culture of O-group 1 serotype *V. cholerae* from local crabs and shrimp. However, incidental contamination of crabs or other fauna with water containing vibrios seems unlikely to cause cholera transmission, because environmental water counts of *V. cholerae* are typically six to eight logs less than that needed to pass the gastric acid barrier and induce cholera in most normal volunteers (Cash *et al*., 1974 and Kaper *et al*., 1979).

From anecological point of view, chitin plays a key role in the biogeochemical cycles of both C and N, and the rates of chitin production and degradation influence C and N pools and their availability (Poulicek *et al.,* 1998). Chitin is, however, rapidly recycled in most environments and the accumulation of chitin in sediment is low (Gooday, 1990). It has been shown that microorganisms, e.g., chitinolytic bacteria that are ubiquitous in the marine environment play a major role in chitin recycling in the ocean (Kirchner, 1995; Poulicek *et al.,* 1998). Adhering bacteria are able to metabolize chitin more efficiently than free-living bacteria, thereby increasing the rate of chitin mineralization in the natural environment (Yu *et al.,* 1991).

Chitin is one of the most abundant and important sources of nutrients and energy in the marine environment (Gooday, 1990). It is distributed throughout all kingdoms, as it is a crucial component of the cell walls of moulds, yeasts, fungi and certain green algae, and is a major component of the cuticles and exoskeleton of worms, mollusks and arthropods (Jeuniaux, 1982).

Vibrio cholerae is an integral part of the aquatic environmentand in addition to heterotrophic protists interacts with a wide range of organisms. The association of *V. cholerae* with zooplankton has been a topic of study since the discovery of cells attached to the surface of copepods in the early 1980s (Huq *et al.,* 1983; Tamplin *et al.,* 1990). Zooplankton is an important part of the aquatic food web, grazing an autotrophic and heterotrophic bacterio, nano, and microplankton and in turn preyed upon by larger plankton, such as insect and crustacean larvae and fish. There is also an interaction between *V. cholerae* and Chitionous zooplankton e.g., copepods and cladocerans (Nalin *et al.,* 1979; Huq *et al.,* 1983, Rawlings *et al*., 2007).

The highly diverse zooplankton community *V. cholerae* serogroup O1 has been reported to attach only to certain groups, notably copepods, cladocerans and rotifers (Tamplin *et al.,* 1990). *Vibrio spp*. produce an extracellular chitinase that aids their adhesion to the integument of planktonic crustaceans (Meilbom *et al*., 2004), explaining the widespread association of these bacteria with these arthropods.

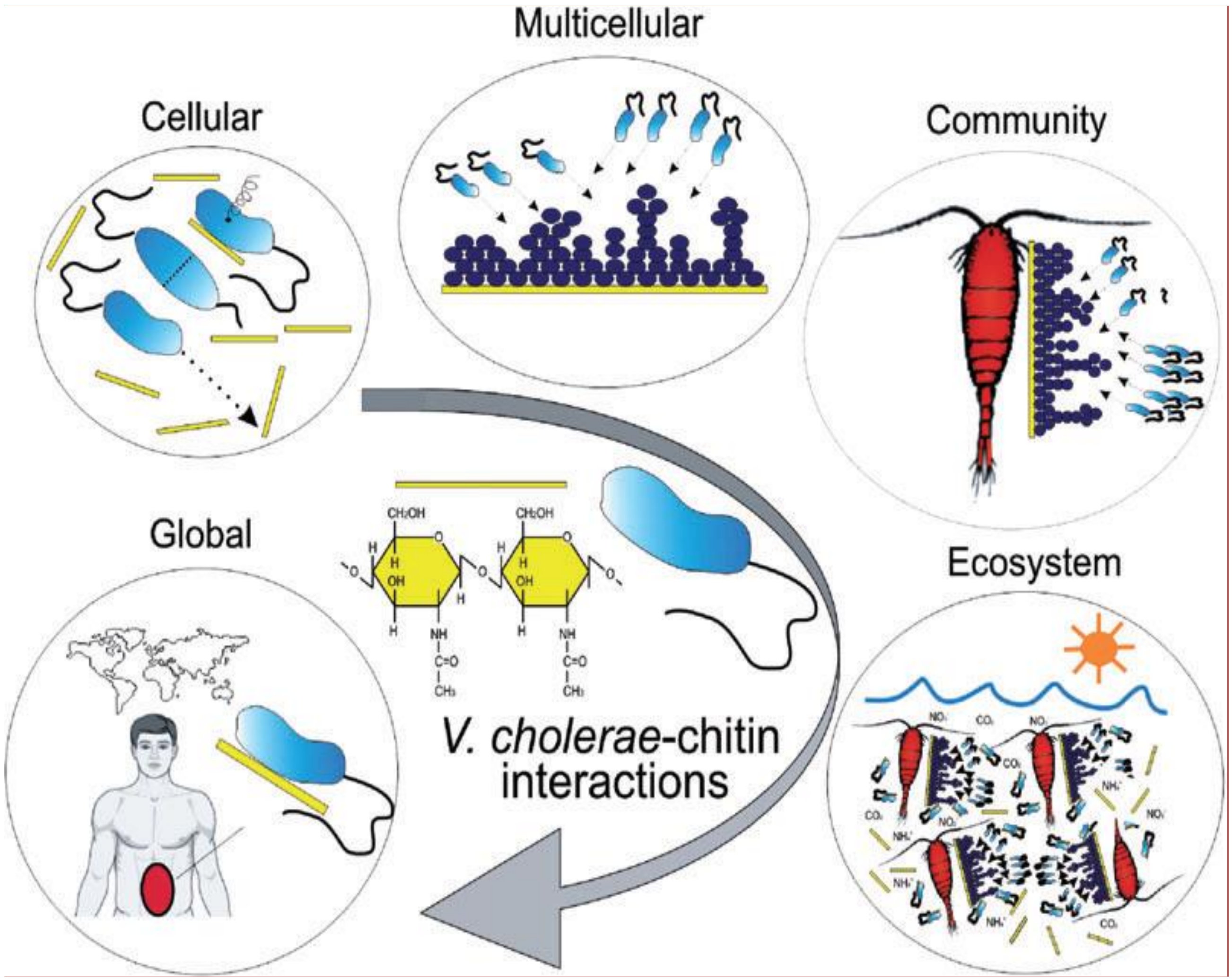


Figure1. *Vibrio cholerae and* chitin binding at different hierarchical scales in the ecosystem, environment andhuman cell response (e.g. cell multiplication, chemotaxis, competence), biofilm formation, association with chitinous organisms, C and N cycling, and pathogenicity for humans (adopted from Carla *et al.*, 2008)

Vibrios favour higher water temperature; consequently, the outbreaks are more frequent during the warmer season (Paz, 2009 and Iwamoto *et al*., 2010). Detection and counts of *Vibrio* spp. have been shown to be correlated with the density of certain zooplankton taxa such as copepods, cirripede nauplii and rotifers (Heidelberg *et al*., 2002). Similarly, the occurance of *V. cholerae* O1 in plankton samples was associated with a high prevalence of juvenile stages of calanoid copepods (Louis *et al.*, 2003).

Outbreaks of cholera over the last several decades in South Asia, Africa, and South America have occurred mostly along coastal areas (Colwell, 1996, de Magny *et al.*, 2008, Jutla *et al.*, 2010). While coastal regions remain the largest natural reservoirs of vibrio bacteria, including *V. cholerae*, epidemiological evidence showed an increase in cholera incidence in inland water regions (Rebaudet *et al.,* 2013). The World Health Organization report database indicates that almost the entire African continent has reported cholera over the past 20 years, with inland regions experiencing massive outbreaks (Jutla *et al*., 2017). However noncoastal regions of Mozambique, Rwanda, Cameroon, and South Sudan reported significant cholera cases in recent decades (Jutla *et al*., 2015). While there is growing evidence of relationships between extreme weather conditions and waterborne infections (Jutla *et al*., 2010, 2015, 2017).

Climate-based early warning systems can provide reliable information on water quality and quantity, natural hazards, and population vulnerability to potential diarrhoeal disease outbreaks. The impact of temperature and rainfall, both associated with climate change, on cholera was studied in Tanzania and the conclusions was that temperature was significantly assocated with cholera, i.e., a one degree celsius increase in air temperature resulted inthe relative risk of cholera by 15-29% (Traerup *et al.*, 2012) .

Studies show that location and intensity of cholera outbreaks can be predicted up to 3 months in advance in the Bay of Bengal Deltic region with understanding of underlying hydroclimatology and satellite-derived environmental variables (Akanda *et al.*, 2012 and Jutla *et al.*, 2013). On the other hand, for Haiti, Pakistan, and Mozambique, different sets of hydroclimatological factors can result in cholera outbreaks (Banddyopadhyay *et al.*, 2012 and Jutla *et al.*, 2013). High temperatures, lack of safe water and sanitation infrastructures play a critical role in the trigger and transmission of *Vibrio cholerae* infection to human populations (Mboera *et al.*, 2012).

A predictive cholera study in Africa examined diarrhoeal incidence in Botswana over a 30-year period, in relation to several climatic variables, including rainfall, minimum temperature and the vapour pressure (Alexander *et. al.*, 2013).

It is not clearly understood what factors in the water body of a particular ecological zone influence *V. cholerae* to excelerate the pathogenic condition and then spreading disease.

1.2 Objectives:

Considering the situation giving emphasis on seasonality and diversity of zooplankton in two different ecological areas (e.g., Mathbaria and Chhatak) in Bangladesh, an attempt had been made to develop artificial habitats in the laboratory to show the nutritional requirement and affinity of *V. cholerae* to a particular zooplankton or anyalternate host. The general aim of the present study is to find out the ecological relationships and association of *V. cholerae* serogroup O1 with plankton occurring in aquatic ecosystem of Mathbaria and Chattak. The present research was undertaken with the following specific objectives;

- Taxonomic identification of zooplankton from the aquatic ecosystem of Mathbaria and Chhatak;
- Observing seasonal dynamics of the plankton population in the selected waterbodies;
- Identifying the host range of plankton for *Vibrio cholerae* bacterium;
- To assess the seasonal variation of *V. cholerae* in the selected water bodies;
- Assessing the influence of physico-chemical variables of water and the incidence of cholera in the coastal aquatic environment;
- Role of copepods and other sources of chitin in laboratory micro ecosystems to show the range of attachment with *Vibrio cholerae*.

Chapter-2. Review of Literatures

2.1 History of Cholera

The cholera causing bacterium *Vibrio cholerae* is the species of the genus *Vibrio* under the family Vibrionaceae. Members of this genus are facultatively anaerobic, a sporogenous, motile, curved or straight gram-negative rods 1.4 to 2.6 µm in length. More than 13 serogroups of *V. cholerae* have been identified. Cholera is the disease caused mainly by the serogroup O1 of *V. cholerae* (Abd, *et al*., 2004 and Alam *et al*., 2006a).

During the historical times of Hippocrates and Buddha, cholera like diseases emerged which was after then reported as first epidemic outbreak across the Indian subcontinent in Southeast Asia. During the 19th century six cholera pandemics took place, ending in 1923 and affecting mostly the continents located in the southern hemisphere, as well as North America and Europe (Pollitzer, 1959 ; Barua, 1991).

In 1961, the seventh pandemic began in Indonesia then spread to the Indian subcontinent and Middle East, then moved on to Africa in the 1970s and finally reached South America in the early 1990s (Blake, 1994; Swerdlow and Issacson 1994; Tauxe *et al*.,1994 ; Faruque *et al.*, 1998).

Epidemic cholera is caused by strains of *Vibrio cholerae* that produce enterotoxin; strains that do not produce the toxin are identified as non-epidemic, although they may cause diarrhoea. The presence of the microorganisms in aquatic environments does not depend solely on the presence of the fecal contamination. No correlation between the presence of fecal coliform bacteria and toxigenic and non-toxigenic *Vibrio cholerae* O1 biotype El Tor in aquatic environments observed in several studies (Colwell *et al.,* 1981; Hood *et al*., 1981; Hood and Ness 1982). Toxigenic *Vibrio cholerae* O1 biotype El Tor has also been detected for extended periods in freshwater where there is no human fecal contamination observed (Roggers *et al.,* 1980; Bourke *et al.,* 1986).

In South Asia two seasonal peaks of cholera coincide with the dry season and rainy season (Emch *et al.,* 2008). In Bangladesh, the freshwater sources become more salty all through dry season and during monsoon the fresh water bodies are inundated by coastal flooding, and this flood water can lead to the contamination of fresh water with brackish water organisms. During this time, the toxigenic strains of cholera bacterium have been isolated from the aquatic ecosystem of Bangladesh in association with diverse groups of arthropods (Alam *et al.,* 2006a, 2006b, 2007; Nahar *et al.,* 2012) as well as unicellular organisms such as protozoan (Abd *et al.,* 2004). Cholera is endemic to Bangladesh and occurs in bimodal seasonal pattern (Glass *et al.,* 1982; Longini. 2002 ; Sack *et al.,* 2003).

2.2 Cholera and it's Ecosystem

Colwell (1996) and Pascual *et al.*, (2002) studied effects of environmental changes on the cholera incidence. It is of interest to understand the mechanisms thataffect the natural populations of *V. cholerae* in the environment and to anticipate the potential impact of extreme climate events such as abnormally hot temperatures or floods on cholera. Changes in the number of *V. cholerae* reservoirs could lead to changes in the number of bacteria in theenvironment. Thus, climatic and/or environmental changes can potentially be responsible for the emergence of cholera in human populations.

According to Yildiiz and Schoolnik (1999); Watnick *et al*. (1999) and Watnick *et al*. (2001) *V. cholerae* O1 El Tor and O139 are both able to form a three-dimensional biofilm on abiotic surfaces. Biofilm formation is likely to be important for the life-cycle of *V. cholerae*, facilitating environmental persistence within natural aquatic habitats during interepidemic periods.

A hierarchial model later been proposed which defines the role of environmental, weather and climatic related variables on the outbreaks of cholera (Colwell and Huq, 1994; Lipp *et al.,* 2002). Coastal regions surrounded by the Bay of Bengal, Bangladesh, the Indian subcontinent, Africa and coastal Latin America now-a-days considered to be the main geographicalregions of cholera endemicity. This is because of the similarity of environmental parameters in these regions. Sunlight, temperature and nutrients affect the growth of phytoplankton and aquatic plants, in addition to affecting the growth of *V. cholerae* population in aquatic ecosystem.

Lipp *et al.,* 2002 revisited this previous model and suggested a scaling up-and-down scenario to interpret the significance of climate and environment on *V. cholerae* population dynamics and its incidence in terms of cholera cases community.

2.3 Environmental Persistence of *Vibrio cholerae*

One of the most dangerous gastroenteric infections is cholera, which is a major health problem in developing countries. Epidemic cholera is caused by enterotoxin-producing *V. cholerae* of serogroup O1and O139. *V. cholerae* O1 consists of the classic and El Tor biotypes, the latter of which is responsible for the seventh pandemic cholera. In humans, *V. cholerae* infection results from ingestion of the bacteria, and depends on the size of pathogen inoculum. The incubation period for *V. cholerae* can range from several hours to five days, and again is dependant in part on the inoculum size (Levine *et al*., 1981).

Singleton *et al.* (1982) observed that optimal growth conditions for *V. cholerae* include 37°C temperature, with persistence in the environment when temperatures reach less than 10°C and as high as 43°C.

V.cholerae is naturally present in the environment and is autochthonous in riverine, coastal and estuarine ecosystems (Alam *et al.*, 2007; Baumann *et al*., 1984 and Baumannand Schubert, 1984). The bacterium is strongly associated with plankton, forming commensal or symbiotic relationships, mainly with copepods (Colwell and Spira, 1992; Huq and Colwell, 1996 and Faruque *et al*., 1998).

Vibrios are abundant in aquatic environments, where they are found free-living in water or in association with plankton. Vibrios favour higher water temperature; consequently, the outbreaks are more frequent during the warmer season (Paz, 2009; Iwamoto *et al*., 2010).

In the aquatic ecosystem, chitin is the most abundant polysaccharide and the principal component of many zooplankton exoskeleton. Chitinous organisms i.e., copepods and other crustaceans are dominant among zooplankton populations. The copepod exoskeleton has been shown to support large populations of vibrios, including the pathogenic species, *V. cholerae* (Islam *et al*., 1989; Colwell and Huq 1994; Shukla *et al*., 1995; Colwell and Huq, 1999).

Previous theory of the survibility of *V. cholerae* O1 in aquatic environment for few hours or days was abandoned because of the proven study that the presence of the microorganisms in aquatic environments does not depend solely on the extent of fecal contamination. There is no correlation between the presence of coliform bacteria and toxigenic and non toxigenic strains of *V. cholerae*

O1 biotype El Tor in aquatic environments (Colwell *et al.*, 1981; Hood *et al.,* 1981; Hood and Ness, 1982). Laboratory research also supported the hypothesis that the microorganism is an autochthonous member of the microbial flora found in brackish waters typical of estuaries and coastal swamps (Singleton *et al*., 1982; Miller *et al*., 1984).

Colwell (1996); Faruque *et al.* (2003) and Huq *et al.* (2005) opined that abundance of *V. cholerae* appears to be triggered by environmental signals. The central role of a climatic factor(s) in the clonal selection of an epidemic strain becomes evident from the reemergence of *V. cholerae* O1, which eventually displaced the epidemic clone of *V. cholerae* O139 and remained the sole causative agent of cholera in Mathbaria.

2.4 Long Term Outbreak of Cholera being observed

Kaneko and Colwell (1975) observed that *Vibrio parahaemolyticus* was absorbed onto copepods which wereaffected by the efficacy of pH and salinity. Kaneko and Colwell (1978) also studied pH and salinity were major factors influencing the distribution of *V. parahaemolyticus* in estuarine ecosystems such as Chesapeake Bay.

According to West (1989), temperature is thought to be the most important ecological parameter governing the survival and growth of *Vibeio cholerae* in aquatic environments. The optimum temperature for growth of this microorganism is 37ºC (Burrows, 1979; Ananthanaryan, 1984; Jawetz *et al.*, 1990).

Environmental factors, e.g., precipitation, salinity, temperature and nutrients, have been shown to be associated with the presence and growth of cholera bacteria (*Vibrio cholerae*) in the aquatic environment (Singleton *et al.,* 1982; Epstein 1993; Alam *et al.,* 2006).

Early ecological studies of cholera by Colwell (1984); Kaysner *et al.* (1987) and Islam *et al.* (1995) showed that *V. cholerae* is readily isolated from brackish, estuarine or marine ecosystems and the biological factors play an important role in the epidemiology of cholera.

Huq *et al.* (1984) studied in laboratory microcosm that at 5‰ salinity value *V. cholerae* survived longer in the presence of live copepods.

Taneja *et al.* (2003, 2005, 2009 and 2010) pointed out that the northern region of the Indian sub-continent, which has no coastal connection, has endured several cholera epidemics.

Pascual *et al.* (2000) observed that changes in climate, classically related to warm temperatures and pre and post heavy rains can directly influence the appearance of cholera.

According to the study of Thomas, Raveendran and Nair (2006), Salinity and temperature are reported to be important parameters controlling growth of *V. cholerae* in estuarine environments.

Bompangue *et al.* (2011) observed the increase in cholera outbreaks following heavy rainfallin epidemic regions of Africa. Similar observations have been reported in Bangladesh earlier (Hashizume *et al.,* 2008; Hashizume *et al.,* 2011; Cash *et al.,* 2009) and later from East Africa (Reyburn *et al.,* 2011).

Mishra *et al.* (2011) studied that isolation of cholera bacteria increased significantly when the temperature was above 25°C in the flatlands of India. He also analyzed several freshwater sites where an early summer season (April-June) with warm temperature was conductive to proliferation *V. cholerae* in the environment.

Gurbanov *et al.* (2012) hypothesized the role of temperature in several recent studies where incidence of cholera peaked when the temperature reached 26°C.

Jutla *et al.* (2013) hypothesized that, in epidemic cholera regions elevated air temperatures create environmental conditions favorable for bacterial growth followed by above normal rainfall in combination with appropriate transmission mechanisms suchas poor availability of safe water and destruction of sanitation (Akanda *et al.*, 2011a) infrastructures aiding in mixing of overflowing sewers with flood waters (Rinaldo *et al.*, 2012), result in an epidemic of cholera.

The survival of *Vibrio cholerae* in aquatic environments is linked to both abiotic and biotic ecological factors, which are likely to be influenced by global climate changes and sea level rise (Colwell, 2005; West, 1989 and Islam *et al.*, 1994).

2.5 Conditions of the *Vibrio cholerae* Contaminated Ponds

Cockburn and Cassanos (1960) first proposed the theory about the main source of infection in several ponds of Bangladesh communityto the community. According to their proposal, if the pH in ponds were sufficiently elevated, *V. cholerae* could outcompete other bacteria and reach infectious dose levels. Experimentally they showed a relationship between elevated pH and onset of cholera cases, which was also related to time of year, light, temperature and precipitation.

Huq (1984) observed that among physical factors temperature perhaps has the most direct and significant effect on the ecology of most bacteria. Warmer temperature in combination with elevated pH and plankton blooms can influence its attachment, growth, and multiplication in the aquatic environment, particularly in association with copepods. An alkaline pH of 8.5, often associated with algal blooms, was found to positively influence the attachment of *V. cholerae* to copepods.

Kaper *et al.* (1979), Lee *et al.* (1984) and Roberts *et al.* (1984) investigated that high temperature during summer months appear to be favourable for survival of *V. cholerae* in water in the environment.

Tamplin and Colwell (1986) observed that physical and chemical parameters of the aquatic environment affected not only the physiological state of *V. cholerae* but also it's potential pathogenecity.

According to Houghton *et al.,* 2001 Climatologists predict a 1.4°C to 5.8°C rise in mean temperature over the next 100 years which will affect the activity of the phytoplankton and the solubility of CO_2 in sea water. Increasing temperature would be expected to expand the range and increase the prevalence of *V. cholerae* and cholera both geographically and temporally, if public health measures are not implemented.

2.6 Zooplankton in Different Aquatic Habitats

A variation of zooplankton in different aquatic habitats was observed by species and numbers. Michael (1968) worked in detail on the ecology of zooplankton population from different waters of India. Rotifers, Cladocerans, Copepods and Ostracods constitute the major groups of Zooplanktons.

Sager and Hasler (1969) stated that the species diversity is influenced by richness and equitability or relative abundance of species.

Cairns (1979) mentioned that fresh water is one of the abundantly available resources which man has utilized for the sustenance of life. Water of good quality is required by living organisms to meet their everyday demands. Increasing level of pollutions into the surface waters has been causing serious disturbances in the aquatic ecosystems which are reflected in the biotic community structure.

Chowdhury *et al.* (1989) observed the occurrence and seasonal variation of zooplankton in relation to some physico-chemical factors. Rotifers appeared at the dominant group (52%), followed by protozoans (16%), ostracods (12%), and cladocerans (4%). Rotifers, the most common zooplankton in the sample were found to occur in abundance in April (22.43%) and least in May (1.07%). Two other groups also attain peak during the month of April (Ostracods, with 26.6% and Nauplius with 29.58%). Cladocerans appeared as the least abundantgroup over the year was absent in the months of April, May, October, December and January. The coefficient of correlation between temperature and occurrence of zooplankton showed an inverse relationship -0.47 when air temperature was considered and -0.33 when water temperature was considered.

Baruah *et al.* (1997) and Gunale (1991) studied that plankton population is very much sensitive to the environment in which they live and alteration in them leads to change in the communities in terms of tolerance, abundance, diversity and dominance in the habitat.

Saha (2004) observed that the evenness showed insignificant relationship with species diversity index, species richness showed negative relationship with species diversity index values in coal field areas of Jharkhand. He got 9 species of cladocerans and rotifers, 7 species of copepods and one species of ostracoda. He explained reason of negative relationship between species diversity index and species richness index as the effect of high alkalinity of water due to fly ash deposition.

Ravi Kumar *et al.* (2005) reported that the management of any aquatic ecosystem is a means of conservation of fresh water habitat with an aim to maintain the water quality or to rehabilitate the physico-chemical and biological setting of water.

Sharma *et al.* (2007) reported that zooplankton communities are typically diverse and are highly sensitive to environmental variation. Due to short life cycle, zooplankton communities often respond quickly to environmental change, the changes in physico-chemical conditions of water can be reflected directly on the biotic community of ecosystem.

Ansari *et al.* (2007) studied on physico-chemical aspects and plankton of Unkal Lake in Karnataka, India. In this study, they revealed the presence of phytoplankton consisted of 13 species of Cyanophycea, 12 species of Chlorophyceae and 3 species of Bacillariophyceae. They are also revealed the occurrence of zooplankton consisted of 3 species of protozoa, 23 species of Rotifera, 16 species of crustacea (including copepoda, cladoceara and ostracoda).

Kedar *et al.* (2007) identified total 61 species of zooplankton by 5 groups such as Protozoa (14 sp.), Rotifera (29 spp.), Copepoda (6 spp.), Ostracoda (5 spp.) and Cladocera (7 spp.) from Rishi Lake of Karanja, Maharastra, India. During the study period, the highest numbers of zooplankton were recorded in summer months and lowest in rainy season.

Nahar *et al.* (2008) found a total of 30 species belonging to 16 genera of rotifers from the ponds of Bakerganj area. Among them most common genus was *Brachionus* with 10 different species which was the most common and viable genus in the coastal ponds.

Rahman and Hussain (2008) studied on the abundance of zooplankton of a culture and a non-culture pond of the Rajshahi University campus and identified 4 groups (Rotifera, Copepoda, Cladocera and Crustacean larvae) of zooplankton, where copepods (1260 units/l in culture and non-culture pond respectively) were most dominant. A total of 9 genera of zooplankton were identified of which *Cyclops* (68.25% and 60.28% of total copepod) was most abundant in both ponds. During study, total zooplankton showed positive correlation with pH, carbonate alkalinity (CO_3) and bicarbonate alkalinity (HCO_3) in both ponds and DO, CO_2 in culture pond. They found that the culture pond showed better result than that of the non-culture pond regarding zooplankton production.

Mozumder *et al.* (2011a) studied the rotifer fauna of Mathbaria in Southern part of Bangladesh and identified a total of 22 species of rotifers. Among them *Polyarthra vulgaris*, *Brachionus caudatus*, *B. falcatus*, *Filinia longiseta*, *F. terminalis*, *Hexarthra intermedia*, *Horaella brehmi*, *Keratella tropica* and *Trichocerca cylindrica* were common species in all three aquatic environments round the year.

Mozumder *et al.* (2011b) investigated seasonal diversirty and abundance of xooplankton species at three ponds of Mathbria from surface water column during January 2008 to December 2008. During the study period, 36 species of zooplankton were identified from the pond. Among these, 25 species belonged to rotifer, 6 species were of protozoans, 3 were copepods and one each from cladocera and ostracoda.

Mozumder *et al.* (2011c) observed 3 genera of protozoan (*Glaucoma*, *Nassula* and *Holophyra*) in 3 ponds of Mathbria. The mean composition of protozoan of ponds was 1,489 ind/l and the percentage composition was *Glaucoma* 74.16%, *Nassula* 12.44% and *Holophyra* 0.96% of total protozoan. The physico-chemical parameters and zooplankton composition showed direct relationship with each other. Water temperature showed direct relationship with air temperature (r= 0.941). Water temperature showed positive relationship with pH (r=0.676) and DO (r=0.348). pH showed positive relationship with DO (r= 0.351). Protozoans showed positive relationship with dissolved oxygen (r= 0.227) while inversely related with water temperature (r= -0.276) and pH (r= -0.397).

2.7 Zooplankton and their Association with *Vibrio cholerae*

Huq *et al.* (1983) studied that copepods play an important role in the survival, multiplication, and transmission of *V. cholerae* and related vibrios in the natural aquatic environment. Results of their study also suggested that the surface and gut of zooplankton are ecosystems that may deter the onset of a non-culturable state and/or provide for improved growth of these bacteria.

Further Huq *et al.* (1984) reported the association of *V. cholerae* with planktonic copepods and Tamplin and colleagues (1990) showed that planktonic copepods play a key role in the survival and distribution of vibrios in the aquatic environment.

Zooplankton is microscopic organisms, which move at the mercy of water currents. Rotifera, cladocerans, copepod and ostracoda constitute the major groups of zooplankton which occupy an intermediate position in the food web. They are also important component in the transfer of energy from primary producers of phytoplankton to higher trophic level such as fish (Stemberger, 1990).

Huq and Colwell (1996) suggested that ingestion of plankton at the time of the spring and autumn blooms was associated with increase in cholera cases in Bangladesh.

Colwell *et al.* (1996) concluded that the association of *V. cholerae* with plankton is a significant factor in the occurrence of cholera in temperate and tropical coastal areas of the world.

Most zooplanktons are filter feeders that use their appendages to strain bacteria, algae and other fine particles of water (Thilak, 2009). Zooplankton also serve as an important host for *V. cholerae* which present throughout the year in and on zooplankton (Huq *et al.,* 1990). It's commensal existence provides protection from grazing by heterotrophic nanoflagellates (Matz and Kjelleberg, 2005) and also from toxic chemicals, including those used to disinfect drinking water, such as alumn and chlorine (Chowdhury *et al.*, 1997).

Louis *et al.* (2003) observed that the occurrence of *V. cholerae* O1 in plankton samples was associated with a high prevalence of juvenile stages of calanoid copepods.

Rawlings *et al.* (2007) observed that *V. cholerae* has a close association with copepods for persistence and multiplication in the natural environment, specifically with the calanoid copepods *Acartia tonsa* and *Eurytemora affinis.*

De Magny *et al*. (2011) suggested the use of different zooplankter to predict cholera epidemics as they demonstrated that the cladocerans, *Moina* spp. and *Diaphanosoma* spp. As well as the rotifer *Brachionus angularis*, were significantly correlated with the presence of *Vibrio cholerae* and with cholera outbreaks.

Oumar *et al.* (2014) observed that *V. cholerae* was isolated from fish mainly during the warm period including March, April and May.

2.8 Environmental Influences on *Vibrio cholerae A*ssociation with Plankton

Kaneko and Colwell (1975) studied that interactions between vibrios and copepods are affected by environmental variables.

Nalin *et al.* (1979) studied that, *V. cholerae* multiplies efficiently on chitinous fauna, including crab, shrimp and zooplankton. Surface and gut of zooplankton are ecosystems that may deter the onset of a non-culturable state and/or provide for improved growth of these bacteria as per suggestion of Huq *et al.* (1983).

Huq (1984) observed that salinity of 15‰ and temperatures ranging from 25° to 30°C have been shown to be important in influencing the attachment of *V. cholerae* to copepods. Salinity values above 34 is known to affect *V. cholerae*, but the influence of salinity on the attachment of vibrios to surface is unknown (Miller *et al.*, 1984).

The relationship of climate with infectious diseases has been reported by Colwell (1996).

Baruah *et al.* (1997) and Gunale (1991) observed that, plankton population is very much sensitive to the environment in which they live and alteration in them leads to change in the communities in terms of tolerance, abundance, diversity and dominance in the habitat and these observations may be used as a reliable tool for biomonitoring studies to assess the pollution status.

Colwell and Patz (1998) observed that, cholera outbreaks are associated with rainfall and warm temperatures of water.

In previous studies environmental connections to cholera epidemics had been established by several investigators (Kelly-Hope *et al.,* 2008; Jutla *et al.,* 2013; Pascual *et al.*, 2000)

According to Lipp *et al.* (2002) and Louis *et al*., 2003, statistically significant empirical relationships have been established between the presence of *V. cholerae* and environmental factors, notably temperature and salinity affecting the growth rates of *V. cholerae*. The exact mechanisms and environmental interactions giving rise to proliferation of *V. cholerae* are poorly understood.

Li and Roseman (2004) reported that binding to chitin in the environment may be either a causal phenomenon or promoted by chitin and/or chitin oligomers.

Temperature is strongly correlated with *V. cholerae* attachment to zooplankton (Turner *et al.*, 2009).

Warm temperature and increased rainfall have been shown to have strong association with cholera in many regions of the world, including Africa (Reyburn *et al.,* 2011), Haiti (Kirpich *et al.,* 2015), Zimbabwe (Jutla *et al.,* 2015), Bangladesh (Hashizume *et al.,* 2008), and India (1885).

2.9 *Vibrio cholerae* and it's Relationships with Chitin

Dastidar and Narayanaswami (1968) isolated a Chitinase in *V. cholerae* O1 and in Kaneko and Colwell (1975) described the absorption of *V. parahaemolyticus* onto the chitin of copepods zooplankton.

Freter (1969); Gibbons and Houte (1971); Guentzel and Berry (1975); Huq *et al.,* (1984) and Jones *et al.,* (1976) observed the multiple recognition sites of *V. cholerae* including the intestinal mucosa, brush border cells and chitin. They also reported the attachment of *V. cholerae* to hindgut mucosa of blue crabs (*Callinectes sapidus*).

Nagy *et al.* (1977) reported the surface-specific attachment and colonization of *V. cholerae*.

According to Costerton *et al.* (1978), attachment of bacteria is considered a prerequisite in the pathogenesis of many bacteria, notably enteric pathogens.

Bauman *et al.* (1980) studied that all pathogenic vibrio species elaborate an extracellular chitinase and also investigated the association between these pathogenic vibrios and the chitin-containing zooplankton in the water column.

Huq *et al.* (1986) reported attachment of *V. cholerae* O1 to the hindgut of the blue crab which (*Callinectes sapidus*) which is an extension of the exoskeleton and is chitin lined. This observation of specific attachment by vibrios in crabs has important implications for the epidemiology and transmission of cholera in the aquatic environment, since ingestion of shell fish is well established as a major factor for cholera in endemic areas.

Costerton *et al.* (1999) studied that chitin interactions at the cellular level can lead to the formation of multicellular complexes, e.g., biofilm formation.

Watnick *et al.,* (1999) observed that, in the aquatic environment diverse substances including suspended mineral particulates, of which the negatively charged silicates are a major component, plants whose surfaces include organic polymers such as cellulose, and the chitinous exoskeletons of crustaceans are available for biofilm formation.

Broza and Halpern (2001) reported that chironomids (non-biting midges) constituted a new important reservoir of *V. cholerae* in the environment. Broza *et al.*, (2005) also found the bacterium to be associated with egg masses and adult midge.

Li and Roseman (2004) reported chemotaxis of *V. cholerae* toward chitin oligosaccharides where binding to chitin in the environment may be either a causal phenomenon or promoted by chitin and/or chitin oligomers.

According to Muller *et al*. (2007), *V. cholerae* strains possess multiple strategies for surface colonization depending upon the presence and expression of both conserved and variable genes. Binding to chitin is a complex process involving hydrophobic and ionic bonds, forces responsible for the primary reversible phase of attachment and specific cell ligands that are responsible for subsequent firm anchoring to substrate.

2.10 Laboratory Based Microcosms of *Vibrio cholerae* and Nutrients

Nutrient requirements of microcosm created in laboratory condition varies. Accordingto Cole (1979) and Huq *et al.* (1984), growth of plankton and aquatic vascular plants depends on temperature, pH and salinity as well as nutrients. The chief nutrients nitrogen and phosphorus in sewage effluents, fertilizers, organic and inorganic pollutants and combined byproducts, together considered tobe the primary cause of eutrofication or coastal algae overgrowth.

West and Lee (1982) in their early studies identified water temperature, salinity and nutrient concentrations by using laboratory microcosms, as abiotic parameters affecting growth and survival of *V. cholerae* in chemically defined aquatic environments. These environmental parameters also were shown to influence the temporal and spatial distribution of *V. cholerae* in freshwater and estuarine envioronments in nature.

Singleton *et al.* (1982) used laboratory microecosystems (microcosms) prepared with a chemically defined sea salt solution, to study effects of selected environmental parameters on growth and activity of *Vibrio cholerae*. Growth responses under simulated estuarine conditions of 10 strains of *V. cholerae*, including clinicand environmental isolates as well as serovers O1 and non-O1, were compared and all strains yielded populations of approximately the same final size. Effect of salinity and temperature on extended survival of *V. cholerae* demonstrated that, at an estuarine salinity (25‰) and a temperature of 10°C, *V. cholerae* survived (i.e., was culturable) for less then 4 days. Salinity was also found to influence activity, as measured by uptake of 14C-amino avids. Studies on the effect of selected ions on growth and activity of *V. cholerae* demonstrated that Na^+ was required for growth.

Huq *et al.* (1984) investigated the influence of water temperature, salinity and pH on the multiplication of toxigenic *V. cholerae* serover O1 cells and their attachment to live planktonic crustaceans, i.e., copepods by using laboratory microcosms. These were measured by culturable counts on agar plates and direct observation by scanning electron microscopy, respectively. Of the three salinities examined (5‰, 10‰ and 15‰).

Borroto (1997) observed that *Vibrio* sometimes requires NaCl and even grows in high saline aquatic environments. An adequate concentration of nutrients in fresh water may meet its salinity requirements. Furthermore, it is facultatively anaerobic, highly sensitive to acidity and has little resistance to solar radiation.

2.11 Viability of *Vibrio cholerae* in Different Ecological Habitats

The viability of *Vibrio cholerae* ecological habitat related to its survival and pathogenecity. Huq *et al.* (1996); Akselman *et al.* (2010); Shikuma and Hadfield, (2010) observed that *V. cholerae* attaches to abiotic and biotic surfaces (chitinous as well as gelatinous zoo and phytoplankton) as biofilms.

According to Nilsson *et al.* (1991); McDougald *et al.* (1998) and Oliver (2010) VBNC cells fail to grow on culture media in contrast to starved cells which are often reduced in size though metabolically active. Factors known to induce VBNC formation in *V. cholerae* include extremes in in temperature and salinity as well as nutrient deprivation (Colwell *et al.*, 1985; Ravel *et al.*, 1995; Carroll *et al.*, 2001; Gonzalez-Escalona *et al.*, 2006; Thomas *et al.*, 2006; Mishra *et al.*, 2012).

McDougald *et al.* (1999) reported that theVBNC cells in unfavorable condition are able to resuscitate and divide when conditions become favorable. Numerous conditions that induce VBNC formation in different species, numerous factors such as temperature upshift (Nilson *et al.,* 1991; Mishra *et al.,* 2012) or an increase in nutrients (Binsztein *et al.,* 2004; Senoh *et al.,* 2010).

Colwell (2000) and Thomas *et al.,* 2006 stated that the evolution of a range of adaptive responses allow *V. cholerae* to survive stressors such as nutrient deprivation, fluctuations in salinity and temperature and to resist predation by heterotrophic protists and bacteriophage. This strategy is the conversion into a viable but non-culturable (VBNC) state during unfavourable conditions.

Islam *et al.,* 2007 suggested that cells of *Vibrio cholerae* in laboratory microcosm experiments form biofilms on biotic and abiotic surfaces for protecting themselves with this exopolymer barrier. Biofilm is a slimy, slippery coat which is formed when the bacteria adhere to the solid surface. It has been suggested that biofilms play a significant role in the transmission and persistence of human disease. Biofilms offer protection to the human pathogenic bacteria from the host immune system and allow those bacteria to withstand killing doses of antibiotics.

2.12 Predicting the Assessibility of Chitin in Water: Biomass of Plankton

The biomass is the mass of living biological organisms in a given area or ecosystem at given time. Biomass can refer to species biomass, which is the mass of one or more species, or to community biomass which is the mass of all species in the community. It can include microorganisms, plants or animals. The mass can be expressed as the average mass per unit area, or as the total mass in the community (Nic *et al.,* 2009). The impact of an environmental variable on population dynamics is typically largest when it is highly variable and affects population growth with a steep and monotonic functional response (Eppley, 1972). Temperature, salinity, stratification and nutrients are key environmental variables for plankton population dynamics, and these variables are also influenced by anthropogenic pressures such as eutrophication and climate change (Suikkanen *et al.,* 2013 and Andersson *et al.,* 2015).

Chapter-3. Materials and Methods

3.1 Study Locations

Fourteen domestic ponds and river sites of coastal Mathbaria and hilly Chhatak ecosystem were used for this study.

3.1.1 Mathbaria:

Mathbaria is a coastal upazila of Bangladesh located adjacent to the Bay of Bengal, approximately 400 km southwest of the capital city Dhaka. It is an administrative unit under the district of Pirojpur district. Samples were collected from seven ponds that were used for household purpose. There were 11 ponds were selected as the first. However, four sampling sites (sites- 1, 3, 4 and 6) were dried during the courses of the sampling. They were abundant due to the discontinuation of the sampling and data. These were named as follows:

i. Jotishkanti Bepari’s Pond: Site-2

ii. BRAC Pond: Site-5

iii. Mathbaria Thana Health Complex (THC) Pond: Site-7

iv. Mathbaria Canal: Site-8

v. Najir’sPond: Site-9

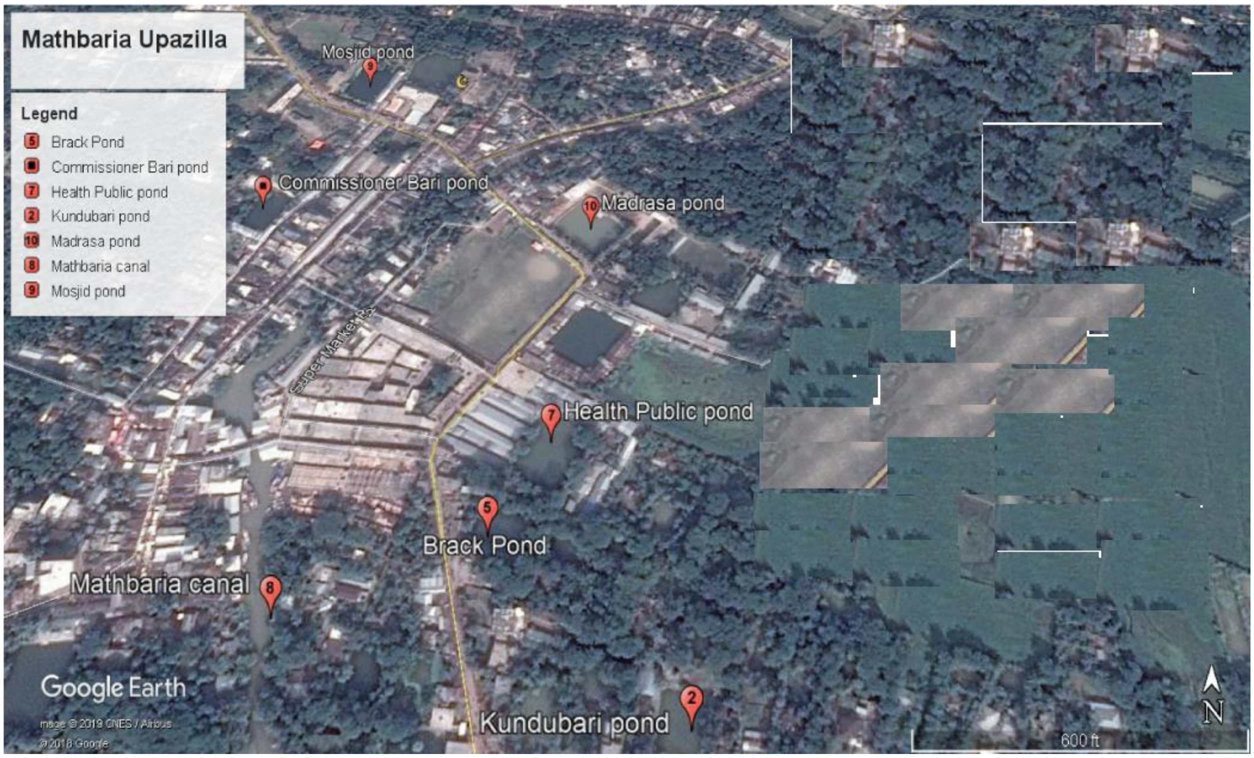
vi. Madrasa Pond: Site-10

vii. Commissioner Bari Pond: Site-11

<u>Description of the studied ponds:</u>

i) Site 2 (Jotish Kanti Beparis’ pond) :

This is one the most important ponds which is situated in the village named Kachichira. It is a very small pond but historically never dried up due to a connection with canal. This pond is about 1 km away from Mathbaria Thana Health Complex (THC). People (women) use this pond water for washing their utensils regularly.



Map 1. GPS Mapping of Mathbaria Upazila showing the location of studied ponds

ii) Site-5 (BRAC Pond):

It is located about 2 km away from Mathbaria Bazar within the village named North Mithakhali. Its area is about 4.5 ha and is perennial. About 2-3 thousand people utilize this pond-water only for drinking where bathing is restricted. A filtration (sand filter) unit has been set by an NGO (Non-government organization). Most of the village people use this filtered water for drinking. However, other villagers use unfiltered water of the same pond for drinking purpose.

iii) Site-7 (Mathbaria Thana Health Complex Pond) :

It is located just 46 m away from the Thana health complex. It is a medium sized (about 2 ha) pond and is perennial because of its connection to a canal leading to the river Baleshwar that flows across the Sunderbans and finally falls into the Bay of Bengal. A sand filtration unit has been set there and most of the people use filtered water for drinking. The staff members (doctors and nurses) of Mathbaria THC living in the government quarter use this water for their daily needs. Patients coming mostly with diarrhoea for the treatment in the hospitals also use this filtered water.

iv) Site-8 (Mathbaria Canal):

This canal faces two times tidal flow daily and washes away nearby houses and fields. It is connected to the Baleshwar River that flows across the Sunderbans and finally falls into the 18 Bay of Bengal by crossing about 10 - 12 km lands and houses. The canal water has high salinity due to the direct connection to the Bay of Bengal. This site was selected to compare and find out any significant ecological differences among the ponds connected directly to this canal and frequently overflowed by its water.

v) Site-9 (Najir's Pond):

It is a domestic pond and is located in the village named Jariper Char which is about 4 km away from Mathbaria THC. This is a unique pond because it is surrounded by the trees. Tidal water logged across the gaps within the trees surrounding the pond. Strong tidal flow results water to enter into this pond and the pond never dries out. About 500-1000 people use its water for drinking and other household works.

vi) Site-10 (Madrasa Pond):

This pond is also within the village named Jariper Char which is about 50 m away from the Samsul Huq Nazir's Pond. This pond is called "Madrasaha pond" as it is situated near a Madrasah (Islamic school). As the aquifer in this locality is salty, people use the water of this pond for their daily use. About 200-500 people (mostly students and teachers of the Madrasaha) use this water for drinking and other daily uses like ablution. The pond water is also used in the nearby village shops for daily use.

vii) Site-11 (Commissioner Bari Pond):

It is situated in the village named Kachichira, as a result local people called it Kachichira pond. It is also well known as Commisionar's pond in this village. It is never dries up due to a connection with canal. This pond is about 1 km away from Mathbaria THC. People (women) use this pond water for washing their utensils regularly. About 200-250 people particularly children use this pond water for bathing regularly.

3.1.2 Chhatak:

Chhatak is a town in northeastern hilly area of Bangladesh, along the Surma River, Sunamganj district, Sylhet Division away from the Bay of Bengal. Seven domestic ponds were selected here for the sampling. There were 12 sites selected initially but 5 dried up (sites- 3, 5, 6, 7, 8) during the long courses of sampling.

The sampled water bodies were named as follows:

i. Govt. Pond near THC: Site-1

ii. River SurmaGhat 1: Site-2

iii. Baghabari Govt. Primary School Pond: Site-4

iv. Commissioner Bari Pond: Site-9

v. Surma River Ghat 2 (Cement factory ghat): Site-10

vi. Mondolibhog Girl’s High School Pond: Site-11

vii. Sarderbari Abdul Khalek’s Pond: Site-12

<u>Description of the Studied ponds:</u>

i. Site-1 (Govt. Pond near THC):

Chhatak Station 1 is a rectangular pond with an area of approx. 0.30 ha and is located about 100 m away from the Chhatak THC. There are a number of houses surrounding this pond and most of the owners are slum dwellers and farmers. Peoples are extensively using this pond water for their household and bathing purposes.

Map 2. GPS Mapping of Chhatak Upazila showing the location of studied ponds

ii. Site-2 (River Surma Ghat 1):

This is a beautiful ghat near the Mosque situated in the village name Tatikona which is about 3 km away from Chhatak THC. About 500-1000 people uses this water for their daily purposes. People usually take bath and perform ablutions with this Ghat water before offering prayers. The site was selected because it is upstream of the River Surma.

iii. Site-4 (Baghabari Govt. Primary School Pond):

This pond is situated at a village called Baghbari. It is a semi rectangular pond with an area of about 0.2 ha, and is about 2 km away from the THC. There are a number of farmers' houses and grocery shops in the vicinity of the pond. People were using this pond water for bathing, washing and other household work. Good evidence of plankton bloom was found in the pond water.

iv. Site-9 (Commissioner Bari Pond):

This pond is located in the village Bashkhola and named as Shamsu Miah pond with the name of Mr. Shamsu Miah, ward commissioner of the Local Government. It is an isolated pond and properly maintained from the extensive contamination by feces (as per comment by the owner of the pond). About 200-350 people use this pond water daily particularly for bathing and other household purposes. A pump connected to the pond supplies water for the daily house hold works of commissioner's house.

v. Site-10 (Surma River Ghat 2 or Cement factory ghat):

This study site is about 3 km away from Chhatak THC which is just opposite to Lafarge Cement Factory, Chhatak, Sunamganj. Ferry communication connects this Ghat to Lafarge Cement Factory. About 4000-5000 people have direct or indirect influence with this Ghat-water for their daily purposes. This station was chosen because it is situated in the downstream compared to the other Ghat (Surma River Ghat 1).

vi. Site-11 (Mondolibhog Girl's High School Pond):

It is located in the village named Modolibhog. It is a semi rectangular pond with an area of about 0.3 ha, and is about half a km away from the THC and is closer to the Surma River. A girl's high school is situated nearby this pond. More than thousand peoples use this pond-water for their daily needs i.e., washing utensils, bathing and other household works.

vii. Site-12 (Sarderbari Abdul Khalek's Pond):

It is situated in a village called Charerban, located about half a km away from Chhatak THC. It's a very small pond and historically perennial. Villagers frequently affected by diarrheal diseases rush to the THC for treatment. Several hanging latrines were seen surrounding this pond, and is beset by poor sanitary conditions. High rainfalls cause fecal wastes to be washed out into this pond water. About 200-250 peoples particularly women use this pond water regularly. Children taking bath in this pond often suffer from diarrhea (2-3 times in a month) most probably by swallowing pond water.

3.2 Weather Parameters

Recorded weather parameters i, e., air temperature and precipitation or rainfall during the study period in the two selected regions (Mathbaria and Chhatak) were collected from the Bangladesh Meteorological Department, Agargaon, Dhaka.

3.3 Water Quality Parameters

The limnological aspects in relation to cholera surveillance of the studied ponds parameters of water quality measurement were taken from the data collected by the ICDDR'B team.

3.4 Crab's Gut Microbes Analysis

Mud crab (*Scylla sp.*) was collected from Joymoni and Chila of Sundarbans for about eight months. Crabs were sacrificed and their digestive systems were analyzed for the detection of microbial flora.

3.5 Zooplankton Sampling and Identification

Water samples were collected from seven ponds of Mathbaria and Chhatak on weekly basis during the peak seasons of cholera and monthly basis in other non-cholera seasons of the year between January 2013 to December 2014. In Mathbaria coastal region there are two seasonal peaks based on the clinical surveillance of that that area. These are Spring (March-May) and Autumn (September-November). Whereas, in Chhatak there is a single peak of cholera in Autumn season (September- November). All samples were collected in 50 ml Nalgene bottles (Nalgene Nune International, St. Louis, Mo. U.S.A.), placed in an insulated plastic box, and transported overnight at ambient air temperature ranging from 20°C to 35°C from the site of collection to the Zoology Department, University of Dhaka. During sampling, 64 µm nylon nets (Milliopore Corp., Bedford, MA. U.S.A.) were used. Samples were poured onto the net and zooplanktons were screened on net. 50 ml of the concentrates was collected initially for the measurement of zooplankton.

Concentrated 10 ml of zooplankton sample was used for identification and characterizations of zooplankton using a Sedgwick-Rafter counting cell following standard methods (Boyd and Tucker, 1992).

3.5.1 Zooplankton Species Composition (%)

Species composition is the percentages of plankton species in a specific zooplankton taxa which was calculated as follows:

Species composition (SC) %= n (100)/N

Here,

n= the total number of zooplankton species in each taxonomic group

N=the total number of zooplankton species in all taxonomic group

3.5.2 Relative Abundance (%)

Relative abundance (%) was calculated by the following formula:

Relative abundance (RA) %= n (100)/N

Here,

n= the total of individuals in each zooplankton taxonomic group

N=the total of individuals in the entire zooplankton taxonomic group

3.5.3 Species Diversity Indices

A. **Diversity indices**: Diversity indices are several mathematical methods of species diversity in a community. In case of Mathbaria several types of indices used as follows:

i) **Shannon-Wiener diversity Index (H′):**

Shannon-Wiener (Williams and Feltmate, 1992) indicates species diversity of a community or area. It takes into account the increasing value as an indication of higher diversity of a community.

$$H' = \sum_{i=1}^{s} \frac{ni}{n} ln \frac{ni}{n}$$

Where,

H′= Index of species diversity

S= Number of species

ni= Proportion of total sample belonging to the ith species

ii) **Simpson's Diversity Index (D):**

Another diversity index is Simpson's index (Krebs, 1994) which gives relatively little weight to the rare species and more weight to the common species. The range of this index is from (0-1). If the value of index is close to 1, it is considered as less diversified.

$$D = \frac{\sum_{i=1}^{s}(ni-1)}{n(n-1)}$$

Where,

D=Index of species diversity

S= Number of species

n_i= Proportion of individuals of the ith taxon in the community

B. Species Richness:

Species richness is the number of different species represented in an ecological community, landscape or region of an ecosystem. The number of species per sample is measuredby richness. The more species present in a sample, the 'richer' the ecosystem. Species richness is a measure which takes no account of the number of individuals of each species present. It gives as much weight to those species numbers i.e., which ecosystem have very few individual species as to those which have many individual species.

Two types of richness are tested:

i) Menhinick's Richness Index (Menhinick, 1964) : S√n and

ii) Margalef's Richness Index (Margalef, 1951): d= S-1/ln (N)

Where,

S= total number of species

N= total number of individuals

C. **Species Evenness:**

Species evenness is a measure of the relative abundance of the different species making up the richness of an area. Evenness is the proportion of species or functional groups present on a site. The more equal species are in proportion to each other, the greater the evenness of the site. If a community has a large disparity between the numbers of individuals within each species, it has low evenness. If the number of individuals within a species is fairly constant throughout the community it shows high evenness. The evenness of a community is represented by Pielou's evenness index. It is expressed as:

$E=H/\ln(R)$

Where,

E= Species Evenness

R= Total no. of distinct taxa in a population

3.6 Microbiological Analysis

3.6.1 Sampling for Biological Analysis

From 50ml of each zooplankton sample 40ml of unfixed sample were used for microbiological analysis after further concentration to 10ml by filtering through a 20 µm mesh nylon filter and homogenizing in Teflon-tipped tissue grinder using a Steadfast stirrer. Appropriate dilutions were used further for plate counts.

3.6.2 Analysis for *Vibrio cholerae*

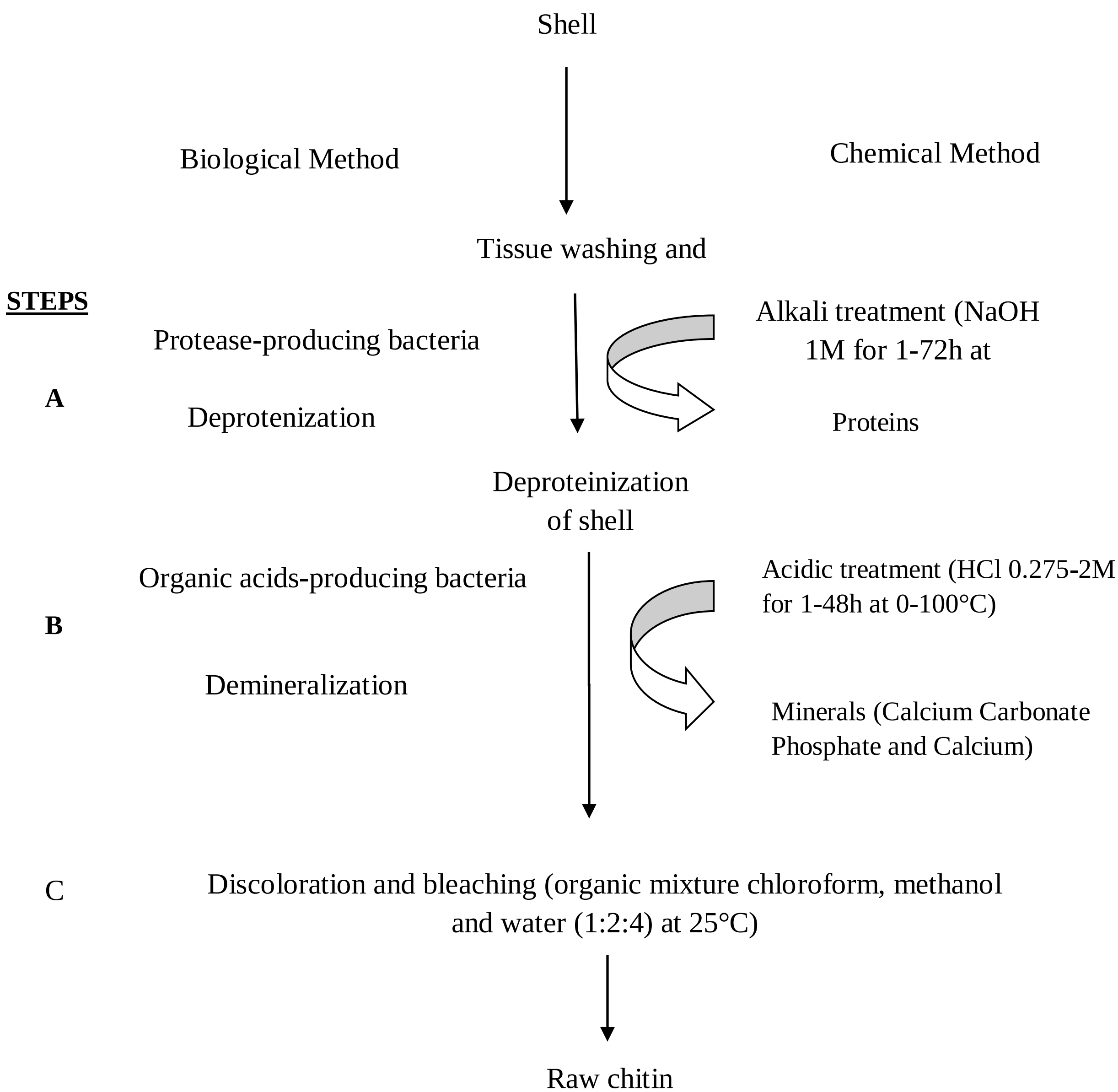
One ml of zooplankton homogenate was enriched in 10 ml (1x) alkaline peptone water. After enrichment, appropriate dilutions were prepared and spread plated on thiosulfate citrate bile salts sucrose (TCBS) agar and telluritetaurocholate gelatin agar (TTGA). These were then incubated at 37ºC overnight. Colonies of presumptive *Vibrio* sp. were characterized using standard procedures (DeWitt *et al*., 1971, Sack *et al*., 1974).

3.6.3 Direct Fluorescent Antibody assay (DFA)

DFA counting was used for detecting the presence of a particular antigen (typically a specific protein on the surface of the virus, bacterium or other microbe). The assay was done according to a method described in Brayton *et al*., 1987. Samples were pre-incubated overnight, in the dark, with 0.025% yeast extract (DIFCO) and 0.002% nalidixic acid (Sigma). The samples were then centrifuged, and the pellet was stained with flurosceinisothiocyanate-labeled antiserum specific for O1 or O139 obtained from new Horizon diagnostic Corp (Columbia, Md.). Stained samples were observed under UV light by using an epifluroscence microscope (Olympus BX51) connected to a digital camera (Olympus DP20).

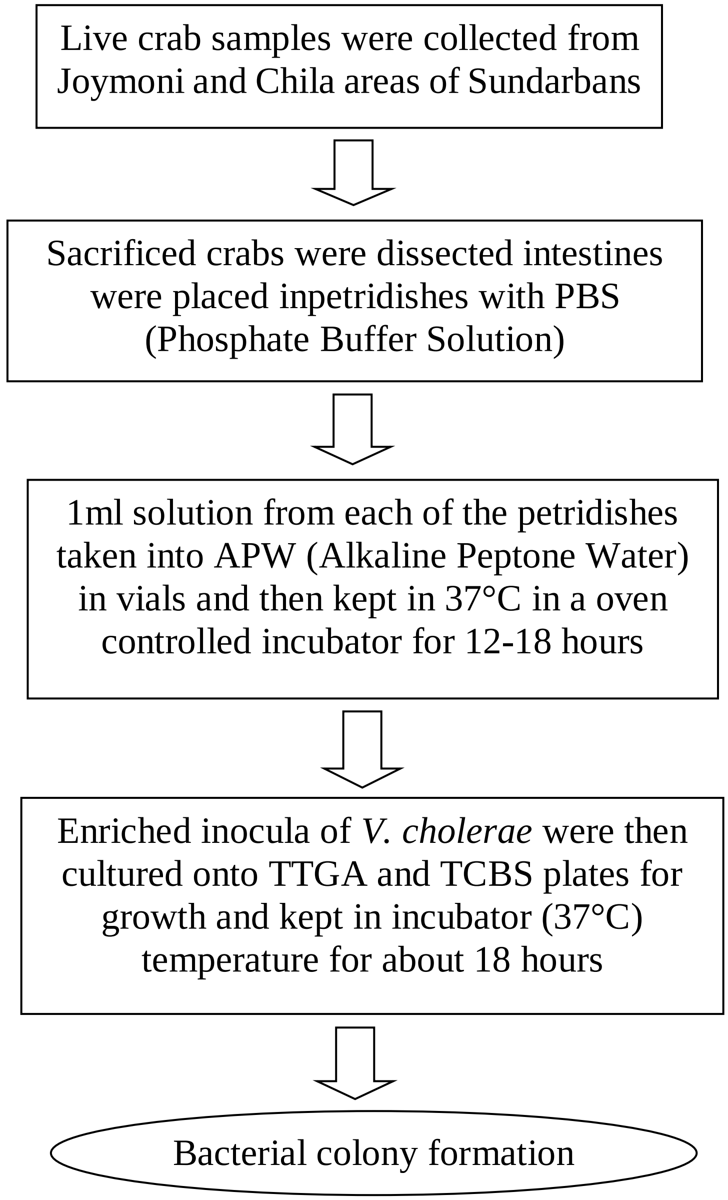
3.7 Crab and Shrimp Shell for Chitin Extraction

Chitin was extracted from crab and shrimp shell following a bio-chemical method in the Department of Zoology, University of Dhaka. At first, shell was separated, washed and dried in oven to decrease the moisture content. The procedure described in a flow diagram as follows:



3.8 Fresh Crab Sample for Microbial Analysis

Digestive system from each crab was kept in a petridish with 5ml PBS (Phosphate Buffer Saline). After dissecting all the crabs the systems in petridishes were melt with PBS separately and 1ml of crab residue was taken in a two drum vial containing APW (Alkaline Peptone Water) which helps to enrich the bacterium *Vibrio cholerae*. The vials were then kept in incubator with 37°C temperature. Following is the Protocol for bacteria culture from crab samples:



3.9 Micro-ecosystem (Microcosm) Study of Chitin for the Attachment of *Vibrio cholerae*

3.9.1 Collection of strain

Isolated strain of *Vibrio cholerae* O1 El Tor (1780) from culture collection of Environmental Microbiology Laboratory of ICDDR'B was used in this study. Identification of the strain was confirmed by a series of biochemical tests and serotyping. After confirmation biochemically and serologically, remaining portion of the isolated colony was streaked onto a gelatin agar (GA) plate and grown overnight at 37°C to get a pure culture.

3.9.2 Water sources and preparation

Water for microcosm was collected from two different sources. One of the two areas was site 2 of Mathbaria which is a cholera infected area and possesses brackish water and another was Paikgacha water reservoir of mud crab with saline water. These two types of water was then filtered through 0.22 µm Millipore filter and used to prepare the microcosms.

3.9.3 Preparation of different microecosystems with crab and shrimp based chitin

Water collected from Mathbaria and Paikgacha were used to prepare the microcosms of crab and shrimp chitin. Raw crab chitin and raw shrimp chitin prepared in the laboratory were used. Water for microcosms were filtered using 0.22 µm Millipore filter to remove all kinds of biotic and abiotic particles. 200 ml of water taken in each of the eight 500 ml conical flasks. Distilled water was taken in two flasks to observe the condition of *V. cholera*e in neutral environment. Flakes of 0.6 gm of small pieced three types of chitin were released into each of the eight flasks randomly. Among 12 microcosms eight were with chitin chips and four were without chitin as control. All the flasks were autoclaved. Water quality variables of these two water sources were as follows:

Table 1: Comparisn of water parameters in Mathbaria and Paikgacca

Parameters	Mathbaria Water	Paikgacha Water
pH	6.77	6.52
Salinity	0.3 ppt	3.9 ppt
Conductivity	658 µs	7.16 mg/l
TDS	329 mg/l	3580 mg/l

3.9.4 Preparation of inoculum

V. cholerae O1 biotype ElTor N-16961 cells in exponential phase were harvested from Luria–Bertan (LB) broth incubated at 37˚C for 18h, washed with phosphate buffer saline (PBS) at pH 7.0. The number of cells per ml had been assessed by using drop plate method as described by Hoben and Somasegoran (1982) cited in Colwell *et al.*, 1995 to ensure about 107 colony forming unit (CFU)/ml. A ten-fold dilution was prepared by using PBS of pH 8.4. Diluted inoculum of 2 ml in PBS of *V. cholerae* O1 El Tor N-16961 was added by a pipette in each microcosm flask so that the final concentration of the strain would be 10^4 (CFU/ml). The number of *V. cholerae* was then monitored by bacteriological culture method.

3.9.5 Two Microcosms supplemented with three chitin flakes from three sources

Chitin was extracted from the exoskeleton of large crustacean animal the golda shrimp (M*acrobrachium rosenbergii*), collected from coastal area of Sundarbans and mud crab (*Scylla serrata*) collected from coastal area of Southern-West district, Paikgachhha, Bangladesh following the described procedures (Sen, 2005). The chitin was washed, autoclaved, and dried at 60°C overnight and cut aseptically into small pieces. Four microcosms were constructed using200 mlfiltered (0.22 µm membrane) and autoclaved which were designed as MW+RCC (Mathbaria Water with Raw Crab Chitin), MW+RSC (Mathbaria Water with Raw Shrimp Chitin), PW+RCC (Paikgacha Water with Raw Crab Chitin) and PW+RSC (Paikgacha Water with Raw Shrimp Chitin. The media were inoculated with *V. cholerae* O1 biotype ElTor N-16961 cells in exponential phase, collected after growth in LB at 37°C and washed with PBS (pH 7.0). These were inoculated to a final concentration of 10^7cfu/ml into Mathbaria water micrococosoms supplemented with crab and shrimp chitin chips (0.3% w/v) as sole source of nutrient. The microcosms designated above were sealed and incubated at room temperature.

3.9.6 Processing of samples

Samples processing were started within few minutes after added the inoculum which was considered as 'zero day' sampling or reading. Sampling was done sequencially at 1st day, 7th day, 15th day, 30th day, 45th day, 60th day, 75th day, 90th day, 105th day, 120th day, 135th day 150th day, 165th day, 180th day, 195th day, 225th day, 255th day 285th day, 300th day, 315th day, 330thday, 345th day, 360th day, 375th day, 390th day, 410th day, 430th day, 450th day and 480th day.

A series of tenfold dilutions were prepared separately for each sample with PBS. The dilutions were homogenically mixed with a vortexer and 100 µl from each serial dilution were inoculated onto TTGA and LB plates using drop plate method and incubated at 37°C for 18-24 h. The counting of the colonies of *V. cholerae* O1 were colony formation. Simple staining, DFA staining and M-PCR were performed to detect and enumerate *V. cholerae* O1.

3.9.7 Counting procedure for *Vibrio cholerae* O1

After incubation, for confirmation of the serotype one colony from the resulted growth in each plate was tested by serological methods (Hoben and Somasegoran, 1982). Bacterial counts were derived from the counts of individual colony and were expressed as colony forming unit in mililitre or gram (CFU/ml or g).

3.9.8 Simple staining

Chitin chips from the microcosms were aseptically collected on clean glass slides, air-dried, stained with 4% crystal violet (Sigma St. Louis, MO, USA), washed and visualized by a light microscope (Axioskop 40, Carl Zeiss AG, Gottingen, Germany). Images were captured with digital camera attached (Axio Cam MRc; Carl Zeiss AG, Gottingen, Germany).

