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Characteristics of managed and unmanaged water bodies influencing their suitability as mosquito breeding habitats in Bahir Dar, Ethiopia

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"*No animal on earth has touched so directly and profoundly the lives of so many human beings. Mosquitoes have felled great leaders, decimated armies, and decided the fates of nations. All this, and she is roughly the size and weight of a grape seed*."

Spielman, 2002

ABSTRACT

Characteristics of managed and unmanaged water bodies influencing their suitability as mosquito breeding habitats in Bahir Dar, Ethiopia

Elin Renstål and Karolina Carlström

Mosquito-borne diseases like malaria, dengue fever, yellow fever and West Nile virus are serious problems in many parts of the world, especially in Africa, and partially in Ethiopia. Millions of people become infected and several hundred thousand people die worldwide from these diseases every year. The most conventional methods for mosquito vector control target female adult mosquitoes in a reactive way using insecticides. However, it is possible to perform proactive vector control through source reduction.

The main focus of this study was to investigate if there are any associations between characteristics of managed still water bodies and the presence of mosquito larvae in Bahir Dar, Ethiopia. This was done by measuring twelve variables, both abiotic and biotic; pH, conductivity, dissolved oxygen, turbidity, biochemical oxygen demand, nitrate, phosphate, sulphate, carbonate, depth and presence of algae as well as collecting and counting mosquito larvae in ten different sites, during a time series of five weeks. The most common managed waters believed to serve as efficient breeding habitat for mosquitoes were ponds and ditches used for irrigation, drainage or cultivation.

A total of 204 mosquito larvae were collected, where 95% were found in four of the ten sites, three of them were managed waters and the last one was unmanaged. Statistical analyses were performed in order to examine potential correlations and differences among the sites. Wilcoxon test was performed to investigate differences between managed and unmanaged waters. Simple linear regression analyses were performed to identify driving variables for the presence of mosquito larvae.

The main results from the group tests were significant differences in mosquito larvae density between managed and unmanaged sites. Significant differences between mosquito sites and zero-sites for dissolved oxygen and sulphate were found. Simple linear regression revealed pH and dissolved oxygen as driving variables for mosquito larvae presence. It was concluded that resources should be put on treatment or manipulation of ponds, which were considered the preferred mosquito breeding habitats. However, if conflicts arise among interests concerning food production, measures must be planned and performed thoughtfully or focus should be put on less controversial breeding habitats. Among the driving variables, dissolved oxygen was the suggested variable to manipulate in order to reduce mosquito larvae populations.

Keywords: Mosquitoes, breeding habitat, vector control, water characteristics, abiotic and biotic variables, Bahir Dar, Ethiopia

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REFERAT

Lämpligheten hos reglerade och oreglerade ytvattensamlingar som mygglarvshabitat baserat på deras egenskaper, i Bahir Dar, Etiopien

Elin Renstål och Karolina Carlström

Myggburna sjukdomar som malaria, denguefeber, gula febern och West Nile viruset orsakar allvarliga problem i många delar av världen, särskilt i Afrika, och till viss del Etiopien. Miljontals människor världen över blir smittade och flera hundratusen dör varje år till följd av dessa sjukdomar. De konventionella metoderna för att kontrollera och minska spridningen av myggburna sjukdomar handlar om att kontrollera vuxna myggor med insektsmedel. Det är dock möjligt att utföra en mer förebyggande vektorkontroll genom att minska populationen.

Fokus för denna studie var att undersöka om det fanns några samband mellan mygglarvsförekomst och egenskaperna hos mänskligt reglerade eller oreglerade vatten i och kring Bahir Dar i Etiopien. Detta gjordes genom att mäta tolv variabler, både abiotiska och biotiska såsom; pH, konduktivitet, löst syre, turbiditet, biokemisk syreförbrukning, nitrat, fosfat, sulfat, karbonat, djup och algförekomst, samtidigt som mygglarver samlades in och räknades, vilket utfördes på tio olika platser under fem veckor. Den vanligaste typen av reglerade vatten som potentiellt skulle kunna utgöra effektiva mygglarvshabitat ansågs vara dammar och diken som används för bevattning, dränering eller odling.

Totalt samlades 204 mygglarver in och 95 % av dem fanns i enbart fyra av de tio etablerade mätplatserna, där tre var reglerade och den sista var oreglerad. Statistiska analyser utfördes för att undersöka potentiella samband och skillnader i mygglarvsförekomst och uppmätta variabler bland de tio mätplatserna. Wilcoxons metod användes för att undersöka om det fanns skillnader mellan reglerade och oreglerade ytvattensamlingars larvförekomst och egenskaper. Enkel linjär regressionsanalys utfördes för att hitta eventuella drivvariabler som därmed anses styra mygglarvsförekomsten.

De huvudsakliga resultaten i studien var att det förekom en signifikant skillnad i mygglarvsdensitet mellan reglerade och oreglerade ytvattensamlingar. Signifikanta skillnader i löst syre och sulfat förekom även mellan myggsiter och nollsiter. Enkel linjär regression visade på att pH och löst syre var de mest drivande variablerna för mygglarvsförekomsten i denna studie. Slutsatsen var att dammar utgjorde de mest tilltalande habitaten i samband med mygghonors äggläggning och därför borde prioriteras med avseende på resursfördelning vid planering och utförande av vektorkontroll. Om det skulle uppstå konflikter mellan olika intressen som kan äventyra matproduktionen bör insatser planeras och genomföras med försiktighet eller istället göras i andra mindre kontroversiella mygglarvshabitat. Den drivvariabel som ansågs vara den mest lämpliga att manipulera var löst syre.

Nyckelord: Mygg, mygglarvshabitat, vektorkontroll, vattenegenskaper, abiotiska och biotiska variabler, Bahir Dar, Etiopien

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PREFACE

The studies of our Masters of Science, in Water and Environmental Engineering, end with this thesis that we have been writing during the last semester of our education. The thesis was a project held by the Swedish University of Agricultural Sciences, with Scientist Richard Hopkins at the Ecology Department as supervisor, and Scientist Olle Terenius also at Ecology Department as subject reviewer.

Karolina has written following sections:
1, 2.1.1, 2.2.1, 2.4.(2, 5, 6, 8), 2.5, 2.6, 2.6.2, 3.5.2.

Elin has written following sections:
2.2.2, 2.3, 2.4.(1, 3, 4, 7), 2.6.1, 3.(1, 2, 3, 4), 3.5.1.

Elin and Karolina have written following sections together:
1.(1, 2, 3, 4, 5), 4, 5, 6.

When we now have completed our thesis we would like to thank all who contributed to make the project possible. First we would like to thank Richard for giving us this project and for believing that we could complete the task. He also joined us to Ethiopia and introduced us to locals in Bahir Dar, which were valuable and appreciated. Thanks also to Olle who contributed with additional trust and support.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Lämpligheten hos reglerade och oreglerade ytvattensamlingar som mygglarvshabitat baserat på deras egenskaper, i Bahir Dar, Etiopien.

Elin Renstål och Karolina Carlström

År 2010 dog cirka 627 000 människor i världen till följd av malaria och 30 000 till följd av gula febern. Utöver dessa två finns ett flertal andra myggburna sjukdomar som alla bidrar till att skörda offer och orsaka avsevärda ekonomiska kostnader. Problemen är globala, men allokerade till tropiska och subtropiska regioner vars klimat är särskilt gynnsamt för de myggarter som sprider sjukdomar. En stor utmaning är således att reducera spridningen av myggburna sjukdomar samt förhindra att människor blir infekterade. Tidigare forskning har fokuserat på mygghonornas bitvanor och fysiskt skydd samt hur den vuxna populationen kan reduceras på ett reaktivt sätt. Istället krävs nu proaktiva metoder som fokuserar på att minska själva populationen i ett så tidigt skede som möjligt och samtidigt inte ge utrymme för anpassning eller möjlighet för resistensutveckling. Nästa steg i utvecklingen av effektiva åtgärder kräver bland annat kunskap om preferenser och urval av habitat. Denna studie syftar till att bidra med kunskap om vilka egenskaper eller typer av vatten som mygghonor föredrar inför äggläggning.

År 2010 kom 90 % (över 550 000) av alla rapporterade dödsfall till följd av malaria från Afrika. Bara i Etiopien rapporterades år 2012 över 1,5 miljoner fall varav drygt 2000 dog till följd av sjukdomen. Etiopien, ett östafrikanskt land vid Afrikas horn, har länge haft problem med myggburna sjukdomar, och nu finns oro att problemen kommer öka. Landets utbredda högland håller kvar stora molnmassor från Indiska Oceanen, vilket skapar lokala väderförhållanden som genererar omfattande och frekvent nederbörd. Regnet är av stor betydelse för Etiopiens välfärd, men bidrar också till att temporära mygglarvshabitat bildas och att permanenta habitat kan upprätthållas. Nu intensifierar dessutom klimatförändringar problematiken med att reducera spridningen av myggburna sjukdomar i länder som Etiopien, där de höglänta områdena fortfarande är malariafria. Klimatförändringen medför ökad temperatur med avseende på altitud vilket i sin tur ökar risken att sjukdomsspridande myggarter kan etablera sig på nya höjder. Ytterligare en aspekt som försvårar situationen är att människorna i dessa områden blir särskilt utsatta eftersom den naturliga immuniteten är låg.

I över 50 år har Etiopien aktivt arbetat med att reducera spridningen och konsekvenserna av malaria genom att investera i olika metoder och strategier. Konventionella metoder för att kontrollera spridningen av myggburna sjukdomar är främst myggnät, täckande kläder och insektsmedel som antingen appliceras direkt på kroppen eller vid besprutning av habitat. Insektsmedel är fortfarande den mest kostnadseffektiva metoden. Det finns dock oundvikliga negativa konsekvenser att ta hänsyn till såsom ökad tolerans och resistens hos mygg, samt miljö- och hälsoaspekter.

Ny forskning fokuserar på alternativa metoder som bidrar till att reducera populationen på ett tidigt stadium genom att utnyttja att mygg lever i två olika typer av habitat under sin livscykel. De inledande utvecklingsstadierna; ägg, larv och puppa är begränsade till akvatiska

habitat. Genom att identifiera egenskaper som honor föredrar inför äggläggning, kan åtgärder införas som antingen minskar det akvatiska habitatets lämplighet eller försvårar överlevnaden. En sådan metod kräver omfattande kunskap om respektive arts habitatpreferenser, något som idag är bristfälligt.

Denna studie är en av flera, vars syfte är att bidra till kunskap om hur reglerade ytvattens kvalitet och egenskaper påverkar mygghonornas val av habitat inför äggläggning. Studien utfördes i Bahir Dar i nordvästra Etiopien under perioden september till oktober 2013. Data samlades in i fält under fem veckor från tio potentiella habitat genom inventering av mygglarver samt mätning av tolv variabler. I syfte att identifiera eventuell konflikt mellan matproduktion och sjukdomsrisk, är det av betydelse att undersöka huruvida mänsklig aktivitet och reglering bidrar till ökad myggpopulation. De undersökta reglerade ytvattnen bestod av bevattningsdammar och dräneringsdiken, därutöver valdes ett antal oreglerade ytvattensamlingar som referens. Statistisk analys utfördes för att utreda eventuella samband mellan mygglarvsförekomst och uppmätta variabler.

Inga signifikanta skillnader i uppmätta variabler mellan reglerade och oreglerade ytvatten erhölls från den statistiska analysen. Resultatet visade däremot på en signifikant skillnad i antalet mygglarver mellan dessa två habitatgrupper. Noterbara skillnader och variationer fanns dock inom respektive grupp, vilket bör beaktas vid tolkning av resultatet. Vidare visade resultatet att förekomsten av mygglarver i undersökningsområdet, var starkast kopplat till vattnets pH-värde och koncentrationen löst syre. Det kan innebära risker med att reducera habitatets lämplighet genom att manipulera dessa två variabler eftersom andra arter också kan missgynnas. Insatser av den typen kan dock tänkas vara motiverade om de leder till minskad risk för människor att insjukna i myggburna sjukdomar. Det är viktigt att försvåra myggens utveckling och därmed överlevnad i dessa habitat genom att tillämpa ekologiskt och ekonomiskt hållbara metoder.

De habitat som visade sig vara mest populära för äggläggning var dammar, både reglerade och oreglerade och borde därför prioriteras med avseende på insatser tänkta att bidra till minskade myggpopulationer. Om det däremot skulle uppstå konflikter mellan olika intressen som kan äventyra matproduktionen i området bör insatser planeras och genomföras med försiktighet eller att insatser istället görs i andra mindre kontroversiella mygglarvshabitat.

TABLE OF CONTENTS

1 INTRODUCTION

Mosquito-borne diseases cause serious problems in many parts of the world, especially in tropical and subtropical regions where climate is favourable for mosquito species that spread diseases (vectors). Economy development and human health are strongly affected by the consequences linked to such diseases. According to the World Health Organization (WHO) (2014a), vector-borne diseases, all types of vectors included, stand for 17% of the total burden of infectious diseases globally. In 2012, approximately 627,000 people died from malaria and another 30,000 died from yellow fever which are two human diseases transmitted by mosquitoes (WHO, 2013a; 2013b).

A major challenge is therefore to reduce the spread of mosquito-borne diseases, and prevent people from becoming infected; these types of actions are called vector control. In order to reach the United Nations' (UN) goal of malaria, immediate and extensive investments and actions are required. The UN goal is to stop and reduce the incidence of malaria by 2015 (UN, 2013). Previous research and methods for vector control has focused on biting habits of female mosquitoes and physical protection and how the adult population can be reduced in a reactive manner. Research today focuses instead on proactive methods that reduce the population at an early stage by utilizing that mosquitoes inhabit two different habitats during their life cycle; one aquatic and one terrestrial.

In 2012, 90% (550,000) of all reported deaths due to malaria came from Africa, which is the worst affected continent (WHO, 2013b). Ethiopia had more than 1.5 million reported cases, of which almost 2000 died from the disease (WHO, 2013c). Ethiopia, an East African country at the Horn of Africa, has had problems with mosquito-borne diseases for a long time and there is concern that the problem is about to increase. The country has a widespread highland with high tops that retains large clouds from the Indian Ocean, creating local weather conditions that generate extensive and frequent rainfall during rainy seasons (Brown, 1967). The rain is vital for the Ethiopian welfare, but it also contributes to create temporary mosquito breeding habitats and maintain permanent ones. Now climate change intensifies the problem of reducing the spread of mosquito-borne diseases in countries like Ethiopia, where the highland areas are still malaria-free. Climate change causes increased temperatures at higher altitudes, which in turn increases the risk of introducing mosquito vector species in new areas (Sokona et al., 2011).

Ethiopia has for more than 50 years tried to reduce the spread and impact of malaria by investing in vector control strategies and methods (Tadesse, Mekonnen and Tsehaye, 2011). Conventional mosquito vector control methods are mainly nets, covering clothing and insecticides applied either direct on the human body or sprayed in adult mosquito habitats (Goddard, 2008). Despite the cost-efficiency in the use of insecticides, there are negative consequences to consider such as increased tolerance and resistance among mosquito species (Goddard, 2008; Tolle, 2009) as well as environmental and human health impacts (Kalipeni, 2007). Instead, more efficient vector control methods should focus on the aquatic stage of the mosquito life cycle in terms of both population and source reduction. By identifying the

preferred characteristics of mosquito breeding habitats, it is possible to implement measures that reduce the suitability of the habitat or inhibit survival. However, actions like this require extensive knowledge about habitat preferences of specific vector species, which is currently insufficient (Lindsay and Martens, 1998).

This study aims to contribute with knowledge about the suitability of managed freshwater as mosquito breeding habitats based on their physical, chemical and biological properties in Bahir Dar, Ethiopia. In order to identify a potential conflict between food production and infection risk, it is important to investigate whether human activity and management lead to increased mosquito populations.

1.1 DEFINITIONS

- Managed water – a surface water body located in either an urban or rural setting, which is utilized as a resource in some way to serve human interests. It is either totally artificial or of natural origin and is regulated or controlled by human and their activities.
- Unmanaged water – a natural or semi-natural surface water body located in either an urban or rural setting, which is not utilized, regulated or controlled to serve human interests as a resource.
- Vector control – methods to reduce and control mosquito populations possible to transmit diseases.
- Mosquito site – a site where at least one mosquito larva was found during the entire measurement period.
- Zero-site – a site where mosquito larvae were absent throughout the entire measurement period.

1.2 OBJECTIVE

The objective of this master thesis was to, in Bahir Dar, Ethiopia examine selected water characteristic variables in relationship to the presence of mosquito larvae in managed and unmanaged surface water bodies. This was done with a view to better understand how water characteristics influence the abundance of vectors. The study aimed to specify which type of water is best suited to vector mosquito reproduction and where efforts should be put in the future to limit the reproduction of disease vector species.

1.3 GOALS

- through statistical analyses examine if there are any significant differences between managed and unmanaged water bodies in mosquito larvae presence and the abiotic and biotic variables; pH, conductivity, dissolved oxygen, biochemical oxygen demand, turbidity, nitrate, phosphate, sulphate and carbonate, and depth,
- through statistical analysis examine if there are significant differences between zero-sites and mosquito sites in the abiotic and biotic variables,
- through statistical analyses determine if there are any significant correlations between the presence of mosquito larvae and the abiotic and biotic variables.

1.4 HYPOTHESES

In the group comparison between managed and unmanaged waters, it is expected to receive significant differences in water characteristics. The expected differences should appear in the biotic variables pH, dissolved oxygen, turbidity.

In the group comparison between managed and unmanaged waters, a significant difference in mosquito abundance is expected, where managed waters are expected to generate the largest number of mosquito larvae.

In the group comparison between zero-sites and mosquito sites significant differences in water characteristics are expected. The expected differences should appear in the biotic variables pH, dissolved oxygen, conductivity and turbidity.

Among the abiotic and biotic variables, pH, dissolved oxygen, conductivity, turbidity and depth are expected to have significant correlations with the presence of mosquito larvae.

1.5 LIMITATIONS

The study is partly based on conclusions from previous studies about water characteristic measurements, statistical analysis, and mosquito breeding habitat preferences as well as additional data collection. Resources and time aspects required a selection between all possible variables to measure. The time series is in many perspectives short and covers only one part of one season. It is therefore not possible to receive fully reliable results and perform appropriate analysis due to lack of data. The data set was insufficient regarding both time and space. Results and conclusions from earlier studies were used in a more important way for the conclusions in this study than would have been needed if more data were collected.

The hypotheses in the study are intended to be used only in forecasts concerning the results from the specific sites chosen, and the conditions predominating in Bahir Dar at the time for the study.

2 BACKGROUND AND THEORY

2.1 AREA OF STUDY

Ethiopia (Figure 1) is a pronounced agricultural country about 2.5 times the size of Sweden. After Nigeria, Ethiopia is the most populated country in Africa with almost 100 million inhabitants (Landguiden, 2013). The landscape and climate vary considerably among neighbouring countries as well as within the borders. The Great Rift Valley traverses the country and divides it into highland and lowland regions. In highland regions a sub-alpine climate generally predominates while lowlands belong to the tropical climate zone (Brown, 1967). The bedrock of the northern highland region consists of limestone and sandstone and the southern parts have volcanic bedrocks. Erosion and weathering processes have left distinct marks on the Ethiopian landscape due to the porous bedrock (Brown, 1967).

Figure 1. The area of study, Ethiopia in eastern Africa (Wikipedia, with permission, 2014).

The climate has seasonal variations with one main rainy season and one dry season. The altitude range at the bottom of the rift valley stretches from 1200 to 1600 m above sea level (m a.s.l.) while the northern highland region has an average altitude between 3000 and 3500 m a.s.l. (Skovitina et al., 2012). Most inhabitants live at altitudes around 1700 to 2400 m a.s.l. where temperatures vary between 16 and 30 °C (Landguiden, 2011). Agriculture is the most important livelihood in the country. Coffee and other crops are the main agricultural produce in highland areas whereas livestock is dominating in lowland areas (Landguiden, 2012). The country is however frequently exposed to drought where famine is a major problem (Landguiden, 2013).

