

Water Quality and Ecological Assessment of Natural Wetlands in Southwest Ethiopia

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List of Abbreviations

APHA	American Public Health Association
ASPT	Average Score Per Taxon
AWWA	American Water Works Association
BOD	Biological Oxygen Demand
BMWP	Biological Monitoring Working Party
CART	Classification and regression tree models
CCA	Canonical Correspondence Analysis
CCI	Correctly Classified Instances
CI	Confidence Interval
COD	Chemical Oxygen demand.
DCA	Detrended Correspondence Analysis
DEM	Digital Elevation Model
DO	Dissolved Oxygen
DSE	Department of Sustainability and Environment
DWAF	Department of Water and Forestry
EOT	Ephemeroptera, Odonata, Trichoptera
EPT	Ephemeroptera, Plecoptera, Trichoptera
EFAP	Ethiopian Forestry Action programme
EWNRA	Ethio Wetlands and Natural Resources Association
FBI	Family Biotic Index
GLWD	Global lakes and wetlands database
IQ	Interquartile Range
IUC	Institutional University Cooperation
JU	Jimma University
MEA	Millennium Ecosystem Assessment
MOA	Ministry of Agriculture
MOH	Ministry of Health
MMI	Multimetric Macroinvertebrate Index

RDA	Redundancy Analysis
SWS	Society of Wetland Scientist
TDS	Total Dissolved Solids
TON	Total Organic Nitrogen
TP	Total Phosphorous
TSS	Total Suspended Solids
WHO	World Health Organization
WPCF	Water Pollution Control Facility
WWF	World Wildlife Fund
USEPA	United States Environmental Protection Agency

Chapter 1: General introduction

1.1 Definition of Wetlands

Wetlands have been defined in many different ways. However, no internationally agreed upon definition of wetlands exists due to the unclear boundary between the aquatic and terrestrial communities and due to the fact that wetland encompasses aquatic and terrestrial biomes interface, which is subjected to seasonal shifts (National Research Council, 1995). The most broad and universally accepted definition is that by the Ramsar convention, which defines wetlands as: “*areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters*” (Ramsar Convention Secretariat, 2013).

The three important characteristics that are associated with and used to constitute a wetland include hydrology, hydrophytic vegetation, and hydric soils (Braack et al., 2000). Water may enter the wetland directly as precipitation, surface flow, interflow (water flowing through the soil profile), groundwater (including deep and/or perched groundwater) or any combination of these (Williams, 1993). Storage of this water occurs in the channel, the basin and ground water table, which coexist with the hydric soils and create specific conditions suitable for growth and establishment of hydrophytic vegetation at least periodically (Denny, 1995; Schuyt and Brander, 2004). The hydrophytic vegetation refers to plants adapted to wet conditions and areas that are covered by water for at least part of the growing season (Bacon, 1997). A soil is considered hydric if it has been flooded or saturated with water long enough to become anaerobic. The hydric soils are volatile and are continually changing with decomposition of the vegetation and the erosion of sediment with river flow and flooding (Kotze, 1994).

1.2 Classification of wetlands

The purpose of wetland classification system is to define or describe the ecological units of a wetland that have certain homogenous natural attributes (Breen, 1988). The system can be used to assist in inventory and mapping, provide uniformity in wetland concepts and terminology and facilitate decisions about resource management (Cowardin et al., 1979;

Breen, 1988). There are a large number of wetland classification systems employed by many countries (Cowardin et al., 1979; Larson et al., 1989; Cowan and Riet, 1998). The most widely used classification system was developed in 1979 for the U.S. Fish and Wildlife Service (USFWS) by Cowardin et al. (1979). This classification approach categorizes wetlands into five major systems: marine, estuarine, lacustrine, riverine, and palustrine, which combine a variety of hydrologic, geomorphic, chemical and biological factors (Semeniuk, 1987). The USFWS classification system is hierarchical and includes several layers of detail for wetlands including a subsystem of water flow; classes of substrate types; subclasses of vegetation types and dominant species (Cowardin et al., 1979).

The Ramsar Convention classifies wetlands habitats into three main categories and these include: (1) marine/coastal wetlands; (2) inland wetlands; (3) man-made wetlands (Figure 1.1). The marine and coastal wetlands include estuaries, inter-tidal marshes, brackish, saline and freshwater lagoons, mangrove swamps, as well as coral reefs and rocky marine shores such as sea cliffs. Inland wetlands refer to such areas as lakes, rivers, streams and creeks, waterfalls, marshes, peat lands and flooded meadows. Lastly, man-made wetlands include canals, aquaculture ponds, water storage areas and wastewater treatment areas (MEA, 2005). Wetlands in Ethiopia are classified based on ecological zones, hydrologic functions, geomorphologic formations and climatic conditions. These categories interlink to form four major biomes. These biomes are the afro-tropical highlands, the Somali-Musai, the Sudan-Guinea and the Sahelian zone groups (Tilahun et al., 1996).