3.9.9 DFA

Vibrio cholerae incubated in Mathbaria water (MW) and Paikgacha water (PW) with Raw Crab Chitin (RCC), Raw Shrimp Chitin (RSC) microcosms were collected aseptically using wide-mouthed tips or sterile forceps and placed on glass slide. They were stained with cholera DFA reagent (New Horizon Diagnostics, Columbia, MD, USA) following the methods, as described earlier (Brayton and Colwell, 1987; Hasan *et al.*, 1994). Stained samples were observed using an epifluroscence microscope connected to a digital camera (Model described earlier).

3.9.10 DNA isolation

One ml water of each microcosm taken in eppendorfs and centrifuged at 8000 rpm for 5 mins. After releasing the supernatant 200 µl clumped colony dissolved vigorously. The samples were subsequently heated inboiling water for 10 mins. The samples were then cooled in ice for 20 min and followed by centrifuged at 13,000 rpm for 10 mins. The supernatants were used as template for the RAPD and PCR for *ctxA* and *rfb*O1 genes. On the other hand five pieces chitin chips from each microcosmswere mortared sequentially with pestle in 300 µl PBS. 200µl mashed chitin was taken byeppendorf from each sample and heated in boiling water for 10 mins. The samples were then cooled in ice for 20 min and centrifuged at 13,000 rpm for 10 mins. The supernatants were used as template for the RAPD and PCR for *ctxA* and *rfb*O1 genes.

3.9.11 M-PCR

Vibrio cholerae O1 serotype specific *rfb*O1genes encoding O-antigen and *ctxA* encoding subunit A of cholera toxin (CT) were amplified using M-PCR, details of the protocol followed after Hoshino *et al*. (1998).

3.10 Micro-ecosystem Study (microcosms) of Copepods in Different Ecological Habitats

3.10.1 Preparation of microcosms of copepoda

Three microcosms were set up with different sources of water collecting from Mathabaria (Cholera infected area), Paikgachha (saline water) and Dhanmondi Lake (Fresh water). Each microcosm was with two different subsets. The microcosms were designated as Mathbaria water microcosm (MW), Mathbaria water microcosm with algal feed (MW+AF), Paikgachha water microcosm (PW), Paikgachha water microcosm with algal feed (PW+AF), Lake water microcosm (LW) and Lake water microcosm with algal feed (LW+AF). Copepods were collected with plankton net of 64µm mesh size from Dhanmondi Lake. Salinity of the microcosms was 0.3 ppt, 3.6 pptand 0 ppt for Mathbaria water, Paikgachha water and lake water respectively. They were then released into the microcosms after counting. All sets of microcosms were kept at room temperature (27°C).

3.10.2. Inoculation of *Vibrio cholerae*

V. cholerae O1 biotype El Tor N-16961 cells isolated from a pond of Mathbaria. Bacteria was grown in Luria-Bertani (LB) broth at 37°C for 18 h. After collection bacterial colony was washed with Phosphate Buffer Saline (PBS). The cells were then inoculated into following combinations, Mathbaria water (MW), Mathbaria water with alagal feed (MW+AF), Paikgachha water (PW), Paikgachha water with algal feed (PW+AF), Lake water (LW) and Lake water with algal feed (LW+AF) to a final concentration of 10^7cfu/ml. Continuous aeration was provided the copepods at the room temperature. Sub samples from the beakers were taken to conduct plate culture, Direct Flouroscent Antibody (DFA) and multiplex Polymerase chain Reaction (mPCR).

3.10.3 Plate Count of *Vibrio cholerae* O1

Samples were diluted 10 fold serially in PBS and 100 µl of diluted samples were spread on the surface of TTGA plates. Inoculated plates were incubated at 37º C for 24 h. After incubation, probable *V. cholerae* O1 colonies on plates were confirmed by slide agglutination test using polyvalent anti-O1 serum (Nandi *et al.*, 2000). The confirmed colonies represented the total viable and culturable count of *V. cholerae.*

3.10.4 Multiplex Polymerase chain Reaction (mPCR)

The colonies confirmed as *V. cholerae* O1 by slide agglutination test (antigen-antibody reaction) were subjected to M-PCR for detection of O1serotype specific *rfb*O1 genes encoding O-antigen and *ctxA* encoding subunit A of cholera toxin (CT) were amplified using M-PCR, details of which are provided elsewhere (Hoshino *et al.*, 1998).

3.11 Statistical Analysis

Statistical analysis was done using SPSS version 22 and Statistics-10 for performing correlation, Student's t-test and Analysis of varience (ANOVA) respectively.

Correlation: Correlation among zooplankton and hydroclimatological factors was done to view the interrelationships among themselves.

Student's t-test: This method of testing hypotheses about the mean of a small sample drawn from a normally distributed population when the population standard deviation is unknown. In order to test the equality of plankton production in two study area (Mathbaria and Chhatak), Independent Sample test has been performed.

ANOVA: Analysis of variance (ANOVA) is a collection of statistical models and their associated estimation procedures (such as the "variation" among and between groups) used to analyze the differences among means. ANOVA was developed Analysis of Variance (ANOVA) tests have been performed to comparison of plankton production in different months of the year. Analysis of Variance (ANOVA) tests have also been performed to comparison of plankton production in different ponds of Mathbaria and Chhatak.

Chapter-4. Results and Observations

4.1 Biological assessment of *Vibrio cholerae* affected ponds

4.1.1 Biological assessment of *Vibrio cholerae* affected ponds in Mathbaria

4.1.1.1 Zooplankton composition of different *Vibrio cholerae* affected ponds

In this study, protozoa, rotifera and nauplii preside over the copepoda and cladocera. Quantitative analysis of zooplankton was shown in Table 2 to Table 8.

Protozoa

At Site-2 protozoan plankton showed increasing mode in rainy season (June-August) in 2013. On the other hand, in 2014 slight increase was found among protozoa in the month of January and September (Table-2).

At site-5, highest percentage of protozoa observed in the month of January (mid of dry season) and July (mid of rainy season). In both months percentage was 100% (Table-3).

At site-7, maximum percentage was in August (86.7%) and November (100%) in 2013 and 2014 respectively (Table-4).

At site-8, highest percentage of protozoa was recorded in July 2013 (100%) and in January 2014 (84.4%) (Table-5).

At site-9, percentage of protozoa was not significant in 2013. On the other hand, in November maximum amount of protozoan plankon was recorded in November (Table-6).

At site-10, protozoan plankton was highest in January 2013 and in 2014 protozoa showed maximum percentage two times a season (September and November) (Table-7).

At site-11, increased amount of protozoa was noticed in January 2013. But in 2014, there was no significant increment of plankton (Table-8).

Rotifera

Rotifera showed a sequential trend of imergence in different seasons of 2013 at site-2 (Table-2). In mid of dry season i.e., January 2013 quantity of rotifer was 66.7%, 63.2% was infirst month of summer (March 2013).   Maximum percentage was observed in October 2013 i.e., mid of autumn (67.4%).

At site-5, highest percentage was recorded in summer 2013 (76.6%) and comparatively minimal quantity of rotifer in December 2014 (33.3%) (Table-3).

At site-7, maximum 87.5% rotifera was observed in February 2013 and 100% was in September 2014 (Table-4).

At site-8, in 2014 significant quantity of rotifera was found than in 2013 and quantity of rotifera was found (33.3% and 40% in 2013 and 2014 respectively) (Table-5).

At site-9, highest amount of rotifera was recorded in April 2013 (44.6%) and February 2014 (54.5%) (Table-6).

The month of January and February at site-10 in 2013 and 2014 showed a winter peak when maximum density of rotifera was 53.7% and 75.4% respectively (Table-7).

At site-11, rotifer quantity was maximum in August 2013 (33.3%) and in January 2014 (67.3%) (Table-8).

Nauplii

At site-2, site-5, Nauplii showed highest peak during March-May in both the year 2013 and 2014 (Table-2).

At site-9 nauplii showed maximum percentage in August-September of 2014 whereas September and November was supposed to be the second peak season of Cholera sometimes (Table-6).

Site-11 also had highest nauplii composition in summer and autumn of 2014 (Table-8).

In spring relationships found on the basis of quantitative analysis amongst the group of zooplankton was: Nauplii>Rotifera>Copepoda>Cladocera>Protozoa

Copepoda

At site-2, copepods dominate the peak season of cholera in both 2013 and 2014. In the year 2013 when peak season of cholera started, at the mid month of the season (April) the number of copepods decreased and again increased as the seasondisappeared. Whereas, in 2014 copepods decreased when the germs of cholera raised in the pond and at the end of the season the number of copepods again increased.

At site-5, the copepods were attacked by the *V. cholerae* during the appearance of cholera season and decreased in number as the season remained in the year 2014 (Summer and Autumn peak seasons) (Table-3).

At site-7, percentage of copepod was highest in winter but among the months in peak season of cholera the percentage was highest during the starting of season and then decreased till the season continued in the year 2013. But in the second peak season and in 2014 the opposite scenario was observed and highest percentage was found in the first month of rainy season (June) of 2014 (Table-4).

At site-8, during the summer peak of cholera copepods were maximum in number but decreased as the season was end in the year 2013 and 2014 (Table-5).

At site-9, copepod plankton was decreased after the arrival of peak season of cholera and was highest in winter of 2013. In 2014 copepoda was maximum in rainy season (Table-6).

At site-10, copepods were supposed to increase from the summer season and maximum percentage was observed in rainy season and again decreased in the second peak season of 2013. In 2014, increased number of copepods were observed after summer and highest was in the first month of rainy season and then decreased in the second peak season of cholera (Table-7).

At site-11, no significant amount of copepods were found in 2013 and in 2014 in summer months copepods were decreased when the season started then reached after the arrival of rainy season (Table-8). In the second peak season, cholera was decreased when the season started.

Cladocera

Cladocera was suppressed at site-2 and site-8 (Table-2 and Table-5) in the year of 2013 and 2014. Except site-5 and site-7 (Table-3 and Table-4) cladocerans were maximum during rainy season such as at site-5 66.7% in September of 2013, 58.3% and 50% in April of 2013 and 2014 at site-7 respectively. On the otherhand, site-9 (Table-6), site-10 (Table-7) and site-11 (Table-8) showed a similar pattern of cladoceran plankton composition during rainy season of 2013 i.e., at site-9 in July (100%), site-10 in June (66.7%) and site-11 in June (66.7%).

Table2. Quantitative analysis of Zooplankton at Mathbaria pond (Site-2) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	900	11200	0	10.7	66.7	66.1	0	18.75	33.3	4.5	0	0
February	900	5600	55.6	1.8	11.1	10.7	0	48.2	0	25	33.3	14.3
March	1900	6500	5.3	3.1	63.2	6.2	15.8	44.6	10.5	36.9	5.3	9.2
April	10400	6500	1.0	4.6	1.0	78.5	73.1	12.3	16.3	4.6	8.7	0.0
May	900	11900	22.2	0.8	11.1	2.5	33.3	59.7	11.1	32.8	22.2	4.2
June	700	400	71.4	0	0	0	0	0	0	75	28.6	25
July	300	800	100	0	0	12.5	0	75	0	12.5	0	0
August	4000	1300	100	0	0	69.2	0	30.8	0	0	0	0
September	2500	400	8.0	25	52	0	32	25	4	50	4	0
October	8600	8100	7.0	0	67.4	2.5	24.4	51.9	1.2	45.7	0	0
November	4400	5400	0	0	13.6	20.4	79.5	64.8	6.8	14.8	0	0
December	-	2000	-	0	-	25	-	60	-	15.0	-	0

Table 3. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-5) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	100	2300	100	0	0	17.4	0	47.8	0	34.8	0	0
February	300	2800	33.3	7.1	33.3	14.3	33.3	57.1	0	17.9	0	3.6
March	5000	1000	2	10	76	20	14	20	4	40	4	10
April	4700	1500	4.3	0	76.6	13.3	14.9	46.7	0	33.3	4.3	6.7
May	1600	4100	18.75	0	25	31.7	43.75	24.4	6.25	17.1	6.25	26.8
June	10900	13500	4.6	0	45.9	9.6	48.6	14.1	0	45.2	0.92	27.4
July	400	1000	100	30	0	10	0	50	0	10	0	0
August	9300	400	9.7	75	29	0	57	25	3.2	0	1.1	0
September	1500	500	20	20	6.7	40	66.7	20	6.7	20	66.7	0
October	4800	5600	2.1	0	66.7	5.4	31.25	80.4	0	14.3	0	0
November	9000	1300	0	15.4	64.4	15.4	35.6	61.5	0	7.7	0	0
December	-	1800	-	16.7	-	33.3	-	33.3	-	16.7	-	0

Table 4. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-7) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1500	5400	0.0	7.4	0	46.3	0	40.7	100	5.6	0	0
February	800	1900	0.0	21.1	87.5	10.5	12.5	36.8	0	26.3	0	5.3
March	6300	3500	1.6	2.9	1.6	8.6	23.8	45.7	46.0	14.3	27.0	28.6
April	3600	5600	0.0	1.8	0	1.8	16.7	19.6	25	26.8	58.3	50
May	2700	5800	7.4	1.7	3.7	1.7	66.7	62.1	7.4	32.8	14.8	1.7
June	1100	3000	0.0	0.0	0	0.0	45.5	10	36.4	63.3	18.2	23.3
July	4200	400	2.4	50.0	0	25.0	69.0	25	16.7	0	11.9	0
August	1500	500	86.7	0	0	40.0	0	60	13.3	0	0	0
September	4000	200	5.0	0	0	100.0	77.5	0	10	0	7.5	0
October	9800	1900	2.0	5.3	10.2	31.6	39.8	52.6	39.8	10.5	8.2	0
November	7100	700	18.3	100.0	9.9	0.0	40.8	0	31.0	0	0	0
December	-	3800	-	0.0	-	0.0	-	52.6	-	39.5	-	7.9

Table5. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-8) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	600	4500	83.3	84.4	16.7	8.9	0	0	0	6.7	0	0
February	300	1900	33.3	26.3	33.3	10.5	0	47.4	33.3	15.8	0	0
March	600	1500	16.7	46.7	16.7	6.7	16.7	13.3	50	33.3	0	0
April	1200	2300	50	60.9	0	0	25	21.7	16.7	17.4	8.3	0
May	1500	2800	73.3	53.6	6.7	3.6	0	25	6.7	17.9	13.3	0
June	4400	500	68.2	0	0	0	18.2	20	13.6	80	0	0
July	700	400	100	25	0	0	0	50	0	0	0	25
August	800	500	37.5	0	12.5	40	37.5	20	12.5	40	0	0
September	1200	100	83.3	0	8.3	0	8.3	100	0	0	0	0
October	1200	1000	25	0	0	0	0	80	66.7	20.0	8.3	0
November	3400	900	64.7	44.4	8.8	11.1	11.8	33.3	14.7	11.1	0	0
December	-	1300	-	0	-	0	-	61.5	-	23.1	-	15.4

Table 6. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-9) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	2900	10100	31.0	4.0	0	33.7	0	56.4	69.0	5.9	0	0
February	700	11200	14.3	4.5	14.3	54.5	14.3	37.5	42.9	3.6	14.3	0
March	1600	15500	6.25	0	31.25	1.9	25	56.1	37.5	34.8	0	7.1
April	5600	3000	12.5	3.3	44.6	3.3	33.9	70	5.4	20	3.6	3.3
May	400	17600	25	0	25	0.6	0	61.9	0	35.8	50	1.7
June	400	1000	0	0	50	0	25	0	25	70	0	3
July	200	200	0	50	0	0	0	50	0	0	100	0
August	2100	100	0	0	4.8	0	76.2	100	14.3	0	4.8	0
September	1500	200	20	50	6.7	0	60	50	0	0	13.3	0
October	8000	5400	3.75	5.6	36.25	0	52.5	92.6	3.75	1.9	3.75	0
November	3000	1300	30	100	0	0	70	0	0	0	0	0
December	-	1900	-	0	-	5.3	-	89.5	-	5.3	-	0

Table 7. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-10) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	600	22400	66.7	7.1	33.3	75.4	0	14.3	0	3.12	0	0
February	4100	15500	9.8	6.5	53.7	58.7	26.8	20.6	7.3	14.2	2.4	0
March	2400	7400	4.2	1.4	33.3	29.7	33.3	48.6	25	16.2	4.2	4.1
April	3800	3600	7.9	2.8	18.4	0.0	50	47.2	18.4	41.7	5.3	8.3
May	1500	4400	6.7	0.0	6.7	2.3	20	43.2	33.3	15.9	33.3	38.6
June	300	200	0	0	0	0	0	0	33.3	100	66.7	0
July	700	200	42.9	0	0	0	0	100	0	0	57.1	0
August	1300	300	0	0	7.7	0	0	33.3	76.9	66.7	15.4	0
September	1900	500	15.8	60	5.3	0	57.9	20	15.8	20	5.3	0
October	5800	3600	8.6	8.3	50	30.6	25.9	50	6.9	5.6	8.6	5.6
November	11700	3000	47.0	60	42.7	13.3	2.6	23.3	1.7	0	6.0	3.3
December	-	3300	-	6.1	-	60.6	-	21.2	-	6.1	-	6.1

Table 8. Quantitative Analysis of Zooplankton at Mathbaria pond (Site-11) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	800	10700	87.5	2.8	0	67.3	0	27.1	12.5	2.8	0	0
February	2700	7300	3.7	0	25.9	53.4	48.1	28.8	11.1	16.4	11.1	1.4
March	3000	2500	0	0	3.3	28	63.3	44	30	24	3.3	4
April	4600	2100	6.5	0	23.9	4.8	58.7	81.0	8.7	9.5	2.2	4.8
May	800	4800	12.5	0	12.5	4.2	37.5	62.5	12.5	29.2	25	4.2
June	200	500	50	0	0	0	0	0	0	60	50	40
July	600	400	16.7	0	16.7	0	0	75	0	25	66.7	0
August	300	200	0	0	33.3	0	0	50	0	50	66.7	0
September	800	500	25	20	0	0	62.5	40	12.5	40	0	0
October	4200	2400	11.9	4.2	11.9	0	59.5	70.8	14.3	25	2.4	0
November	2000	1400	35	7.1	20	7.1	45	64.3	0	7.1	0	14.3
December	-	3200	-	0	-	9.37	-	68.75	-	9.37	-	12.5

4.1.1.2 Zooplankton at Mathbaria ponds: A qualitative approach

The coastal region Mathbaria exhibited in total86 species of zooplankton of which 27 species belonged to the phylum protozoa under 9 families and 7 orders. Rotifer had 43 species of planktonunder 8 families and 3 orders. Among crustacean plankton 8 species of copepods were foundunder 2 families and 2 orders. Another group of planktonic crustacean, cladocera was identified in Mathbaria ponds, which was represented by 8 species and 5 families and single order (Table 9).

Table 9. Zooplankton species identified from Mathbaria ponds

Order	Family	Species
Protozoa		
Amoebiae	Mayorellidae	*Astramoeba radiosa*
Testacealobosa	Arcellidae	*Arcella sp.*
		Arcella discoides
		Arcella vulgaris
	Centropyxidae	*Centropyxis sp.*
		Centropyxis aculeata
		Centropyxis constricta
		Centropyxis ecornis
		Ceratium hirudinella
	Difflugidae	*Difflugia sp.*
		Difflugia acuminata
		Difflugia lebes
		Difflugia lobostoma
		Difflugia oblonga
		Difflugia tuberculata
		Difflugia urceolata
Euglenoidina	Euglenaceae	*Euglena acus*
		Euglena oxyuris
		Euglena tripteris
		Phacus acuminata
		Phacus longicauda
		Phacus pleuronectes
Volvocales	Chlamydomonadaceae	*Polytoma sp.*
Euglyphida	Trinematidae	*Trinema complanatum*
Holotrichida	Frontoniidae	*Glaucoma sp.*
Tubilinia	Heleoperidae	*Heleopera rosea*
		Unidentified Protozoa
Rotifera		
Ploima	Asplanchnidae	*Asplanchna sp.*
		Asplanchna priodonta

Order	Family	Species
	Brachionidae	*Anuraeopsis sp.*
		Brachionus sp.
		Brachionus angularis
		Brachionus calcyflorus
		Brachionus caudatus
		Brachionus diversicornis
		Brachionus falcatus
		Brachionus forficula
		Brachionus nilsoni
		Brachionus plicatilis
		Brachionus quadridentatus
		Brachionus urceolaris
		Eothinia elongata
		Euclanis dilata
		Keratella sp.
		Keratella cochlearis
		Keratella taurocephala
		Keratella tropica
		Platyias patulus
	Lecanidae	*Lecane luna*
		Lepadella imbricata
		Monostyla bula
	Synchaetidae	*Polyarthra sp.*
		Polyarthra multiappendiculata
		Polyarthra vulgaris
	Tricocerchidae	*Tricocerca cylindrica*
		Tricocerca longiseta
		Tricocerca similis
Flosculariacea	Filinidae	*Filinia sp.*
		Filinia camascela
		Filinia longiseta
		Filinia opolienesis
		Filinia terminalis
	Testudinellidae	*Pompholyx sp.*
		Pompholyx sulcata
		Horaella brehmi
		Testudinella sp.
		Testudinella patina
Bdelloida	Phylodinidae	*Rotaria sp.*
		Rotaria neptunia
		Rotaria rotatoria
Copepoda		
Cyclopoida	Cyclopidae	*Cyclops sp.*
		Cyclops nanus
		Cyclops vernalis

Order	Family	Species
		Mesocyclops sp. *Mesocyclops hyalinus* Unidentified copepods
Eucopepoda	Diaptomidae	*Diaptomus sp.* *Diaptomus gracilis*
Cladocera		
Diplostraca	Bosminidae	*Bosmina sp.*
	Daphnidae	*Ceriodaphnia sp.* *Ceriodaphnia laticaudata* *Daphnia sp.* *Daphnia lumholtzi*
	Sididae	*Diaphanosoma sp.*
	Chydoridae	*Chydorus sp.*
	Simocephalidae	*Kurzia latissima*

4.1.1.3 Species composition of zooplankton in Mathbaria ponds

In Mathbaria, seven domestic ponds had shown a greater diversity of zooplankton species in the year 2013 and 2014. Pond at site-2 had 36 species of plankton in total, on the other hand site-5 had 38 species of plankton, site-7 had 31 species, site-8 had 22 species, site-9 had 42 species, site-10 had 40 species and site-11 had 30 species. All of them were recorded in the year 2013. In the year 2014, these ponds showed another numerical amount of plankton species, such as site-2 had 34 species, site-5 had 29 species, site-7 had 38 species, site-8 had 18 species, site-9 had 24 species, site-10 had 42 species and site-11 had 25 species of zooplankton. The zooplanton diversity in summary are as follows,

In 2013: Pond site 9 (42 sps)> site 10 (40 sps)> site 5(38 sps)> site 2(36 sps)>site 7(31sps)> site 11(30 sps)>site 8(22 sps)

In 2014: Pond site 10 (40 sps)> site 7 (38 sps)> site 2(34 sps)> site 5(29 sps)> site 11(25 sps)> site 9(24 sps)>site 8(18 sps)

Comparison of zooplankton number diversity in 2013 and 2014 at Mathbaria ponds

Sites	2	5	7	8	9	10	11
2013	36	38	31	22	42	40	30
2014	34	29	38	18	24	42	25

In case of protozoan taxa highest composition observed in site-8 (44%) in the year 2013 and in site-9 (35%) during the sampling periods in 2014 (Figure 5 and Figure 6).

Highest species composition in rotifera taxa was shown in site-5 (51%) in the year 2013 and in site-10 (62%) in 2014 (Figure 3 and Figure 7).

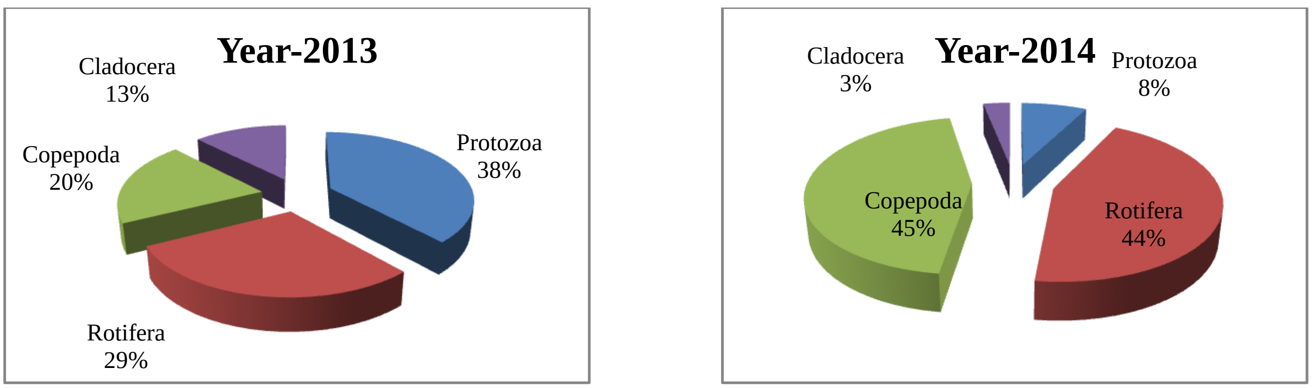


Figure 2. Pie-chart showing species composition in the Year-2013 and 2014 at Site-2

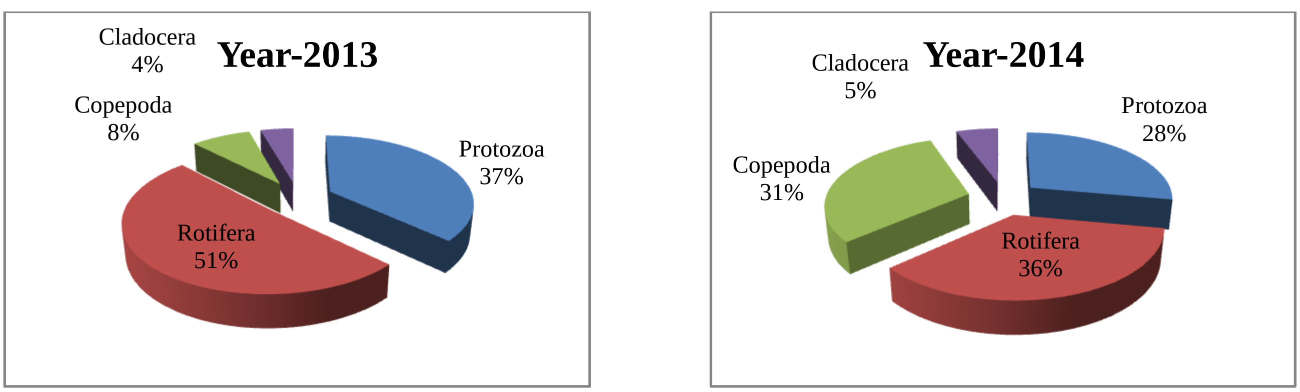
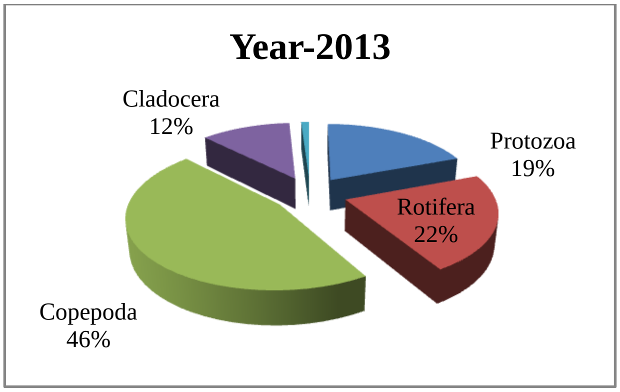


Figure 3. Pie-chart showing species composition in the Year-2013 and 2014 at Site-5



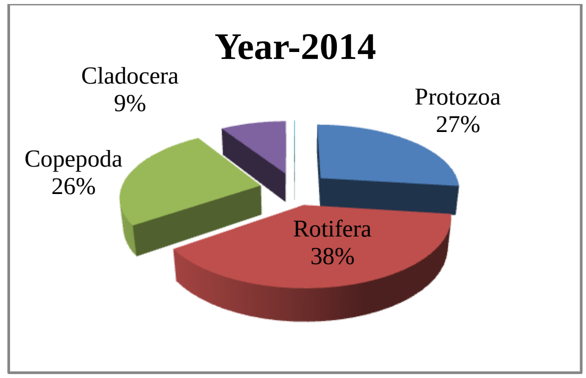
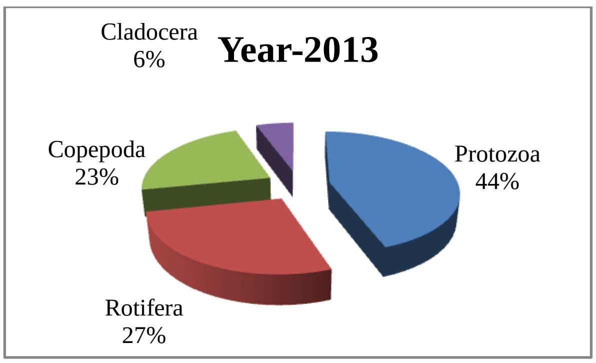


Figure 4. Pie-chart showing species composition in the Year-2013 and 2014 at Site-7



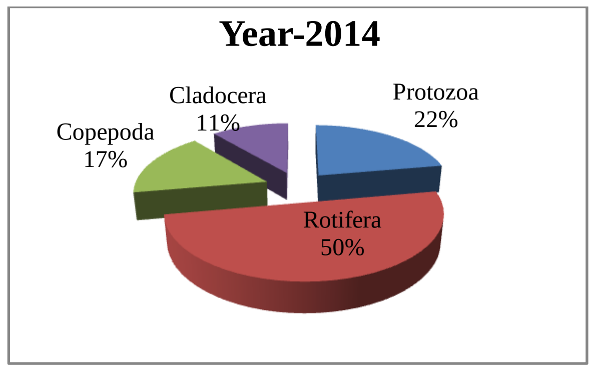
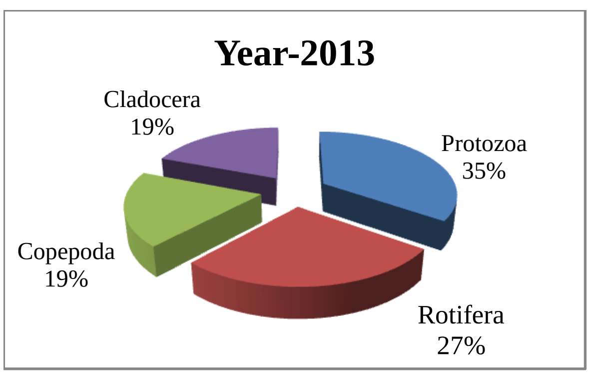


Figure 5. Pie-chart showing species composition in the Year-2013 and 2014 at Site-8



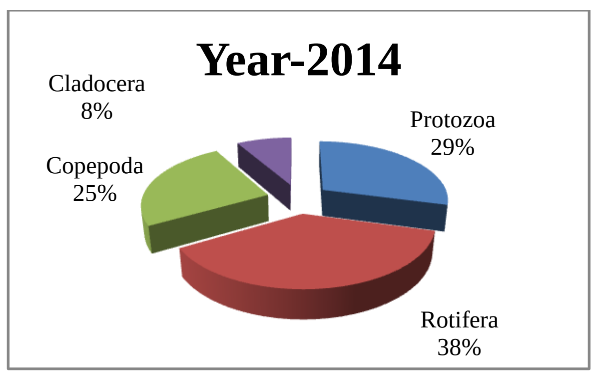
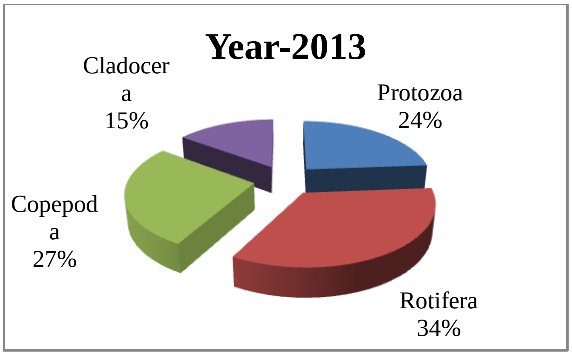


Figure 6. Pie-chart showing species composition in the Year-2013 and 2014 at Site-9



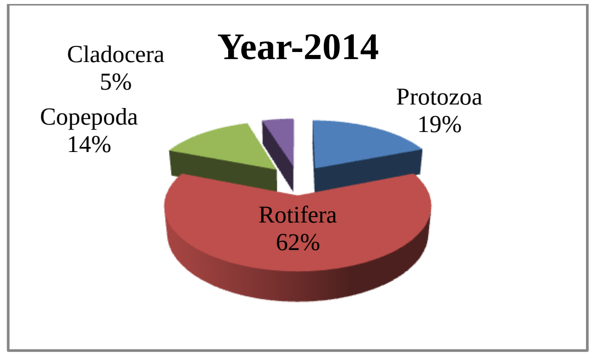
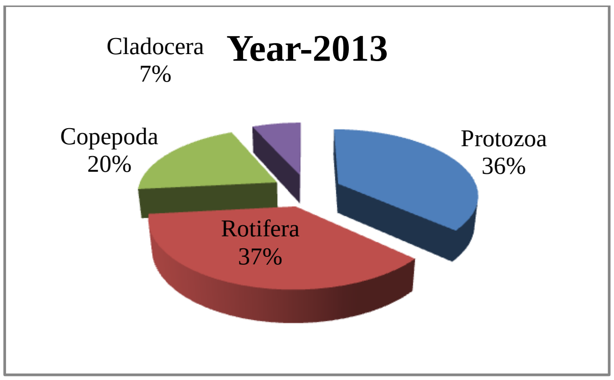


Figure 7. Pie-chart showing species composition in the Year-2013 and 2014 at Site-10



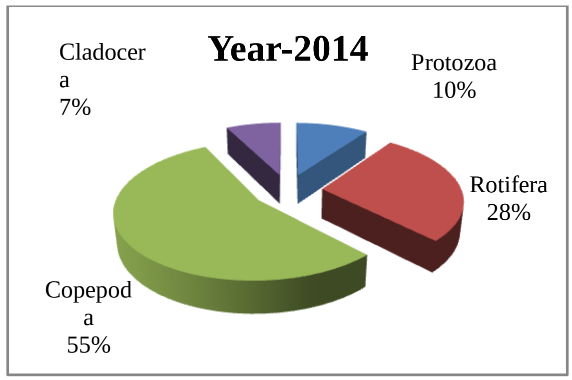


Figure 8. Pie-chart showing species composition in the Year-2013 and 2014 at Site-11

Copepoda taxa had maximum species composition (46%) in the year 2013 in site-7 (Figure 4) and was represented in site-11 (55%) in 2014 (Figure 8).

Among cladocera site-9 had highest species composition (19%) in 2013 (Figure 6) and in 2014 site-8 had highest composition of 11% (Figure 5).

4.1.1.4 Distribution of zooplankton in seven Mathbaria ponds

In the first year of study (2013), among protozoa the most dominant taxa in the selected ponds of Mathbaria were *Arcella discoides, Centropyxis sp., Difflugia sp., D. tuberculata, Glaucoma sp., Phacus longicauda* and *P. pleuronectes. Brachionus sp., B. angularis, B. diversicornis, Keratella sp., K. tropica, P. vulgaris* were found dominantly in Mathbaria. Some unknown stalked rotifer also found to be distributed in those ponds. Some copepod species are commonly recorded all through the year in Mathbaria which were *Cyclops sp., Cyclops vernalis, Diaptomus sp., Diaptomus gracilis. Diaphanosoma sp.* among cladoceran plankton was the only species that dominated over the other species in Mathbaria (Table 10).

Table 10. Diversity of Zooplankton at seven study sites during January 2013-December 2013

	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Protozoa							
Arcella sp.	-	-	-	-	-	-	+
Arcella discoides	+	-	+	+	+	-	+
Arcella vulgaris	+	-	-	+++	-	-	-
Astramoeba radiosa	-	-	-	-	-	+	-
Centropyxis sp.	++	+	+	+	+	-	-
Centropyxis aculeata	-	-	+	++	-	-	-
Centropyxis constricta	-	+	+++	+	-	-	-
Centropyxis ecornis	++	-	+	++	+	-	-
Ceratium hirudinella	+	-	-	-	+	-	-
Difflugia sp.	+++	+++	++	+	+	+	+++
Difflugia acuminata	-	-	-	-	+	++	-
Difflugia lebes	-	-	+	-	+	+	+
Difflugia lobostoma	-	+	+	-	-	-	-
Difflugia oblonga	-	-	-	-	+	-	-
Difflugia tuberculata	+++	+++	-	++	++	++	++
Difflugia urceolata	-	-	-	-	+	-	-
Euglena acus	-	-	-	-	+	-	+
Euglena oxyuris	+	+	-	-	+	+	+++
Euglena tripteris	-	-	-	-	-	+	-
Glaucoma sp.	-	++	+	++	+	++	++
Heleopera rosea	-	-	-	-	+	-	-
Phacus acuminata	-	-	-	-	+	-	+
Phacus longicauda	+	+	-	-	++	++	++
Phacus pleuronectes	-	+	+	-	+	+	+
Polytoma sp.	-	-	-	-	+	-	-
Trinema complanatum	+	-	-	-	-	-	-
Unidentified Protozoa	-	+	-	+	-	-	-
Rotifera							
Anuraeopsis sp.	-	-	+	-	-	-	+
Asplanchna sp.	-	++	-	+	-	++	-
Asplanchna priodonta	-	+	-	-	+	+	-
Brachionus sp.	++	+	++	-	++	-	-
Brachionus angularis	++	++	+	-	++	++	++

	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Brachionus calcyflorus	-	++	-	-	-	-	-
Brachionus caudatus	++	++	-	-	-	-	-
Brachionus diversicornis	++	++	-	-	+	+	+
Brachionus falcatus	+	+	-	-	-	-	-
Brachionus forficula	+	++	-	-	+	+	-
Brachionus nilsoni	-	+	-	-	-	-	-
Brachionus plicatilis	-	+	-	-	-	-	-
Brachionus quadridentatus	-	+	-	-	-	++	-
Brachionus urceolaris	-	+	-	-	-	+	-
Eothinia elongata	+	-	+	-	-	+	-
Filinia sp.	+	++	+	+	-	+	+
Filinia camascela	+	-	-	-	-	-	-
Filinia longiseta	-	+	-	-	-	-	-
Filinia opolienesis	+	+	-	-	+	-	-
Filinia terminalis	+	++	-	-	-	+	-
Keratella sp.	+	-	-	-	++	-	+
Keratella cochlearis	++	-	-	+	++	+	-
Keratella taurocephala	-	-	-	+	-	-	-
Keratella tropica	++	+	-	+	++	++	++
Lecane luna	-	-	-	-	+	+	-
Monostyla bula	-	+	+	-	-	-	-
Polyarthra sp.	+	+	-	+	-	++	+
P. multiappendiculata	+	+	+	-	-	-	-
P. vulgaris	-	++	++	-	+	++	+
P. sulcata	-	-	+	-	-	+	-
Rotaria sp.	-	++	-	-	+	++	++
Rotaria neptunia	-	-	-	-	+	+	+
Rotaria rotatoria	-	-	-	-	-	+	-
Testudinella sp.	-	-	-	-	-	+	-
Testudinella patina	-	-	-	-	-	+	-
Trichocerca cylindrica	-	+	-	-	-	-	-
Trichocerca longiseta	-	-	-	-	+	-	-
Trichocerca similis	-	++	-	-	++	+	-
Unidentified rotifer	+	++	+	+	+	+	++
Copepoda							
Cyclops sp.	++	+	+++	++	++	+++	++
Cyclops nanus	+	-	-	-	-	++	-
Cyclops vernalis	++	-	+++	+	+	++	++
Diaptomus sp.	++	++	+++	-	++	-	+
Diaptomus gracilis	-	++	++	++	++	-	+
Mesocyclops sp.	+	-	++	+	+	+	+
Mesocyclops hyalinus	-	-	+	-	-	-	-

	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Unidentified copepods	-	-	++	+	+	++	++
Cladocera							
Bosminasp.	-	-	-	-	+	-	-
Ceriodaphnia laticaudata	+	-	-	-	-	-	-
Chydorus sp.	+	-	-	-	-	-	+
Daphnia sp.	-	-	+	-	-	-	-
Diaphanosoma sp.	++	++	++	++	++	+++	+++
Simocephalus sp.	-	-	+	-	-	-	-

+++ = Most Abundant; ++ = Fairly Present; + =Present ; - = Absent

In the second year (2014), among protozoa *Difflugia sp.*, *Euglena oxyuris*, *Phacus acuminata* and *P. pleuronectes* were found to be dominated in the studied ponds. *Brachionus sp*., *B. angularis, B. caudatus, Monostyla bula, Polyarthra sp., P. vulgaris* and some unknown rotifera species were commonly distributed taxa among rotifera. Among copepods *Cyclops sp., Cyclops vernalis, Diaptomus sp., Diaptomus gracilis* and some unidentified copepod plankton were available throughout the year in our coastal region. *Diaphanosoma sp.* was the only abundant species that represents the cladocerans (Table 11).

Table 11. Diversity of Zooplankton at seven study sites during January 2014-December 2014

Species	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Arcella sp.	-	+	+	-	-	+	-
Arcella discoides	+	-	+	+	-	-	-
Arcella vulgaris	-	-	++	++	-	-	-
Astramoeba radiosa	-	-	+	-	-	-	-
Centropyxis sp.	-	+	+	-	-	-	-
Centropyxis aculeata	-	-	+	-	-	-	-
Centropyxis ecornis	-	-	-	+	-	-	-
Difflugia sp.	+	++	+	-	+	+	-
Difflugia tuberculata	+	-	-	-	-	-	-
Difflugia urceolata	-	-	-	-	+	-	-
Euglena acus	-	+	-	-	+	++	-
Euglena oxyuris	-	++	++	-	+	++	-
Euglena tripteris	-	-	+	-	+	++	-
Glaucoma sp.	+	-	-	+	+	-	-
Phacus acuminata	+	+	++	-	-	++	-
Phacus longicauda	+	-	+	-	-	++	+

Species	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Phacus pleuronectes	+	+	++	-	++	+	+
Polytoma sp.	-	-	-	-	-	-	++
Rotifera							
Anuraeopsis sp.	-	-	-	+	-	-	-
Asplanchna sp.	-	-	-	-	-	+	-
Asplanchna priodonta	-	-	+	-	-	++	+
Brachionus sp.	++	+	++	+	++	++	-
Brachionus angularis	+	++	+	+	++	++	++
Brachionus calcyflorus	-	+	-	+	-	++	+
Brachionus caudatus	+	+	+	-	-	+	++
Brachionus diversicornis	+	-	-	-	-	++	++
Brachionus falcatus	-	++	+	-	-	-	-
Brachionus forficula	-	++	-	-	-	-	-
Brachionus nilsoni	-	-	-	-	-	-	-
Brachionus plicatilis	-	-	-	-	-	-	-
Brachionus quadridentatus	++	+	-	-	-	-	-
Brachionus urceolaris	++	++	-	-	-	+	++
Eothinia elongata	-	-	-	-	-	+	-
Euclanis dilata	-	-	-	-	-	+	-
Filinia sp.	-	+	-	-	-	+	+
Filinia camascela	-	-	-	-	-	-	-
Filinia opolienesis	-	+	-	-	-	+	++
Filinia terminalis	-	-	-	-	++	+	+
Horaella brehmi	-	-	+	-	-	-	-
Keratella sp.	++	-	+	-	-	+	+
Keratella cochlearis	-	-	+	++	-	++	+
Keratella tropica	-	-	+	-	-	-	+
Lecane luna	+	+	+	+	-	-	-
Lepadella imbricata	-	-	-	-	-	+	-
Monostyla bula	+	-	+	+	+	++	-
Platyias patulus	-	-	-	-	-	+	-
Polyarthra sp.	++	++	+	-	-	++	+
Polyarthra multiappendiculata	+	-	-	-	-	+	+
Polyarthra vulgaris	+	+	++	+	++	+	++
Pompholyx sulcata	+	-	+	+	-	-	-
Rotaria sp.	-	-	-	-	+	-	-
Rotaria neptunia	-	+	-	-	-	-	-
Testudinella sp.	-	-	+	-	+	++	-
Testudinella patina	-	-	+	-	-	+	-
Tricocerca similis	-	+	-	-	+	+	-
Unidentified rotifer	++	++	+		++	++	+

Species	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Copepoda							
Cyclops sp.	++	++	++	++	++	+++	++
Cyclops vernalis	-	-	+	-	++	+	++
Diaptomus sp.	+++	+++	++	++	+++	++	+++
Diaptomus gracilis	++	++	++	-	++	+	++
Mesocyclops sp.	+	-	-	-	+	+	++
Unidentified copepods	++	++	++	+	++	++	++
Cladocera							
Ceriodaphnia sp.	-	-	-	+	-	-	-
Chydorus sp.	-	-	+	-	-	-	-
Daphnia lumholtzi	-	-	-	-	+	-	-
Diaphanosoma sp.	++	++	++	+	++	++	++
Kurzia latissima	-	+	-	-	-	+	-
Unidentified Cladocerans	-	-	+	-	-	-	-

+++ = Most Abundant; ++ = Fairly Present; + =Present; - = Absent

4.1.1.5 Frequency of occurrence of zooplankton in Mathbaria

Considering the occurrence constancy in the studied ponds of Mathbaria protozoa was shown to have two absolute constant species (*Arcella vulgaris* and *Difflugia sp.*) at site-5 and site-8 and two constant species (*Difflugia sp.*and *D. tuberculata*) at site-2. Rotifera had one constant species (*B. angularis*) at site-5 and site-10. Two absolute constant taxa were observed among copepods (*Cyclops sp.* and *Diaptomus sp.*) at site-7, site-10 and site-11. They have also some constant species such as, *Cyclops sp.*, *Cyclops vernalis*, *Diaptomus sp.*, *Diaptomus gracilis and* some unknown copepods at site-2, site-5, site-7 and site-8. *Diaphanosoma sp.* was the unique representing the cladoceran group and was absolutely constant at site-9 and site-11. It was also constantly present at site-2, site-5, site-7 and site-10 (Table 12).

Among protozoan plankton, *Arcella discoides* (85.7%), *Centropyxis sp.* (71.4%), *Difflugia sp.* (100%), *Difflugia tuberculata* (100%), *Euglena oxyuris* (85.7%), *Phacus acuminata* (85.7%), *Phacus longicauda* (85.7%), *Phacus pleuronectes* (85.7%) and *Glaucoma sp.*(85.7%) were frequently distributed in Mathbaria ponds.

Among rotifers, *Brachionus sp.* (85.7%), *B. angularis* (100%), *B. calcyflorus* (100%), *B. caudatus* (71.4%), *B. diversicornis* (71.4%), *Filinia sp.* (85.7%), *Filinia opolienesis* (71.4%), *Keratella sp.*(71.4%), *K. cochlearis* (85.7%), *K. tropica* (100%), *Polyarthra sp.*(85.7%), *Polyarthra* *multiappendiculata* (71.4%) were distributed frequently in most of the ponds of Mathbaria.

Cyclops sp. (100%), *Diaptomus sp.* (100%), *Diaptomus gracilis* (100%) and *Mesocyclops sp.* (85.7%) among macro crustacean plankton copepods were frequently distributed.

On the otherhand, *Diaphanosoma sp*. (100%) was the only cladoceran plankton found to be distributed in almost all ponds of Mathbaria.

Table 12. Frequency of Occurrence of particular zooplankton species in Mathbaria on a four degree scale; Absolute Constant Species (AS)- >75%, Constant Species (S)- 51-75%, Absolute Species (A)- 26-50% and Accidental Species (P)- < 25%

Group	**Species**	**Sites**							**Frquency (%)**
		S-2	**S-5**	**S-7**	**S-8**	**S-9**	**S-10**	**S-11**	
Protozoa	*Astramoeba radiosa*	-	-	P	-	-	P	-	28.6
	Arcella sp.	-	-	P	-	-	P	P	42.8
	A. discoides	P	P	P	P	P		P	85.7
	A. vulgaris	P	-	P	AS	-	-	-	42.8
	Centropyxis sp.	P	P	P	P	P	-	-	71.4
	C. aculeata	-	-	P	P	-	-	-	28.6
	C. constricta	-	P	A	P	-	-	-	42.8
	C. ecornis	P	-	P	A	P	-	-	51.1
	C. hirudinella	P	-	-	-	P	-	-	28.6
	Difflugia sp.	S	AS	A	P	P	P	A	100
	D. acuminata	-	-	-	-	P	P	-	28.6
	D. lebes	-	-	P	-	P	P	P	57.1
	D. lobostoma	-	P	P	-	-	-	-	28.6
	D. oblonga	-	-	-	-	P	-	-	14.2
	D. tuberculata	S	A	P	A	P	P	P	100
	D. urceolata	-	-	-	-	P	-	-	14.2
	Euglena acus	-	P	-	-	P	P	P	57.1
	E. oxyuris	P	A	P	-	A	A	P	85.7
	E. tripteris	-	-	P	-	P	A	-	42.8
	Phacus acuminata	P	P	P	-	P	P	P	85.7
	P. longicauda	P	P	P	-	P	A	A	85.7
	P. pleuronectes	P	P	P	-	A	P	P	85.7
	Polytoma sp.	-	-	-	-	P	-	P	28.6
	Trinema complanatum	P	-	-	-	-	-	-	14.2
	Glaucoma sp.	-	P	P	A	P	P	P	85.7
	Heleopera rosea	-	-	-	-	A	-	-	14.2
	Unidentified Protozoa	-	P	-	P	-	-	-	28.6
Rotifera	*Asplanchna sp.*	-	P	P	P	-	A	-	57.1
	A. priodonta	-	P	-	-	P	A	P	57.1
	Anuraeopsis sp.	-	-	P	P	-	-	P	42.8

Group	Species	Sites							Frquency (%)
		S-2	S-5	S-7	S-8	S-9	S-10	S-11	
	Brachionus sp.	A	P	S	P	A	P	-	85.7
	B. angularis	A	S	P	P	A	S	A	100
	B. calcyflorus	-	-	-	P	-	P	P	42.8
	B. caudatus	A	A	P	-	-	A	P	71.4
	B. diversicornis	A	A	-	-	P	A	A	71.4
	B. falcatus	P	A	P	-	-	-	-	42.8
	B. forficula	P	A	-	-	P	P	-	57.1
	B. nilsoni	-	P	-	-	-	-	-	14.2
	B. plicatilis	-	P	-	-	-	-	-	14.2
	B. quadridentatus	A	P	-	-	-	P	-	42.8
	B. urceolaris	P	A	-	-	-	P	P	57.1
	Eothinia elongata	P	-	P	-	-	P	-	42.8
	Euclanis dilata	-	-	-	-	-	P	-	14.2
	Filinia sp.	P	A	P	P	-	P	P	85.7
	F. camascela	P	-	-	-	-	-	-	14.2
	F. longiseta	-	P	-	-	-	-	-	14.2
	F. opolienesis	P	P	-	-	P	P	P	71.4
	F. terminalis	P	P	-	-	P	P	P	71.4
	Horaella brehmi	-	-	P	-	-	-	-	14.2
	Keratella sp.	A	-	P	-	P	P	P	71.4
	K. cochlearis	P	-	P	A	P	A	P	85.7
	K. taurocephala	-	-	-	P	-	-	-	14.2
	K. tropica	P	P	P	P	P	P	P	100
	Platyias patulus	-	-	-	-	-	P	-	14.2
	Lecane luna	P	P	P	P	P	P	-	85.7
	Lepadella imbricata	-	-	-	-	-	P	-	14.2
	Monostyla bula	P	P	P	P	P	P	-	85.7
	Pompholyx sp.	-	-	P	P	-	-	-	28.6
	P. sulcata	P	-	P	-	-	P	-	42.8
	Polyarthra sp.	A	A	P	P	-	A	P	85.7
	P. multiappendiculata	P	P	P	-	-	P	P	71.4
	P. vulgaris	P	A	A	P	A	A	A	100
	Rotaria sp.	-	A	-	-	P	P	P	57.1
	R. neptunia	-	P	-	-	P	P	P	57.1
	R. rotatoria	-	-	-	-	-	P	-	14.2
	Testudinella sp.	-	-	P	-	P	A	-	42.8
	T. patina	-	-	P	-	-	P	-	28.6
	Tricocerca cylindrica	-	P	-	-	-	-	-	14.2
	T. longiseta	-	-	-	-	P	-	-	14.2
	T. similis	-	A	-	-	A	P	-	42.8
Copepoda	*Cyclops sp.*	S	A	AS	S	P	AS	AS	100
	C. nanus	P	-	-	-	-	P	-	28.6
	C. vernalis	P	-	S	-	P	A	A	57.1

Group	Species	Sites							Frquency (%)
		S-2	S-5	S-7	S-8	S-9	S-10	S-11	
	Mesocyclops sp.	P	-	P	P	P	P	A	85.7
	M. hyalinus	-	-	P	-	-	-	-	14.2
	Unidentified copepods	A	A	S	A	P	A	A	100
	Diaptomus sp.	S	S	S	A	A	AS	AS	100
	D. gracilis	S	S	S	P	P	P	A	100
Cladocera	*Bosmina sp.*	-	-	-	-	P	-	-	14.2
	Ceriodaphnia sp.	-	-	-	P		-	-	14.2
	C. laticaudata	P	-	-	-	-	-	-	14.2
	Daphnia sp.	-	-	P	-	-	-		14.2
	D.lumholtzi	-	-	-	-	P	-	-	14.2
	Diaphanosoma sp.	S	S	S	A	AS	S	AS	100
	Chydorus sp.	P	-	P	-	-	-	P	42.8
	Kurzia latissima	-	P		-	-	P	-	28.6
	Simocephalus sp.	-	-	P	-	-		-	14.2
	Unidentified Cladocerans	-	-	P	-	-	-	-	14.2

4.1.1.6 Seasonal abundance of zooplankton species at Mathbaria ponds

Protozoa

Among protozoa most abundant taxa that dominated in different seasons at different sites of Mathbria were *Arcella discoides, Arcella vulgaris, Centropyxis sp., Centropyxis constricta, Difflugia sp., Difflugia lebes, Difflugia tuberculata, Euglena oxyuris, Glaucoma sp., Phacus acuminata* and *Phacus pleuronectes*. *Arcella discoides* was dominated in summer season at site-8 whereas *Arcella vulgaris* was influential during summer, autumn and winter season in the same pond. One unique species *Trinema comlanatum* at site-2 was only found in rainy season (Table 13).

Table 13. Relative abundance of Protozoa at different sites of Mathbaria according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-2				
Arcella discoides	5	2	nd	nd
A. vulgaris	nd	nd	1	nd
Centropyxis sp.	1	nd	nd	11
C. ecornis	nd	2	1	nd
Ceratium hirudinella	nd	2	nd	nd

	Summer	Rainy Season	Autumn	Winter
Difflugia sp.	1	2	1	11
D. tuberculata	3	8	2	4
Euglena oxyuris	nd	nd	2	nd
Glaucoma sp.	nd	nd	nd	8
Phacus acuminata	nd	nd	2	nd
P. longicauda	nd	nd	1	3
P. pleuronectes	1	nd	nd	nd
Trinema complanatum	nd	79	nd	nd
Site-5				
Arcella sp.	2	nd	nd	nd
Centropyxis sp.	nd	nd	3	nd
C. constricta	nd	1	nd	nd
Difflugia sp.	1	5	3	21
D.lobostoma	1	nd	nd	nd
D. tuberculata	3	3	6	nd
Euglena acus	nd	2	nd	nd
E. oxyuris	2	2	nd	13
Glaucoma sp.	1	3	nd	nd
Phacus acuminata	nd	nd	nd	3
P. longicauda	nd	nd	1	nd
P. pleuronectes	5	nd	nd	5
Site-7				
Arcella sp.	1	nd	nd	nd
A. discoides	nd	6	4	nd
A. vulgaris	2	nd	nd	4
Astramoeba radiosa	1	nd	nd	nd
Centropyxis sp.	nd	11	2	nd
C. aculeata	nd	nd	1	2
C. constricta	6	19	1	nd
C. ecornis	nd	3	nd	nd
Difflugia sp.	nd	6	4	nd
D. lebes	nd	nd	1	nd
D. lobostoma	1	nd	nd	nd
D. tuberculata	1	nd	nd	nd
Euglena oxyuris	1	nd	9	nd
Euglena tripteris	nd	nd	4	nd
Glaucoma sp.	nd	nd	1	5
Phacus acuminata	nd	nd	7	5
P. longicauda	nd	nd	4	nd
P. pleuronectes	1	nd	4	nd
Site-8				
Arcella discoides	28	nd	nd	nd
A. vulgaris	33	14	31	28
Centropyxis sp.	nd	nd	nd	9
C. aculeata	3	nd	3	nd

	Summer	Rainy Season	Autumn	Winter
C. constricta	3	nd	nd	nd
C. ecornis	3	2	nd	2
Difflugia sp.	5	nd	nd	13
D. tuberculata	3	2	3	nd
Glaucoma sp.	nd	9	19	8
Unidentified Protozoa	nd	nd	3	nd
Site-9				
Arcella discoides	nd	nd	1	nd
Centropyxis sp.	1	nd	nd	nd
C. ecornis	nd	nd	1	nd
Ceratium hirudinella	6	nd	nd	nd
Difflugia sp.	2	nd	5	nd
D. acuminata	nd	nd	nd	7
D. lebes	nd	nd	nd	5
D. oblonga	2	nd	nd	nd
D. tuberculata	3	nd	3	5
D. urceolata	nd	nd	nd	2
Euglena acus	1	nd	3	nd
E. oxyuris	1	nd	17	10
E. tripteris	nd	nd	4	nd
Glaucoma sp.	nd	nd	8	3
Heleopera rosea	1	nd	nd	nd
Phacus acuminata	5	nd	nd	nd
P. longicauda	2	nd	3	nd
P. pleuronectes	4	20	8	nd
Polytoma sp.	nd	nd	nd	2
Site-10				
Arcella sp.	nd	nd	nd	1
Astramoeba radiosa	nd	13	nd	nd
Difflugia sp.	6	nd	nd	1
D. acuminata	nd	nd	1	2
D. lebes	nd	7	nd	nd
D. tuberculata	1	nd	nd	9
Euglena acus	nd	nd	8	1
E. oxyuris	2		3	2
E. tripteris	nd	nd	2	1
Glaucoma sp.	nd	nd	19	4
Phacus acuminata	nd	nd	11	2
P. longicauda	4	nd	2	2
P. pleuronectes	nd	nd	7	nd
Site-11				
Arcella sp.	1	nd	nd	nd
A. discoides	nd	nd	2	nd
Difflugia sp.	6	14	4	8
D. lebes	nd	14	nd	nd

	Summer	Rainy Season	Autumn	Winter
D. urculata	2	nd	nd	2
Euglena acus	nd	nd	nd	nd
E. oxyuris	nd	nd	4	nd
Glaucoma sp.	nd	nd	2	10
Phacus acuminata	nd	nd	12	4
P. longicauda	nd	nd	4	nd
P. pleuronectes	nd	nd	4	2
Polytoma sp.	nd	nd	6	2

nd=Not detected

Rotifera

Rotifera taxa were mostly present in summer season in most of the ponds. Among the recorded plankton *Asplanchna sp*., almost all species of *Brachionus, Keratella sp, Filinia sp. Polyarthra sp., Rotaria sp.* and *Trichocerca sp.* were abundant in peak season of cholera. But *Asplanchna sp., Polyarthra sp., Pompholyx sulcata, Trichocerca similis* were dominant in autumn and winter season (Table 14).

Table 14. Relative abundance of Rotifera at different sites of Mathbaria according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-2				
Brachionus sp.	1	19	1	1
B. angularis	4	nd	6	nd
B. caudatus	2	6	4	nd
B. diversicornis.	nd	nd	2	3
B. falcatus	nd	nd	8	nd
B. forficula	35	nd	nd	nd
B. quadridentatus	3	19	nd	2
B. urceolaris	2	nd	nd	18
Eothinia elongata	nd	nd	6	nd
Filinia sp.	nd	nd	nd	15
F. camascela	nd	nd	1	nd
F. opoliensis	nd	nd	1	nd
F. terminalis	1	nd	nd	nd
Keratella sp.	1	nd	2	2
K. cochlearis	nd	nd	2	8
K. tropica	1	nd	12	nd
Lecane luna	1	nd	nd	nd
Monostyla bula	nd	nd	nd	2

	Summer	Rainy Season	Autumn	Winter
Polyarthra sp.	2	nd	13	7
P. multiappendiculata	nd	nd	13	8
P. vulgaris	2	nd	nd	nd
Pompholyx sulcata	nd	19	nd	nd
Unidentified rotifera	2	nd	6	2
Site-5				
Asplanchna sp.	8	1	nd	nd
A. priodonta	2	nd	nd	nd
Brachionus sp.	1	nd	3	nd
B. angularis	14	9	15	nd
B. calcyflorus	nd	nd	nd	12
B. caudatus	1	nd	1	nd
B. diversicornis.	13	nd	1	nd
B. falcatus	nd	11	5	nd
B. forficula	2	nd	1	3
B. nilsoni	2	nd	nd	nd
B. plicatilis	2	nd	nd	nd
B. quadridentatus	1	3	nd	nd
B. urceolaris	4	2	nd	nd
Filinia sp.	13	3	nd	nd
F. longiseta	3	nd	nd	nd
F. opoliensis	2	nd	nd	10
F. terminalis	4	nd	1	nd
K. tropica	1	nd	nd	nd
Lecane luna	3	nd	nd	nd
Monostyla bula	2	nd	nd	nd
Polyarthra sp.	31	nd	33	7
P. multiappendiculata	nd	nd	2	nd
P. vulgaris	nd	18	9	nd
Rotaria sp.	1	4	1	nd
R. neptunia	nd	3	nd	nd
Trichocerca cylindrica	nd	nd	1	nd
T. similis	3	nd	7	28
Unidentified rotifer	3	1	6	7
Site-7				
Anuraeopsis sp.	nd	nd	nd	3
A. priodonta	nd	nd	nd	3
Brachionus sp.	nd	8	3	16
B. angularis	nd	nd	4	nd
B. caudatus	nd	nd	nd	2
B. falcatus	nd	nd	4	nd
Eothinia elongata	nd	nd	3	nd
Filinia sp.	nd	nd	nd	3
Horaella brehmi	nd	nd	nd	3
Keratella sp.	nd	nd	7	nd

	Summer	Rainy Season	Autumn	Winter
K. cochlearis	nd	nd	nd	7
K. tropica	nd	nd	nd	6
Lecane luna	nd	8	nd	nd
Monostyla bula	1	nd	1	nd
Polyarthra sp.	2	nd	nd	nd
P. multiappendiculata	nd	nd	3	nd
P. vulgaris	1	nd	9	11
Pompholyx sp.	nd	11	nd	nd
P. sulcata	nd	nd	nd	22
Testudinella sp.	nd	nd	4	3
T. patina	nd	nd	nd	nd
Unidentified rotifer	1	nd	nd	7
Site-8				
Anuraeopsis sp.	3	nd	nd	nd
Asplanchna sp.	nd	nd	3	nd
Brachionus sp.	nd	nd	nd	6
B. angularis	nd	nd	nd	14
B. calcyflorus	nd	nd	nd	2
Filinia sp.	nd	2	nd	nd
Keratella cochlearis	3	nd	10	9
K. taurocephala	nd	nd	nd	9
K. tropica	5	nd	nd	nd
L. luna	nd	12	nd	nd
Monostyla bula	3	nd	nd	nd
Polyarthra sp.	nd	nd	nd	9
P. vulgaris	nd	12	nd	nd
Pompholyx sp.	nd	12	nd	nd
Site-9				
A. priodonta	13	nd	nd	nd
Brachionus sp.	2	nd	3	4
B. angularis	1	nd	5	5
B. caudatus	nd	nd	nd	nd
B. diversicornis	1	nd	nd	nd
B. forficula	nd	nd	6	nd
Filinia opolienesis	nd	nd	1	nd
F. terminalis	1	nd	nd	1
Keratella sp.	1	nd	nd	2
K. cochlearis	nd	7	nd	3
K. tropica	nd	14	nd	nd
Lecane luna	nd	nd	1	nd
Monostyla bula	1	nd	nd	nd
Polyarthra vulgaris	15	nd	nd	10
Pompholyx sulcata	nd	nd	nd	nd
Rotaria sp.	1	nd	nd	nd
R. neptunia	2	nd	nd	nd

	Summer	Rainy Season	Autumn	Winter
Testudinella sp.	1	nd	nd	nd
Trichocerca longiseta	nd	nd	3	nd
T. similis	2	nd	25	2
Unidentified rotifer	1	nd	1	2
Site-10				
Asplanchna sp.	3	nd	5	31
A. priodonta	1	nd	10	4
Brachionus sp.	2	nd	nd	1
B. angularis	16	nd	2	9
B. caudatus	1	nd	nd	nd
B. calcyflorus	10	nd	3	17
B. diversicornis	2	nd	nd	7
B. forficula	1	nd	nd	nd
B. quadridentatus	1	nd	nd	10
B. urceolaris	3	nd	nd	nd
Eothinia elongata	nd	nd	9	1
Euclanis dilata	nd	nd	4	nd
Filinia sp.	1	nd	nd	1
F. opolienesis	nd	nd	nd	4
F. terminalis	nd	nd	1	3
Keratella sp.	nd	nd	nd	2
K. cochlearis	5	nd		1
K. tropica	nd	nd	1	1
Lecane luna	nd	7		nd
Lepadella imbricata	nd	nd	6	nd
Monostyla bula	nd	nd	3	1
Platyias patulus	nd	nd	nd	1
Polyarthra sp.	6	nd	1	13
P. multiappendiculata	nd	nd		1
P. vulgaris	1	nd	8	10
Pompholyx sulcata	nd	nd	1	nd
Rotaria sp.	6	nd	nd	7
R. neptunia	nd	nd	nd	2
R. rotatoria	nd	nd	1	nd
Testudinella sp.	nd	nd	6	2
T. patina	nd	nd	5	3
Tricocerca similis	nd	nd	9	1
Unidentified rotifer	nd	nd	3	4
Site-11				
Anuraeopsis sp.	nd	nd	nd	4
Asplanchna priodonta	nd	nd	nd	3
Brachionus sp.	nd	nd	nd	nd
B. angularis	4	nd	7	6
B. calcyflorus	nd	nd	nd	1
B. caudatus	9	nd	nd	2

	Summer	Rainy Season	Autumn	Winter
B. diversicornis	3	nd	10	2
B. urceolaris	2	nd	nd	6
Filinia sp.	nd	14	nd	3
F. opolienesis	3	nd	nd	3
F. terminalis	nd	nd	nd	4
Keratella sp.	nd	14	nd	7
K. cochlearis	nd	nd	nd	6
K. tropica	1	nd	15	9
Polyarthra sp.	27	nd	nd	nd
P. multiappendiculata	4	nd	nd	nd
P. vulgaris	nd	nd	5	13
Rotaria sp.	3	nd	nd	2
R. neptunia	1	nd	nd	nd
Unidentified rotifer	2	nd	nd	2

nd= Not detected

Copepoda

Copepods are the crustacean plankton that from the ancient period supposed to be responsible for carrying the germ of cholera. In peak season of cholera (summer) all the experimental ponds had the abundance of copepod species than the other seasons. Among them *Diaptomus sp.* had the highest abundance in rainy season in most of the ponds when the period of cholera disappears (Table 15).

Table 15. Relative abundance of Copepoda at different sites of Mathbaria according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-2				
Cyclops sp.	3	nd	4	7
C. nanus	2	nd	nd	nd
C. vernalis	3	nd	nd	nd
Diaptomus sp.	12	6	12	5
D. gracilis	6	nd	5	6
Mesocyclops sp.	2	nd	nd	1
Unidentified copepod	3	6	nd	3
Site-5				
Cyclops sp.	1	nd	20	7
Diaptomus sp.	4	43	3	7
D. gracilis	3	5	nd	5
Unidentified copepod	5	2	nd	7

	Summer	Rainy Season	Autumn	Winter
Site-7				
Cyclops sp.	8	2	4	16
C. vernalis	2	5	6	16
Diaptomus sp.	6	4	7	5
D. gracilis	7	9	9	9
Mesocyclops sp.	3	nd	4	nd
M. hyalinus	nd	nd	1	nd
Unidentified copepod	7	42	5	7
Site-8				
Cyclops sp.	4	13	20	4
Diaptomus sp.	10	nd	15	6
D. gracilis	nd	8	nd	nd
Mesocyclops sp.	6	nd	3	nd
Unidentified copepod	8	2	13	16
Site-9				
Cyclops sp.	9	nd	nd	8
C. vernalis	nd	nd	1	3
Diaptomus sp.	5	60	3	7
D. gracilis	4	10	3	2
Mesocyclops sp.	3	nd	nd	nd
Unidentified copepod	14	nd	nd	30
Site-10				
Cyclops sp.	3	nd	3	5
C. nanus	2	nd	1	nd
C. vernalis	3	20	1	nd
Diaptomus sp.	6	23	2	2
D. gracilis	8	nd	nd	nd
Mesocyclops sp.	2	nd	nd	nd
Unidentified copepod	6	nd	1	1
Site-11				
Cyclops sp.	5	nd	15	3
C. vernalis	2	nd	nd	1
Diaptomus sp.	5	40	5	5
D. gracilis	5	20	nd	2
Mesocyclops sp.	3	40	nd	2
Unidentified copepod	7	nd	2	3

nd= Not detected

Cladocera

Among crustacean plankton *Diaphanosoma sp.* is the cladoeran species that was found frequently in all ponds of Mathbria. In summer and rainy season this plankton species most commonly observed in Mathbaria (Table 16).