This study took place in Bahir Dar, which is the third largest city in Ethiopia with approximately 220,000 citizens (Landguiden, 2011). The city is located at the southern end of Lake Tana at an altitude around 1800 m a.s.l.. Both the altitude and the proximity to Lake

Tana affect the climate in the area, which keeps it stable with relatively low temperatures most of the year. In the dry season, day temperatures vary between 24 and 32 °C and during rainy season, temperature drops to 14 up to 16 °C. Night temperatures vary between 14 and 16 °C all year round (NMA, 2013). The proximity to Lake Tana also intensifies storms due to its large surface area and heat storage. Thunderstorms are particularly common during the main rainy season, which extends from June to September. The amount of rainfall peaks in July and August with an average precipitation of 230 mm per month. In the end of the rainy season, September and October, the average rainfall decreases to 200 mm and 130 mm respectively. During December and January there is no precipitation and the weather is dry until March when the amount of rainfall gradually increases every month until the peak in July (NMA, 2013).

2.1.1 Climate change

The average global air and sea temperatures are about to increase due to climate change. In the fourth assessment report, AR4, the Intergovernmental Panel on Climate Change (IPCC) affirmed that anthropogenic impacts on climate-controlling systems cause global warming (Sokona et al., 2011). In Africa, global warming and climate change will affect the possibilities of reaching the Millennium Development Goals (MDGs) since stress on already limited water resources and insufficient food production will increase along with human health impacts. Environmental issues like increased coastal inundation due to raised sea levels and drought will enhance the negative consequences of climate change (Sokona et al., 2011). All of these complex effects combined with limited capacity and pronounced poverty is what makes Africa particularly sensitive to climate change (Sokona et al., 2011). According to AR4, Africa is one of the most vulnerable regions in the world in this matter.

Climate change in highland areas

Temperature changes along with altitude; temperature decreases with an increased altitude, and as climate change temperatures will increase also at higher altitudes (Lindsay and Martens, 1998). Warmer climates in highland areas result in more frequent and extensive rainfalls along with introduction of new flora and fauna (Sokona et al., 2011). Negative consequences for human health may arise when diseases such as the mosquito-borne are spread into these new areas. Highland areas with low temperatures may not be suitable habitats for either mosquito vector species or the parasites causing the disease. However, as temperature increases, these areas could become efficient mosquito habitats, hence the risk of infection will increase. Additional and intensified rainfalls enhance this problem since it contributes to create and maintain efficient mosquito breeding habitats (Sokona et al., 2011).

According to Lindsay and Martens (1998) the number of malaria cases increases in African highland areas due to climate change. In Ethiopia, there are still highland areas considered to be malaria-free (WHO, 2013c). However, the AR4 declare that these areas by the year 2050 may have “incursions of malaria”, and by 2080 transmission and potential epidemics could have established (Sokona et al., 2011). Further, Sokona et al. (2011) highlights the importance of improving vector control strategies and disease treatment but also consider changes in land use, human activities and management, especially in countries where poverty is a problem.

2.2 MOSQUITOES

Mosquitoes together with flies belong to the order Diptera, which in turn is divided into more than 140 families. The number of mosquito species exceeds 3000 and all of them are included in the Culicidae family. Culicidae constitutes of two subfamilies; Anophelinae and Culicinae, and all species are divided into genus and subgenus levels (Becker et al., 2003). The three genera *Anopheles*, *Aedes* and *Culex* contain species responsible for the transmission of infectious diseases such as malaria, yellow fever and West Nile virus (Becker et al., 2003; Goddard, 2008).

Mosquitoes are found almost all over the world in a range of altitudes from sea level up to nearly 2500 m a.s.l.. The extensive geographical distribution is a result of great adaptability to exploit and inhabit new areas and climate zones (Becker et al., 2003). Some mosquitoes change their biting and resting habits depending on their preferred blood meal. For example, certain species are anthropophilic which means that humans are the preferred blood meal and thus have behavioural adjustments been performed to match human activities (Becker et al., 2003). As it turns out, some of the most significant human vector species are also the most anthropophilic, like *Aedes aegypti*, *Aedes albopictus*, *Anopheles funestus*, *Anopheles gambiae sensu stricto*, and *Anopheles arabiensis* (Becker et al., 2003). Most mosquito species are active at night especially around dusk and dawn. In other words, this is the time when female mosquitoes usually have their blood meal. However, some species such as *Ae. aegypti* and *Ae. albopictus* are day active and prefer having blood meals in broad daylight (Goddard, 2003).

2.2.1 Mosquito life cycle - from egg to imago

All mosquitoes undergo total metamorphosis that include four stages; egg, larvae, pupae and imago, during their development into adult individuals. The initial three stages of the lifecycle occur in aquatic habitats whereas the final stage is terrestrial. Temperature is an important factor during the aquatic stages since an increased temperature speed up the biological processes (Becker et al., 2003; Patz and Reisen, 2001). In addition, biotic and abiotic conditions as well as length of the breeding season also affects development and survival rate (Becker et al., 2003).

Egg

Female mosquitoes require extra protein during egg production and therefore feed on human or animal blood as a complement to their main nourishment, which consist of nectar and other sources of sugar (Spielman, 2002). Oviposition occurs after two to four days after the blood meal and females generally lay between 50 and 500 eggs. Species within genera *Anopheles* and *Culex* deposit eggs singly and in batches respectively, directly on the water surface (Becker et al., 2003). The eggs will hatch one or two days after oviposition during favourable conditions (Goddard, 2008), otherwise it might take a week or more (Becker et al., 2003). Species within *Aedes* genera are called floodwater mosquitoes, which have a different oviposition strategy; to lay eggs with resting-behaviour. The eggs are laid singly in breeding habitats with moist soil, which is subsequently flooded.

The female breeding habitat selecting behaviour is not fully understood for any mosquito species and the choice differs significantly among different genera. However, some general

breeding habitat preferences has been identified for the genera *Aedes*, *Anopheles* and *Culex* along with preferences of specific species. There are many types of aquatic habitats and basically any water body could serve as mosquito breeding habitats. Oviposition may occur in very small, temporary and commonly artificial habitats like tyres, leaf axils, tree holes, buckets, cans, animal footprints and rain puddles (Becker et al., 2003), as well as in large permanent habitats such as swamps, ponds, lakes and ditches (Goddard, 2008). The preferences in breeding habitats are divided into three main groups, those preferring permanent habitats, those preferring artificial habitats, and floodwater mosquitoes (Goddard, 2008). The wide ranges of preferences as well as the characteristics of breeding habitats are further results of the great adaptability of mosquitoes (Becker et al., 2003).

Species within subfamily Anopheline or the *Culex* genera prefer permanent habitats such as swamps, ponds, lakes and ditches (Gerberg, 2008; Goddard, 2008). These species choice of breeding habitat is affected by characteristics like water quality, light conditions, food availability, already existing eggs and vegetation (Becker et al., 2003). Typically preferred characteristics for Anopheline species are clear and still (Kenea, Balkew and Gebre-Michael, 2011), open and sun-lit waters. The anthropophilic species within *An. gambiae sensu lato* prefer habitats located close to inhabited human dwellings (Minakawa et al., 1999). Some mosquitoes within *Culex* genera are able to assess the habitat suitability and food availability based on the leakage of ammonium, methane and carbon dioxide from decomposition processes. Other species selects breeding habitats exclusively on vegetation type (Becker et al., 2003). Species in the *Culex pipiens* complex prefer the same habitats as *Culex* species in general (Goddard, 2008) but could as well inhabit storm sewer catch basins, cess-pools, polluted waters and artificial habitats (Amerasinghe, 2008).

Species of *Aedes* genera are dependent of a subsequent and satisfactory flooding to secure egg development. Absence of predators in the breeding habitat is preferred but not required (Becker et al., 2003). Both *Ae. aegypti* and *Ae. albopictus* breeds commonly in artificial habitats especially tires located close to human dwellings since these species are highly anthropophilic (Goddard, 2003).

Larva

When hatched the eggs turn into larvae, a stage that undergoes four moults in a period of between two and four days (Becker et al., 2003). A way to visually distinguish the larvae of the two subfamilies Anophelinae and Culicinae is to compare their resting positions relative the water surface. Larvae within Anophelinae subfamily always lie horizontally just beneath the water surface (Figure 2) whereas Culicinae larvae hang head down from the water surface (Figure 3) (Goddard, 2008). If the larvae become disturbed it dives towards the bottom where it remains for a short time before it returns to the water surface (Becker et al., 2003).

Figure 2. The horizontal position of Anophelinae larvae (Phillips, with permission, 2014).

The food sources for mosquito larvae are carbohydrates, animal proteins, yeast and other nutrients from algae, invertebrates, protozoa, detritus (Becker et al., 2003) and bacteria (Tadesse, Mekonnen and Tsehaye, 2011). Larvae of both *Culex* and *Anopheles* genera are generally suspension feeders meaning that they feed on suspended particles through water filtration. Most larvae within *Aedes* genera are browsers, which mean they shred or scrape the food from the surface of submerged substrates or the microbial film at water surface, or collect the food by re-suspension (Becker et al., 2003). In addition to suspension feeders and browsers, larvae could also be predators.

Figure 3. The vertical position of Culicinae larvae (Thaika, with permission, 2014).

Pupa

The stage after larvae is pupa, which floats at the water surface but is able to swim or dive when disturbed (Goddard, 2008). The pupae do not feed and the stage endures around two days. They are not very sensitive to desiccation and it can survive even in dried out habitats (Becker et al., 2003).

Imago

The transition from pupa stage into the final imago stage (adult) is the most critical phase during the development. After emergence the adults needs to dry its wings, groom its head appendages and stretch the legs before it can fly away. During emergence, the water has to be quite still since wind or wave action could tip the mosquito over into the water and risk drowning. For this reason oviposition generally take place in sheltered areas in proximity to the shore or emergent vegetation instead of open waters (Goddard, 2008).

2.2.2 Mosquito-borne diseases

Diseases transmitted by mosquitoes make up huge global problems and puts billions of people at risk of infection, with sometimes high morbidity and mortality as consequences. The affected countries have to handle extensive financial burdens connected to treatment, vector control and source reduction. Mosquito-borne diseases are caused by arboviruses or parasites. The word arbovirus means that the virus is transmitted by arthropods in which mosquitoes are included (Gubler, 2004). Dengue fever, yellow fever, West Nile virus and Rift Valley fever are examples of such viral mosquito-borne diseases. Malaria and lymphatic filariasis are two examples of parasitic diseases caused by protozoa and nematodes respectively.

Malaria

Malaria is one of the most important human diseases with a wide geographical distribution that covers the tropical and subtropical parts of the world. This mosquito-borne disease puts 2.6 billion people at risk of infection worldwide (Goddard, 2008). According to WHO (2013b) there were 207 million cases of malaria in 2012, and the mortality was 627,000 during that same year. The continent most affected both according to morbidity and mortality was Africa. In Ethiopia alone, there were over 1.5 million confirmed cases and nearly 2000 deaths in malaria during year 2012 (WHO, 2013c). The mosquito species responsible for human transmission of malaria belong to the genera *Anopheles* exclusively. There are about 400 species of *Anopheles* where approximately 40 to 75 are considered to be important vectors (Gerberg, 2008; Goddard, 2008). In Africa, species within *An. gambiae s.l.* are considered the most efficient human malaria vectors (Goddard, 2008). In Ethiopia the most common malaria vector species are *An. arabiensis* and *Anopheles pharoensis* (Kenea, Balkew and Gebre-Michael, 2011).

Dengue fever

Dengue fever is found in more than 100 countries and has about the same global distribution as malaria putting about 2.5 billion people at risk of becoming infected (WHO, 2013d). There are two mosquito vectors for the transmission of the disease; *Ae. aegypti* and *Ae. albopictus* (Goddard, 2008). The classic dengue is rarely fatal, but there is a much more severe form of dengue fever called dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) which mostly affects children up to 15 years (Gubler, 1998). The mortality of severe dengue is rather high (Kautner, Robinson and Kuhnle, 1997) and according to Kirch (2008) it varies between 6% and 30%.

Yellow fever

Today, yellow fever is found in 44 countries in the tropical parts of Africa and the Americas, which covers more than 900 million of the human population (WHO, 2013b). There are two

transmission cycles to be considered when it comes to the transmission of the yellow fever virus, the jungle and urban cycle (Tabachnick, 2008). The main vector species of the jungle cycle is *Aedes africanus* (Goddard 2008). The most important vector in the urban cycle is *Ae. aegypti* followed by *Ae. albopictus* and *Aedes simpsoni* (Rogers et al., 2006). The mortality of the classic yellow fever is less than 5% whereas the mortality can be over 50% during epidemics or if severe cases of the disease are left untreated (Goddard, 2008).

West Nile virus

West Nile virus is found in Africa, the Americas, Europe, Middle East and Central Asia (WHO, 2011) where the mortality is between 4 and 16% (Goddard, 2008). The disease is transmitted by species from the *Culex* genera (Kramer, Li and Shi, 2007). The most significant vector in case of human transmission in Africa is *Culex univittatus* (Campbell et al., 2002). Another significant vector species is *Cx. p. pipiens*, which is found in East Africa as well as South Africa, Europe, Australia and South America (Becker et al., 2010).

Rift Valley fever

The Rift Valley fever is found in sub-Saharan Africa, Egypt and the Arabian Peninsula. Outbreaks of the disease are strongly affected by climatic factors like precipitation and especially the El Niño phenomena (Anyamba et al, 2009). The disease causes big economic problems due to high mortality in domestic animals, but the disease also has a significant morbidity and mortality among humans. In East Africa, Rift Valley fever is mainly transmitted by mosquito species within the *Culex* genera (Anyamba et al., 2009).

Lymphatic filariasis

Lymphatic filariasis is distributed in tropic and sub-tropic regions and believed to be found in between 76 to 81 countries, which put between 750 million and 1.4 billion people at risk of infection. The number of people already infected varies between almost 80 million and 120 million. (Goddard, 2008; Nayar, 2008; Shiferaw et al., 2012; WHO 2014b). One country where the disease is known to exist is Ethiopia, however, the morbidity and the full geographical distribution is not fully known due to lack of data (Shiferaw et al., 2012). Lymphatic filariasis is transmitted by species within both sub-families Anophelinae and Culicinae. In rural parts of Africa, species within *An. gambiae s.l.* and *An. funestus* are the main vectors and species within *Culex* genera are the main vectors in urban areas (Shiferaw et al., 2012).

2.3 VECTOR CONTROL

The fight to reduce human transmission, morbidity and mortality of mosquito-borne diseases requires huge financial resources worldwide. Even though the costs are high it is not only a question of funding or human health. Conventional control methods may cause problems with significant environmental impact (Watkins, 2003). In Ethiopia vector control has been performed for over 50 years and the main focus has been to reduce malaria transmission. Nowadays, Ethiopian vector control programme involves indoor residual spraying, insecticides targeting larvae and epidemic control, but also diagnosis and treatment at an institution-basis performed by community health workers (Tadesse, Mekonnen and Tsehaye, 2011). Consequently adult female mosquitoes have so far been the main targets and the use of insecticides has been the common control method in order to reduce vector populations

(Tadesse, Mekonnen and Tsehaye, 2011). However, there are other ways to control vector species. Since mosquitoes have two distinct habitats during their lifetime, one aquatic and one terrestrial, it is possible to apply vector control methods and techniques in both habitats.

The most effective vector control is a combination of knowledge and common sense. The use of biological or chemical products along with understanding about habitats and life cycles has increased the possibility of efficient vector control (Watkins, 2003). There are three main control strategies; adulticiding, larviciding and source reduction. The first two are reactive measures whereas the last one is more proactive. Adulticiding target adult mosquitoes and involves indoor and habitat spraying with insecticides. The use of insecticides is the most cost-effective method even though it has its consequences (Watkins, 2003). Mosquitoes are very adaptable and it has been found that vector species become more tolerant or resistant over time against conventional insecticides (Goddard, 2003; Tolle 2009). Other concerns are potential environmental impacts and human health concerns due to persistent and toxic properties of the insecticide (Kalipeni, 2007). Dichloro-diphenyl-trichloroethane (DDT) is one such insecticide, which has been forbidden in many countries since it is a neurotoxin with major ecologic and environmental impacts. One country that uses DDT for vector control again is South Africa, which reintroduced it in 2000 (van den Berg, 2009). In Ethiopia the use of DDT stopped recently (2009) due to resistance (Asale et al., 2014).

Moreover it is important to apply the right control method based on the characteristics and behaviour of the species to be controlled. However, since malaria have been the main focus in terms of mosquito-borne diseases and vector control, the importance and methods of controlling other vector species have been neglected. Indoor insecticide spraying is for example not a suitable method to control most *Aedes* and *Culex* species since they tend to rest outdoors and the latter has also an intrinsic tolerance against insecticides (Pates and Curtis, 2005).

Larviciding has proven to be a successful vector control approach that involves both chemical and biological control methods (Rutledge, 2008). The chemical method has similarities to adulticiding but in this case the insecticides are sprayed over aquatic habitats. The biological method involves an introduction of predators like tadpoles, fish or various invertebrates, such as water beetles, dragonfly larvae or backswimmer bugs (WHO, 1975a; Wanji et al., 2009). Introduction of predators is an effective method when applied in permanent habitats where there is a continuous mosquito production. However, if the method is supposed to target floodwater mosquitoes there is a risk that the predators cannot handle the rapid larval development during the actual flooding (Pates and Curtis, 2005).

The final vector control strategy is called source reduction and aims to reduce the mosquito population without the use of insecticides. One crucial point of this approach is to identify breeding habitats of the species that are supposed to be controlled and then eliminate the habitat. These actions focus on removing stagnant water bodies, where drainage projects could be one such action. It is also important to avoid creating open artificial reservoirs that may serve as habitats. Everything from small cans or jars up to big dams could serve as

efficient breeding habitats since mosquito development is depending on the presence of water (Rutledge, 2008).

The source reduction method has proven to be especially effective in controlling *Ae. aegypti*. The presence of adult mosquitoes of this species tends to be greater in areas where artificial habitats are found in proximity to human dwellings compared to areas where such habitats have been removed. Presence of *Ae. aegypti* is proven to be driven by their search of breeding habitats (Pates and Curtis, 2005). Elimination of artificial habitats in proximity to human settlements is also effective for controlling *An. gambiae s.s.,* which is the most effective malaria vector for human transmission (Wanji et al., 2009). Included in the source reduction approach are genetic control methods where a mechanism or pest is introduced into a wild population of mosquitoes through mating. One such action is sterilization of male mosquitoes by irradiation, which results in oviposition of sterile eggs (Pates and Curtis, 2005).

Other methods to reduce the spread of mosquito-borne diseases, particularly malaria, are the use of different personal protection and preventive measures, which reduce the risk of being bitten by mosquitoes. Repellents and covering clothes are two interventions to consider during daytime but the most effective measure has proven to be the use of impregnated nets during night time (Watkins, 2003; Pates and Curtis, 2005). Many mosquito species are night active, especially around dusk and dawn. According to Pates and Curtis (2005), the biting frequency decreases with 75% if people use nets while sleeping, this is based on human sleeping hours in rural areas which normally occur around 22:00 and 05:00. However, in areas where temperature is low night time, the biting habits of mosquitoes change. The biting occurs earlier in the night or later in the morning, and the usage of nets is therefore less effective in such areas.

2.4 ABIOTIC AND BIOTIC VARIABLES

2.4.1 pH and carbonate

pH is important to any aquatic ecosystem since it affects nutrient supply like phosphate and ammonia concentrations as well as iron concentration or trace metals (Horne and Goldman, 1994). The range of pH for water bodies located in natural settings lies normally between 6.5 and 7.5 (Lampbert and Sommer, 2007). Carbonate is also an important factor but it is not toxic in itself for aquatic organisms but the ion is involved in processes that may have secondary toxic effects (Gustafsson et al., 2007).