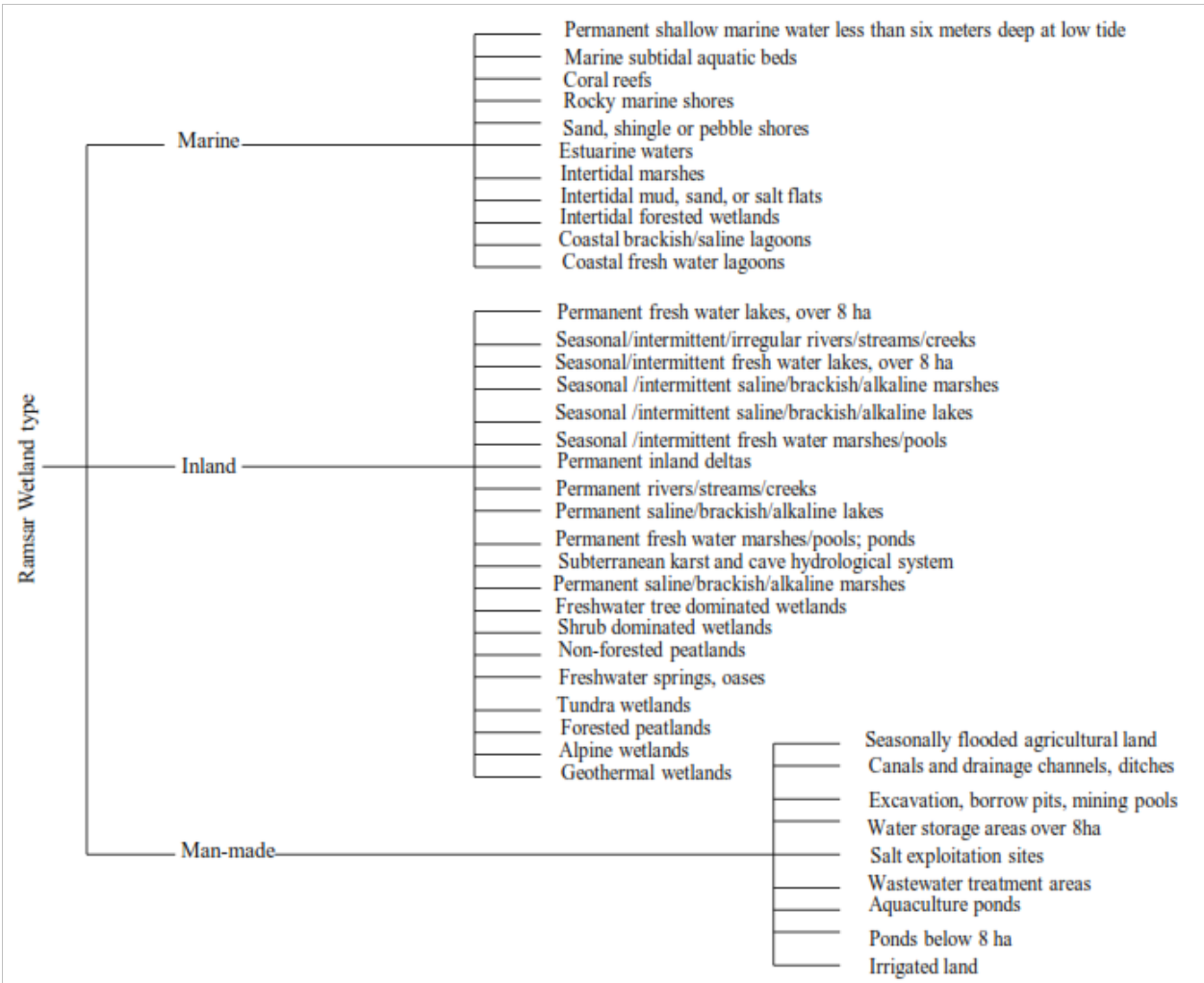


Figure 1.1 Ramsar Classification System for Wetland Type.

1.3 Distribution of wetlands

Wetlands are distributed all over the world and are estimated to cover 12.8 million km², approximately 6% of the land surface (Schuyt and Brander, 2004) (Table 1.1). This estimate includes inland and coastal wetlands (including lakes, rivers, and marshes), near-shore marine areas (to a depth of 6 meters below low tide), and human-made wetlands such as reservoirs and rice fields and was derived from multiple information sources (MEA, 2005). However, the global extent of wetlands is most likely underestimated, because of lack of concise definition agreed upon commonly by international parties, detailed national inventories and seasonality of some wetland habitats (seasonally inundated wetlands) (MEA, 2005).

Table 1.1 Estimates of global wetland area, with percentage area in parentheses for each of the six geopolitical regions used by the Ramsar Convention on Wetlands.

Region	Global Lakes and Wetlands database (Lehner and Döll, 2004) Million km ² (% area)	Global review of wetland resources (Finlayson et al., 1999) Million km ² (% area)
Africa	1.31 (14)	1.25 (10)
Asia	2.86 (32)	2.04 (16)
Europe	0.26 (3)	2.58 (20)
Neotropics	1.59 (17)	4.15 (32)
North America	2.87 (31)	2.42 (19)
Oceania	0.28 (3)	0.36 (3)
Total	9.17	12.8

According to Lehner and Döll (2004), African wetlands occupy 1.3 million Km², which is approximately 4% of the continent's land surface. This estimate is extracted from the Global lakes and wetlands database (GLWD) and includes inland wetlands associated with rivers and lakes such as the Congo River swamps, the Sudd in the upper Nile, the Lake Victoria basin, the Chad basin, the Okavango Delta, the Bangweulu swamps, the Lake Tanganyika basin, the Lake Malawi/Nyasa/Niassa basin, and the floodplains and deltas of the Niger and Zambezi rivers (Denny, 1995; Kansiime et al., 2007; Kaggwa et al., 2009) (Figure 1.2).