Table 16. Relative abundance of Cladocera at different sites of Mathbaria according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-2				
Ceriodaphnia laticaudata	nd	nd	1	nd
Chydorus sp.	nd	nd	nd	15
Diaphanosoma sp.	5	5	nd	10
Site-5				
Chydorus sp.	nd	nd	nd	5
Diaphanosoma sp.	6	4	nd	2
Kurzia latissima sp.	nd	nd	nd	nd
Site-7				
Chydorus sp.	nd	nd	nd	2
Daphnia sp.	21	nd	nd	nd
Diaphanosoma sp.	30	8	6	4
Simocephalus sp.	1	nd	nd	nd
Cladocerans	nd	6	nd	nd
Site-8				
Ceriodaphnia sp.	nd	12	nd	nd
Diaphanosoma sp.	9	nd	6	4
Site-9				
Bosmina sp.	nd	nd	nd	3
Daphnia lumholtzi	nd	nd	nd	1
Diaphanosoma sp.	4	11	3	10
Site-10				
Diaphanosoma sp.	9	18	4	2
Kurzialatissima sp.	1	nd	nd	nd
Site-11				
Chydorus sp.	2	nd	nd	nd
Diaphanosoma sp.	7	43	7	6

nd= Not detected

4.1.1.7 Zooplankton community structures in Mathbaria

In Mathbaria, three indices were applied to estimate the species diversity, species richness and species evenness according to different seasonal environment in our country.

A. Diversity Indices:

i) Simpson's Diversity Index:

In summer, the value of index ranges between (0.1454-0.8437) where minimum value was in site-10 (0.1454) which indicates highest diversity and maximum was in site-5 (0.8437) indication of lowest biodiversity (Table 17).

In rainyseason, the value of index ranges between (0.107-0.9284) where minimum value was in site -7 (0.107) and maximum was in site-10 (0.9284). Higher diversity found in site-7 than other sites (Table 18).

In autumn, the value of index ranges between (0.05904-0.9147) where maximum value was in site -8 (0.9147) and minimum was in site-7 (0.05904). So, diversity was high in site-7 (Table 19).

In winter, the value of index ranges between (0.0581-0.9324) where maximum value was in site -8 (0.9324) and minimum was in site-7 (0.0581). That means plankton diversity was high in site-7 (Table 20).

ii) Shannon-Weiner Diversity Index:

In summer, the value of index ranges between (0.5233-2.079) where maximum value was in site -10 (2.079) and minimum was in site-5 (0.5233). Diversity was high in site- 10 (Table 17).

In rainy season, the value of index ranges between (0.2306-2.466) where maximum value was in site -7 (2.466) and minimum was in site-10 (0.2306). Higher diversity was in site-7 (Table 18).

Table 17. Diversity Indices of Zooplankton in Summer

Diversity Indices	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Simpson's Index	.2158	.8437	.5008	.4699	.2719	**.1454**	.5907
Shannon-Weiner's Index	1.754	.5233	.9298	1.048	1.514	**2.079**	1.16
Menhinick's Index	.0439	.084	.0307	.0814	.06611	.04834	**.1366**
Margalef's Richness Index	1.958	**2.89**	1.352	1.498	2.37	2.122	2.546
Species Evenness	1.239	.336	.693	.852	1.025	**1.437**	.852

Table18. Diversity Indices of Zooplankton in Rainy Season

Diversity Indices	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Simpson's Index	.4146	.5457	**.107**	.9284	.1942	.9243	.1792
Shannon-Weiner's Index	1.518	1.211	**2.466**	.236	1.713	.2306	1.834
Menhinick's Index	.17	.094	.2509	.0536	.1897	.04203	.2214
Margalef's Richness Index	1.383	1.711	**1.731**	1.093	.7238	.5865	.8686
Species Evenness	1.363	.931	2.097	.212	**2.202**	.273	2.17

Table 19. Diversity Indices of Zooplankton in Autumn

Diversity Indices	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Simpson's Index	.5161	.638	**.05904**	.9147	.1232	.4749	.5935
Shannon-Weiner's Index	1.072	.8439	**3.057**	.2687	2.595	1.53	1.085
Menhinick's Index	.08	.065	**.3074**	.04663	.2602	.1548	.1137
Margalef 's Richness Index	2.089	1.661	**3.079**	.9909	2.366	2.907	1.434
Species Evenness	.767	.648	**2.091**	.249	1.934	1.017	.923

Table 20. Diversity Indices of Zooplankton inWinter

Diversity Indices	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
Simpson's Index	.9239	0.0753	**.0581**	.9324	.08486	.6093	.689
Shannon-Weiner's Index	.2772	2.668	**2.961**	.2332	2.754	1.212	.7824
Menhinick's Index	0.051	0.2177	**.2544**	.0496	.2297	.1126	.07201
Margalef's Richness Index	1.799	1.745	**3.442**	1.226	2.388	3.249	2.334
Species Evenness	0.203	**2.216**	2.174	.198	2.022	.762	.535

In autumn, the value of index ranges between (0.2687-3.057) where maximum value was in site-7 (3.057) and minimum was in site-8 (0.2687). So, zooplankton diversity was high in site-7 (Table 19).

In winter, the value of index ranges between (0.2332-2.961) where maximum value was in site -7

(2.961) and minimum was in site-8 (0.2332) which indicates the higher diversity in site-7 (Table 20).

In both case of diversity index the results are same.

iii) Species Richness:

In both types of richness index maximum value for Menhinick's index was (0.1366) and for Margalef's index was (2.546). Highest species richness was shown in site-11 in summer (Table 17).

In rainyseason, site- 7 was rich in species and the index was high for Menhinick's index (0.2509) and (1.731) for Margalef's index (Table 18).

Site-7 in Mathbaria was rich in species and the value was (0.3074) and (3.079) for Menhinick's and Margalef's index respectively in autumn (Table 19).

In winter site-7 also had maximum richness of species with (0.2544) for Menhinick's index and (3.442) for Margalef's index (Table 20).

iv) Species Evenness:

In summer, zooplankton species evenness was found to be high (1.437) in site-10 (Table 17).

In rainyseason, species evenness was maximum (2.202) in site-9 (Table 18).

In autumn, highest value (2.091) of evenness was in site-7 (Table 19).

In winter, maximum value (2.216) of species evenness was found in site-5 (Table 20).

4.1.2 Biological assessment of *Vibrio cholerae* affected ponds in Chhatak

4.1.2.1 Zooplankton composition of different *Vibrio cholerae* affected ponds

In Chhatak, percentage of protozoa and copepodawere maximum preside over the copepoda and cladocera. Quantitative analysis of zooplankton was shown in table (21-27).

Protozoa

Percentage of Protozoa was shown highest at summer, rainy season and winter months in some selected ponds of Chhatak (site- 2, 9, 10, 11, 12). At site-2 mid of rainy season had the highest percentages of plankton in the year 2013.

At site-9, highest percentage of protozoa observed in the month of February (End of dry season) and April (Mid of Summer) in 2013. In 2014, maximum percentages of plankton was found in both summer and rainy season.

At site-10, protozoans were prominent in second year of study (2014) where the highest percentage was shown in May-July and in 2013 the percentage was highest at June.

At site-11, highest percentage of protozoa was recorded in April and May (88.7% and 100%) of 2013 and in 2014 highest percentage was recorded in the month of May (87.5%) and January (81%).

At site-12, percentage of protozoa was not significant in 2013. On the other hand, at the end of winter when the hot summer started composition of protozoan was recorded to be maximum.

Rotifera

Rotifer was most dominantly recorded in almost all ponds of Chhatak in the two years of study period. At site-1, percentage of rotifer was maximum during summer months and in some period of rainy season and winter in 2013. But in 2014 winter months had highest compositin of rotifer (94.3-99.7) %.

At site-2, highest percentage was recorded in February- 2013 (100%) and comparatively lower quantity of rotifer in rainy season (64-67) %. In 2014 maximum quantity was recorded in winter (64-75) %.

At site-4, rotifera was observed mostly in summer and rainy season and occasionally in autumn and winterof those two years of study period.

At site-9, percentage of rotifer was highest March (100%) in 2013. No significant presence of rotifers was found in 2014.

At site-10, highest quantity of rotifera was noticed in February- 2013 (100%) that was increased from January month and no significant percentage observed in 2014.

In the year 2013, rainy season showed the maximum percentage of rotifera at site-11 which decreased and again evolved in the month of October. In 2014, June had the maximum percentage (50%) of rotifer which is comparatively lower.

At site-12, rotifer quantity was maximum in September 2013 (100%).

Nauplii

At site-2, site-11 and site-12 larval stage of crustacean plankton i.e., nauplii was shown scatterdly in different months to be highest. In 2013 maximum percentage was shown in case of site-2 and site-11. And at site-12 rainy season had the significant percentage (69-75) % of rotifera in 2014.

Copepoda

Copepods the principal crustacean group of plankton was showed to be distributed during summer, rainy season and winter months of the study period.

Maximum percentages of copepods were recorded in summer at site-4, site-11 and site-12 of 2013.

Site-2 and site-12 in 2014 showed the maximum percentage in winter (100%) and rainy season (65%) respectively.

Cladocera

Cladocerans were not so prominent in Chhatak in comparison to other plankton groups. At site-10 and site-12 their percentage was significant in the month of April and May of 2013. In the year 2014 only site-11 had the maximum 55% cladoceran plankton.

Table 21. Quantitative Analysis of Zooplankton at Chhatak pond (Site-1) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	8300	15700	20.5	1.3	19.3	94.3	0	4.5	47.0	0	13.3	0
February	12200	353400	0	0.03	11.5	99.7	0	0	88.5	0	0	0
March	17900	800	0	12.5	22.9	25	25.7	25	51.4	37.5	0	0
April	5400	3700	1.9	8.1	94.4	40.5	0	48.6	3.7	0	0	2.7
May	6500	52000	7.7	0.8	86.2	77.7	0	17.1	6.2	1.3	0	3.1
June	2500	1300	40	0	4	61.5	8	38.5	28	0	20	0
July	5400	4900	1.9	20.4	0	65.3	0	8.2	72.2	0	25.9	6.1
August	20800	59200	3.4	0	90.9	38.0	0	21.1	1.9	14.0	3.8	26.9
September	40500	5700	0.5	8.8	84.7	28.15	2.0	14.0	2.0	10.5	10.9	38.6
October	6600	5700	3.0	1.8	36.4	12.3	31.8	17.5	24.2	63.2	4.5	5.3
November	14000	1500	1.4	60	47.9	13.3	28.6	13.3	22.1	13.3	0	0
December	1900	1900	-	31.6	-	15.8	-	52.6	-	0	-	0

Table 22. Quantitative Analysis of Zooplankton at Chhatak pond (Site-2) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1600	1400	18.8	21.4	56.3	64.3	0	14.3	12.5	0	12.5	0
February	1700	9400	0	2.1	100	74.5	0	5.3	0	0	0	18.1
March	9500	2000	4.2	0	31.6	10	33.7	25	28.4	55	2.1	10
April	8400	3800	7.1	2.6	16.7	21.1	45.2	65.8	19.0	7.9	11.9	2.6
May	1000	10000	20	65	30	14	0	13	0	6	50	2
June	1000	7000	70	28.6	20	32.9	0	24.3	10	11.4	0	2.9
July	700	600	100	16.7	0	50	0	0	0	0	0	33.3
August	300	3600	0	2.8	66.7	33.3	0	33.3	33.3	5.6	0	25
September	1100	700	9.1	14.3	63.6	14.3	9.1	42.9	9.1	14.3	9.1	14.3
October	1300	1400	30.8	14.3	38.5	35.7	15.4	42.9	7.7	7.1	7.7	0
November	3300	2700	54.5	0	21.2	33.3	9.1	37.0	0	7.4	15.2	22.2
December	-	200	-	0	-	0	-	0	-	100	-	0

Table 23. Quantitative Analysis of Zooplankton at Chhatak pond (Site-4) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1000	7500	0	20	80	80	0	0	10	0	10	0
February	800	4600	0	4.3	100	10.9	0	32.6	0	41.3	0	10.9
March	1000	400	0	0	90	100	0	0	0	0	10	0
April	19700	14300	0	2.1	63.5	65.7	0	16.1	0	7.7	36.5	8.4
May	2000	84800	5	12.9	5	78.8	0	6.4	90	1.9	0	0.1
June	2800	57300	21.4	1.9	64.3	92.0	0	1.0	7.1	2.3	7.1	2.8
July	1100	18200	0	13.7	54.5	63.7	0	3.8	0	7.7	45.5	11.0
August	400	900	0	0	0	66.7	0	33.3	50	0	50	0
September	14900	2000	1.3	40	90.6	30	4.7	5	0.7	5	2.7	20
October	500	3900	40	2.6	20	46.2	20	30.8	20	20.5	0	0
November	6600	4800	28.8	2.1	60.6	60.4	9.1	25	1.5	6.25	0	6.25
December	-	4300	-	9.3	-	65.1	-	18.6	-	0	-	7.0

Table 24. Quantitative Analysis of Zooplankton at Chhatak pond (Site-9) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1900	11400	0	6.1	5.3	14.0	0	62.3	89.5	16.7	5.3	0.9
February	1300	14400	100	7.6	0.0	45.1	0	24.3	0	21.5	0	1.4
March	300	1400	0	57.1	100.0	14.3	0	14.3	0	0	0	14.3
April	1000	2823300	80	99.2	20.0	0.7	0	0.1	0	0.04	0	0.0
May	4100	5050200	36.6	99.8	63.4	0	0	0.1	0	0.02	0	0.0
June	2400	2023700	66.7	98.8	20.8	0.6	0	0.5	8.3	0.03	4.2	0.0
July	45400	2109500	11.2	99.6	13.4	0.2	22.9	0.2	24.0	0.03	28.4	0.0
August	4400	37700	25	0.8	34.1	24.1	25	49.1	9.1	14.59	6.8	11.4
September	15700	17100	17.8	4.1	30.6	30.4	36.9	38.0	7.0	21.05	7.6	6.4
October	2900	31200	44.8	59.6	20.7	11.9	27.6	16.3	6.9	9.62	0	2.6
November	11300	140100	13.3	92.4	60.2	3.1	5.3	1.9	14.2	1.21	7.1	1.5
December	-	464400	-	99.5	-	0.2	-	0.3	-	0.04	-	0

Table 25. Quantitative Analysis of Zooplankton at Chhatak pond (Site-10) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1100	5000	0	36	72.7	6	0	18	0	18	27.3	22
February	4000	3300	0	6.1	100	36.4	0	42.4	0	6.1	0	9.1
March	32600	200	0	50	16.9	50	44.2	0	38.0	0	0.9	0
April	400	11300	0	49.6	0	8.0	0	31.9	0	4.4	100	6.2
May	400	18700	50	90.4	50	2.7	0	1.6	0	3.7	0	1.6
June	600	18200	66.7	87.9	16.7	3.8	16.7	3.8	0	1.6	0	2.7
July	300	10700	33.3	93.5	66.7	3.7	0	1.9	0	0	0	0.9
August	4500	1000	48.9	0	26.7	60	15.6	40	4.4	0	4.4	0
September	900	500	33.3	0	44.4	20	22.2	40	0	20	0	20
October	600	1300	16.7	7.7	33.3	15.4	16.7	46.2	16.7	23.1	16.7	7.7
November	2400	8300	4.2	37.3	58.3	18.1	20.8	16.9	4.2	12.0	12.5	15.7
December	-	1300	-	100	-	0	-	0	-	0	-	0

Table 26. Quantitative Analysis of Zooplankton at Chhatak pond (Site-11) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	3200	2100	9.4	81.0	0	9.5	0	4.8	81.25	4.8	9.4	0
February	40100	2100	1.2	0	20.4	28.6	55.1	57.1	22.9	14.3	0.2	0
March	37200	300	0.0	33.3	0	33.3	0	0	98.1	0	1.9	33.3
April	16800	115800	88.7	35.6	11.3	16.4	0	45.8	0	1.4	0	0.9
May	200	2400	100.0	87.5	0	8.3	0	4.2	0	0	0	0
June	10900	600	26.6	50	70.6	50	1.8	0	0.92	0	0	0
July	4600	5200	15.2	42.3	84.8	46.2	0	7.7	0	0	0	3.8
August	1500	6500	0.0	0	100	44.6	0	0	0	0	0	55.4
September	17900	2500	11.7	16	30.2	20	19.6	4	5.6	0	32.96	60
October	6400	9300	1.6	4.3	85.9	47.3	1.6	9.7	6.25	22.6	4.7	16.1
November	1900	2300	26.3	13.0	31.6	17.4	15.8	21.7	10.5	43.5	15.8	4.3
December	-	23500	-	0	-	28.1	-	20.9	-	51.1	-	0

Table 27. Quantitative Analysis of Zooplankton at Chhatak pond (Site-12) in 2013 and 2014

Sampling Months	Total No. of Zooplankton/L		Composition of Zooplankton Groups (%)									
			Protozoa		Rotifera		Nauplii		Copepoda		Cladocera	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	3900	8700	15.4	5.7	53.8	42.5	0	0	30.8	11.5	0	40.2
February	20100	300	0	66.7	100	0	0	0	0	0	0	33.3
March	400	900	50	100	0	0	0	0	50	0	0	0
April	700	96500	0	62.7	0	19.7	0	15.5	85.7	0.2	14.3	1.9
May	1500	24400	6.7	66.0	26.7	32.4	0	0.8	0	0.8	66.7	0
June	22100	19700	12.2	0	14.0	36.0	69.2	51.3	3.6	10.2	0.9	2.5
July	22100	23000	11.3	6.1	4.5	43.5	74.7	43.0	9.5	5.2	0	2.2
August	1200	14200	8.3	0	0	4.2	8.3	23.9	66.7	64.8	16.7	7.0
September	13400	2600	15.7	3.8	75.4	30.8	1.5	42.3	4.5	15.4	3.0	7.7
October	4400	18400	13.6	1.6	27.3	9.2	38.6	74.5	18.2	8.75	2.3	6.0
November	8200	12100	39.0	19.8	45.1	9.1	12.2	41.3	2.4	17.4	1.2	12.4
December	-	17700	-	1.7	-	88.7	-	9.0	-	0.6	-	0

4.1.2.2 Zooplankton at Chhatak ponds: A qualitative approach

Freshwater zone Chhatak exhibited in total 100 species of zooplankton of which 14 species belonged to the phylum protozoa under 3 families and single order. Rotifera had 58 species of plankton under 11 families and 3 orders. Among crustacean plankton 9 species of copepods were found under 2 families and 2 orders. Another group of planktonic crustacean, cladocera was identified in Chhatak ponds, which was represented by 19 species and 7 families and 2 orders (Table 28).

Table 28.Zooplankton species identified from Chhatak ponds

Order	Family	Species
Protozoa		
Testacealobosa	Arcellidae	*Arcella sp.*
		Arcella discoides
		Arcella vulgaris
	Centropyxidae	*Centropyxis sp.*
		Centropyxis aculeata
		Centropyxis constricta
		Centropyxis ecornis
		Ceratium hirudinella
	Difflugidae	*Difflugia sp.*
		Difflugia acuminata
		Difflugia lebes
		Difflugia rubescens
		Difflugia tuberculata
		Unidentified Protozoa
Rotifera		
Ploima	Asplanchnidae	*Asplanchna sp.*
		Asplanchna priodonta
	Brachionidae	*Anuraeopsis sp.*
		Brachionus sp.
		Brachionus angularis
		Brachionus bidentata
		Brachionus calcyflorus
		Brachionus caudatus
		Brachionus diversicornis
		Brachionus donneri
		Brachionus falcatus
		Brachionus forficula
		Brachionus havanensis
		Brachionus nilsoni
		Brachionus quadridentatus

Order	Family	Species
		Brachionus urceolaris
		Euclanis dilata
		Keratella sp.
		Keratella cochlearis
		Keratella edmondsoni
		Keratella procurva
		Keratella tecta
		Keratella tropica
		Mytilina mucronata
		Platyias patulus
		Platyias polyacanthus
		Platyias quadricornis
	Dicranophoridae	*Myersinella sp.*
	Lecanidae	*Lecane sp.*
		Lecane halychysta
		Lecane luna
		Lepadella sp.
		Lepadella imbricate
		Monostyla sp.
		Monostyla bula
		Monostyla hamata
		Monostyla sinuate
	Notommatidae	*Monommata sp.*
	Synchaetidae	*Polyarthra sp.*
		Polyarthra multiappendiculata
		Polyarthra vulgaris
	Tricocerchidae	*Trichocerca cylindrica*
		Trichocerca longiseta
		Trichocerca similis
Flosculariacea	Filinidae	*Filinia sp.*
		Filinia camascela
		Filinia longiseta
		Filinia opolienesis
		Filinia terminalis
	Testudinellidae	*Pompholyx sulcata*
		Testudinella sp.
		Testudinella mucronata
		Testudinella patina
	Hexarthidae	*Hexartha intermedia*
		Unidentified rotifer
Bdelloida	Phylodinidae	*Rotaria sp.*
		Rotaria citrinus
		Rotaria neptunia

Order	Family	Species
Copepoda		
Cyclopoida	Cyclopidae	*Cyclops sp.*
		Cyclops nanus
		Cyclops vernalis
		Cyclops vicinis
		Mesocyclops sp.
		Unidentified copepods
		Calanoid copepods
Calanoida	Diaptomidae	*Diaptomus sp.*
		Diaptomus gracilis
Cladocera		
Cladocera	Bosminidae	*Bosmina sp.*
		Bosmina coregoni
		Bosmina longirostris
	Daphniidae	*Ceriodaphnia sp.*
		Ceriodaphnia pulchella
		Daphnia sp.
		Daphnia lumholtzi
		Daphnia magna
		Daphnia similis
		Scapholeberis kingi
	Chydoridae	*Chydorus sp.*
	Macrothricidae	*Macrothrix sp.*
	Moinidae	*Moina sp.*
		Moina brachiata
Diplostraca	Sididae	*Diaphanosoma sp.*
		Pseudosida bidentata
	Simocephalidae	*Kurzia latissima*
		Simocephalus sp.
		Unidentified Cladocera

Fig. Arcella sp.

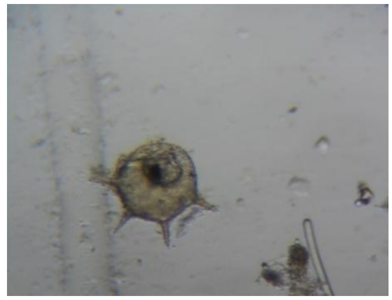


Fig. Centropyxis sp.

Fig. Glaucoma sp.

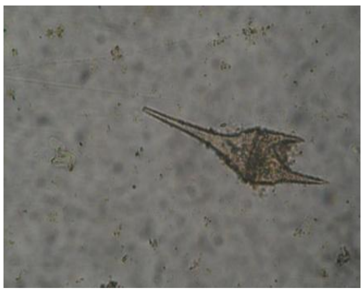


Fig. Ceratium hirudinella

Fig. Euglena oxyuris

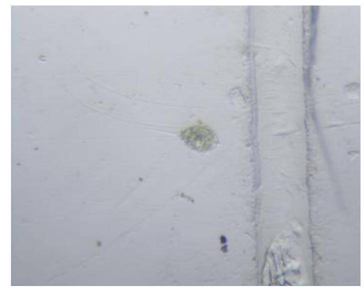


Fig. Phacus longicauda.

Plate 1. Some Protozoan Plankton Identified in Mathbaria and Chhatak

Fig. *Asplanchna priodonta*

Fig. *Brachionus caudatus*

Fig. *Brachionus falcatus*

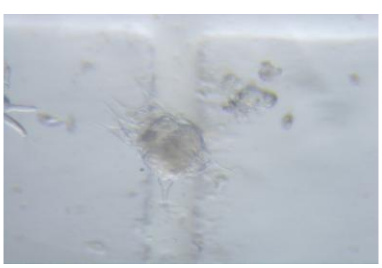


Fig. *Brachionu. quadridentatus*

Fig. *Polyarthra vulgaris*

Fig. *Filinia longiseta*

Plate 2. Some Rotifers in Mathbaria and Chhatak

Fig. Cyclops sp.

Fig. Diaptomus sp.

Fig. Diaphanosoma sp.

Fig. Bosmina longirostris

Fig. Simocephalus sp.

Plate 3. Copepoda and Cladocera species recorded in Mathbaria and Chhatak

4.1.2.3 Species composition of zooplankton in Chhatak ponds

In Chhatak, seven domestic ponds had shown diversified zooplankton species in the year 2013 and 2014. Pond at site-1 had 45 species of plankton in total, on the other hand site-2 had 45 species of plankton, site-4 had 45 species, site-9 had 53 species, site-10 had 38 species, site-11 had 57 species and site-12 had 48 species. All of them were recorded in the year 2013. In the year 2014, these ponds showed another numerical amount of plankton species, such as 47 species site-1 had, 40 species at site-2, 50 species at site-4, site-9 had 49 species, side-10 had 41 species, site-11 had 50 species and site-12 had 50 species of zooplankton.

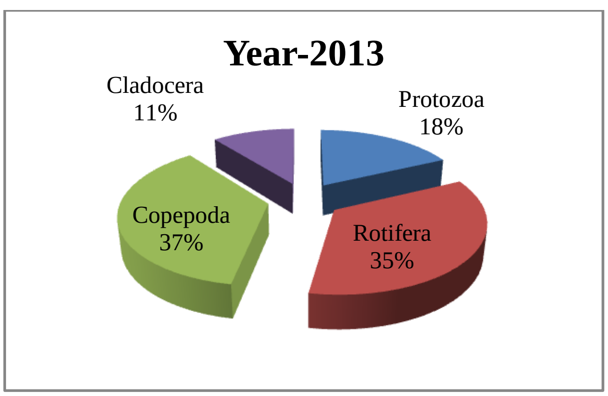
The zooplanton diversity in summery are as follows,

In 2013: Pond site 11 (57 sps)> site 9 (53 sps)>site 12(48 sps)>site 1, site 2 and site 4 (45 sps)> site 10 (38sps)

In 2014: Pond site 4, site 11 and site 12 (50 sps)> site 9 (49 sps)>site 1 (47 sps)>site 10 (41 sps)> site 2 (40 sps)

Comparison of zooplankton number diversity in 2013 and 2014 at Chhatak ponds

Sites	1	2	4	9	10	11	12
2013	45	45	45	53	38	57	48
2014	47	40	50	49	41	50	50



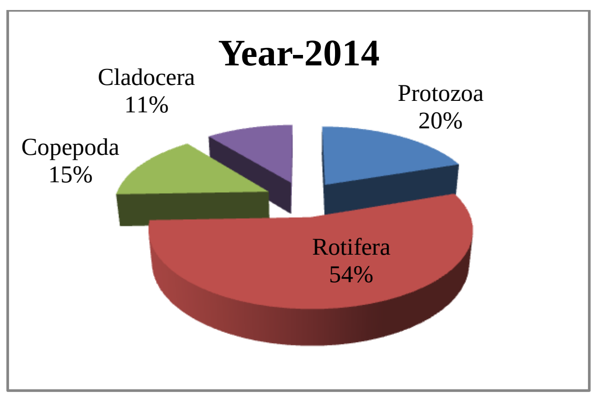
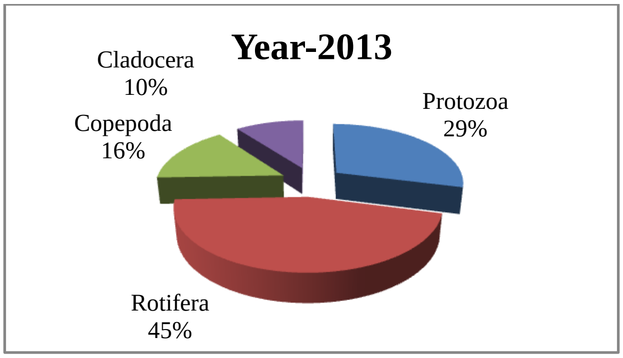


Figure 9. Pie-chart showing species composition in the year 2013 and 2014 at Site-1



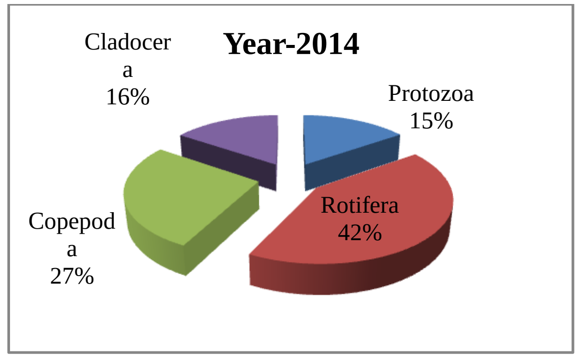
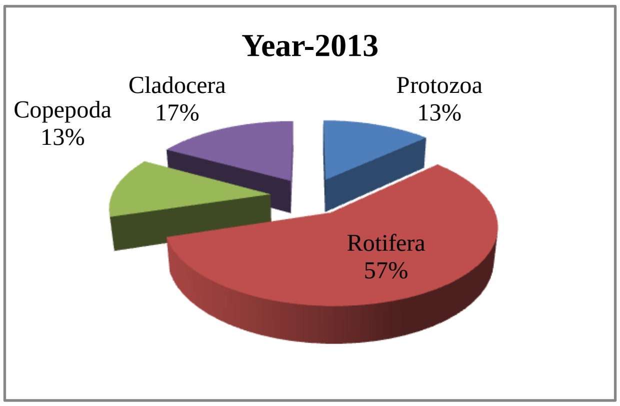


Figure 10. Pie-chart showing species composition in the year 2013 and 2014 at Site-2



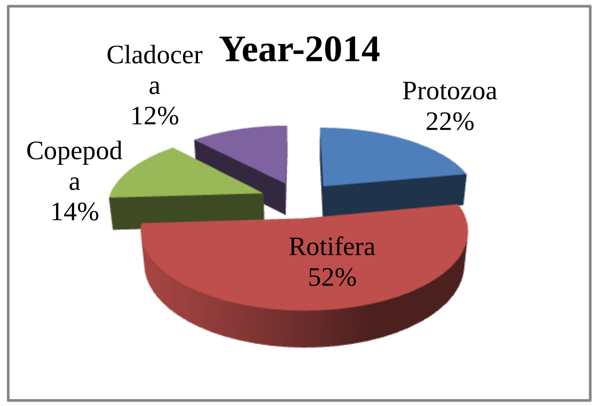
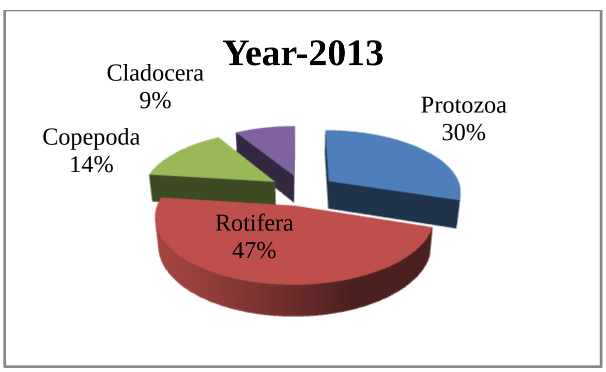


Figure 11. Pie-chart showing species composition in the year 2013 and 2014 at Site-4



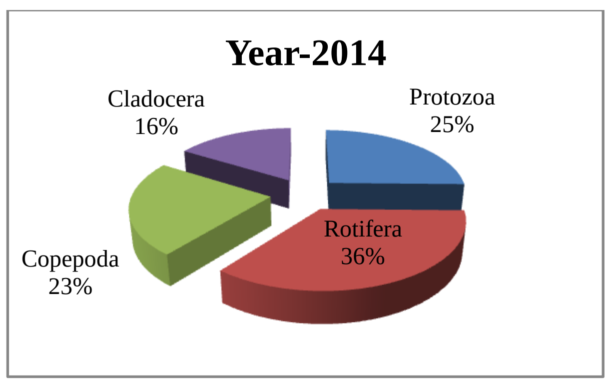
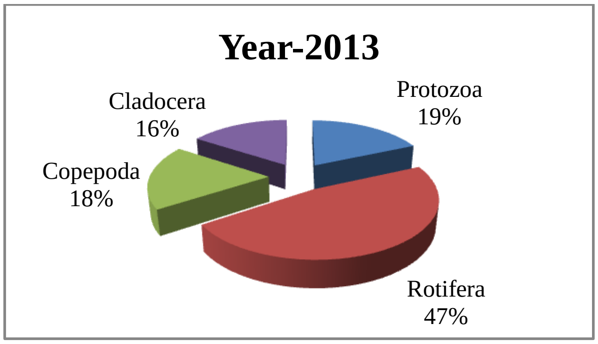


Figure 12. Pie-chart showing species composition in the year 2013 and 2014 at Site-9



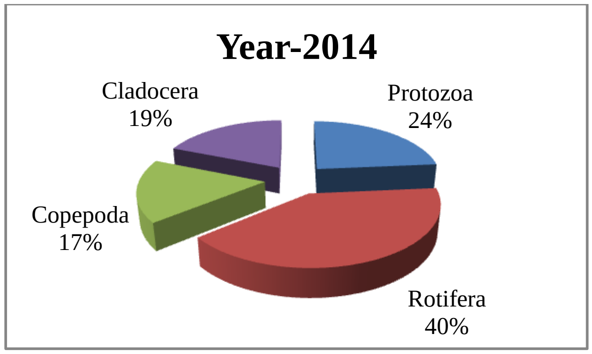
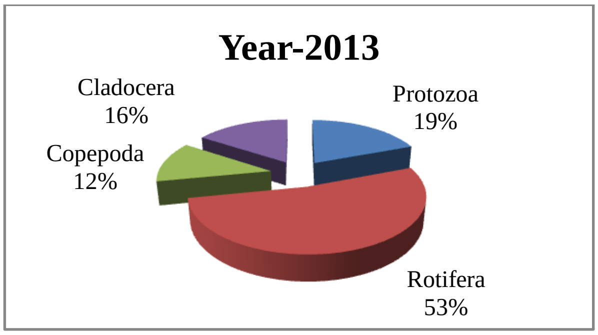


Figure 13. Pie-chart showing species composition in the year 2013 and 2014 at Site-10



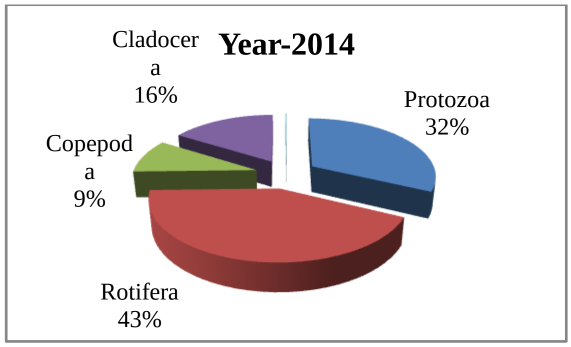
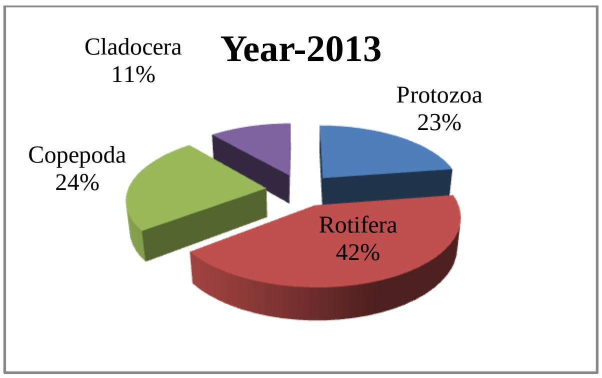


Figure 14. Pie-chart showing species composition in the year 2013 and 2014 at Site-11



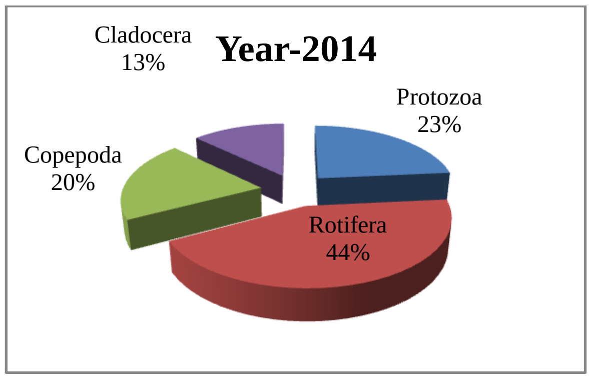


Figure 15. Pie-chart showing species composition in the year 2013 and 2014 at Site-12

In case of protozoan taxa highest composition observed in site-9 (30%) in the year 2013 and in site-11 (32%) during the sampling periods in 2014 (Figure 12 and Figure 14).

Highest species composition in rotifera taxa was recorded in site-4 (57%) in the year 2013 (Figure 11) and in site-1 (54%) in 2014 (Figure 9).

Copepoda taxa had maximum species composition (37%) in the year 2013 at site-1 and (27%) at site-2 in 2014 (Figure 9 and Figure 10).

Among cladocera site-4 had highest species composition (17%) in 2013 and in 2014 site-10 had (19%) highest composition (Figure 11 and Figure 13).

4.1.2.4 Distribution of Zooplankton in seven Chhatak ponds

In the first year of study (2013), among protozoa the most dominant taxa in some selected ponds of Chhatak were *Centropyxis sp., Ceratium hirudinella, Difflugia sp., Euclanis dilata, Phacus acuminata, Phacus longicauda* and *P. pleuronectes, Brachionus sp., B. angularis, B. calcyflorus, B. caudatus, B. falcatus, B. forficula,Filinia camascela, Filinia longiseta, Filinia terminalis, Keratella sp., K. cochleria, K. tropica, P. vulgaris, Rotaria sp.* and *Trichocerca similis*were the rotifer plankton in Chhatak ponds. Some copepod species are commonly recorded all through the year in Chhatak were *Cyclops sp., Cyclops nanus, Cyclops vernalis, Diaptomus sp.* and some unidentified copepods. *Bosmina sp.* and *Diaphanosoma sp.* among cladoceran plankton were commonly distributed in Chhatak (Table 29).

Table 29. Diversity of Zooplankton at seven study sites during January 2013-December 2013

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Arcella sp.	-	-	+	+	+	+	+
Arcella discoides	-	+	-	-	+	-	-
Arcella vulgaris	-	+	-	-	-	-	-
Centropyxis sp.	+	++	++	++	++	+	+
Centropyxis aculeata	+	+	-	+	-	-	
Centropyxis constricta	+	-	-	-	-	-	-
Centropyxis ecornis	-	-	-	-	-	+	-
Ceratium hirudinella	+	++	-	++	++	-	-
Difflugia sp.	++	++	++	+++	-	++	++
Difflugia acuminata	+	-	-	-	-	+	-

Species	**Site-1**	**Site-2**	**Site-4**	**Site-9**	**Site-10**	**Site-11**	**Site-12**
Difflugia tuberculata	-	+	-	+	-	-	+
Euglena sp.	-	-	-	-	-	-	+
Euglena acus	-	+	+	++	+	+	++
Euglena oxyuris	-	++	-	+	-	-	+
Euglena tripteris	-	-	-	-	-	+	+
Glaucoma sp.	-	-	-	-	+	-	-
Paramecium sp.	+	-	+	-	-	-	-
Phacus acuminata	+	+	+	++	-	++	+
Phacus longicauda	-	++	-	++	-	++	+
Phacus pleuronectes	++	++	-	++	+	++	++
Unidentified Protozoa	-	-	+	-	-	+	-
Rotifera							
Anuraeopsis sp.	-	-	-	-	+	-	-
Asplanchna sp.	+	+	+	-	-	-	+
Asplanchna priodonta	+	-	+	-	-	+	-
Brachionus sp.	++	++	++	++	+	++	++
Brachionus angularis	+++	+++	++	++	+	++	++
Brachionus bidentata	-	-	+	-	-	-	-
Brachionus calcyflorus	++	+	++	+	-	++	+
Brachionus caudatus	++	+	++	++	+	+	+
Brachionus diversicornis	-	+	++	+	-	-	-
Brachionus falcatus	+	+	+	++		++	++
Brachionus forficula	++	-	+	++	+	++	-
Brachionus havanensis	+	-	-	-	-	-	-
Brachionus nilsoni	+	-	++	+	-	-	+
Brachionus quadridentatus	+	-	++	++	-	+	-
Brachionus urceolaris	++	-	++	+	-		-
Euclanis dilata	-	-	-	-	-	+	-
Filinia sp.	-	+	+	-	-	++	-
Filinia camascela	+	+	-	++	++	++	+
Filinia longiseta	++	+	+	-	-	++	+
Filinia opolienesis	+	-	-	-	-	-	++
Filinia terminalis	+	-	+	++	+	++	+
Hexartha intermedia	-	+	-	-	+	+	+
Keratella sp.	-	++	+	+	+	+	-
Keratella cochlearis	-	+++	+	++	++	-	+
Keratella edmondsoni	-	-	-	+	-	-	-
Keratella procurva	-	-		+	-	-	-
Keratella tecta	++	++	+	+	++	+	++
Keratella tropica	+	++		++	+++	+	++
Lecane sp.	-	-	-	-	+	+	-

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Lecane halychysta	-	-	-	-	-	+	-
Lecaneluna	-	-	+	++	-	++	++
Lepadella sp.	-	-	+	+	-	-	-
Lepadella imbricata	-	-	-	-	+	++	+
Monostyla sp.	-	-	-	-	-	+	-
Monostyla bula	-	-	++	+	-	+	-
Monostyla hamata	-	-	-	-	-	+	-
Monostyla sinuata	-	-	-	-	-	+	-
Platyias patulus	+	+	-	+	-	+	+
Platyias polyacanthus	-	-	+	-	-	-	-
Platyias quadricornis	+	-	-	+	-	-	-
Polyarthra sp.	+	+	+	++	+	-	+
Polyarthra multiappendiculata	-	++	-	-	-	-	-
Polyarthra vulgaris	+	-	++	+	++	+	+
Pompholyx sulcata	-	-	-	+	+	+	+
Rotaria sp.	++	+	++	+	-	+	+
Rotaria neptunia	-	-	+	-	-	-	-
Testudinella mucronata	-	-	+	-	-	-	-
Trichocerca cylindrica	-	+	+	+	-	+	+
Tricocerca similis	-	+	++	+	++	-	+
Unidentified rotifer	+	-	-	++	+	+	+
Copepopda							
Cyclops sp.	+++	+++	+	++	++	++	++
Cyclops nanus	++	+	+	++	++	++	++
Cyclops vernalis	++	++	+	++	++	++	++
Cyclops vicinis	-	-	-	-	+	+	-
Diaptomus sp.	+	++	-	++	+	++	++
Diaptomus gracilis	-	+	-	-	-	-	+
Mesocyclops sp.	++	-	-	-	+	++	+
Unidentified copepods	++	++	++	++	+	++	++
Calanoid copepods	+	-	-	+	-	-	-
Cladocera							
Bosminasp.	+	+	-	++	+	+	+
Bosmina coregoni	-	+	-	-	++	-	-
Bosmina longirostris	-	+	-	-	+	-	-
Ceriodaphnia sp.	-	-	-	-	-	+	-
Chydorus sp.	-	+	-	-	-	-	-
Daphnia sp.	-	+	-	-	+	-	
Diaphanosoma sp.	++	++	+++	++	++	++	++
Kurzia latissima	-	-	+	-	-	-	-
Moina sp.	-	-	-	-	-	++	+

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Simocephalus sp.	+	-	+	+	+	+	-
Unidentified Cladocera	++	+	++	++	-	+	+

+++ = Most Abundant; ++ = Fairly Present; + =Present; - = Absent

In the second year (2014), among protozoa *Ceratium hirudinella, Euglena acus* and *P. pleuronectes* were prominent in Chhatak. *Asplanchna priodonta*, *Brachionus sp*., *B. angularis, B. caudatus, B. calcyflorus, B. falcatus, Filinia terminalis, Keratella cochlearis, Keratella tropica, P. vulgaris, Rotaria sp. Testudinella sp.* and some unknown rotifera species were commonly distributed taxa among rotifera. Among copepods *Cyclops sp., Cyclops nanus, Cyclops vernalis, Diaptomus sp., Diaptomus gracilis* and some unidentified copepod plankton were available throughout the year in Chhatak. *Ceriodaphnia sp., Chydorus sp*. and *Diaphanosoma sp.* were the most abundant species that represents the presence of cladocerans (Table 30).

Table 30. Diversity of Zooplankton at seven study sites during January 2014-December 2014

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Arcella sp.	-	+	+	-	-	++	+
Centropyxis sp.	-	-	-	+	++	++	-
Centropyxis aculeata	-	-	+	-	-	-	
Centropyxis ecornis	-	+	-	-	-	-	-
Ceratium hirudinella	++	++	++	+++	+++	++	++
Difflugia sp.	++	-	+	+	+	+	+
Difflugia acuminata	+	-	-	+	-	-	-
Difflugialebes	-	-	+	+	-	-	-
Difflugia rubescens	+	-	-	-	-	-	-
Difflugia tuberculata	-	-	-	+	-	-	-
Euglena acus	++	++	++	-	+	++	++
Euglena oxyuris	-	+	+	+	-	+	++
Euglena tripteris	+	+	-	-	-	+	++
Glaucoma sp.	-	+	-	-	-	-	-
Paramecium sp.	-	-	-	-	-	+	-
Phacus acuminata	+	-	+	-	-	++	++
Phacus longicauda	+	-	+	+	-	++	+
Phacus pleuronectes	++	-	+	++	-	++	++
Unidentified Protozoa	-	-	+	-	-	-	-
Rotifera							
Asplanchna sp.	++	+	+++	-	-	+	++

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Asplanchna priodonta	++	++	+	++	-	++	+
Brachionus sp.	+	-	++	+	++	+	+
Brachionus angularis	+++	+++	+++	++	++	+++	++
Brachionus calcyflorus	++	+	+++	+	+	+	+
Brachionus caudatus	+	++	++	+	+	++	++
Brachionus diversicornis	-	-	++	+	+	+	-
Brachionus donneri	-	-	-	+	-	+	-
Brachionus falcatus	+	+	++	++	++	-	-
Brachionus forficula	-	+	+	+	-	-	-
Brachionus havanensis	-	-	-	+	-	-	-
Brachionus nilsoni	-	-	-	-	-	-	+
Brachionus quadridentatus	-	+	+	-	+	++	-
Brachionus urceolaris	-	-	-	-	-	-	+
Euclanis dilata	-	-	-	-	-	+	-
Filinia sp.	-	+	++	-	-	-	+
Filinia longiseta	++	-	+	+	+	+	+
Filinia opolienesis	+	-	-	-	-	-	-
Filinia terminalis	++	++	++	+	-	+	++
Hexartha intermedia	++	-	+	-	+	+	+
Keratella sp.	+	+	-	-	-	-	+
Keratella cochlearis	-	+++	-	++	+++	+	++
Keratella tecta	+	+	-	++	+	-	++
Keratella tropica	++	++	+	++	+++	++	++
Lecane sp.	-	+	-	-	-	-	-
Lecane luna	+	-	++	+	-	++	-
Lepadella sp.	-	+	+	-	-	-	-
Lepadella imbricata	-	-	+	+	-	-	+
Monommata sp.	+	-	-	-	-	+	-
Monostylabula	-	-	-	+	-	+	-
Myersinellasp.	+	-	-	-	-	-	-
Mytilina mucronata	+	+	-	-	-	+	-
Platyias patulus	++	-	-	+	-	++	-
Platyias quadricornis	-	+	-	+	+	+	+
Polyarthra sp.	++	-	++	+	+	-	+
Polyarthra multiappendiculata	-	-	-	+	-	-	+
Polyarthra vulgaris	+	++	++	+	++	+	++
Rotaria sp.	+	+	++	-	-	++	++
Rotaria citrinus	-	-	+	-	-	-	-
Testudinella sp.	++	+	+	+	+	++	+
Testudinella patina	-	-	+	-	-	-	-
Trichocerca cylindrica	-	-	+	-	-	+	-
Trichocerca longiseta	-	-	-	-	+	-	-

Species	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Tricocerca similis	+	-	-	-	+	-	-
Unidentified rotifer	+	-	++	+	+	++	+
Copepoda							
Cyclops sp.	++	++	++	++	++	++	++
Cyclops nanus	+	++	++	+	+	-	++
Cyclops vernalis	+++	+++	++	++	+++	++	++
Diaptomus sp.	+	++	++	++	++	+	++
Diaptomus gracilis	-	-	-	++	+	-	-
Mesocyclops sp.	+	-	++	+	-	++	+
Unidentified copepods	++	+	++	+	++	-	-
Calanoid copepods	+	+	+	-	-	-	+
Cladocera							
Bosminasp.	-	++	-	-	++	++	-
Bosmina coregoni	-	+	-	+	+	-	+
Bosmina longirostris	-	+	-	+	+	-	-
Ceriodaphnia sp.	++	+	+	+	++	++	+
Chydorus sp.	+	+	++	+	++	++	+
Daphnia sp.	-	+	-	-	+	+	-
Daphnia lumholtzi	-	-	-	-	-	-	+
Daphnia magna	-	-	-	+	-	+	-
Daphnia similis	-	-	-	-	-	-	+
Diaphanosoma sp.	++	+++	+++	++	+++	++	++
Kurzia latissima	-	-	-	-	+	-	-
Macrothrix sp.	+	-	-	-	-	-	-
Moina sp.	++	-	-	++	+	++	+
Pseudosida bidentata	-	-	-		+	-	+
Scaphaloberis kingi	-	-	+	-	-	-	-
Simocephalus sp.	-	+	+	+	+	-	+
Unidentified Cladocera	++	+	++	-	++	++	++

+++ = Most Abundant; ++ = Fairly Present; + =Present; - = Absent

4.1.2.5 Frequency of occurrence of zooplankton in Chhatak

Considering the occurrence constancy in the studied ponds of Chhatak, protozoa was shown to have two absolute constant species (*Arcella vulgaris* and *Difflugia sp.*) at site-5 and site-8 and two constant species (*Difflugia sp.*and *D. tuberculata*) at site-2. Rotifera had one constant species (*B. angularis*) at site-5 and site-10. Two absolute constant taxa were observed among copepods (*Cyclops sp.* and *Diaptomus sp.*) at site-7, site-10 and site-11. They have also some constant species such as, *Cyclops sp.*, *Cyclops vernalis*, *Diaptomus sp.*, *Diaptomus gracilis and* some unknown copepods*)* at site-2, site-5, site-7 and site-8. *Diaphanosoma sp.* was the unique representing the cladoceran group and was absolutely constant at site-9 and site-11. It was also constantly present at site-2, site-5, site-7 and site-10 (Table 31).

Among protozoan plankton, *Arcella sp.* (85.7%), *Centropyxis sp.* (100%), *Centropyxis aculeata* (71.4%), *Difflugia sp.* (100%), *Euglena acus* (100%), *Euglena oxyuris* (71.4%), *Phacus acuminata* (71.4%), *Phacus pleuronectes* (85.7%) and *Glaucoma sp.* (85.7%) were frequently distributed in Chhatak ponds.

Among rotifers, *Asplanchna sp.*(85.7%), *A. priodonta* (85.7%), *Brachionus sp.* (100%), *Brachionus angularis* (100%), *B. calcyflorus* (100%), *B. caudatus* (100%), *B. diversicornis* (71.4%), *B. falcatus*

(100%), *B. forficula* (85.7%), *B. quadridentatus* (85.7%), *Hexartha intermedia* (85.7%), *Keratella sp.* (100%), *K. cochlearis* (85.7%), *K. tecta* (100%), *K. tropica* (100%), *Platyias patulus* (71.4%), *Platyias quadricornis* (85.7%), *Lecane luna* (71.4%), *Lepadella imbricata* (71.4%), *Polyarthra sp.*(85.7%), *P. vulgaris* (100%), *T. cylindrica* (71.4%), *T. similis* (85.7%), *Filinia camascela* (85.7%), *Filinia longiseta* (85.7%), *Testudinella sp.*(100%), *Rotaria sp.* (85.%7) were distributed frequently in most of the ponds of Chhatak.

Cyclops sp. (100%), *Cyclops nanus* (100%) and *Cyclops vernalis* (100%), *Diaptomus sp.* (100%) and *Mesocyclops sp.* (85.7%) among copepods were frequently distributed in Chhatak

Among cladoceran plankton *Bosmina sp*. (71.4%), *Ceriodaphnia sp*. (85.7%), *Chydorus sp*. (85.7%), *Diaphanosoma sp*. (100%), *Moina sp.* (71.4 %) and *Simocephalus sp.* (100%) were most frequently distributed in almost all ponds of Chhatak.

Table 31. Frequency of Occurrence of particular zooplankton species in Chhatak on a four degree scale; Absolute Constant Species (AS)- >75%, Constant Species (S)- 51-75%, Absolute Species (A)- 26-50% and Accidental Species (P)- < 25%

Group	Species	Sites							Frequency
		S-1	S-2	S-4	S-9	S-10	S-11	S-12	(%)
Protozoa	*Arcella sp.*	-	P	P	P	P	A	P	85.7
	A. discoides	-	P	-	-	P	-	-	28.6
	A. vulgaris	-	P	-	-	-	-	-	14.2
	Centropyxis sp.	P	P	A	A	A	A	P	100
	C. aculeata	P	P	P	P	-	-	P	71.4
	C. constricta	P	-	-	-	-	-	-	14.2
	C. ecornis	P	P	-	-	-	P	-	42.8
	Ceratium hirudinella	P	-	-	-	P	-	-	28.6
	Difflugia sp.	S	P	P	S	P	A	A	100
	D. acuminata	P	-	-	P	-	-	-	28.6
	D. lebes	-	-	P	P	-	-	-	28.6
	D. rubescens	P	-	-	-	-	-	-	14.2
	D. tuberculata	-	P	-	P	-	P	P	57.1
	Unidentified Protozoa	-	-	P	-	-	P	-	28.6
	Paramecium sp.	P	-	P	-	-	P	-	42.8
	Euglena acus	P	A	A	P	P	A	A	100
	E. oxyuris	-	A	P	P	-	P	A	71.4
	E. tripteris	P	P	-	-	-	P	A	57.1
	P. acuminata	P	P	P	P	-	S	-	71.4
	P. longicauda	P	P	P	A	-	S	-	57.1
	P. pleuronectes	S	A	P	A	P	S	-	85.7
	Glaucoma sp.	-	P	P	A	P	P	P	85.7
Rotifera	*Asplanchna sp.*	A	P	S	-	P	P	A	85.7
	A. priodonta	A	P	P	P	-	A	P	85.7
	Brachionus sp.	A	P	S	A	A	A	A	100
	B. angularis	AS	AS	AS	S	A	AS	S	100

B. bidentata	-	-	P	-	-	-	-	14.2
B. calcyflorus	S	P	S	P	P	A	P	100
B. caudatus	A	A	S	S	P	A	A	100
B. diversicornis	-	P	A	P	P	P	-	71.4
B. donneri	-	-	-	-	-	P	-	14.2
B. falcatus	A	P	A	A	P	A	A	100
B. forficula	A	P	P	A	P	P	-	85.7
B. havanensis	A	-	-	P	-	-	-	28.6
B. nilsoni	A	-	P	P	-	-	P	57.1
B. quadridentatus	A	P	A	P	P	A	-	85.7
B. urceolaris	A	-	P	P	-	-	P	57.1
Euclanis dilata	-	-	-	-	-	P	-	14.2
Keratella sp.	P	A	P	P	P	P	P	100
K. cochlearis	-	AS	P	A	AS	P	A	85.7
K. edmondsoni	-	-	-	P	-	-	-	14.2
K. procurva	-	-	-	P	-	-	-	14.2
K. tecta	A	A	P	A	A	P	S	100
K. tropica	A	S	P	S	AS	A	A	100
Mytilina mucronata	P	P	-	-	-	P	-	42.8
Platyias patulus	A	P	-	P	-	A	P	71.4
P. polyacanthus	-	-	P	-	-	-	-	14.2
P. quadricornis	A	P	-	P	P	P	P	85.7
Myersinella sp.	P	-	-	-	-	-	-	14.2
Lecane sp.	-	P	-	-	P	P	-	42.8
L. halychysta	-	-	-	-	-	P	-	14.2
L. luna	P	-	A	A	-	S	P	71.4
Lepadella sp.	-	P	P	P	-	-	-	42.8
L. imbricata	-	-	P	P	P	P	P	71.4
Monostyla sp.	-	-	-	-	-	P	-	14.2
M. bula	-	-	P	P	-	P	-	42.8
M. hamata	-	-	-	-	-	P	-	14.2

	M. sinuata	-	-	-	-	-	P	-	14.2
	Monommata sp.	P	-		-	-	P	-	28.6
	Polyarthra sp.	A	P	A	A	P	-	P	85.7
	P. multiappendiculata	-	-	-	P	-	-	P	28.6
	P. vulgaris	P	A	S	P	A	P	A	100
	T. cylindrica	-	P	P	P	-	P	P	71.4
	T. longiseta	-	-	-	-	P	-	-	14.2
	T. similis	P	P	P	P	A	-	P	85.7
	Filinia sp.	-	P	A	-	-	A	P	57.1
	F. camascela	P	P	-	P	P	P	P	85.7
	F. longiseta	A	-	P	P	P	A	P	85.7
	F. opolienesis	P	-	A	-	-	-	P	42.8
	F. terminalis	A	-	-	A	P	-	A	57.1
	Pompholyx sulcata	-	-	-	P	P	P	P	57.1
	Testudinella sp.	P	P	P	P	P	P	P	100
	T. mucronata	-	-	P	-	-	-	-	14.2
	T. patina	-	-	P	-	-	-	-	14.2
	Hexartha intermedia	P	P	P	-	P	P	P	85.7
	Unidentified rotifer	P	-	P	A	P	A	P	85.7
	Rotaria sp.	A	P	S	P	-	A	A	85.7
	R. citrinus	-	-	P	-	-	-	-	14.2
	R. neptunia	-	-	P	-	-	-	-	14.2
Copepoda	*Cyclops sp.*	S	S	A	S	A	S	A	100
	C. nanus	A	A	A	A	A	A	A	100
	C. vernalis	AS	S	A	A	S	S	S	100
	C. vicinis	-	-	-	-	P	P	-	28.6
	Mesocyclops sp.	A	-	A	P	P	S	P	85.7
	Unidentified copepods	S	A	S	A	A	A	P	100
	Calanoid copepods	P	P	P	P	-	-	P	71.4
	Diaptomus sp.	P	S	P	S	A	A	A	100

	D. gracilis	-	P	-	P	P	-	P	57.1
Cladocera	*Bosmina sp.*	P	A	-	-	A	A	P	71.4
	B. coregoni	-	P	-	A	A	-	P	57.1
	B. longirostris	-	P	-	P	P	-	-	42.8
	Ceriodaphnia sp.	P	P	P	P	P	A	-	85.7
	C. pulchella	-		-	P	-	P	-	28.6
	Daphnia sp.	-	P	-	-	P	P	-	42.8
	D. lumholtzi		-	-	-	-	-	P	14.2
	D. magna	-	-	-	P	-	P	-	28.6
	D. similis	-	-	-	-	-	-	P	14.2
	Scapholeberis kingi	-	-	P	-	-	-	-	14.2
	Chydorus sp.	P	P	P	P	P	P	-	85.7
	Macrothrix sp.	P	-	-	-	-	-	-	14.2
	Moina sp.	P	-	-	A	P	A	P	71.4
	M. brachiata	P	-	-	-	-	P	-	28.6
	Diaphanosoma sp.	S	S	AS	S	S	S	S	100
	Pseudosida bidentata	-	-	-	-	P	-	P	28.6
	Kurzia latissima	-	-	P	-	P	-	-	28.6
	Simocephalus sp.	P	P	P	P	P	P	P	100
	Unidentified Cladocera	S	P	A	P	A	A	-	85.7

4.1.2.6 Seasonal abundance of zooplankton species at Chhatak ponds

Protozoa

Among protozoa most abundant taxa that dominated in different seasons at different sites of Chhatak were *Centropyxis sp., Centropyxis aculeata, Ceratium hirudinella, Euglena acus* and *Phacus pleuronectes*. At site-9 and site-10 *Ceratium hirudinella* was dominant in almost all seasons. *Euglena acus* was present in spring season at site-2, site-11 and site-12. One unique species *Trinema comlanatum* at site-2 was only found in rainy season (Table 32).

Table 32. Relative abundance of Protozoa at different sites of Chhatak according to Fourseasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-1				
Centropyxis sp.	nd	1	nd	nd
C. aculeatata	1	nd	nd	nd
C. constricta	1	nd	nd	nd
C. ecornis	1	nd	nd	nd
Ceratium hirudinella	1	1	nd	nd
Difflugia sp.	1	1	3	1
D. acuminata	1	nd	1	nd
D. rubescens	nd	nd	nd	nd
Euglena acus	nd	1	nd	1
E. tripteris	nd	nd	4	nd
Paramecium sp.	nd	nd	1	nd
Phacus acuminata	nd	nd	1	nd
P. longicauda	nd	nd	2	nd
P. pleuronectes	nd	2	2	11
Site-2				
Arcella sp.	nd	nd	nd	2
A. discoides	nd	nd	2	nd
A. vulgaris	1	nd	nd	nd
Centropyxis sp.	8	nd	8	nd
C. aculeatata	1	nd	nd	nd
C. ecornis	nd	1	nd	nd
Ceratium hirudinella	8	8	2	4
Difflugia sp.	7	nd	2	nd
D. tuberculata	3	nd	nd	nd
Euglena acus	53	11	12	nd
E. oxyuris	nd	nd	3	3.8
E. tripteris	nd	nd	7	nd
Glaucoma sp.	nd	nd	2	nd
Phacus acuminata	nd	nd	2	nd

	Summer	Rainy Season	Autumn	Winter
P. longicauda	1	nd	3	nd
P. pleuronectes	18	nd	5	8
Site-4				
Arcella sp.	nd	nd	1	1
Centropyxis sp.	1	7	1	nd
C. aculeatata	nd	nd	1	nd
Ceratium hirudinella	14	5	nd	3
Difflugia sp.	1	7	1	nd
D. lebes	nd	nd	1	nd
Euglena acus	nd	1	1	7
E. oxyuris	nd	nd	1	nd
Paramecium sp.	nd	nd	1	nd
Phacus acuminata	nd	nd	4	nd
P. longicauda	1	nd	nd	nd
P. pleuronectes	nd	nd	2	nd
Unidentified Protozoa	nd	nd	1	1
Site-9				
Arcella sp.	nd	nd	1	nd
Centropyxis sp.	nd	3	1	3
C. aculeata	28	nd	nd	nd
Ceratium hirudinella	99	51	39	92
Difflugia sp.	9	2	4	34
D. aculeata	nd	nd	1	nd
D.lebes	nd	nd	1	nd
D. tuberculata	6	nd	1	nd
Euglena acus	nd	5	2	nd
E. oxyuris	nd	1	1	nd
Phacus acuminata	nd	2	3	nd
P. longicauda	nd	1	1	1
P. pleuronectes	nd	7	1	1
Site-10				
Arcella sp.	nd	nd	7	nd
A. discoides	1	nd	nd	nd
Centropyxis sp.	1	6	2	2
Ceratium hirudinella	59	67	10	21
Difflugia sp.	nd	nd	2	nd
Euglena acus	nd	2	2	nd
Glaucoma sp.	nd	nd	2	nd
P. pleuronectes	nd	nd	4.8	nd
Site-11				
Arcella sp.	1	1	1	1
Centropyxis sp.	1	8	3	nd
C. ecornis	1	nd	nd	nd
Ceratium hirudinella	1	10	2	nd
Difflugia sp.	1	6	1	nd

	Summer	Rainy Season	Autumn	Winter
D. tuberculata	1	nd	nd	nd
Euglena acus	48	nd	1	3
E. oxyuris	nd	nd	1	nd
E. tripteris	26	nd	1	nd
Paramecium sp.	nd	nd	nd	1
Phacus acuminata	1	9	2	1
P. longicauda	1	nd	2	1
P. pleuronectes	1	nd	3	4
Unidentified Protozoa	1	nd	nd	nd
Site-12				
Arcella sp.	1	nd	2	nd
Centropyxis sp.	nd	nd	3	nd
C.aculeata	nd	nd	1	nd
Ceratium hirudinella	2	nd	1	4
Difflugia sp.	6	3	1	nd
D. tuberculata	5	nd	4	nd
Euglena acus	45	4	3	2
Euglena oxyuris	1	nd	4	nd
Euglena tripteris	6	nd	2	nd
Phacus acuminata	2	1	3	nd
P. longicauda	1	7	nd	nd
P. pleuronectes	3	3	4	2

nd= Not detected

Rotifera

Maximum species of rotifer was recorded in Chhatak. Among them *Brachionus* angularis was observed in almost all seasons. At site-2, 9, 10, 11 and 12 *Keratella tropica* was dominant in four seasons of the study period. Another plankton *Keratella cochlearis* was also present atsite-2 and site-10all the year round. Sometimes, *Asplanchna sp*. and *Polyarthra vulgaris* were noticed to be distributed in some ponds of Chhatak (Table 33).