In aquatic ecosystems, pH is affected by on-going processes such as photosynthesis, which cause pH to increase as carbon dioxide (CO_2) is consumed. Another process is respiration performed by various organisms where CO_2 is added to the system and results in a decrease of pH (Wetzel and Likens, 1991). The carbonate-bicarbonate-carbon dioxide equilibrium system is a pH dependent chain of reactions where CO_2 from the air dissolves into the water and forms carbonic acid (H_2CO_3). This ionic compound is almost instantly dissociated into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, while hydrogen ions are simultaneously released (Equation 1) (Horne and Goldman, 1994). Based on the prevailing pH-value the equilibrium reactions will be forced in one direction or the other. An increased hydrogen ion concentration will shift the equilibrium to the right and the response of the system is to even

out the imbalance by the formation of bicarbonate and further on. The reaction will be shifted in the opposite direction as the hydrogen ions are decreased. As long as the supply of carbonate and bicarbonate is sufficient, an addition of hydrogen ions from external processes will not result in a change of pH since these equilibrium reactions serve as a buffer system.

$$CO_2(g) \leftrightarrow CO_2(aq) \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \quad (1)$$

pH is also affected by depositions from air pollutants and runoff water. Generally, it is difficult to separate which effect are directly or indirectly linked to variations in pH since there are many on-going processes (Lampbert and Sommer, 2007). This reasoning is also applicable the other way around, where water chemistry is influenced by pH. According to Horne and Goldman (1994) the abundance of many aquatic animals such as snails, amphibians, fish and zooplanktons become reduced if the pH value is lower than 6 for a longer time and if it falls below 5 most animals will die. There are two potential toxic effects when the pH is either high or low. The first situation can occur when there is a significant concentration of ammonium (NH_4^+) in the water together with an ongoing increase of pH. The consequence is that non-toxic ammonium will be transformed into ammonia (NH_3) as the pH increases. Ammonia is toxic to most aquatic organisms and when pH exceeds 10.5, ammonia is the dominant form, but if pH is lower than 8, ammonium becomes the dominant form. The second situation of toxic effects could arise when pH is decreasing and metallic compounds like aluminium, iron, copper, lead and cadmium are present in sediments, ground or soil. The solubility of these metallic compounds will increase as pH decreases which result in higher concentrations of toxic metals in the water column.

2.4.2 Dissolved oxygen and biochemical oxygen demand

Dissolved Oxygen

The majority of aquatic organisms are dependent on concentration of dissolved oxygen, DO, in the water. DO concentrations under 3 mg/l make it difficult for many species to survive, but the critical value varies among species in terms of optimal concentrations and tolerance limits (Naturvårdsverket, 2007). In order to decide whether an obtained DO concentration is insufficient, the value has to be compared to a specific reference value for the current water. From the comparison it is possible to determine whether the low DO concentration is due to natural processes or anthropogenic activities (Naturvårdsverket, 2007). Biological and chemical decomposition are natural processes that consume/require DO. Anthropogenic activities like sewage discharge, leaching from farmland and agricultural, as well as other emissions increases the decomposition rate, which affects DO in aquatic systems.

Oxygen infuses to water from the atmosphere through wind and wave actions, and from photosynthesis by green plants and organisms like algae (Bydén, Larsson and Olsson, 2003). High DO concentrations could thus indicate a well-functioning photosynthesis. The processes of producing and consuming oxygen divides a water body into two vertically parts. The infusion of oxygen occurs at the surface where sun and winds are active, and the respiration is held at the bottom where organic matter is present (Lampert and Sommer, 2007). Oxygen saturation in water is dependent on atmospheric pressure, water temperature and salinity. DO increase when temperature or salinity decreases (Bydén, Larsson and Olsson, 2003).

Biochemical oxygen demand

Biochemical oxygen demand, BOD, is a measure of the amount oxygen consumed during microbial oxidation of organic material (biologic decomposition). Decomposition is limited by organic material, a decrease in organic content result in a reduced decomposition rate (Bydén, Larsson and Olsson, 2003).

Long-time standing water, isolated waters, or eutrophic waters with an excessive amount of dead organic matter, can easily develop anaerobic conditions at the bottom. The anaerobic condition is a result of when decomposition and respiration together outpace oxygen-producing processes. Microbial decomposition does not stop even though water conditions are anoxic, instead of DO the microorganisms utilize oxygen from other chemical compounds (Bydén, Larsson and Olsson, 2003).

2.4.3 Nitrate

In natural freshwater bodies, the most common compounds of nitrogen are dissolved nitrogen gas (N_2), nitrate (NO_3^-), ammonia (NH_4^+) and nitrite (NO_2^-). Organic compounds of nitrogen are less common but still important for aquatic organisms (Horne and Goldman, 1994). The major inorganic nitrogen component in aquatic systems is dissolved nitrogen gas. However, it is only cyanobacteria that can benefit from this form of nitrogen as a nutrient source, through nitrogen fixation (Lampert and Sommer, 2007). The second most abundant and most important form of inorganic nitrogen is nitrate, which is a nutrient source for microscopic autotrophic and photosynthetic organisms that belong to the group of primary producers (Horne and Goldman, 1994). Nitrate enters aquatic systems through various sources like runoff, groundwater and precipitation. The amount and infusing rate of nitrate that end up in aquatic systems are highly depending on the type of land use in the area. According to Horne and Goldman (1994) it has been shown that acid rain contains more nitrogen than sulphur components in areas with heavy traffic. The deposit of nitrate can thus be linked to the presence of vehicles. Another significant source of nitrogen transport into surface waters is runoff or leachate from arable lands, especially fertilized ones and sewage effluents. Nitrate is a highly mobile ion and thus easily transported through the soil into aquatic systems.

Nitrate is a key component of the nitrogen cycle, which is formed and consumed in various ways and processes. Decomposition of organic material will result in a release of ammonium that can be transformed into nitrate by microorganisms. This transformation process is called nitrification and can only occur during aerobic conditions. Anoxic respiration of nitrate will release either ammonium or nitrate, depending on what microorganisms that performs the transformation. The process where ammonium is released is called ammonification and the release of nitrate is called denitrification (Lampert and Sommer, 2007).

Nitrate is generally not toxic at normal concentrations of natural water bodies such as lakes and streams. Toxic effects on aquatic organisms may arise due to both high concentrations and duration of exposure. According to Camargo, Alonso and Salamanca (2005) the survival of freshwater animals like fish, amphibians and various invertebrate species can be negatively affected at concentrations of 10 mg/l. This concentration is also a limit for drinking water suitable for humans (Horne and Goldman, 1994).

2.4.4 Phosphate

In aquatic ecosystems phosphorus occurs in different forms; dissolved inorganic compounds, dissolved organic compounds and both organic and inorganic particulate compounds (Wetzel and Likens, 1991). Generally in natural freshwaters, the amount of particle bound phosphorus exceeds the amount of dissolved organic phosphorus, which in turn is greater than the amount of dissolved inorganic phosphorus. The major supply of phosphorus into aquatic systems is through erosion processes but weathering and runoff processes as well as decomposition of organic material are also significant sources. Phosphorus does not occur naturally in the atmosphere and phosphate tends to be retained in the ground, by adsorption to clay minerals or organic material (Horne and Goldman, 1994). Aquatic systems lose phosphorus through sedimentation of particle bound compounds. However, phosphorus may be reintroduced in the system due to bioturbation and chemical exchanges between the water column and sediment, this process is referred to as the internal load (Lampert and Sommer, 2007).

During aerobic conditions, dissolved organic phosphorus is present in three different compounds; dihydrogen phosphate ($H_2PO_4^-$), monohydrogen phosphate (HPO_4^{2-}) and phosphate (PO_4^{3-}) which constitute the group of orthophosphates. These three are the only forms of phosphorus that plants and phytoplankton can directly assimilate (Lampert and Sommer, 2007). In fresh waters, phosphate is generally the limiting nutrient for plants, algae and other primary producers (Horne and Goldman, 1994).

Phosphate concentration is somewhat a measure of plant accessible phosphorus in water, where a high concentration could be an indication of eutrophication (Horne and Goldman, 1994). Phosphorus has no direct toxic effects to aquatic organisms at concentrations of natural freshwaters but there might be secondary effects connected to oxygen deficiency and eutrophication (CCME, 2004).

Phosphate releases and recirculates within an aquatic system as dead plants and animals are decomposed. This process mostly takes place at the bottom, which makes water near the bottom and sediments rich in phosphorus. During aerobic conditions, the released phosphate is bound in sediments due to reactions with metallic ions, especially iron forming low soluble compounds (Lampbert and Sommer, 2007). Phosphorus is therefore removed from the internal circulation of the aquatic system. However, if oxygen conditions are poor, metallic ions are reduced and phosphate is released into the water, increasing the concentration (Lampert and Sommer, 2007). The oxygen condition at the bottom of the water is thus of great importance regarding nutrient supply of the aquatic system.

2.4.5 Sulphate

Dissolved sulphur can occur naturally in quite high concentrations in surface waters, especially in oceans. Sulphate (SO_4^{2-}) enters freshwater bodies mostly through atmospheric deposition like sea spray and precipitation as dissociated sulphuric acid (H_2SO_4) (Bydén, Larsson and Olsson, 2003; Lampbert and Sommer, 2007). The deposition of sulphate may be enhanced by anthropogenic emissions. One of the largest sources of acidic sulphur linked to human activity is combustion of fossil fuels where released sulphur dioxide is transformed into sulphuric acid in the atmosphere. Most sulphuric ions are easily dissociated in water and

concentrations in natural freshwater vary considerable (Lampbert and Sommer, 2007). Biologic processes only require small amounts of sulphate (Lampbert and Sommer, 2007) and the excess sulphate contributes to acidification of water (Naturvårdsverket, 2007). Periods of drought and lowered groundwater levels may increase the sulphate concentration in the soil due to oxidation processes. As rainy season or flooding occur, sulphate ions will be transported through the soil into surface waters and thus increase the concentration (Naturvårdsverket, 2007). A guideline limiting value for high levels of sulphate in aquatic systems is 50 mg/l SO_4 and levels should never exceed 100 mg/SO_4 (Environmental Protection Division, 2000).

In anoxic waters, bacteria reduce sulphate during microbial decomposition, which result in a production of toxic hydrogen sulphide (H_2S). In other words, high sulphate concentrations could have indirect toxic effects on aquatic ecosystems during anoxic conditions due to production of hydrogen sulphide (Bydén, Larsson and Olsson, 2003). Despite the direct toxic effects of increased concentration of hydrogen sulphide, there may also be indirect effects linked to the concentration of potential toxic trace elements. Another aspect is impacts on the interchange between sediments and the water column and the internal load of phosphorus, which could extend the duration of the anaerobic condition if phosphate is released (Bydén, Larsson and Olsson, 2003). One way to avoid this toxic scenario is to re-oxygenize the water, resulting in oxidation of hydrogen sulphide into sulphate (Bydén, Larsson and Olsson, 2003).

2.4.6 Turbidity

Turbidity is an optic measure of suspended particles and non-dissolved substances in water, where an increased particle density results in higher turbidity. Common units for turbidity are either Formazine Turbidity Units (FTU), Formazine Nephelometric Units (FNU) (Bydén, Larsson and Olsson, 2003) or Nephelometric Turbidity Units (NTU) (Auckland Regional Council, 2002). The suspended material could be sludge, microorganisms, plankton, organic matter and inorganic particles like clay, which all have different reflective index (Bydén, Larsson and Olsson, 2003).

Runoff and erosion are natural processes that affect turbidity in surface water bodies (Horne and Goldman, 1994). In fairly stagnant water, lakes for example, the dominating particles are organic while streaming water contains more inorganic particles. Human activities like farming and forestry as well as building and road constructions may enhance transport of particles and thus contribute to increased turbidity in surface waters (Horne and Goldman, 1994).

Limiting turbidity levels for the survival of organisms fairly differs between species why only one limit value is venturous to appoint. According to a study performed by Kerr (1995), an increase of turbidity with 25 NTU in shallow clear waters may decrease the primary production with 13 to 50% (Kerr, 1995). Another study concerning turbidity, from Auckland Regional Council (2002), reveals how some species react on an expose of high turbidity. One finding in the study was that the survival of two invertebrate species investigated was affected at a turbidity of 2000 and 5000 NTU respectively. The invertebrates were chosen due to their presence in non-urban catchments (Auckland Regional Council, 2002).

2.4.7 Conductivity

Conductivity is a measure of total dissolved ionic content of water, where more ions result in a higher conductivity (Lerman et al., 1995). In natural freshwaters the most abundant cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) and the anions carbonate (CO_3^{2-}), bicarbonate (HCO_3^{-}), sulphate (SO_4^{2-}), nitrate (NO_3^{-}) and chloride (Cl^{-}). Together these ions generally represent 99% of the total dissolved ions in natural surface waters. Conductivity varies mostly due to weathering processes but also through atmospheric deposition and the balance between evaporation and precipitation (Wetzel and Likens, 1991). Unlike seawater, conductivity in fresh water is highly variable between different water bodies, and over time. The ion composition also varies considerably between different freshwaters. In some systems one pair of cations and anions, like calcium and bicarbonate could be totally dominant whereas in other systems there is an evenly distributed mixture of various ions (Lerman et al., 1995).

In aquatic systems conductivity is both a direct and indirect factor regarding toxicity. High conductivity can be toxic to aquatic organisms itself or due to influences on the oxygen concentration and increased exposure of toxic metallic compounds e.g. chrome (Naturvårdsverket, 2008). According to Dunlop, McGregor and Horrigan (2005) the upper tolerance level of conductivity for many invertebrate species is around 1500 μS/cm, a higher value normally affects the survival negatively. That same study also concluded that flies of the Diptera order along with water beetles and crustaceans generally have rather high tolerance to increased conductivity values.

2.4.8 Algae

Algae are unicellular, colonial or filamental aquatic organisms, which may be found suspended in the water column or attached to submerged rocks, plants or debris. In most aquatic ecosystems algae are the dominating phytoplankton in the primary production. Many algae are photoautotrophs, i.e. perform photosynthesis just like plants. Photosynthesis is a complex process in which sunlight is utilized as an energy source and carbon dioxide and water are transformed into carbohydrates (Horne and Goldman, 1994).

Suspended algae exist mostly in large lakes and tranquil streams while attached algae are common in shallow, clear lakes and rapid streams (Horne and Goldman, 1994). In tropical climates algal growth may occur continuously throughout the year. The population density may vary due to seasonal as well as daily variations. Blue-green algae which sometimes cause extensive and toxic algae blooms thrive in well stratified waters with temperatures above 20 °C (Horne and Goldman, 1994).

Several algae can move either with help of vacuoles or flagellates. Some algae have random movements, while others move as a reaction of changes in light conditions. The transportation is an adaption of lack of nutrients and energy as well as avoiding grazing zooplankton. High reproduction rate is also a way to secure the existence of algae even though zooplankton is present (Horne and Goldman, 1994).

2.5 EARLIER STUDIES

There are several studies previously conducted with similar purpose, objective or method as this particular study. The summarized studies in the following sections (2.5.1-7) are all studies from Africa, mostly Ethiopia or neighbouring countries. The overall aims of the studies were to determine what affects the choice of breeding habitat as well as mosquito development, e.g. water properties, food supply, co-habitation, or crowding. One common aspect was altitude, which for most of the studies was over 1500 m a.s.l..

2.5.1 Enhancement of development of larval *Anopheles arabiensis* by proximity to flowering maize (Zea mays) in turbid water and when crowded

This study was conducted in Zwai in the Great Rift Valley in central Ethiopia by the American Society of Tropical Medicine and Hygiene (Ye-Ebiyo et al., 2003). The altitude of Zwai is 1640 m a.s.l.. The main conclusion was that mosquito breeding habitats located close to flowering maize give favours development and survival of *An. arabiensis*. In the experiments, larvae were reared into pupae and adults in arranged habitats with specified water characteristics. The main factors affecting the development of larvae in the study were the proximity to flowering maize, turbidity and crowding by mosquito larvae.

It was found that mosquito larvae become more tolerant to high turbidity and crowding if the breeding habitat is located close to flowering maize or its pollen. In addition to the increased survival of the larvae living in these habitats, the study also showed that adult mosquitoes generally grew larger than those not exposed to maize pollen.

2.5.2 Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia

This study was also executed in the Great Rift Valley in central Ethiopia, south of Zwai at an altitude of 1700 m a.s.l., as collaboration between the universities in Wollega and Addis Abeba (Kenea, Balkew and Gebre-Michael, 2011). One of the main observations was that *An. arabiensis* and *An. pharoensis* are the two most common species within the *Anopheles* genera in Ethiopia. Presence of *An. arabiensis* was found to be highest in sand pits and the larvae were mostly found in clear and still waters with no aquatic vegetation. The relative presence of *An. arabiensis* larvae had a significant negative association with aquatic vegetation and water current. The *An. pharoensis* was densely populated in natural swamps and the larvae were found in permanent and natural habitats with clear and still water as well as presence of algal mats. For the relative presence of *An. pharoensis* there was a significant positive association with water temperature and the presence of algae.

2.5.3 Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats in Minna, north central Nigeria

The study was conducted by the Department of Biological Sciences at the Federal University of Technology in Minna, a lowland area around 300 m a.s.l. in north central Nigeria (Olayemi et al., 2010). Five potential mosquito breeding habitats types; ponds, swamps, rivers, drains and domestic containers were defined and analysed based on 10 different physico-chemical variables. The variables were; total dissolved solids (TDS), DO, conductivity, pH, nitrate,

phosphate, sulphate, carbonate, temperature and transparency. The data collection was performed weekly during the rainy season of 2008.

The water characteristics of the different habitat types were significantly different among each other. Significant correlations between three mosquito genera (*Culex*, *Anopheles* and *Aedes*) and different habitat types were also found. Species within *Culex* genera were mostly found in ponds followed by swamps and domestic containers and had strongest correlation with temperature and conductivity. The highest amount of *Anopheles* larvae was observed in swamps followed by domestic containers and ponds. The strongest correlations were found with phosphate and conductivity, negatively and positively respectively. *Aedes* mosquitoes preferred domestic containers before ponds and swamps and showed the strongest positive correlation with DO and carbonate. No mosquitoes were found in drains and rivers. According to Olayemi et al. (2010) these observations and results combined strengthens the idea that these variables explain the mosquito distribution.

Conclusions in the study are based on the findings about heterogeneous distribution of mosquito larvae, the significant differences in water chemistry between habitats, as well as the strong correlation between specific mosquitoes and some of the physico-chemical variables. The main conclusion communicated in the article is that larvicide interventions must be targeted in order to get an effective larval control in terms of cost and effort.

2.5.4 Characterization of mosquito breeding sites in and in the vicinity of Tigray microdams

The study was conducted by Department of Biology; College of Natural and Computational Sciences and Department of Microbiology; Immunology and Parasitology; and College of Health Sciences at the Mekelle University in Ethiopia (Tadesse, Mekonnen and Tsehaye, 2011). It was performed in the Tigray area in northern Ethiopia at altitudes within an interval of 2000 up to 2070 m a.s.l.. Mosquito larvae were collected from six dams at five occasions within a five-month period covering various seasons. The measured characteristics were DO, temperature, pH and conductivity, depth, turbidity, vegetation and bottom surface of the habitat, fauna and microhabitat.

The result showed that the distribution between the two genera *Anopheles* and *Culex* was heterogeneous. In 32.5% of the 301 analysed sites only *Anopheles* species were present and in 28% only *Culex* were found. A mix of the two genera was found in 21.5% of the sites whereas in 18% no larvae were found at all. The statistical analysis revealed seasonal variations in population density for both genera. During September and November, the highest density was observed for both genera. The lowest densities were observed during February for *Culex* and in May for *Anopheles*. Positive significant associations between *Culex* species and the variables DO, pH, conductivity, vegetation, microhabitat, fauna and bottom surface were obtained. For *Anopheles* positive and significant associations was obtained for DO, pH, vegetation, transparency, rainfall and fauna. One conclusion drawn based on the results was that both abiotic and biotic factors are important regarding the suitability of breeding habitats for *Culex* and *Anopheles* species.

2.5.5 Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya

This study was conducted in Gwassi Hills in western Kenya by the International Centre of Insect Physiology and Ecology in Nairobi; Kenya Medical Research Institute also in Nairobi; Department of Tropical Medicine at Tulane University Medical Center in New Orleans, Louisiana and the Department of Biological Sciences at the State University of New York in Buffalo, New York (Minakawa et al., 1999). The purpose of the study was to analyse anopheline distribution and characterize breeding habitats based on eight variables; pH, turbidity, surface area, algal coverage, canopy coverage, debris coverage, emergent plant coverage and water depth. The district was located at a highest altitude of 2270 m a.s.l..

The result showed no significant associations between the abundance of anopheline larvae and the variables. For culicine larvae significant associations were found for pH, canopy and debris coverage (no sign was declared in the article). Anopheline larvae exclusively were found in 32.8% of the habitats whilst only culicine larvae were found in 8.6%. Co-existence between larvae from the two subfamilies was found in 58.6% of the habitats. Significant differences were obtained between habitats where anopheline larvae and culicine larvae were found exclusively. Among the anopheline larvae, only species from *An. gambiae s.l.* were found. *An. arabiensis* (63.4%) was the most common species followed by *An. gambiae s.s.* (31.4%). Anopheline larvae were most abundant in puddles whereas culicine larvae were mostly found in ponds.