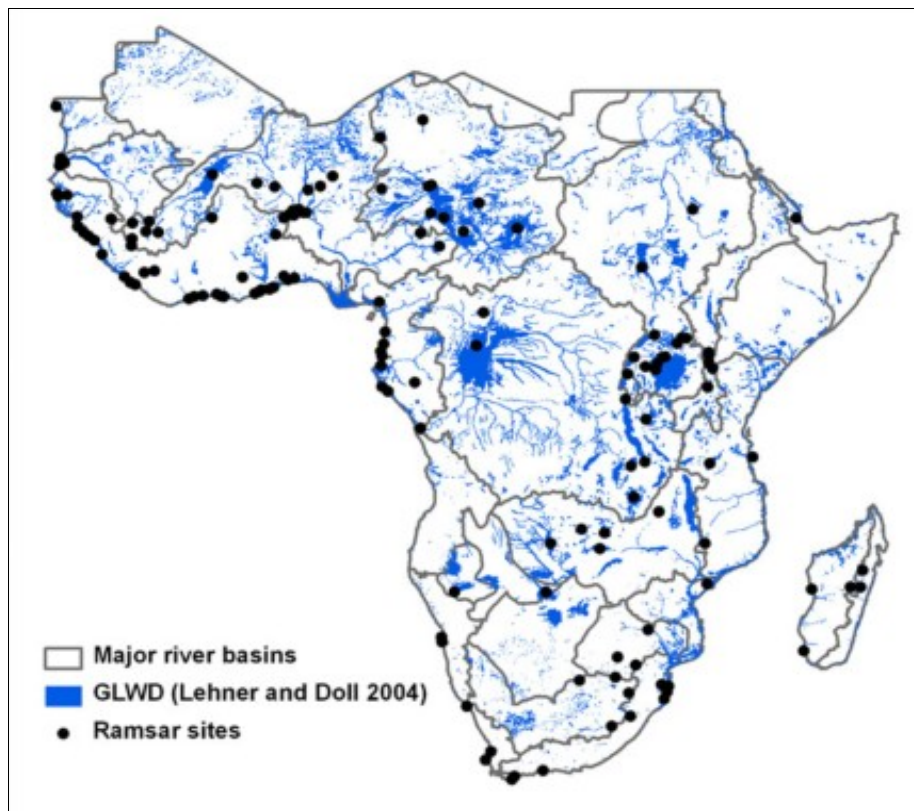


Figure 1.2. GLWD (Lehner and Döll, 2004). Wetland distribution and location of Ramsar wetland sites across major river basins in sub-Saharan Africa.

Ethiopia is endowed with abundant water resources and wetland ecosystems, including twelve river basins, eight major lakes, many swamps, floodplains and man-made reservoirs (Abunie, 2003). Studies estimated that about 110 billion cubic meters of water runs off annually from the above sources, of which 74% flows into rivers draining into Sudan, Egypt, Kenya and Somalia (EFAP, 1989). These water resources occur over a wide range of altitudes, from 125 meters below sea level in the Danakil depressions of the Afar region to 4,620 meters above sea level at Ras Dashen in the Amhara region (Abebe and Geheb, 2003). Despite a wide distribution across the country, inventory of wetland resources in Ethiopia is not complete. According to Hillman and Abebe (1993), Ethiopian wetlands occupy an area of 13,700 km², approximately 1.14% of the country's land surface. This estimate includes shallow lakes and the margin of the rift valley and other lakes, ponds, the floodplain of major rivers and many swamps (Abunie, 2003).

1.4 Functions and values of wetlands

Historically wetlands were designated as breeding places for disease vectors and as impediments to civilization (Day et al., 2006). However, wetlands are now well recognized for their ecological functions and services they provide to human society (Dugan, 1990; Dixon and Wood, 2007). Wetlands perform a wide variety of ecological functions including provisioning of habitat for wildlife, purification of catchment surface water, floodwater attenuation, groundwater recharge, climate regulation and erosion control (Hey and Philippi, 1995; Costanza et al., 1997; Bunn et al., 1999; Mitsch and Gosselink, 2000; Adhikari and Bajracharaya, 2009; Jacobs et al., 2009). Furthermore, wetlands play a vital role in providing a wide range of ecosystem services for millions of people mainly living in developing countries (Shewaye, 2008; Teferi et al., 2010).

Wetlands are important in biogeochemical cycling, involves the biological, physical, and chemical transformations of various nutrients within the biota, soils, water, and air (Yang et al., 2008). Wetlands are very important in this regard, particularly relating to nitrogen, phosphorous and carbon. Nitrogen transformations in wetlands are complex due to the multiple oxidation states of nitrogen molecule (Davidsson and Mattias, 2000; Bohlen and Gathumbi, 2007; Vymazal, 2007). The major transformations include mineralization of organic nitrogen, ammonia volatilization, nitrification, nitrogen fixation, plant uptake, denitrification, anaerobic ammonia oxidation, fragmentation, sorption, desorption, burial and leaching (Davidsson and Mattias, 2000; Vymazal, 2007). Mineralization of organic nitrogen in sediments provides the major source of nitrogen to wetland plants and is responsible for the high rates of productivity of many wetlands ecosystems (Bohlen and Gathumbi, 2007). Phosphorous has no significant atmospheric flux and has a much longer temporal biogeochemical cycle than nitrogen (White et al., 2000). Slow water flow through a wetland is essential for settling of particulate phosphorous (Van der Valk et al., 1978).

Wetlands are one of the most effective ecosystems for storing soil carbon (Schlensinger, 1997). It has been estimated that different kinds of wetlands contain 20-25% of world's organic soil carbon (Gorham, 1998). The mechanisms by which carbon store in wetland ecosystem include photosynthesis, wetland trees and other plants convert atmospheric carbon dioxide into biomass. Carbon may be temporarily stored in wetlands as trees and plants and

the living material which feed upon them, and detritus including fallen plants and animals which feed upon them. Many wetland plants are known to use atmospheric carbon dioxide for their main carbon source, and their death/decay and ultimate settlement at a wetland bottom can have profound effect on carbon sequestration (Adhilari et al., 2009).