Table 33. Relative abundance of Rotifera at different sites of Chhatak according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-1				
Asplanchna sp.	1	nd	1	nd
A. priodonta	nd	2	2	nd
Brachionus sp.	nd	6	2	1
B. angularis	43	17	14	26
B. calcyflorus	nd	12	3	2
B. caudatus	nd	19	12	nd
B. falcatus	nd	5	2	nd
B. forficula	17	nd	2	nd
B. havanensis	nd	nd	3	nd
B. nilsoni	nd	nd	1	nd
B. quadridentatus	nd	nd	2	nd
B. urceolaris	nd	nd	1	9
Filinia camascela	nd	nd	nd	nd
F. longiseta	nd	1	6	4
F. opolienesis	nd	nd	1	nd
F. terminalis	nd	1	1	4
Hexartha intermedia	nd	1	nd	2
Keratella sp.	5	nd	nd	nd
K. tecta	nd	nd	11	16
K. tropica	nd	1	1	nd
Lecane luna	nd	1	nd	nd
Monommata sp.	nd	nd	1	nd
Myersinella sp.	nd	1	nd	nd
Mytilina mucronata	nd	3	nd	nd
Platyias patulus	nd	1	1	nd
P. quadricornis	nd	nd	1	nd
Polyarthra sp.	nd	1	1	1
P. vulgaris	nd	nd	2	nd
Rotaria sp.	1	1	3	nd
Testudinella sp.	nd	4	2	nd
Trichocerca similis	nd	nd	nd	5
Unidentified rotifera	nd	nd	2	nd
Site-2				
Asplanchna sp.	4	nd	9	nd
A. priodonta	nd	1	2	nd
Brachionus sp.	1	nd	2	nd
B. angularis	5	8	3	9
B. calcyflorus	nd	8	nd	nd
B. caudatus	2	nd	3	nd
B. diversicornis	nd	nd	nd	4

	Summer	Rainy Season	Autumn	Winter
B. falcatus	nd	nd	2	nd
B. forficula	nd	nd	2	nd
B. quadridentatus	nd	nd	5	nd
Filinia sp.	1	nd	2	nd
F. camascela	nd	nd	3	nd
F. longiseta	nd	nd	2	nd
Filinia opolienesis	nd	nd	2	nd
F. terminalis	nd	2	3	nd
Hexartha intermedia	nd	nd	nd	nd
Keratella sp.	1	nd	nd	23
K. cochlearis	7	10	4	7
K. tecta	2	nd	2	20
K. tropica	1	11	3	nd
Lecane sp.	nd	1	nd	nd
Lepadella sp.	nd	1	nd	nd
Mytilina mucronata	nd	2	nd	nd
Platyias patulus	nd	nd	2	nd
P. quadricornis	nd	nd	7	nd
Polyarthra sp.	4	nd	nd	nd
P. vulgaris	3	3	4	nd
Rotaria sp.	nd	1	8	nd
Testudinella sp.	nd	2	nd	nd
Trichocerca cylindrica	nd	nd	2	nd
Tricocerca similis	nd	nd	2	nd
Site-4				
Asplanchna sp.	2	1	13	2
A. priodonta	nd	nd	2	nd
Brachionus sp.	6	3	1	25
B.angularis	9	15	11	10
B.bidentata	3	nd	nd	nd
B. calcyflorus	3	2	12	12
B.caudatus	49	35	18	nd
B. diversicornis	1	nd	1	4
B. falcatus	8	1	4	nd
B. forficula	nd	2	nd	nd
B. nilsoni	nd	4	5	nd
B. quadridentatus	1	nd	nd	15
B. urceolaris	nd	nd	4	19
Filinia sp.	1	1	1	nd
F. longiseta	1	nd	1	nd
F. terminalis	9	nd	nd	5
Hexartha intermedia	nd	nd	nd	5
Keratella sp.	1	nd	nd	nd
K. cochlearis	nd	nd	1	nd

	Summer	Rainy Season	Autumn	Winter
K. tecta	nd	nd	1	nd
K. tropica	nd	nd	2	nd
Lecane luna	1	2	nd	nd
Lepadella sp.	nd	1	1	nd
L. imbricata	nd	nd	1	nd
Monostyla bula	nd	4	1	nd
P. polyacanthus	nd	nd	1	nd
Polyarthra sp.	1	1	nd	4
P. vulgaris	1	4	14	3
Rotaria sp.	3	2	4	14
R. citrinus	nd	nd	3	nd
R. neptunia	3	nd	nd	nd
Testudinella sp.	nd	nd	2	nd
T. mucronata	16	nd	nd	nd
T. patina	nd	nd	1	nd
Trichocerca cylindrica	nd	7	2	nd
T. similis	nd	nd	1	6
Unidentified rotifera	nd	nd	2	3
Site-9				
Asplanchna priodonta	nd	nd	1	1
Brachionus sp.	20	1	1	2
B. angularis	6	1	2	1
B. calcyflorus	nd	nd	2	nd
B. caudatus	7	2	4	1
B. diversicornis	6	nd	1	nd
B. donneri	nd	nd	1	nd
B. falcatus	2	4	3	nd
B. forficula	2	1	1	nd
B. havanensis	nd	nd	1	nd
B. nilsoni	44	nd	nd	nd
B. quadridentatus	4	nd	nd	3
B. urceolaris	nd	6	nd	nd
Filinia camascela	nd	10	2	nd
F. longiseta	nd	nd	1	nd
F. terminalis	nd	11	1	nd
Keratella sp.	4	nd	nd	nd
K. cochlearis	nd	1	7	1
K. edmondsoni	nd	2	nd	nd
K. procurva	nd	1	nd	nd
K. tecta	nd	nd	6	1
K. tropica	1	1	6	nd
Lecane luna	nd	1	7	nd
Lepadella sp.	nd	nd	1	nd
L. imbricata	nd	nd	1	nd

	Summer	Rainy Season	Autumn	Winter
Monostyla bula	nd	nd	1	nd
Platyias patulus	nd	nd	1	1
P. quadricornis	nd	nd	1	nd
Polyarthra sp.	nd	nd	4	nd
P. multiappendiculata	nd	nd	1	nd
P. vulgaris	nd	nd	2	nd
Pompholyx sulcata	nd	1	nd	nd
Rotaria sp.	nd	nd	2	nd
Testudinella sp.	nd	nd	1	nd
Trichocerca cylindrica	nd	nd	2	nd
T. similis	nd	nd	2	nd
Unidentified rotifera	4	nd	1	nd
Site-10				
Anuraeopsis sp.	nd	nd	nd	3
Brachionus sp.	2	2	nd	13
B. angularis	nd	2	2	4
B. calcyflorus	nd	nd	nd	nd
B. caudatus	2	nd	2	nd
B. diversicornis	nd	nd	1	nd
B. falcatus	nd	2	1	nd
B. forficula	nd	9	nd	nd
B. quadridentatus	nd	nd	2	nd
Filinia camascela	nd	2	2	nd
F. longiseta	nd	nd	2	nd
F. terminalis	nd	nd	2	nd
Hexartha intermedia	nd	nd	5	4
Keratella sp.	nd	nd	nd	21
K. cochlearis	1	3	3	36
K. tecta	1	nd	2	8
K. tropica	11	4	3	4
Lecane sp.	nd	nd	2	nd
Lepadella imbricata	nd	nd	nd	5
Platyias quadricornis	nd	nd	3	nd
Polyarthra sp.	nd	nd	2	2
P. vulgaris	8	nd	3	4
Pompholyx sulcata	nd	2	nd	nd
Testudinella sp.	nd	nd	nd	2
Trichocerca longiseta	nd	nd	3	nd
T. similis	nd	2	6	3
Unidentified rotifer	nd	nd	2	2
Site-11				
Asplanchna sp.	nd	nd	2	nd
A. priodonta	nd	8	3	28
Brachionus sp.	nd	40	7	1

	Summer	Rainy Season	Autumn	Winter
B. angularis	9	3	3	1
B. calcyflorus	1	nd	3	1
B. caudatus	1	1	1	nd
B. diversicornis	nd	nd	2	nd
B. donneri	nd	nd	1	nd
B. forficula	nd	5	6	nd
B. falcatus	1	nd	2	1
B. quadridentatus	1	nd	1	1
Euclanis dilata	1	14	nd	nd
Filinia sp.	nd	1	1	10
F. camascela	nd	nd	1	1
F. longiseta	nd	nd	9	2
F. terminalis	1	nd	6	2
Hexartha intermedia	nd	2	1	nd
Keratella sp.	nd	nd	1	nd
K. cochlearis	nd	nd	2	nd
K. tecta	nd	nd	3	nd
K. tropica	1	3	1	nd
Lecane sp.	1	nd	nd	nd
L. halychysta	2	nd	nd	nd
L. luna	1	12	2	nd
Lepadella imbricata	nd	6	1	nd
Monostyla sp.	nd	2	nd	nd
M. bula	3	nd	1	nd
M. hamata	1	nd	nd	nd
M. sinuata	1	nd	nd	nd
Myersinella mucronata	nd	nd	3	nd
Monommata sp.	nd	nd	5	nd
Platyias patulus	nd	3	1	nd
P. quadricornis	nd	nd	1	nd
Polyarthra vulgaris	nd	nd	1	nd
Pompholyx sulcata	nd	nd	nd	4
Rotaria sp.	1	1	1	nd
Testudinella sp.	3	nd	3	nd
Tricocerca cylindrica	nd	nd	1	nd
Unidentified rotifer	1	nd	4	nd
Site-12				
Asplanchna sp.	1	nd	2	nd
A. priodonta	nd	nd	1.5	nd
Brachionus sp.	1	nd	1	54
B. angularis	12	1	3	3
B. calcyflorus	nd	nd	1	nd
B. caudatus	7	nd	2	nd
B. falcatus	nd	1	2	nd

	Summer	Rainy Season	Autumn	Winter
B. nilsoni	nd	1	2	nd
B. urceolaris	nd	nd	3	nd
Filinia sp.	1	nd	nd	nd
F. camascela	nd	nd	1	nd
F. longiseta	1	nd	1	nd
F. opolienesis	nd	2	1	nd
F. terminalis	3	nd	1	nd
Hexartha intermedia	nd	nd	31	1
Keratella sp.	nd	nd	nd	1
K. cochlearis	5	nd	2	5
K. tecta	1	nd	1	3
K. tropica	16	4	3	48
Lecane luna	nd	1	1	nd
Lepadella imbricata	1	1	nd	nd
Platyias patulus	nd	3	nd	nd
P. quadricornis	nd	nd	1	nd
Polyarthra sp.	nd	nd	1	1
P. multiappendiculata	nd	nd	1	nd
P. vulgaris	1	nd	3	nd
Pompholyx sulcata	nd	nd	nd	33
Rotaria sp.	3	nd	2	nd
Testudinella sp.	1	nd	nd	nd
Tricocerca cylindrica	nd	nd	1	nd
T. similis	nd	nd	1	nd
Unidentified rotifer	nd	nd	4	6

nd= Not detected

Copepoda

Different species of Cyclops among copepods were abundant in Chhatak. *Diaptomus* sp. was recorded just before the arrival of peak season (autumn) wheres in winter some cylops copepods were observed in some water bodies (Table 34).

Table 34. Relative abundance of Copepoda at different sites of Chhatak according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-1				
Cyclops sp.	1	7	5	15
C. nanus	2	nd	1	26
C. vernalis	6	2	7	20
Diaptomus sp.	3	nd	2	nd
Mesocyclops sp.	18	nd	1	2
Unidentified copepods	1	8	2	5
Calanoid Copepods	1	nd	1	nd
Site-2				
Cyclops sp.	11	7	3	6
C. nanus	33	6	nd	9
C. vernalis	2	3	nd	7
Diaptomus sp.	2	nd	5	24
D. gracilis	4	nd	2	nd
Unidentified copepods	4	7	nd	nd
Calanoid Copepods	nd	nd	nd	4
Site-4				
Cyclops sp.	1	1	2	nd
C. nanus	1	2	1	nd
C. vernalis	1	2	3	nd
Diaptomus sp.	1	1	nd	nd
Mesocyclops sp.	1	1	1	nd
Unidentified copepods	6	4	1	6
Calanoid Copepods	1	nd	nd	nd
Site-9				
Cyclops sp.	nd	2	4	16
C. nanus	nd	3	2	1
C. vernalis	nd	20	1	1
Diaptomus sp.	nd	1	3	28
D. gracilis	nd	nd	1	1
Mesocyclops sp.	nd	nd	1	nd
Unidentified copepods	nd	1	2	10
Calanoid Copepods	nd	1	nd	nd
Site-10				
Cyclops sp.	3	nd	5	2
C. nanus	16	5	2	2
C. vernalis	5	3	5	14
C. vicinis	3	nd	nd	nd
Diaptomus sp.	4	nd	7	2
D. gracilis	nd	nd	3	nd
Mesocyclops sp.	1	nd	nd	nd
Unidentified copepods	1	nd	4	nd

	Summer	Rainy Season	Autumn	Winter
Site-11				
Cyclops sp.	5	nd	4	28
C. nanus	29	nd	1	4
C. vernalis	18	nd	7	6
C. vicinis	1	nd	nd	nd
Diaptomus sp.	7	nd	4	nd
Mesocyclops sp.	1	nd	1	1
Unidentified copepods	nd	11	1	3
Site-12				
Cyclops sp.	8	nd	3	1
C. nanus	10	3	3	1
C. vernalis	1	4	5	2
Diaptomus sp.	26	nd	1	1
D. gracilis	nd	nd	2	nd
Mesocyclops sp.	nd	nd	2	nd
Unidentified copepods	nd	nd	1	7
Calanoid copepoda	nd	nd	nd	2

nd= Not detected

Cladocera

In Chhatak *Diaphanosoma sp.* was the specified cladoeran species that was found frequently in almost all. Sometimes *Bosmina sp.* (*Bosmina coregoni and Bosmina longirostris*) and *Ceriodaphnia sp*. also found in autumn season (Table 35).

Table 35. Relative abundance of Cladocera at different sites of Chhatak according to four seasons during two years of study

	Summer	Rainy Season	Autumn	Winter
Site-1				
Bosmina sp.	nd	nd	1	nd
Ceriodaphnia sp.	nd	9	7	nd
Chydorus sp.	nd	nd	2	nd
Diaphanosoma sp.	7	11	3	7
Macrothrix sp.	nd	nd	16	nd
Moina sp.	nd	2	1	nd
M. brachiata	nd	nd	2	nd
Simocephalus sp.	nd	nd	1	nd
Cladocerans	1	1	2	nd
Site-2				
Bosmina sp.	1	nd	4	4

	Summer	Rainy Season	Autumn	Winter
B. coregoni	nd	nd	10	8
B. longirostris	4	nd	3	nd
Ceriodaphnia sp.	nd	nd	5	nd
Chydorus sp.	nd	nd	nd	nd
Daphnia sp.	2	nd	nd	4
Diaphanosoma sp.	3	7	3	2
Simocephalus sp.	1	nd	nd	nd
Cladocerans	nd	2	nd	3.8
Site-4				
Ceriodaphnia sp.	nd	nd	4	nd
Chydorus sp.	1	3	nd	nd
Diaphanosoma sp.	12	5	4	15
Kurzia latissima	nd	nd	1	nd
Scaphaloberis kingi	nd	2	nd	nd
Simocephalus sp.	nd	1	1	nd
Cladocerans	nd	5	3	nd
Site-9				
Bosmina coregoni	nd	7	1	nd
Bosmina longirostris	nd	nd	1	nd
Ceriodaphnia sp.	nd	nd	1	nd
Ceriodaphnia pulchella	nd	1	nd	nd
Chydorus sp.	nd	nd	1	nd
Daphnia magna	nd	nd	1	nd
Diaphanosoma sp.	nd	2	4	3
Moina sp.	nd	2	1	nd
Simocephalus sp.	nd	nd	1.5	nd
Cladocerans	nd	1	1	nd
Site-10				
Bosmina sp.	2	nd	4	nd
B. coregoni	nd	5	3	8
B. longirostris	1	nd	3	nd
Ceriodaphnia sp.	nd	nd	6	4
Chydorus sp.	nd	nd	3	2
Daphnia sp.	1	nd	nd	7
Diaphanosoma sp.	1	2	2	3
Kurzia latissima	nd	nd	1	nd
Pseudosida bidentata	nd	nd	2	nd
Moina sp.	0.8	nd	nd	nd
Simocephalus sp.	1	1	nd	nd
Cladocerans	4	1	7	nd
Site-11				
Bosmina sp.	nd	nd	2	1
Ceriodaphnia sp.	nd	9	5	nd
C. laticaudata	nd	nd	2	nd
C. pulchella	nd	nd	1	nd

	Summer	Rainy Season	Autumn	Winter
Chydorus sp.	nd	8	2	nd
Daphnia sp.	nd	3	nd	nd
D. magna	nd	nd	2	nd
Diaphanosoma sp.	2	5	6	1
Moina sp.	1	4	2	1
M. brachiata	nd	nd	1	nd
Simocephalus sp.	nd	nd	4	nd
Cladocerans	nd	3	2	nd
Site-12				
Bosmina sp.	nd	nd	1	nd
Bosmina coregoni	nd	nd	1	nd
Ceriodaphnia sp.	nd	nd	3	nd
C. pulchella	nd	1	nd	nd
Chydorus sp.	nd	nd	1	nd
Daphnia lumholtzi	nd	nd	nd	1
D. similis	nd	nd	nd	10
Diaphanosoma sp.	16	1	3	1
Moina sp.	nd	nd	1	nd
Pseudosida bidentata	nd	nd	3	nd
Simocephalus sp.	nd	nd	nd	nd
Cladocerans	1	nd	1	nd

nd= Not detected

4.1.2.7 Zooplankton community structure in Chhatak

A. Diversity Indices:

In Chhatak, three indices were applied to estimate the species diversity, specie richness and species evenness according to different seasonal environment in our country.

i) Simpson's Diversity Index:

In summer, the value of index ranges between (0.1618-0.9655) where minimum value was in site -10 (0.1618) which indicates highest diversity and maximum was in site-9 (0.9655) indication of lowest biodiversity (Table 36).

In rainy season, the value of index ranges between (0.07287-0.6471) where minimum value was in site -2 (0.07287) and maximum was in site-11 (0.6471). Higher diversity found in site-2 than other sites (Table 37).

In autumn, the value of index ranges between (0.2238-3.195) where maximum value was in site -1 (3.195) and minimum was in site-11 (0.2238). So, diversity was high in site-11 (Table 38).

In winter, the value of index ranges between (0.07683-0.8712) where maximum value was in site -9 (0.8712) and minimum was in site-10 (0.07683). That means plankton diversity was high in site-10 (Table 39).

ii) Shannon-Weiner Diversity Index:

In summer, the value of index ranges between (0.1169-2.308) where maximum value was in site -10 (2.308) and minimum was in site-9 (0.1169). Diversity was high in site- 10 (Table 36).

In rainy season, the value of index ranges between (0.8903-2.819) where maximum value was in site -2 (2.819) and minimum was in site-1 (0.8903). Higher diversity was in site-2 (Table 37).

In autumn, the value of index ranges between (0.9269-1.979) where maximum value was in site-11 (1.979) and minimum was in site-9 (0.9269). So, zooplankton diversity was high in site-11 (Table 38).

In winter, the value of index ranges between (0.4031-2.859) where maximum value was in site -10 (2.859) and minimum was in site-9 (0.4031) which indicates the higher diversity in site-10 (Table 39).

iii) Species Richness:

In both types of richness index maximum value for Menhinick's index was (0.1542) and for Margalef's index was (3.278). Highest species richness was shown in site-10 in summer (Table 36).

In rainy season, site- 2 was rich in species and the index was high for Menhinick's index (0.2663) and (3.379) for Margalef's index (Table 37).

Site-2 in Chhatak was rich in species and the value was (0.1917) and (3.879) for Menhinick's and Margalef's index respectively in autumn season (Table 38).

Table 36.Diversity Indices of Zooplankton in Summer

Diversity Indices	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Simpson's Index	0.963	0.3175	0.6378	0.9655	**0.1618**	0.1901	0.2894
Shannon Index	0.1323	1.644	0.9152	0.1169	**2.308**	2.192	1.912
Menhinick's Index	0.0213	0.1309	0.0541	0.01901	**0.1542**	0.1185	0.12
Margalef's Index	1.451	2.744	2.428	1.028	**3.278**	2.278	2.626
Species Evenness	0.1001	1.103	0.608	0.078	**1.672**	1.377	1.294

Table 37.Diversity Indices of Zooplankton in Rainy Season

Diversity Indices	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Simpson's Index	.5582	**.07287**	.303	.4094	.4886	.6471	.1534
Shannon Index	.8903	**2.819**	1.493	1.073	1.155	1.05	2.636
Menhinick's Index	.03493	**.2663**	.07352	.02896	.09933	.09589	.2104
Margalef's Index	2.083	**3.379**	2.482	2.037	1.635	2.451	3.085
Species Evenness	.609	**2.1005**	1.001	.674	.92	.718	1.751

Table 38.Diversity Indices of Zooplankton in Autumn

Diversity Indices	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Simpson's Index	.3195	.3316	.4804	.6789	.3382	**.2238**	.3295
Shannon Index	1.423	1.708	.9549	.9269	1.662	**1.979**	1.793
Menhinick's Index	.05331	**.1917**	.0673	.02966	.1726	.1404	.0492
Margalef's Index	3.705	**3.879**	3.641	3.132	3.731	3.801	3.662
Species Evenness	.829	1.046	.565	.519	1.024	**1.117**	1.045

Table 39. Diversity Indices of Zooplankton in Winter

Diversity Indices	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
Simpson's Index	.106	.08953	.8594	.8712	**.07683**	.1381	.1809
Shannon Index	2.484	2.674	.458	.4031	**2.859**	2.346	2.161
Menhinick's Index	.1273	.2556	.0572	.04918	**.2916**	.1385	.1136
Margalef's Index	1.798	2.089	1.622	1.581	**2.696**	2.231	2.071
Species Evenness	1.944	**2.092**	0.352	0.309	2.045	1.7	1.586

In winter site-10 also had maximum richness of species with (0.2916) for Menhinick's index and (2.696) for Margalef's index (Table 39).

iv) Species Evenness

In summer, zooplankton species evenness was found to be high (1.672) in site-10 (Table 36).

Inrainy season, species evenness was maximum (2.1005) in site-2 (Table 37).

In autumn, highest value (1.117) of evenness was in site-11(Table 38).

In winter, maximum value (2.092) of species evenness was found in site-2 (Table 39).

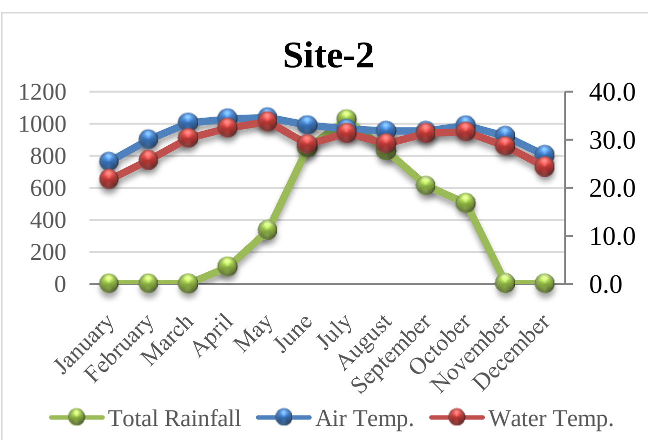
4.2 Climatic Factors and It's Relationships with Pond's Limnological Dynamics

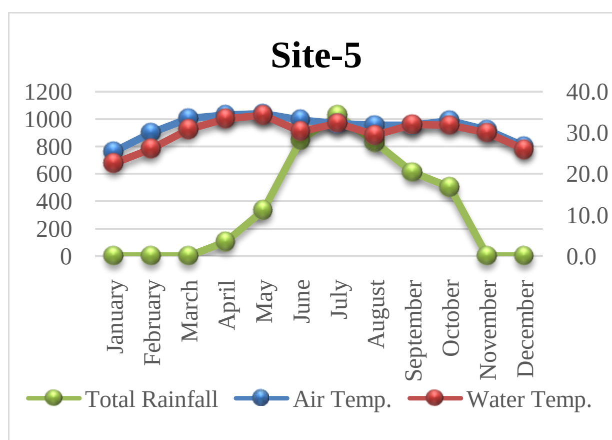
Previous limnological data of the studied ponds inMathbaria and Chhatak during 2010-2012 were analyzed to show the effects of total rainfall at different sites.

4.2.1 Limnological dynamics of ponds in Mathbaria

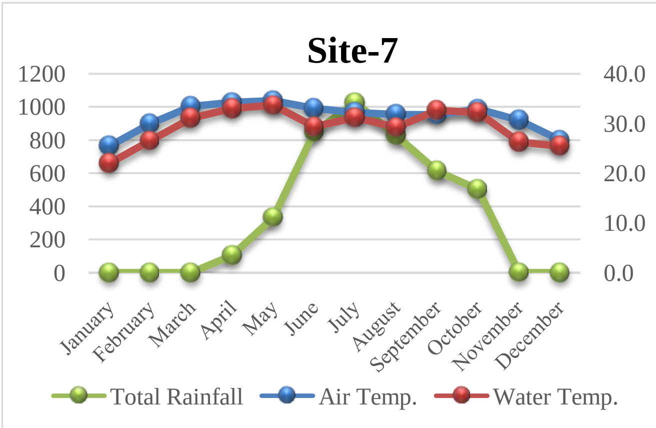
4.2.1.1 Interrelationsips of air and water temperature with rainfall

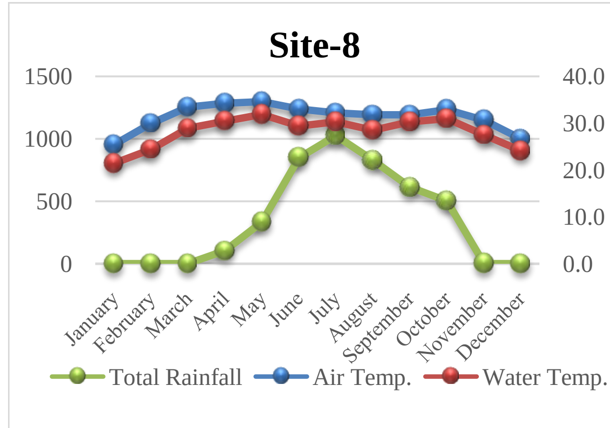
On the onset of summer season (March-May) air and water temperature started to raise when the amount of precipitation or rainfall was lower. During rainy season (June-August) heavy rainfall occurred and at the same time air temperature became lower at most of the ponds than the other seasons. Wheares, water temperature of the studied ponds were also high at site-2, site-5 and site-7 in comparison to air temperature.





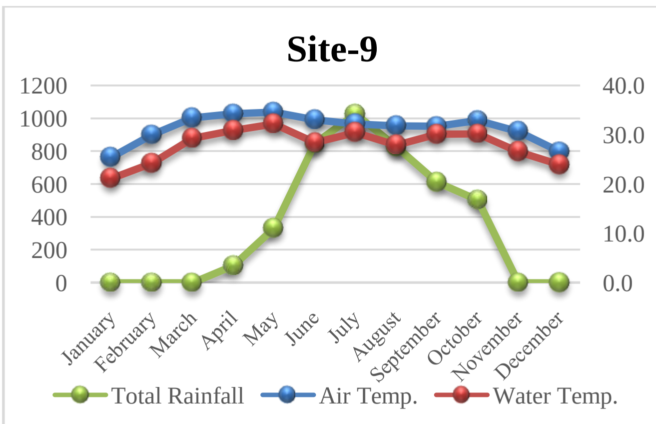
A **B**

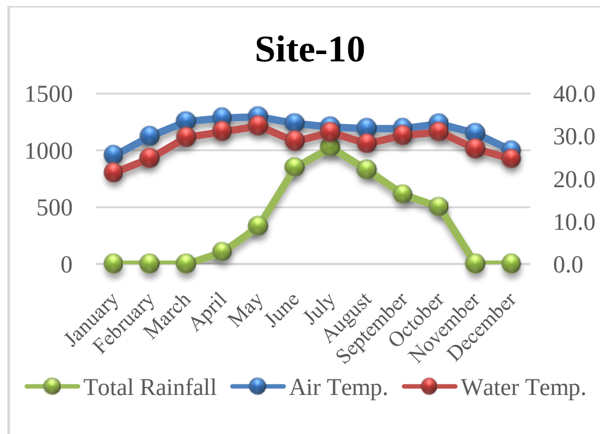




C

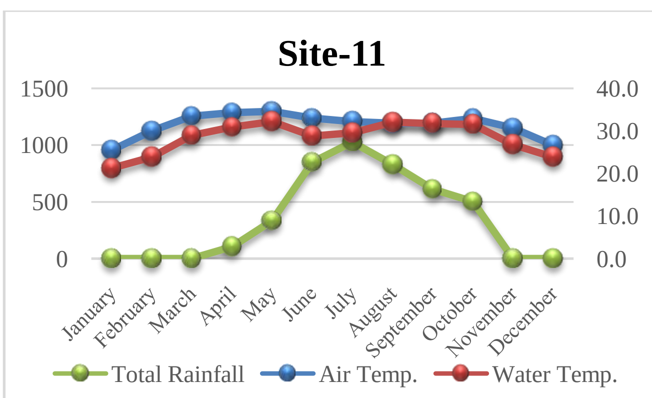
D





E

F



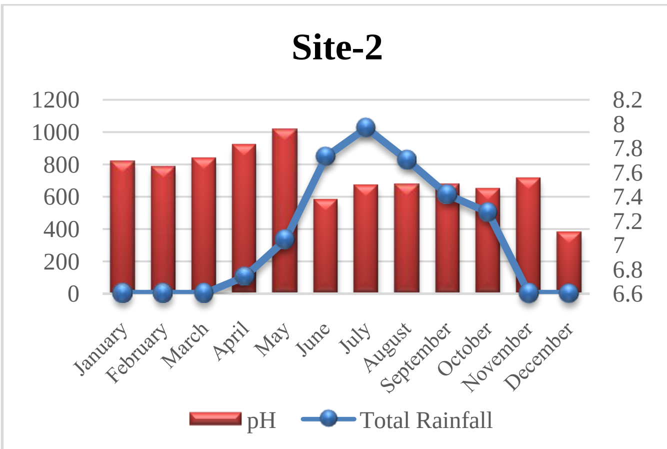
G

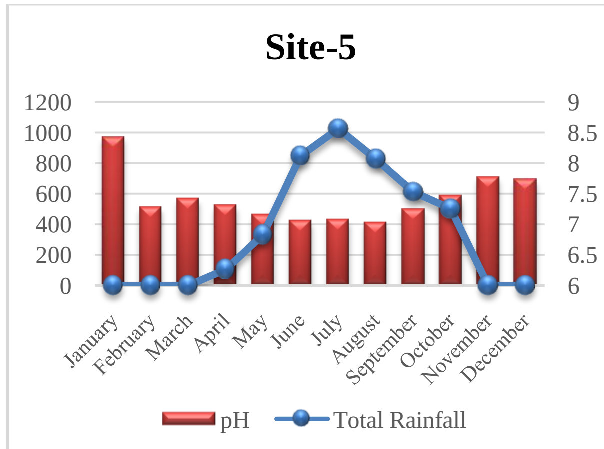
Figure 16. Interrelationships among air and water temperature and total rainfall in Mathbaria

ponds

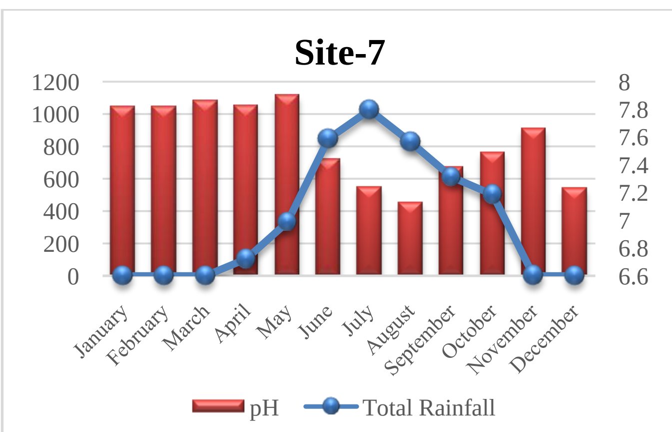
4.2.1.2 Total Rainfall and pH at Mathbaria ponds

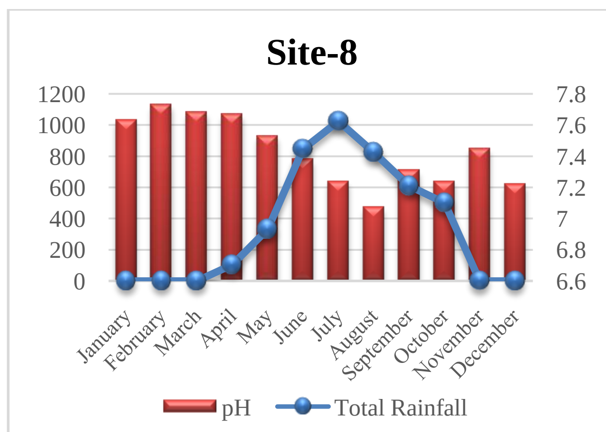
pH in most of the ponds were high during peak season of cholea with a few exception at site-5. During heavy rainfall pH became lower during monsoon season but at selected ponds (site-9 and site-11) higher range of pH was recorded.



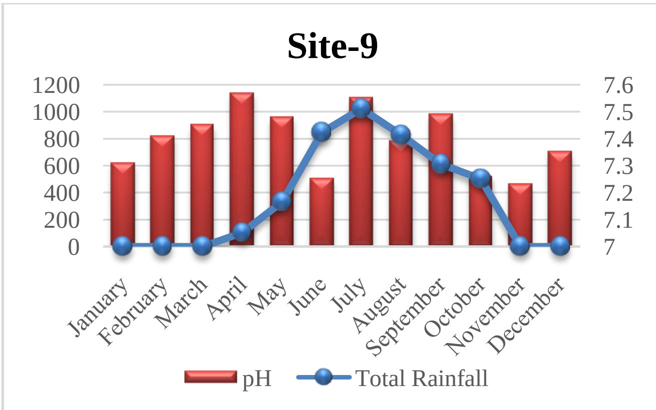


A **B**

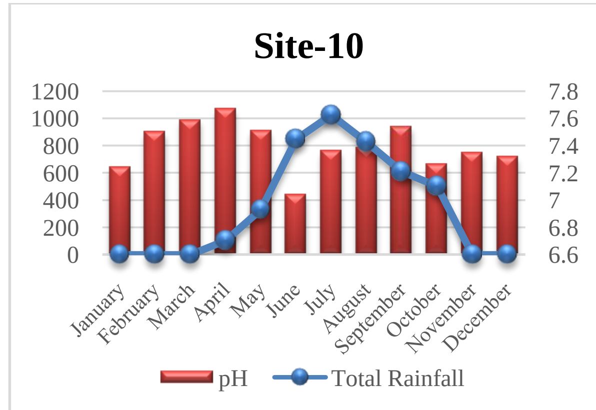




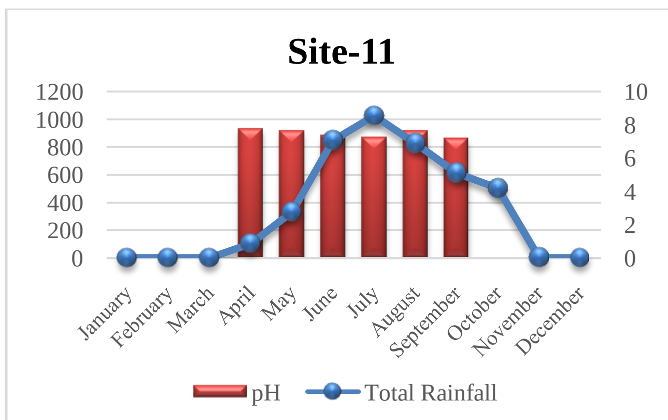
C **D**



E



F

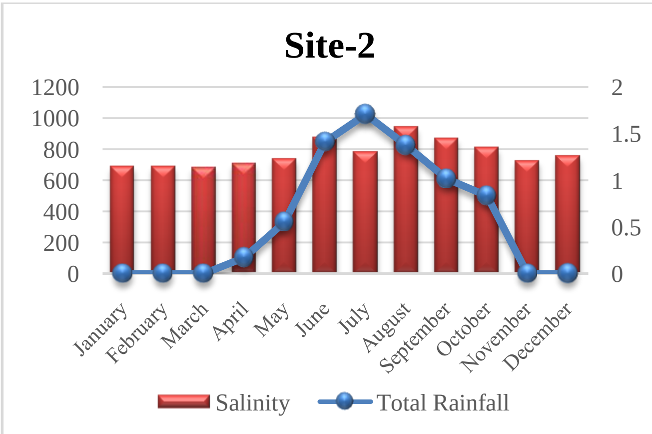


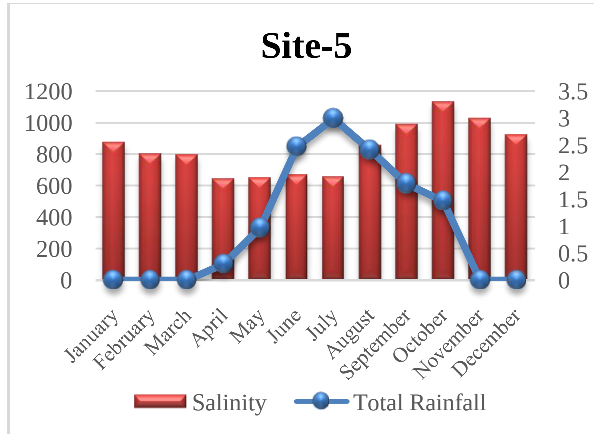
G

Figure 17. pH of seven Mathbaria ponds and it's realationships with rainfall of that region

4.2.1.3 Total rainfall and it's relation with salinity

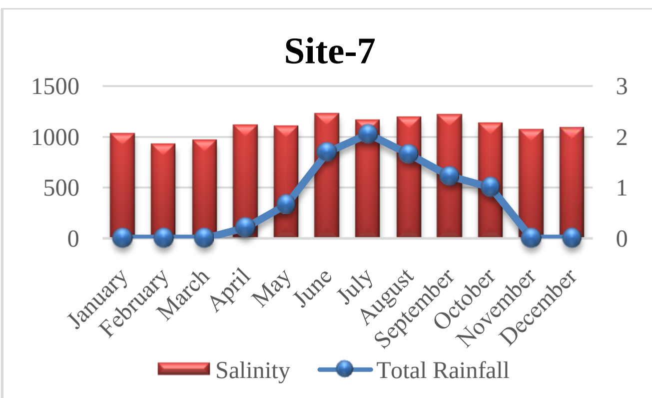
Salinity in most of the ponds (site-2, site-5, site-9, site-10, site-11) in Mathbaria was recorded to be lower during summer (March-May) than the other seasons. At site-7 exceptionally high salinity was recorded but there was no salinity at site-8 throughout the year.

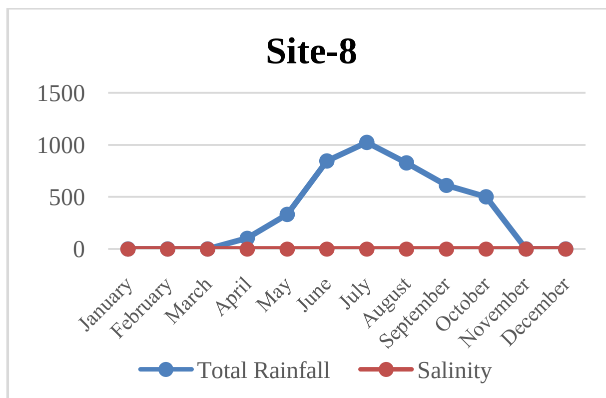

Site-2
1200
1000
800
600
400
200
0
2
1.5
1
0.5
0
January
February
March
April
May
June
July
August
September
October
November
December
Salinity
Total Rainfall


Site-5
1200
1000
800
600
400
200
0
3.5
3
2.5
2
1.5
1
0.5
0
January
February
March
April
May
June
July
August
September
October
November
December
Salinity
Total Rainfall

A

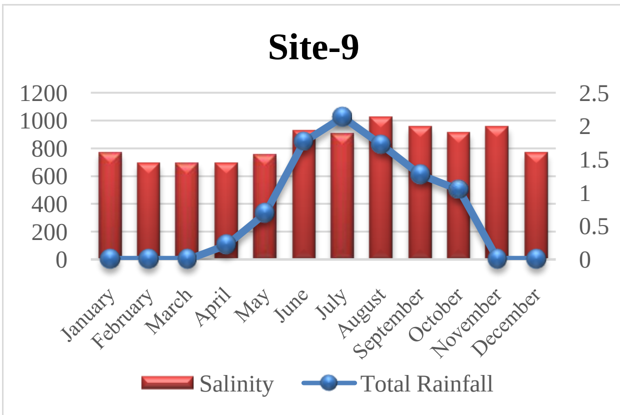
B


Site-7
1500
1000
500
0
3
2
1
0
January
February
March
April
May
June
July
August
September
October
November
December
Salinity
Total Rainfall

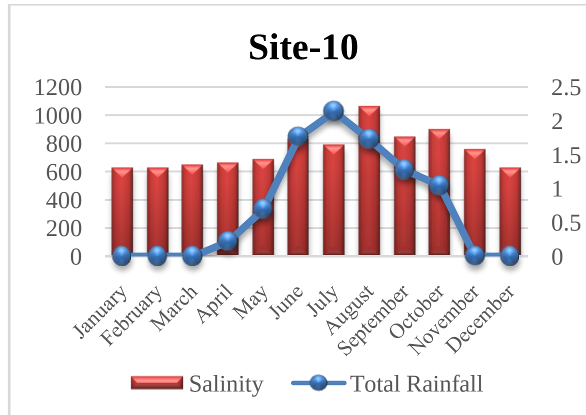

Site-8
1500
1000
500
0
January
February
March
April
May
June
July
August
September
October
November
December
Total Rainfall
Salinity

C

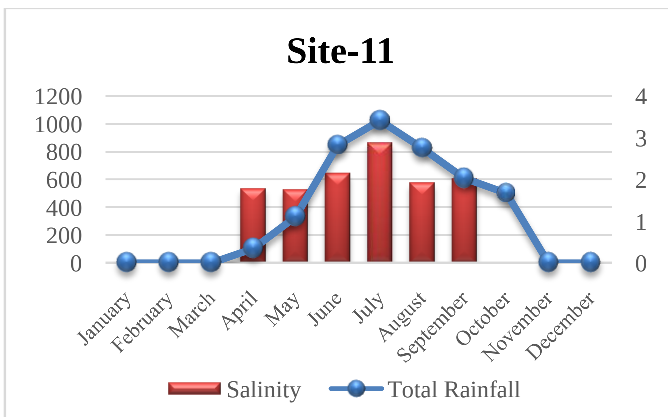
D



E



F



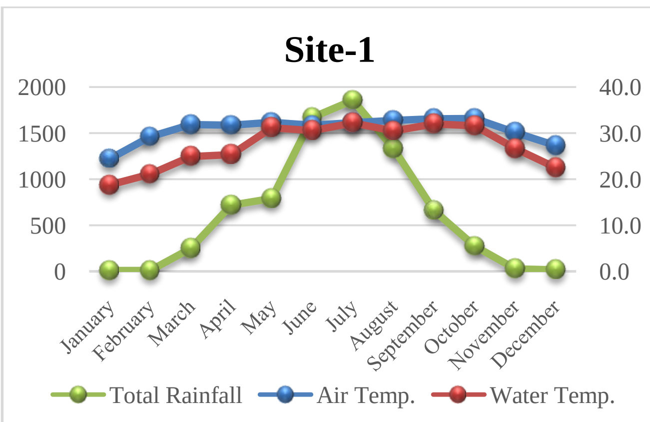
G

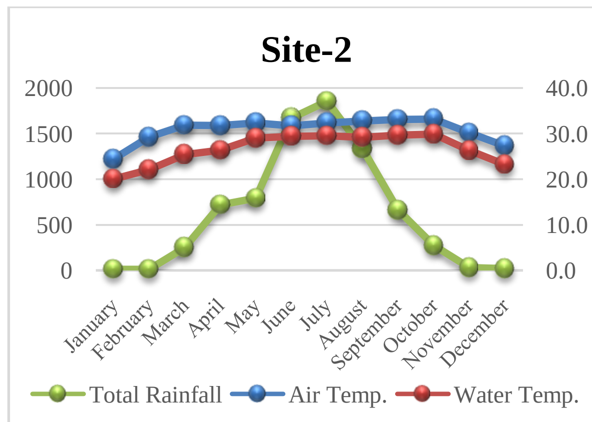
Figure 18. Salinity of seven Mathbaria ponds and it's realationships with rainfall of that region

4.2.2 Limnological dynamics of ponds in Chhatak

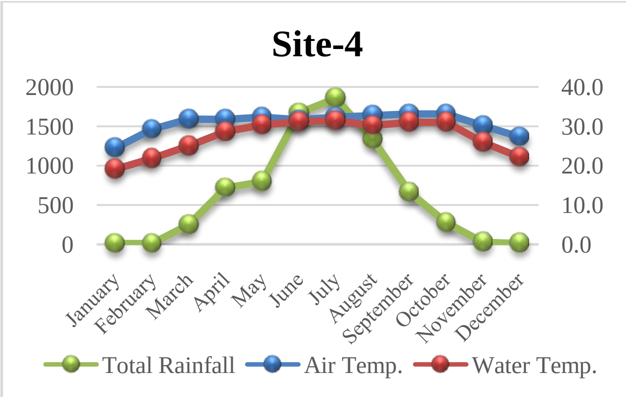
4.2.2.1 Interrelationsips of air and water temperature with rainfall

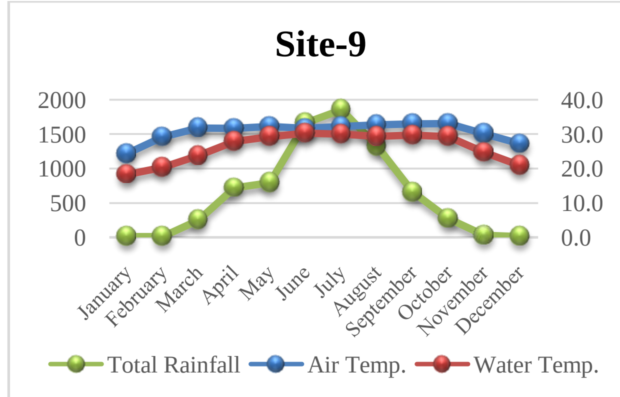
In Chhatak, peak season of cholera was (September-November) when air temperature of that region and water temperature of the studied ponds were maximum and then became decrease. Precipitation or rainfall of that region at the same time was lower and maximum amount of rainfall was recorded during Rainy season (June-August).





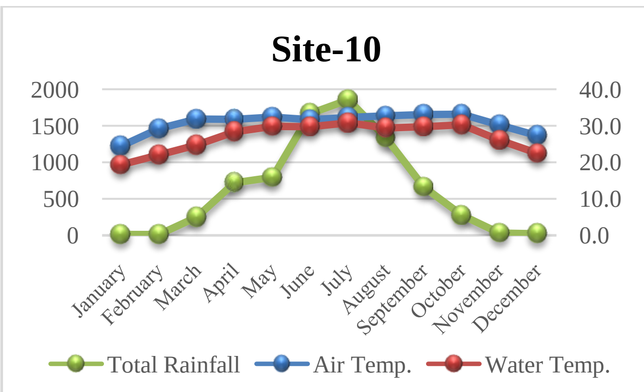
A **B**

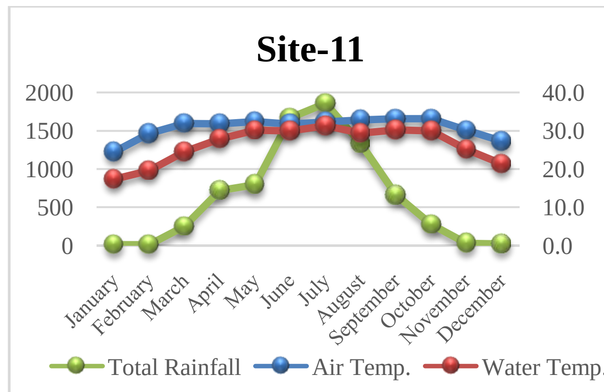




C

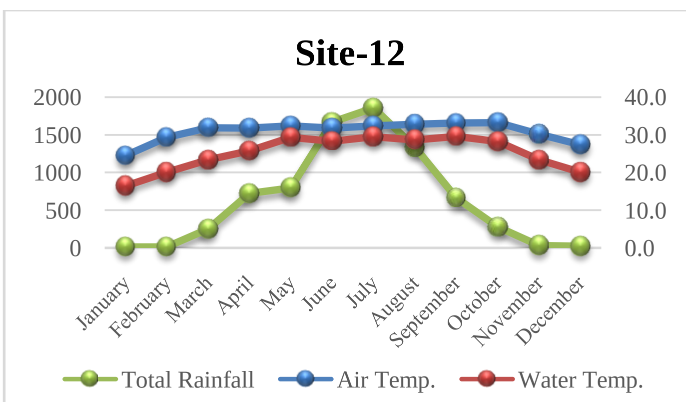
D





E

F

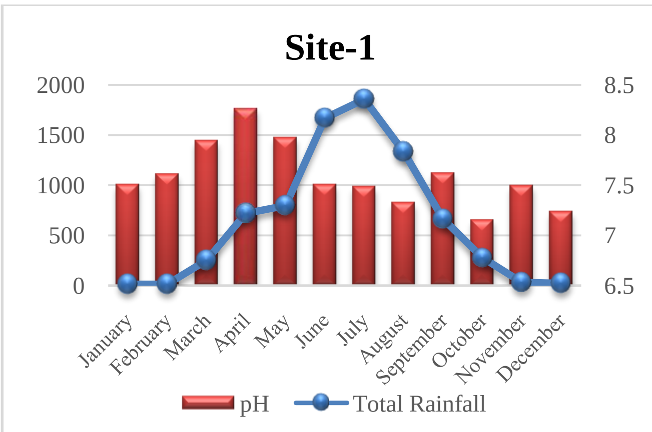


G

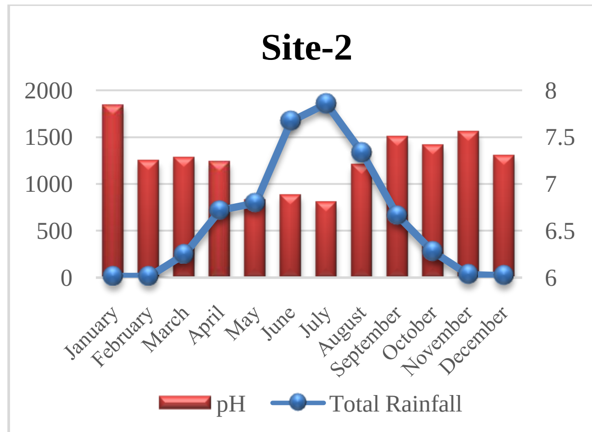
Figure 19. Interrelationships of air and water temperature with rainfall in seven Chhatak ponds

4.2.2.2 Total rainfall and pH at Chhatak ponds

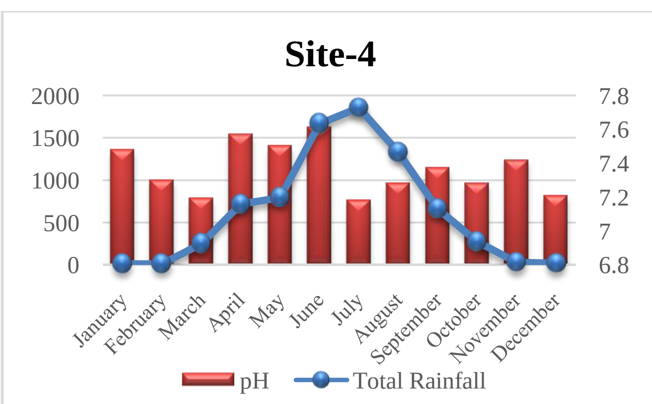
During peak season of cholera pH was lower in Chhatak ponds (site-1, site-4, site-9 and site-11). During heavy rainfall pH became lower during monsoon season but at selected ponds (site-9 and site-10) higher range of pH was recorded. During heavy rainfall pH of the studied ponds was lowest exceptionally a different case at site-11.



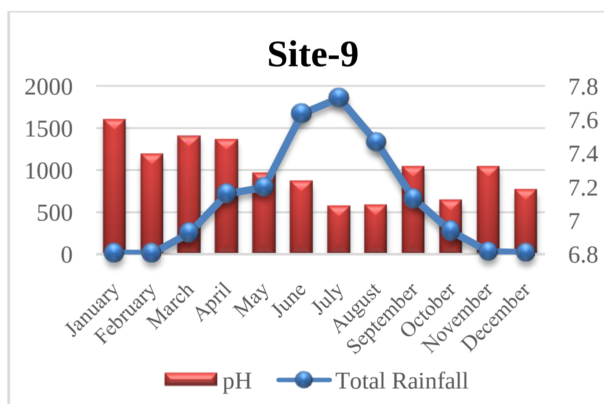
A



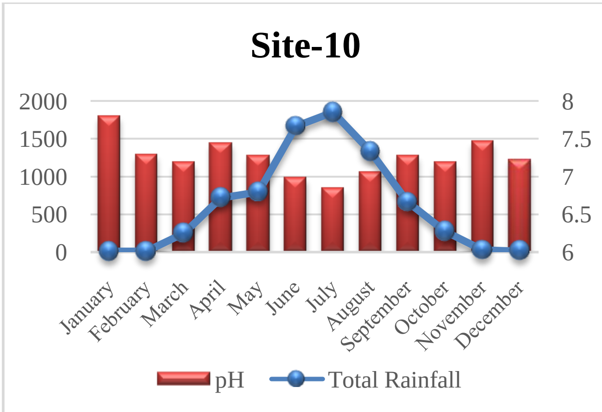
B



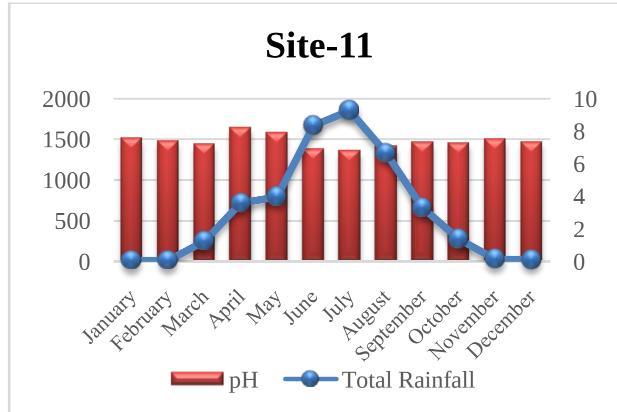
C



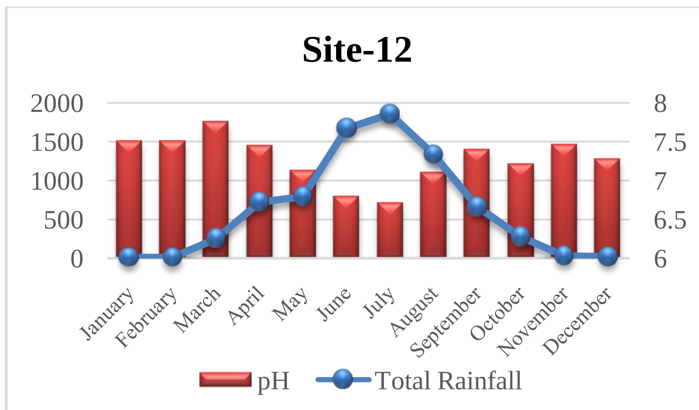
D



E



F

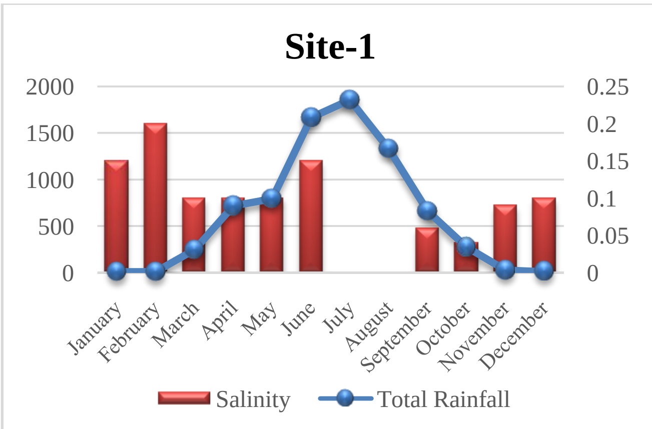


G

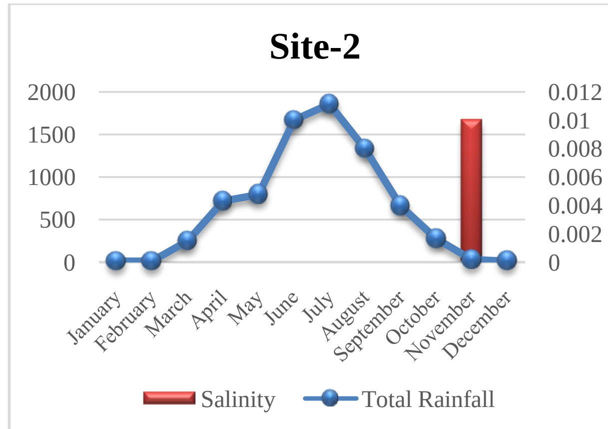
Figure 20. pH of seven Chhatak ponds and it's realationships with rainfall of that region

4.2.2.3 Total rainfall and it's relation with salinity

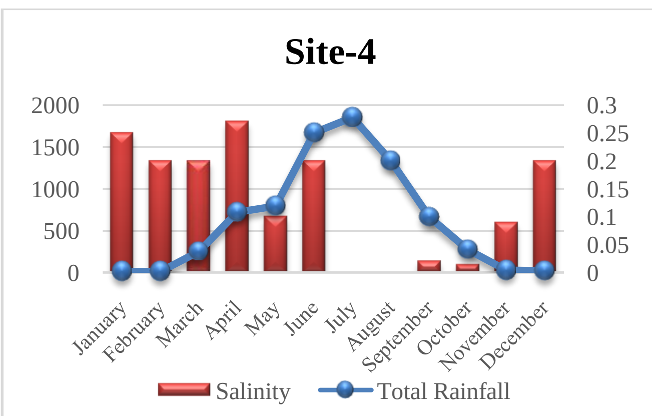
Maximum salinity was reorded during the peak season of cholera at site-2 and site-10.Whereas, during rainy season minimum or no salinity recorded in most of the ponds of Chhatak.



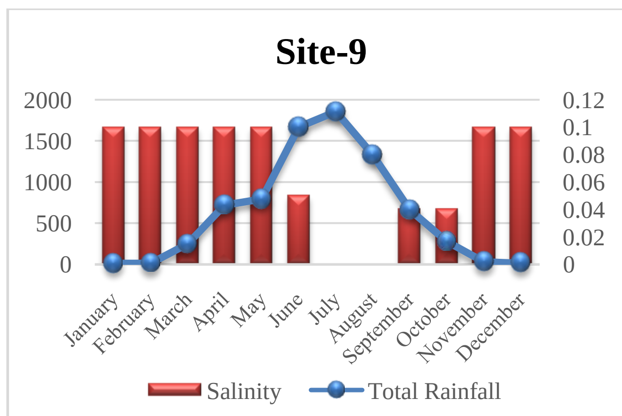
A



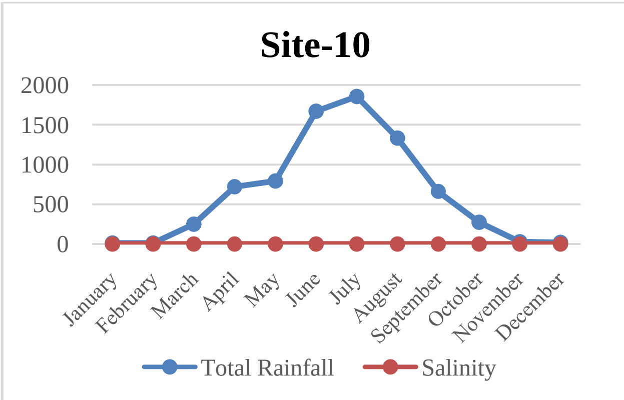
B

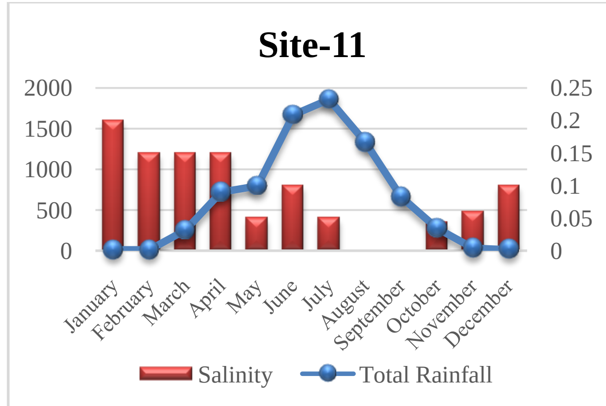


C

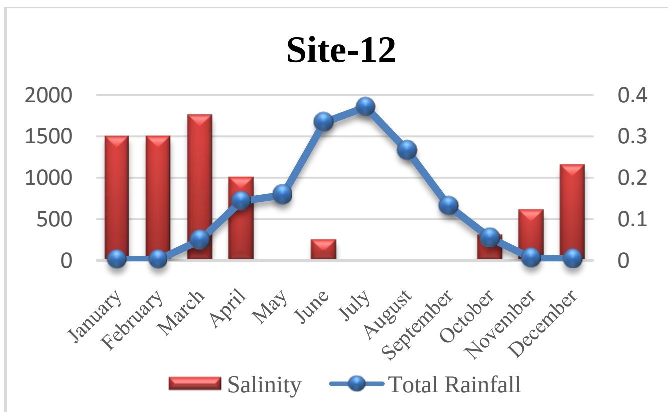


D





E **F**



G

Figure 21. Salinity of seven Chhatak ponds and it's realationships with rainfall of that region

4.3 Estimating Zooplankton Species of *Vibrio cholerae* Affected Ponds underTwo Geographical Conditions (Mathbaria and Chhatak)

Table 40. A comparison of the plankton production in Mathbaria and Chhatak (n=161)

Plankton	Place	Mean	Std. Deviation
Protozoa	Mathbaria	3.71	7.241
	Chhatak	797.54	5054.562
Rotifera	Mathbaria	8.78	20.408
	Chhatak	63.90	287.791
Nauplii	Mathbaria	12.99	17.702
	Chhatak	22.83	56.254
Copepoda	Mathbaria	5.75	10.249
	Chhatak	13.48	36.243
Cladocera	Mathbaria	2.05	4.702
	Chhatak	6.84	18.536

**The above table shows that the average production of protozoa, rotifera, nauplii, copepoda and cladocera of Chhatak was far more than that of Mathbaria pond. Deviation of plankton production of Chhatak were maximum than that of Mathbaria ponds.

Table 41. Testing the equality of plankton production in two study areas i.e., Mathbaria and Chhatak

		Levene's Test for Equality of Variance		t-test for equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. Error Difference	95% Confidence of the Difference Lower	Upper
Protozoa	Equal variances assumed	15.417	**.000	-1.993	320	.047	-793.832	398.356	-1577.559	-10.105
	Equal variances not assumed			-1.993	160.001	.048	-793.832	398.356	-1580.546	-7.119
Rotifera	Equal variances assumed	10.325	**.001	-2.424	320	.016	-55.124	22.738	-99.859	-10.389
	Equal variances not assumed			-2.424	161.609	.016	-55.124	22.738	-100.026	-10.222
Nauplii	Equal variances assumed	19.997	**.000	-2.117	320	.035	-9.839	4.648	-18.983	-.694
	Equal variances not assumed			-2.117	191.380	.036	-9.839	4.648	-19.006	-.671
Copepoda	Equal variances assumed	16.929	**.000	-2.605	320	.010	-7.733	2.968	-13.573	-1.893
	Equal variances not assumed			-2.605	185.426	.010	-7.733	2.968	-13.589	-1.877
Cladocera	Equal variances assumed	21.150	**.000	-3.182	320	.002	-4.795	1.507	-7.760	-1.830
	Equal variances not assumed			-3.182	180.507	.002	-4.795	1.507	-7.769	-1.821

** Highly significant at 1% level.

**In order to test the equality of plankton production in two study area (Mathbaria and Chhatak), Independent Sample t test has been performed. In fact, this test has two parts: Levene's test for equality of variances and independent sample t-test.

Levene's test showed that, variances of production of all other planktons are significantly unequal ($p<0.05$) at 5% level of significance. So, for t-tests unequal variance has been assumed.

It is evident from the Independent Sample t-test that the average production of protozoa, rotifera, nauplii and copepoda in Mathbaria is significantly different than that in Chhatak ($p<0.05$).

Table 42. Comparison of plankton production in different months of the year

Plankton	Source of Variations	Sum of Squares	df	Mean Square	F	Sig.
Protozoa	Between Months	104464823.352	11	9496802.123	.730	.710
	Within Months	4034048210.179	310	13013058.743		
	Total	4138513033.531	321			
Rotifera	Between Months	453062.888	11	41187.535	.974	.470
	Within Months	13109959.214	310	42290.191		
	Total	13563022.102	321			
Nauplii	Between Months	17679.848	11	1607.259	.912	.529
	Within Months	546577.357	310	1763.153		
	Total	564257.205	321			
Copepoda	Between Months	12289.230	11	1117.203	1.578	.104
	Within Months	219498.786	310	708.061		
	Total	231788.016	321			
Cladocera	Between Months	2304.245	11	209.477	1.118	.346
	Within Months	58059.357	310	187.288		
	Total	60363.602	321			

**Analysis of Variance (ANOVA) tests have been performed to comparison of plankton production in different months of the year. It is evident that there is no significant difference between production of plankton (Protozoa, Rotifera, Nauplii, Copepoda and Cladocera) (p>0.05) in different months of the year.

Table 43. Comparison of plankton groups between ponds

Plankton	Source of Variations	Sum of Squares	df	Mean Square	F	Sig.
Protozoa	Between Ponds	292977685.205	6	48829614.201	4.000	.001
	Within Ponds	3845535348.326	315	12208048.725		
	Total	4138513033.531	321			
Rotifera	Between Ponds	398748.689	6	66458.115	1.590	.149
	Within Ponds	13164273.413	315	41791.344		
	Total	13563022.102	321			
Nauplii	Between Ponds	10898.379	6	1816.396	1.034	.403
	Within Ponds	553358.826	315	1756.695		
	Total	564257.205	321			
Copepoda	Between Ponds	5668.385	6	944.731	1.316	.249
	Within Ponds	226119.630	315	717.840		
	Total	231788.016	321			
Cladocera	Between Ponds	938.298	6	156.383	.829	.548
	Within Ponds	59425.304	315	188.652		
	Total	60363.602	321			

**Analysis of Variance (ANOVA) tests have also been performed to comparison of plankton production in different ponds of Mathbaria and Chhatak. It is evident that there is no significant difference between production of plankton other than Protozoa at (p>0.05), in the selected ponds.

4.4 Climatic Influx on Changing Environmental Condition of Pond

Hydroclimatic factors are profoundly related with the outbreak of cholera in the coastal and fresh water region of Bangladesh. Zooplanktons identified and counted are supposed to be related with the arrival of associated bacteria during the peak season (March-May and November-December in Mathbaria and only November-December in Chhatak).

4.4.1 Climatic influx on changing environmental condition of Mathbaria

4.4.1.1 Hydroclimatic influence on the plankton count in Mathbaria ponds

From the graphs (Fig. 22-35), it has been shown that, maximum temperature was highest in the month of April and highest minimum temperature was observed during May-September. Whereas total amount of precipitation or rainfall started to increase in the month of April. Count of total zooplankton was maximum in April and among them nauplii showed maximum abundance at site-2, site-7, site-9, site-10 and site-11. Wheel organ bearing plankton group rotifera had the highest abundance at site-5 and site-8 was abundant with protozoan plankton during the peak season of cholera. So, from analysis it has been showed that maximum average temperature had profound influence on the nauplii of crustacean plankton in most of the ponds of Mathbaria during the peak season of cholera.