The results of this study did not reveal specific environmental variables that determine the distribution of anopheline larvae. The conclusion highlights the importance of further research for the possibility to find such associations between anopheline larval abundance and explanatory variables. According to Minakawa et al. (1999), further studies should include additional variables, considerations about ecology of predators, more thorough analysis about chemical composition of the water, and integration of remote sensing technology.

2.5.6 Effects of co-habitation between *Anopheles gambiae s.s.* and *Culex quinquefasciatus* aquatic stages on life history traits

The study was conducted in Iguhu village in western Kenya highlands by Centre for Global Health Research at Kenya Medical Research Institute; the Kilimanjaro Christian Medical College at the Tumaini University in Tanzania and Program in Public Health and Ecology, and Evolutionary Biology at University of California, USA (Kweka et al., 2012). The purpose was to investigate how co-habitation between *An. gambiae s.s.* and *Culex quinquefasciatus* affect development and survival of each species.

The results revealed that co-habitation between the two species affected the adult size of *An. gambiae s.s.*, but not the size of *Cx. quinquefasciatus*. The survival of the two species was not affected even though the development was significantly slower during co-habitation. Females were the predominating sex during co-habitation, while males were more common when the two species lived separately. According to Kweka et al. (2012) effects of co-habitation has to be more thoroughly investigated to possibly understand how such results could increase the efficiency of mosquito vector control strategies and methods.

2.5.7 Spatial distribution, environmental and physico-chemical characterization of *Anopheles* breeding sites in the Mount Cameroon region

In the Mount Cameroon region in western Africa a study was conducted by the Research Foundation for Tropical Diseases and the Environment; Department of Biochemistry and Microbiology, University of Buea; Department of Animal Biology and Physiology, University of Yaounde; and Institut de Recherche en Agronomie et Développement (IRAD) Ekona in order to characterize *Anopheles* breeding habitats (Wanji et al., 2009). The objective of the study was to find environmental and physico-chemical characteristics, which described the distribution of mosquito larvae. The area of interest, where six localities were seated, had an altitude over 1000 m a.s.l.. The environmental and physico-chemical variables analysed were temperature, exposure to sunlight, pH, calcium, potassium, sodium, chloride, carbonate, bicarbonate, ammonium, nitrate, phosphate, magnesium, sulphate ions and conductivity. The localities were classified as either temporary or permanent, which made it possible to identify potential differences based on the measured variables between the two groups.

The results showed significant differences in potassium, bicarbonate, nitrate, sulphate and conductivity between temporary and permanent habitats. Of all 287 habitats investigated, almost 81% contained *Anopheles* larvae. From the total amount of found *Anopheles* habitats, almost 93% of the breeding sites were temporary. Permanent sites included streams and fishponds with bottoms of sand or mud. Temporary sites included roadside ditches, rain pools, shallow drainage, footprints, hoof prints, artificial holes and building foundations. The permanent sites generally held fish and had clear water and shading aquatic or terrestrial vegetation. Temporary sites had shallow waters with high turbidity due to muddy bottoms, and stagnant rain pools often had green algae. Sites that had plenty of predators generally had few or no *Anopheles* mosquito larvae. All the temporary sites were located within a distance of 50 m to nearest inhabited house; the permanent sites were all located more than 50 m from nearest inhabited house. In permanent habitats there were a range of *Anopheles* species from different complexes; in the temporary habitats only species within *An. gambiae s.l.* were found.

The final conclusion was that resources should be allocated to eliminate or avoid creating man-made temporary habitats close to human settlements. According to Wanji et al. (2009) this would be an effective vector control of especially *An. gambiae s.l.* species.

2.5.8 Summary of conclusions

Results and conclusions from the previous studies reveal that more resources and studies are required in order to obtain a better understanding of mosquito larvae, their aquatic habitats, and their preferences. Increased knowledge would enable improved and more effective mosquito vector control. The previous studies concluded targeted interventions as the most effective way to control larvae in their habitats, both according to efforts and costs. Abiotic and biotic variables are considered to have important implications for the choice of breeding habitat even though the details are not fully defined. However, several studies have commonly found that DO, pH, conductivity and vegetation seem to be driving variables for larval abundance of several mosquito species (Minakawa et al., 1999; Olayemi et al., 2010;

Kenea, Balkew and Gebre-Michael, 2011; Tadesse, Mekonnen and Tsehaye, 2011). Two of the studies (Minakawa et al., 1999; Olayemi et al., 2010) further declare that ponds are the most common habitat for *Culex* larvae. According to three of the studies (Minakawa et al., 1999; Olayemi et al., 2010; Kenea, Balkew and Gebre-Michael, 2011), *Anopheles* prefers swamps, sandpits or puddles as breeding habitats. A first choice of breeding habitat for *Aedes* was concluded by one of the studies (Olayemi et al., 2010) as domestic containers.

It was moreover found that co-habitation among certain mosquito species can affect development and size of the inferior species (Kweka et al., 2012). These observations and conclusions however, require more investigation before it is possible to implement the insights in vector control. Differences in abiotic variables between permanent and temporary habitats were determined in one study (Wanji et al., 2009) where *Anopheles* species were found in highest density in temporary habitats, which all had a distance less than 50 m to nearest settled house.

2.6 STATISTICAL ANALYSES

Despite visual differences between water bodies, various characteristics in collected data are generally common. Typical characteristics of water data are e.g. non-normal distribution, outliers, autocorrelation, and dependences with other not analysed factors (Helsel and Hirsch, 2002).

Assuming wrong characteristics of an obtained data set could have considerable consequences in the results from an analysis that requires specific characteristics, and mislead to incorrect interpretations (Helsel and Hirsch, 2002). However, there are several tests to statistically analyse data leading to the same or similar result. It is thus possible to choose an appropriate method with requirements consistent with the collected data that yields the requested result.

Hypothesis testing is a way in statistical analysis to test whether certain assumptions of collected data are true or not. The assumption believed to be true constituting the alternative hypothesis even though the null-hypothesis will be valid until evidence proves the alternative (Helsel and Hirsch, 2002). Depending on what data is being tested and in what purpose, the performance and hypotheses are set up differently. Common is the significance tests where the outcome often is a p-value that should exceed α for the null-hypothesis to be rejected, which is desired. α is the significance level and according to Helsel and Hirsch (2002) “the probability of incorrectly reject the null-hypothesis”. Commonly in computer statistics tools the default value of α is 0.05, which consequently means that in 1 out of 20 samples the null-hypothesis will be rejected even though it is true (Andersson, Jorner and Ågren, 2007).

2.6.1 Group tests

Wilcoxon test (Wilcoxon-Mann-Whitney test or Wilcoxon rank-sum test) is a nonparametric test used to determine whether or not two independent groups of data have similar characteristics. Nonparametric methods are statistical methods that have little or no requirements on the populations from which the obtained data come (Siegel and Castellan Jr., 1988). Another facilitative aspect is that the data is not required to follow a specific distribution like normal or binomial. Other general advantages of nonparametric tests are e.g.

easy calculations, and their versatility and thus applicability on small sample sizes (Conover, 1980).

The motive of using Wilcoxon test can be described by a comparison with the t-test, the parametric equivalent. The t-test requires certain characteristics of the data; data should be normally distributed and the standard deviation of the variable needs to be known or possible to estimate. Wilcoxon test has not as extensive requirements, the only requirement is that the data should be possible to rank (Conover, 1980). First, data from two groups are ranked together in an ascending order followed by calculation of the sum of ranks for the group with the least number of values. The sum calculated is the test statistic W. If the sample size is large (n>10) or if it contains ties, calculations will be based on a normal approximation instead of the exact method (Siegel and Castellan Jr., 1988).).When two or more equal values appear during the ranking they are called tied values or ties which could affect the test results. If this error is not corrected, the size of W will be affected and so will the p-value, on which the whole hypothesis test is based (Siegel and Castellan Jr., 1988).

The common application of Wilcoxon test is using a two tailed hypothesis test to determine whether two groups are significantly different from each other in regard to a single variable. Data from two independent groups X and Y generates a null-hypothesis, H_0, which implies that X and Y have the same distribution meaning that there is no difference between the two groups. The alternative hypothesis H_1 implies the opposite situation that there is a difference between the two groups (Conover, 1980).

2.6.2 Correlations

To determine whether a correlation exists between two variables, a correlation test can be conducted where a correlation coefficient is calculated. The correlation coefficient is a standardized measure of the linear fit of two variables. However, it does not tell whether a significant association exists (a further significance test is required) or if one of the variables explains the other in causality. The range of values, which the correlation coefficient can obtain, lies within the interval of -1 to 1, where the ends declare strong negative or positive correlation. If the coefficient reaches -1 or 1 the data points all lie on a straight line with a perfect linear relationship. Moreover, the correlation coefficient does not reveal any information about the slope of the line or where it is located, only how well the points fit a straight line (Andersson, Jorner and Ågren, 2007). Three examples of various correlation coefficients are Kendall's tau, Spearman's rho and Pearson's r (Helsel and Hirsch, 2002). All three are easily calculated in a statistics or calculation computer program.

Linear regression analysis is a way to further determine more details about the linear relationship between the variables. In linear regression the equation is determined through minimizing calculations and estimations of the true intercept and slope. When there is only one driving variable expected to explain the response variable simple linear regression (SLR) is used, hence the name simple. When more than one variable is believed to explain the response variable and when certain requirements are fulfilled, multiple linear regressions can be used. If the relation is not linear, a nonlinear regression method can be used instead (Helsel and Hirsch, 2002). The driving variables and the response variable must be identified

(theoretically) before the SLR analysis. The analysis results in a description of the covariance of the variables through the equation of the regression line, which depends on what variable is set as y or x. Regression analysis is easiest conducted with a statistics or calculation computer tool. The coefficient of determination, R^2, which by Helsel and Hirsch (2002) is described as "the fraction of the variance explained by regression", is obtained together with other measures of the actual linear fit.

There are three main reasons to use regression analysis; to analyse the relationship between two or more variables, to test and emphasize important variations within the data, or to, based on knowledge of another variable, predict values of a less known variable (Helsel and Hirsch, 2002). The model in SLR shows in Equation 2.

$$y_i = b_0 + b_1 x_i + \varepsilon_i, \qquad i = 1,2,\dots,n, \tag{2}$$

where y_i=i^{th} observation of the response variable, x_i=i^{th} observation of the driving variable, b_0=intercept (where the regression line intersect with the y axis), b_1= slope of regression line, ε_i=i^{th} residual or random error, and n= sample size. The residuals (or random variable of error), $\varepsilon_i = y_i - \hat{y}_i$, comes from the natural variability of the system and are defined as the difference between the real value of the i^{th} observation of y, and the estimated value, $\hat{y}$, of the ith observation of y. Residuals always have a mean value of zero and a constant variance (Helsel and Hirsch, 2002).

If the purpose is to study a relationship exclusively the two hypotheses H_0 and H_1 are set up. The null-hypothesis, H_0, implies that $b_1=0$, i.e. the slope of the regression line is zero and therefore does no linear relationship exist. The alternative hypothesis, H_1, says that $b\neq0$, the slope of the regression line is not zero and a relationship does exist. When all values were calculated significance of both coefficients b_0 and b_1 were tested with a t-ratio test statistic (Helsel and Hirsch, 2002). Software programs often present both the p-value and the t-ratio. The t-ratios for coefficients b_0 and b_1 should both lie outside an interval -2 to 2 for the possibility to reject the null-hypothesis at $\alpha=0.05$ (Helsel and Hirsch, 2002). If the t-value does not exceed the interval in the higher or lower end, the relationship is most likely a random event caused by chance (Helsel and Hirsch, 2002).

Consequently following assumptions have to be applied when testing a hypothesis to determine whether a driving variable explains the response variable for real or by chance; y is linearly related to x, data used are representative, the variance of residuals are constant and does not depend on x or something else, e.g. time, and the residuals are normal distributed. Concerning the response and driving variables no assumptions are made about distribution. Further is the regression line sensitive to outliers, which are values that differ heavily from the others (Helsel and Hirsch, 2002).

3 MATERIAL AND METHODS

3.1 SAMPLING STRATEGY

The fieldwork of the study was carried out in and around the city of Bahir Dar, Ethiopia. In order to establish adequate sampling sites it was necessary to explore the area by car and foot. The routes for the expedition were discussed and planned with locals who had good knowledge about the area. It was found out that the most common managed waters that could serve as efficient breeding habitats for mosquitoes were ponds and ditches used for irrigation, drainage or cultivation.

Ten sampling sites were established with the water bodies classified as either managed or unmanaged, mostly in rural areas of Bahir Dar (Figure 4). The number of sites in each category was equal to enable comparisons. Field measurements and laboratory chemical analysis were performed to determine the water characteristics in each site. In order to analyse changes in abiotic and biotic factors within the sites over time five consecutive sampling occasions were performed weekly, which created a time series. The measurement period stretched from 2013-09-25 until 2013-10-30, which covered the end of the rainy season and the very beginning of the subsequent dry season. All measurements were performed by the two students conducting this study and held as close in time as possible to approximate synoptic measures.

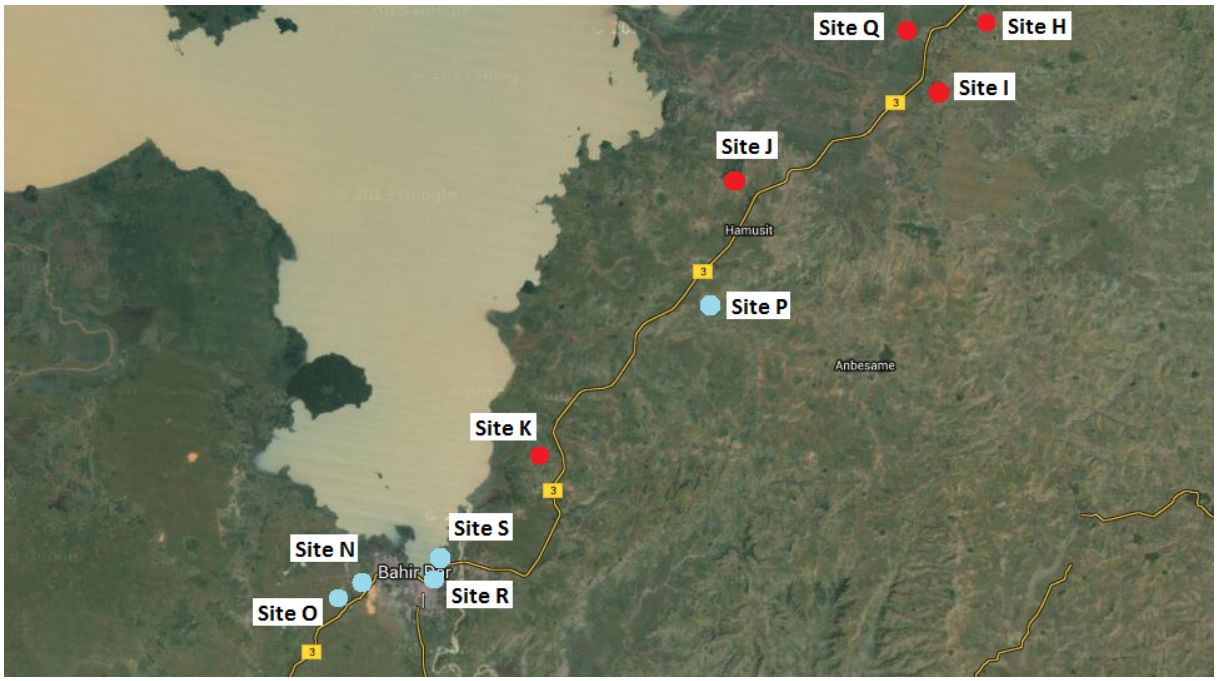

Figure 4. The ten established sampling sites in and around the city of Bahir Dar (Google Maps, edited, 2013). Red dots points out managed sites and blue points out unmanaged sites.

At the first measurement occasion, a representative sub-area (sampling area) was defined for each site. All the sampling procedures took place within this area at every sampling occasion. The full sampling schedule involved field measurements, water sample collection, landscape descriptions and mosquito larvae sampling.

3.2 WATER CHARACTERISTICS

Field measurements of five abiotic and one biotic variable were conducted in all ten sampling sites. The variables were dissolved oxygen (DO), pH, electrical conductivity, temperature, depth and presence of algae. The extended analysis of the water characteristics involved chemical analysis of six additional variables in a laboratory. These variables were turbidity, biochemical oxygen demand (BOD_5), nitrate, phosphate, sulphate and carbonate.

3.2.1 Field measurements

Dissolved oxygen (DO) was measured using an ExStik DO600 meter (Extech Instruments, 2008). This instrument had a built-in feature that compensated for both temperature and altitude (1800 m a.s.l. in this case). For pH, electrical conductivity and temperature an Omega Water proof Tester model number 99720 was used (Omega, 2007). Depth was measured using a plumb and a measuring tape. All abiotic variables were measured at three representative locations within the sampling area. The presence of algae was approximated visually and recorded as present or not present.

3.2.2 Extended chemical analysis in laboratory

A water sample was collected at the water surface from all ten sampling sites at every measurement occasion. Approximately one litre of water was filled in empty drinking water bottles of either a half or one litre each. To reduce contamination, the bottle was rinsed five times in sample water before the actual sample was taken. Immediately after collection the water was kept in a cooling bag with ice during transport and in a deep freezer during the storage period before the extended chemical analysis. All chemical analyses were performed by the School of Chemical and Food Engineering at the Technology Institution of Bahir Dar University.

Turbidity

The turbidity analysis was performed using a Portable Turbidity Meter 430-260 (ELE International, 2004), which is a photometer recording non-absorbed light. Measurement unit for this instrument was FTU. The instrument was tested with a blank sample containing deionized water before the actual analyse of the sample. Since the meter displayed 0 FTU for the blank, calibration was not necessary. To analyse the sample, a clean cuvette was filled with fully stirred sample water up to 0.5 cm from its threading and then put at rest to remove air bubbles. The cuvette was sealed with a screw cap and cleaned using a lint-free tissue (ELE International, 2005). After air bubbles were removed the sample was put into the turbidity meter for analysis and the result was recorded.

Biochemical oxygen demand (BOD_5)

The BOD-analysis was carried out according to a standard procedure where the initial DO concentration of the water sample was measured using a SX716 portable Dissolved Oxygen Meter (Samsan Korea, n.d.). 100 ml of sample water was diluted with 200 ml of deionized water before it was incubated. The incubation took place in a dark location at a constant temperature of 20 °C for five days, hence the index 5 in BOD_5. After the incubation period the final DO concentration was measured and the BOD was calculated as the difference between the initial and the final DO concentration (Bydén, Larsson and Olsson, 2003).

Carbonate, nitrate, phosphate and sulphate

Chemical analyses of carbonate, nitrate, phosphate and sulphate were conducted using a Wagtech WTD 8000 photometer (Palintest Ltd, 2003). All water samples were prepared according to the manufacturer's test instructions for each variable respectively. The sample preparations involved addition of different reagent tablets to colour and flocculate the sample, which increased the turbidity. The specific chemical compound was measured photometrically where colour intensity of the sample was proportional to its concentration. Before each measurement, the photometer was reset using a blank sample of deionized water.

Carbonate was the only exception from the standard procedure described above, which was a multi-step process that required both laboratory work and analytical calculations. Initially the total alkalinity was measured photometrically similar to the description above. The subsequent step was to calculate carbonate concentration using a standard analytical procedure divided in two steps (HP Technical Assistance, 1999). The bicarbonate concentration was first calculated based on the pH value and the recently measured total alkalinity (Equation 3).

$$[HCO_3^-] = \frac{Alk_{tot} - 5.0 \cdot 10^{(pH-10)}}{1 + 0.94 \cdot 10^{(pH-10)}} \quad (3)$$

where Alk_{tot} is total alkalinity. The concentration of bicarbonate [HCO_3^-] was expressed as milligram $CaCO_3^-$ per litre.

The carbonate concentration was finally calculated based on previously calculated bicarbonate concentration (Equation 4). Carbonate concentration, [CO_3^{2-}] was expressed as milligram $CaCO_3^-$ per litre.

$$[CO_3^{2-}] = 0.94 \cdot [HCO_3^-] \cdot 10^{(pH-10)} \quad (4)$$

3.3 LANDSCAPE AND SITE DESCRIPTION

The location of all sampling sites was recorded using a Garmin Oregon 550 global positioning system (GPS) device. This device recorded coordinates and elevation (Garmin Ltd, 2010). The landscape surrounding the sites was described according to their land use, type of vegetation, terrain and slope. The spatial range of the landscape description was a circle with a 50 m radius around the sampling area. Distance to nearest tree, building or house was noted if the object was located within a distance of 50 m from the sampling area.