Wetland macrophytes are effective sediment traps, generally intercepting and retaining suspended sediments (Fennessy et al., 1994; Christopher and David, 2004). Sedimentation helps not only to improve water quality, but also retain many toxic substances such as pesticides through sorption processes (Clausen and Johnson, 1990; Cooper et al., 2000; Noe and Hupp, 2009). Furthermore, wetlands play a vital role in retaining and sequestering large amounts of organic matter in the soil, representing a significant terrestrial carbon pool and playing an important role in global carbon cycles and climate change. For example, peatlands, which occupy only 3–4% of the world's land area, are estimated to hold 540 gigatons of carbon, representing about 1.5% of the total estimated global carbon storage (MEA, 2005).

Wetlands provide water and nutrients upon which species of mammals, birds, fish, amphibians, invertebrates, microbial and plant species are depend on (Schuyt and Brander, 2004; MEA, 2005). It has been estimated that freshwater wetlands harbouor more than 40% of world's species and 12% of all animal species (Ramsar, 2002). The Congo river basin, probably the most diverse area in Africa in terms of fishes, has over 700 identified species of which 560 are endemic to the basin (Ramsar, 2000). Many wetlands are renowned because of their diversity of birds. Around 12% of all African bird species are found in and around wetlands (Mafabi, 1995). In Ethiopia, 204 bird species (around 25% of all bird species) are wetland-dependent (Wondafrash, 2003). Over 140 bird species are found in wetlands surrounding Jimma area (Mereta et al., unpubl. data). The Wattled Crane (*Bugeranus carunculatus* Gmelin, 1789), which is included in the IUCN red list as vulnerable, and two endemic species (Wattled ibis, *Bostrychia carunculata* Rüppel, 1837 and Rouget's Rail, *Rougetius rougetii* Guérin-Méneville, 1843) have breeding grounds in these wetlands.

The economic value of goods and services provided by wetlands are not well quantified because many wetland functions do not have a market price. According to Costanza et al. (1997), the global economic value of wetlands is estimated to be \$4.9 trillion. The valuation

of wetland services were conducted based on non-market valuation techniques, since many wetland services such as habitat for species, protection against erosion, water purification, amenities and recreational opportunities have no market prices (Table 1.2).

Table 1.2. Ecosystem services and functions used to value of the world's ecosystem (Costanza et al., 1997).

Ecosystem services	Ecosystem functions	Technique(s) typically used to quantify the values of the service(s)
Gas regulation	Regulation of atmospheric chemical composition	Replacement cost
Climate regulation	Regulation of global temperature, precipitation and other biologically mediated climatic processes at global or local levels	Replacement cost
Disturbance regulation	Capacitance, dumping and integrity of ecosystem response to environmental fluctuations	Avoided cost or Replacement cost
Water regulation	Regulation of hydrological flows	Factor income or Replacement cost
Water supply	Storage and retention of water	Factor income
Erosion control and sediment retention	Retention of soil within an ecosystem	Avoided cost or Replacement cost
Soil formation	Soil formation processes	Replacement cost
Nutrient cycling	Storage, internal cycling, processing and acquisition of nutrients	Replacement cost

Waste treatment	Recovery of mobile nutrients and removal or breakdown of excess nutrients or compounds	Replacement cost
Pollination	Movement of flora gametes	Replacement cost
Biological control	Trophic-dynamic regulation of population	Replacement cost
Refugia	Habitat for resident and transient populations	Factor income, Replacement cost, Contingent valuation
Food production	That portion of primary production extractable as food	Factor income
Raw materials	That portion of primary production extractable as raw materials	Net factor income or Replacement cost
Genetic resources	Sources of unique biological materials and products	
Recreation	Providing opportunities for recreational activities	Travel cost
Cultural	Providing opportunities for non-commercial uses	Hedonic pricing

However, the precision and accuracy of Costanza et al. (1997) calculation was heavily criticized, since no distinctions were made between economic values of wetlands in different geographical regions and values of different wetland types or values of different wetland goods and services (Schuyt and Brader, 2004). On the other hand, Schuyt and Brader (2004) estimated that the total economic value of the world's wetlands is in the order of \$70 billion per year. This estimation is based on extrapolation of data collected from 89 wetland sites, occupying 63 million hectares in all the continents except Antarctica (Table 1.3).

Table 1.3. Total Economic Value of Global Wetlands by Continent and Wetland Type (Thousands of US\$ per year, 2000, Schuyt and Brader, 2004).

	Mangrove	Unvegetated sediment	Salt/Brackish Marsh	Freshwater Marsh	Freshwater woodland	Total
North America	30,014	550,980	29,810	1,728	64,315	676,846
Latin America	8,445	104,782	3,129	531	6,125	123,012
Europe	0	268,333	12,051	253	19,503	300,141
Asia	27,519	1,617,518	23,806	29	149,597	1,818,534
Africa	84,994	159,118	2,466	334	9,775	256,687
Australia	34,696	147,779	2,120	960	83,907	269,462
Total	185,667	2,848,575	73,382	3,836	333,223	3,444,682

1.5 Threats to Wetlands

Wetlands all over the world are under heavy pressure, in spite of their critical role in providing social, economic and ecological benefits (Dahl, 1990; Wolfson et al., 2002; Finlayson and D’Cruz, 2005). The Millennium Ecosystem Assessment (MEA) declared that the degradation and loss of wetlands globally is more rapid than those of any other ecosystem (MEA, 2005). A conservative estimate indicates that approximately 50% of the world’s wetlands have been lost in the last century due to rapid expansion in human population and urbanization, demanding increased resources (Shine and Klemm, 1999). The loss and degradation of wetlands has been driven by expansion of human settlement, irrigation agriculture, water withdrawal, industrial pollution, overexploitation and introduction of invasive alien species (MEA, 2005; McCartney et al., 2010).