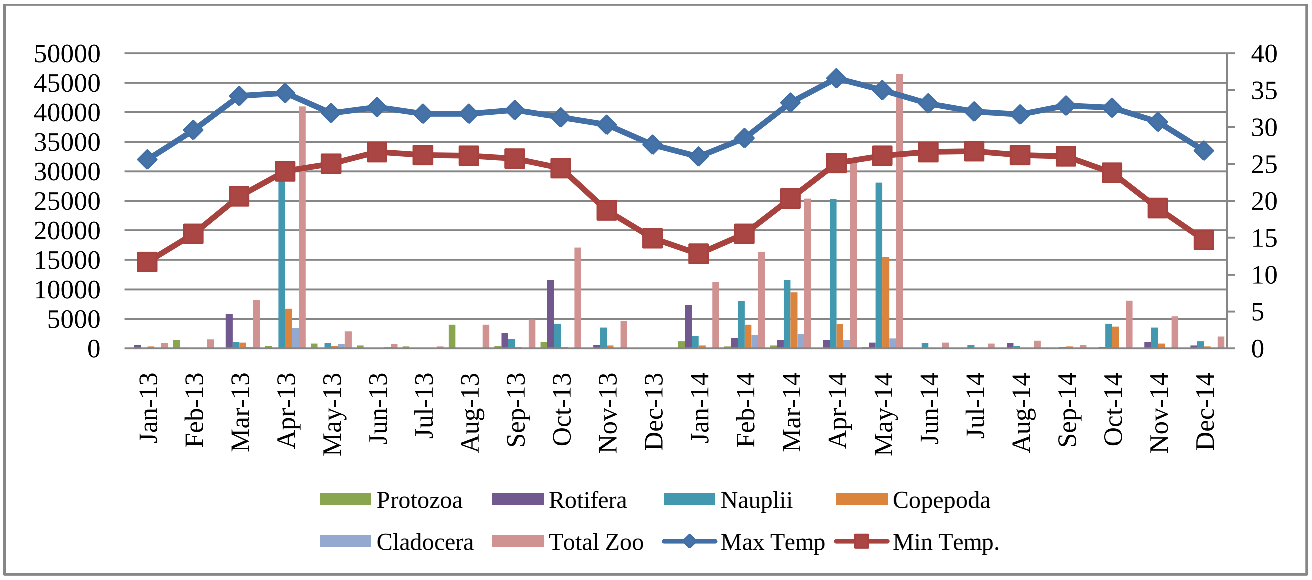


Figure 22. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-2

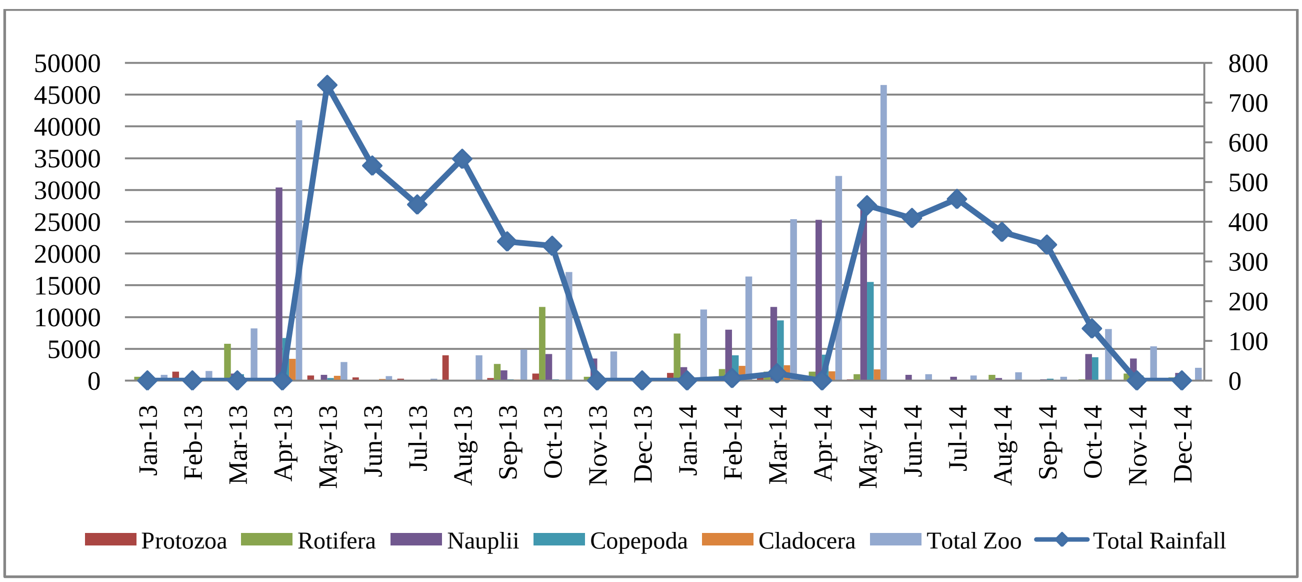


Figure 23. Impact of Precipitation on the abundance of Zooplankton at site-2

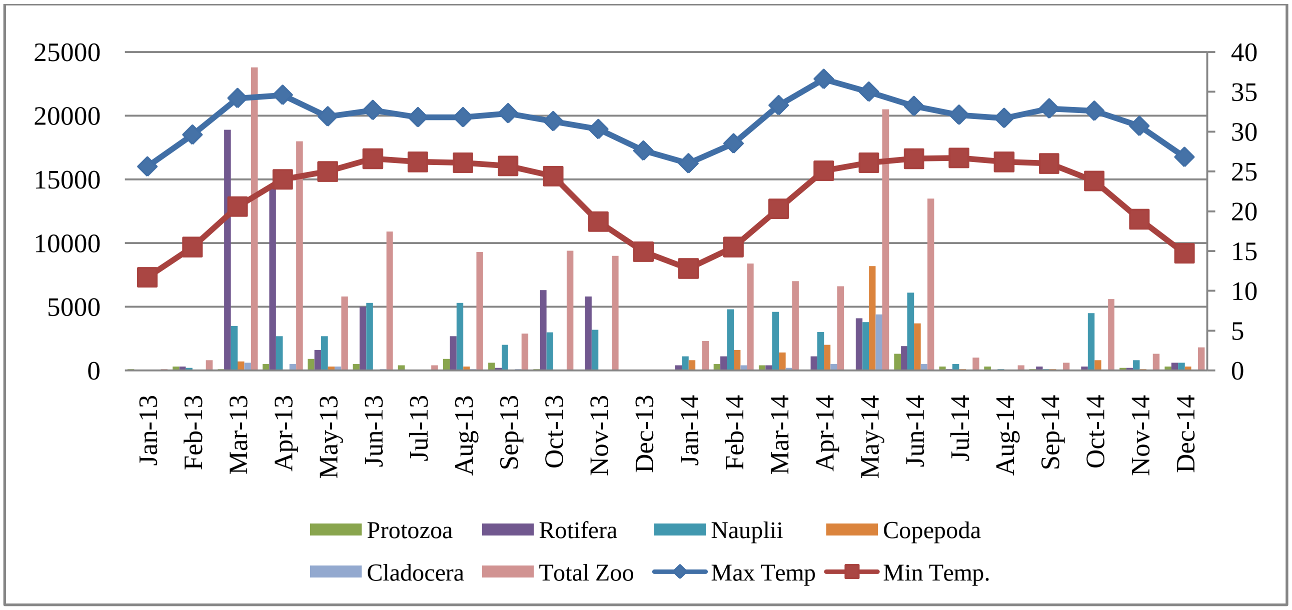


Figure 24. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-5

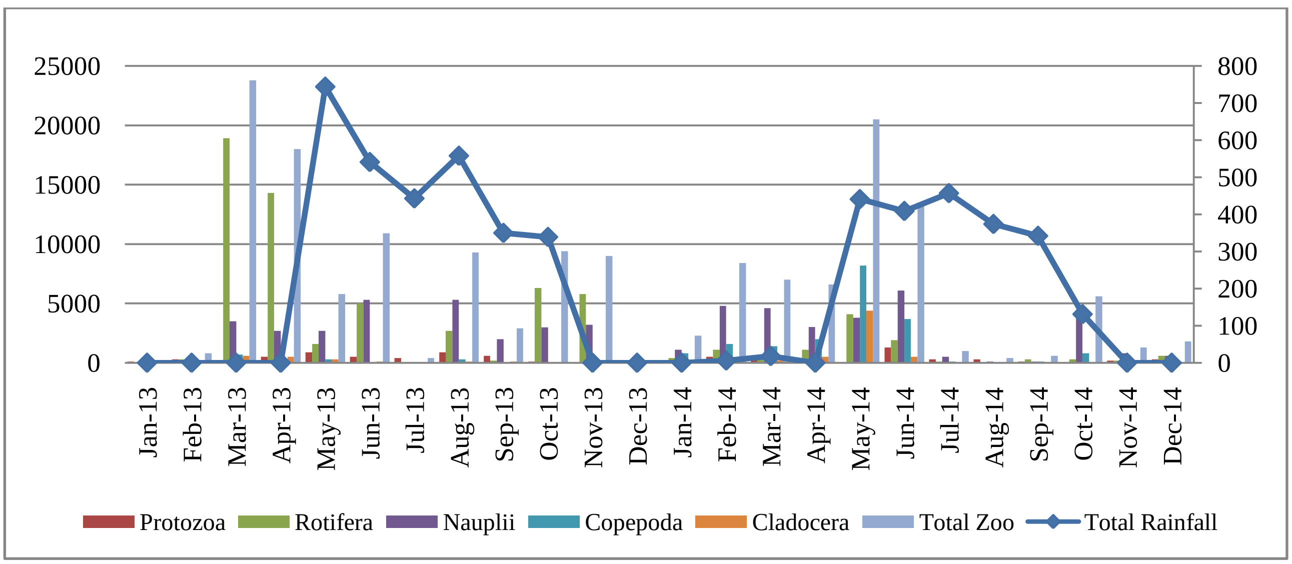


Figure 25. Impact of Precipitation on the abundance of Zooplankton at site-5

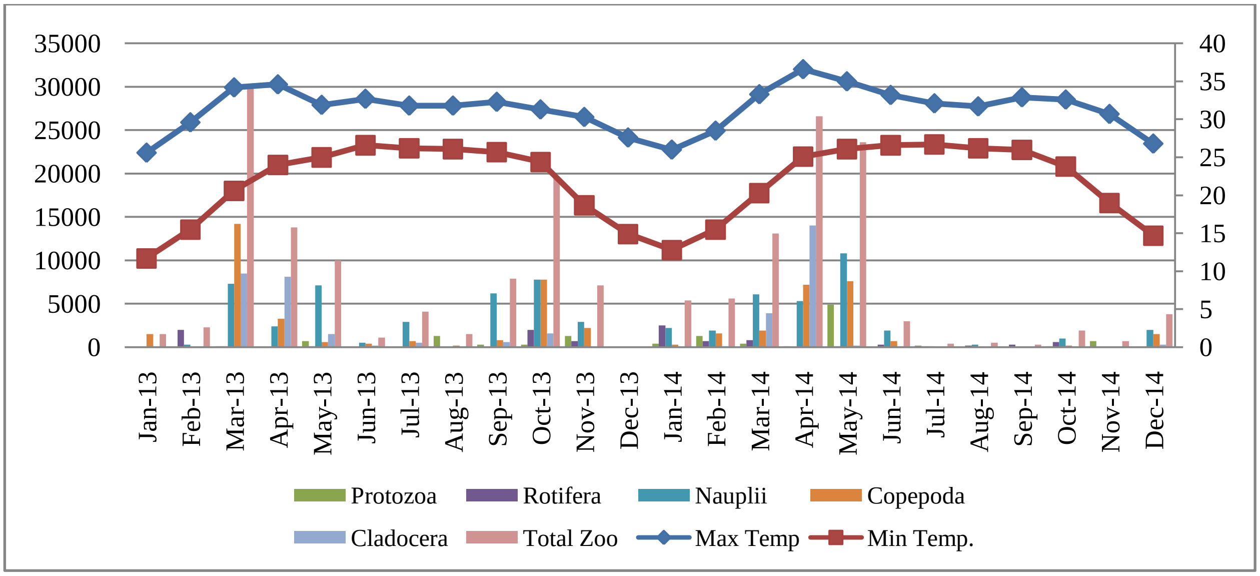


Figure 26. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-7

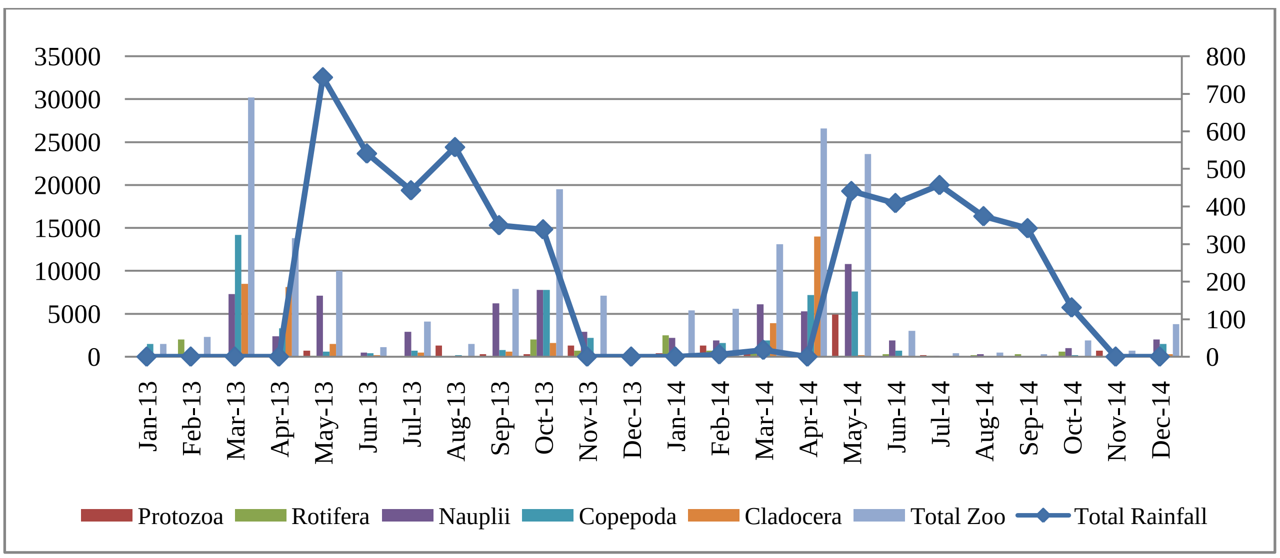


Figure 27. Impact of Precipitation on the abundance of Zooplankton at site-7

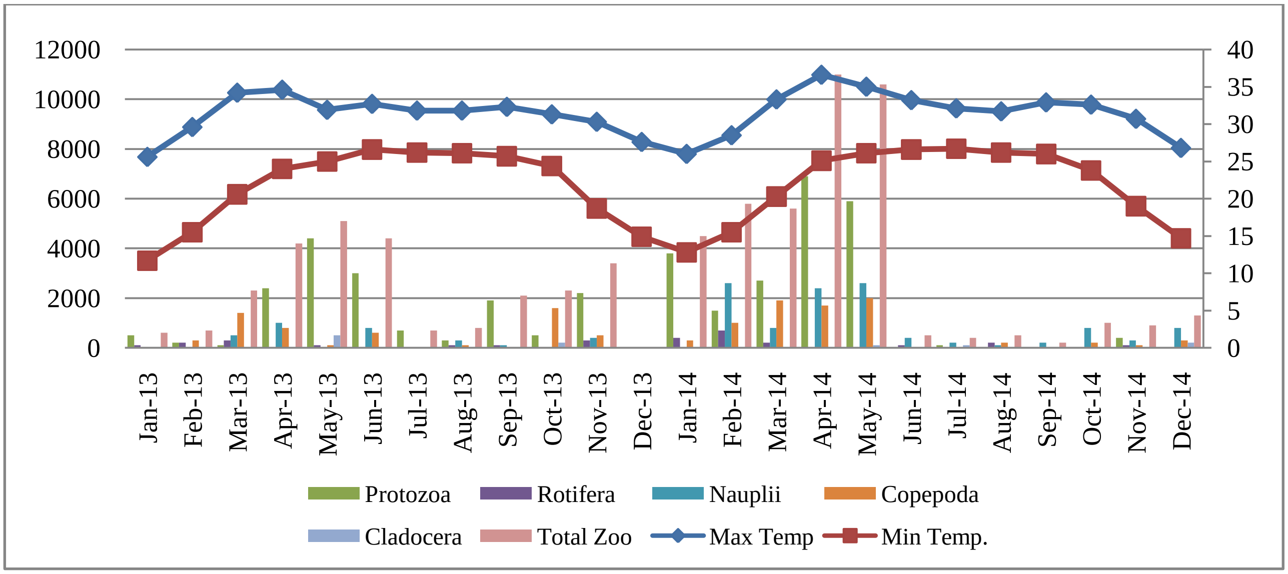


Figure 28. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-8

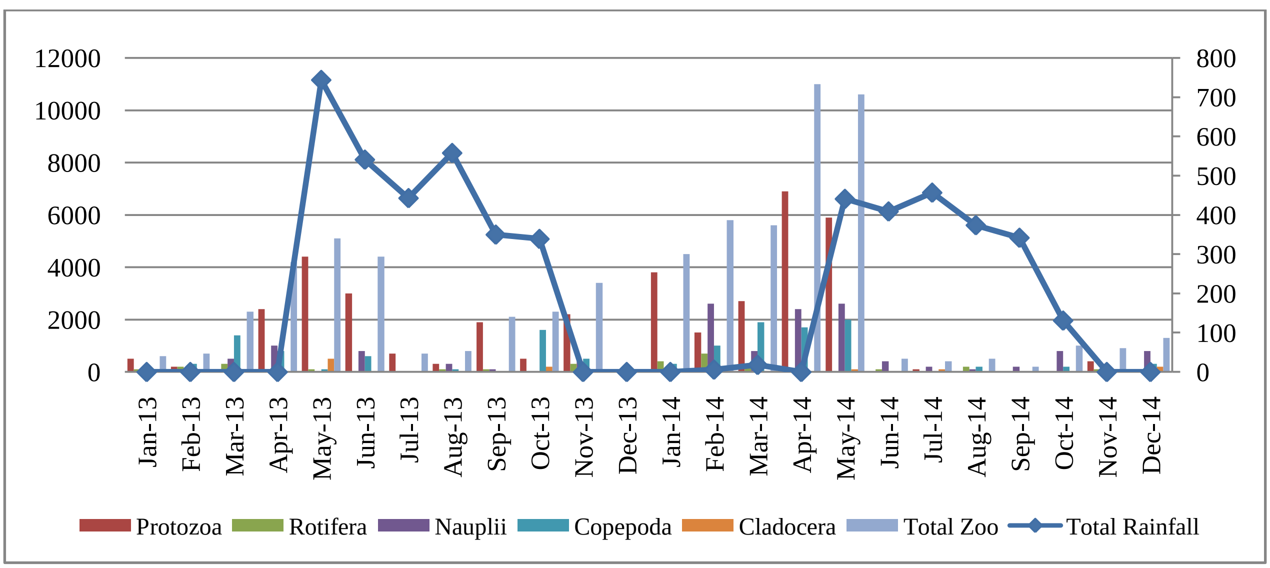


Figure 29. Impact of Precipitation on the abundance of Zooplankton site-8

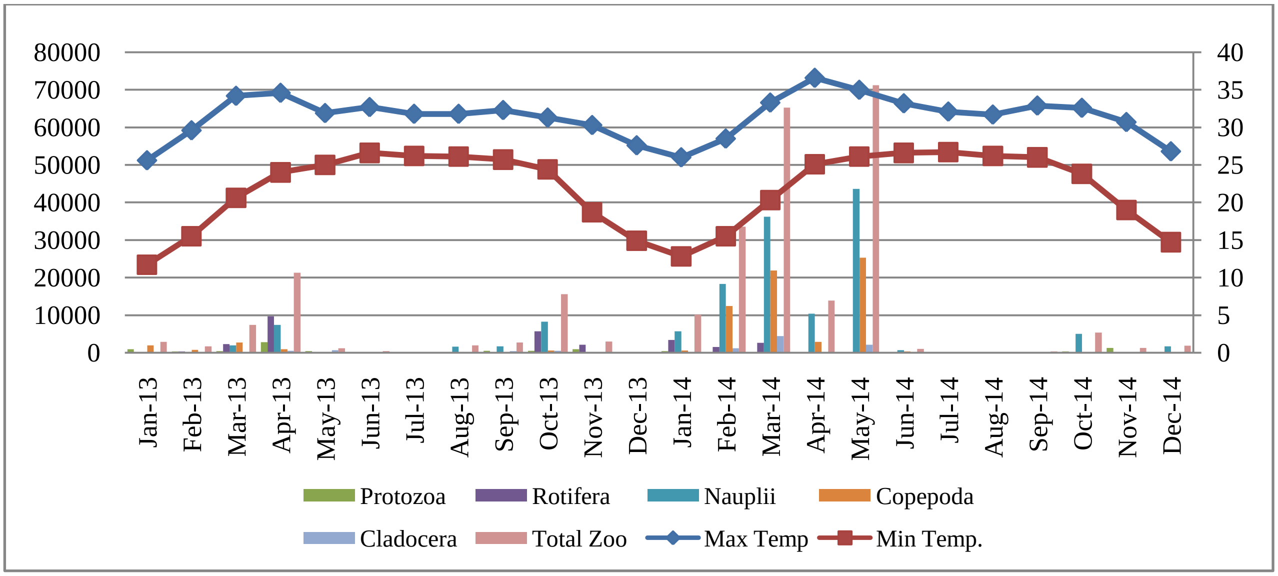


Figure 30. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton site-9

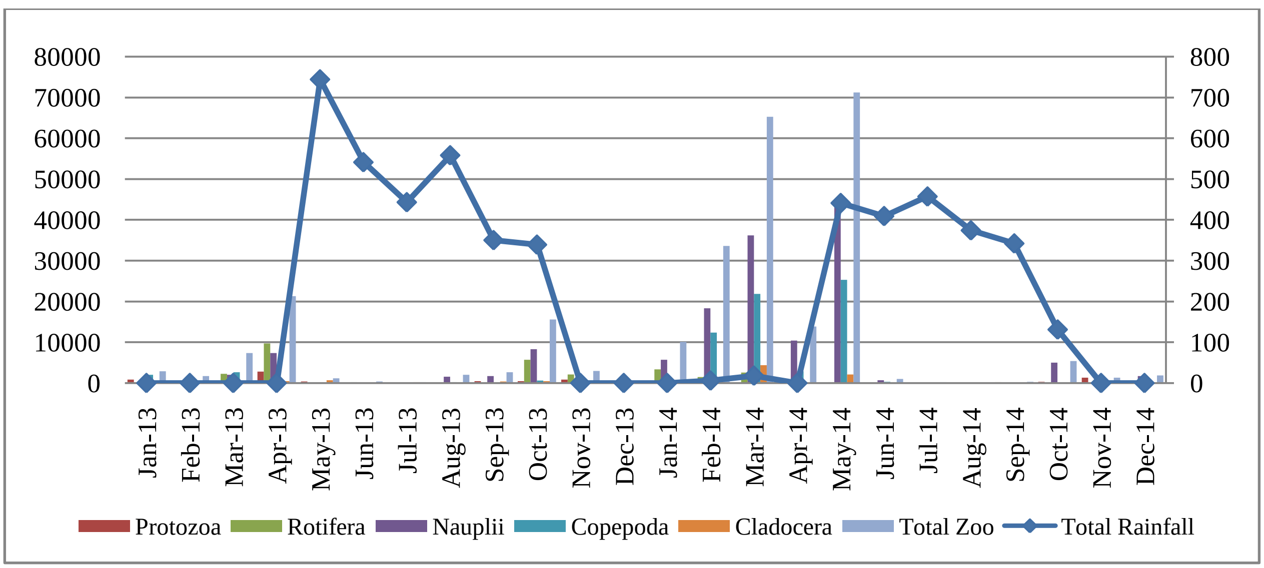


Figure 31. Impact of Precipitation on the abundance of Zooplankton site-9

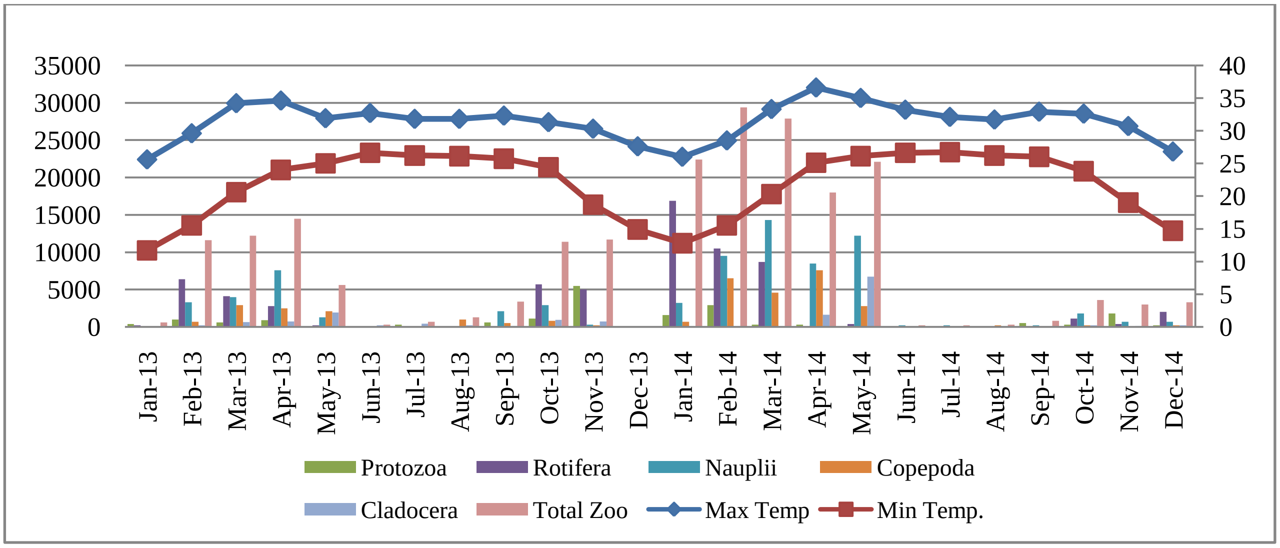


Figure 32. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-10

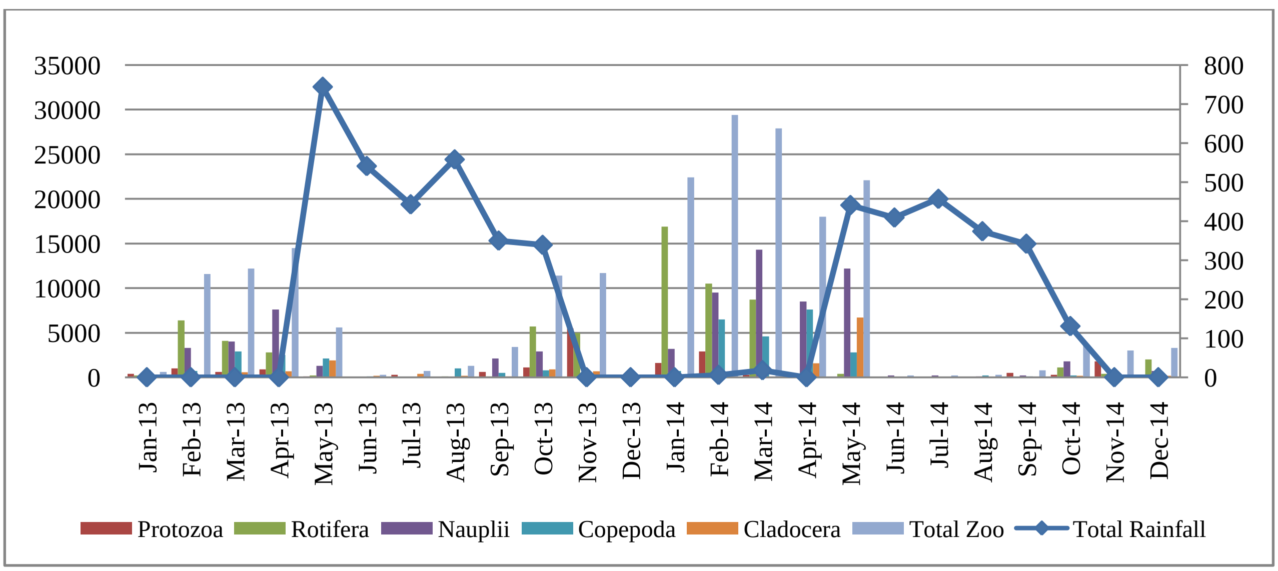


Figure 33. Impact of Precipitation on the abundance of Zooplankton site-10

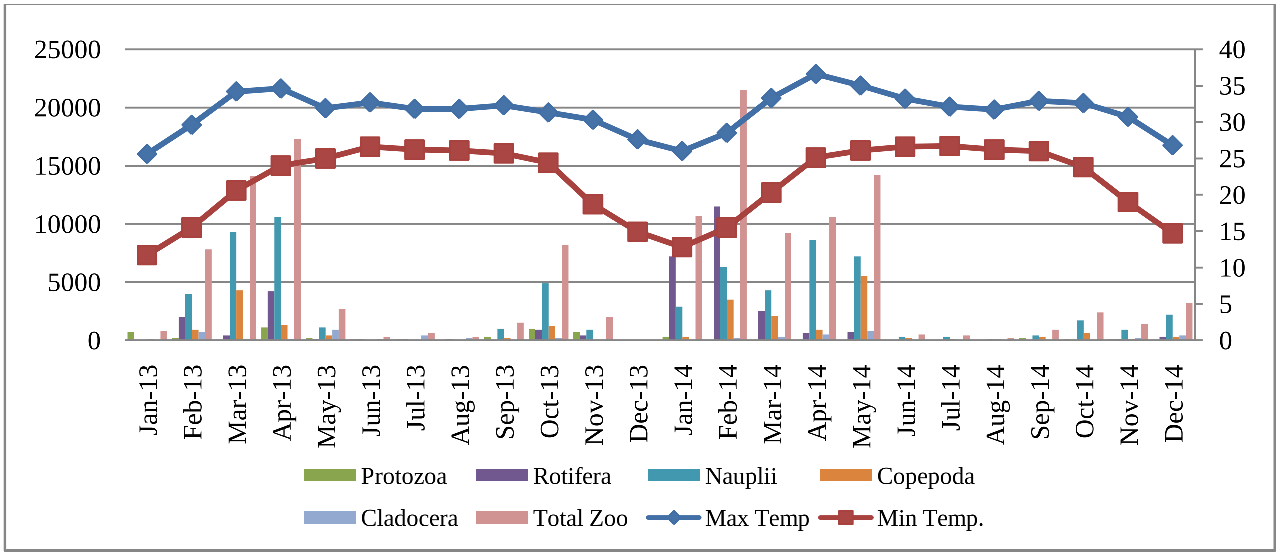


Figure 34. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton site-11

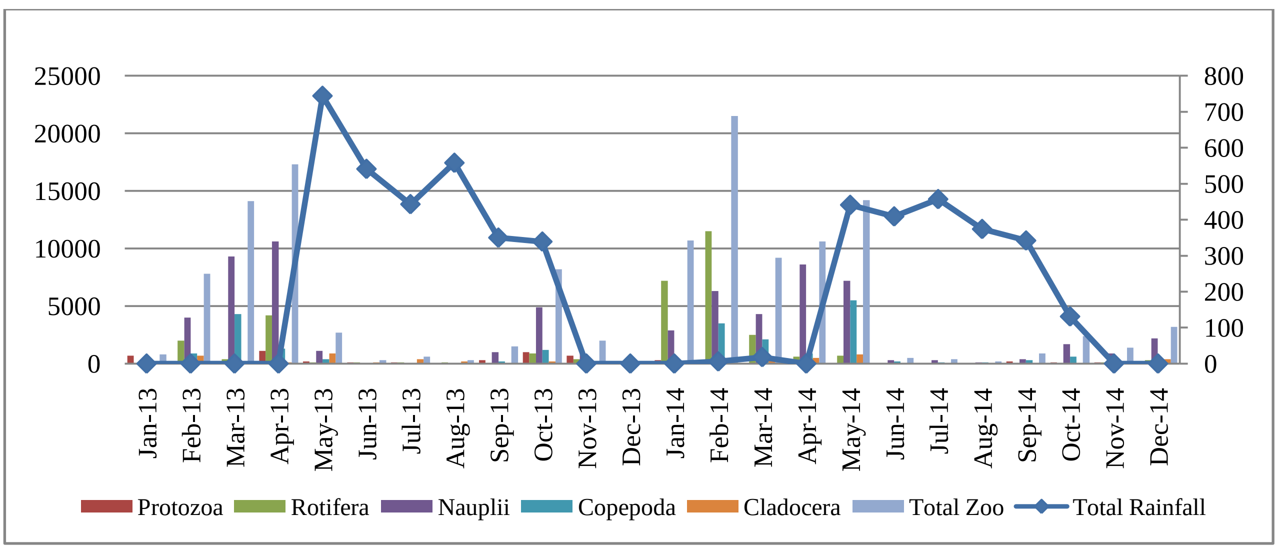


Figure 35. Impact of Precipitation on the abundance of Zooplankton site-11

4.4.1.2 Interrelation between plankton group and major climatic factors in seven ponds of Mathbaria

Interrelation by correlation was computed among the major climatic factors i.e., maximum air temperature, minimum air temperature and total rainfall and major zooplankton groups (Table 44). Significant relation among nauplii, copepoda, cladocera and total zooplankton and maximum air temperature was observed through correlationduring the study period (January 2013-December 2014) in almost allstudied ponds of Mathbaria. Protozoa and Rotifera showed positive correlation with max-min temperature and total rainfall at site-5, site-8 and site-9. Strong positive correlation existed between protozoa and total rainfall and total zooplankton and maximum temperature at site-5.

Table 44. Correlation among zooplankton groups and some hydroclimatic factors that can influence the abundance of plankton in seven domestic ponds of Mathbaria

Interrelationships between	Correlation between plankton and climatic factors in seven ponds of Mathbariastudy (2013-2014)													
	Site-2		**Site-5**		**Site-7**		**Site-8**		**Site-9**		**Site-10**		**Site-11**	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
Protozoa-Max Temp	0.138	-0.488	**0.443**	-0.012	0.070	0.228	0.366	0.375	0.257	-0.171	-0.054	-0.546	-0.008	-0.411
Protozoa-Min Temp	0.336	-0.608	**0.671**	0.128	0.223	0.093	**0.453**	-0.016	-0.052	-0.317	-0.201	-0.686	-0.085	-0.352
Protozoa-Total Rainfall	0.442	-0.359	**0.781**	0.257	0.302	0.263	**0.508**	-0.208	-0.428	-0.462	-0.392	-0.578	-0.325	-0.206
Rotifera-Max Temp	0.190	-0.537	**0.653**	0.375	-0.136	-0.536	0.018	-0.536	**0.464**	-0.444	0.114	-0.621	0.364	-0.536
Rotifera-Min Temp	0.145	-0.646	0.161	0.238	-0.192	-0.573	-0.340	-0.600	0.178	-0.649	-0.243	-0.770	0.006	-0.670
Rotifera-Total Rainfall	-0.038	-0.443	-0.321	0.344	-0.196	-0.340	-0.383	-0.387	-0.312	-0.498	-0.498	-0.540	-0.399	-0.458
Nauplii-Max Temp	**0.446**	**0.593**	**0.612**	0.333	**0.495**	**0.437**	**0.626**	0.334	**0.431**	0.325	**0.557**	0.318	**0.568**	0.288
Nauplii-Min Temp	0.167	0.195	**0.613**	0.090	0.415	0.099	0.321	-0.006	0.309	-0.010	0.104	-0.115	0.047	-0.153
Nauplii-Total Rainfall	-0.292	-0.072	**0.443**	-0.056	0.246	0.029	-0.124	-0.175	-0.102	-0.032	-0.367	-0.268	-0.436	-0.368
Copepoda-Max Temp	**0.462**	**0.454**	**0.413**	**0.440**	**0.403**	**0.551**	**0.473**	**0.449**	-0.022	0.295	**0.661**	0.312	**0.470**	0.233
Copepoda-Min Temp	0.122	0.137	0.131	0.286	0.022	0.203	0.169	0.010	-0.437	-0.006	0.245	-0.118	-0.032	-0.007
Copepoda-Total Rainfall	-0.323	0.060	0.102	0.361	-0.296	-0.017	-0.199	-0.214	-0.528	0.011	-0.018	-0.428	-0.346	0.076
Cladocera-Max Temp	**0.484**	0.278	**0.691**	0.398	**0.644**	**0.533**	0.096	-0.255	**0.401**	0.251	0.380	**0.444**	0.100	0.329
Cladocera-Min Temp	0.209	-0.1221	0.244	0.274	0.145	0.158	0.261	-0.145	0.512	-0.068	0.355	0.288	0.158	-0.017
Cladocera-Total Rainfall	-0.161	-0.2760	-0.083	0.383	-0.315	-0.351	**0.577**	0.118	0.462	-0.140	0.419	0.322	0.453	-0.132
Total Zoo-Max Temp	**0.515**	**0.4687**	**0.740**	**0.438**	**0.574**	**0.549**	**0.591**	0.376	**0.437**	0.285	**0.422**	-0.047	**0.544**	-0.096

Total Zoo-Min Temp	0.237	0.0618	0.328	0.250	0.199	0.136	**0.492**	-0.044	0.170	-0.046	-0.042	-0.448	0.027	-0.429
Total Zoo-Total Rainfall	-0.250	-0.1146	-0.114	0.276	-0.161	-0.137	0.351	-0.236	-0.287	-0.051	-0.443	-0.467	-0.435	-0.392

**Interpretation of the correlation:

0<r<0.39 is considered low positive correlation

0.39<r<0.69 is considered moderate positive correlation

0.70<r<0.99 is considered strong positive correlation

-0.39 < r < -0.1 is considered to be low negative correlation

-0.69 < r < -0.40 is considered to be moderate negative correlation

4.4.2 Climatic Influx on changing environmental condition of Chhatak

4.4.2.1 Hydroclimatic influence on the plankton count in seven ponds and river of Chhatak

Environmental conditons of Chhatak is opposite to Mathbaria. From the graphs (Fig.36, Fig.38, Fig. 40, Fig. 42, Fig.44, Fig. 46 and Fig. 48) drastic fluctuations in maximum average temperature was shown in comparison to that of Mathbaria. During the first year of study(2013), highest of maximum temperature was recorded in the month of March and then decreased. The trend again increased in June showing a slow rate decrease till December. In the second year of study (2014), average of maximum temperature began to rise in April and this line with slow and steady up down continued till December.

Total rainfall started to increase from March month and continued till May in 2013 (Fig. 37, Fig. 39, Fig. 41, Fig. 43, Fig. 45, Fig. 47 and Fig. 49). Then showing up and down slope the precipitationamount increased in September. In the year 2014, the amountof precipitation was much higher than that of 2013. In the month of June and September the quantity of rainfall was highest.In Chhatak, the peak season of cholera incidence was September-November. Total count of zooplankton was maximum in May-June, September-October in most of the ponds whereas sometimes highest amount was recorded in February-March. Rotifera was abundantly recorded at site-1, site-4and site-12.Maximum percentage of protozoa was observed at site-2, site-9, site-10 and site-12. Sometimes, nauplii was observedin high abundance at site-10 and site-12 during September.

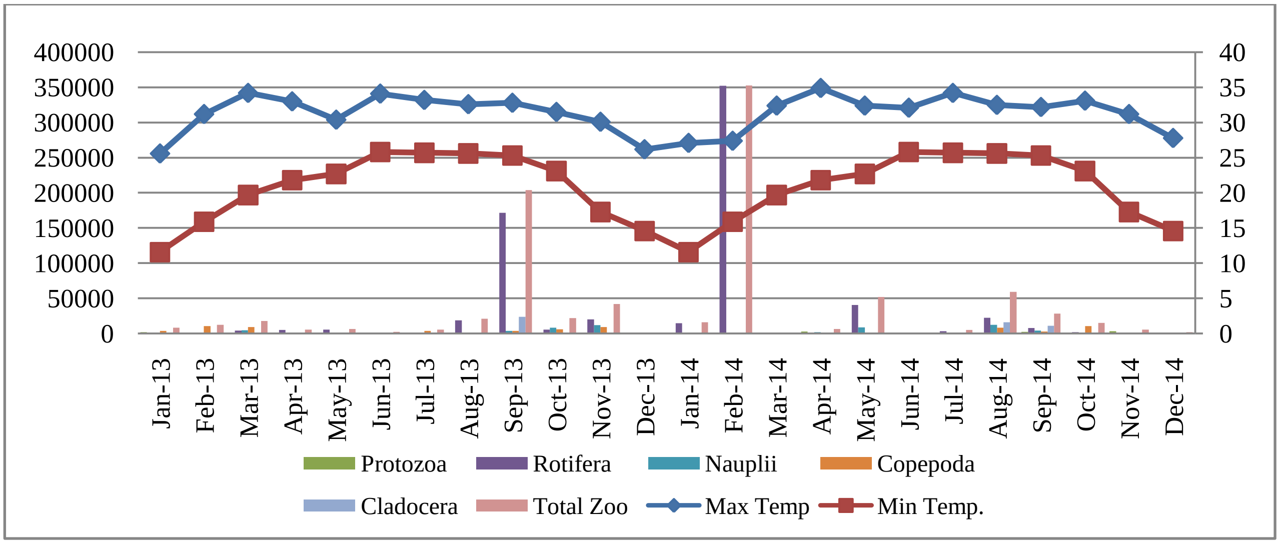


Figure 36. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-1

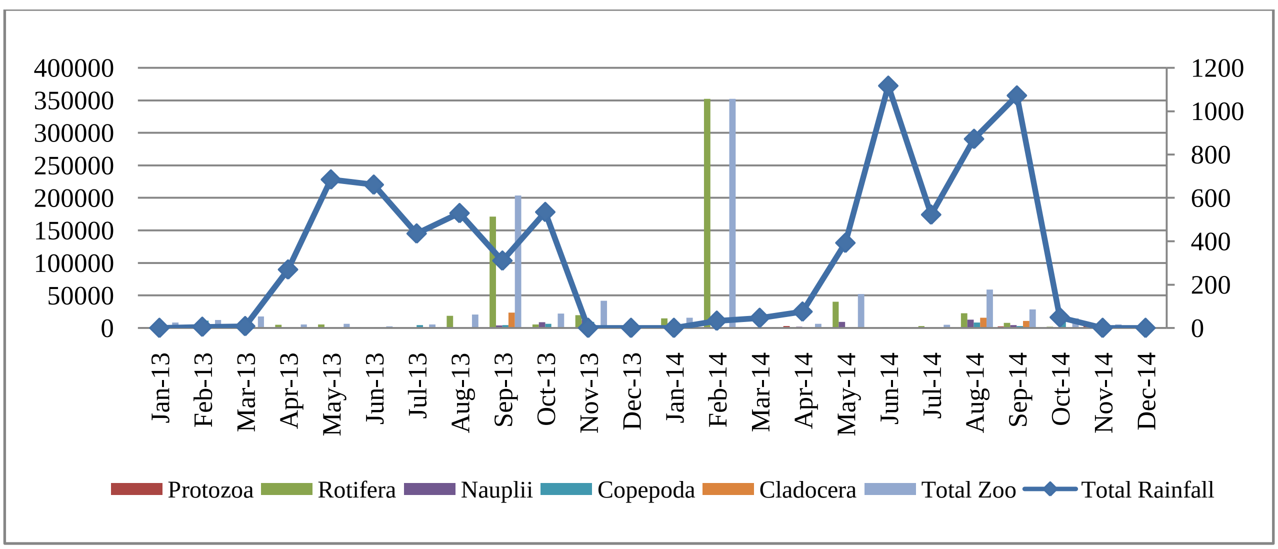


Figure 37. Impact of Precipitation on the abundance of Zooplankton at site-1

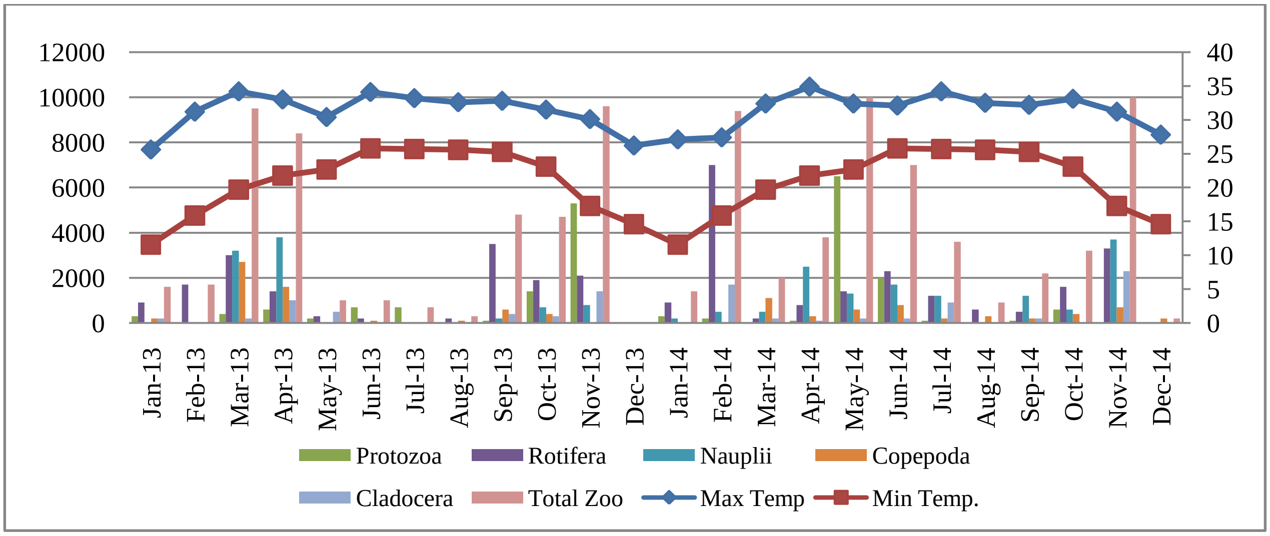


Figure 38. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-2

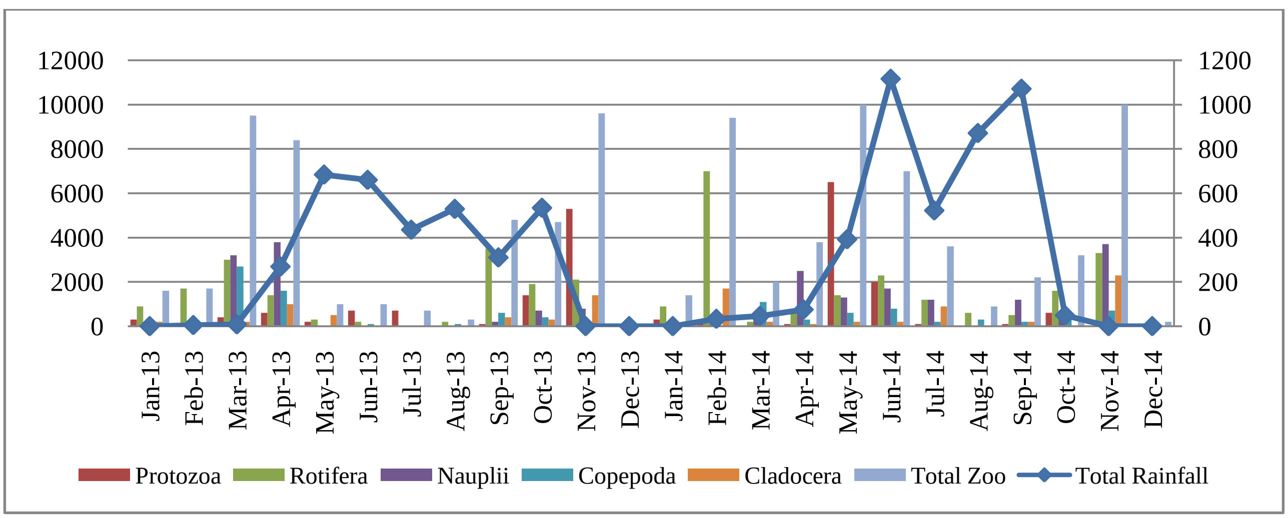


Figure 39. Impact of Precipitation on the abundance of Zooplankton at site-2

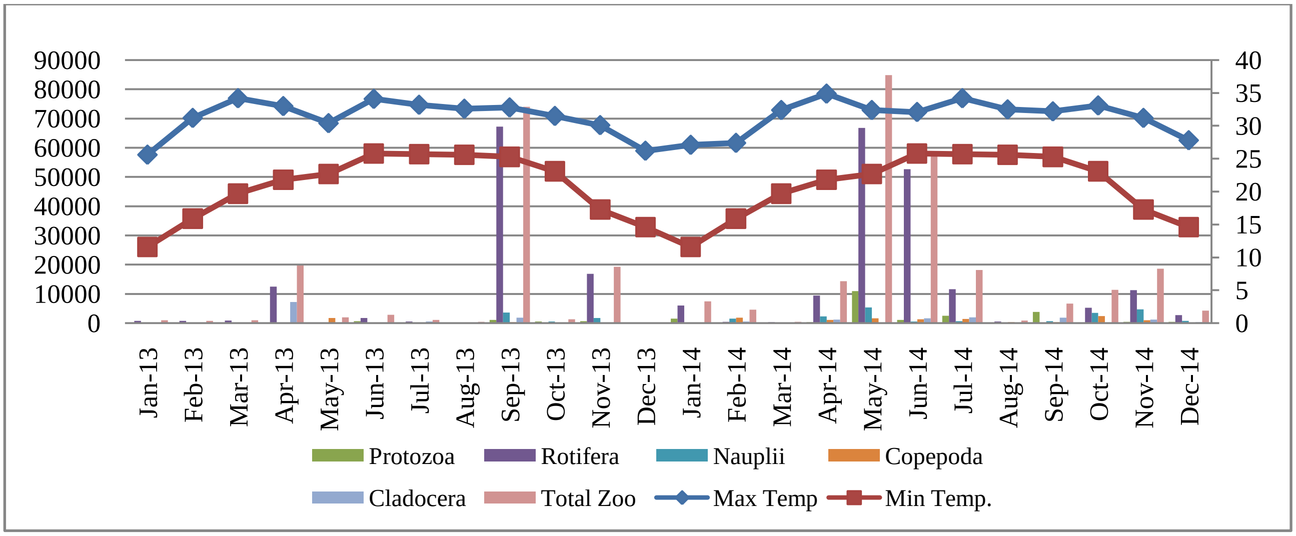


Figure 40. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-4

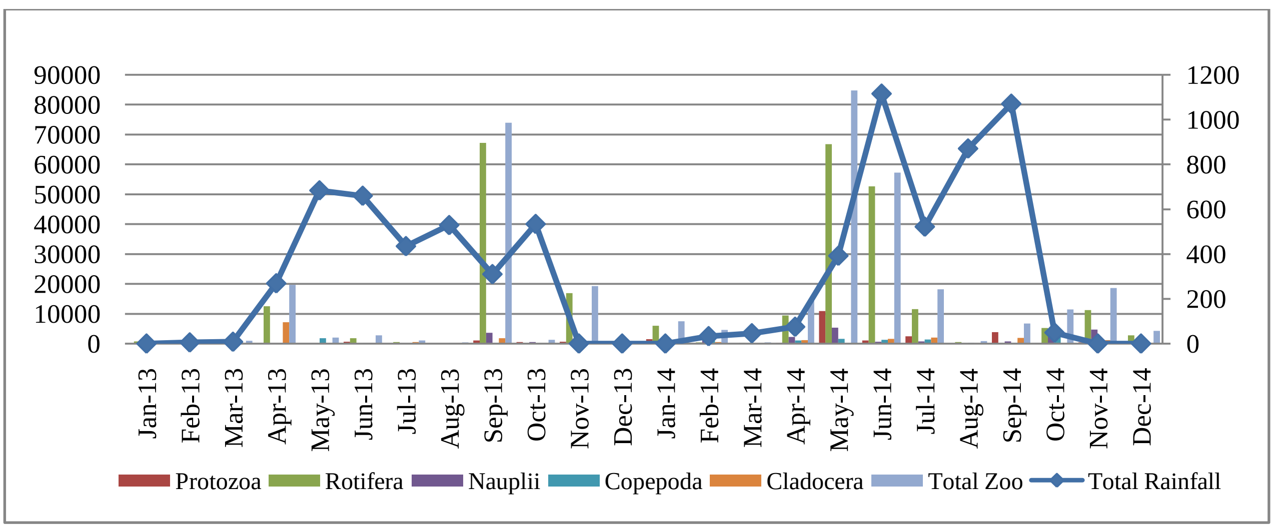


Figure 41. Impact of Precipitation on the abundance of Zooplankton at site-4

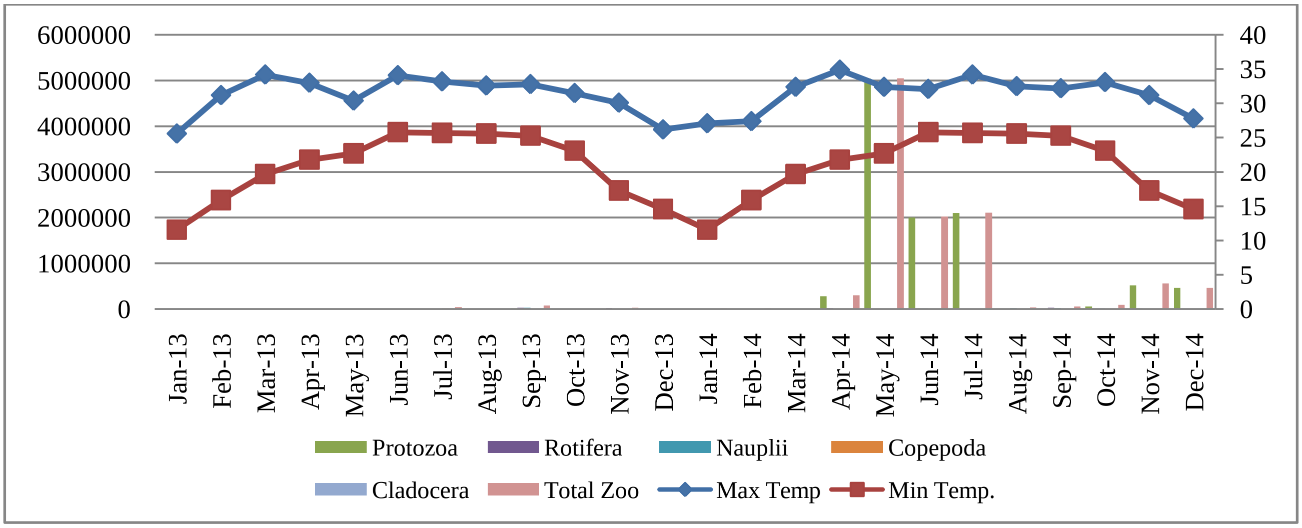


Figure 42. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-9

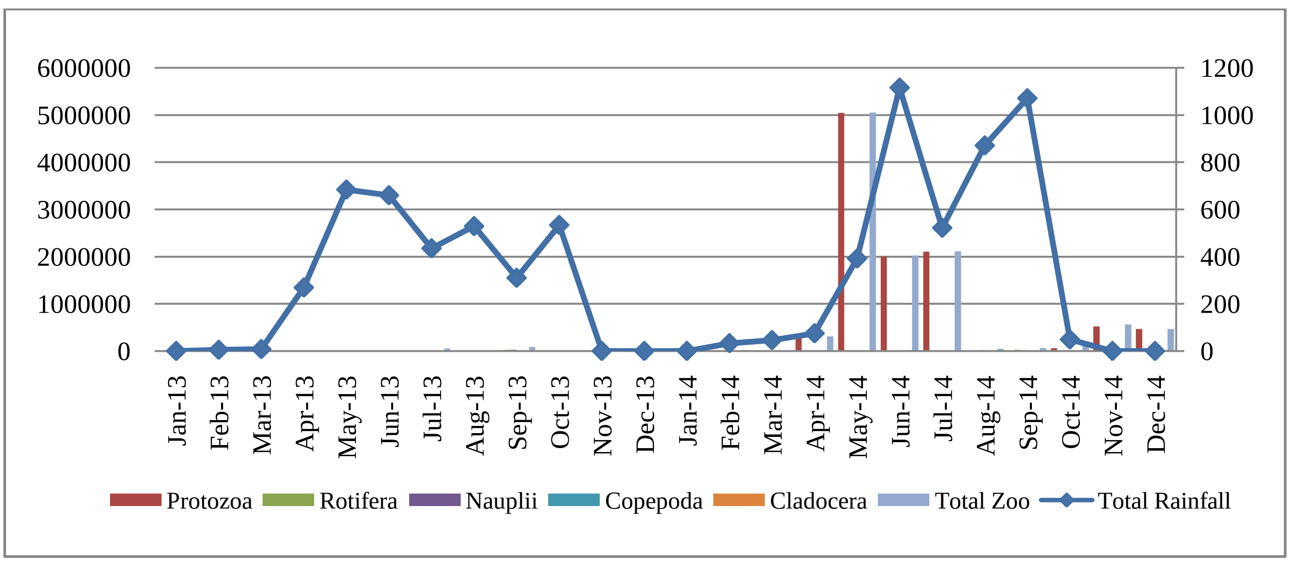


Figure 43. Impact of Precipitation on the abundance of Zooplankton at site-9

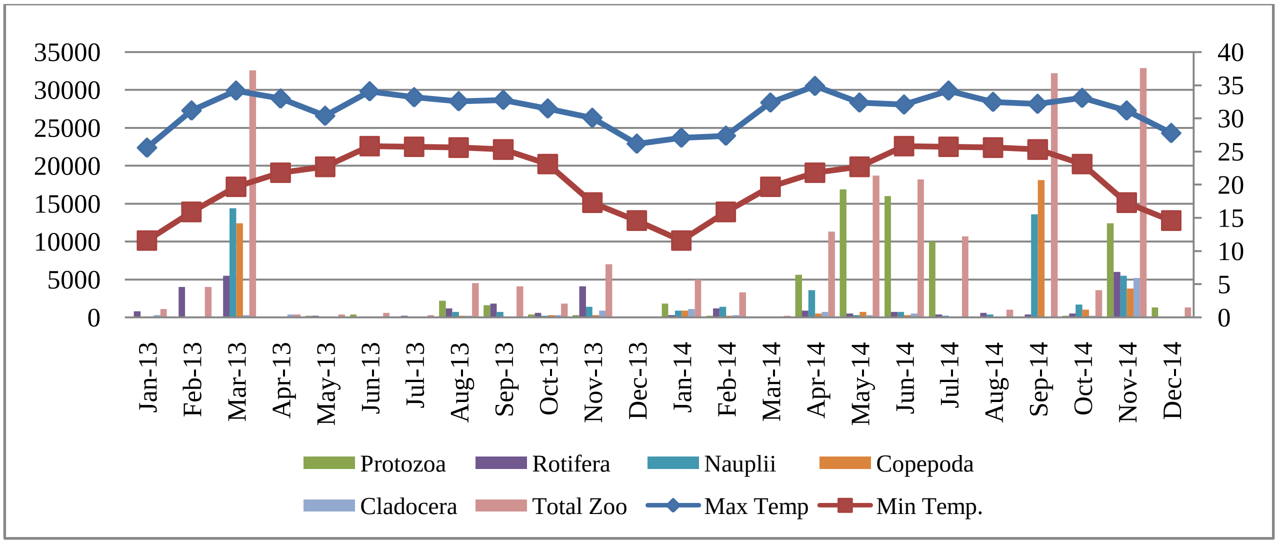


Figure 44. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-10

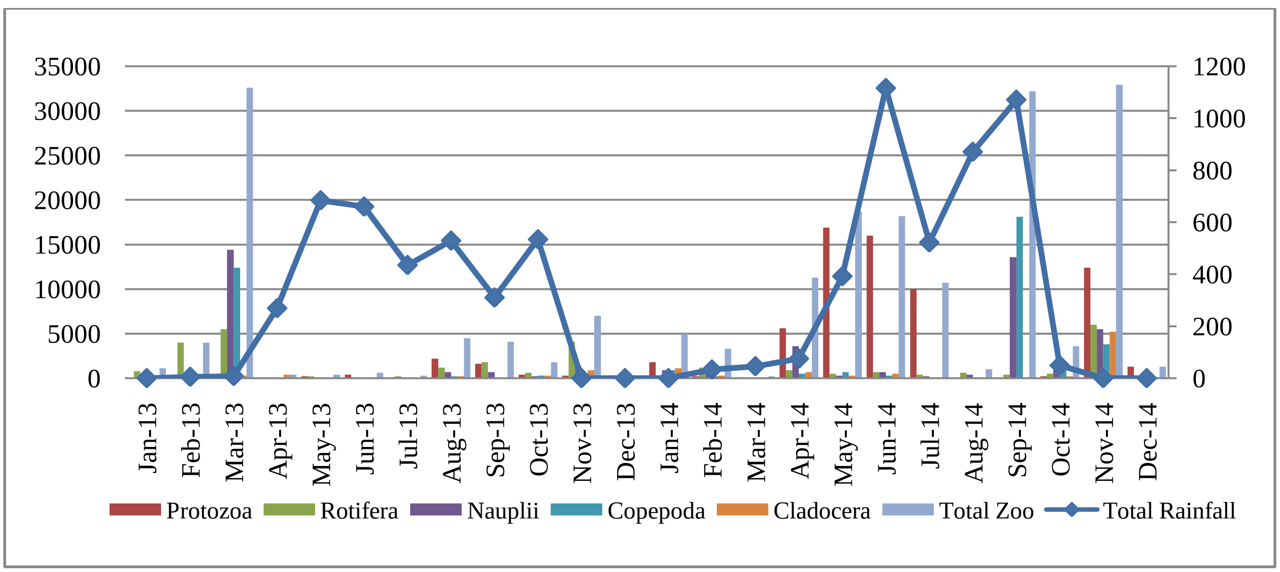


Figure 45. Impact of Precipitation on the abundance of Zooplankton at site-10

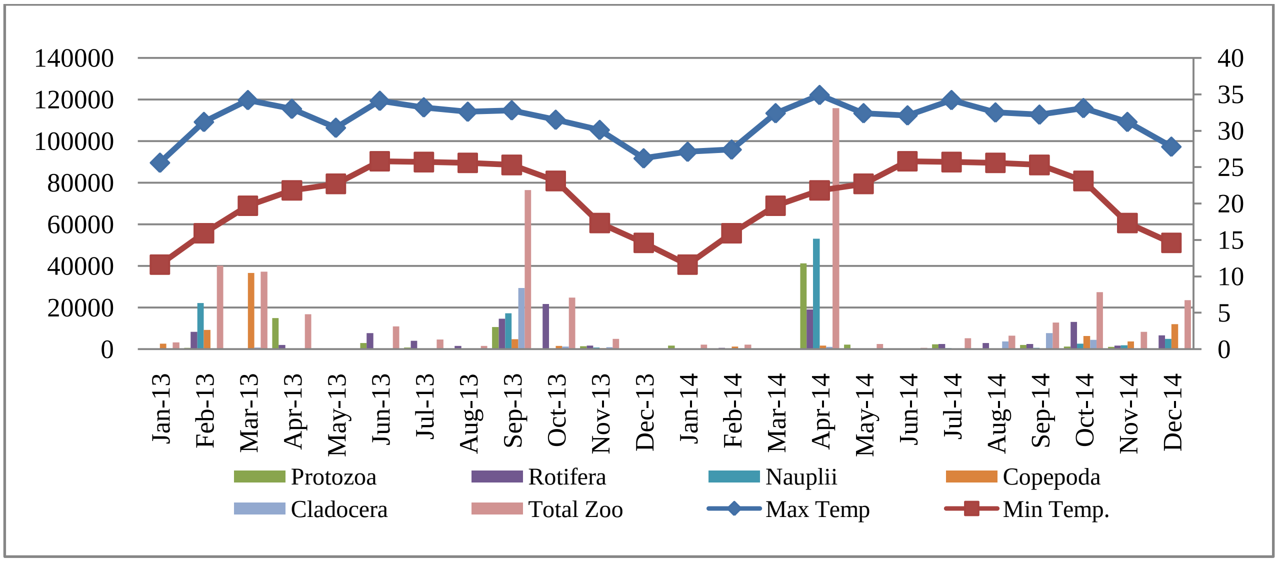


Figure 46. Impact of Maximum and Minimum Temperature as Hydroclimatological factors on the abundance of Zooplankton at site-11

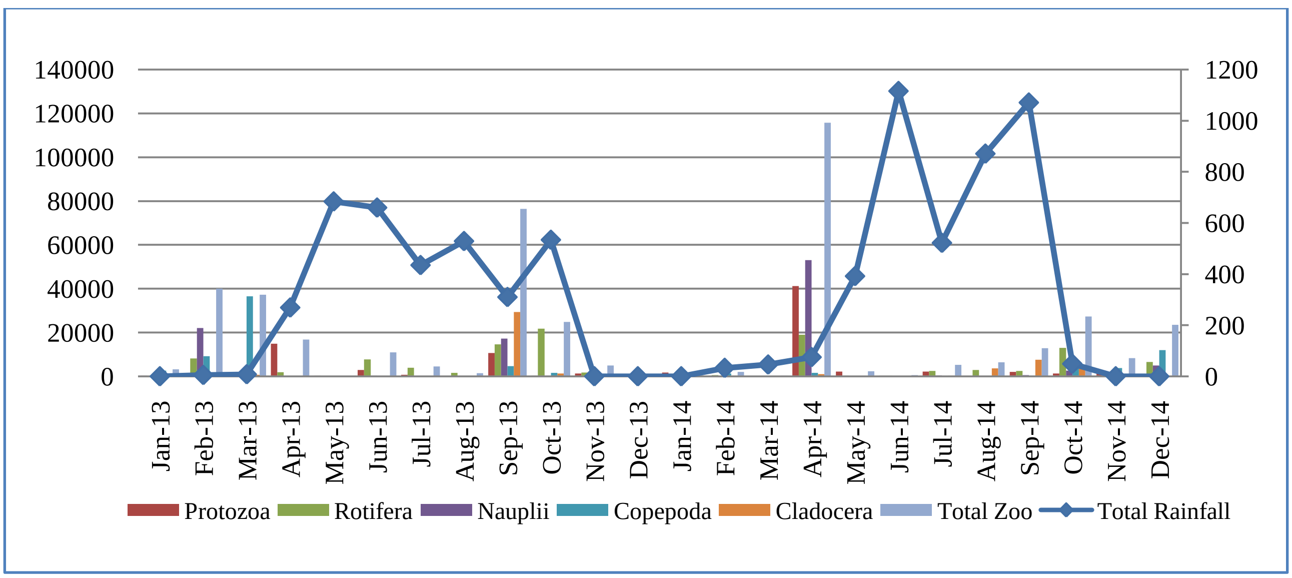


Figure 47. Impact of Precipitation on the abundance of Zooplankton at site-11

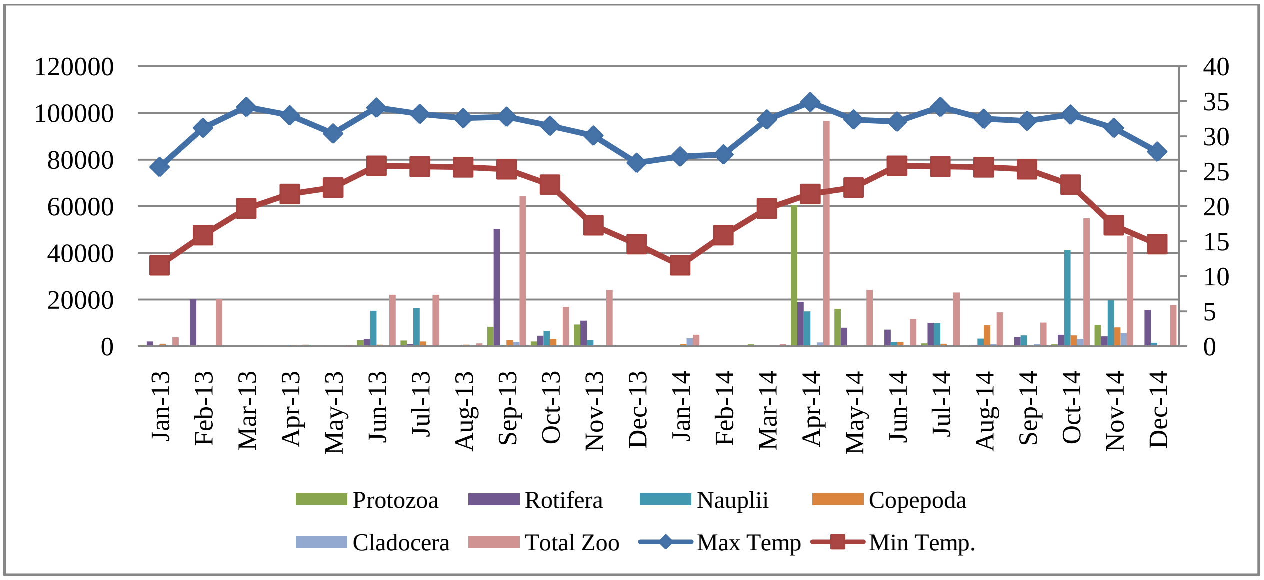


Figure 48. Impact of Maximum and Minimum Temperature as Hydroclimatologicalfactors on the abundance of Zooplankton at site-12

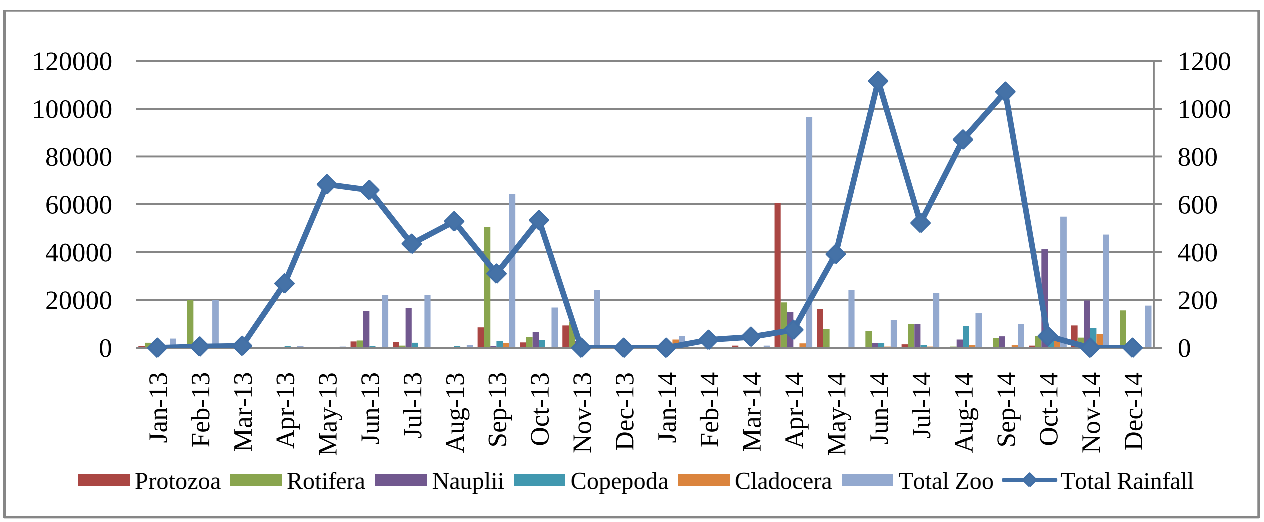


Figure 49. Impact of Precipitation on the abundance of Zooplankton at site-12

4.4.2.2 Interrelation between plankton groups and climatic factorsin the studied ponds and river of Chhatak

Very few correlationswere observed in the selected ponds and river of Chhatak. Moderate positive correlation was observed among crustacean plankton (nauplii, copepoda and cladocera) and maximum-minimum temperature and rainfall at site-1, site-2, site-4, site-9, site-11 and site-12 (Table 45). On the other hand, some protozoan plankton showed moderate relation with minimum temperature at site-9 and site-10.