All ten sites were spread out over a distance of approximately 50 km (Figure 4). Three of the five managed sites (H, I, Q), were located in rice fields, two of them were controlled irrigation ditches (I, Q). The remaining two managed sites (J, K) were irrigation and drainage ditches connected to cornfields. To enable comparison between managed and unmanaged waters, five additional unmanaged sampling sites were established. Two of these (N, P) were natural ponds, one (O) was located in a swamp surrounded by eucalyptus trees, and the two remaining sites (R, S) were located in Lake Tana; see a summary in Table 1. The exact location of each site is listed in Appendix A.

Table 1. Site descriptions. Columns indicate group (Managed/Unmanaged), location (Rural/Urban), habitat type and altitude.

Site	Group	Location	Habitat type	Altitude [m a.s.l.]
H	Managed	Rural	Ricefield	1714
I	Managed	Rural	Irrigation ditch	1793
J	Managed	Rural	Irrigation ditch	1901
K	Managed	Rural	Irrigation ditch	1907
N	Unmanaged	Urban	Pond	1742
O	Unmanaged	Urban	Ditch/swamp	1752
P	Unmanaged	Rural	Pond	1723
Q	Managed	Rural	Irrigation ditch	1797
R	Unmanaged	Urban	Micro-habitat of Lake Tana	1770
S	Unmanaged	Urban	Micro-habitat of Lake Tana	1735

Seasonal variations in the area affect the duration of drought, heat and precipitation periods. Rice is only possible to grow in the study area during rainy seasons. As rainfall decreases the fields will no longer be suitable for rice production since no artificial irrigation systems are installed. During dry season, when temperatures are high and precipitation low, farmers focus more on other less water-demanding crops.

The swamp surrounded by eucalyptus trees (site O), was at the last measurement occasion (2013-10-30) completely dry. It is likely that the sites located in or around the rice fields also got dried out shortly after the rain season. However, site I could be an exception since the ditch was relatively deep and a more long-term storage of water might be possible if the dam was reconstructed. This was not observed during the study due to the short measurement period. The irrigation ditches connected to corn fields (J, K) also risk drying out due to their narrow and shallow character. Other sites that might be at risk of drying out are the unmanaged ponds (N, P) since both of them are shallow. Sites R and S, which are located in Lake Tana, could possible outstand a prolonged drying process since big aquatic systems respond slowly to variations, but they might also be at risk of drying out as the water level drops.

Another seasonal aspect is runoff, which affects aquatic systems with increased inflow of water and addition of particulate and dissolved organic and inorganic material as well as pollutants. Transport of various physical and chemical compounds are intensified during rainy seasons and the extent of this transport depends on slope and soil properties. If the ground contains erosive soil types, the risk of a significant supply of mineral particles could arise. In this study some sites may be more affected by runoff than others, e.g. the unmanaged ponds N and P due to their location in sloping landscapes with erosive soils.

3.3.1 Managed waters

All of the five managed water sampling sites were located outside Bahir Dar along highway number 3 in the north-east direction (Figure 4). The road was elevated and lined with short-

grown grass on both sides. The strips of grass serve as pasture for cattle, sheep, and goats and during the time of this study it was clearly overgrazed.

Site H

Site H was located directly within a rice field (Figure 5). The surrounding landscape was flat and consisted mainly of rice paddies. In this particular part of the field, rice was not growing very tight, so there were no shading but some open water which was fairly clear except for a foggy appearance. In the slightly lower land between the road and the actual rice field there was a shallow and 10 m wide ditch that stretched along the road (Figure 6).

Figure 5. Site H was located right into a rice field (Renstål, 2013).

Figure 6. The surroundings of site H, where the rice fields and the ditch between the actual site and the highway is visible (Renstål, 2013).

Site I

Site I was an irrigation ditch in an area where the primarily land use was rice cultivation and secondly teff cultivation. The water in the ditch was regulated by a dam made out of rocks and clay (Figure 7 and 8). During dry periods, farmers close the dam to enable irrigation, and during wet periods and temporary heavy rainfalls the dam is opened to avoid flooding of the fields. The dam was deconstructed at some point between the first and second measurement occasion (2013-09-25 and 2013-10-08) and remained deconstructed during the rest of the sampling period.

Figure 7. The water level at the first measurement occasion (2013-09-25) when the dam was still present (Renstål, 2013).

Figure 8. The last measurement occasion (2013-10-29) showing the effects of dam deconstruction (Renstål, 2013).

The water was fairly muddy with a reddish colour (Figure 9). While the dam was closed, the water was still but due to the deconstruction of the dam the water had a turbulent flow. Barely any emergent vegetation grew on the edges and sloping sides of the ditch or in the water itself. This prevented shading of the water surface since there were no trees or other shading obstacles around.

Figure 9. The water in site I at the last measurement occasion (2013-10-29) when the water level was the lowest (Renstål, 2013).

Site J

Site J was a shallow hand-dug irrigation ditch in connection to a corn field which the farmers seemed to control carefully due to weekly changes in the watercourse. The ditch branched off from the main irrigation ditch and was led straight into the cornfield (Figure 10). The water was clear and there were no algae and barely any vegetation present, therefore no shading of the water surface. Distance to the highway was about 20 m. The farmer's dwellings and barn facilities were located about 50 m from the sampling area. The main ditch stretched along the road and was unevenly lined with bushes, short grass and other vegetation (Figure 11).

Figure 10. The water of site J (Renstål, 2013).

Figure 11. The environs of site J with parts of the cornfield, main irrigation ditch, farmer's dwellings and the highway (Renstål, 2013).

Site K

Site K was a narrow drainage ditch running through corn, chat and teff cultivation fields (Figure 12). The ditch was quite deep, about 0.5 m, which gave the impression that it has been designed to handle large seasonal flow variations. The presence of rocks and vegetation within the watercourse slowed the water down and caused standing water in the sampling area. Vegetation also caused an extensive shading of the water surface. The water was clear and algae were present (Figure 13).

Figure 12. The surroundings of site K with the teff field on the left side of the ditch and chat on the right side. The cornfield on both sides of the ditch behind the teff and chat (Renstål, 2013).

Figure 13. The water of site K and its shading vegetation (Renstål, 2013).

Site Q

Site Q was a wide and shallow irrigation ditch right between the road and a rice field (Figure 14 and 15). Between the second and third measurement occasion (2013-10-02 and 2013-10-15) the water got controlled by a dam made out of clay and organic substrate, which merged several separate puddles into one larger body of water. Around the site the ground was covered with short grass. The water was rather clear and no algae were present. In the latter part of the measurement period grass was growing in the water due to an increased surface area probably caused by the dam construction.

Figure 14. Site Q at the first measurement occasion (2013-10-02) (Renstål, 2013).

Figure 15. Site Q at the final measurement occasion (2013-10-29) (Renstål, 2013).

3.3.2 Unmanaged waters

The five unmanaged sampling sites were mostly ponds with various properties and locations. Four of these sites were located in more urban areas of Bahir Dar, whilst the last one was located in the same rural area as the managed sites.

Site N

Site N was a natural pond located in the south-western outskirts of Bahir Dar. The sampling area was a sub-area of a much larger pond (Figure 16) with shallow and muddy water (Figure 17). Clusters of algae were present at the bottom of the water and the surface. There was no vegetation either in or around the water, and therefore no shading of the water surface. The surrounding landscape was mainly bare ground and short grass scattered with various sized rocks and bushes. Nearby the site there was an elevated and non-heavy traffic road with a concrete culvert crossing beneath it. The far side of this road was lined with large trees, mainly eucalyptus and bushes. In the first part of the measurement period the water level was high enough for water to flow through the culvert but as time went the water level dropped.

Figure 16. The surroundings of site N. The narrow waterway linking the site and the larger pond together (Renstål, 2013).

Figure 17. The water of site N was linked with a ditch on the other side of the road through a concrete culvert (Carlström, 2013)

Site O

Site O was located in the south-western outskirts of Bahir Dar across the road from site N. This area was flooded during the rainy season, which created a still water body in a somewhat swampy environment (Figure 18). The main vegetation type growing both in and around the water was eucalyptus trees (Figure 19), which caused an extensive shading of the water. The water had like site H a foggy appearance and a greyish colour. A strong odour was exuded from the water, which also contained a lot organic material, mostly fallen leafs and branches. Algae grew on organic material but were not present at the water surface.

Figur 18. The surroundings of site N. The narrow waterway linking the site and the larger pond together (Renstål, 2013).

Figur 19. Eucalyptus tree barrier separating site O from the road (Renstål, 2013).

Site P

Site P was a small natural pond surrounded by a sloping landscape, possible created from a combination of landslide and erosion processes (Figure 20). The land closest to the water was covered with short grass whereas the slopes were mainly bare ground with rocks mixed up with smaller grain sizes such as sand and clay. In the upper part, which had not yet been subjected for landslides, the land was used for cereal cultivation, e.g. corn and teff. The water was fairly clear and clusters of algae were present scattered along the edge of the pond at the water surface (Figure 21).

Figure 20. Surrounding landscape of site P including erosion and landslide zones that stretched around the pond (Renstål, 2013).

Figure 21. The water of site P (Renstål, 2013).

Site R

Site R was a sub-part and perhaps a micro-habitat of Lake Tana basin located in connection with the urban parts of Bahir Dar. The area had plenty of vegetation, both on land and as emergent plants in the water. Water lilies and reed or grass-like plants covered or shaded parts of the water surface (Figure 22). Surrounding trees, mainly eucalyptus, also contributed to the shading. A walkway was directly adjacent to the water, which many pedestrians used for recreation (Figure 23). On the far side of the walkway there was a slightly sloping landscape covered in brushwood, followed by a forest-like area. The water was clear and algae were growing on organic material in the water, but not in the water column or at the surface.

Figure 22. The water of site R (Renstål, 2013).

Figure 23. The surroundings of site R. The open water of the main part of Lake Tana is visible in the upper left corner (Renstål, 2013).

Site S

Site S was also a sub-part and a possible micro-habitat of the Lake Tana basin. The area was cut off from the rest of the lake by papyrus grass, which created a physical barrier that enabled standing water on the inside of this wall (Figure 24). Close to the water, the same walkway passed the site as for site R. The water was fairly clear and no algae were present but some organic matter such as fallen leaves and branches (Figure 25). Shading was mainly caused by papyrus and other emergent grass-like plants in the water. The extent of the shading varied during the day with most shade in the morning and least around lunchtime.

Figure 24. The water and papyrus grass barrier of site S (Renstål, 2013).

Figure 25. Papyrus grass barrier viewed from behind site S (Renstål, 2013).

3.4 MOSQUITO LARVAE SAMPLING AND COLLECTION

The mosquito larvae sampling was performed according to a standard collection method by using a dipper (WHO, 1975b). For the first measurement occasion, (2013-09-25, site H, I, J and K) a 350 ml standard dipper was used. For the next four measurement occasions a bowl shaped stainless steel dipper with a diameter of 14 cm and a volume of 540 ml was used. Light reflections in the metal made it difficult to spot the larvae. The water was therefore poured into a white plastic bowl for the counting of larvae. Ten dips were made within the sampling area in each habitat at every measurement occasion. If larvae were present, the number of larvae in each dip was recorded separately. Larvae identification was performed visually based on literature studies of mosquito biology and a short practically oriented course in mosquito larvae identification. The course was held by a PhD student at Addis Ababa University in the study area outside Bahir Dar.

3.5 STATISTICAL ANALYSES

3.5.1 Group tests

The purpose of performing group tests was to examine whether there were any significant differences between groups in regard to water characteristics or mosquito larvae presence. Three such tests were performed in R version 3.0.2 where the two-sample Wilcoxon test was used (Field, Miles and Field, 2012). The significance test was based on the potential rejection of the null-hypothesis, H_0. The null-hypotheses implied that there were no differences between the two groups, which became rejected if $p<0.05$. The alternative hypotheses, H_1, implied that the two groups are different.

The first pair to be tested was managed sites versus unmanaged sites, the second pair was mosquito larvae sites versus zero-sites and the third test were a pair of sites where larvae were found consequently (sites H, K, P and Q) during the measurement period versus zero-sites.

H_0 and H_1 for the three groups were:

Group test 1, H_0: managed and unmanaged waters are not different in the abiotic and biotic variables or mosquito larvae presence, and H_1: there are differences between managed and unmanaged water.

Group test 2, H_0: there are no differences between mosquito larvae sites and zero-sites regarding the abiotic and biotic variables, and H_1: there are differences between mosquito larvae sites and zero-sites.

Group test 3, H_0: there are no differences between sites where mosquito larvae were found consequently and zero-sites regarding the abiotic and biotic, and H_1: there are differences between consequent mosquito larvae sites and zero-sites.

Data included in the tests had both spatial and time aspects since all data from all sites at all measurement occasions were included. The three pseudo-replicate values of the variables measured directly in field (pH, conductivity, DO and depth) were used separately instead of taking the mean values. The remaining variables (turbidity, BOD, nitrate, phosphate, sulphate and carbonate) had one measurement value per site and measurement occasion.

3.5.2 Correlations

Pearson's correlation coefficients (r) were calculated in Microsoft Excel 2011 by using the commando KORREL to determine whether any of the abiotic and biotic variables correlated with each other. The calculations were based on data sets from each measurement occasion, with all values from all sites for every variable. Non-logarithmic data was used due to the presence of zero-values.

To determine the driving variables that explained the presence of mosquito larvae simple linear regression was performed. The aim of the regression analyses was mainly to determine significant associations but also to state equations for the driving variables. Prediction can later on be performed to state what level of the driving variable that results in a sufficient decreased mosquito larvae population. Simple linear regression was chosen instead of multiple regressions due to lack of degrees of freedom because of the high number of variables and low number of sites. Residuals were assumed to be approximately normally distributed and no transformations of the data were made.

The regression analyses were based on each measurement occasion separately since it was known that seasonal variations occur in the study area and that water chemistry varieties have time dependency. No extra term was added in the regression model to include the time aspect. Internal influences among the driving variables were not analysed or taken into account since the analysis was conducted using simple linear regression. However, as the significance analysis was performed, the correlation coefficient and determination coefficient were compared in order to determine the strongest correlation.

The null-hypothesis, H_0, was defined as $b_1=0$; the slope of the regression line is zero and therefore no relationship between the variables exists. The alternative hypothesis, H_1, was defined as $b_1\neq0$; the slope of the regression line is not zero and a relationship exists.

The regression analysis was performed in Excel using the tool for statistical analyses StatPlus®:mac version 2009. In each analyse the data set consisted of one biotic or abiotic variable from all sites at one measurement occasion as the driving variable (x-axis), and number of mosquito larvae as the response variable (y-axis). The most frequent significant associations ($p<0.05$) were decided to be the most driving variables for larval abundance.

Mean values, medians and standard deviations were calculated based on the data sets used for the regression analysis to describe the measures of location and spread in each set.

4 RESULTS

4.1 MOSQUITO LARVAE ABUNDANCE

Mosquito larvae were found in eight of the ten sites and a total of 204 larvae were collected throughout the study. Nearly all larvae (192) were collected in four of the sites (H, K, P, Q) exclusively. Site P was the site where most larvae were found with a total of 87, followed by site K where 45 larvae were collected. The sites H and Q held a similar numbers of larvae, 29 and 31 respectively. Two of the sites, N and S, were zero-sites meaning that no larvae were found throughout the measurement period (Table 2). Mosquito larvae were found in all five managed sites (H, I, J, K, Q). Unmanaged sites included three mosquito sites (O, P, R) as well as the two zero-sites (N, S) of the study. The majority of collected mosquito larvae belonged to the subfamily Culicinae. Only a few larvae of the Anophelinae subfamily were collected in site H and K always during coexistence with Culicinae larvae.

Table 2. Sampling site results. Columns of group membership, managed sites (M), unmanaged sites (U); total number of larvae found; algae presence; canopy shading; distance to nearest house; distance to nearest tree and presence of other invertebrates.

Site	Group	Total number of larvae	Algae (Yes/No)	Canopy coverage (Yes/No)	Distance to nearest house [m]	Distance to nearest tree [m]	Other invertebrates (Yes/No)
H	M	29	No	No	-	>50	* Yes
I	M	2	No	No	-	-	Yes
J	M	3	No	No	50	20	Yes
K	M	45	Yes	Yes	-	20	Yes
N	U	0	Yes	No	>50	25	* Yes
O	U	1	Yes	Yes	>50	1	Yes
P	U	87	Yes	No	-	40	* Yes
Q	M	31	No	No	-	-	* Yes
R	U	6	No	Yes	40	2	* Yes
S	U	0	No	Yes	30	2	* Yes

In Table 2, algae were considered present (Yes) if algae were found consistently at every measurement occasion. Canopy shading was recorded as present (Yes) if the shading of the water surface was caused by surrounding terrestrial vegetation and trees. Other aquatic invertebrate species were recorded as present (Yes) if such animals were found at one measurement occasion or more. The marking * represent sites where mosquito larvae predators were found (Section 2.3).

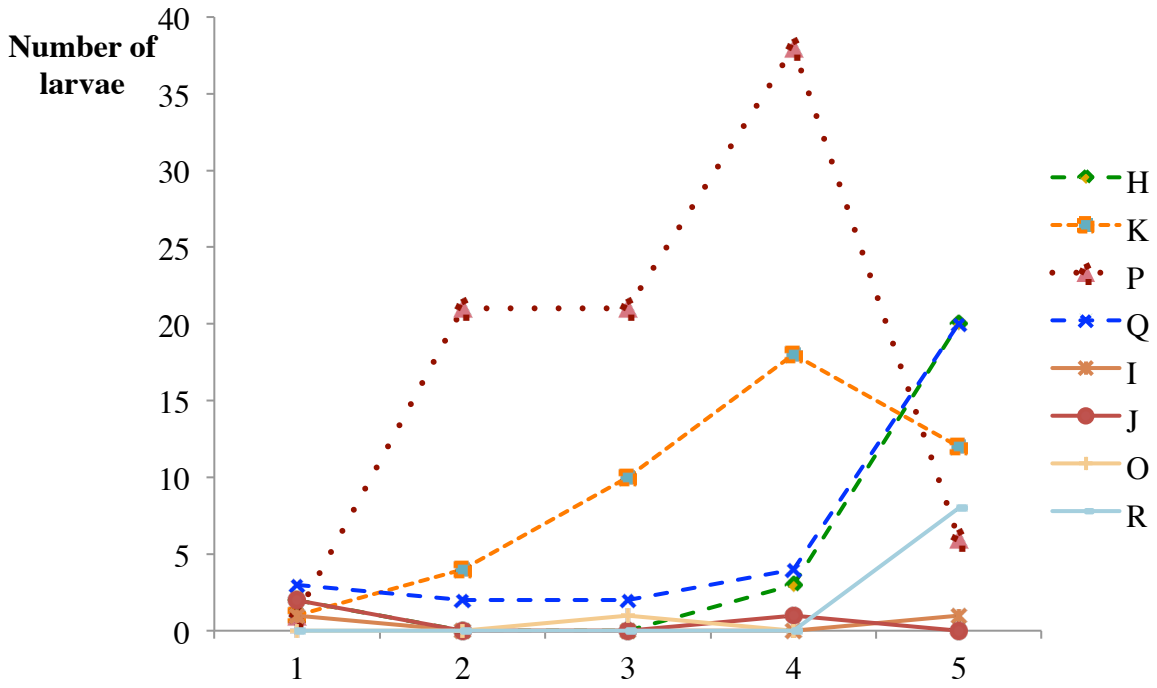

Figure 26. The number of mosquito larvae collected for sites H, I, J, K, O, P, Q and R at each sampling occasion (x-axis).

Two different trends in the number of mosquito larvae were observed in the four mosquito sites that consequently held larvae. In site H, Q and R the number of mosquito larvae increased considerably at the end of the measurement period. In site K and P a peak was observed for both sites at measurement occasion 4 (Figure 26).

4.2 VARIABLES

In this section a selection of sites and variables are present that were considered most important for the results of the study. Sites were chosen where mosquito larvae were found consequently throughout the entire measurement period; H, K, P and Q. Mosquito sites are compared with the zero-site S. Site N was excluded from the results because of its extreme characteristics high turbidity and high sulphate (Appendix B). The selected variables were pH, DO, conductivity and turbidity.