High population growth, degradation of upland fields and prolonged drought as a result of climate change triggers wetland agriculture in many developing countries (McCartney et al, 2010). Intensification of agriculture with chemical fertilizers, pesticides, irrigation and mechanization has undoubtedly caused adverse effects to wetland ecosystems and has

profound social and economic effects for people dependent on wetland ecosystem services (MEA, 2005).

Drainage for agriculture recognized as the primary cause of global wetland loss (Xu et al., 2010). The agricultural benefits from drained wetlands are often difficult to sustain (Wood, 2001). Wetland soils may lose their fertility after drainage because of oxidation, acidification and other processes that take place once the anaerobic conditions are removed (Wood, 2001). Furthermore, in heavily agricultural areas, riparian transport has been shown to contribute to a large input of sediment to riparian wetlands, which contributes to the degradation of downstream water quality (Heimann and Roell, 2000).

Wetlands modification through deforestation and drainage farming creates stagnant pools of water and allows more sunlight to reach water surfaces, which is ideal breeding ground for anopheles mosquito, principal vector of malaria (Vittor et al., 2006; Blumenfeld et al, 2009). Studies have shown that an increase in human-biting rates of the anopheles mosquito related to deforestation (Vittor et al., 2006). Furthermore, habitat fragmentation has a detrimental impact on both native flora and fauna causing changes in species distribution and abundances and ecosystem functions (Yimer and Mengistu, 2009). Croonquist and Brooks (1993) reported a 50% decrease in the number of neotropical migratory birds in riparian wetland habitats located in disturbed watersheds compared with those in undisturbed landscapes.

In Ethiopia, wetlands are often perceived as impediments to development and progress or as productive lands suitable for agriculture. The government of Ethiopia has been encouraging farmers to cultivate wetlands in order to compensate for more drought-induced food shortages (Dixon and Wood, 2003a). The Rural Agricultural Development Department of the MOA also developed its own programmes for draining some of the larger wetlands for agriculture (Wood, 2000). In Southwest Ethiopia, in the Illubabour zone for example, the area of wetlands converted to agricultural land increased from 28% in 2003 to 66% in 2006 (Legesse, 2007). Similarly, several microfinance initiative groups established in several towns to cultivate peri-urban wetlands and making bricks in the wetlands. Consequently, several wetlands in Ethiopia, either disappeared or are on the verge of drying out (Shewaye, 2008), while others rapidly decline in water quality (Mereta et al., 2012).

The impact of wetland loss on biodiversity was verified by the decline of populations of several wetland-dependent species (MEA, 2005). The rapid degradation of wetlands and the insufficient status of scientific knowledge on patterns of species richness in such systems bring the urgent need for ecological studies to provide scientific support to management and conservation programs of biodiversity.

1.6 Wetland inventory, monitoring and assessment

There are three steps to evaluate the condition of wetlands. These include wetland inventory, monitoring and assessment (Finlayson et al., 2002) (Figure 1.3). Wetland inventory is the collection of data that provides managers and/or policy makers with the information that they require not only to manage individual wetlands, but also to undertake conservation actions (Dugan, 1990; Scott and Jones, 1995; Finlayson and Spiers, 1999). Wetland inventory helps to identify the distribution and status of wetlands. Moreover, inventory can also provide information on the distribution of various taxa inhabiting these wetlands, on natural resources, on the functions and values of each wetland, which can be used as a baseline for specific assessment and monitoring activities (Finlayson et al., 2001; DSE, 2007).

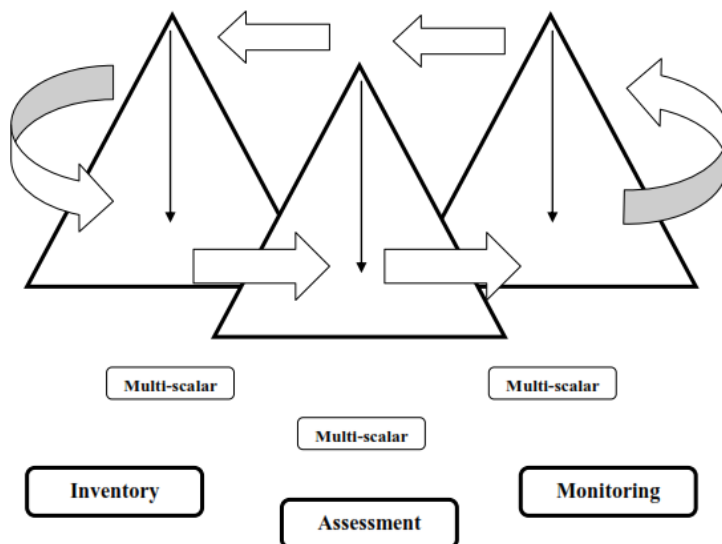


Figure 1.3. Framework for wetland inventory, assessment and monitoring proposed by Finlayson and Lukacs (2003).