Table 45. Correlation among zooplankton groups and some hydroclimtic factors that can influence the abundance of plankton in seven domestic ponds and river of Chhatak

Interrelationships between	Correlation between plankton and climatic factors in seven ponds of Chhatak study (2013-2014)													
	Site-1		Site-2		Site-4		Site-9		Site-10		Site-11		Site-12	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Protozoa-Max Temp	-0.320	0.380	-0.039	0.132	0.244	0.151	0.278	0.257	0.289	0.303	0.324	0.439	0.125	0.451
Protozoa-Min Temp	-0.094	0.094	-0.123	0.212	0.369	0.231	**0.444**	0.319	**0.539**	0.282	0.277	0.082	0.179	0.070
Protozoa-Total Rainfall	0.169	-0.037	-0.207	0.169	0.216	0.233	0.162	0.230	0.381	0.239	0.048	-0.185	-0.071	-0.229
Rotifera-Max Temp	0.186	-0.489	0.277	-0.366	0.187	0.209	0.129	0.393	0.202	-0.012	0.285	0.416	0.142	0.330
Rotifera-Min Temp	0.309	-0.298	-0.039	-0.231	0.251	0.307	0.228	0.400	-0.286	-0.185	0.374	0.116	0.133	0.123
Rotifera-Total Rainfall	0.031	-0.20	-0.382	-0.183	-0.056	0.322	-0.066	0.396	-0.587	-0.224	0.320	-0.287	-0.125	-0.094
Nauplii-Max Temp	0.069	0.271	0.380	**0.409**	0.111	0.216	0.249	0.217	0.341	0.146	0.108	0.393	0.399	0.414
Nauplii-Min Temp	-0.037	**0.446**	0.004	0.150	0.197	0.022	**0.415**	**0.501**	-0.056	0.202	-0.073	0.035	0.512	0.199
Nauplii-Total Rainfall	-0.198	**0.461**	-0.247	-0.016	-0.083	-0.299	0.106	0.632	-0.331	0.341	-0.251	-0.234	0.487	-0.258
Copepoda-Max Temp	0.100	0.240	**0.421**	**0.413**	-0.056	0.284	0.149	0.065	0.330	0.083	0.311	-0.321	0.198	0.169
Copepoda-Min Temp	-0.334	0.364	0.036	0.275	0.204	0.307	0.275	0.039	-0.065	0.237	-0.148	-0.385	**0.431**	0.188
Copepoda-Total Rainfall	-0.546	0.145	-0.258	0.080	**0.511**	-0.072	0.019	-0.207	-0.315	0.450	-0.412	-0.447	0.315	0.115
Cladocera-Max Temp	0.174	0.194	-0.007	-0.180	0.265	0.388	0.264	0.157	-0.093	-0.085	0.174	0.280	0.221	-0.037
Cladocera-Min Temp	0.3141	**0.473**	-0.0977	-0.234	0.172	**0.458**	0.397	0.168	-0.282	-0.310	0.286	**0.474**	0.351	-0.294
Cladocera-Total Rainfall	0.055	0.634	-0.182	-0.260	0.009	**0.506**	0.125	0.194	-0.378	-0.291	0.017	**0.478**	0.077	-0.338
Total Zoo-Max	0.189	-0.460	0.308	-0.001	0.203	0.234	0.234	0.261	0.348	0.246	0.394	**0.404**	0.284	0.552

Temp														
Total Zoo-Min Temp	0.2767	-0.244	-0.064	0.009	0.260	0.317	0.388	0.325	-0.077	0.268	0.183	0.063	0.342	0.161
Total Zoo-Total Rainfall	-0.018	-0.145	-0.377	-0.061	-0.038	0.295	0.061	0.236	-0.379	0.381	-0.157	-0.244	0.069	-0.283

**Interpretation of the correlation:

0 <r < 0.39 is considered low positive correlation

0.39 < r < 0.69 is considered moderate positive correlation

0.70 < r < 0.99 is considered strong positive correlation

-0.39 < r < -0.1 is considered to be low negative correlation

-0.69 < r < -0.40 is considered to be moderate negative correlation

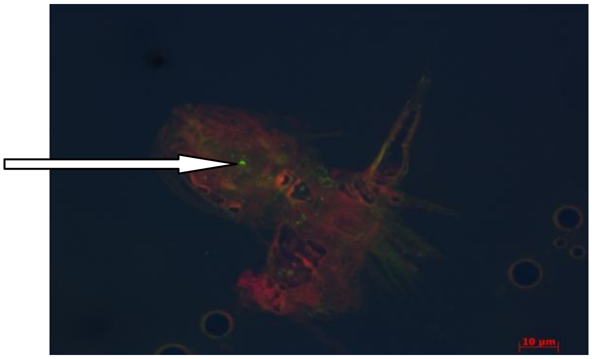
4.5 *Ex-situ* Experiments of *Vibrio cholerae* Growth with Zooplankton and Chitin Extraction

4.5.1 Association of *Vibrio cholerae* with planktonic chitin

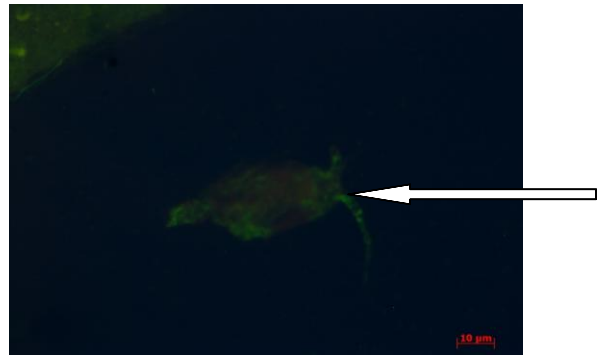
4.5.1.1 Growth of *Vibrio cholerae* in Mathbaria water micro-ecosystem (microcosm)

In Mathbaria water two sets of microcosms i.e., microcosm supplemented with feed and without any supplemented feed was set. 150copepods were released into the microcosms. Count of *V. cholerae* that was inoculated from a pure culture into the microcosm was ($1.9X10^6$) at day 0. Number of bacteria decreased with the decreased number of copepods such as after seven days of inoculation number of adult copepods was minimum. At the same time bacterial count was poor onto the counting plates. Nauplii emerged in the microcosm after eights days of rearing the plankton. Bacterial count again increased in number with the increased number of nauplii. At the end of the experiment, no. of bacteria totally depleted with the declinining no of nauplii and adult copepods (Figure 50 and Figure 51).

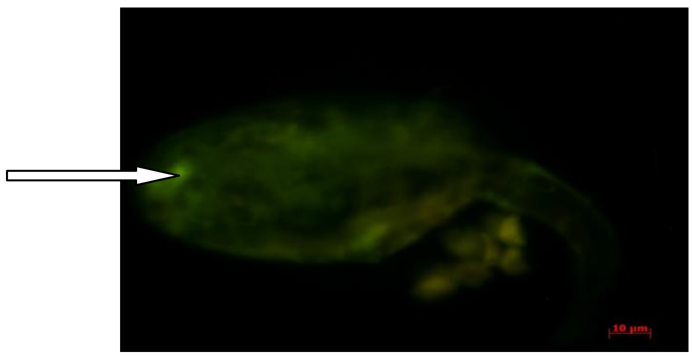
DFA count of bacteria was (Plate f) and epifluorescent micrographs showed the abundance of *V. cholerae* with the cephalothorax and antennae of copepods.



A



B



C

Plate 4. DFA images of copepods to view the attachment position of *V. cholerae* to their carrier

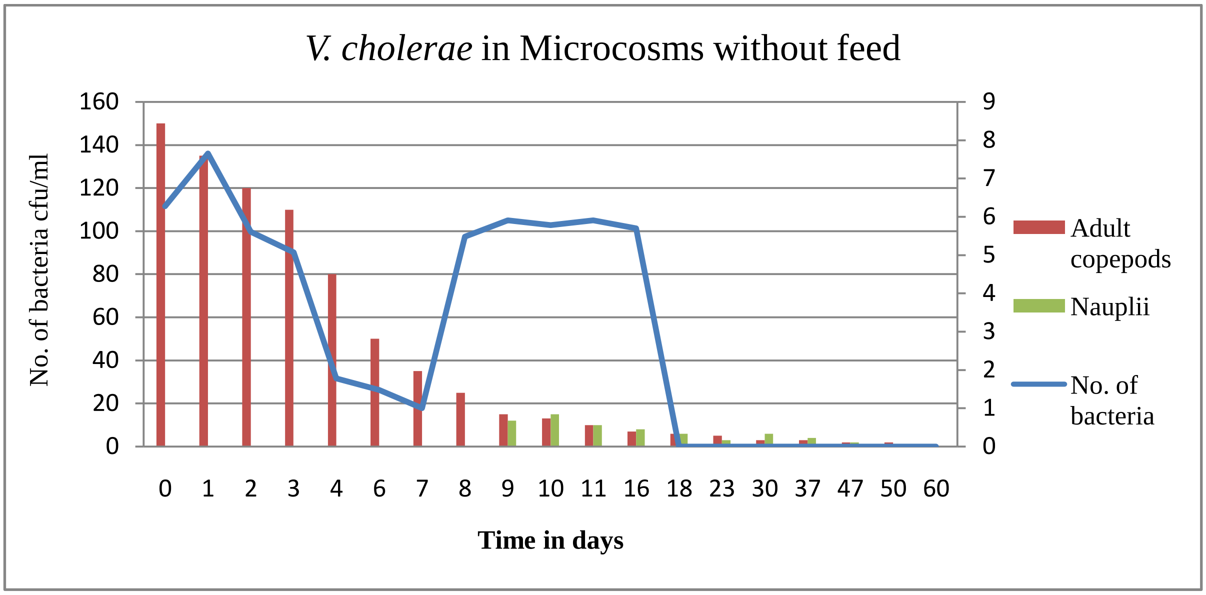


Figure 50. Growth of *V. cholerae* in Mathbaria water micrococsm (Without feed)

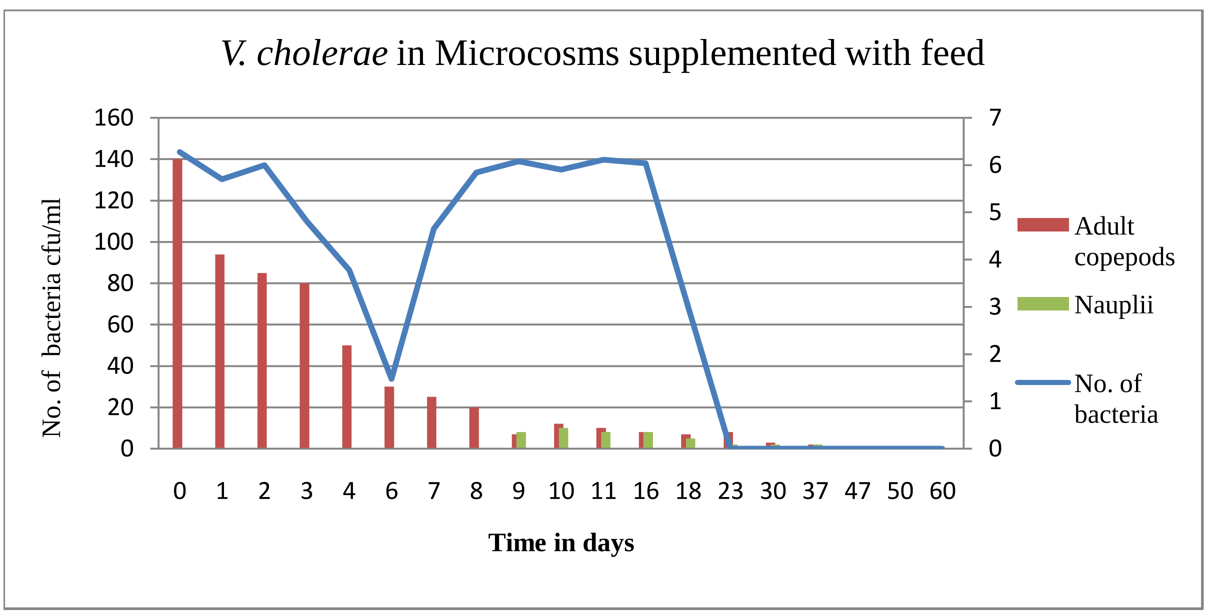


Figure 51. Growth of *V. cholerae* in Mathbaria water micrococsm (With feed)

4.5.1.2 Growth of *Vibrio cholerae* in Paikgachha water micro-ecosystem (microcosm)

Paikgachha pond water in comparison to Mathbaria had higher water salinity. Number of copepods declined quickly in both set of microcosms (PW and PW+F). Number of bacterial cells at first increased with time and then decreased. After seven days of rearing bacterial count on TTGA plate was ($4X10^5$ cfu/ml) and ($3X10^5$cfu/ml) (Figure 52 and Figure 53).

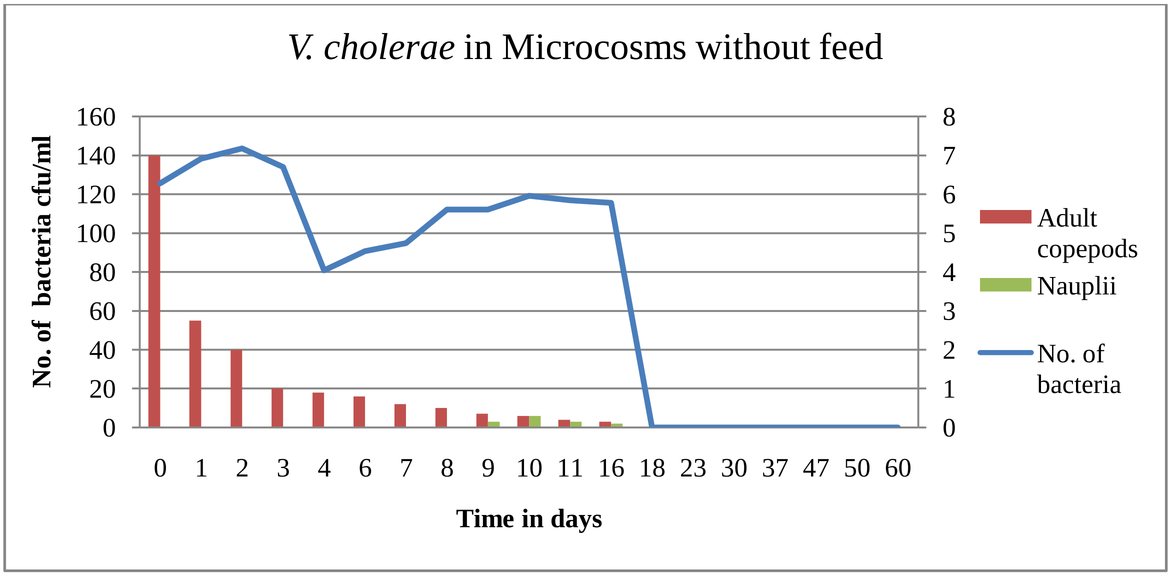


Figure 52. Growth of *V. cholerae* in Paikgachha water micrococsm (Without feed)

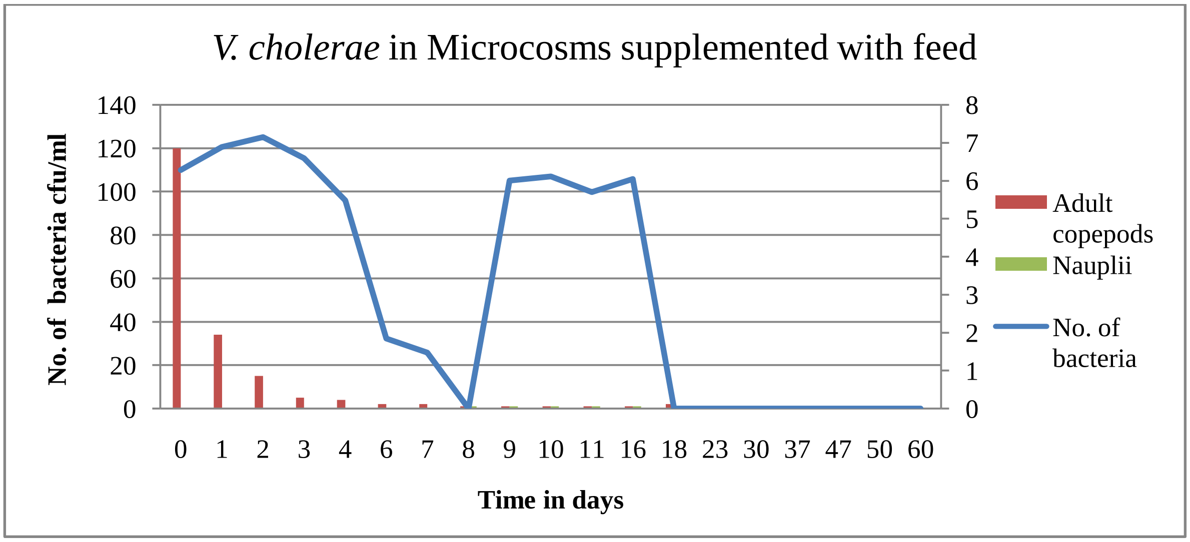


Figure 53. Growth of *V. cholerae* in Paikgachha water micrococsm (With feed)

4.5.1.3 Growth of *Vibrio cholerae* in Lake watermicro-ecosystem (microcosm)

Microcosms prepared with Dhanmondi lake water had more or less similar results with that of Mathbaria water microcosms. Number of copepods and bacterial count declined after seven days of experiment. Then the bacterial cells increased with emerging number of nauplii. This condition continued upto 16thday and then decresed (Figure 54 and Figure 55).

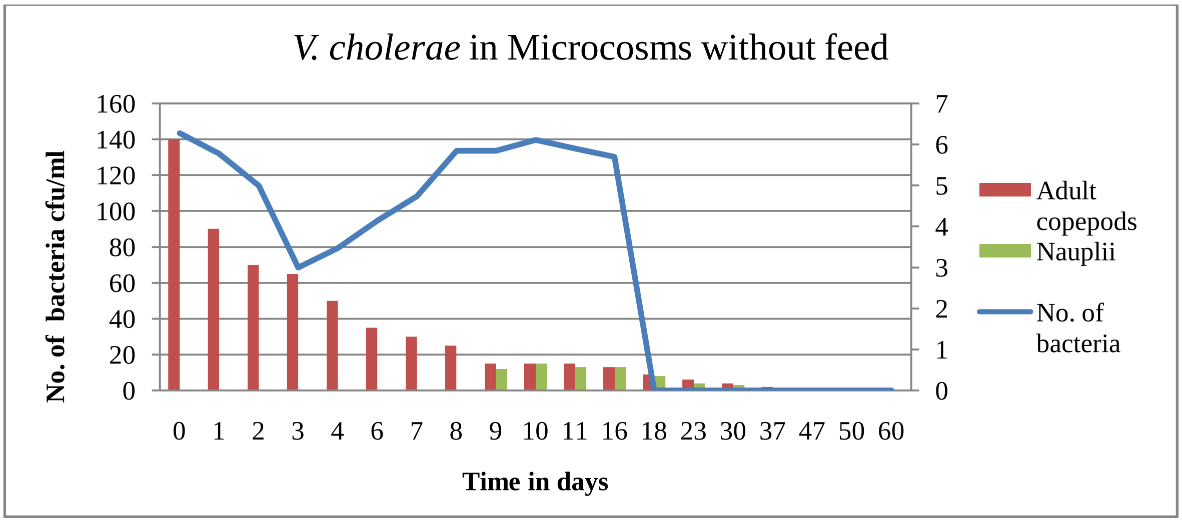


Figure 54. Growth of *V. cholerae* in Lake water micrococsm (Without feed)

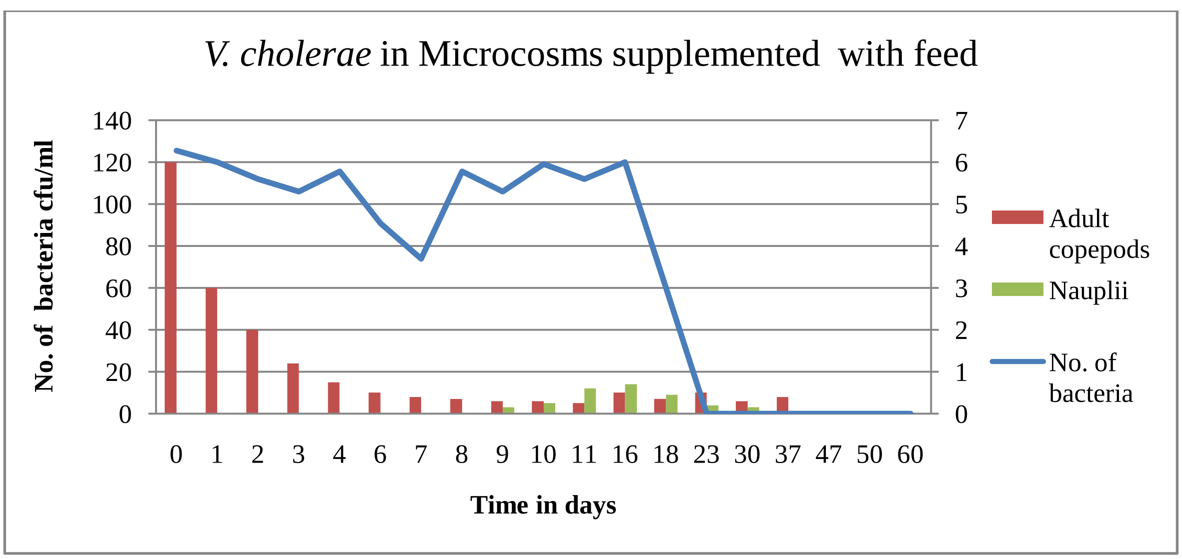


Figure 55. Growth of *V. cholerae* in Lake water micrococsm (With feed)

4.5.2 Association of *Vibrio cholerae* with crustacean chitin in micro-ecosystem study

4.5.2.1 Growth of *Vibrio cholerae* in microcosms of crab and shrimp chitin

Study of microcosms with different chitin chips were observed until the chips were fully degraded. At selected time intervals count of *V. cholerae* from each microcosm taken onto TTGA and LBagar at interval of 15 days and counts were 10^7cfu/ml on LB agar and 10^5 cfu/ml on TTGA agar 8). Bacterial plate counts differed in case of different microcosms. As, Matrhbaria and Paikgachha were two different sources of water used in the microcosms salinity of these two areas were also different. So, salinity showed an impact on the growth of *V. cholerae* in these microcosms. Bacteria in microcosms prepared with Paikgachha water had enormous population than those with Mathbaria water. On the other hand, higher growth of *V. cholerae* was observed on raw crab chitin than raw shrimp chitin, commerecial shrimp chitin and chitin powder.

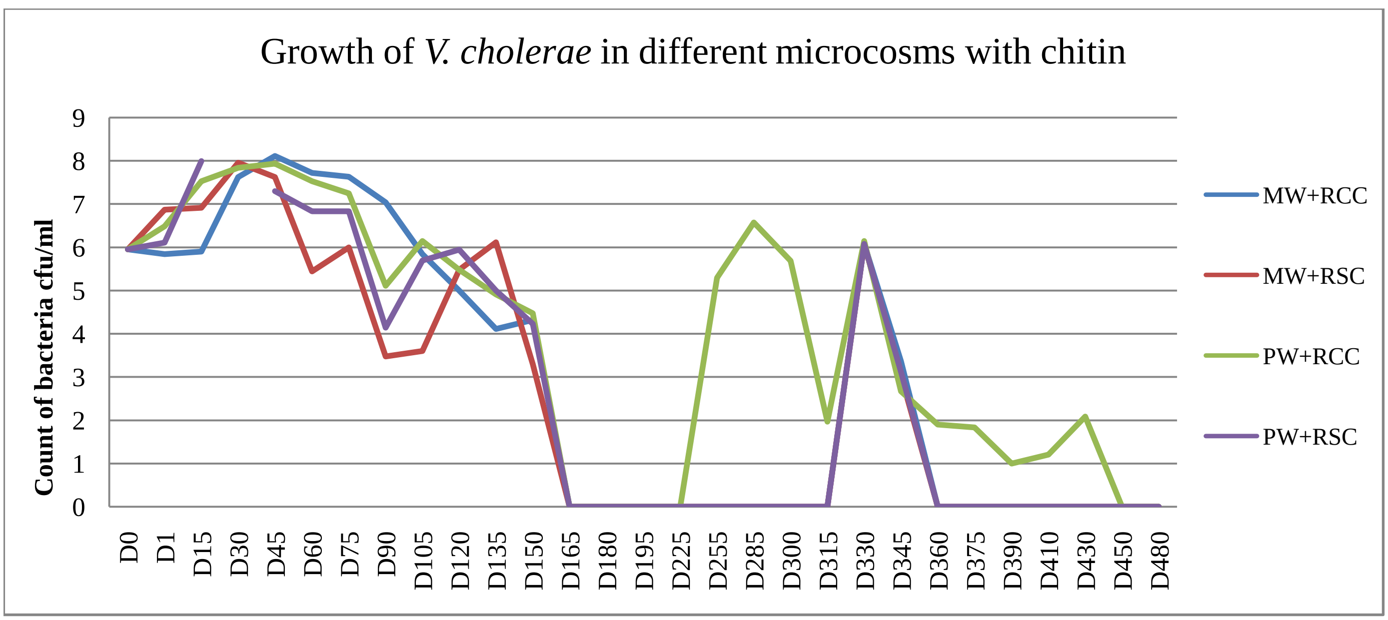


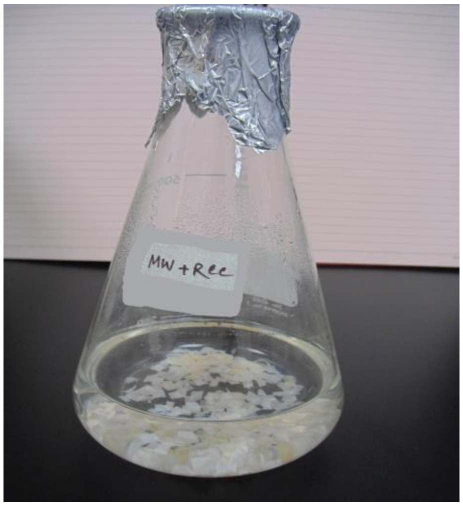
Figure 56. Association of *V. cholerae* in two different microcosms supplemented with Raw Crab chitin and Raw Shrimp chitin

At initial stage (Day 0) bacterial count on TTGA plate was $9X10^5$ cfu/ml. Counts of *V. cholerae* declined the following state. This VBNC condition found in all types of microcosms till 225th day. After seven and fifteen days later bacterial population increased. But at day 30 bacterial count continued to decrease on TTGA and LB agars. In Mathbaria water without chitin chips at day sixty no bacterial growth observed and this condition continued upto 135 days of the experiment. From 150thday bacterial growth in all microcosms were in non-culturable 255th day, 285th day and 300th day bacterial population revive and showed a better count on TTGA and LB agars.

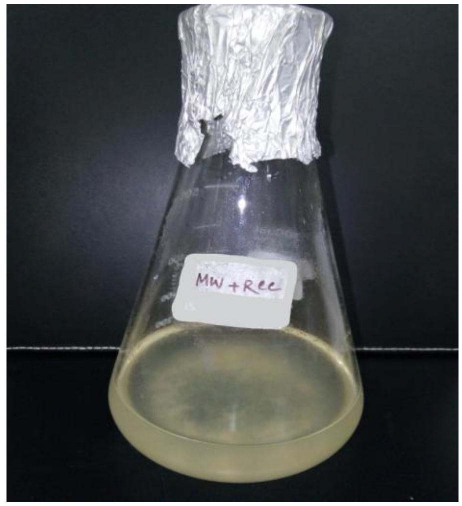
4.5.2.2 Physical observations of microcosms

Physical appearences of all microcosms in which *V. cholerae* was cultured in protected environment were clearly observed at different time interval. At initial stages, all the microcosms with chitin were transparent (Plate-5 and Plate-6). The microcosms supplemented with crab and shrimp chitin became turbid when the bacteria grew with time and this phase continued untill the chitins were fully degraded (Plate-5 and Plate-6). These conditions continued upto the day of 450 and at the last moment the degraded chips became slimy.

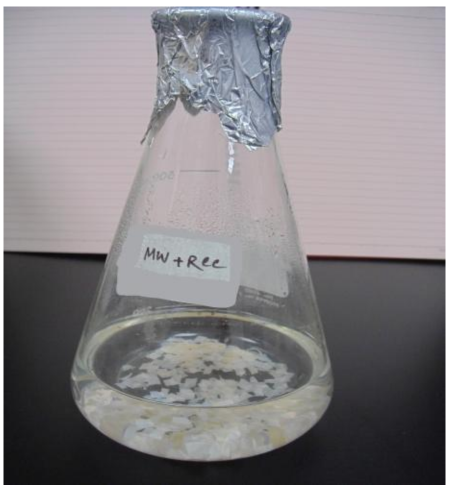
Notable thing that happened in the prepared microcosms supplemented with chitin was the earlier deterioration of the shrimp chitin (in 300 days) than the crab chitin (450 days).



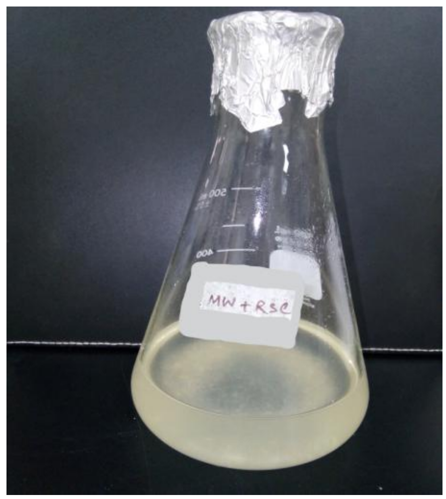
A



B



C



D

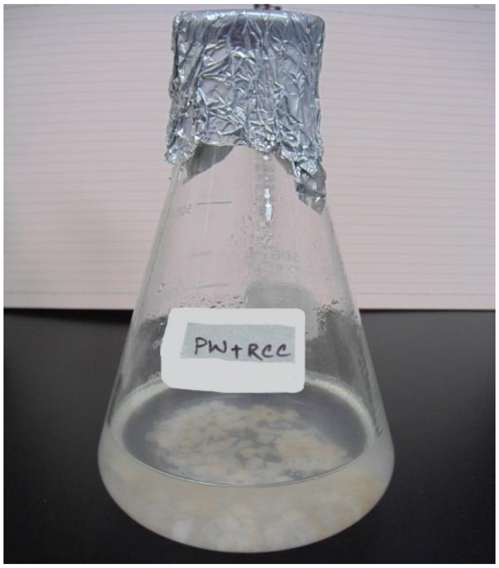
Plate 5. Physical observation of microcosms supplemented with chitin in Mathbaria water

A- Initial stage of Mathbaria Water Microcosm with Raw Crab Chitin

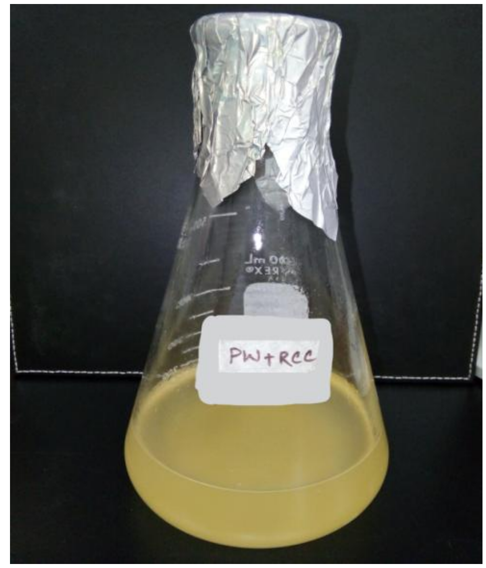
B- Final stage of Mathbaria Water Microcosm with Raw Crab Chitin

C- Initial stage of Mathbaria Water Microcosm with Raw Shrimp Chitin

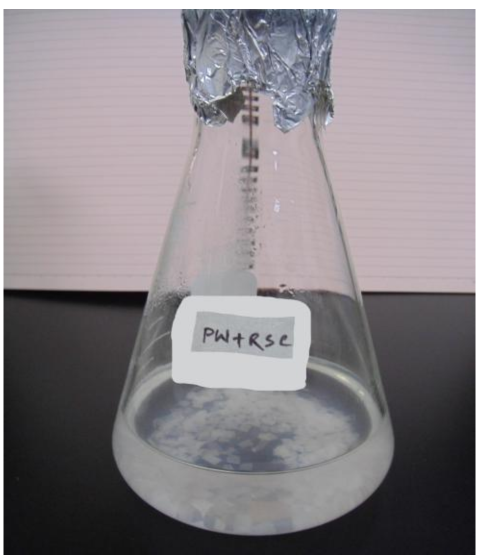
D- Final stage of Mathbaria Water Microcosm with Raw Shrimp Chitin



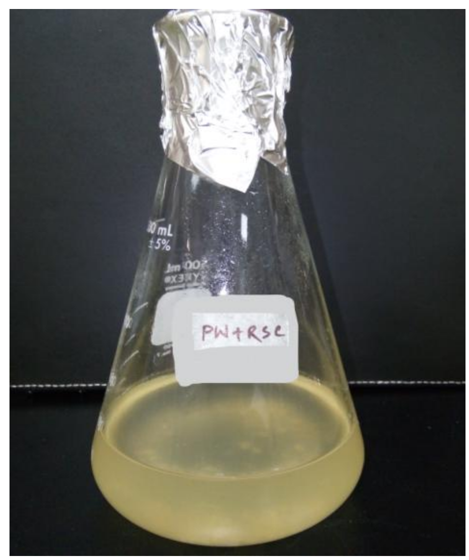
A



B



C



D

Plate 6. Physical observation of microcosms supplemented with chitin in Paikgachha water

A- Initial stage of Paikgachha Water Microcosm with Raw Crab Chitin

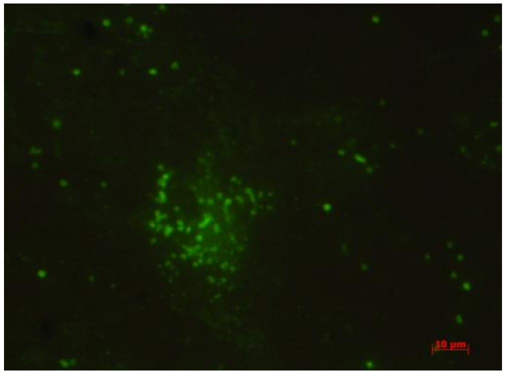
B- Final stage of Paikgachha Water Microcosm with Raw Crab Chitin

C- Initial stage of Paikgachha Water Microcosm with Raw Shrimp Chitin

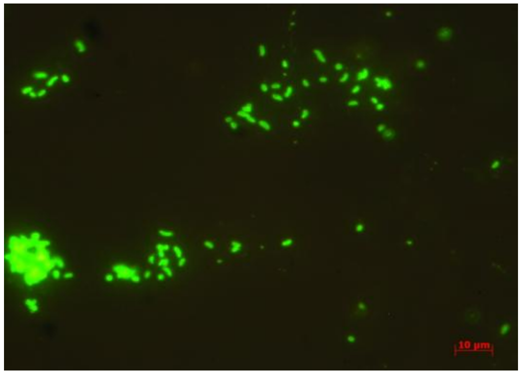
D- Final stage of Paikgachha Water Microcosm with Raw Shrimp Chitin

4.5.2.3 *Vibrio cholerae* in chitin supplemented micro-ecosystems: A DFA image study

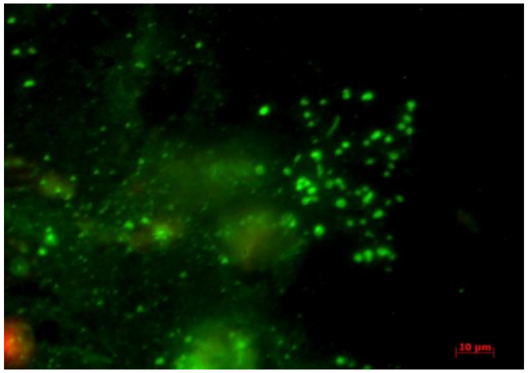
Biofilm formation is the ultimate stage of the lifecycle of *V. cholerae* where they remain non-culturable and dormant in any unfavourable environmental conditions. In the four experimental microcosms *V. cholerae* O1 became dormant and protected from the outer adverse conditions of the aquatic media.



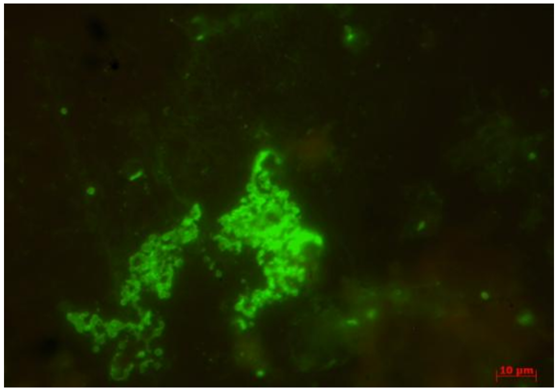
Stage-1



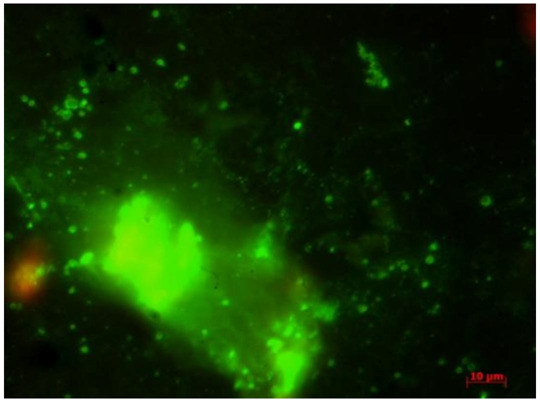
Stage-2



Stage-3

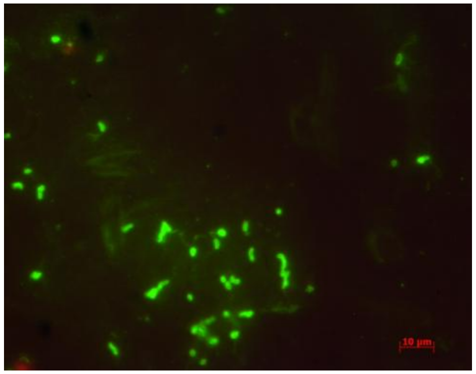


Stage-3

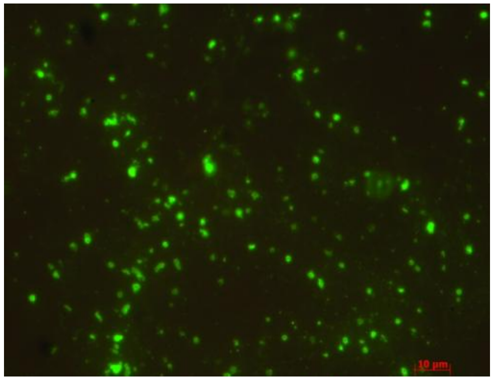


Stage-4

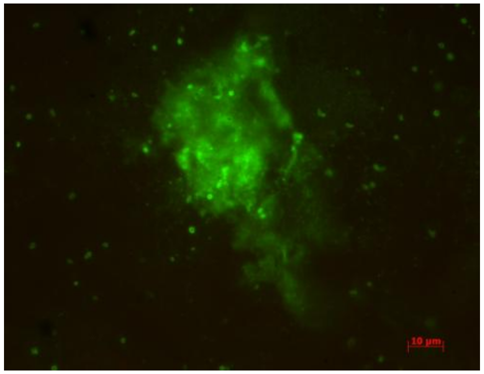
Plate 7. Epifluorescent micrographs of different stages of Biofilm formation of *V. cholerae* in Mathbaria water microcosms supplemented with Raw Crab Chitin



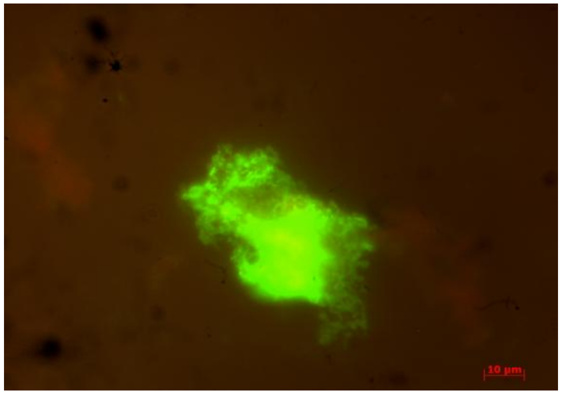
Stage-1



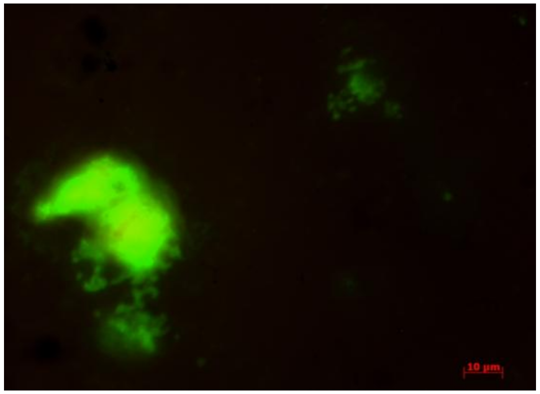
Stage-2



Stage-3

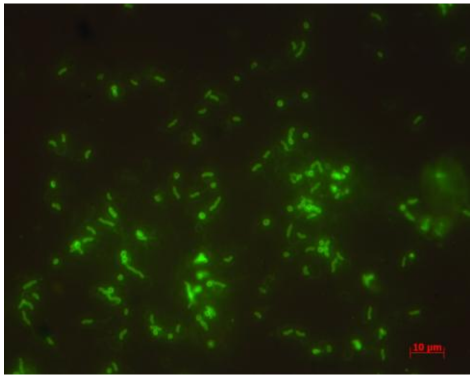


Stage-3

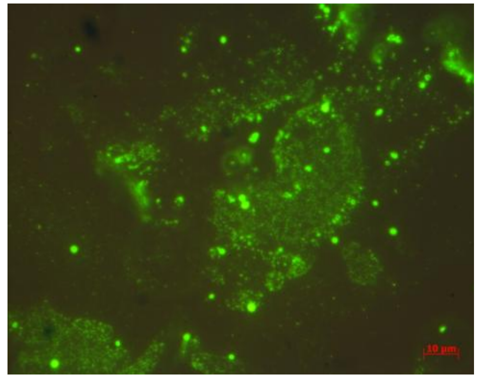


Stage-4

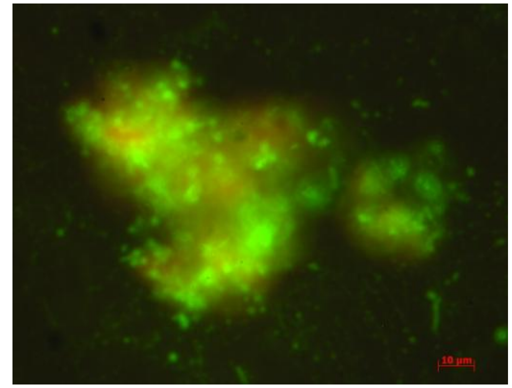
Plate 8. Epifluorescent micrographs of different stages of Biofilm formation of *V. cholerae* in Mathbaria water microcosms supplemented with Raw Shrimp Chitin



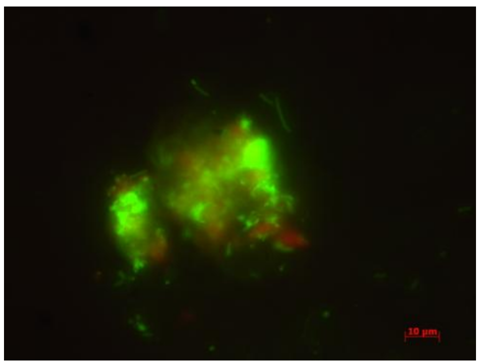
Stage-1



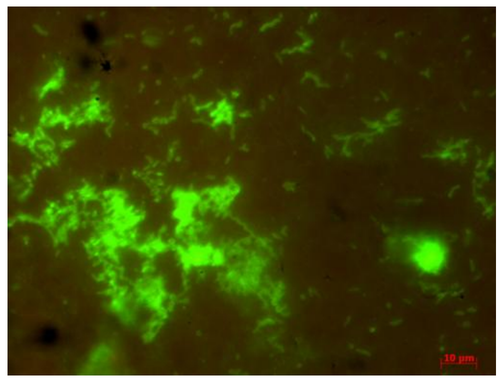
Stage-2



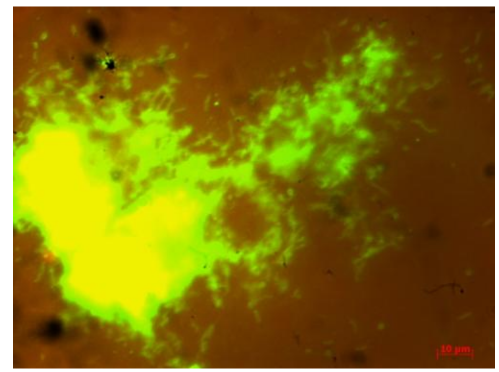
Stage-3



Stage-4

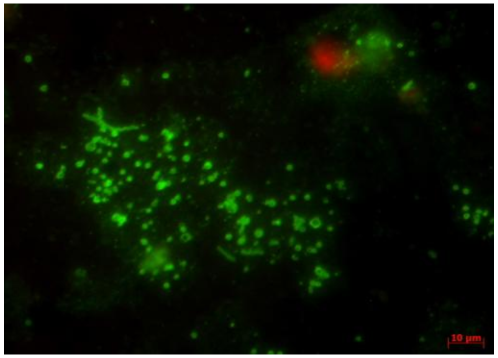


Stage-5

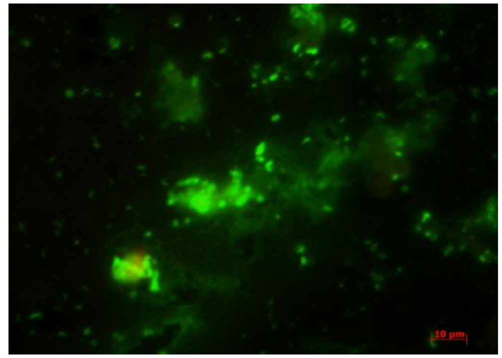


Stage-6

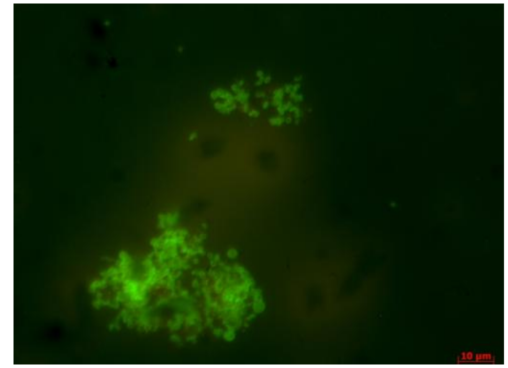
Plate 9. Epifluorescent micrographs of different stages of Biofilm formation of *V. cholerae* in Paikgachha water microcosms supplemented with Raw Crab Chitin



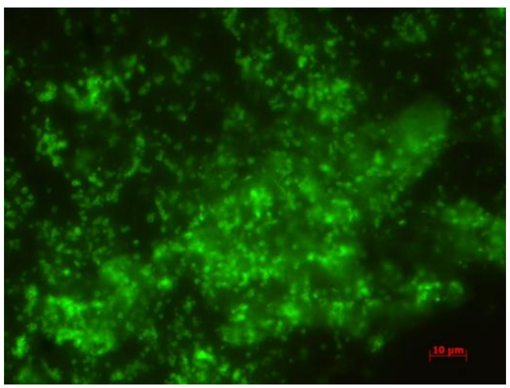
Stage-1



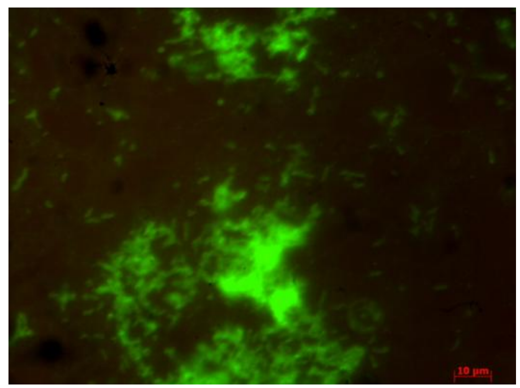
Stage-2



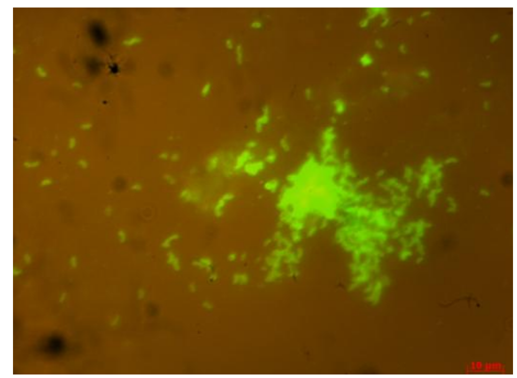
Stage-3



Stage-4



Stage-5



Stage-6

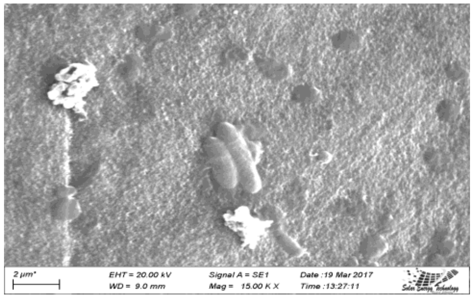
Plate 10. Epifluorescent micrographs of different stages of Biofilm formation of *V. cholerae* in Paikgachha water microcosms supplemented with Raw Shrimp Chitin

4.5.2.4 Association of *Vibrio cholerae* with different chitin structures in micro-ecosystems: A Scanning Electron Microscope (SEM) image study

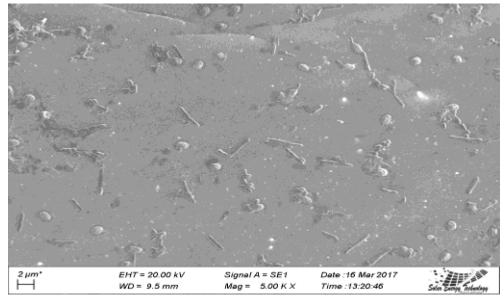
Scanning Electron Microspic Images (Plate-11-14) of the cultured *V. cholerae* in thye microcosms were taken where the attachment were visibly proved. Type of attachment was different in different microcosms as the medium and the supplemented chitin was also from two different sources as mentioned earlier.

Attached *V. cholerae* with crab and shrimp chitin in microcosms took different time duration to form biofilm. After about 450 days of culture in microcosms (Mathbaria water and Paikgachha water) supplemented with raw crab chitin *V. cholerae* O1 became non-culturable.

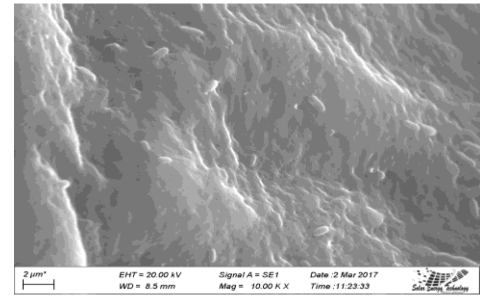
On the otherhand, *V. cholerae* in the microcosms attached with raw shrimp chitin had the formation of biofilm in about 300 days of culture which is 100 days less than that of crab chitin. So, it is assumed that degradation of shrimp chitin is faster than that of crab chitin.



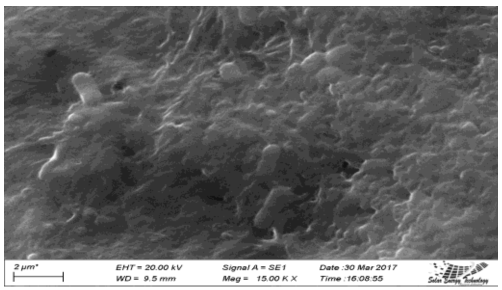
Day-1



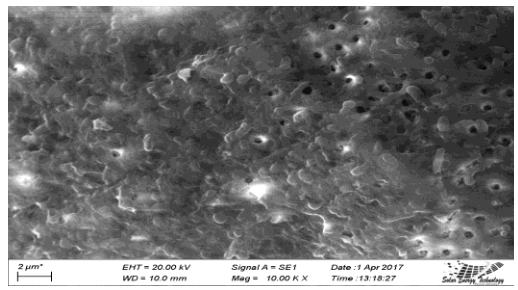
Day-15



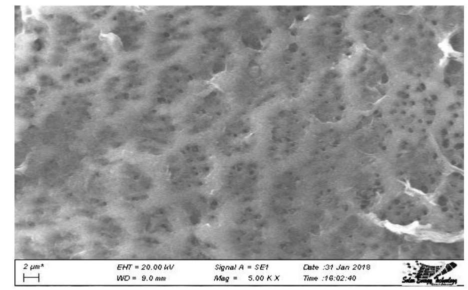
Day-30



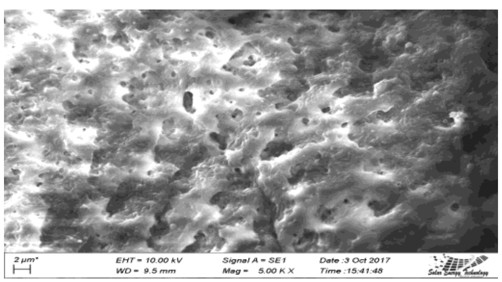
Day-60



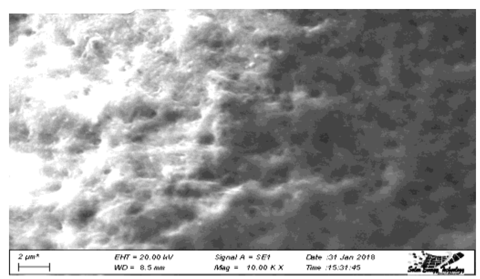
Day-120



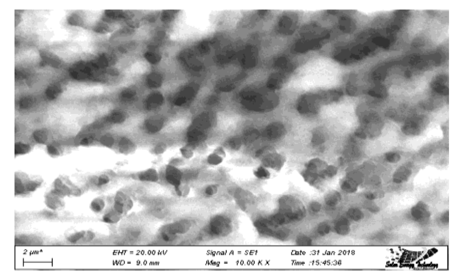
Day-210



Day-280

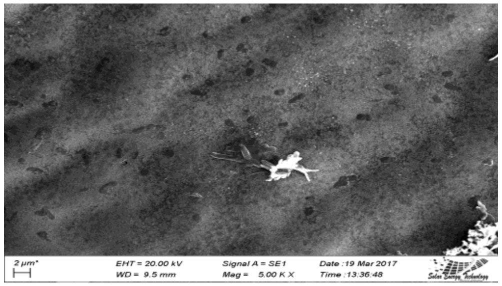


Day-365

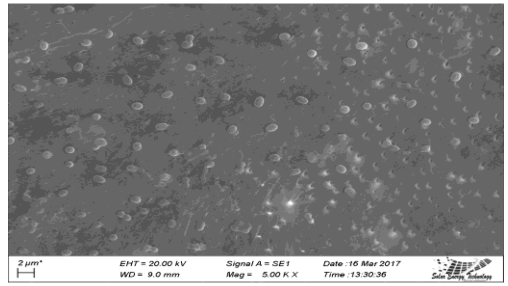


Day-450

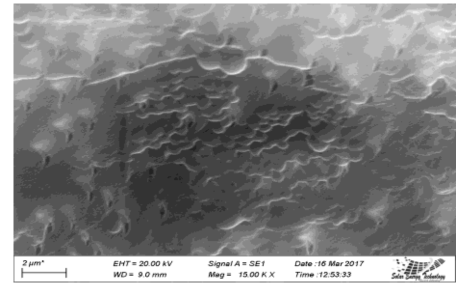
Plate 11. Scanning Micoscopic Images of the Attachment of *V. cholerae* with Raw Crab Chitin in Mathbaria water microcosm at room temperature



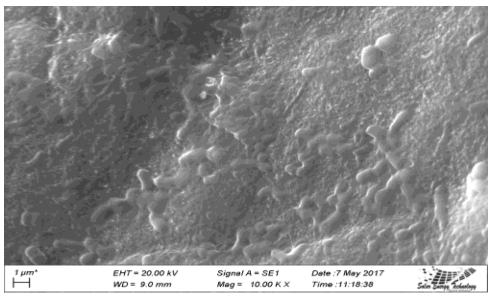
Day-1



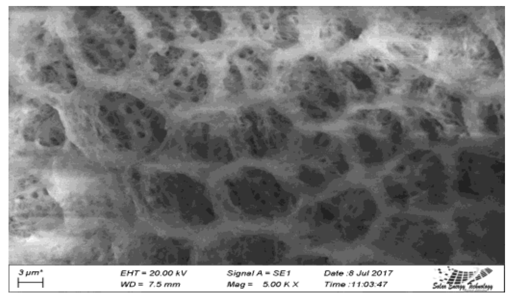
Day-15



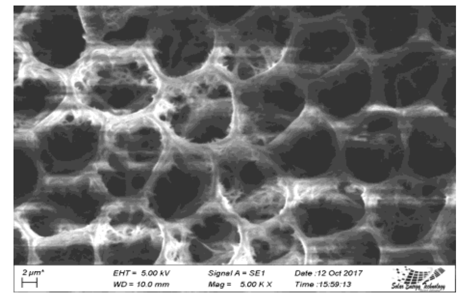
Day-30



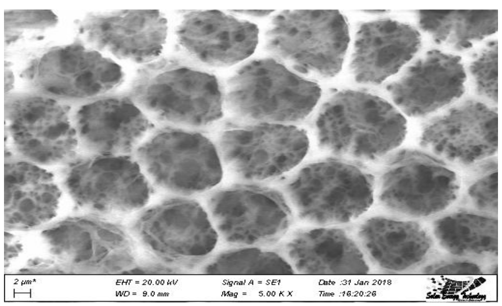
Day-60



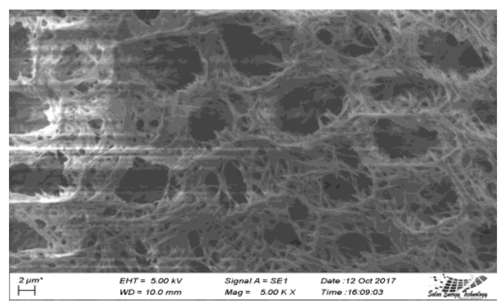
Day-120



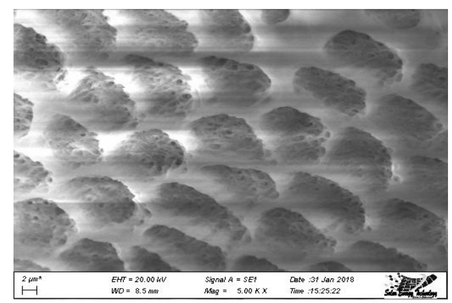
Day-165



Day-280

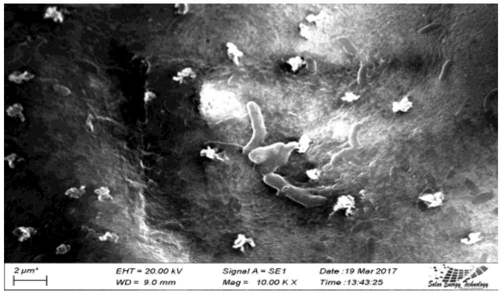


Day-300

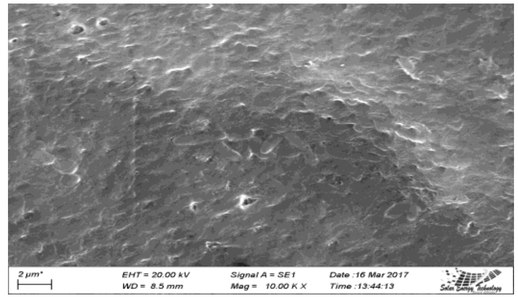


Day-365

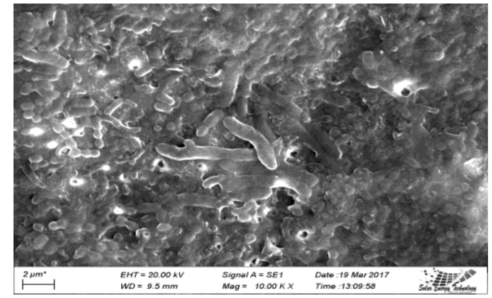
Plate 12. Scanning Micoscopic Images of the Attachment of *V. cholerae* with Raw Shrimp Chitin in Mathbaria water microcosm at room temperature



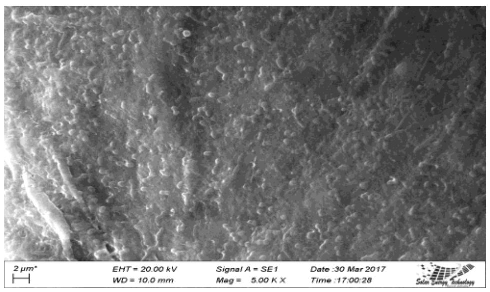
Day-1



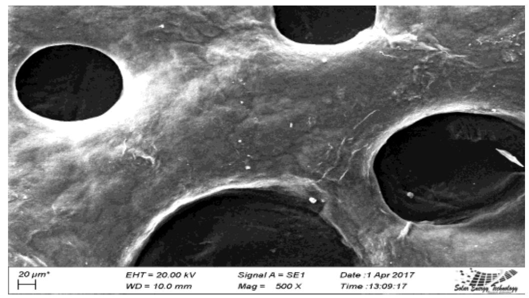
Day-15



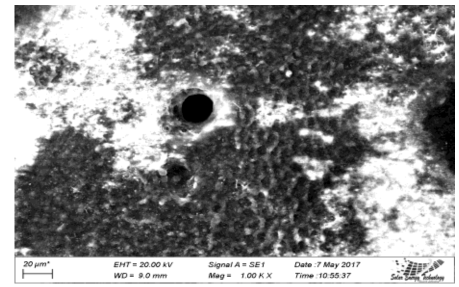
Day-45



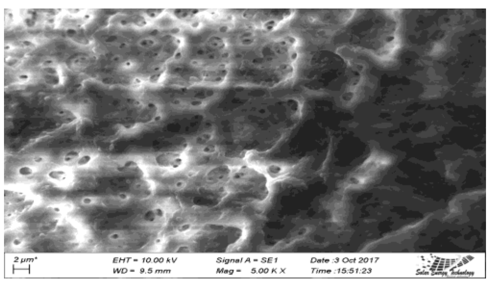
Day-90



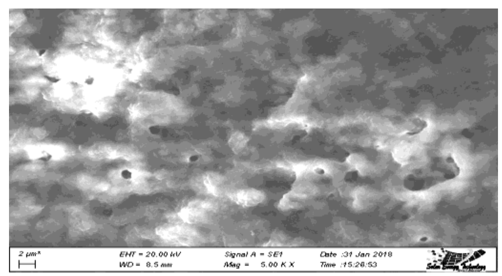
Day-150



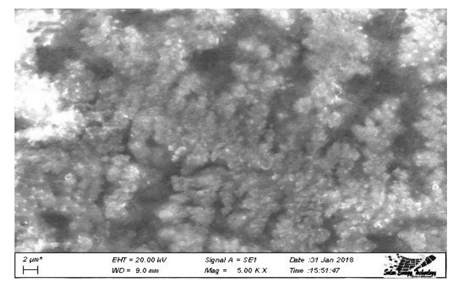
Day-180



Day-280

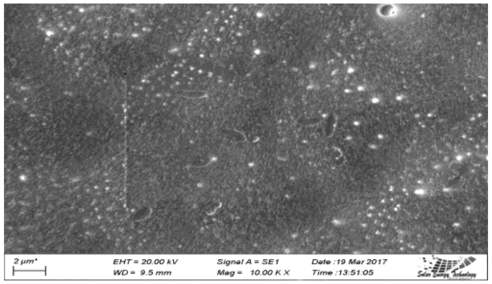


Day-365

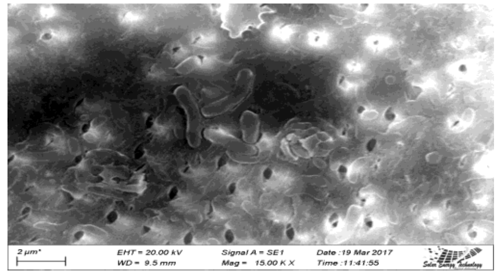


Day-450

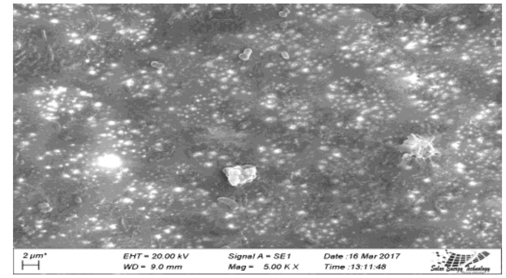
Plate 13. Scanning Micoscopic Images of the Attachment of *V. cholerae* with Raw Crab Chitin in Paikgachha water microcosm at room temperature



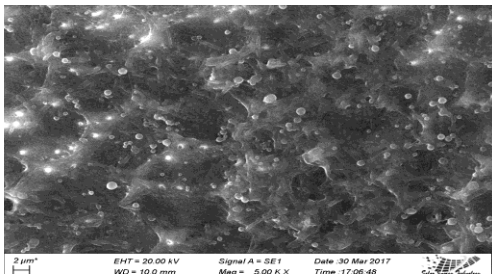
Day-1



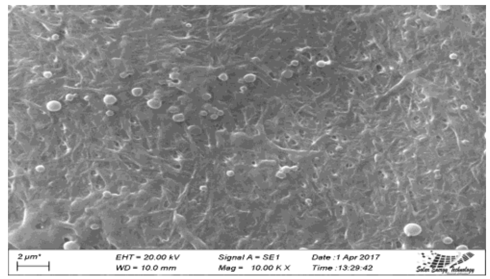
Day-15



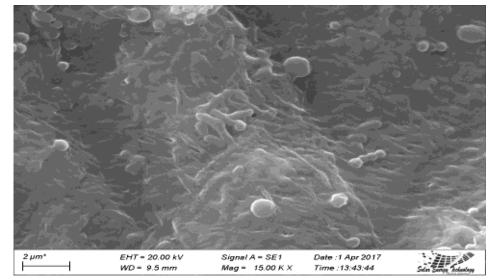
Day-30



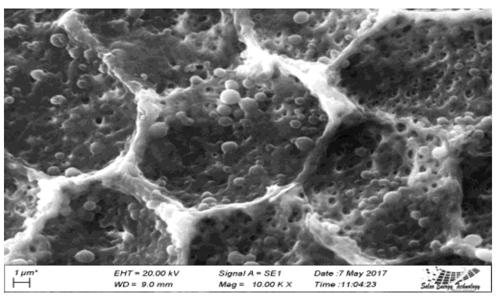
Day-90



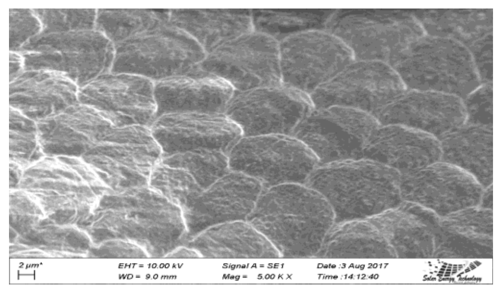
Day-120



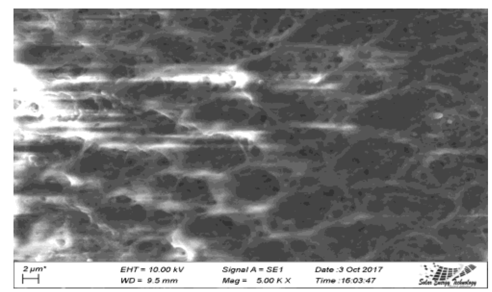
Day-150



Day-180



Day-210



Day-300

Plate 14. Scanning Micoscopic Images of the Attachment of *V. cholerae* with Raw Shrimp Chitin in Paikgachha water microcosm at room temperature

4.6 Bacterial Colony Growth in Some Crab Samples

Some colony of bacteria was observed on TTGA plates which were tested with *Vibrio cholerae* O1 antiserum for the confirmation of toxic vibrio causing cholera disease. But no toxic vibrio i.e., *Vibrio cholerae* O1 found in the crab samples from Sundarbans but few non toxic vibrio i.e., *Vibrio cholerae* non O1 were found to exist in the collected crab samples which are responsible for diarrheal disease among the fisherman in those villages.