4.2.1 pH

Four of the five selected sites (H, K, Q, S) had fairly even pH values throughout the measurement period with values within the interval of pH-values of natural water bodies (6.5-7.5) (Figure 27). Site P had large variations in pH with respect to both time and space. The values varied considerably from one measurement occasion to another. Between measurement occasion 1 and 2, the mean pH-value dropped from 9.5 to 6.6 in five days and between the

measurement occasions 2 and 4 the pH increased from 6.6 to 10.5 in 13 days (Figure 27). Local variations in pH within the sampling area at specific measurement occasions were also observed. At measurement occasion 1 for example, the largest spatial difference among the replicates was 1 pH-unit (Appendix C).

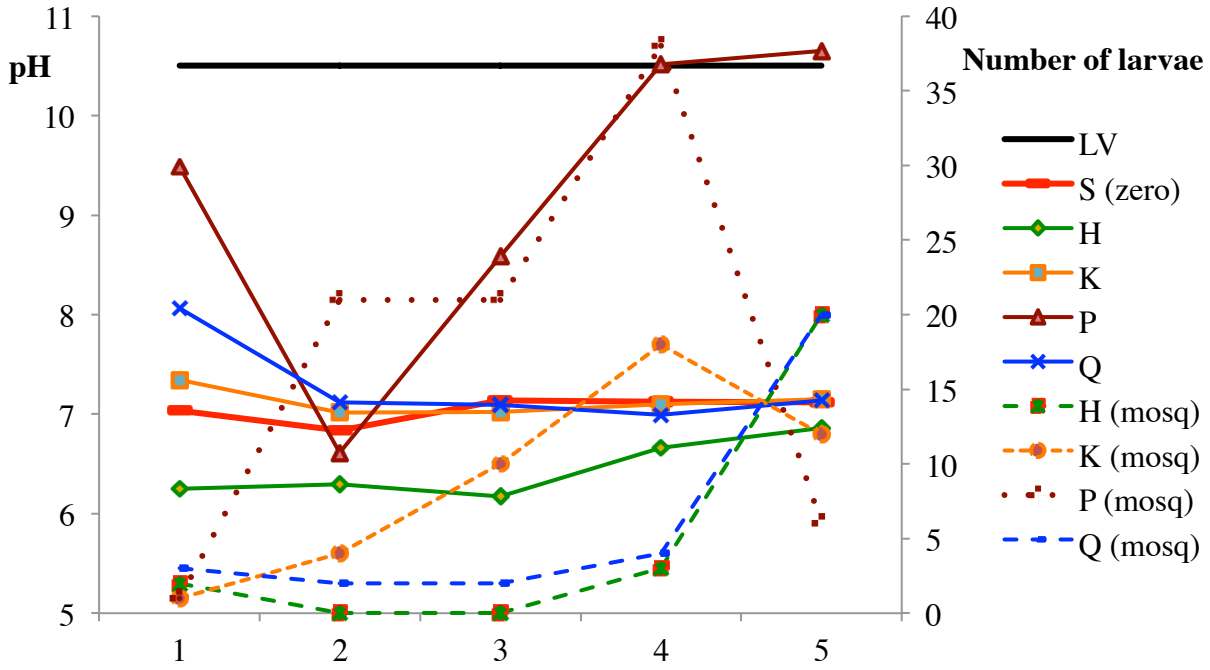

Figure 27. pH (solid lines), biologic limit value for pH (solid black line) and number of mosquito larvae (dotted lines) for sites H, K, P, Q and S at each measurement occasion.

4.2.2 Dissolved oxygen

All mosquito sites (H, K, P, Q) had DO levels well over the limit value of 3 mg/l throughout the measurement period. Sites H, K and Q had even DO levels between 5 and 6 mg/l while site P had an increasing DO level from about 6.7 to almost 13.8 mg/l during the measurement period (Figure 28). Site S also had an even DO level, but the oxygen concentration was close to the limit value during the entire measurement period (Figure 28).

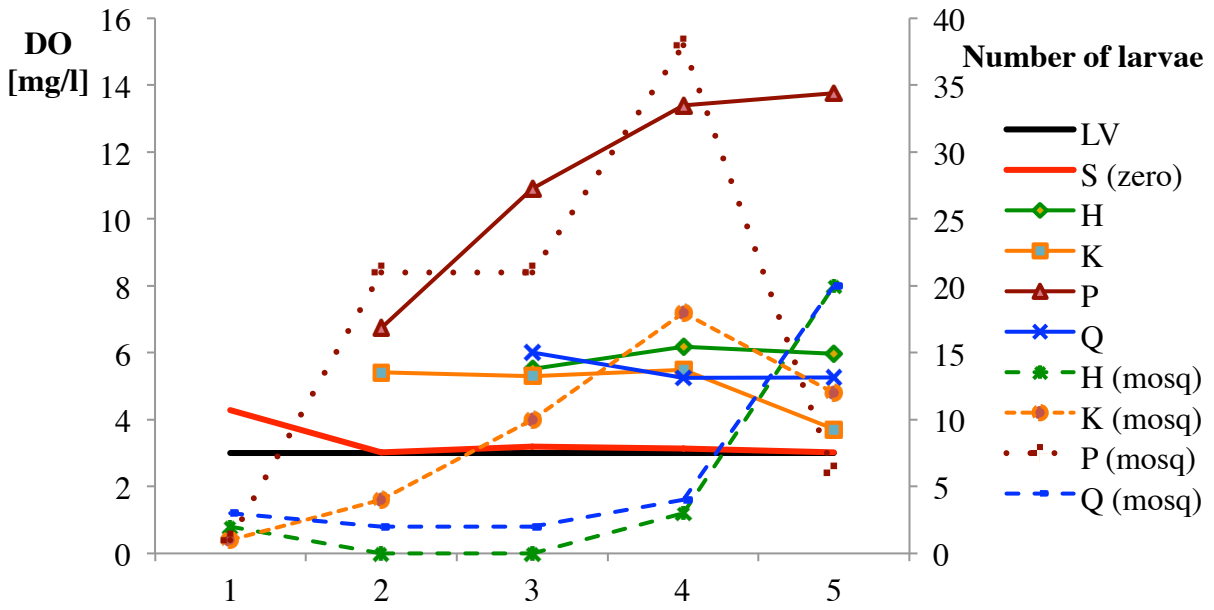

Figure 28. DO (solid lines), biologic limit value for DO (solid black line) and number of mosquito larvae (dotted lines) for sites H, K, P, Q and S at each measurement occasion (x-axis).

4.2.3 Conductivity

All measured conductivity values in all sites were well below the biological limit value of 1500 μS/cm. Site H had relatively low conductivity below 35 μS/cm at all measurement occasions, except for the first one where a value of 86 μS/cm was obtained (Figure 29). Site K and S had quite even conductivities within the intervals 200 to 250 μS/cm and 150 to 210 μS/cm respectively. In site P and Q there had considerable variations in conductivity. The conductivity in site P varied within an interval of 108 and 215 μS/cm while the interval in site Q was 78 up to 371 μS/cm (Figure 29).

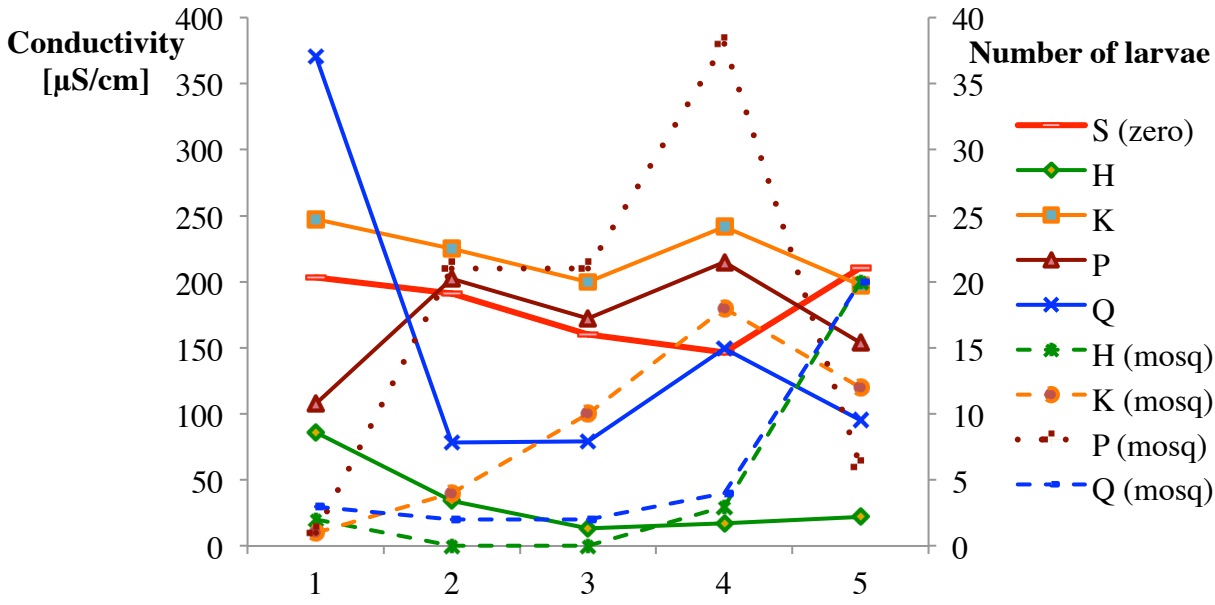

Figure 29. Conductivity (solid lines) and number of mosquito larvae (dotted lines) for sites H, K, P, Q and S at each measurement occasion (x-axis).

4.2.4 Turbidity

The sites H, P and S had a relatively low and even turbidity with values around 50, 40 and 30 FTU respectively during the entire measurement period (Figure 30). Also site K had low and even turbidity values but within an interval of 2.5 to 13.3 FTU during the first four measurement occasions. Between measurement occasions 4 and 5 the turbidity increased from 6.6 up to 85 FTU in seven days. In site Q, the turbidity varied between 50 and 150 FTU except during the third measurement occasion where a peak was observed around 430 FTU (Figure 30).

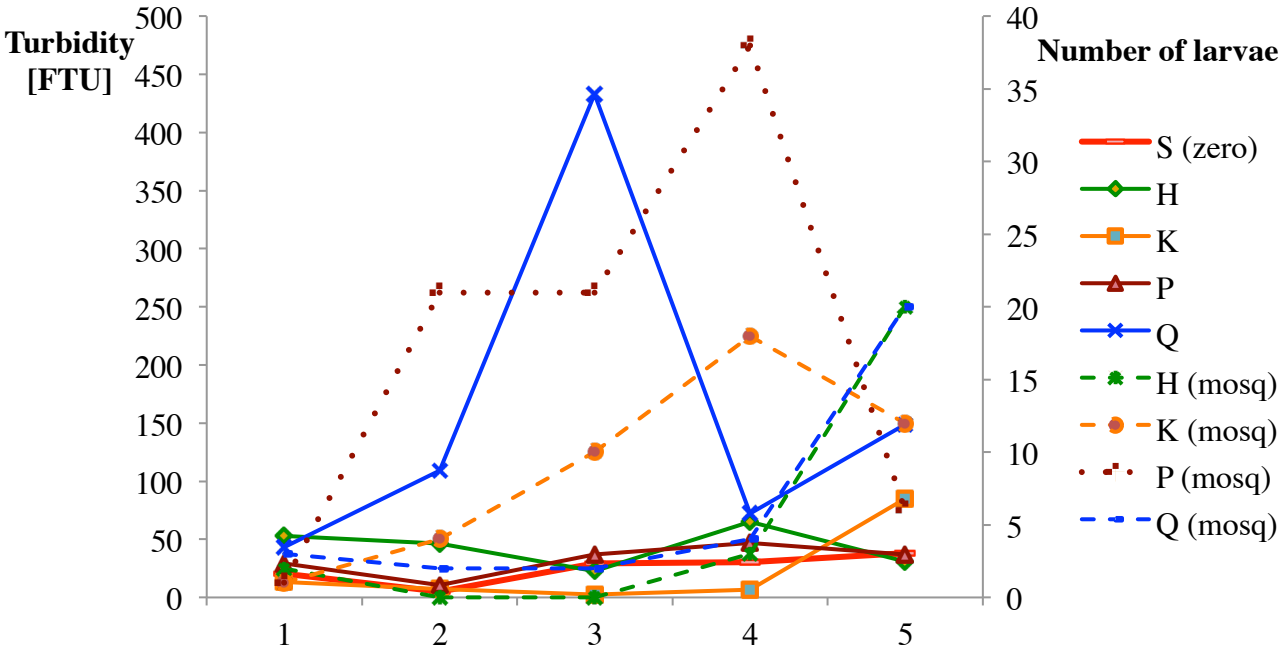

Figure 30. Turbidity (solid lines) and number of mosquito larvae (dotted lines) for sites H, K, P, Q and S at each measurement occasion (x-axis).

4.3 STATISTICAL ANALYSES

4.3.1 Group tests

The number of mosquito larvae showed a significant difference between the two groups managed and unmanaged water bodies (Table 3). Further was phosphate the only abiotic (or biotic) variable that showed a significant difference between the groups.

The pair of groups; zero-sites and sites that (at least once) held mosquito larvae, showed significant differences regarding dissolved oxygen, conductivity and sulphate.

When the group with sites that held mosquito larvae only included sites where larvae were consequently found (H, K, P, Q), differences compared to zero-sites were found in number of larvae, DO and sulphate.

Table 3. p-values from group tests using the Wilcoxon test. Yellow markings show significant differences between two groups for regarding specific variables.

Variables	Managed sites/ unmanaged sites p-value	All mosquito sites/non mosquito sites p-value	Consequent mosquito sites/non mosquito sites p-value
Number of larvae	0.0225	<0.0010	<0.0001
BOD	0.3076	1	0.8950
Depth	0.0872	0.8437	0.4797
DO	0.0781	0.0466	0.0015
Phosphate	0.0356	0.0780	0.0583
Carbonate	0.2936	0.2916	0.2348
Conductivity	0.1533	0.0044	0.0968
Nitrate	0.3843	0.1844	0.8950
pH	0.4102	0.3598	0.0750
Sulphate	0.2665	0.0024	0.0059
Turbidity	0.6745	0.1399	0.2865

4.3.2 Correlations

Strong correlations were found between some abiotic variables, and were set to have a value of r equal to or more than 0.9 (Table 4). Turbidity and sulphate was the only pair of abiotic variables that correlated strongly at all measurement occasions. Carbonate and pH were found to strongly correlate at three out of the five occasions. At one occasion only did pH and DO, turbidity and phosphate, as well as phosphate and sulphate correlate strongly.

Table 4. The measurement occasions (1-5) where variables correlated strongly ($r \geq 0.9$).

r ≥ 0.9	Variable	pH	Turbidity	Phosphate
	Sulphate		All	5
	DO	5		
	Carbonate	2, 4, 5		
	Phosphate		5	

Correlations with values of r equal to or more than 0.8 (less than 0.9) were found for the abiotic variables DO and carbonate at three occasions, carbonate and pH at two occasions, and pH and DO at one occasion only (Table 5).

Table 5. The measurement occasions (1-5) where variables correlated with $r \geq 0.8$.

r ≥ 0.8	Variable	pH	DO
	Carbonate	1, 3	3, 4, 5
	DO	4	

pH and DO were the two variables that resulted in most significant associations with the number of mosquito larvae in the regression analysis (Table 6). The significant association for both pH and DO were found for two subsequent measurement occasions, the third and fourth.

Two additional variables, nitrate and carbonate, showed significant associations with presence of larvae at measurement occasions one and three respectively. Carbonate was the variable showing the highest association with larvae.

Table 6. The significant associations ($p<0.05$) between mosquito larvae and abiotic variables. Std. is the short form of standard deviation, b_0 and b_1 are coefficients in the regression equations.

Measurement occasion	Variable	R^2	p-value	Median	Mean value	Std.	b_0	b_1
1	Nitrate	0.5854	0.0099	0.93	2.47	3.63	0.4516	0.2220
3	pH	0.4528	0.0330	7.12	7.24	0.62	-50.997	7.5147
3	Carbonate	0.7323	0.0016	0.17	0.43	0.77	0.1161	7.6648
3	DO	0.7332	0.0016	5.15	5.19	2.28	-10.078	2.5977
4	pH	0.4252	0.0443	7.33	7.80	1.18	-46.281	6.7546
4	DO	0.4654	0.0298	5.72	6.40	3.27	-10.161	2.5896

5 DISCUSSION

One of the main results of the study was the difference in mosquito larval abundance between managed and unmanaged water bodies (1). Further, differences in variables DO, conductivity and sulphate were detected between mosquito sites and zero-sites during the time series (2). Finally, the driving variables of larval abundance were considered to be DO and pH since those two most consequently explained the presence of larvae during specific measurement occasions (3).

5.1 MOSQUITO ABUNDANCE IN MANAGED AND UNMANAGED SITES (1)

The hypothesis that more mosquito larvae should be found in managed sites than unmanaged sites was rejected based on the total number of collected larvae. Important to remark is that the unmanaged sites had highest total number of collected mosquito larvae between the two groups (Table 2). Even though this result was unexpected it could possibly be explained by the fact that all unmanaged sites (N, O, P, R, S) had relatively stagnant water.

First, both theory about mosquitoes and previous studies, pointed out stagnant water as a preferred habitat characteristic particularly for species within *Anopheles* and *Culex* genera (Tipping and Weber, 2008; Tadesse, Mekonnen and Tsehaye, 2011). Theory also states that *Anopheles* and *Culex* species generally prefer permanent habitats like ponds, lakes, ditches and swamps (Tipping and Weber, 2008), which all are comparable with sites N, O, P, R and S, in one way or another. Secondly, it is important to notice that the distribution of collected mosquito larvae was unevenly distributed among the unmanaged sites. The majority of all larvae were found in site P while both N and S were zero-sites. According to Tadesse, Mekonnen and Tsehaye (2011) dams are significant habitats for *Anopheles* larvae. The results

and conclusions of this earlier study were considered comparable to site P even though it was categorized as a natural pond and not a dam. An interesting similarity was the conclusions of the study by Olayemi et al. (2010) where it was concluded that *Culex* larvae prefer ponds as breeding habitats.

The fact that most larvae were found in site P while site N and S were zero-sites increases the significance of the difference in collected larvae between managed and unmanaged sites. It is remarkable that larvae were found more consequently among the managed sites even though the number of larvae was lower overall.

5.2 DIFFERENCES IN BREEDING HABITAT CHARACTERISTICS (2)

The first hypothesis regarding differences in habitat characteristics was that significant differences in pH, DO and turbidity were expected between managed and unmanaged waters. The hypothesis was rejected since phosphate was the only variable where a significant difference was obtained. The second hypothesis was about expected significant differences in pH, DO, conductivity and turbidity between mosquito sites and zero-sites. The hypothesis could thus be partly confirmed since significant differences were obtained for DO, conductivity and sulphate (Table 3). The reason why DO and pH were expected to be different between mosquito sites and zero-sites was mainly based on results of earlier studies (Minakawa et al., 1999; Olayemi et al., 2010, Tadesse, Mekonnen and Tsehaye, 2011). All of these studies concluded that DO and pH are the two most driving variables of mosquito larvae abundance.

5.3 DO AND pH AS DRIVING VARIABLES (3)

The hypothesis that the variables pH, DO, conductivity and turbidity were expected to be the most driving variables for the mosquito larvae presence was partly confirmed. The two variables pH and DO turned out to be the most common driving variables during the study. This result was supported by earlier studies by Minakawa et al. (1999), Olayemi et al. (2010) and Tadesse, Mekonnen and Tsehaye (2011). The DO levels in site S could also support this result since it was a zero-site, which had consequently low DO throughout the entire measurement period.

In this study most pH-values were between 6.5 and 7.5, which are normal values for natural water bodies. Site P was the only site that exceeded the limit value of 10.5 and the distinct clusters of algae at the water surface was believed to cause the rise in pH in the end of the measurement period (Figure 27). Two explanations for the drop in number of larvae in site P from the fourth to the final measurement occasion have been considered. First, it was possible that toxic effects, linked to formation of ammonia during high pH-values (>10.5) (Section 2.4.1), affected the number of mosquito larvae negatively. Secondly, it may be possible that most larvae completed the development into adult mosquitoes and that no additional larvae were introduced by the time of the last sampling occasion.