Monitoring includes the assessment of how, and to what extent, the ecological character of a wetland has been changed, and is therefore dependent on having baseline inventory data (Finlayson and Mitchell, 1999; Finlayson et al., 2002; DWAF 2004; Cole, 2006). The purpose of monitoring must be clearly stated, and the variables used for monitoring must respond in some way to the proposed changing influences (Yen and Butcher, 1997). Monitoring programs should be designed in order to assess wetland condition with statistical rigor and at the same time ensuring that adequate information is collected to allow management decisions (Butcher, 2003). Wetland assessment is the identification of the status of, and threats to, wetlands as a basis for the collection of more specific information through monitoring activities (Finlayson et al., 2002). Unlike rivers and lakes, wetland assessment techniques are not well researched (Rader and Shiozawa, 2001; Brooks et al., 2004). The USEPA recognizes two broad approaches to wetland assessment: biological assessment and functional assessment (USEPA, 1998a).

Wetland functional assessments are tools specifically developed to evaluate wetland functions and predict potential changes to a wetland's functions that may result from proposed activities. The approach is based on combining variables that are typically structural measures or indicators that are associated with one or more ecosystem functions. Functions normally fall into one of three major categories: (1) hydrogeomorphic (HGM) (2) biogeochemical and (3) physical habitat (USEPA, 1998b). The HGM approach includes consideration of the landscape (geomorphic setting), hydrology (water dynamics) and the use of reference sites and condition against which to benchmark monitoring programs (Butcher, 2003). HGM uses the concept of functional indices composed of different combinations of physical and biological indicators that can be quantified on a scale developed from reference wetlands to evaluate wetland functions. On the other hand, a fundamental understanding of the biogeochemical processes regulating the functions of the ecosystem is critical to evaluating nutrient impacts and successes of restoration efforts. Biogeochemical processes are also likely to be highly reliable indices in the sense that ecological changes at such a fundamental level will affect all species utilizing the ecosystem. Furthermore, relationships between indicators and processes may provide a more reliable estimate of ecosystem health for assessment at a landscape level (USEPA, 2008)

Biological assessment provides information about the condition of wetlands and determines whether these wetlands are maintaining biological integrity (DWAF, 2004). The biological assemblages living in a wetland reflect the cumulative effects of multiple stressors (Gerritsen, 1995; Rader et al., 2001; Chipps et al., 2006). In general, the diversity and species composition of these biological assemblages often decreases in disturbed systems and may be dominated by few tolerant taxa (USEPA, 2002a). In pristine or minimally disturbed sites, the proportion of sensitive taxa increases while the proportion of tolerant taxa decreases (Figure 1.4).

Biological assemblages employed in wetland assessment include macrophytes (e.g. USEPA, 1998a; DeKeyser, 2000; Cronk and Fennessy, 2001; DeKeyser, et al 2003; Mack, 2004; Reiss, 2006; Hargiss et al., 2008), macroinvertebrates (e.g. Adamus and Brandt, 1990; Butcher, 2003; Chessman et al., 2002; USEPA, 2002b; DWAF, 2004), birds (e.g. USEPA, 2002c; Mistry et al., 2008; Petersen and Westmark, 2013), fish (e.g. Galastowitsch et al., 1998; Schulz et al., 1999; Bhagat et al., 2007), amphibians (e.g. Pollet and Bendell-Young, 2000; Adamus et al., 2001; Hecnar, 2004, Brazner et al., 2007). Among these biological assemblages, macrophytes and macroinvertebrate are the most commonly used indicators in wetland health assessment.

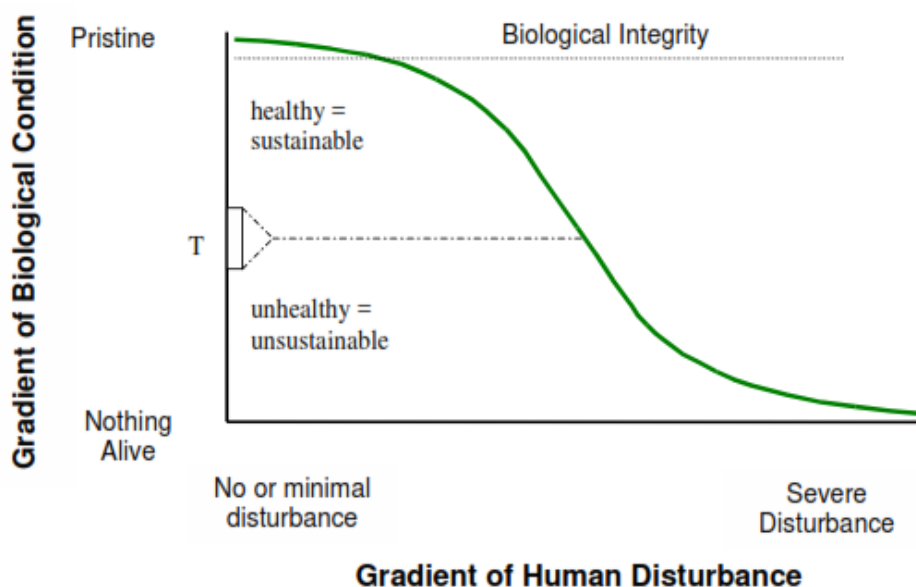


Figure 1.4. Continuum of human disturbance on biological condition of wetlands (Adapted from Karr, 2000).

Macrophytes are aquatic plants, growing in or near water that can be emergent, submergent, or floating (USEPA, 1998a, Madsen, 2001). They are regarded as the most popular biotic assemblage for use in wetland bioassessment worldwide, since their spatial distribution in the landscape results from a multitude of factors, including substrate type, water chemistry, and hydroperiod, as well as climatic conditions (Bedford, 1996; Reiss, 2006). The macrophyte assemblage plays a vital role in supporting the structure and function of wetlands by providing food and habitat for other assemblages including algae, macroinvertebrates, fish, amphibians, reptiles, birds, and mammals (Bacon, 1997; Cronk and Fennessy, 2001).