4.7 Availability of Nutrients in Three *Vibrio cholerae* Inhabiting Ponds of Mathbaria

V. cholerae the prime agent for causing horrible cholera needs some sort of nutrients for their survival and infectious activity. In Mathbaria, Nitrogen and Phosphorus amount as well as some other micronutrients were available in those infectious ponds of Mathbaria during peak season of cholera. During the study perid, water sample of three heavily infected ponds were analyzed to observe the nutrients level for the production of primary producers (blue-green algae) which influence the growth and abundance of zooplankton to act as the reservoir of *V. cholerae.*

Total nitrogen content during the peak season of cholera was (0.812-0.504), (0.448-0.478) and (0.523-0.578) for site-2, site-8 and site-11 respectively. Phosphorus amount ranged between (28.8-34.4), (29.6-29.56) and (28.3-31.2) in the site-2, site-8 and site-11 sequentially. These amounts were higher than those measured in another peak season of cholera. On the otherhand, zinc, iron and manganese were minimum or below detection limit during the seasonal abundance of cholera. Magnesium was higher ranging (8.96-11.13), (17.78-18.50) and (10.16-10.35) at site-2, site-8 and site-11 respectively.

Table 46. Amount of micronutrients analyzed of some infected ponds in Mathbaria during the peak season of cholera

Contaminated ponds	Cholera Infection period	Total Nitrogen (ppm)	Phosphorus (mg/100g)	Zinc (ppm)	Iron (ppm)	Magnesium (ppm)	Manganese (ppm)
Site-2	April	0.812	28.8	<0.01	0.011	11.13	<0.01
	May	0.504	34.4	<0.01	<0.01	8.96	<0.01
	October-1st week	0.315	25.2	0.672	1.159	4.466	0.001
	October-3rd week	0.238	25.20	.0217	1.150	3.654	0.072
Site-8	April	0.448	29.6	<0.01	0.014	18.50	<0.01
	May	0.478	29.56	<0.01	<0.01	17.78	<0.01
	October-1st week	0.175	29.10	0.011	0.937	3.641	0.774
	October-3rd week	0.175	18.60	BDL	0.129	3.826	0.085
Site-11	April	0.523	28.3	<0.01	0.013	10.35	<0.01
	May	0.578	31.2	0.089	0.0335	10.16	<0.01
	October-1st week	0.280	21.00	0.0117	0.967	3.633	0.096
	October-3rd week	0.252	22.50	0.0117	0.397	3.612	0.023

4.8 Relationships with Nauplii Biomass in Ponds During Infection Periods

In Mathbaria, site-2 (Jotishkanti Bepari's Pond), site-8 (Mathbaria Canal) and site-11 (Commissioner Bari Pond) were identified as contaminated water sources for *Vibrio cholerae* infection. They are shown in solid lines in the Figures 57 and 58 for sampling year 2013 and 2014. Except Mathbaria canal (Site 8) in 2013 the nauplii were available in highest in biomass in the contaminated and non-contaminated waterbodies.

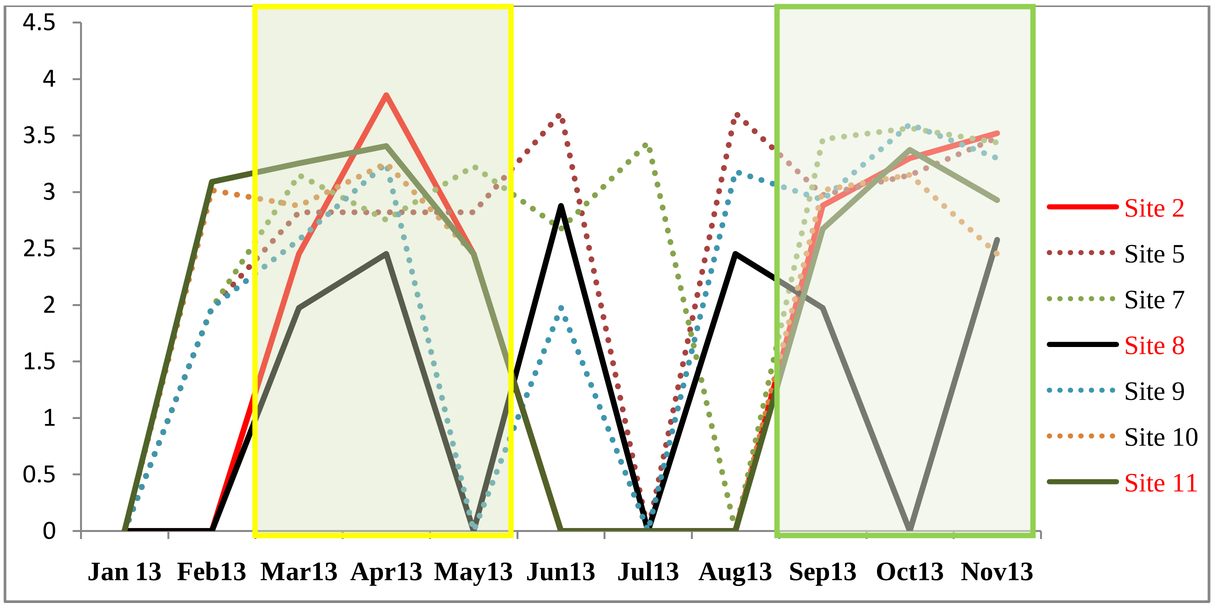


Figure 57. Mathbaria nauplii data, showing the monthly weight (log) distributions and their relationships with two infection seasons and the ponds (solid line sites 2, 8 and 11 were the contaminated ponds) in 2013. The non-contaminated pond data were shown as in dotted line. March to May and September to November were the infected season of Mathbaria. The December sampling was missing due to strike in communication. The highlighted zone is the disease outbreak period of the sampling sites.

In 2014, the biomass of Nauplii showed the similar trends for abundance. The contaminated and non-contaminated ponds water sources were evitable at peak for spreading the bacteria. Thus the occurrence of nauplii biomass can initiate the infection of *Vibrio* in the ecosystem along with other factors. As the other water bodies (in dotted lines) were found to be rich in nauplii biomass, the contamination could aid in spreading the *V.cholarae* infection in the area.

Except Mathbariacanal in October 2013, minimum biomass was available at 94.3 g per cubic meterof nauplii in contaminated and non-contaminated ponds. There may be some other reasons in lowering the nauplii in the canal (site 8) in Mathbaria.

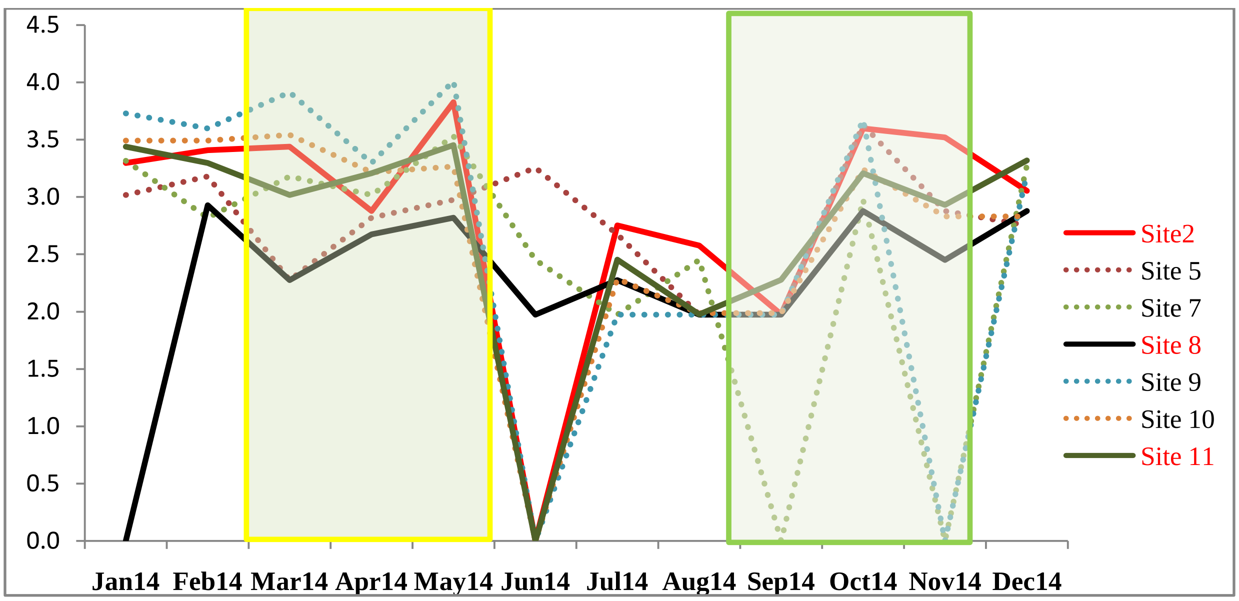


Figure 58. Mathbaria nauplii data, showing the monthly weight (log) distributions and their relationships with two infection seasons and the ponds (solid line sites 2, 8 and 11 were the contaminated ponds) in 2014. The non-contaminated pond data were shown as in dotted line. March to May and September to November were the infected season of Mathbaria. The highlighted zone is the disease outbreak period of the sampling sites.

In Chattak, site-1 (Govt. Pond near THC), site 10 (Surma River Ghat-2: Cement factory ghat) and site-12 (Mondolibhog Girl's High School Pond) were identified as contaminated water sources for *Vibrio cholerae* infection. They are shown in solid lines in the Figures 59 and 60 for sampling year for 2013 and 2014. All contaminated and non-contaminated ponds showed single highest peak of biomass in the seasons.

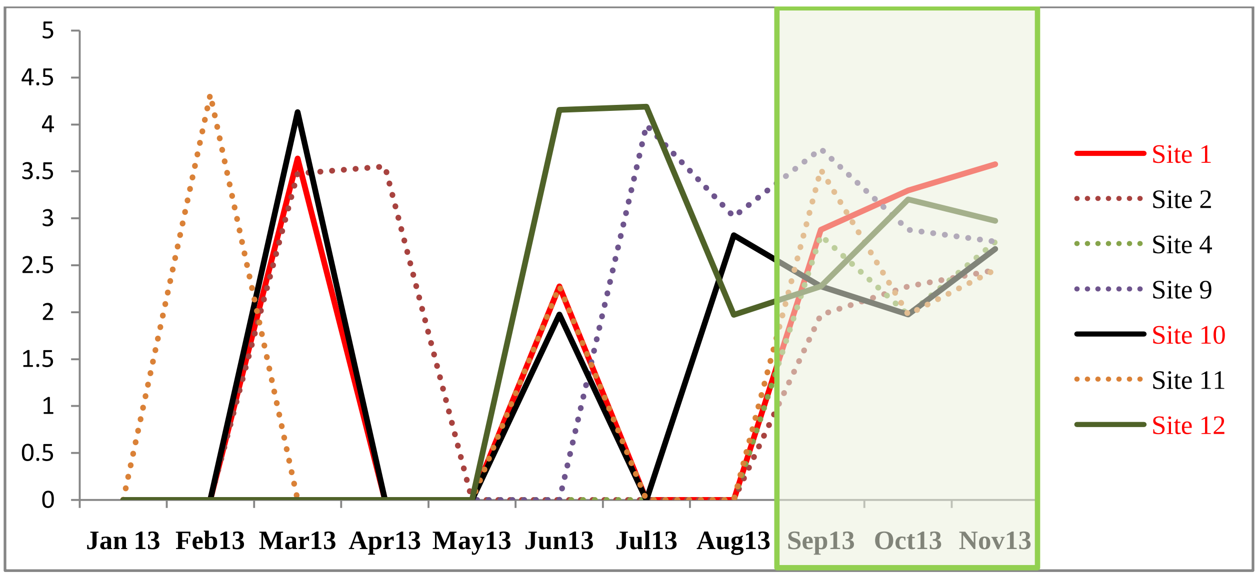


Figure 59. Chattak nauplii data, showing the monthly weight (log) distributions and their relationships with single infection season and the ponds (solid line sites 1,10 and 12 were the contaminated ponds) in 2013. The non-contaminated pond data were shown as in dotted line. September to November were the infected season of Chattak. December sampling was missing due to communication strike. The highlighted zone is the disease outbreak period of the sampling sites.

In 2014, the biomass of Nauplii showed the same trends for abundance in all waterbodies. The contaminated and non-contaminated ponds water sources were evitable to be at peak in naupliibiomass in September to November, the cholera spreading season. However, the peak was again varied throughout the year in the waterbodies in the Chattak area. In 2013, two earlier peak were observed in March and June, while in 2014 were in April and onwards. The occurrence of nauplii biomass can only be maintained in September to November to initiate the infection of *Vibrio* in the ecosystem along with other factors. As the ecosystem was fresh water, the occurance of infection may take other factors to initiate to the process in the vicinity. Other water bodies (in dotted lines) were also found to be rich in nauplii biomass, any contamination in the non-infectious pond ecosystem could aid in spreading the *V. cholarae* infection in the area. In Chattak, minimum biomass of naupliiwas available at 94.3g per cubic meterin contaminated and non-contaminated ponds.

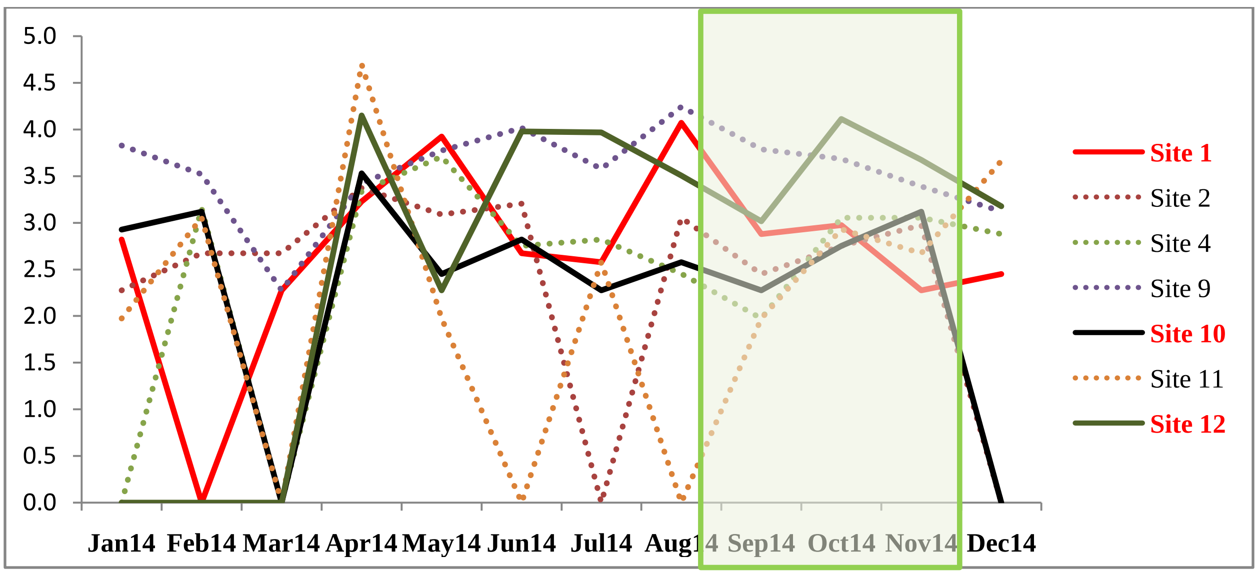


Figure 60. Chattak nauplii data, showing the monthly weight (log) distributions and their relationships with single infection season and the ponds (solid line sites 1, 10 and 12 were the contaminated ponds) in 2014. The non-contaminated pond data were shown as in dotted line. September to November were the infected season of Chattak. The highlighted zone is the disease outbreak period of the sampling sites.

Chapter-5. Discussions

While it is likely to have been responsible for human infections and mortality throughout human history, cholera outbreaks have only been formally known to science since 1817 (Pollitzer, 1959). Sir John Snow was credited in 1849 as being the first person to connect contaminated water with cholera outbreaks and to use that information as an infection control strategy (Snow, 1855). It took 120 years for *V. cholerae* to be recognized as an autochthonous aquatic bacterium rather than a human pathogen that is a transient resident of the aquatic environment (Colwell *et al.*, 1977) though Sir John Snow was the first to study on the ecology of *V. cholerae.*

Vibrio cholerae inhabits a vast geographical range from the tropics (e.g., the Bay of Bengal where pandemics still occur (Albert *et al.,* 1993; Huq *et al.,* 2005; de Magny *et al.,* 2011). To temperate waters world-wide e.g., USA, South America, Australia, Sweden, and Italy (Vezzulli *et al.*, 2011; Islam *et al.*, 2013; Tall *et al.*, 2013).

A variety of biological surfaces in water can bind bacteria. Bacteria associated with surfaces have been shown to survive in aquatic environments for longer times than suspended forms (Kirchman and Mitchell, 1982; Pedros and Brock, 1983), possibly as an adaptation of a bacterium to the stressful effects of low nutrient levels (Dawson *et al.*, 1981).

Huq *et al.* (1984) hypothesized that an important aspect of the ecology of *V. cholerae* O1 in cholera-endemic regions of Bangladesh may involve a relationship with plankton, supporting previous hypothesis that interepidemic reservoirs of *V. cholerae* O1 in Bangladesh are influenced by seasonal plankton blooms that accompanies cholera epidemics.

Vibrio cholerae is among the most intensively studied of those bacteria pathogenic for humans including its genetics, physiology and ecology (Faruque *et al.*, 1998). The *V. cholerae* connection with chitin is an extensively documented phenomenon and for microbial ecology, one of the most abundant biopolymers in nature, and perhaps the most abundant in the marine environment (Gooday, 1990). *V. cholerae* strains possess multiple strategies for surface colonization depending upon the presence and expression of both conserved and variables genes (Mueller *et al.*, 2007).

Limnological factors of the ponds are related to the cholera infection. According to Roberts *et al.*(1984) during the peak seasons of cholera water temperature was high as it is favourable for survival of *V. cholerae* in water in the environment. Previous study also shows the similar observations regarding the water temperature of that region. pH of Mathbaria during the study period showed positive correlation with the onset ofcholera but salinity was negative during cholera infectious period. Jutla *et al.* (2017), showed that about 50% or more cholera outbreaks occurred when the air temperature is >31ºC which was also accompanied by poor water quality, lack of sanitation infrastructure andrainfall as well.

During the study period 27 species of protozoan plankton, 43 species of rotifer, 8 species of copepoda and 8 species of cladoceran plankton were recorded in the coastal region of Mathbaria. Mozumder *et al.* (2011) identified total 46 species of zooplankton from Mathbaria ponds where 6 species were protozoan, 34 species were rotiferan, 3 were copepods and 2 were cladoceraans. Mozumder *et al.* (2010) found 4 taxa of protozoa, 31 rotiferan taxa, 5 taxa of copepod and 5 taxa of cladocera in another coastal region of Bangladesh, Bakerganj. Fresh water area of Chhatak had 14 species of protozoa, 58 species of rotifer, 9 species of copepod and 19 species cladoceran plankton which is diversified than Mathbraia.

In Mathbaria, among planktonic protozoan highest abundance was found for *Trinema complanatum* (79%) at Kundubari pond (site-2) in the rainy season followed by *Arcella vulgaris* (33%) and (31%) during summer and autumn and *Arcella discoides* (28%) during summer respectively at Mathbaria canal (site-8). The wheel organ bearing plankton rotifera was distributed diversely in almost all ponds of Mathbaria. Highest abundand rotifer was found as Polyarthra sp. in autumn (33%) and summer (31%) at Brack pond (site-5). Crustacean copepods showed maximum abundance in rainy season at Mosjid pond (site-9) which was60%, though in other ponds their percentage was high than the other copepod species. *Diaphanosoma sp.*, another crustacean plankton also showed maximum abundance (43%) in rainy season at Commissioner Bari pond (site-11). Mozumder *et al.* (2011) found the maximum relative abundance (31.56%) for *Difflugia sp.* at South Mithakhali pond (site-1) and minimum (0.02%) *Trichotria tetractis* at Kachishori pond (site-2) in Mathbaria. In contrast, the relative abundance of species in Bakerganj, was maximum (19.23%) for *Polyarthra vulgaris* at Harun Dakua's pond (site-3) and minimum (0.02%) for *Rotaria neptunia* and *Cyclops vernalis* at Thana health complex pond (site-1) and Harun Dakua's pond (site-3) respectively.

In the present study with Chhatak, maximum abundance was shown in *Ceratium hirudinella* (99%) at Commissioner Bari pond (site-9) in summer. *C. hirudinella* was also distributed promptly in other seasons of the same pond. Second highest abundant species in Chhatak was *Euglena acus* in summer season.Among rotifer highest abundance was shown in *Brachionus sp.* (54%) during winter at Chorer bondo pond (site-12). *Cyclops nanus* was most abundantly found (33%) at River Surma (site-2) in summer.

In the present study, highest species composition was observed from protozoan (54.5%) then in copepoda (46.1%) and lowest was cladoceran (19.2%) which did not match with the study of Oscar (2013).Again relative abundance of the current study showed that the highest abundance was recorded in protozoa and rotifer in some ponds of Mathbaria. In Chhatak, protozoa had the highest abundance (100%) in particular pond whereas rotifer was the second in position. According to Oscar (2013) species composition was found to be highest in Copepod (45.45%) followed by Cladocera (25%). On the other hand, relative abundance was also measured as 64.52% in copepod and 29.46% in Cladocera.

Dipankar and Biswas (2014) found copepod and rotifer to be dominated during monsoon in Oxbow Lake. And two species of copepoda (*Diaptomus sp.* with 45.45% and *Eucyclops sp.* with 27.27%) and three species of rotifera (*Keratella sp.*, *Platyias sp.* and *Ascomorpha sp*. each with 9.09%) among total zooplankton were found in monsoon.

Two *Diaptomus sp.* were abundant than the species of *Cyclops sp.* and *Diaphanosoma sp.* among cladoceran in Mathbaria. In Chhatak, abundance of *Cyclops sp.* was strong enough than the *Diaptomus* among the crustacean copepods. Several species of planktonic cladoceran were recorded in Chhatak, of them two species of *Bosmina* (*B. coregoni* and *B. longirostris*) and *Diaphanosoma sp.* was commonly distributed in all studied ponds. This is similar to the findings of Tamplin *et al.* (1990) who also recorded that *Diaptomus sp.* were abundant and *Cyclops sp.* were lower in numbers. A *Bosminopsis sp.* was the dominant cladoceran, with some *Daphnia sp.* This species indicate the fact of occurance of cholera for this pond as De Magny *et al.* (2011)

earlier observed the association of *Diaphanosoma sp*. and *Moina sp.* with the occurrence of cholera caused by *V. cholerae* O1 in Mathbaria ponds.

Among rotifera, *B. angularis, B. calcyflorus, B. forficula, Filinia sp*. and *Polyarthra sp*. were found to be abundant during the peak season of cholera (Summer) in Mathbaria. Constantin De Magny *et al.* (2011) opined that *Brachionus forficula* in Mathbaria was significantly associated with occurrence of cholera caused by *V. cholerae* O1. In Bakerganj, another rotifer species *B. angularis* was found to be associated with the occurrence of cholera. Two other rotifer species *B. diversicornis* and *B. forficula was also* associated with *V. cholerae* O1 cases of cholera in Mathbaria. Tamplin *et al.* (1990) found that, *V. cholerae* serogroup O1 attached preferentially to exuviae of zooplankton, including the rotifer *Brachionus sp.,* in samples collected from the rivers and ponds of Matlab earlier.

Diversity indices were used to calculate the species diversity, species richness and species evenness of Mathbaria and Chhatak on seasonal basis during the study period. In Mathbaria, ranges of species diversity was following in different seasons:

Autumn>Winter>Rainy season > Summer

During the peak season of cholera (summer), species diversity and species evenness was highest at site-10 and species richness was shown to be maximum at site-11.

Among the four different seasons of Chhatak, ranges of species diversity measured with diversity indices were as follows:

Winter > Rainy season > Summer > Winter

Rotifera showed an annual increasing trend (47%-67%) in the month of May-August 2006 and 2007 when rains were abundant (Pradhan, 2014). He also observed lowest (12% and 13%) abundance of rotifer in winter. Pradhan (2014) proposed for any water reservoir i.e., pond or lake Shannon-Weiner Diversity Index should be used to assess the impact of pollution depending on plankton diversity. He proposed an index value of 1: indicates maximum impact of pollution; while value between 1-2: indicates medium impact of pollution and value> 2: indicates lowest impact of pollution.

In the present study with coastal area of Mathbaria maximum pollution in most of the studied ponds were detected in summer and rainy season rather than the autumn and winter. Winter is the pollution free season in comparison to other seasons. On the other hand, in fresh water zone of Chhatak most pollution in the water bodies were observed in summer and Autumn whereas winter was free from pollution.

During the peak season of cholera (autumn), highest diversity and evenness was measured at site-11 (Commissioner Bari Pond) and species richness was measured at site-2 (Jotishkanti Bepari's Pond).

In the present study with Mathbaria summer season showed lowest plankton diversity which periodically increased and winter season was reached with zooplankton. In Chhatak, the highest diversity was also in winter season and lowest in summer which is dissimilar with the results of Tripathi*et al.* (2006). Tripathi *et al.* (2006) suggested that increase in zooplankton diversity was highest in summer and lowest in winter at Seetadwar Lake of Uttar Pradesh, India.

Boxshall and Jaume (2000) opined that cyclopoids are one of the most conspecious and diverse group of freshwater copepods which tend to have wide distributional patterns with many species being cosmopolitan in nature. Gliwicz (1969); Patalas (1972); Straile and Geller (1998); Anneville *et al.* (2007) suggested that species of the family cyclopoida tend to increase stronger with eutrophication than species of Calanoida.

Huq *et al.* (1986) in his study with blue crab observed that the attachment of *V. cholerae* to hindguts of blue crab which did not match with the present where no significant result of the presence of *V. cholerae* was found in mud crabs of Sundarbans. John and Ronald (1982) also worked on blue crabs of Galveston Bay where pathogenic *V. cholerae* were detected in the hemolymph of collected crabs. The present study is opposite to the mentioned experiment in the Galveston Bay.

Study on the association of chitin from two crustacean sources and *V. cholerae* revealed the fact that the growth of the bacteria was proliferated in raw crab chitin more extensively in Paikgacca water microcosm than that of Mathbaria in comparison to raw shrimp chitin in the same microcosms. Nahar *et al.* (2012) in her study on *V. cholerae* in association with shrimp chitin in Mathbaria water showed better growth of cholera bacteria in brackish and estuarine water.

DFA images of copepods to show the position of the attachment of *V. cholerae* after inoculation of the bacteria colony gave the conception of the preference of the oral region of the plankton for aggregation. In the earlier study of Huq (1984), highest concentration of copepods were found around the oral region and on the mouth parts which is similar to the findings of the present study.

The experimentation on chitin and *V. cholerae* relationships in two different sources of water showed that the shape of attached Vibrios were exhibited in various shapes (coccoid and rod shapes) through SEM images. Xu *et al.* (1982) and Colwell and Huq (1994) studied that *V. cholerae* O1 becomes coccoid and enteres into a non-culturable state in the environment when conditions are not favourable for active growth.

Mud crab collected from Sundarbans when dissected in this study period did not show any colony of *V. cholerae* but some non O1 count of the bacterium which was identified onto TTGA plates as well as with mPCR. Ashiru *et al.* (2012) isolated *V. cholerae* from the gut of the swimming crabs, *Callinectus sp.*, after growth on the selective agar media. According to Benenson (1992), non-O1 strains that do not agglutinate with serogroup O1 antiserum can express the enterotoxin, producing sporadic cases and small outbreak of diarrhoeal diseases, but do not cause large epidemics.

In *ex-situ* experiments of laboratory microcosms prepared with three different sources of water (Mathbaria, Paikgachha and Dhanmondi Lake water) *V. cholerae* was found to attach with the adult *Cyclops sp.* and their larval nauplii. Kogure *et al.* (1980) earlier reported that zooplankton promote the growth of *Vibrio* species. Huq *et al.* (1983, 1984) showed that the survival of *V. cholerae* O1 is enhanced when it is grown with laboratory-grown planktonic copepods isolated from fresh and estuarine waters. Large numbers of *V. cholerae* was noted by those authors to be attached to plankton structures.

In this study, microcosm study of *V. cholerae* O1 and their association with copepods in the present study revealed that they are influenced by the larval stages of the copepods than the adults. This activity was observed regarding the three different water made microcosms in the laboratory and the process continued upto four weeks. Huq *et al.* (1983) showed that *V. cholerae* associated with living copepods remained culturable at least 10 days or longer than *V. cholerae* associated with dead copepods.

DFA assessment at present on the *V. cholerae*-copepod attachment showed that the preference of the oral region of the reservoir (copepods) to be perfect zone for the bacteria for association. Huq *et al.* (1983) in his experiment confirmed the specificity of attachment of *V. cholerae* by scanning electron microscopy, which revealed that the oral region and egg sac were the heavily colonized areas of the copepods. He also showed that the survival of *V. cholerae* in water was extended in the presence of live copepods. Huq *et al.* (1990) documented the presence of *V. cholerae* O1 year-round via it's commensal association with plankton established by Colwell and co-workers (1996) using direct detection methods.

In *V. cholerae*–chitin microcosm study, growth of the bacteria after inoculation was increased and for long period shown the enhancement of the progeny especially in Paikagachha water microcosm with pH 6.52 which was mild acidic in nature. Nalin *et al.* (1979) proposed that chitin protects *V. cholerae* O1 from the lethal effect of low pH and promote pathogenecity of *V. cholerae* O1 by protecting it from the acidic environment of the human gastrointestinal tract. Later Tamplin *et al.* (1990) in their studies showed that chitinous surfaces of plankton concentrate *V. cholerae* O1 and may increase the number of *V. cholerae* in a given unit of water.

Studying the abundance of zooplankton in relation to the hydroclimatic factors of the experimental zone revealed that nauplii was found to be related with maximum temperature in the month of April in Mathbaria. Statistically this relationship was also proved. Mendelsohn and Dawson (2008) observed that two years of cholera outbreak data from KwaZulu-Nata in South Africa were shown to be statistically associated with sea surface temperature, precipitation and coastal phytoplankton, the latter being the surrogate indicator of zooplankton, the natural host of the cholera vibrio. In Chhatak, no synchronized relationships were observed among the plankton and climatic factors. Satatistically, minimum relationship was observed among plankton and the environmental factors. Perhaps the relationship with phytoplankton was related as basic food for zooplankton to emerge in the pond population cycle.

Alexander *et al.,* (2013) examined a predictive cholera study in Africa where diarrhoeal incidence in Btswana over a 30-year period occurred in relation to several climatic variables, including rainfall, minimum temperature, and vapor pressure.

Xu *et al.,* (1982) and Colwell *et al.,* (1995) stated that *V. cholerae* O1 becomes coccoid and enters into a non-culturable state in the environment when conditions are not favourable for active growth. This condition was visualized in the chitin supplemented microcosms in association with *V. cholerae* O1 under the Scanning Electron Microscope where coocoid shape are mostly evidents from the microcosms supplemented raw shrimp chitin after four months of culture. And after that they enterd into the dormant state that is inactive on the culture media.

During periods of reduced nutrient levels, such as those encountered in aquatic environments, *V. cholerae* O1 and other *Vibrio spp*. undergo physiological and morphological changes. According to Huq *et al.* (1990); Colwell and Huq (1994); Miller*et al.* (1985) and Huq *et al.,* 1984, seasonal blooms of plankton may increase the presence of *V. cholerae* O1 in waters, reaching concentrations high enough to cause the death. Re Velle, P. (1982) stated that, warm waters are optimum for growth of green and blue-green algae species, which require temperatures ranging from 25°C through 35°C for optimum growth. These algal production is controlled by the supply of nutrients (mainly nitrogen, phosphorus and iron) to the sunlit layers. During the current study nitrogen and phosphorus amount was higher in the infectious ponds of Mathbaria when there was peak season of cholera.

In conclusion, from the study there were some observations regarding the emergence of cholera in Mathbaria and Chhatak. Mathbaria as a coastal habitat is in the more risk position than Chhatak. Tidal ups and downs directly influence the aquatic environment of Mathbaria. There crustacean plankton and their larval stages were abundant with some particular species of rotifera during the peak season of cholera. In Chhatak, protozoan plankton along with diversified species of rotifer were evident. Parallel observations of some crab samples collected from Sundarbans did not show any significant results as Non O1 *V. cholerae* in the fresh intestines of crab. On the other hand, microcosm study with plankton and chitin revealed the fact that *V. cholerae* need chitins for their nutrition and survival. Also salinity is an additional important parameter to grow *V. cholerae* as in coastal water microcosm they survive for long time with crab chitin. So, further study needed to observe the other coastal zones of Bangladesh to understand the ecological relationships between the environmental role as the reservoir of *V. cholerae* and biological factors responsible to carry on the epidemicity of cholera.

Chapter-6. Summary and Conclusion

From the ancient period cholera was recognized as deadly threatened disease to the human being as it caused severe mortality and also was epidemic in nature. With time this disease was disappeared from the developed country due to the preventive measures taken to protest cholera and infections caused by the primitive agent *V. cholerae*. In poorly developed and developing countries this scenario is different as people in the coastal regions are still suffering from the severe diarrheal disease and also killed in extreme cases. In the current study two different regions were selected (Mathbaria and Chhatak) during the period January 2013 to December 2014. Mathbaria is a coastal zone situated near Bay of Bengal and heavily washed during monsoon which is the main issue to spread cholera. Chhatak in Sunamganj district is a fresh water zone where in some ponds *V. cholerae* was identified during autumn and cholera patients were recorded in health complex.

Quarterly sampling was done from the fourteen pristine ponds in Mathbaria and Chhatak. In Mathbaria 86 species of plankton were identified including 27 protozoan species, 43 rotifera species, 8 copepoda species and 8 cladoceran species. Chhatak was rich in zooplankton in comparison to Mathbaria which had 100 species. Among them 14 species of protozoa, 58 species of rotifer, 9 species of copepod and 19 species of cladoceran plankton were identified during the study period. Most dominant group recorded in Mathbaria were rotifera, copepod and cladocera. On the other hand, Freshwater zone Chhatak exhibited in total 100 species of zooplankton of which 14 species belonged to the phylum protozoa under 3 families and single order. Rotifera had 58 species of plankton under 11 families and 3 orders. Among crustacean plankton 9 species of copepods were found under 2 families and 2 orders. Another group of planktonic crustacean, cladocera was identified in Chhatak ponds, which was represented by 19 species and 7 families and 2 orders.

In Mathbaria two peak seasons of cholera exist of which one is summer peak and another is winter peak. Site-2 (Jotishkanti Bepari’s Pond), site-8 (Mathbaria Canal) and site-11 (Commissioner Bari Pond) were recognized as infectious ponds where nauplii (larval stage of crustacean plankton) was recorded in high percentage than the other plankton both in summer and autumn peak. Copepoda was second highest group of plankton at site-8 in autumn peak. In other non-contaminated ponds (Site-5, 7 and 9) protozoa, rotifer and nauplii were dominant in the peak season of cholera. Species composition of copepod was maximum at site-2 (Jotishkanti Bepari's Pond) and site-11 (Commissioner Bari Pond) in the year 2014. Protozoa and rotifera on the other hand had highest species composition at non-infectious ponds. Among protozoa *Arcella discoides, Centropyxis sp., Difflugia sp., D. tuberculata, Euglena oxyuris, Glaucoma sp., Phacus acuminata, Phacus longicauda* and *P. pleuronectes* commonly observed in most of the ponds. *Brachionus sp., B. angularis, B. caudatus, B. diversicornis, Keratella sp., K. tropica, Monostyla bula* and *P. vulgaris* were the abundant species of rotifera. *Cyclops sp., Cyclops vernalis, Diaptomus sp., Diaptomus gracilis* and some unidentified copepod were dominant among copepods in the year 2013 and 2014. *Diaphanosoma sp.* among cladoceran plankton was the only species that dominated over the other species in Mathbaria. Some species were frequently distributed in all of the ponds in Mathbaria. Among them *Arcella discoides* (85.7%), *Difflugia sp.* (100%), *D. tuberculata* (100%), *E. oxyuris* (85.7%), *Phacus acuminate* (85.7%), *P. longicauda* (85.7%), *P. pleuronectes* (85.7%), *Glaucoma sp.* (85.7%), *Brachionus sp.* (85.7%), *B. angularis* (100%), *Filinia sp.* (85.7%), *K. cochlearis* (85.7%), *K. tropica*(100%), *Lecane luna* (85.7%), *Monostyla bula* (85.7%), *Polyarthra sp.* (85.7%), *P. vulgaris* (100%), *Cyclops sp.* (100%), *Mesocyclops sp.* (85.7%), *Diaptomus sp.* (100%), *D. gracilis* (100%), *Diaphanosoma sp.* (100%) were most frequently observed. Seasonally, among protozoan plankton *Arcella discoides, C. ecornis, Difflugia sp., D. tuberculata, Euglena oxyuris, Glaucoma sp., Phacus acuminata, P. longicauda, P. pleuronectes* were abundant in the infectious ponds (site-2, site-8 and site-11) of Mathbaria at peak seasons (Summer and Autumn) of cholera. *Asplanchna sp., Monostyla bula, B. angularis, B. caudatus, B. diversicornis, B. forficula, B. urceolaris, Keratella sp., Keratella cochlearis, K. tropica, Polyarthra sp., P. vulgaris, Rotaria sp.* and*R. Neptunia* were mostly abundant plankton of rotifer in the infectious ponds at peak seasons. *Cyclops sp., Cyclops vernalis, Diaptomus sp., D. gracilis* and *Mesocyclops sp.* of copepod and *Diaphanosoma sp.* of cladocera were found to be abundant in those selected ponds. Though zooplankton species diversity is lowest in the infectious ponds but those are rich in species among other ponds of Mathbaria. In summer peak site-11 was highly reached with species and in autumn site-2 had more volume of species considering Menhinick's (0.1366 at site-11) and Margalef's index (2.089 at site-2 and 2.546 at site-11). Chhatak is a freshwater zone in geographical position. So, the abundance of plankton and their association with *Vibrio cholerae* is slightly different than that of Mathbaria. Here only autumn peak exists and the infectious pond from the earlier studies was recognized as site-1 (Govt. Pond near THC), site-10 (Surma River Ghat 2: Cement factory ghat) and site-12 (Sarderbari Abdul Khalek's Pond). Nauplii was the dominant group of plankton considering the stages of crustacean copepods in the infectious ponds of Chhatak during peak season (autumn) of cholera. Other dominant group was rotifera and the copepod plankton was also found at site-1 (Govt. Pond near THC). On the other hand, protozoa and rotifer were dominantly found in the non-contaminated ponds (site-2, site-4, site-9 and site-11) Chhatak at the peak season. Species composition of crustacean plankton (copepod and cladoceara) was lowest in the contaminated and non-contaminated ponds than that of rotifera in both of the studied year (2013 and 2014). *Centropyxis sp., Ceratium hirudinella, Difflugia sp., Euglena acus, Phacus acuminata, Phacus longicauda* and *P. pleuronectes* of protozoa were abundant. Among rotifer *Asplanchna priodonta, Brachionus sp., B. angularis, Brachionus calcyflorus, B. caudatus, Brachionus falcatus, Brachionus forficula, Filinia camascela, Filinia terminalis, Keratella sp., Keratella cochlearis, Keratella tecta, K. tropica, Tricocerca similis* and *P. vulgaris* were the abundant species of rotiferan plankton. *Cyclops sp., Cyclops nanus, Cyclops vernalis* and *Diaptomus sp.* and some unidentified copepod species were dominant among copepods in the year 2013 and 2014. Among cladoceran plankton only few species that was recorded from Chhatak ponds were *Bosmina sp.* and *Diaphanosoma sp.* Among protozoan plankton, *Arcella sp.* (85.7%), *Centropyxis sp.* (100%), *Difflugia sp.* (100%), *Euglena acus* (100%), *Phacus pleuronectes* (85.7%) and *Glaucoma sp.* (85.7%) were frequently distributed in Chhatak ponds. Among rotifers, *Asplanchna sp.*(85.7%), *A. priodonta* (85.7%), *Brachionus sp.*(100%), *Brachionus angularis* (100%), *B. calcyflorus* (100%), *B. caudatus* (100%), *B. falcatus* (100%), *B. forficula* (85.7%), *B. quadridentatus* (85.7%), *Hexartha intermedia* (85.7%), *Keratella sp.* (100%), *K. cochlearis* (85.7%), *K. tecta* (100%), *K. tropica* (100%), *Platyias quadricornis* (85.7%), *Polyarthra sp.* (85.7%), *P. vulgaris* (100%), *T. similis* (85.7%), *Filinia camascela* (85.7%), *Filinia longiseta* (85.7%), *Testudinella sp.*(100%), *Rotaria sp.* (85.%7) were distributed frequently in most of the ponds of Chhatak. Seasonally, among protozoan plankton *Arcella sp., Centropyxis sp., Ceratium hirudinella, Difflugia sp., D. acuminata, Euglena acus, Glaucoma sp. Phacus acuminata, P. longicauda, P. pleuronectes,* were abundant in the infectious ponds (site-1, site-10 and site-12) of Chhatak at peak season (Autumn) of cholera. *B.*

angularis, B. caudatus, Filinia longiseta, K. tecta, Hexartha intermedia and *P. vulgaris* were abundant species among rotifers in those infectious ponds during autumn. *Cyclops sp., Cyclops vernalis, Diaptomus sp., D. gracilis* and *Mesocyclops sp.* of copepoda and *Bosmina sp., Bosmina coregoni, Ceriodaphnia sp.* and *Diaphanosoma sp.* of cladocera were common in the selected ponds at that time. Species diversity was high at site-12. In autumn peak site-10 of Chhatak was highly rich with species considering Menhinick's (0.17260) and Margalef's index (3.731).

During summer peak air and water temperature of the studied ponds was maximum with lower precipitation in the studied ponds of Mathbaria. In Chhatak, air temperature of that region and water temperature of the studied ponds were maximum and then became decrease during peak season of cholera was (September-November) in the selected ponds. pH in most of the ponds were high during peak season of cholera. On the other hand, in peak season pH was lower in Chhatak ponds. Salinity in most of the ponds (site-2, site-5, site-9, site-10, site-11) in Mathbaria was recorded to be lower during summer (March-May) than the other seasons. Maximum salinity was recorded during the peak season of cholera at one of the infectious ponds (site-10) whereas in other ponds there was minimum salinity.

Statistical analysis of the two areas in plankton showed that the average production of protozoa, rotifera, nauplii, copepoda and cladocera of Chhatak was far more than that of Mathbaria. From the Independent Sample t-test it is evident that the average production of protozoa, rotifera, nauplii and copepod in Mathbaria is significantly different than that in Chhatak ($p<0.05$). Analysis of Variance (ANOVA) tests have been performed to comparison of plankton production in different months of the year. It is evident that there is no significant difference between production of plankton (Protozoa, Rotifera, Nauplii, Copepoda and Cladocera) ($p>0.05$) in different months of the year. There is no significant difference between plankton production other than Protozoa at ($p>0.05$), in the selected ponds of Mathbaria and Chhatak.

Hydroclimatological factors are profoundly related with the outbreak of cholera in the coastal and fresh water region of Bangladesh and recorded zooplankton are supposed to be related with the arrival of associated bacteria during the peak season of cholera in Mathbaria and Chhatak. Count of total zooplankton was maximum in April and among them nauplii showed maximum abundance at two infectious ponds of Mathbaria (site-2 and site-11) when highest maximum temperature was recorded and total amount of precipitation or rainfall started to increase in this month. Moderate positive correlation among nauplii, copepoda, cladocera and total zooplankton and maximum air temperature was shown during the study period in Mathbaria ponds. In the infectious ponds of Mathbaria total rainfall had positive effects on the zooplankton mostly copepod and cladocera. On the other hand, maximum temperature was moderately correlated to the most of the plankton. In Chhatak, total count of zooplankton was maximum in May-June, September-October in most of the ponds when precipitation amount is high. In two infectious ponds (site-10 and site-12) nauplii was observed in high abundance during September. Protozoa, nauplii and copepoda showed moderate positive correlation with maximum temperature, minimum temperature and total rainfall at site-1, site-2, site-4, site-9, site-10, site-11 and site-12 (site-1, site-10 and site-12 are infectious ponds).

Here in the experimental design two types of experiment were performed including *In-situ* experiment and *Ex-situ* experiment. Studying the zooplankton in different aquatic reservoirs of Mathbaria and Chhatak, their composition, distribution and seasonal abundance were accomplished the conditions of *In-situ* experiment as these are the natural habitual measurement of the plankton of that regions. On the other hand, *ex-situ* experiment comprising of the study with microcosms in the laboratory setup.

Micro ecosystem study of copepods in three water sources in laboratory condition inoculated with the pure culture of toxigenic *Vibrio cholerae* O1 revealed that the growth of bacteria increased with the increased production of nauplii. Association of *V. cholerae* O1 was strongly positive in microcosms prepared with Paikgachha water and crab and shrimp chitin. Growth of the bacteria was continued till they formed biofilm in 480 days of culture. Which is evident from the DFA (Direct Fluorescent Antibody and SEM (Scanning Electron Microscope Image) study.

Some nutrients at the infectious ponds of Mathbaria was analyzed during peak season of cholera which may influence the production of primary producers (blue-green algae) in the ponds which may influence the growth and abundance of zooplankton to act as the reservoir of *V. cholerae.* Amount of nitrogen and phosphorus was higher than the other micronutrients in the ponds during peak season.

From the study, it is evident that hydroclimatological factors in association with the limnological parameters of the ponds in coastal region is favorable for the availability of cholera bacteria to survive and emergence when the favorable conditions are available. Though, zooplankton diversity was maximum in the freshwater zone Chhatak. From the overall study this is revealed that if contamination occurs in Chhatak ponds cholera will explore as crustacean planktonic diversity was maximum from Chhatak. On the other hand, though zooplankton quantity was less in Mathbaria, if contamination occurs by vomiting or extraction of feces then the selected ponds would show the maximum effect of cholera in that region.

Chapter-7. Recommendations

Considering the overall biological assessments and hydroclimatological factors in the two geographically different locations of Bangladesh following preventive measures should be considered:

- Hydroclimatological factors of the two locations are favorable for the diversion of *Vibrio cholerae* which is alarming and necessary steps should be taken prior to the choler season.
- The cholera ecology of a coastal upazilla needs to be understood.
- People of the coastal bed should aware of the two peak seasons of cholera in the vicinity.
- Plankton bloom in the aquatic bodies could be an indicator for cholera outrage. If precautions are taken as filtering or boiling the pond water before drinking it would be the savior of the local people.
- Zooplankton is the prime food for the other crustacean such as shrimp, crab and some other white fishes culturing in the coastal belt. So, the farmers engaged in fishing should take necessary steps before handling these fishes as shrimp and crab chitin could be the reservoirs of *Vibrio cholerae* for transmitting them from one place to another.
- Freshwater zone of Chhatak is diversified with various zooplankton species and could be the zone of prevalence for the infection in near future.

REFERENCES

ABD, H., WEINTRAUB, A. and SANDSTROM, G. 2004.Interaction between *Vibrio cholerae* and *Acanthamoeba castellanii*. *Microbial Ecology in Health and Disease*. **16:** 51-57.

AKANDA, A. S., JUTLA, A. S., DEMAGNY, G. C., ALAM, M., SIDDIQUE, A. K. SACK, R.B. HUQ, A. COLWELL, R. R.and ISLAM, S. 2011a. Hydroclimatic influences on seasonal and spatial cholera transmission cycles: implications for public health intervention in the Bengal Delta. *Water. Resour. Res*. **47:** W00H07.

AKANDA, A. S., JUTLA, A. S., GUTE, D. M., EVANS, T. and ISLAM, S. 2012. Reinforcing cholera intervention through prediction aided prevention. Bull world Healthy Organ. **90:** 243-44.

AKSELMAN, R., JURQUIZZA, V., NCOSTAGLIOLA, M. C., FRAGA, S. G., PICHEL, M., HOZBOR, C., *et al.,* 2010. *Vibrio cholerae* O1 found attached to the dinoflagellateNoctilucascintillans in Argentine shelf waters. *Mar. Biodivers. Rec*. **3,** e120.

ALAM, M., HASAN, N. A., SADIQUE, A., BHUIYAN, N. A., AHMED, K. U., NUS-RIN, S., NAIR, G. B., SIDDIQUE, A. K., SACK, R. B., SACK, D. A., HUQ, A. and COLWELL, R. R.2006a. Seasonal cholera caused by *Vibrio cholerae* serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. *Appl. Environ. Microbiol.***72:**4096-4104.

ALAM, M., SULTANA, M., NAIR, G. B., SIDDIQUE, A. K., HASAN, N. A., SACK, R. B., SACK, D. A., AHMED, K. U., SADIQUE, A., WATANABE, H., GRIM, C. J., HUQ, A.andCOLWELL, R. R. 2007. Viable but non-culturable *Vibrio cholerae* O1 in biofilms in the aquatic environment and their role in cholera transmission.Proc. Natl. Acad. Sci**.** USA. **104**: 17801-17806.

ALAM, M., SULTANA, M., NAIR, G. B., SACK, R. B., SACK, D. A., SIDDIQUE, A. K., ALI, A., HUQ, A. and COLWELL, R. R. 2006b. Toxigenic *Vibrio cholerae* in the aquatic environment of Mathbaria, Bangladesh. *Appl. Environ. Microbiol.***72:** 2849–2855.

ALBERT, M., ANSARUZZAMAN, M., BARDHAN, P., FARUQUE, A., FARUQUE, S. and ISLAM, M., *et al.,* 1993. Large epidemic of cholera-like disease in Bangladesh caused by *V. cholerae* O139 synonym Bengal. *Lancet*.**342:** 387-390.

ALEXANDER, K. A., CARZOLIO, M., GOODIN, D. and VANCE, E. 2013. Climatic change is likely to Worsen the Public Health Threat to Diarrheal Disease in Botswana. *Int J Environ Public Health*.**10:** 1202-1230.

ANANTHANARAYAN, R. and JAYARAM, PANIKER, C. 1984. Textbook of Microbiology. 2nd. Ed. New Delhi: Orient Longman.

Anderson, A.; Meier, H. E. M. ; Ripszam, M., Rowe, O; Wikner, J; Haglund, P; Eilola, K; Legrand, C. *et al.*, 2015. Projected future climate change and Baltic Sea ecosystem management. Ambio 44. 345-356.

ANDERSON, A.; MEIER, H. E. M. ; RIPSZAM, M., ROWE, O; WIKNER, J; HAGLUND, P; EILOLA, K; LEGRAND, C. *et al.*, 2015. Projected future climate change and Baltic Sea ecosystem management. Ambio 44. 345-356.

ANNEVILLE, O., MOLINERO, J. C., SOUISSI, S., BALVAY, G. and GEARDEAUX, D. 2007. Long term changes in the copepod community of Lake Geneva. *Journal of Plankton Research.***29:** 49-59.

ANSARI, M. F., ANKALGI, R. F. and ANKALGI, S. R. 2007. Studies on physic-chemical aspects and plankton of Unkal Lake at Hubli (Karnatka, India). *Proceedings of Tall*: *The 12th world lake Conference:* 1687-1694.

ASHIRU, A. W., UABOI-EGBENI, P. O., ODUNLADE, A.K., ASHADE, O. O., OYEGOKE, T. M. and IDIKA C.N. 2012. Isolation of *Vibrio* species from the gut of swimming crabs (*Callinectus* sp.)and theirantibiotic susceptibility. *Pakistan Journal of Nutrition*. **11** (6): 536-540.

BAKER, R. M., SINGLETON, F. L., and HOOD, M. A. 1983. Effects of nutrient deprivation on *Vibrio cholerae*. *Appl. Environ. Microbiol*.**4 6**: 930-940.

BANDDYOPADHYAY, S., KANJI, S. and WANG, L. 2012. The impact of rainfall and temperature variation on diarrhea prevalence in sub-Saharan Africa. *Appl. Geogr*. **33**: 63-72.

BARUA, D. 1991. History of cholera. In: cholera (Barua, D. and Grenough III, W.B., Eds), pp. 1-35. Plenum, New York.

BARUAH, B.K., CHAUDHURY, M. and DAS, M., 1997.Plankton as index of water quality with reference to paper mill pollution. Poll. Res. **16**(4): 259-263.

BAUMAN, P., BAUMAN, L., BANG,S. S. and WOLKALIS, M. J. 1980.Reevaluation of the taxonomy of the vibrio, *Beneckea,* and *Photobacterium*-abolition of the genus *Beneckea*.*Curr.Microbiol*. **4**: 127-133.

BAUMANN, P., FURNISS, A. L., and LE,J. V. 1984.Genus I. *Vibrio Pacini*. 1854, 411AL.*In*Krieg, N. R. and Holt, J. G. (ed.), Bergey’s Manual of systematic Bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.p. 518-538.

BAUMANN, P. and SCHUBERT, R. H. W. 1984. Family II, VibrionaceaeVeron 1965, 5245[AL]. *In* Krieg, N. R. and Holt, J. G. (ed.), Bergey's Manual of systematic Bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.p. 516-555

BENENENSON, A. 1992. The control of transmission disease in man. 15ta ed. Washington, DC. Organization Panamcricana de la Salud.

BIGGS, J., WILLIAMS, P., WHITFIELD, M., NICOLET, P. and WEATHERBY, A. 2005. 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquatic Consv. Mar. Freshw. Ecosyst*. **15**: 693-714.

BINSZTEIN, N., COSTAGLIOLA, M. C., PICHEL, M., JURQUIJA, V., RAMEIZ, F. C., AKSELMAN, R., *et al.,* 2004.Viable but non-culturable *Vibrio cholerae* O1 in the aquatic environmemnt of Argentina. *Appl. Environ. Microbiol*.v**70**:7481-7486.

BLAKE, P. A. 1994.Historical perspectives on pandemic cholera. In: *Vibrio cholerae* and Cholera: Molecular to Global Perspectives (Wachsmuth, K.I., Blake, P.A., and Olsik, O., Eds.). American Society of Microbiology Press, Washington, DC.pp. 293-295.

BOMPANGUE, N. D., GIRAUDOUX, P., PISNIER, P. D., TINDA, A. M., PIARROUX, M., SUDRE, B., HORION, S., TAMFUM, J. J., ILUNGA, BK. and PIARROUX, R. 2011. Dynamics of cholera outbreaks in great lake region of Africa. 1978-2008. *Emerg. Infect. Dis.***17**: 2026-2034.

BORROTO, R. J. 1997. Ecology of *Vibrio cholerae* serogroup O1 in aquatic environment**. 2:** 328-333.

BOURKE, A., COSSINS, Y., GRAY, B. 1986. Investigation of cholera acquired from the riverine environment in Queensland. *Med J Aust.* **144**: 229-234.

BOXSHALL, G. A, JAUME, D. 2000. Making waves: The reapeted colonization of fresh water by copepod crustaceans. *Advances in Ecological Research*.**31**: 61-79.

BRAYTON, P.R., TAMPLIN, M. L., HUQ, A., and COLWELL,R. R. 1987. Enumeration of *Vibrio cholerae* O1 in Bangladesh waters by fluorescent antibody direct viable count. *Appl. Environ. Microbiol*. **53**: 2862-2865.

BRAYTON, P. R.and COLWELL, R. R.1987.Fluorescent antibody staining method for enumeration of viable environmental *Vibrio cholerae* O1.*J. Microbiol. Methods.* **6**:309–314.

BRENNER D.J., KRIEG, N. R.and STALEY, J. T. 2005. Bergey's Man Systematic Bacteriology. **2**:491-546.

BROZA, M. and HALPERN, M. 2001. Pathogen reservoirs- Chironomid egg masses and *Vibrio cholerae*. *Nature.* 412: 40.

BROZA, M, GANCZ, H., HALPERN M. and KASHA, Y. 2005. Adult non-biting midges: possible windborne carriers of *Vibrio cholerae* non-O1 and non-O139. *Environ Microbiol*.**7**: 576-585.

BURROWS, W. 1979. Textbook of Microbiology. 21 ma. Ed. Philadelphia: Saunders.

CAIRNS, J. Jr.1979. Final Summary, In: James, A and Evison,L. Biological Indicators and Water Quality, John Wiley and Sons, New York: 226-227.

CARROLL, J., MATEESCU, M., CHAVA, K., COLWELL, R. R. and BEJ, A. 2001. Response and tolerance of toxigenic *Vibrio cholerae* O1 to cold temperatures. *Antnie Van Leeuwenhoek*. **79**: 377-384.

CASH,R. A., MUSIC, S., LIBONATI, J. P., SNYDER, M. J., WENZEL, R. P. and HORNICK, R. B. 1974. Response of man to infection with *V. cholerae*. I. Clinical, serologic and bacteriologic responses to known inoculums. *J. Infect. Dis.* **129**: 45-52.

CASH, B.A., RODO, X., KINTER, J. L., III, 2009. Links between tropical pacific SST and cholera incidence in Bangladesh: role of the western tropical and central extratropical pacific. *J.Clim*. **22**: 1641-1660.

Center for Disease Control. 1978. Follow-up on *Vibrio cholerae* infection, Lousiana. Morbid. Mortal.Weekly Rep. **27**: 367.

CHOWDHURY, A. N., BEGUM, S. and SULTANA, N. 1989. Occurrence and seasonal variation of zooplankton in a fish pond in relatipon to some physico-chemical factors. *Bangladesh J. Zool*. **17**(2): 101-106.

COCKBURN, T. A. and CASSANOS, J. G. 1960. Epidemiology of endemic cholera. Public Health Rep.**75**: 791-803.

COLWELL, R. R., KAPER, J., and JOSEPH, S. W. 1977. *Vibrio cholerae, Vibrio parahaemolyticus*and other vibrios: occurrence and distribution in Chesapeake Bay. Science. **198:** 394-396.

COLWELL, R. R, SEIDLER, R. J, KAPER, J., *et al*., 1981.Occurance of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuarie. *Appl Environ Microbiol*. **41**(2):555-558.

COLWELL, R. R. 1984. *Vibrios in the Environment*. New York, NY, USA. John Wiley & Sons.

COLWELL, R., BRAYTON, R. P.R., GRIMES, D. J.,ROSZAK, D.R.,HUQ, S. A., and PALMER, L. M. 1985. Viable but nonculturable *Vibrio cholerae* and related pathogens in the environment: implications for release of genetically engineered microorganisms. Bio/Technology **3**:817-820.

COLWELL, R. R., and SPIRA, W. M. 1992. "The ecology of *Vibrio cholerae*," in *Cholera*, edsBarua, D. andGreenouh, W. B. I (New York: Plenum Press Inc.), 107–127.

COLWELL, R. R. and HUQ, A. 1994. *V. cholerae* and Cholera: Molecular to Global perspectives, ed, Wachsmuth IK, Blake PA, Olsvik O. Am Sos. Microbiol, Washington DC. 117-133.

COLWELL, R. R. and HUQ, A. 1994. Vibrios in the environment: Viable but non-culturable*V. cholerae*. In: Wachsmuth, I., Blake, P., Olsvik, O., Editors. *Vibrio cholerae* and Cholera: Molecular to Global Perspectives. Washington, D.C.: ASM Press. Pp.117-33.

COLWELL, R. R. and HUQ, A. 1995. Environmental reservoirs of *V. cholerae*. *Ann N.Y. Acad. Sci.* **740**: 44-54.

COLWELL, R. R., HUQ, A., CHOWDHURY, M. A. R, BRYTON, P. R., and XU, B. 1995.Serogroup Conversion of *Vibrio cholerae*.*Can J Microbiol*. **41:** 946-950.

COLWELL, R. R. 1996. Global climate and infectious disease: the cholera paradigm. Science. **274**: 2025–31.[PubMed]

COLWELL, R. R.,BRAYTON, P., HERRINGTON, L., TALL, B., HUQ, A. and LEVINE, M. M. 1996. Viable but non-culturable*Vibrio cholerae* O1 revert to a cultivable state in the human intestine. *World J. Microbiol. Biotechnol.***12**: 28–31.

COLWELL, R. R. and PATZ, J. A. (eds).1998. Climate, Infectious disease and Health: An Interdisciplinary perspective. Washington DC, USA: American academy of Microbiology.

COLWELL, R. R. and HUQ, A. 1999.Global microbial ecology: biogeography and diversity of vibrios as a model. *J of App MicrobiolSymp Suppl*. **85**: 134S-7S.

COLWELL, R. R. 2000. Viable but non-culturable bacteria: a survival strategy. J. Infect. Chemother. 6: 121-125.

COLWELL, R. R. 2005. Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. *Appl. Environ Microbiol.***71**:4645–4654.

CHOWDHURY, M. A., HUQ, A., XU, B., MADEIRA, F. J., and COLWELL, R. R., 1997. Effect of alumn on free-living and copepod associated *V. cholerae* O1 and O139. *Appl Environ Microbiol.* **63**: 3323-3326.

COLE, G. A. 1979. Textbook of limnology. 2nded St Louis; CV Mosby Co, 1979.

CONSTANTIN DE MAGNY, G. *et al.*, 2008. Environmental signatures associated with cholera epidemics. Proc. Natl. Acad. Sci. **105** (46): 17676-17681.

COSTERTON, J. W., GEESEY, G. G. and CHENG, K. J. 1978. How bacteria stick. Sci. Am. **238**: 86-95.

COSTERTON, J. W., STEWART, P. S., and GREENBERG, E. P.1999. Bacterial biofilms: a common cause of persistant infections. Science. **284**: 1318-1322.

DASTIDAR, S.G. and NARAYANSWAMI, A. 1968. The occuranc of chitinase in vibrios. *Indian J. Med. Sci.* **56**:654-658.

DAWSON, M. P., HUMPHREY, B. A. and MARSHALL, K. C 1981. Adhesion, a tactic in the surviaval strategy of a marine vibrio during starvation. *Curr. Microbial*. **6**: 195-198.

DE MAGNY, G. C., MOZUMDER, P. K., GRIM, C. J., HASAN, N. A., NASER, M. N., ALAM, M., *et al.,* 2011. Role of zooplankton diversity in *Vibrio cholerae*population dynamics and in the incidence of cholera in the Bangladesh Sundarbans. *Appl. Environ. Microbiol*. **77**: 6125-6132. doi: 10.11258/AEM.01472-10.

Editorial. 1976. Cholera research: what next? Lancet ii: 1283-1284.Emch, M; Fedacker, C.; Islam, S. M. Ali, M. Seasonality of cholera from 1974 to 2005: a review of global patterns. International Journal of Health Geographics, v. 7, 13p., 2008. Available from: http://www. j-healthgeographics. com/content/7/1/31.Accessed in 12 Oct, 2014.

EMCH, M., FELDACKER, C., YUNUS, M., STREATFIELD, P. K., THIEM, V. D., CANTH, D. G. and ALI, M. 2008. 'Local Environmental Predictors of Cholera in Bangladesh and Vietnam.'*American Journal of Tropical Medicine and Hygiene.* **78**(5): 823-832.

EPPLEY (1972). Temperature and phytoplankton growth in the sea. Fish B-NOAA, **70**, 1063-1085.

EPSTEIN, P. R. 1993. Algal blooms in the spread and persistence of cholera. Bio Syst. **31** (2-3): 209-221.

FARUQUE, S. M., ASADUL, G., SAHA, M. N., *et. al.,*1988. Analysis of clinical and environmental strains of nontoxigenic*Vibriocholerae* for susceptibility to CTXPhi: molecular basis for origination of new strains with epidemic potential. *Infect Immune*. **66**(12): 5819-25.

FARUQUE, S.M., ALBERT, M. J. and MEKALANOS, J. J. 1998. Epidemiplogy, genetics, and ecology of toxigenic *Vibrio cholerae*. Microbiol. *Mol. Biol. Rev*. **62**: 1301-1314.

FARUQUE, S.M., CHOWDHURY, N., KAMRUZZAMAN, M., AHMED, Q. S., FARUQUE, A. S. G., SALAM, M. A., RAMAMURTHY, T., NAIR, G B., WEINTRAUB, A. and SACK, D. A. 2003. Reemergence of epidemic *Vibrio cholerae* O139, Bagladesh. *Emerg. Infect. Dis*. **9**: 1116-1122.

FARUQUE, S. M., CHOWDHURY, N., KAMRUZZAMAN, M., DZIEJMAN, M., RAHMAN, M. H., SASK, D. A., *et al*. 2004.Genetic diversity and virulence potential of environmental *V. cholerae* population in a cholera endemic area.*Proc. Natl. Acad. Sci*. U.S.A. **101**: 2123-2128.

FRETER, R. 1969. Studies on the mechanism of action of intestinal antibody in experimental cholera. *Tex. Rep. Biol. Med*. **27**: 299-316.

GIBBONS, R. J., and VAN HOUTE, J. 1971. Selective bacterial adherenceto oral epithelial surfaces and its role as ecological determinant. *Infect. Immun*. **3**: 567-573.

GLASS, R. I., HUQ, M. I., STOLL, B. J., KHAN, M. U., MERSON, M. H., LEE, J. V. and BLACK, R. E. 1982. Endemic cholera in rural Bangladesh, 1966–1980.*Am. J. Epidemiol.* **116:** 959–970.

GLASS, R. I., BECKER, S., HUQ, M. I., STOLL, B. J.,KHAN, M. U.,MERSON, M. H.,LEE, J. V., and BLACK, R. E. 1982. Endemic cholera in rural Bangladesh, 1966-1980. *Am J. Epidemiol*. **116**: 959-70.

GLIWICZ, Z. M. 1969 studies on the feeding of pelagic zooplankton in lakes of varying trophy. *EcologiaPolska.* **17**: 663-708.

GOODAY, G.W. 1990. The ecology of chitin degradation.*AdvMicrob Ecol*. **11**: 387-430.

GONZALEZ-ESCALON, N., FEY, A., HOFLE, M. G., ESPEJO, R. T. and GUZMAN, C. 2006. Quantitative reverse transcription polymerase chain reaction analysis of *Vibrio cholerae* cells entering the viable but non-culturable state and starvation in response to cold shock. *Environ. Microbiol.* **8**: 658-666.

GUENTZEL, M. N. and BERRY, L. J. 1975.Motility as virulence factor for *V. cholerae*. *Infect. Immun*. **11**: 890-897.

GUNALE, V. R., 1991. Algal communities as indicators of pollution. *J. Environ. Biol*. 223-232.

GURBANOV, S., AKHMADOV, R., SHAMKHALOVA, G., AKHMADOVA, S.,HALEY, B. J., COLWELL, R. R. and Huq,A. 2012. Occurrence of *Vibrio cholerae* in municipal and natural waters and incidence of cholera in Azerbaijan. *Eco Health*. **8**: 468-477.

HALEY, B. J., CHEN, A., GRIM, C. J., CLARK, P., DIAJ, C. M., TAVIANI, E.,*et al.*, 2012.*Vibrio cholerae* in a historically cholera-free country.*Environ. Microbiol*.Rep. **4**: 381-389.

HASAN, J.A., BERNSTEIN, K.D., HUQ, A., LOOMIS, L., TAMPLIN, M. L., and COLWELL, R.R.1994. Cholera DFA: an improved direct fluorescent mono-clonal antibody staining kit for rapid detection and enumeration of *V. cholerae* O1. *FEMS Microbiol.Lett.* **120**: 143–148.

HASHIZUME, M., ARMSTRONG, B., HAJAT, S., WAGATSUMA, Y., FARUQUE, A.S., HAYASHI, T. and SACK, D. A. 2008. The effect of rainfall in the incidence of cholera in Bangladesh. *Epidemiology*. **19** (1): 103-110.

HASHIZUME, M., FARUQUE, A.S., TERAO, T., YUNUS, M., STREATFIELD, K., YAMAMOTO, T. and MOJII, K. 2011. The Indian Ocean dipole and cholera incidence in Bangladesh: a time-series analysis. *Environ. Health. Prospect*. **119**: 239-244.

HEIDELBERG, J.F., HEIDELBERG, K. B. and COLWELL, R.R., 2002. Bacteria of the γ-Subclass Proteobacteria associated with zooplankton in Cheasapeake Bay. *Appl. Environ. Microbiol.* **68**: 5498-5507.

HOBEN, H. J &SOMASEGORAN, P. 1982. Comparison of the pour, spread and drop platemethod for enumeration of *Rhizobium* spp. in inoculants made from prestilized peat. *Appl. Environ.Microbiol*. **44**:1242-1247.

HOOD, M., NESS, G. and RODRICK, G. 1981. Isolation of *Vibrio cholerae* serotype o1 from the eastern oyster, *Crassostreavirginica. Appl Environ Microbiol*. **41**: 559-560.

HOOD, M. and NESS, G. 1982.Survival of *Vibrio cholerae* and *Eischerechia coli* in estuarine waters and sediments. *Appl Environ Microbiol.* **43**:578-584.

HOOD, M. A., GUCKERT, J. B. and WHITE, D. C. 1986. Effect of nutrient deprivation on lipid, carbohydrate, DNA, RNA, and protein levels in *Vibrio cholerae*. *Appl. Environ. Microbiol*. **52:** 788-793.

HOSHINO, K. S., YAMASAKI, A. K., MUKHOPADHYAY, S.,CHAKRABORTY, A., BASU, S. K., BHATTACHARYA, S. K., NAIR,G. B., SHIMADA, T. and TAKEDA,Y.1998. Development and evaluation of a multiplex PCR assay for rapid detection of toxi-genic *Vibrio cholerae*O1 and O139. *FEMS Immunol. Med. Microbiol.* **20**: 201–207.