In site P algae were found in distinct clusters on the water surface and it was found that the pH values among the three pseudo-replicates varied according to the presence of algae on an utter most local scale. The fact that these replicates varied to that extent might result in a

misleading mean value, which has been used in the regression analysis. However, it is perhaps possible that this mean takes the algae presence into account.

5.4 VARIATIONS IN BREEDING HABITAT CHARACTERISTICS OVER TIME

A putative explanation of why significant associations were not obtained consequently among the five measurement occasions was the prevailing conditions of the area during the study. One such aspect was the potential climatic influences on breeding habitat characteristics. It was likely that the changing climate that included frequent and extensive rainfall affected all sites considerably. As the measurement period went, the rainfall gradually decreased and the expected response was a stabilization of biotic variables and characteristics in the sites. Another aspect that limited the possibility to draw general conclusions from the obtained results was that the timeframe of the study only covered a portion of one season. These limitations mean that associations obtained from the analysis only are valid for the area and specific conditions at the time of study.

There are probably other factors than climate that affected the site characteristics between different measurement occasions. Biological as well as chemical processes could have changed the relationship between the measured variables. The dynamics in aquatic systems are complex and many of the on-going processes interact or affect each other to some extent, directly or indirectly. It is difficult to draw relevant and accurate conclusions about the cause of variations due to the complexity of aquatic systems.

5.5 IMPACT ON MOSQUITO LARVAE PRESENCE

5.5.1 Vegetation types, coverage and shading

All sites had different types of vegetation growing in and around the water. Three of the sites, I, J, and N, lacked vegetation in the water, as well as trees and bushes in the surroundings which were tall or big enough to cause shading of the water surface. There were not many mosquito larvae found in these sites and moreover is site N one of the two zero-sites of this study. The low amount of larvae present in these sites is not very surprising due to the fact that mosquitoes tend to avoid breeding in open water; they prefer to stay rather close to vegetation and the shoreline.

The remaining seven sites all had some kind of vegetation growing directly in the water or in connection to it. All of the four sites where most larvae were collected (H, K, P, Q) had some kind of shading of the water surface. Shading was caused by either canopy coverage or from emergent plants. This result may not be surprising; according to Tadesse, Mekonnen and Tsehaye (2011) vegetation is an important factor that influences the mosquito larvae abundance. These four sites, with most larvae present, approximately provided the same amount of protection for the mosquito larvae in terms of hiding spots by existing vegetation. In site P and Q grass was growing in the water close to the shoreline. The rice plants in site H were a bit grass-like and could probably be considered having the same properties as grass when it comes to foliage and coverage of the water surface. Site K had a mixed variety of vegetation but most of the plants did not grow in the water, instead they were found on the sloping sides of the ditch.

There is however other sites that probably provide more protection for the larvae than the four discussed above. Site R and S are both sites with canopy coverage as well as vegetation growing in the water. In fact it seems as the coverage and shading in these cases might help to prevent presence of larvae since few larvae were found in site R, and no larvae were present in site S. Perhaps the most important purpose of vegetation is not to provide protection against predators but rather slowing down the water so that still or nearly still water appears.

Previous studies have shown that mosquito larvae presence is affected by the extent of shading caused by vegetation. According to Minakawa et al. (1999) there is a significant correlation between the presence of the larvae of the subfamily Culicinae and canopy coverage as well as the surface aquatic plant coverage. Fillinger et al. (2009) describes that mosquito larvae presence decreases as the vegetation shading of a habitat exceeds 25%, which perhaps could explain why barely any mosquito larvae were found in site O and R, both of which were subjected to extensive shading from trees in the surrounding area.

Fillinger et al. (2009) also described that the presence of *Anopheles* larvae were associated with the presence of emergent plants like grass or reed-like vegetation. Greenway, Dale and Chapman (2003) also received similar results. These types of plants may not cause as much coverage and shading compared to plants with larger foliage, but these plants could provide some protection for the larvae and also reduce the water movement. This could possibly explain why sites H, Q and P are among the most efficient breeding habitats in this study.

5.5.2 Presence of algae, microorganisms and other food sources

Several studies conclude that the presence of algae is affecting the mosquito larvae presence in different habitats (Minakawa, 1999; Fillinger et al., 2009; Mala and Irungu, 2011). Some sites in this study consistently held algae while others never had algae present, either floating at the water surface or growing on physical objects such as vegetation or rocks. Site Q and H stands out regarding their absence of algae even though mosquito larvae were found frequently throughout the measurement period. In site K and P, sites which frequently held larvae, algae were present during the same period. Site N and O are two examples of when algae are present but few or no mosquito larvae were found. In site N there were other factors that probably could explain the absence of larvae, such as the turbidity, the frequent and sometimes extensive number of predators, and perhaps sulphate high concentrations. The algal observations indicate that the presence of algae alone could not be the reason why some sites were effective breeding habitats while others were not.

Minakawa et al. (1999) points out that the algae coverage is partly dependent on the substrate type and the habitat itself. The study showed that rocks were more suitable for algal growth than clay or soil, and that ponds and other permanent habitats tend to be decent for especially algae. This may explain why algae were present in most of the unmanaged sites but barely in the managed sites in this study. The only managed site where algae were present at every measurement occasion was site K. However, this could maybe be explained by the substrate type, which contained rocks. The remaining managed sites had all mainly clay as substrate type.

In addition to algae, bacteria and other microorganisms are also important food sources for mosquito larvae (Tadesse, Mekonnen and Tsehaye, 2011). The presence of microorganisms are depending on temperature but also on the supply of degradable organic material (Minakawa et al., 1999). Speculations about microorganisms being a driving variable for mosquito larvae could be relevant but no association was found in this study between BOD, which is a measure of microorganism activity, and presence of larvae.

Another study has shown that mosquito larvae living in habitats with proximity to corn fields increases their tolerance to factors that normally would affect their survival negatively, such as water turbidity, and crowding with regard to other larvae (Ye-Ebiyo et al., 2003). According to Ye-Ebiyo et al. (2003) the study, mosquito larvae are only affected by such factors when the food sources are scarce. Two of the sites, J and K, were located in connection to cornfields but the result does not show any evidence of such relationship discussed above. However, none of the two had fairly turbid water and there were no crowding, which made it hard to include this aspect even though it is interesting. It appeared that one of these two sites were an effective breeding habitat for mosquito larvae while the other was not. One explanation could be that this study was not conducted during the pollen season.

5.5.3 Predators

Mosquito larvae were not the only insects to be found in the sites during this study. The two most common types of mosquito larvae predators were diving water beetles of the family Dytiscidae (Greenway, Dale and Chapman, 2003) and backswimmer bugs of the genera *Notonecta* (WHO, 1975a). These predators were found in almost every site but not necessarily at every measurement occasion, and in some sites insects were found more regularly than in others. Sites H, N, P, R and S were the ones where predators were most numerous and frequently found throughout the entire measurement period. Site H and P stands out from the other five in this matter due to the fact that these were two of the most efficient mosquito breeding habitats in this study even though predators were present. The remaining three sites mentioned above includes the only zero-sites (N, S) and also a site where mosquito larvae were only found at the very last measurement occasion (site R). One explanation might be the presence of numerous predators along with other possible factors. This discussion is somewhat controversial due to conflicts among previous studies. Fillinger et al. (2009) for example, concluded that anopheline larvae generally were found in habitats with high insect diversity whereas another study by Greenway, Dale and Chapman (2003) received the total opposite result concerning the presence of other insects and mosquito larvae. Minakawa et al. (1999) point out that predator might play an important role in terms of controlling species within *An. gambiae s.l.* in rice fields but this reasoning should perhaps also be valid for other genera and species of mosquitoes as well.

5.5.4 Altitude, climate change, and seasonal variations

A limiting factor that consistently may affect the conditions in the sites is the altitude they all are at. At least one previously study done say that the Ethiopian malaria border lays around 2000 m a.s.l., but is about to rise (Lindsay and Martens, 1998). In this study altitude could have a major impact on the sites due to the fact that they all lay at almost 2000 m a.s.l.. On the

other hand, since the limit is likely to rise as climate change with warmer climate brought to higher altitudes, the malaria border might have been moved higher than before. If this is the case in Bahir Dar, other factors as seasonal variations could limit the mosquito abundance (Lindsay and Martens, 1998). The seasonal variations with periods of heavily rainfall and drought have impact on larvae presence, with more larvae present after rainy season than throughout the dry ones. Since the rain was partly rather heavy during the beginning of the time series in this study, the water amounts and streaming might have prevented larval breeding.

5.6 ASPECTS INHIBITING LARVAL PRESENCE

5.6.1 Habitat rejection by female mosquitoes before oviposition

A comparison was made between the sites of this study based on their classification as well as characteristics and the breeding habitat preferences of the two most common *Anopheles* species in Ethiopia. *An. pharoensis* prefer natural swamps as breeding habitats (Kenea, Balkew and Gebre-Michael, 2011) and it was only site O that could be considered a swamp. However, only one mosquito larva was found during the measurement period and the site did apparently not meet the preferred habitat characteristics. According to Kenea, Balkew and Gebre-Michael (2011) *An. arabiensis* was found in highest density in sand pits. The only site that could possibly be classified as a sand pit was site N, one of the two zero-sites in this study. However, site N had except for the first measurement extreme high turbidity, together with high sulphate concentrations, which might explain the fact that no larvae were found there.

The characteristics of sites H and K where *Anopheles* larvae actually were found did also not meet the habitat preferences of either *An. pharoensis* or *An. arabiensis*. Both sites had relatively clear water, although the foggy water and lack of algae in site H and the flowing water in site K may have decreased their habitat suitability. Even though sites K and H were not natural swamps or sand pits these could possibly serve as sufficient breeding habitats for *Anopheles* species when no better option is available. It is also possible that external conditions of the study area were not suitable for *Anopheles* breeding at the time of the study.

5.6.2 Temperature

The climate of Bahr Dar may have limited the mosquito larvae development since night temperatures did generally not exceed 16 °C. Temperature is the most important factor that affects biological process rates which are involved in the aquatic phase of the mosquito development. If temperature falls below 16 °C, the development of anopheline species is inhibited (Lindsay and Martens, 1998). This might partly explain why only a few larvae of Anophelinae subfamily were collected in the study.

5.6.3 Distance to inhabited houses

Anthropophilic mosquito species prefer to breed close to human inhabited houses (Section 2.2.1). Earlier studies have concluded that there are significant negative associations between larval abundance of species within *An. gambiae s.l.* and the distance to the nearest house (Minakawa et al., 1999; Wanji et al., 2009; Kenea, Balkew and Gebre-Michael, 2011). However, no such association were found in this study. The distance to the nearest house was

less than 50 m for sites J, R and S while the distance was over 50 m for sites N and O but none of these sites was efficient breeding habitats (Table 2).

5.6.4 Coexistence between anopheline and culicine larvae

If external conditions and site characteristics were less favourable for larvae within the Anophelinae subfamily (Section 5.6.1-2) then coexistence with Culicinae larvae could have limited the abundance and survival even more (Kweka et al., 2012). According to Kweka et al. (2012) the species *An. gambiae s.s.* is not as competitive as *Cx. quinquefasciatus* during coexistence. Even though the experiments were done with two specific species within Anophelinae and Culicinae respectively analogue assumptions could probably be made for other mosquito species as well.

5.6.5 Flowing and non-stagnant water

The water in sites I, J and K were not stagnant and instead the water was flowing. Mosquitoes generally prefer stagnant waters for their oviposition (Section 2.2.1). The water flow and active control and management were therefore considered important factors explaining why site I and J were less efficient as mosquito breeding habitats. Site K was probably a more suitable breeding habitat because the control of the ditch was not active like sites I and J.

5.6.6 Exudation from vegetation

The mosquito larvae abundance in sites H, O, R and S has been speculated to be affected by exudation from surrounding vegetation. The rice in site H grew directly in the water which foggy appearance could maybe be due to exudations from the rice plants. Mosquito larvae were found consequently in the site throughout the measurement period, but less than expected.

Eucalyptus trees grew directly in the swamp of site O and close to site R. The water in site O also had a foggy appearance (similar to site H) and a distinct odour believed to be caused by exudations from the eucalyptus trees. The presence of eucalyptus trees has been considered one of the reasons for the low number of collected larvae in both sites O and R due to the canopy coverage as well as the putative exudation.

Site S was expected to provide larvae based on visual as well as chemical characteristics, but this site turned out to be a zero-site. The foggy look of the water in site S water might come from exudations from the papyrus grass that grew in the water. If papyrus grass generated a special odour this might have affected female mosquitoes to reject the site as a suitable breeding habitat. The speculation about papyrus grass exudations might be supported by Imbahale et al. (2011) who declare in a study about *Anopheles* mosquito larval abundance; “natural and undisturbed papyrus swamps were found unsuitable for anopheline breeding”.

5.6.7 Mosquito development and survival

Variations in the number of collected mosquito larvae from one sampling occasion to another may be consequences connected to mosquito development and survival. However, since either eggs or pupae were collected in the study was it difficult to determine whether variations were due to oviposition, completion of larval development or death. The larval stage lasts for about two to four days and the sampling occasions occurred once a week which

means it was possible that whole populations of mosquito larvae could have developed between two sampling occasions. It is also be possible that oviposition occurs consequently within a site creating an evenly distributed time series of the number of mosquito larvae.

5.7 OTHER OBSERVATIONS

One consistent but not foreseen association was found between turbidity and sulphate during the correlation coefficient analysis of this study. The correlation coefficients had all values above 0.93, which were considered strong correlations in this study. However, the reason or explanation for this covariance remains unclear but it seems most likely that it is not the dissolved sulphate ions themselves that affect turbidity but rather natural processes or chemical reactions linked to the formation or supply of the ion in the water. Reactions where dissociation of sulphate occurs at the same time as creation of precipitation, flocks or other particles, which remain suspended in the water column, might explain this result.

5.8 STATISTICAL ANALYSES

5.8.1 Group tests

The significant difference of sulphate between the groups of zero-sites and mosquito sites might be misleading due to the fact that there were only two sites included in the zero-site group and that one of them held the highest sulphate concentration. The high concentration of sulphate in site N (Appendix B) could have had a large impact on the outcome of the test. This effect is however applicable on all of the variables of this test, but sulphate is probably the most extreme.

Tied values can affect the outcome of the Wilcoxon test, some variables had many tied values spanning across the two groups. This has inevitable affected the sum of ranks which is the statistic of the group tests. The impact of ties varies depending on how many tied values are present from each group. If there is only one value from one of the groups and the rest is from the other, the sum of ranks will be more affected than the case of equal numbers of ties evenly distributed within respective group.

5.8.2 Regression analysis and driving variables

A statement was made that no transformations would be done on the data since not all residual plots showed non-normal distribution. All residuals are therefore approximated to be normal distributed although this approximation might be misleading. Log transformations could not be done due to zero-values. Neither could any of the obtained data be removed since the extremely high levels of some variables analysed generally followed a high trend throughout the time series. Deleting suspected outliers would change relationships drastically and the regression equations would be others. Because of deviating values, there might be resulting underestimations from the regression that exclude significant associations. The statistical analyses need further and deeper consideration by a more experienced analyst for more trustworthy and rigorous results.

The study of residuals in the regression analysis indicates a need of additional variables in the equation. A multiple regression method did not fit in the time duration for the study though, neither within the limitations. However, lack of data anyway inhibited the possibility to use

multiple regressions but in the analysis of the result multiple variables has still been considered.

No driving variable can obviously be totally confirmed based on the results obtained in the statistical analyses. The significant correlations are not consistently throughout the measurement period, which indicates influences on the choice of breeding habitat from other factors than the variables analysed in this study. However, it is probably a combination of additional factors together with those analysed that explains the choice of breeding habitat.

5.9 SOURCES OF ERROR

The time series was limited to consist only a period of five weeks due to lack of time, which resulted in five measurement occasions at each site. The duration is short in time and a proper sampling approach would have been to include all seasonal variations through en extend of the time series to at least one year. The time series in the study ended up covering only a short period of the end of the rainy season, and a short part of the start of the preceding dry period.

An ideal sampling strategy would have been to measure all sites synoptically, at the exact same time and day, which would have minimized the risk of misleading results due to changes in weather conditions, air temperature, and other factors. The time spent on each site varied from 30 minutes up to just over an hour depending on the amount of mosquitoes collected and how quickly the field measurement equipment stabilized. The measurement of all sites were performed in two days where site H, I, J, K, Q and P were measured one day and the remaining sites N, O, R and S were measured the subsequent day. The error appears when the data is used in the regression analysis where it is assumed that all sites from each measurement occasion have been measured the same day and time.

During the dipping performance when mosquito larvae were collected no regard was paid to where larvae actually were located. Only a visual judgement was done to determine the sampling area before the dipping. Investigation of where the larvae actually were seated in the habitat should have been done to surely collect mosquitoes from a representable area in the habitat of interest.

Depending on the person in charge of the measurement tools, different result could have been obtained. The tools often took time to stabilize, and since the measurement position was not ergonomic appropriate the waiting time could differ between both person and place. Sometimes the water in the sites was not easily accessible, which made it difficult to measure direct into the water and the stabilizing time was sometime shorten too much. Results would have been more reliable if the tools were surely stabilized before reading. A better way to perform the measurements would have been to use a tripod for the tools to stabilize without having ergonomic related problems hindering.

In the beginning of the mosquito sampling it was hard to identify and separate mosquito larvae from other aquatic invertebrates due to insufficient preparations and practice before starting the actual study. This source of error was however reduced as time went due to the increased larval sampling skill as a result of practical performance.

The comparisons with previous studies throughout the report are sometimes done with studies at different altitude as the one in this study. Since altitude is a factor impacting mosquitoes the differences in altitude has to be considered. Headmost is temperature the direct factor and altitude the indirect factor, but since temperature lower with higher altitudes mosquito abundance inhibits in highlands. Conditions in highlands and lowlands need thus to be distinguished for a proper comparison, even though some conclusions could be applied in both situations.

5.10 SUMMARY

It was determined in the study that DO and pH were the most frequent driving variables for mosquito larval abundance, followed by carbonate and nitrate. DO had the highest value of R^2 and the lowest value of p, which indicates DO as the variable most critical for the survival of larvae. Most mosquitoes were collected from the group unmanaged sites, but the explanation is the pond in site P, which held most larvae of all sites. Graphs of DO and pH together with larval presence for site P showed a co-variation that may confirm the importance of them to have proper levels. The reason why most mosquitoes were found in P is probably stagnant water, the presence of algae and suitable pH and DO values, preferable temperature and sufficient vegetation. Managed water constitute among others of ponds since they are common to use for irrigation. Tadesse, Mekonnen and Tsehaye (2011) declare that irrigation dams are preferable breeding sites for mosquitoes.

In site S, where no larvae were found at any of the measurement occasions despite expectations of larvae presence due to visual qualities, DO levels were relatively low through the entire measurement period. pH held values far below the limit value which confirms that pH was not preventing the larval presence. DO, on the other hand could be expected to be the abiotic variable preventing the larvae presence in the site. Even though for all sites pH and DO correlated at, at least, two measurement occasions ($r>0.9$ and $r>0.8$) they should both be considered as important factors in larval breeding since they could be critical at different occasions. Both S and P are unmanaged water bodies, which could be an indication of the unimportance of group membership.

The absence of mosquito larvae at several measurement occasions in a majority of the sites could have many different explanations. It is hard to establish the main factors inhibiting larvae abundance since it seems to be a complex combination of several factors. Some of the probable most important factors in Bahir Dar but also specifically in the analysed sites should be altitude, distance to inhabited houses, streaming water, exudation from papyrus grass and eucalyptus trees, and predators.

Conclusions about relationships between mosquitoes and biotic and abiotic factors should be drawn at a localized level since conditions vary both locally and between sites, sometimes significantly as with phosphate between the groups managed and unmanaged where both groups provided mosquitoes. Before starting the vector control with manipulation of explanatory variables careful research must be made in advance for the exception of unnecessary efforts or disruption.

Based on the results from this study recommendations could be done by applying precautionary due to lack of data, there should have been more replicates of sites analysed for a better explanation of the relationships found in the statistical analysis. However, implications could be drawn that resources should prior to others be put on manipulation of ponds. Even though most mosquitoes were found in the group unmanaged sites, there is not enough evidence to say that priority should be focused on unmanaged sites. Since site P is included in the unmanaged sites, all kind of ponds has to be in focus of the efforts put in larval control. The recommendation that all kind of ponds should be in focus of this sort of vector control support by several previous studies, e.g. Tadesse, Mekonen and Tsehaye (2011) and Minakawa et al. (1999).