Macroinvertebrates are regarded as the second most useful group for wetland bioassessment worldwide, next to vascular plants (Adamus and Brandt, 1990; Butcher, 2003; DWAF, 2004). Macroinvertebrates play an important role in the overall functioning of wetland ecosystems as they occupy a central position in the food web of wetlands (Batzer et al., 1999). Macroinvertebrates have been used successfully as biotic indicators of wetland condition and health (King and Richardson, 2002). However, some studies indicate that the ecology, toxicity tolerance and pollution sensitivity is considerably less understood for wetland invertebrates than of their river counterparts (Chessman et al., 2002; Bonada et al., 2006). Two main approaches have been applied in ecological assessment of wetlands using macroinvertebrates: the multivariate approach, which describes patterns and relationships between macroinvertebrate communities and the environment (Hawkins et al., 2000; Clarke et al., 2003) and multimetric approach, which describes the state of an ecosystem by means of a combination of several individual metrics (Karr and Chu, 1999; Ofenböck et al., 2004; Applegate et al., 2007).

Multivariate approaches involve statistical analysis of an array of environmental variables together with biotic data. These methods aim to identify the type of environmental variables or impacts that best explain variations in the abundance/composition of biotic data. Gradients of disturbance are often revealed by ordination (Davis et al., 1999). The relationship between biota and environmental factors can be analyzed by e.g. principal component analysis (Lencioni et al., 2007), artificial neural networks (Olden et al., 2004), multilayer perceptron with backward–forward propagation algorithm (MLP) (Gevrey et al., 2004), multiple linear regression (Lencioni et al., 2007) and self-organizing maps (Giraudel and Lek, 2001).

A multimetric index was first used to assess biological integrity of fish communities in Illinois streams (Karr, 1981; Karr et al. 1986). A multimetric index integrates different individual biological measures into a single value that can potentially reflect multiple effects of human impact on the structure and function of aquatic ecosystems (Barbour et al., 1995; Menetrey et al., 2011). The development of a multimetric index often requires the comparison of biological metrics between impaired and reference sites (Bates-prins and Smith, 2007). Multimetric indices simplify more complex biological data and yield policy relevant information for regulatory agencies and decision makers (Karr and Chu, 1999) and hence, they become a popular tool for regional assessment of aquatic resources in Europe (Hering et al., 2006) and the United States (Stoddard et al., 2008). However, multimetric indices developed for one region may not work for another due to local peculiarities in reference conditions, anthropogenic pressures and regional species pools.

1.7 Wetlands management and conservation

In spite of its critical role in providing countless ecological and socio-economic benefits to humans, wetlands all over the world are under heavy pressure (Wolfson et al., 2002; Finlayson and D’Cruz, 2005). As a result, half of the world’s wetlands were lost during the twentieth century (Shine and Klemm, 1999). This loss and degradation asks for an urgent need for cooperation for the conservation and wise use of wetlands and their resources. In this regard, the convention on wetlands of international importance, called the Ramsar convention is a major turning point in wetland conservation. The Convention on Wetlands (Ramsar 1971) is an intergovernmental treaty whose mission is *“the conservation and wise use of all wetlands through local, regional and national actions and international cooperation, as a contribution towards achieving sustainable development throughout the world”*. The treaty was signed by 162 nations, until July 2012. More than 2040 wetlands around the world, covering over 193 million hectares, have been designated for inclusion in the Ramsar list of wetlands of international importance (Ramsar, 2012).

Ethiopia has not yet ratified the Ramsar Convention on wetlands and, therefore, none of the numerous wetlands in the country is designated in the list of wetlands of international importance. Regardless of their vital role in food security and rural livelihood, the extent, diversity, distribution and conservation status of wetlands in Ethiopia is not well documented.

Furthermore, there are no clear policies and strategies that protect wetlands in the country. In fact, wetland related issues are included in Ethiopian water resources, agricultural and environmental policies. However, the implementation of wetland management and conservation in the context of the above policies is compounded by a more pressing wetland task force, extension package and food security policies that may seek to convert wetlands for agricultural purposes (Hailu, 2001).

1.8 Research gaps and objectives

The growing awareness about the adverse ecological, social and economic impacts of the unwise use of wetlands has fostered the development of tools to understand the extent, diversity and distribution of wetlands. Consequently, several monitoring and assessment tools are being developed and used by developed nations for the management and conservation of these resources (Armitage, et al., 1983, Barbour et al., 1995, Karr and Chu, 1999). However, there is a lack of information on the use of these tools and eventually a lack of management decisions in developing countries. Thus in the present work, the most important environmental factors that are useful for community structure of wetland communities were identified and a multimetric index based on macroinvertebrates to assess the ecological condition of wetlands in Southwest Ethiopia was developed. The specific objectives were:

1. To analyse the relationship between habitat and water quality of wetlands and the occurrence and diversity of macroinvertebrates.
2. To assess the effect of abiotic and biotic environmental factors on the abundance and distribution of anopheline mosquito larvae in order to determine their preferred habitat.
3. To determine the sediment and nutrient removal capacity of natural wetlands using a mass balance approach, and to identify the factors, that influence sediment and nutrient retention capacity of wetlands.
4. To develop and test a multimetric index based on macroinvertebrates and to test the capacity of this index to assess a wetlands ecological condition.