HOUGHTON, J. T., DING, Y., GRIGGS, D. J., NOGUER, M., LINDEN, P. J. and XIAOSU, D. (ed.). 2001. *Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom.

HUQ, A. 1984. The role of planktonic copepods in the survival and multiplication of *Vibrio cholera* in the aquatic environment. College Park, MD: University of Maryland.

HUQ, A., SMALL, E., WEST, P., HUQ, M., RAHMAN, R., COLWELL, R. 1983. Ecological Relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Appl Environ Microbiol*. **45**: 275 -283.

HUQ, A., SMALL, E. B., WEST, P. A. and COLWELL, R. R. 1984. The role of planktonic copepods in the survival and multiplication of *Vibrio cholerae* in the aquatic environment. In Vibrios in the Environment. , Colwell, R.R. (ed.). New York, NY, USA: John Wiley and Sons pp. 521-534.

HUQ, A., PAUL, A. W., EUGENE, B. S., HUQ, M. I. and RITA, R. C. 1984. Influence of water temperature, salinity, and pH on survival and growth of toxigenic *Vibrio cholerae* serovar O1 associated with live copepods in laboratory microcosms. *Applied Environmental Microbiology*. **48**(2): 420-424.

HUQ, A., HUQ, S. A, GRIMES, D. J, O'BRIEN, M., CHU, K. H, CAPUZZO, J. M. and COLWELL, R. R. 1986.Colonization of the gut of the blue crab (*Callinectessapidus*) by *Vibrio cholerae*. *Appl. Environ. Microbiol*. **52**(3):586-588.

HUQ, A., COLWELL, R. R., RAHMAN, R., ALI, A., CHOWDHURY, M. A. R., PARVEEN, S., *et al.,* 1990. Detection of *V. cholerae* O1 in the aquatic environment by fluorescent-monoclonal antibody and culture methods. *Appl Environ Microbiol*. **56**: 2370-2373.

HUQ, A., XU, B., CHOWDHURY, M. A., ISLAM, M. S., MANTILLA, R. and COLWELL, R. R. 1996. A simple filtration method to remove plankton-associated Vibrio cholerae in raw water supplies in developing countries. *Appl. Environ. Microbial*. **62**: 2508-2512.

HUQ, A. and COLWELL, R. R. 1996.Vibrios in the Marine and Estuarine Environment. Tracking of *Vibrio cholerae*. *J. Ecosystem Health*. **2**: 198-214.

HUQ, A., SACK, R. B., NIZAM, A., LONGINI, I. M., NAIR, G. B., ALI, A., MORRIS, J. G., KHAN, M. N. H., SIDDIQUE, A. K., YUNUS, M., ALBERT, M. J., SACK, D. A., and ISLAM, M.S. 2005. Seasonality and toxigenicity of *V. cholerae* non-O1 isolated from different components of pond ecosystem of Dhaka city, Bangladesh. *World J. Microbiotech.* **8**: 160-163.

ISLAM, M. S., DRASAR, B. S and BRADLEY, D. J. 1989. Attachment of toxigenic *V. cholerae* O1 to various freshwater plants and survival with a filamentous green algae *Rizocloniumfontanum*. *J. Trop. Med. Hyg*. **92**: 396-401.

ISLAM, M., DRASAR, B. and BRADLEY SACK, R. 1994. The aquatic flora and fauna as reservoirs of Vibrio cholera: a review. *Journal of Diarrhoeal Disease Research*. **12**(2): 87-96.

ISLAM, M. S., ALAM, M. J., and KHAN, S. I. 1995. Occurance and distribution of culturable*Vibrio cholerae* O1 in aquatic environments of Bangladesh. *Int J Environ Stud.* **47**: 217-223.

ISLAM, M., JAHID, M RAHMAN M, RAHMAN, M., ISLAM M., KABIR, M., SACK, D. and SCHOOLNIK G. 2007. Biofilm acts as a microenvironment for plankton associated *Vibriocholerae* in the aquatic environment of Bangladesh. *Microbiol Immunol*. **51**: 369-379.

ISLAM, A., LABBATE, M., DJORDJEVIC, S. P., ALAM, M., DARLING, A., MELVOID, J., *et al.,* 2013. Indigenous *Vibrio cholerae*strains from a non-endemic region are pathogenic. *Open Biol.* 3, 120181.

IWAMOTO, M., AYERS, T., MAHON, B. E. and SWERDLOW, D. L. 2010. Epidemiology of seafood-associated infections in the United States. *Clin. Microbiol.* **23**: 399-411.

JAWETZ, E., MELNICK, J. ADELBERG, E., BROOKS, G., BUTEL, J. and Nickolas Ornston, N. 1990. *Medical microbiology*, 13ra ed. Mexico, D. F.

JOHN, W.D. and RONALD, K. S. 1982. Incidence of Vibrio species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas.

JONES, G.W., ABRAMS, G. D. and FRETER. R. 1976. Adhesive properties of *V. cholerae*: adhesion to isolated rabbit brush border membranes and hemagluttinating activity. *Infect. Immun.* **14**: 232-239.

JUTLA, A. S., AKANDA, A. S. and ISLAM, S. 2010. Tracking cholera in coastal regions using satellite observations. JAWRA J. Am. Water Resour. Assoc. **46**(4): 651-662.

JUTLA, A. S., HASAN, N. A., WHITCOMBE, E. and AKANDA, A. S. 2013. Environmental factors influencing epidemic cholera. *Am J Trop Med Hyg*. **89**: 597-607.

JUTLA, A. S., BALDAACH, H. AKANDA, A. S. HUQ, A. and Colwell R. R. 2015. Satellite based assessment of hydroclimatic conditions related to cholera in Zimbabwe. *PLOS* One. **10**(9): e0137828.

JUTLA, A. S., KHAN, R. and COLWELL R. R. 2017. Natural disasters and cholera and cholera outbreaks: Current understanding and future outlook. *Cur. Environ. Health Rep*. **4** (1):99-107.

KAPER, J., LOCKMAN, H., COLWELL, R. R. and JOSEPH, S.W. 1979. Ecology, serology and enterotoxin production of *Vibrio cholerae* in Chesapeake Bay. *Appl. Environ. Microbiol.***37**: 91-103.

KAPER, J. B., MOSELEY, S.L. and FALKOW, S.1981. *Infect Immun.* **32**: 661-667.

KAPER, J. B., MORRIS, J.G. Jr. and LEVINE, M. M. 1995. *ClinMicrobiol*. **8**: 48-86.

KANEKO, T., and COLWELL, R. R. 1975a. Absorption of *Vibrio parahaemolyticus* onto chitin and copepods. *Appl. Microbiol*. **29:** 269-274.

KANEKO, T. and COLWELL, R. R. 1975b. Incidence of *Vibrio parahaemolyticus* in Cheapeake Bay.*Appl. Microbiol*. **30**: 251-257.

KANEKO, T. and COLWELL, R. R. 1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. *Microb. Ecol*. **4**: 135-155.

KAYSNER, C.A., ABEYTA, C., WEKELL, M.M., DE PAOLA, A., STOTT, R.F., and LEITCH, J. M.1987. Incidence of *Vibrio cholerae* from estuaries of the United States West coast. *Appl Environ Microbiol*. **53**: 1344-1348.

KEDAR, G. T., PATIL, G. P. and YEOLE, S. M. 2007. Effect of physic-chemical factors on the seasonal abundance of zooplankton population in Rishi Lake. *Proceedings of Tall: The 12th world Lake conference:* 88-89.

KELLY-HOPE, L. A., ALONSO, W. J., THIEM, V.D., CANCH, D. G., ANH, D. D., LEE, H. and MILLER, M. A. 2008. Temporal trends and climatic factors associated with bacterial enteric diseases in Vietnam, 1991-2001. *Environmenta Health Perspectives*. **116**: 7-12.

KIRCHMAN, D. and MITCHELL, R. 1982. Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. *Appl. Environ. Microbial*. **43**: 200-209.

KIRPICH, ALEXANDER, WEPPELMANN, T. A., TANG, Y., ALI, A., MORRIS, J. G., Jr and LONGINI, I. M. 2015. Cholera transmission in Quest department of Haiti: Dynamic modeling and the future of the epidemic. PLOS Neglected Tropical Diseases. **10** (9): e0004153.

KIRCHNER, M. 1995. Microbial colonization of copepod body surface and chitin degradation in the sea. *Helgol Meeresunters.* **49**: 201-212.

KOGURE, T. and COLWELL, R. R. 1975.Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods.*Appl. Environ. Microbiol*. **29**: 269-274.

KOGURE, K., Simidu, U. and Taga, N. 1980. Effect of phyto and zooplankton on the growth of marine bacteria in filtered seawater. *Bull. Jpn. Soc. Sci. Fish.* **46**: 323-326.

LEE J. V., BASHFORD D. J., DONOVAN, T. J., FURNISS, A. L. and WEST, P. A. 1984. An incidence and distribution of *Vibrio cholerae* in waters England. *In* R. R. colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.p. 427-450.

LEVINE, M. M. BLACK, R.E., CLEMENTS, M. L., NALIN, D.R. CISNEROS, L. and FINKELSTEIN, R. A. 1981. Volunteer studies in development of vaccine against cholera and enterotoxigenic*Escherichia coli:* a review. In Holme, T. J., Holmgren, M. Merson, H. and Mollby, R. editors, *Acute enteric infection in children*. New prospects for treatment and prevention, Elsevier/North-Holland Biomedical Press, Amsterdam.pages 443-459.

LEVINE, M. M., BLACK, R. E., CLEMENTS, M. L., CISNEROS, L., SAAH, A. D.,NALIN, R., GILL, D. M., CRAIG, J. P., YOUNG, C. R. and RISTAINO, P. J. 1982. *Infect Dis.* **145**: 296-299.

LIPP, E., HUQ, A. and COLWELL, R. R. 2002.Effects of global climate on infectious disease: the cholera model. *Clin. Microbiol.* **15** (4): 757-70. [PMC free article] [Pubmed].

LONGINI, I. M., YUNUS, JR., ZAMAN, M. K., SIDDIQUE, A. K., SACK, R. B. and NIZAM, A. 2002. Epidemic and endemic cholera trends over a 33-year period in *Bangladesh. J. Infect Dis.* **186**: 246-51.

LOUIS, V. R., RUSSEK-COHEN E., CHOOPUN, N. *et al.*, 2003. Predictability of *Vibrio cholerae* in Chesapeake Bay. *Appl. Environ. Microbiol*. **69**: 2773-2785.

LI, X. B., and ROSEMAN, S. 2004. The chitinolytic cascade in Vibrios is regulated by chitin oligosaccharides and a two component chitin catabolic sensor/kinase. *ProcNatlAcadSci USA* **101**: 627-631.

MATZ, C. and KJELLEBERG, S. 2005. Off the hook-how bacteria survive protozoan grazing. Trends microbial. **13**: 302-307.

MBOERA, L. E., MAYALA, B. K., KWEKA, E. J. and MAZIJO, H. D. 2012. Impact of climate change on human health systems in Tanzania: A Review. *Tanzania Journal of Health Research.* **13**: 5.

MCDOUGALD, D., RICE, S. A. and KJELLEBERG, S. 1999. New perspectives on the viable but non-culturable response. *Biologia-bratislava*. **54**: 617-624.

MCDOUGALD, D., RICE, S. A., WEICHART, D. and KJELLEBERG, S. 1998. Non-culturability: Adaptation or debilitation? *FEMS Microbiol. Ecol.* **25**: 1-9.

MEILBOM, K. L., LI, X. B., NIELSON, A.T., *et al.*, 2004.The *Vibrio cholerae* chitin utilization program. *PNAS.* **101:** 2524-2529.

MENDELSOHN, J. and DAWSON, T. 2008. Climate and cholera in KwaZulu-Natal, South Africa. The role of environmental factors and implications for epidemic preparedness. *Int J Hyg Environmental Health. 2***11**: 156-162.

MICHAEL, R.G., 1968a. Studies on zooplankton of a tropical fish pond.*Hydrobiologia*.**32**: 47-68.

MILLER, C.J., DRASAR, B.S. and FEACHEM, R.G., 1984.Response of toxigenic *Vibrio cholerae* O1 to physico-chemical stresses in aquatic environment. *J. Hyg. Camb*. **93**:475-495.

MISHRA, A., TANEJA, N., SHARMA, R. K., KUMAR, R., SHARMA, N. C. and SHARMA M, 2011. Amplified fragment length polymorphism of clinical and environmental *Vibrio cholerae* from a freshwater environment in a cholera-endemic area, India. *BMC Infect Dis*. **11**: 249.

MISHRA, A., TANEJA, N. and SHARMA M, 2012. Viability kinetics, induction, resuscitation and quantitative real-time polymerase chain reaction analyses of viable but non-culturable*Vibriocholerae* O1 in freshwater microcosm. *J. Appl. Microbiol*. **112**: 945-953.

MORRIS, J. G., PICARDI, JR. J. L., LIEB, S., LEE, J.V., ROBERTS, A., HOOD, M., GUNN, R. A. and BLAKE, P. A. 1984. *J Clin. Microbiol.* **19**: 296-297.

MOZUMDER, K. M., NASER, M. N., ALI, M. S., ALAM, M., HUQ, A. SACK, R. B. and COLWELL, R. R. 2010.Qualitative and quantitative analysis of zooplankton of some coastal water bodies of Bakerganj, Bangladesh. *Bangladesh J. Zool*. **38**(1): 127-132.

MOZUMDER, P. K., NASER, M. N., ALAM, M., SACK, R. B., COLWELL, R. R. and HUQ, A. 2011a. Taxonomical study of the Rotifera fauna of Mathbaria in southern part of Bangladesh. *Bangladesh J. Zool*. **39**(1): 01-10.

MOZUMDER, P. K., NASER, M. N., ALAM, M. and HUQ, A. 2011b. Abundance and seasonal diversity of zooplankton in coastal aquatic environments of Mathbaria, Bangladesh. *Dhaka Univ. J. Biol. Sci.* **20**(2): 163-171.

MOZUMDER, P. K., BANU, M. A., NASER, M. N., ALI, M. S., ALAM, M., SACK, R. B., COLWELL, R. R. and HUQ, A. 2011c. Occurrence of protozoans & their limnological relationships in some ponds of Mathbaria, Bangladesh. *Univ. J. Rajshahi*. **29**: 01-03.

MUELLER, R. S., MCDOUGALD, D., CUSUMANO, D., SODHI, N., KJELLEBERG, S., AZAM, F. and BARTLETT, D. H. 2007. *Vibrio cholerae* strains possess multiple strategies for abiotic and biotic surface colonization. *J Bacteriol.* **189**: 5348-5360

NAGY, B., MOON, H. W. and ISAACSON, R. E. 1977. Colonization of porcine intestine by enterotoxigenic*Eshcherichia coli*: selection of piliated forms in vivo, adhesion of piliated forms to epithelial cells in vitro, and incidence of a pilus antigen among porcineenteropathogenic*E. coli Infect. Immun*. **16**: 344-352.

NAHAR, S.,ALI, S., NASER, N. M., ALAM, M. and MOZUMDER, P. K. 2008. Rotifer Fauna of three Pristine Coastal Ponds from South-Western Bangladesh. *Bangladesh J. Zool.* **36**(2): 305-307.

NAHAR, S., SULTANA, M., NASER, N. M., NAIR, B. G., WATANABE, H., *et al.,* 2012.Role of shrimp chitin in the ecology of toxigenic *Vibrio cholerae* and cholera transmission.*Frontiers in Microbiology*. **2**:120-125.

NALIN, D.R. 1976. Cholera, Copepods and chitinase, lancet ii: 958.

NALIN, D. R., DAYA, V., REID, A., and LEVINE, M.M.C.L. 1979. Adsorption and growth of *Vibrio cholerae* on chitin. *Infect Immun.* **25**: 768-770.

NANDI, B., NANDY, R. K., MUKHOPADHYAY, S., NAIR, G. B., SHIMADA, T. and GHOSE, A. C. 2000. Rapid method for species-specific identification of *V. cholerae* using primers targeted to the gene of outer membrane protein Ompw. *J. Clin. Microbiol*. **38**: 4145-4151,

NIC, M., JIRAT, J., KOSATA, B., JENKINS, A and MCNAUGHT, A. 2009. IUPAC Compandium of Chemical Terminology. Pp. 165.

NILSON, L, OLIVER, J. and KJELLEBERG, S. 1991. Resuscitation of *Vibrio vulnificus* from the viable but non-culturable state. *J. Bacteriol*. **173**: 5054-5059.

OLIVER, J. D. 2010. Recent findings on the viable but non-culturable state in pathogenic bacteria. *FEMS Microbiol. Rev*. **34:** 415-425.

PAJ, S. 2009. Impact of temperature variability on cholera incidence in southeastern Africa, 1997-2006. *Ecohealth***.** **6**: 340-345.

PASCUAL, M., RODO, X., ELLNER, S. P., COLWELL, R. R. and BOUMA, M. J. 2000. Cholera dynamics and El Nino-Southern Oscillation. *Science*. **289**: 1766-1769.

PASCUAL, M., BOUMAN, M. J. and DOBSON, A. P. 2002. Cholera and climate: revisiting the quantitative evidence. *Microbes Infect*. **4**(2):237–45. [PubMed]

PATALAS, K. 1972. Crustacean plankton and the eutrophication of St. Lawrence Great lakes. *J. Fish. Res. Bd. Can.* **29**: 1451-1462.

PEDROS, A. C. and BROCK, T. D. 1983. The importance of attachment to particles for planktonic bacteria. Arch. *Hydrobiol.* **98**: 354-379.

POLLITZER, R. 1959. History of the disease. Cholera. Bull. World Health Organ. **10:** 421.

POULICEK, M., GAILL, F. and Goffinet, G. 1998. Chitin biodegradation in marine environments. ACS *Symp Ser.* **707**: 163-210.

PRADHAN, V. P. 2014. Zooplankton diversity in freshwater Wunna Lake. *Int. J. of Life Sciences*, 2014. Vol. **2**(3): 268-272.

RAHMAN, S. and HUSSAIN, M.A. 2008. A study on the abundance of zooplankton of a culture and non-culture pond of Rajshahi University campus. *Univ. J. Zool. Rajshahi Univ*. **27**: 35-41.

RAMAMURTHY, T., GARG, S., SHARMA, R., BHATTACHARYA, S.K., NAIR, G.B., SHIMDA, T., TAKEDA, T., KARASAWA, T., KURAZANO, H., PAL, A. and TAKEDA, Y.1993. *Lancet*. **341**: 703-704.

RAVIKUMAR, M., MANJAPPA, S., KIRAN, B.R., PUTTAIAH, E. T. and RAMESH, I. 2005. Hydrography of Bengali tank Harapanahali, Devangere District, *Indian J. Environmental Prot*.**27** (5): 454-458.

RAWLINGS, T. K., RUIZ, G. M. and COLWELL, R. R. 2007.Association of *Vibrio cholerae* O1 El Tor and O139 Bengal with the copepods *Acartia tonsa* and *Eurytemora affinis. Appl. Environ. Microbiol*. **73**: 7926-7933.

REBAUDET, S., SURDRE, B., FAUCHER, B. and PIARROUX, R. 2013. Environmental determinants oif cholera outbreaks in inland Africa: A systematic review of main transmission foci and propagation routes. Supplement, *J. Infect. Dis*. **208**(S1): 46-54.

RE VELLE, P. and RE VELLE, C. 1982. The Environment: Issues and Choices for Society. 1[st]ed. New York. D. Van Nostrand Company.

REYBURN, R., KIM, D.R., EMCH, M., KHATIB, A., VON, S, L. and ALI, M. 2011. Climate variability and the outbreaks of cholera in Zanzibar, East Africa: A time series analysis. *Am J Trop Med Hyg.* **84**: 862-869.

RINALDO, A., BERTUZZO, E., MARI, L., BLOKESCH, M., GATTO, M., RODRIGUEZ-ITURBE, I. 2012. Reassessment of the 2010-2011 Haiti cholera outbreak and rainfall-driven multiseason projections. Proc. Natl. Acad. Sci. USA. **109:** 6602-6607.

ROBERTS, N. C., BRADFORD, JR., H. B. and BARBAY, J. A. 1984. Ecology of *Vibrio cholerae* in Lousiana Coastal waters. *In* R. R. colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.Pp. 389-398.

ROGGERS, R., CUFFE, R., COSSINS, Y., MURPHY, D., and BOURKE, A. 1980.The Queensland Cholera incident of 1977; II, the epidemiological investigation. Bull World Health Organ.**58**: 665-669.

SACK, R. B., SIDDIQUE, A. K., LONGINI, I. M., NIZAM, JR., YUNUS, A. M., ISLAM, M. S., MORRIS, J. G. Jr., ALI, A., HUQ, A. NAIR, G. B.,QADRI, F. S.,FARUQUE, M. D., SACK, A. and COLWELL, R. R. 2003. A 4-year study of the epidemiology of *V. cholerae* in four rural areas of Bangladesh. *J. Infect Dis*. **187**: 96-101.

SAGER, R. E. and HASLER, A. D. 1969. Species diversity in lacustrine phytoplankton 1. The components of the index of diversity from Shannon's formula. *American Naturalist*. **103:** 51-59.

SAHA, T. K. 2004. Net plankton diversity in coal mining areas of Jharkhand. *Ecol. Environ. Conserve.* **10**: 11-16.

SENOH, M., GHOSH-BANERJEE, J., RAMAMURTHY, T., HAMABATA, T., KURAKAWA, T., TAKEDA, M. *et al.*, 2010. Conversion of viable but non-culturable*Vibriocholerae* to the culturable state by co-culture with eukaryotic cells. *Microbial. Immunol*. **54**: 502-507.

SHARMA, M. S., SHARMA, V. and MALARA, H. 2007. Biodiversity of zooplankton in relation to different types of aquatic pollution. C. P. 46. NSL 2007. pp. 300-302.

SHIKUMA, N. J. and HADFIELD, M. G. 2010. Marine biofilms on submerged surfaces are a reservoir for *Escherichia coli* and *Vibrio cholerae*. *Biofouling.* **26**: 39-46.

SHUKLA, B. N., SINGH, D. V. and SANYAL, S. C. 1995. Attachment of non-culturable toxigenic *Vibrio cholerae* O1 and non O1 and *Aeromonas* spp. To the aquatic arthropod *Gerrisspinolae* and plants in the River Ganga. Varanasi. *FEMS Immunol MED Microbiol*. **12**(2):113-20.

SINGLETON, F., ATTWELL, R., JANGI, M. and COLWELL, R. R. 1982. Influence of salinity and organic nutrient concentration on survival and growth of *V. cholerae* in aquatic microcosms. *Appl Environ Microbiol*. **43**:1080-1085.

SINGLETON, F. L., ATTWELL, R., JANGI, S. and COLWELL, R. R. 1982.Effects of temperature and salinity on *Vibrio cholerae* growth. *Appl. Environ. Microbiol*. **44**: 1047-1058.

SNOW, J. 1855. On the Mode of Communication of Cholera. New Burlington Street, London: John Churchill.

SPIRA, W.M., HUQ, A., AHMED, Q.S. and SAYEED, Y.A. 1981. Uptake of *V. cholerae* biotype El Tor from contaminated water by water hyacinth (*Eichorniacrassipis*). *Appl. Environ. Microbiol*. **42**: 550-553.

STEMBERGER, R.S. 1990. “An inventory of rotifer species diversity of northern Michigan inland lakes”, Archiv fur Hydrobiologie. **118**: 283-302.

STRAILE, D. and GELLER, W. 1998. Crustacean zooplankton in Lake Constance from 1920 to 1995: Response to eutrophication and reoligotrophication. Archive fur Hydrobiol. (Special issue Advances in Limnology) **53**: 255-274.

SUIKKANEN, S., PULINA, S; ENGSTROM-OST, J; LEHTINIEMI, M; LEHTIMEN, S and BRUTEMARK, A. 2013. Climate change and eutrophication induced shifts in northern summer plankton communities. PLoS one 8, C66475.

SWERDLOW, D. L. and ISAACSON, M. 1994.The epidemiology of cholera in Africa.In: *Vibrio cholerae* and Cholera: Molecular to Global Perspectives (Wachsmuth, K.I., Blake, P.A. and Olsik, O, Eds.). American Society of Microbiology Press, Washington, DC.pp. 297-307.

TALL, A., HERVIO-HEALTH, D., TEILLON, A., BOISSET-HELBERT, C., DELESMONT, R., BODILIS, J., *et al.*, 2013. Diversity of *Vibrio spp*. isolated at ambient environmental temperature in the eastern English Channel as determined by pyrH sequencing. *J. Appl. Microbiol*. **114**: 1713-1724.

TAMPLIN, M. L. and COLWELL, R. R. 1986. Effects of Microcosm Salinity and Organic Subtrate Concentration on Production of *Vibrio cholerae* Enterotoxin. *Appl. Environ. Microbiol*. **52**(2): 297-301.

TAMPLIN, A. L, GAUJENS, A. L, HUQ, A., SACK, D. A. and COLWELL, R. R. 1990.Attachment of *Vibrio cholerae*serogroup O1 to zooplankton and phytoplankton of Bangladesh waters.*Appl Environ Microbiol.* **56**(6): 1977-80.

TANEJA, N., KAUR, J., SHARMA, K., SINGH, M., KALRA, J. K., SHARMA, N. M. and SHARMA, M. 2003. A recent outbreak of cholera due to *Vibrio cholerae* O1 Ogawa in and around Chandigarh, North India. *Indian J Med Res*. **117**: 243-246.

TANEJA, N., BISWAL, M., TARAI, B. and SHARMA, M. 2005. Emergence of *Vibrio cholerae* O1 biotype ElTor serotype Inaba in North India. *JPNJ Infect Dis*. **58**: 238-240.

TANEJA, N., MISHRA, A., SANGAR, G., SINGH, G. and SHARMA, M. 2009. Cholera outbreaks in North India due to new variants of *Vibrio cholerae* O1 El Tor. *Emerg Infect Dis.***15:** 352.

TANEJA, N., SAMANTA, P., MISHRA, A. and SHARMA, M. 2010. Emergence of tetracycline resistance in *Vibrio cholerae* O1 biotype ElTor serotype Ogawa from North India. *Indian J PatholMicrobiol.* **53**: 865-866.

TAUXE, R., SEMINARIO, L., TAPITA, R. and LIBEL, M. 1994. The Latin American epidemic. In: *Vibrio cholerae* and Cholera: Molecular to Global Perspectives (Wachsmuth, K.I., Blake, P. A. and Olsik, O. Eds.). American Society of Microbiology Press, Washington, DC.pp. 321-344.

THILAK, J. 2009. On the zooplankton diversity in Gandhi sagar reservoir, MandsaurDistt, Madhya Pradesh Bionotes. **11**(2): 54-55.

THOMAS, K., JOSEPH, N., RAVEENDRAN, O. and NAIR, S. 2006. Salinity-induced survival strategy of *Vibrio cholerae* associated with copepods in Cochin backwaters. *Mar. Pollut. Bull*.**52**: 1425-1430.

TRAERUP, S. L. M., ORTIZ, R. A. and MARKANDYA, A. 2012. The costs of Climate Change: the study of cholera in Tanzania. *Int J Environ Res Public Health*. **9**: 831-854.

TRIPATHI, R. B, SINGH I and TIWARI D. D. 2006. Qualitative and Quantitative study of zooplankton in Seetadwar Lake of Shravasti, U.P. India. Flora and Fauna. **12**: 37-40.

TURNER, J. W., GOOD, B., COLE, D., *et al.,* 2009. Plankton composition and environmental factors contribute to *Vibrio* seasonality. ISME J. **3**: 1082-1092.

VEZZULLI, L., BRETTAR, I., PEZZATI, E., REID, P. C., COLWELL, R. R., HOFLE, M. G.,*et al*., 2011. Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios. *ISME J.* **6**: 21-30.

WATNICK, P. I., FULLNER, K. J. and KOLTER, R.1999. A role for the mannose sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* ElTor. *J. Bacteriol.* **181:**3606–3609.

WATNICK, P. and KOLTER, R. 1999. Steps in the development of a *Vibrio cholerae* El Tor biofilm. *Mol. Microbiol*. **34**: 586-595.

WATNICK, P. I., LAURIANO, C.M., KLOSE, K.E., CROAL, L. and KOLTER, R. 2001. The absence of a flagellum leads altered colony morphology, biofilm development and virulence in *Vibrio cholerae* O139. *Mol. Microbiol*. **39**: 223-235.

WEST, P. A. and LEE, J. V. 1982. Ecology of *Vibrio* species including *Vibrio cholerae* in natural waters of kent. England. *J. Appl. Bacteriol*. **52**: 435-448.

WEST, P. 1989. The human pathogenic vibrios- A public health update with environmental perspectives. *Epidemiology and Infection*. **103**(1): 1-34.

XU, H.S., ROBERTS, N., SINGLETON, F. L., ATTWELL, R. W., GRIMES, D.J. and COLWELL, R.R. 1982. Survival and viability of non-culturable *Escherichia coli* and *V. cholerae* in estuarine and marine environment. *Microbiol. Ecol*. **8**: 313-323.

YILDIZ, F.H. and SCHOOLNIK, G.K. 1999. *Vibrio cholerae* O1 El tor: Identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. Proc. *Natl. Acad. Sci.* USA. **96**: 4028-4033.

YU, C., LEE, A. M., BASSLER, B. L. and ROSEMAN, S. 1991. Chitin utilization by marine bacteria- a physiological function for bacterial adhesion to immobilized carbohydrates. *J Biol Chem*. **266**: 24260-24267.

ANNEXURE

Annexure 1. Total number of zooplankton/ml in Mathbaria ponds during 2013 study

Months	Plankton Group	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
January 2013	Protozoa	0	1	0	5	9	4	7
	Rotifera	6	0	0	1	0	2	0
	Nauplii	0	0	0	0	0	0	0
	Copepoda	3	0	15	0	20	0	1
	Cladocera	0	0	0	0	0	0	0
February 2013	Protozoa	5	1	0	1	1	4	1
	Rotifera	1	1	7	1	1	22	7
	Nauplii	0	1	1	0	1	11	13
	Copepoda	0	0	0	1	3	3	3
	Cladocera	3	0	0	0	1	1	3
March 2013	Protozoa	1	1	1	1	1	1	0
	Rotifera	12	38	1	1	5	8	1
	Nauplii	3	7	15	1	4	8	19
	Copepoda	2	2	29	3	6	6	9
	Cladocera	1	2	17	0	0	1	1
April 2013	Protozoa	1	2	0	6	7	3	3
	Rotifera	1	36	0	0	25	7	11
	Nauplii	76	7	6	3	19	19	27
	Copepoda	17	0	9	2	3	7	4
	Cladocera	9	2	21	1	2	2	1
May 2013	Protozoa	2	3	2	11	1	1	1
	Rotifera	1	4	1	1	1	1	1
	Nauplii	3	7	18	0	0	3	3
	Copepoda	1	1	2	1	0	5	1
	Cladocera	2	1	4	2	2	5	2
June 2013	Protozoa	5	5	0	30	0	0	1
	Rotifera	0	50	0	0	2	0	0
	Nauplii	0	53	5	8	1	0	0
	Copepoda	0	0	4	6	1	1	0
	Cladocera	2	1	2	0	0	2	1
July 2013	Protozoa	3	4	1	7	0	3	1

	Rotifera	0	0	0	0	0	0	1
	Nauplii	0	0	29	0	0	0	0
	Copepoda	0	0	7	0	0	0	0
	Cladocera	0	0	5	0	2	4	4
August 2013	Protozoa	40	9	13	3	0	0	0
	Rotifera	0	27	0	1	1	1	1
	Nauplii	0	53	0	3	16	0	0
	Copepoda	0	3	2	1	3	10	0
	Cladocera	0	1	0	0	1	2	2
September 2013	Protozoa	2	3	2	10	3	3	2
	Rotifera	13	1	0	1	1	1	0
	Nauplii	8	10	31	1	9	11	5
	Copepoda	1	1	4	0	0	3	1
	Cladocera	1	0	3	0	2	1	0
October 2013	Protozoa	6	1	2	3	3	5	5
	Rotifera	58	32	10	0	29	29	5
	Nauplii	21	15	39	0	42	15	25
	Copepoda	1	0	39	8	3	4	6
	Cladocera	0	0	8	1	3	5	1
November 2013	Protozoa	0	0	13	22	9	55	7
	Rotifera	6	58	7	3	0	50	4
	Nauplii	35	32	29	4	21	3	9
	Copepoda	3	0	22	5	0	2	0
	Cladocera	0	0	0	0	0	7	0

Annexure 2. Total number of zooplankton/ml in Mathbaria ponds during 2014 study

	Plankton Group	Site-2	Site-5	Site-7	Site-8	Site-9	Site-10	Site-11
January 2014	Protozoa	12	0	4	38	4	16	3
	Rotifera	74	4	25	4	34	169	72
	Nauplii	21	11	22	0	57	32	29
	Copepoda	5	8	3	3	6	7	3
	Cladocera	0	0	0	0	0	0	0
February 2014	Protozoa	1	2	4	5	5	10	0
	Rotifera	6	4	2	2	61	91	39
	Nauplii	27	16	7	9	42	32	21
	Copepoda	14	5	5	3	4	22	12

	Cladocera	8	1	1	0	0	0	1
March 2014	Protozoa	2	1	1	7	0	1	0
	Rotifera	4	2	3	1	3	22	7
	Nauplii	29	2	16	2	87	36	11
	Copepoda	24	4	5	5	54	12	6
	Cladocera	6	1	10	0	11	3	1
April 2014	Protozoa	3	0	1	14	1	1	0
	Rotifera	51	2	1	0	1	0	1
	Nauplii	8	7	11	5	21	17	17
	Copepoda	3	5	15	4	6	15	2
	Cladocera	0	1	28	0	1	3	1
May 2014	Protozoa	1	0	1	15	0	0	0
	Rotifera	3	13	1	1	1	1	2
	Nauplii	71	10	36	7	109	19	30
	Copepoda	39	7	19	5	63	7	14
	Cladocera	5	11	1	0	3	17	2
June 2014	Protozoa	0	0	0	0	0	0	0
	Rotifera	0	13	0	0	0	0	0
	Nauplii	0	19	3	1	0	0	0
	Copepoda	3	61	19	4	7	2	3
	Cladocera	1	37	7	0	3	0	2
July 2014	Protozoa	0	3	2	1	1	0	0
	Rotifera	1	1	1	0	0	0	0
	Nauplii	6	5	1	2	1	2	3
	Copepoda	1	1	0	0	0	0	1
	Cladocera	0	0	0	1	0	0	0
August 2014	Protozoa	0	3	0	0	0	0	0
	Rotifera	9	0	2	2	0	0	0
	Nauplii	4	1	3	1	1	1	1
	Copepoda	0	0	0	2	0	2	1
	Cladocera	0	0	0	0	0	0	0
September 2014	Protozoa	1	1	0	0	1	3	1
	Rotifera	0	2	2	0	0	0	0
	Nauplii	1	1	0	1	1	1	2
	Copepoda	2	1	0	0	0	1	2
	Cladocera	0	0	0	0	0	0	0
October 2014	Protozoa	0	0	1	0	3	3	1

	Rotifera	2	3	6	0	0	11	0
	Nauplii	42	45	10	8	50	18	17
	Copepoda	37	8	2	2	1	2	6
	Cladocera	0	0	0	0	0	2	0
November								
2014	Protozoa	0	2	7	4	13	18	1
	Rotifera	11	2	0	1	0	4	1
	Nauplii	35	8	0	3	0	7	9
	Copepoda	8	1	0	1	0	0	1
	Cladocera	0	0	0	0	0	1	2
December								
201	Protozoa	0	3	0	0	0	2	0
	Rotifera	5	6	0	0	1	20	3
	Nauplii	12	6	20	8	17	7	22
	Copepoda	3	3	15	3	1	2	3
	Cladocera	0	0	3	2	0	2	4

Annexure 3. Total number of zooplankton/ml inChhatak ponds during 2013 study

	Plankton Group	**Site-2**	**Site-5**	**Site-7**	**Site-8**	**Site-9**	**Site-10**	**Site-11**
January								
2014	Protozoa	17	3	0	0	0	3	6
	Rotifera	16	9	8	1	8	0	21
	Nauplii	0	0	0	0	0	0	0
	Copepoda	39	2	1	17	0	26	12
	Cladocera	11	2	1	1	3	3	0
February		0	0	0	13	0	5	0
2014	Protozoa							
	Rotifera	14	17	8	0	40	82	201
	Nauplii	0	0	0	0	0	221	0
	Copepoda	108	0	0	0	0	92	0
	Cladocera	0	0	0	0	0	1	0
March		0	4	0	0	0	0	2
2014	Protozoa							
	Rotifera	41	30	9	3	55	0	0
	Nauplii	46	32	0	0	144	0	0
	Copepoda	92	27	0	0	124	365	2
	Cladocera	0	2	1	0	3	7	0
April		1	6	0	8	0	149	0
2014	Protozoa							
	Rotifera	51	14	125	2	0	19	0

	Nauplii	0	38	0	0	0	0	0
	Copepoda	2	16	0	0	0	0	6
	Cladocera	0	10	72	0	4	0	1
May		5	2	1	15	2	2	1
2014	Protozoa							
	Rotifera	56	3	1	26	2	0	4
	Nauplii	0	0	0	0	0	0	0
	Copepoda	4	0	18	0	0	0	0
	Cladocera	0	5	0	0	0	0	10
June		10	7	6	16	4	29	27
2014	Protozoa							
	Rotifera	1	2	18	5	1	77	31
	Nauplii	2	0	0	0	1	2	153
	Copepoda	7	1	2	2	0	1	8
	Cladocera	5	0	2	1	0	0	2
July		1	7	0	51	1	7	25
2014	Protozoa							
	Rotifera	0	0	6	61	2	39	10
	Nauplii	0	0	0	104	0	0	165
	Copepoda	39	0	0	109	0	0	21
	Cladocera	0	0	0	0	0	0	0
August		7	0	0	11	22	0	1
2014	Protozoa							
	Rotifera	189	2	0	15	12	15	0
	Nauplii	0	0	0	11	7	0	1
	Copepoda	4	1	2	4	2	0	8
	Cladocera	8	0	2	3	2	0	2
September2014	Protozoa	2	1	2	28	3	21	21
	Rotifera	343	7	135	48	4	54	101
	Nauplii	8	1	7	58	2	35	2
	Copepoda	8	1	1	11	0	10	6
	Cladocera	44	1	4	12	0	59	4
October		2	4	2	13	1	1	6
2014	Protozoa							
	Rotifera	24	5	1	6	2	55	12
	Nauplii	21	2	1	8	1	1	17
	Copepoda	16	1	1	2	1	4	8
	Cladocera	3	1	0	0	1	3	1
November2014	Protozoa	2	18	19	15	1	5	32
	Rotifera	67	7	40	68	14	6	37
	Nauplii	40	3	6	6	5	3	10
	Copepoda	31	0	1	16	1	2	2
	Cladocera	0	5	0	8	3	3	1

Annexure 4. Total number of zooplankton/ml in Chhatak ponds during 2014 study

Months	Plankton Group	Site-1	Site-2	Site-4	Site-9	Site-10	Site-11	Site-12
January 2014	Protozoa	2	3	15	7	18	17	5
	Rotifera	148	9	60	16	3	2	37
	Nauplii	7	2	0	71	9	1	0
	Copepoda	0	0	0	19	9	1	10
	Cladocera	0	0	0	1	11	0	35
February 2014	Protozoa	1	2	2	11	2	0	2
	Rotifera	3524	70	5	65	12	6	0
	Nauplii	0	5	15	35	14	12	0
	Copepoda	0	0	19	31	2	3	0
	Cladocera	0	17	5	2	3	0	1
March 2014	Protozoa	1	0	0	8	1	1	9
	Rotifera	2	2	4	2	1	1	0
	Nauplii	2	5	0	2	0	0	0
	Copepoda	3	11	0	0	0	0	0
	Cladocera	0	2	0	2	0	1	0
	Ostracoda	0	0	0	0	0	0	0
April 2014	Protozoa	3	1	3	28000	56	412	605
	Rotifera	15	8	94	193	9	190	190
	Nauplii	18	25	23	28	36	530	150
	Copepoda	0	3	11	11	5	16	2
	Cladocera	1	1	12	1	7	10	18
May 2014	Protozoa	4	65	109	50411	169	21	161
	Rotifera	404	14	668	16	5	2	79
	Nauplii	89	13	54	63	3	1	2
	Copepoda	7	6	16	11	7	0	2
	Cladocera	16	2	1	1	3	0	0
June 2014	Protozoa	0	20	11	20000	160	3	0
	Rotifera	8	23	527	120	7	3	71
	Nauplii	5	17	6	110	7	0	101
	Copepoda	0	8	13	6	3	0	20
	Cladocera	0	2	16	1	5	0	5
July 2014	Protozoa	10	1	25	21004	100	22	14

	Rotifera	32	3	116	35	4	24	100
	Nauplii	4	0	7	40	2	4	99
	Copepoda	0	0	14	7	0	0	12
	Cladocera	3	2	20	9	1	2	5
August 2014	Protozoa	0	1	0	3	0	0	0
	Rotifera	225	12	6	91	6	29	6
	Nauplii	125	12	3	185	4	0	34
	Copepoda	83	2	0	55	0	0	92
	Cladocera	159	9	0	43	0	36	10
September 2014	Protozoa	5	1	8	7	0	4	1
	Rotifera	16	1	6	52	1	5	8
	Nauplii	8	3	1	65	2	1	11
	Copepoda	6	1	1	36	1	0	4
	Cladocera	22	1	4	11	1	15	2
October 2014	Protozoa	1	2	1	186	1	4	3
	Rotifera	7	5	18	37	2	44	17
	Nauplii	10	6	12	51	6	9	137
	Copepoda	36	1	8	30	3	21	16
	Cladocera	3	0	0	8	1	15	11
November 2014	Protozoa	9	0	1	1294	31	3	24
	Rotifera	2	9	29	43	15	4	11
	Nauplii	2	10	12	26	14	5	50
	Copepoda	2	2	3	17	10	10	21
	Cladocera	0	6	3	21	13	1	15
December 2014	Protozoa	0	0	4	4621	13	0	3
	Rotifera	6	0	28	7	0	66	157
	Nauplii	3	0	8	14	0	49	16
	Copepoda	10	2	0	2	0	120	1
	Cladocera	0	0	3	0	0	0	0

Annexure 5. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-2) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.061	1.386	.3573	.4015	.294	.9897	.1	.106	.966	0.602
February	1.692	2.397	.1971	.1079	.6671	1.509	.1414	.1888	.944	0.908
March	1.433	2.169	.4316	1.335	1.369	1.295	.1625	.1714	.558	0.873
April	1.605	2.157	.239	.1399	.7114	1.23	.1032	.1886	.825	0.899
May	1.557	1.43	.2192	.3393	.5791	.7894	.1581	.09494	0.967	0.688
June	.5983	0	.5913	1	.1526	0	.0756	0.1	0.863	0
July	1.099	.6931	.3311	.4975	.3506	.1887	.1732	.1414	1.0001	1.0001
August	.3141	1.099	.8587	.3326	.2411	.294	.04743	.1	0.286	1.001
September	1.714	1.04	.2615	.3734	.9874	.3338	.1567	.15	0.780	0.947
October	2.126	1.018	.1484	.4358	1.455	.3628	.1606	.06405	0.805	0.734
November	1.633	1.117	.2216	.3762	.7052	.4002	.1732	.09428	0.911	0.806
December	1.03	1.082	.3794	.3429	.2895	.2992	.09487	.1061		0.985

Annexure 6. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-5) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	.1	1.334	1	.275		.4141		.1069	.144	0.962
February	.6931	2.09	.4992	.1345	.1563	.9912	.08165	.1591	10001	0.951
March	2.16	1.733	.1923	.2018	1.863	.7947	.1877	.1606	0.747	0.890
April	1.789	1.908	.2458	.1641	1.233	.9043	.1386	.1668	0.7199	0.917
May	2.497	1.978	.09431	.1864	1.645	1.233	.2694	.1386	0.946	0.796
June	1.527	1.702	.2927	.244	.8145	.8993	.1089	.1053	0.734	0.775
July	.5623	1.332	.6241	.2786	.1669	.4827	.1	.1789	0.811	0.961
August	1.431	.6365	.3208	.5541	.7327	.1753	.1167	.1155	0.735	0.918
September	.7356	1.386	.5932	.2481	.2992	.5007	.1061	.2	0.669	1
October	1.581	1.089	.262	.4194	.9438	.4343	.1299	.1265	0.719	0.786
November	1.345	1.332	.3697	.2786	.6924	.4827	.09191	.1789	0.691	0.961
December	1.321	1.04	.2804	.3745	.4488	.2821	.1414	.0866	0.953	0.947

Annexure 7. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-7) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	.8532	1.985	.4752	.1501	.2735	.8673	.07746	.1414	0.777	0.955
February	1.332	2.031	.3121	.1594	.5422	1.145	.125	.1961	0.828	0.882
March	1.867	1.511	.1842	.3395	1.117	.9737	.1254	.148	0.778	0.688
April	1.426	1.021	.311	.5218	.6011	.5054	.0937	.04264	0.796	0.569
May	1.603	1.4	.2435	.3193	.7796	.6359	.1492	.1177	0.824	0.781
June	1.33	1.304	.2766	.3486	.469	.5579	.1633	.1387	0.959	0.810
July	1.413	1.099	.2776	.3311	.5579	.3506	.1387	.1732	0.878	1.001
August	1.137	0	.3774	1	.4102	0	.1033	.07	0.8203	0
September	1.574	1.099	.2546	.3311	.6902	.3506	.1604	.1732	0.878	1.001
October	2.245	1.894	.1305	.1563	1.435	.8568	.151	.2111	0.851	0.973
November	1.794	1.277	.2345	.3051	.9589	.4579	.1389	.1512	0.816	0.921
December	1.079	1.026	.346	.3762	.3053	.2668	.1134	.07071	0.983	0.934

Annexure 8. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-8) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.011	.9728	.3879	.5555	.3126	.5944	.1225	.08944	0.921	0.543
February	1.475	1.249	.2643	.3147	.6106	.3947	.189	.08944	0.917	0.901
March	1.288	1.279	.3052	.333	.4284	.5229	.1206	.1091	0.929	0.795
April	1.704	.8033	.2546	.5816	.9209	.3732	.1789	.07184	0.819	0.579
May	1.482	1.117	.3532	.4373	.9043	.5139	.1668	.1021	0.713	0.694
June	.9427	0	.4921	1	.3664		.0667	.1	0.680	0
July	.7963	.6931	.5504	.4975	.3053	.1887	.1134	.1414	0.725	1.0001
August	1.332	1.04	.2786	.3734	.4827	.3338	.1789	.15	0.961	0.947
September	.837	0	.583	0	.4231	0	.1155	0	0.604	0
October	1.342	0	.3149	1	.5229		.1091	.07	0.834	0
November	1.337	.8676	.3109	.4992	.4996	.3126	.0913	.1225	0.831	0.790
December	.6365	1.082	.5548	.3429	.1563	.2992	.0816	.1061	0.918	0.985

Annexure 9. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-9) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	.9486	1.39	.5099	.3449	.3763	.596	.07428	.09045	0.6844	0.776
February	1.82	1.918	.1799	.1946	.8463	1.108	.2021	.1207	0.935	0.8001
March	1.984	1.84	.1949	.2375	1.225	1.073	.1859	.1039	0.828	0.828
April	1.782	1.631	.3531	.2406	1.876	.7947	.1941	.7295	0.617	0.916
May	1.277	1.563	.3051	.2561	.4579	.6858	.1512	.08819	0.921	0.767
June	.6365	0	.5541	1	.1753	0	.1155	0.06	0.918	0
July	0	0	1	1	.07	0	0	0.1	0	0
August	1.04	0	.3734	0	.3338	0	.15	0	0.947	0
September	1.494	.6931	.2491	.4975	.5984	.1887	.1768	.1414	0.928	1.0001
October	2.109	.5623	.2032	.6241	1.523	.1669	.196	.1	0.799	0.811
November	.5297	1.157	.6539	.3723	.147	.4184	.06667	.1109	0.764	0.834
December	1.079	.6931	.346	.4975	.3053	.1887	.1134	.1414	0.983	1.0001

Annexure 10. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-10) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	.6365	2.252	.5548	.1453	.1563	1.518	.08165	.1143	0.918	0.812
February	1.939	2.865	.189	.07882	1.108	2.83	.1207	.2061	0.809	0.851
March	.6931	2.631	.4975	.08287	.1887	1.843	.1414	.2213	1.0001	0.928
April	1.187	1.704	.501	.2299	.8914	.8608	.1013	.1372	0.540	0.815
May	1.768	1.268	.2393	.4195	1.028	.7526	.1837	.13	0.805	0.651
June	.6365	0	.5541	0	.1753	0	.1155	0	0.918	0
July	.9557	0	.4278	0	.3053	0	.1134	0	0.870	0
August	1.525	.6931	.2302	.4975	.5579	.1887	.1387	.1414	0.948	1.0001
September	2.245	1.561	.1117	.2209	1.255	.6253	.2774	.2041	0.975	0.970
October	2.254	2.245	.1387	.1106	1.564	1.201	.1709	.2357	0.832	0.975
November	1.818	1.892	.2641	.1868	1.287	1.034	.1228	.1877	0.708	0.861
December	1.525	1.735	.235	.2038	.547	.763	.1291	.1373	0.948	0.891

Annexure 11. Monthwise Diversity indices of monthly zooplankton abundance in Mathbaria pond (site-11) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	.9003	1.662	.4681	.3135	.2992	.9793	.1061	.101	0.819	0.722
February	2.221	2.557	.1222	.09357	1.254	1.785	.2043	.1925	0.926	0.902
March	1.878	2.313	.1796	.1064	.9272	1.249	.1835	.2008	0.903	0.965
April	1.264	1.523	.4904	.2337	1.154	.588	.1444	.1667	0.527	0.946
May	1.834	1.88	.1792	.1904	.8686	1.013	.2214	.1732	0.942	0.856
June	.6931	0	.4975	1	.1887	0	.1414	.07	1.0001	0
July	.8676	0	.4992	1	.3126	0	.1225	.1	0.790	0
August	.6365	0	.5541	1	.1753	0	.1155	.1	1.01	0
September	1.609	1.332	.1984	.2786	.6436	.4827	.2236	.1789	1	0.961
October	1.905	1.004	.1953	.3869	1.111	.3053	.1741	.1134	0.827	0.914
November	.6555	1.332	.5368	.2786	.1428	.4827	.0603	.1789	0.946	0.961
December	1.079	1.28	.346	.2993	.3053	.4343	.1134	.1265	0.983	0.923

Annexure 12. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-1) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.733	1.122	.2102	.3951	.6649	.5152	.077	.04685	1.578	0.626
February	1.345	1.667	.2966	.2339	.4251	.8133	.0453	.175	0.836	0.857
March	1.496	1.33	.2492	.2766	.5266	.469	.05203	.1633	0.835	0.960
April	.2499	.7335	.8937	.6397	.2327	.3974	.04082	.09177	0.228	0.529
May	.6344	.4377	.7462	.8295	.6834	.5623	.08682	.03372	0.326	0.225
June	1.839	2.43	.1868	.1255	.9043	1.779	.1668	.2359	0.885	0.877
July	.6589	2.43	.5891	.1255	.2327	1.779	.04082	.2359	0.600	0.877
August	1.368	2.132	.3272	.1539	.6156	1.116	.05353	.06016	0.703	0.831
September	2.134	2.582	.2193	.09807	2.45	2.009	.134	.1768	0.647	0.862
October	2.593	2.052	.09291	.1845	1.804	1.471	.2018	.1685	0.915	0.778
November	2.01	2.1	.1925	.1477	1.51	1.134	.1457	.189	0.742	0.912
December	1.816	.9743	.1775	.4059	.7494	.2711	.1278	.075	0.933	0.887

Annexure 13. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-2) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.787	.9949	.2245	.4044	.9411	.2856	.194	.09045	0.860	0.906
February	1.103	1.984	.3771	.1814	.4141	1.115	.1069	.1768	0.796	0.862
March	1.896	1.712	.1921	.1898	1.035	.6722	.1291	.1455	0.824	0.955
April	2.59	1.992	.08959	.1473	1.897	.9763	.2507	.2219	0.914	0.958
May	1.609	1.117	.2392	.5009	.7238	.8865	.1897	.09879	0.898	0.508
June	1.609	2.042	.2392	.1446	.7238	.9309	.1897	.1225	0.898	0.929
July	0	1.561	1	.2209		.6253	.037	.2041	0.000	0.970
August	.6365	1.613	.5541	.2497	.1753	.7709	.1155	.1429	0.918	0.829
September	2.5	1.778	.09463	.1946	1.689	.8368	.2985	.1941	0.947	0.914
October	2.685	2.012	.07361	.142	1.908	.8994	.3138	.1633	0.969	0.968
November	2.427	2.599	.1206	.08797	1.947	1.845	.2286	.2744	0.840	0.938
December	1.321	0	.2804	1	.4488		.1414	.071	0.953	0.000

Annexure 14. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-4) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.643	1.877	.2192	.1925	.7238	.8927	.1897	.1019	0.917	0.854
February	.9743	2.034	.4055	.1626	.2992	1.107	.1061	.12	0.887	0.849
March	1.768	0	.2059	1	.8568		.2111	.05	0.909	0.000
April	1.743	1.722	.2296	.2621	1.016	1.28	.08001	.1197	0.727	0.671
May	.3944	1.596	.8149	.3117	.2631	1.254	.06708	.05657	0.359	0.589
June	2.31	.8459	.1451	.7123	1.638	1.622	.2646	.0954	0.875	0.293
July	.9165	2.265	.4375	.1583	.2856	1.846	.09045	.1449	0.835	0.769
August	.6931	1.061	.4987	.3573	.1669	.294	.1	.1	1.000	0.966
September	2.046	2.428	.2286	.1189	2.51	1.774	.1786	.2334	0.628	0.876
October	1.792	2.183	.1653	.1283	.7816	1.154	.2449	.1444	1.000	0.911
November	1.917	2.515	.1747	.1218	1.031	2.266	.127	.2137	0.833	1.203
December	.9557	1.418	.4278	.3615	.3053	.7352	.1134	.1183	0.870	0.729

Annexure 15. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-9) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.307	1.785	.3237	.247	.5298	1.199	.1147	.1697	0.812	0.745
February	.4293	1.617	.7394	.2762	.1395	.971	.05547	.09713	0.619	0.702
March	.6365	1.119	.5541	.4023	.1753	.4231	.1155	.1155	0.918	0.807
April	1.03	.0487	.3794	.9855	.2895	.6733	.09487	.00655	0.938	0.020
May	1.848	.00799	.2054	.9985	.9536	.7775	.1357	.0058	0.841	0.003
June	1.398	.04234	.3116	.987	.6459	.4134	.1251	.00493	0.780	0.022
July	2.632	.02175	.1096	.9951	2.448	.7555	.1576	.0083	0.808	0.009
August	2.082	1.768	.1464	.2571	1.099	1.014	.1667	.07939	0.904	0.738
September	3.209	2.335	.05837	.1803	3.437	2.551	.2795	.1652	0.910	0.708
October	2.667	1.657	.08578	.4222	2.032	2.372	.2745	.1339	0.923	0.509
November	2.907	.5022	.07017	.8516	2.572	2.39	.2352	.06954	0.903	0.148
December	1.352	.01603	.2643	.9961	.4579	.3066	.1512	.0073	0.975	0.010

Annexure 16. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-10) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.295	1.561	.2886	.3006	.4284	.951	.1206	.1342	0.934	0.711
February	1.302	2.434	.3636	.102	.6028	1.589	.09487	.2982	0.727	0.949
March	1.955	.6931	.1714	.4975	1.027	.1887	.08462	.1414	0.816	1.000
April	.6931	1.187	.4987	.501	.1669	.8914	.1	.1013	1.000	0.540
May	.6931	.357	.4987	.8726	.1669	.612	.1	.05203	1.000	0.183
June	.6365	.5295	.5541	.8064	.1753	.7165	.1155	.06047	0.918	0.255
July	.5004	.2367	.6794	.908	.1609	.324	.08944	.03904	0.722	0.171
August	1.422	1.011	.3819	.3879	.852	.3126	.1315	.1225	0.684	0.921
September	2.265	1.55	.1288	.2234	1.438	.6106	.2619	.189	0.911	0.963
October	2.206	2.014	.1218	.1415	1.242	.9339	.2673	.1886	0.958	0.969
November	2.467	2.737	.1103	.1136	1.756	2.715	2.785	.225	0.911	0.830
December	.6365	0	.5541	1	.1753		.1155	.027	0.918	0.000

Annexure 17. Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-11) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.389	1.155	.3181	.4362	.7434	.6272	.1237	.1114	0.714	0.645
February	2.052	1.099	.17	.3311	1.428	.3506	.1115	.1732	0.758	1.001
March	1.479	1.099	.2837	.3311	.7617	.3506	.04717	.1732	0.673	1.001
April	.8759	1.369	.6572	.3695	1.026	1.519	.08412	.0667	0.365	0.474
May	.5983	.5305	.5913	.7617	.1526	.3876	.07559	.08341	0.863	0.383
June	1.746	1.242	.2588	.3322	1.075	.469	.1049	.1633	0.728	0.896
July	.8845	1.975	.4867	.196	.2371	1.298	.04423	.1732	0.806	0.795
August	.5004	2.245	.6798	.1194	.1367	1.139	.05164	.1364	0.722	0.937
September	3.105	2.351	.06632	.1307	3.727	1.706	.2654	.1969	0.854	0.848
October	1.546	2.862	.3311	.07249	1.151	2.529	.1011	.2544	0.622	0.901
November	2.375	2.076	.1025	.1931	1.392	1.5	.2309	.1838	0.956	0.787
December	1.33	.6483	.2766	.5442	.469	.1018	.1633	.0147	0.960	0.935

Annexure 18.Monthwise Diversity indices of monthly zooplankton abundance in Chhatak pond (site-12) during 2013 and 2014

Months	Diversity Indices				Richness Indices				Pielou Evenness (J)	
	Shannon-Weiner (H′)		Simpson (D)		Margalef (R_1)		Menhinick's (R_2)			
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	1.317	1.777	.3134	.2128	.4838	.7857	.08006	.093	0.819	0.855
February	.6928	1.825	.5003	.1796	.1009	.7894	.01411	.1565	1.000	0.938
March	.6931	.3768	.4987	.781	.1669	.1496	.1	.07071	1.000	0.544
April	.7963	.9696	.5504	.5651	.3053	.8845	.1134	.03858	0.725	0.405
May	.9533	2.157	.493	.1535	.4102	1.685	.1033	.1159	0.688	0.746
June	2.378	1.76	.1145	.3101	1.612	1.54	.1953	.159	0.878	0.650
July	1.686	2.324	.202	.1142	.5793	1.276	.08018	.1182	0.941	0.906
August	.7595	.8933	.5699	.6056	.2856	.7537	.09045	.07698	0.692	0.430
September	1.981	2.547	.3469	.09356	2.726	1.77	.1706	.2309	0.588	0.919
October	2.8	2.634	.07418	.09719	2.286	2.21	.2646	.2278	0.920	0.865
November	2.568	2.98	.104	.05859	2.023	2.591	.2224	.2088	0.872	0.915
December	1.557	.7225	.2192	.6727	.5791	.6223	.1581	.05641	0.968	0.371

Annexure 19. Monthwise weather data of Mathbariae.g., Patuakhali Metrological Station (Actual Station used) in the year 2013 and 2014

Months	Maximum Temperature		Minimum Temperature		Total Rainfall	
	2013	2014	2013	2014	2013	2014
January	25.65	26.0	11.7	12.8	1	0
February	29.63	28.5	15.5	15.5	4	3
March	34.2	33.3	20.6	20.3	0	2
April	34.64	36.6	24.0	25.1	27	14
May	31.95	35.0	25.0	26.1	637	301
June	32.73	33.2	26.6	26.6	333	406
July	31.86	32.1	26.2	26.7	552	543
August	31.85	31.7	26.1	26.2	411	349
September	32.35	32.9	25.7	26.0	412	279
October	31.3	32.6	24.4	23.8	281	131
November	30.3	30.7	18.7	19.0	0	0
December	27.6	26.8	14.9	14.7	0	0

Annexure 20. Month wise weather data of Chhatak (Actual Station used) in the year 2013 and 2014

Months	Maximum Temperature		Minimum Temperature		Total Rainfall	
	2013	2014	2013	2014	2013	2014
January	25.6	27.1	11.6	13.1	0.0	0.0
February	31.2	27.4	15.9	14.1	5	33
March	34.2	32.4	19.7	18.0	8	46
April	33.0	34.9	21.8	21.9	269	75
May	30.4	32.4	22.7	23.3	684	392
June	34.1	32.1	25.8	25.5	660	1116
July	33.2	34.2	25.7	25.9	435	522
August	32.6	32.5	25.6	25.5	529	871
September	32.8	32.2	25.3	24.9	310	1071
October	31.5	33.1	23.1	23.1	534	49
November	30.1	31.2	17.3	18.9	0.0	0.0
December	26.2	27.8	14.6	15.2	0.0	0.0

Annexure 21. Comparison of *Vibrio cholerae* O1 counts in Mathbaria and Paikgachha water microcosms

Days	Microcosm Types	TTGA	Agglutinate	rfbO1	ctxA
0	ALL Types	$9 X 10^5$	+	+	+
1	MW+RCC	$7 X 10^5$	+	+	+
	MW+RSC	$7.5 X 10^6$	+	+	+
	PW+RCC	$3.1 X 10^6$	+	+	+
	PW+RSC	$1.3 X 10^6$	+	+	+
15	MW+RCC	$8 X 10^5$	+	+	+
	MW+RSC	$8.2 X 10^6$	+	+	+
	PW+RCC	$3.4 X 10^7$	+	+	+
	PW+RSC	$1 X 10^8$	+	+	+
30	MW+RCC	$4.2 x 10^7$	+	+	+
	MW+RSC	$9.1 X 10^7$	+	+	+
	PW+RCC	$5.3 X 10^7$	+	+	+
	PW+RSC	$7.5 X 10^7$	+	+	+
45	MW+RCC	$1.3 X 10^8$	+	+	+
	MW+RSC	$4.2 X 10^7$	+	+	+
	PW+RCC	$8.7 X 10^7$	+	+	+
	PW+RSC	$2 X 10^7$	+	+	+
60	MW+RCC	$5.3 X 10^7$	+	+	+
	MW+RSC	$3 X 10^5$	+	+	+
	PW+RCC	$3.4 X 10^7$	+	+	+
	PW+RSC	$6.9 X 10^6$	+	+	+
75	MW+RCC	$4.3 X 10^7$	+	+	+
	MW+RSC	$1 X 10^6$	+	+	+
	PW+RCC	$1.8 X 10^7$	+	+	+
	PW+RSC	$6.9 X 10^6$	+	+	+
90	MW+RCC	$1.1 X 10^7$	+	+	+
	MW+RSC	$3 X 10^3$	+	+	+
	PW+RCC	$1.3 x 10^5$	+	+	+
	PW+RSC	$1.4 X 10^4$	+	+	+
105	MW+RCC	$7 X 10^5$	+	+	+
	MW+RSC	$4 X 10^3$	+	+	+
	PW+RCC	$1.4 X 10^6$	+	+	+
	PW+RSC	$5 X 10^5$	+	+	+
120	MW+RCC	$1 X 10^5$	+	+	+
	MW+RSC	$3 X 10^5$	+	+	+
	PW+RCC	$3 X 10^5$	+	+	+
	PW+RSC	$9 X 10^5$	+	+	+
135	MW+RCC	$1.3 X 10^4$	+	+	+

	MW+RSC	1.3 X 10^6	+	+	+
	PW+RCC	8.2 X 10^4	+	+	+
	PW+RSC	1 X 10^5	+	+	+
150	MW+RCC	2.1 X 10^4	+	+	+
	MW+RSC	2 X 10^3	+	+	+
	PW+RCC	3 X 10^4	+	+	+
	PW+RSC	1.7 X 10^4	+	+	+
165	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	VBNC	-	-	-
	PW+RSC	VBNC	-	-	-
180	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	VBNC	-	-	-
	PW+RSC	VBNC	-	-	-
195	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	VBNC	-	-	-
	PW+RSC	VBNC	-	-	-
225	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	VBNC	-	-	-
	PW+RSC	VBNC	-	-	-
255	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	**2 X 10^5**	+	+	+
	PW+RSC	VBNC	-	-	-
285	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	**3.8 X 10^6**	+	+	+
	PW+RSC	VBNC	-	-	-
300	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	**5X10^5**	+	+	+
	PW+RSC	VBNC	-	-	-
315	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	9.4X10^1	+	+	+
	PW+RSC	VBNC	-	-	-
330	MW+RCC	1.2X10^6	-	+	+
	MW+RSC	1.2X10^6	-	+	+
	PW+RCC	1.4X10^6	+	+	+
	PW+RSC	1.2X10^6	-	+	+
345	MW+RCC	2.34X10^3	-	+	+
	MW+RSC	1.08X10^3	-	+	+

	PW+RCC	$4.7X10^2$	+	+	+
	PW+RSC	$1.32X10^3$	-	+	+
360	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	$8X10^1$	+	+	+
	PW+RSC	VBNC	-	-	-
375	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	$6.8X10^1$	+	+	+
	PW+RSC	VBNC	-	-	-
390	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	$1X10^1$	+	-	-
	PW+RSC	VBNC	-	-	-
410	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	$1.6X10^1$	+	+	+
	PW+RSC	VBNC	-	-	-
430	MW+RCC	VBNC	-	-	-
	MW+RSC	VBNC	-	-	-
	PW+RCC	$1.22 X10^2$	+	+	+
	PW+RSC	VBNC	-	-	-

Annexure 22. Growth of *Vibrio cholerae* 01 in Mathbaria water micro-ecosystems under no algal feed supplement

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	150	0
1	7.653	135	0
2	5.602	120	0
3	5.071	110	0
4	1.778	80	0
6	1.477	50	0
7	1	35	0
8	5.477	25	8
9	5.903	15	12
10	5.778	13	15
11	5.903	10	10
16	5.699	7	8
18	0	6	6
23	0	5	3
30	0	3	6
37	0	3	4
47	0	2	2
50	0	2	0
60	0	0	0

Annexure 23. Growth of *Vibrio cholerae* in Mathbaria water micro-ecosystems supplemented with algal feed

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	140	0
1	5.699	94	0
2	6	85	0
3	4.813	80	0
4	3.778	50	0
6	1.477	30	0
7	4.653	25	0
8	5.845	20	0
9	6.079	7	8
10	5.903	12	10
11	6.114	10	8
16	6.041	8	8
18	3	7	5
23	0	8	2
30	0	3	2
37	0	2	2
47	0	1	0
50	0	0	0
60	0	0	0

Annexure 24.Growth of *Vibrio cholerae* in Paikgachha water micro-ecosystems under no algal feed supplement

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	140	0
1	6.914	55	0
2	7.176	40	0
3	6.699	20	0
4	4.041	18	0
6	4.531	16	0
7	4.74	12	0
8	5.602	10	0
9	5.602	7	3
10	5.954	6	6
11	5.845	4	3
16	5.778	3	2
18	0	0	0
23	0	0	0
30	0	0	0
37	0	0	0
47	0	0	0
50	0	0	0
60	0	0	0

Annexure 25. Growth of *Vibrio cholerae* in Paikgachha water micro-ecosystems supplemented with algal feed

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	120	0
1	6.886	34	0
2	7.146	15	0
3	6.592	5	0
4	5.477	4	0
6	1.845	2	0
7	1.477	2	0
8	6	1	1
9	6.114	1	1
10	5.699	1	1
11	6.041	1	1
16	4.356	1	1
18	0	2	0
23	0	1	0
30	0	0	0
37	0	0	0
47	0	0	0
50	0	0	0
60	0	0	0

Annexure 26. Growth of *V. cholerae* in Lake Water micro-ecosystems under no algal feed supplement

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	140	0
1	5.778	90	0
2	5	70	0
3	3	65	0
4	3.477	50	0
6	4.146	35	0
7	4.74	30	0
8	5.845	25	0
9	5.845	15	12
10	6.114	15	15
11	5.903	15	13
16	5.699	13	13
18	0	9	8
23	0	6	4
30	0	4	3
37	0	2	1
47	0	0	0
50	0	0	0
60	0	0	0

Annexure 27. Growth of *Vibrio cholerae* in Lake water micro-ecosystems supplemented with algal feed

Days	No. of bacteria	Adult copepods	Nauplii
0	6.278	120	0
1	6	60	0
2	5.602	40	0
3	5.301	24	0
4	5.778	15	0
6	4.544	10	0
7	3.699	8	0
8	5.778	7	0
9	5.301	6	3
10	5.954	6	5
11	5.602	5	12
16	6	10	14
18	3	7	9
23	0	10	4
30	0	6	3
37	0	8	1
47	0	0	0
50	0	0	0
60	0	0	0