An important aspect of the decisions about where to put efforts and resources has to consider the conditions in the area at the time for the vector control, in regard to other social aspects. Ethiopia is a country where a lot of people go hungry and a major famine problem is not too long in the past. Consideration about whether to put resources at food sources or disease vector control could be critical. With these perspectives unmanaged sites should probably have priority within the issue of vector control, to not adventure agriculture. By mistake failed agriculture would have even more direct and short-term consequences than those from mosquito borne diseases, even if both are severe, and a choice where to put sometimes few available resources nobody wants to make.

6 CONCLUSIONS

When a disease is of great significance to human settlements, and the most efficient breeding habitats are identified, a manipulation of the DO levels could be considered. It might be concluded that the importance of reducing the mosquito population exceeds the interest of being protective and considered of other aquatic organisms. If lowered DO levels could result in decreased human morbidity and mortality an oxygen reduction would be a solution where no chemicals are used.

However, if treatment of habitats with manipulation of this variable is considered, more thoroughly investigations of possible effects of the aquatic system must be performed. Control of biologic factors like the algae presence could be performed to limit the food sources of mosquito larvae. Introduction of predators are as well a possible measure to reduce the larvae abundance in breeding habitats. However, more research on the subject is required in order to be able to determine which predators should be introduced for a possible and efficient reduction of the mosquito larvae population.

Implications could be drawn that resources should be put on treatment of ponds, with no regard whether the pond is managed or unmanaged. Ponds are concluded to be the preferred habitat for mosquito oviposition both according to the result of this study and conclusions from previous studies. Within areas where agriculture is based on controlled irrigation dams, depending on local conditions, mosquito reducing efforts should probably be treatment or manipulation of unmanaged ponds to avoid jeopardizing crop yields. Small cultivations are

the common livelihood for families in Ethiopia and adequate priority and management regarding food production is of utmost importance in this country.

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APPENDIX A: COORDINATES

The altitude and coordinates of all sites.

Site	Altitude [m a.s.l.]	Position/coordinates N	E
H	1714	11°49.988'	037°37.503'
I	1793	11°49.728'	037°36.661'
J	1901	11°48.096'	037°34.208
K	1907	11°39.001'	037°27.701'
N	1742	11°35.624'	037°21.619'
O	1752	11°35.592'	037°21.570'
P	1723	11°44.250'	037°31.115'
Q	1797	11°49.963'	037°37.094'
R	1770	11°38.986'	037°27.735'
S	1735	11°35.965'	037°23.728'

APPENDIX B: DATA SET OF ALL MEASUREMENT OCCASIONS

Data set of all measurement occasions *X*01 to *X*05, for all sites and variables. The field variables pH, conductivity, DO and depth are presented as mean values of the three replicates.

Site	Date	pH	Cond	DO	Turbidity	BOD	Nitrate	Phosphate	Sulphate	Carbonate	Depth	Number
			[µS/cm]	[mg/l]	[FTU]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[m]	of larvae
H01	2013-09-25	6.25	86	-	53.00	13.30	4.40	0.21	23	0.00185	0.14	2
H02	2013-10-08	6.29	34	-	46.34	0.20	2.02	0.07	7	0.0046	0.15	0
H03	2013-10-15	6.17	13	5.52	22.39	5.65	7.44	0.15	12	0.0035	0.15	4
H04	2013-10-22	6.66	17	6.18	65.00	6.85	14.04	0.13	16	0.0085	0.14	3
H05	2013-10-29	6.86	22	5.96	30.93	16.90	8.05	0.18	17	0.02	0.13	20
I01	2013-09-25	7.74	205	-	23.30	1.75	4.14	0.21	9	0.21	0.99	1
I02	2013-10-08	7.32	86	-	161.00	6.35	36.30	0.09	7	0.05	0.13	0
I03	2013-10-15	7.24	94	3.75	161.00	3.65	33.66	0.14	25	0.089	0.18	0
I04	2013-10-22	7.79	173	5.09	99.00	13.00	14.52	0.15	10	0.37	0.13	0
I05	2013-10-29	7.96	152	5.05	155.00	8.25	20.90	0.72	34	0.98	0.11	1
J01	2013-09-25	7.89	299	-	22.69	10.95	41.58	0.50	6	0.40	0.10	2
J02	2013-10-08	7.46	228	-	21.50	6.65	38.94	0.09	0	0.14	0.11	0
J03	2013-10-15	7.76	226	5.56	15.90	4.85	29.70	0.27	9	0.46	0.10	0
J04	2013-10-22	9.03	255	10.48	9.83	6.85	24.86	0.11	4	6.67	0.03	1
J05	2013-10-29	8.71	210	8.89	142.00	4.30	23.10	0.22	14	4.13	0.06	0
K01	2013-09-25	7.35	247	-	13.34	2.25	2.51	0.12	4	0.084	0.11	1
K02	2013-10-09	7.01	225	5.41	7.37	4.70	1.50	0.19	7	0.10	0.08	4
K03	2013-10-15	7.02	200	5.30	2.50	5.25	1.62	1.85	3	0.073	0.11	10
K04	2013-10-22	7.10	241	5.49	6.64	8.25	9.33	0.18	5	0.12	0.07	18
K05	2013-10-29	7.15	197	3.72	85.00	1.10	6.38	0.14	10	0.14	0.09	12
N01	2013-09-30	7.56	34	-	66.00	0.60	2.97	0.62	46	0.21	0.08	0
N02	2013-10-09	8.42	36	7.60	756.00	7.60	39.38	1.30	126	5.90	0.03	0
N03	2013-10-16	7.10	34	5.01	687.00	9.00	24.20	1.90	330	0.41	0.04	0
N04	2013-10-23	8.21	36	6.53	684.00	12.90	6.47	1.90	190	4.95	0.06	0
N05	2013-10-30	7.89	56	6.48	737.00	10.60	11.75	2.25	200	2.82	0.08	0
O01	2013-09-30	6.96	242	-	16.30	16.80	4.36	1.50	3	0.043	0.20	0
O02	2013-10-09	6.96	150	2.76	85.00	0.70	4.40	3.20	37	0.12	0.16	0
O03	2013-10-16	7.38	123	3.76	59.00	2.20	32.34	4.80	35	0.28	0.13	1
O04	2013-10-23	7.53	143	5.95	187.00	18.25	20.11	2.41	66	0.60	0.07	0
O05	2013-10-30	-	-	-	-	-		-	-	-	-	-
P01	2013-10-04	9.49	108	-	29.32	7.89	4.07	0.80	16	11.00	0.05	1
P02	2013-10-09	6.61	202	6.74	10.59	0.75	4.44	0.13	2	0.028	0.17	21
P03	2013-10-15	8.59	172	10.91	36.90	6.85	17.34	0.83	6	2.58	0.11	21
P04	2013-10-22	10.51	215	13.40	46.68	14.15	9.86	0.10	3	6.64	0.14	38
P05	2013-10-29	10.65	154	13.76	36.74	11.15	9.50	0.85	14	26.36	0.08	6
Q01	2013-10-04	8.07	371	-	43.19	2.25	40.70	0.26	25	1.73	0.06	3
Q02	2013-10-08	7.12	78	-	109.00	2.35	3.74	0.12	13	0.05	0.08	2
Q03	2013-10-15	7.09	79	6.00	433.00	4.85	27.72	1.00	95	0.25	0.07	2
Q04	2013-10-22	6.99	150	5.25	72.00	8.35	17.25	0.03	27	0.04	0.05	4
Q05	2013-10-29	7.14	95	5.26	149.00	9.00	10.87	0.29	58	0.12	0.05	20
R01	2013-10-09	6.79	151	4.05	2.53	5.05	3.06	0.07	5	0.028	0.17	0
R02	2013-10-14	7.13	190	6.17	9.83	7.80	27.50	0.22	5	0.082	0.15	0
R03	2013-10-16	6.90	125	3.14	3.84	7.15	20.55	0.12	9	0.044	0.17	0
R04	2013-10-23	7.04	155	2.44	12.57	7.35	20.86	0.10	7	0.072	0.20	0
R05	2013-10-30	7.06	172	3.36	21.50	9.80	17.60	0.14	16	0.065	0.12	6
S01	2013-10-10	7.03	203	4.28	20.68	0.55	0.95	0.34	5	0.080	0.18	0
S02	2013-10-14	6.83	191	3.03	4.87	4.05	3.01	0.11	27	0.047	0.25	0
S03	2013-10-16	7.14	160	3.19	29.52	2.90	17.34	0.15	16	0.10	0.19	0
S04	2013-10-23	7.12	146	3.14	30.44	13.15	3.34	0.18	18	0.11	0.16	0
S05	2013-10-30	7.12	211	3.0	38.20	7.10	4.14	0.22	22	0.093	0.2	0

APPENDIX C: DATA SET SITE BY SITE

The data sets of the three replicates and mean values of the field variables pH, conductivity, DO and depth for each site separately.

SITE H		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	H01	1	6.31	82	-	0.05
Date:	2013-09-25	2	6.20	89	-	0.185
Time:	10:10	3	6.24	87	-	0.175
		Mean value	6.25	86	-	0.137
ID:	H02	1	6.66	44	-	0.13
Date:	2013-10-08	2	6.12	23	-	0.095
Time:	11:10	3	6.10	36	-	0.22
		Mean value	6.29	34	-	0.15
ID:	H03	1	6.27	13	5.56	0.21
Date:	2013-10-15	2	6.17	15	5.35	0.155
Time:	13:00	3	6.07	12	5.64	0.07
		Mean value	6.17	13	5.52	0.15
ID:	H04	1	6.92	20	6.13	0.1
Date:	2013-10-22	2	6.54	16	6.23	0.15
Time:	13:15	3	6.53	16	6.19	0.18
		Mean value	6.66	17	6.18	0.14
ID:	H05	1	7.05	21	5.39	0.16
Date:	2013-10-29	2	6.78	24	6.52	0.11
Time:	14:30	3	6.74	21	5.96	0.105
		Mean value	6.86	22	5.96	0.13

SITE I		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	I01	1	7.59	191	-	1.09
Date:	2013-09-25	2	7.77	210	-	0.64
Time:	11:10	3	7.85	214	-	1.23
		Mean value	7.74	205	-	0.99
ID:	I02	1	7.16	90	-	0.14
Date:	2013-10-08	2	7.33	94	-	0.11
Time:	12:30	3	7.48	75	-	0.13
		Mean value	7.32	86	-	0.13
ID:	I03	1	6.99	100	3.70	0.21
Date:	2013-10-15	2	7.25	85	3.90	0.195
Time:	12:30	3	7.48	97	3.65	0.13
		Mean value	7.24	94	3.75	0.18
ID:	I04	1	7.78	165	4.78	0.1
Date:	2013-10-22	2	7.83	164	4.98	0.195
Time:	12:45	3	7.75	191	5.52	0.08
		Mean value	7.79	173	5.09	0.13
ID:	I05	1	8.02	159	4.90	0.09
Date:	2013-10-29	2	7.98	136	5.15	0.155
Time:	13:15	3	7.88	160	5.11	0.075
		Mean value	7.96	152	5.05	0.11

SITE J		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	J01	1	7.80	306	-	0.10
Date:	2013-09-25	2	7.90	296	-	0.095
Time:	12:10	3	7.97	295	-	0.105
		Mean value	7.89	299	-	0.10
ID:	J02	1	7.64	226	-	0.11
Date:	2013-10-08	2	7.49	229	-	0.11
Time:	13:25	3	7.26	230	-	0.11
		Mean value	7.46	228	-	0.11
ID:	J03	1	7.79	230	5.55	0.10
Date:	2013-10-15	2	7.72	217	5.51	0.095
Time:	12:00	3	7.77	230	5.61	0.11
		Mean value	7.76	226	5.56	0.10
ID:	J04	1	8.99	249	9.94	0.03
Date:	2013-10-22	2	8.99	252	10.39	0.05
Time:	12:15	3	9.12	264	11.10	0.02
		Mean value	9.03	255	10.48	0.03
ID:	J05	1	8.83	216	10.29	0.06
Date:	2013-10-29	2	8.76	217	8.50	0.075
Time:	12:30	3	8.55	198	7.89	0.055
		Mean value	8.71	210	8.89	0.06

SITE K		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	K01	1	7.33	239	-	0.075
Date:	2013-09-25	2	7.33	252	-	0.16
Time:	13:00	3	7.38	251	-	0.095
		Mean value	7.35	247	-	0.11
ID:	K02	1	7.00	214	5.21	0.075
Date:	2013-10-09	2	7.03	224	5.41	0.075
Time:	11:00	3	7.01	237	5.60	0.08
		Mean value	7.01	225	5.41	0.077
ID:	K03	1	7.00	199	5.02	0.1
Date:	2013-10-15	2	7.03	200	4.86	0.14
Time:	10:45	3	7.04	201	6.02	0.095
		Mean value	7.02	200	5.30	0.112
ID:	K04	1	7.00	233	4.69	0.07
Date:	2013-10-22	2	7.07	243	4.54	0.115
Time:	10:20	3	7.22	248	7.23	0.02
		Mean value	7.10	241	5.49	0.07
ID:	K05	1	7.05	172	3.78	0.06
Date:	2013-10-29	2	7.24	215	3.72	0.11
Time:	10:30	3	7.16	205	3.66	0.11
		Mean value	7.15	197	3.72	0.09

SITE N		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	N01	1	7.66	35	-	0.1
Date:	2013-09-30	2	7.51	32	-	0.065
Time:	13:00	3	7.52	36	-	0.08
		Mean value	7.56	34	-	0.082
ID:	N02	1	8.07	27	7.00	0.02
Date:	2013-10-09	2	8.68	46	7.63	0.04
Time:	13:20	3	8.50	35	8.18	0.035
		Mean value	8.42	36	7.60	0.032
ID:	N03	1	7.12	35	4.40	0.045
Date:	2013-10-16	2	7.13	30	5.27	0.04
Time:	13:30	3	7.04	36	5.35	0.03
		Mean value	7.10	34	5.01	0.038
ID:	N04	1	8.55	35	6.35	0.075
Date:	2013-10-23	2	8.13	41	6.48	0.065
Time:	12:55	3	7.96	31	6.75	0.03
		Mean value	8.21	36	6.53	0.057
ID:	N05	1	7.86	51	6.46	0.085
Date:	2013-10-30	2	7.90	54	6.31	0.08
Time:	11:15	3	7.91	62	6.68	0.06
		Mean value	7.89	56	6.48	0.075

SITE O		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	O01	1	6.89	228	-	0.19
Date:	2013-09-30	2	7.00	249	-	0.335
Time:	13:30	3	7.00	248	-	0.06
		Mean value	6.96	242	-	0.195
ID:	O02	1	6.79	155	1.94	0.15
Date:	2013-10-09	2	7.09	146	2.60	0.27
Time:	13:50	3	7.00	150	3.74	0.07
		Mean value	6.96	150	2.76	0.16
ID:	O03	1	7.39	126	4.20	0.10
Date:	2013-10-16	2	7.45	117	4.47	0.19
Time:	14:00	3	7.29	125	2.62	0.09
		Mean value	7.38	123	3.76	0.13
ID:	O04	1	7.52	147	5.76	0.075
Date:	2013-10-23	2	7.54	135	6.64	0.065
Time:	13:30	3	7.52	147	5.45	0.06
		Mean value	7.53	143	5.95	0.07
ID:	O05	1	-	-	-	-
Date:	2013-10-30	2	-	-	-	-
Time:	-	3	-	-	-	-
		Mean value	-	-	-	-

SITE P		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	P01	1	10.07	97	-	0.06
Date:	2013-10-04	2	9.06	121	-	0.05
Time:	10:00	3	9.33	105	-	0.05
		Mean value	9.49	108	-	0.053
ID:	P02	1	6.64	193	6.30	0.165
Date:	2013-10-09	2	6.52	214	7.06	0.165
Time:	12:00	3	6.68	200	6.86	0.19
		Mean value	6.61	202	6.74	0.173
ID:	P03	1	9.08	161	11.83	0.15
Date:	2013-10-15	2	9.33	166	14.38	0.15
Time:	11:30	3	7.35	190	6.52	0.04
		Mean value	8.59	172	10.91	0.113
ID:	P04	1	10.43	193	13.58	0.18
Date:	2013-10-22	2	10.50	213	11.59	0.14
Time:	11:10	3	10.61	239	15.04	0.10
		Mean value	10.51	215	13.40	0.14
ID:	P05	1	10.70	163	15.36	0.105
Date:	2013-10-29	2	10.65	151	13.69	0.075
Time:	11:35	3	10.61	148	12.23	0.045
		Mean value	10.65	154	13.76	0.08

SITE Q		Replicate number	pH	Cond [µS/cm]	DO [mg/l]	Depth [m]
ID:	Q01	1	7.93	366	-	0.065
Date:	2013-10-04	2	8.19	385	-	0.05
Time:	11:00	3	8.08	361	-	0.075
		Mean value	8.07	371	-	0.063
ID:	Q02	1	7.01	80	-	0.08
Date:	2013-10-08	2	7.23	72	-	0.07
Time:	12:00	3	7.12	83	-	0.08
		Mean value	7.12	78	-	0.077
ID:	Q03	1	6.99	81	5.50	0.05
Date:	2013-10-15	2	7.09	81	6.50	0.07
Time:	13:30	3	7.20	76	6.01	0.08
		Mean value	7.09	79	6.00	0.067
ID:	Q04	1	6.96	146	5.35	0.04
Date:	2013-10-22	2	7.00	149	5.40	0.045
Time:	13:50	3	7.02	154	5.00	0.06
		Mean value	6.99	150	5.25	0.048
ID:	Q05	1	7.01	88	4.86	0.03
Date:	2013-10-29	2	7.17	98	5.52	0.06
Time:	14:55	3	7.24	99	5.4	0.07
		Mean value	7.14	95	5.26	0.053

SITE R		Replicate	pH	Cond	DO	Depth
		number		[µS/cm]	[mg/l]	[m]
ID:	R01	1	6.33	147	3.40	0.18
Date:	2013-10-09	2	7.02	153	3.97	0.135
Time:	15:30	3	7.02	154	4.78	0.18
		Mean value	6.79	151	4.05	0.165
ID:	R02	1	7.02	179	5.74	0.10
Date:	2013-10-14	2	7.13	194	6.35	0.13
Time:	14:00	3	7.23	197	6.43	0.21
		Mean value	7.13	190	6.17	0.147
ID:	R03	1	6.84	128	3.19	0.11
Date:	2013-10-16	2	6.96	121	3.33	0.195
Time:	13:00	3	6.91	126	2.90	0.19
		Mean value	6.90	125	3.14	0.165
ID:	R04	1	7.04	166	1.90	0.245
Date:	2013-10-23	2	7.05	149	2.40	0.195
Time:	12:00	3	7.04	151	3.02	0.15
		Mean value	7.04	155	2.44	0.197
ID:	R05	1	6.98	164	2.97	0.12
Date:	2013-10-30	2	7.10	174	3.72	0.09
Time:	10:20	3	7.11	177	3.39	0.15
		Mean value	7.06	172	3.36	0.120

SITE S		Replicate	pH	Cond	DO	Depth
		number		[µS/cm]	[mg/l]	[m]
ID:	S01	1	7.03	198	4.46	0.14
Date:	2013-10-10	2	7.07	198	4.44	0.25
Time:	13:25	3	7.00	214	3.95	0.155
		Mean value	7.03	203	4.28	0.182
ID:	S02	1	6.54	184	3.18	0.26
Date:	2013-10-14	2	6.94	191	3.33	0.22
Time:	14:40	3	7.02	198	2.58	0.265
		Mean value	6.83	191	3.03	0.248
ID:	S03	1	7.05	146	3.44	0.18
Date:	2013-10-16	2	7.19	172	3.24	0.27
Time:	11:50	3	7.18	162	2.88	0.13
		Mean value	7.14	160	3.19	0.193
ID:	S04	1	7.24	144	3.67	0.095
Date:	2013-10-23	2	7.11	147	2.94	0.25
Time:	11:10	3	7.02	148	2.82	0.13
		Mean value	7.12	146	3.14	0.158
ID:	S05	1	7.06	192	2.7	0.17
Date:	2013-10-30	2	7.16	212	3.61	0.16
Time:	09:40	3	7.14	228	2.73	0.165
		Mean value	7.12	211	3.01	0.165