1.9 Organization of the chapters

The thesis is organized in six chapters. **Chapter 1** gives a general background about what wetlands are and how they can be classified. The chapter further explains wetlands global distribution and its role in providing ecological, social and economic benefits to humans. The major threats that have resulted in widespread loss and degradation of wetlands, the use of several monitoring and assessment techniques to evaluate the ecological integrity of wetlands, and management needs are discussed.

Chapter 2 deals with the relationship between habitat quality and the occurrence and diversity of macroinvertebrates in wetlands of Southwest Ethiopia. This approach allows informing decision makers to identify the most important environmental factors that are structuring the macroinvertebrate community and secondly provides a guideline for habitat conservation of wetlands and their related ecosystem services.

In **Chapter 3** habitat suitability models for anopheles mosquito larvae, (the vector of malaria) are developed in order to assess the effect of abiotic and biotic environmental factors on the abundance and distribution. This can allow decision makers to identify priority habitats for the control of anopheline larvae. We also specifically addressed the question whether permanent marshlands in the neighbourhood of Jimma, which are biodiverse areas that are under serious threat by land encroachment and are by the general public perceived as mosquito breeding grounds, are indeed a preferred habitat for mosquito larvae.

Chapter 4 addresses the research question whether natural wetlands in Southwest Ethiopia, which are embedded by agricultural and urban land use, are important for the retention of sediment and nutrients. The influence of habitat disturbance on sediment and nutrient retention potential of wetlands is evaluated. In addition, the role of wetland's vegetation on sediment and nutrients trapping is discussed.

Chapter 5 describes the development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia. This approach integrates information from the various features of a community to give an overall

classification of degradation without losing the information provided by the individual metrics. We tested to what extent the multimetric macroinvertebrate index (MMI) is capable of discriminating reference from impaired wetland sites and validated its performance on a separate subset of the data.

Chapter 6 provides a general discussion of results of the previous chapters. It also gives limitations and recommendation for further research.

Chapter 2: Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia

Based on:

Mereta, S.T., Boets, P., Bayih, A. A., Malu, A., Ephrem, Z., Sisay, A., Endale, H., Yitbarek, M., Jemal, A., De Meester, L. , Goethals, P. L. M., 2012. Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia. *Ecol. Inf.* 7: 52–61.

Chapter 2: Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia

Abstract

In Ethiopia, wetland resources play a vital role in the lives of adjacent communities by helping them to achieve food security and livelihoods. However, many wetlands throughout the country are facing degradation as high population growth rate increased the need for more fertile agricultural land. Lack of awareness and logistic constraints are important reasons for the weak consideration of wetland ecosystems by country's development planners. In this paper, we set out to develop methods for predicting species-environment relationships. Decision tree models and Canonical Correspondence Analysis (CCA) were used to identify factors influencing macroinvertebrate community structure in natural wetlands of Southwest Ethiopia. The models were based on a dataset of 109 samples collected from 57 sites located in eight different wetlands. Sixteen macroinvertebrate taxa were selected based on their frequency of occurrence to determine the status of the wetlands. It was found that Corixidae, Baetidae and Hydrophilidae had the highest predictive model performance. This indicates that these taxa have clear requirements regarding their environmental conditions. The low Kappa value combined with the high number of Correctly Classified Instances of Chironomidae may be related to their high frequency of occurrence, so that their presence is of little predictive power. The Canonical Correspondence Analysis (CCA) also further illustrated this where the family of Chironomidae, common at nearly every sampling station in the wetlands, was plotted in the centre of the CCA axis. Vegetation cover, water depth, and conductivity were the most important variables determining the presence or absence of macroinvertebrate taxa. These variables were selected in more than 80% of the classification tree models and played a critical role in the ordination analyses. The conditional analysis, based on the regression tree models, also showed that vegetation cover and conductivity were affecting the abundance of some macroinvertebrate taxa. Information on habitat quality and environmental factors preserving a high diversity are essential to develop conservation and management programs for wetlands in Ethiopia, where wetland resources have already been lost, and are still at high risk due to expansion of agricultural and other development activities.

2.1 Introduction

Wetlands are one of the most biologically productive natural ecosystems on earth (Dixion and Wood, 2003b; Rolon and Maltchik, 2008). While they occupy about 6% of the world's land surface, they contribute up to 40% of the annual globe's ecosystem services (Bonell et al., 1993; Costanza et al., 1997). Wetlands perform a wide variety of ecological functions including nutrient cycling (Bunn et al, 1999), carbon storage (Adhilari, 2009), reducing flood (Hey and Philippi, 1995) and providing habitat for wild life (Jacobs et al., 2009). Moreover, wetlands play a vital role in ensuring food security and livelihoods for millions of people living in developing countries (Shewaye, 2008).

During the last decades, wetlands have undergone extensive exploitation worldwide (Xu, 2010). Studies have shown that about 50% of the world's wetlands have disappeared in the last century due to agriculture and urban development (Mitsch and Gosselink, 1993; Shine and Klemm, 1999). Drainage for agriculture has been recognized as the primary cause of global wetland loss (Xu, 2010). In Ethiopia, rapid population growth triggers expansion of agricultural areas, resettlement of landless people, and exploitation activities in wetland areas (Shewaye, 2008). Consequently, several wetlands either disappeared or are on the verge of drying out (Shewaye, 2008), while others rapidly decline in water quality. In response to the rapid degradation of wetlands in Ethiopia, a number of studies on wetland hydrology (Dixon, 2002; Dixion and Wood, 2003a) and socio-economic aspects (Solomon, 2004) have been initiated. However, little is known about the overall ecological condition of wetlands in Ethiopia. The diversity and abundance of macroinvertebrates known to provide considerable information on ecosystem impairment (Feio et al., 2007; Liston et al., 2008). In the present study, we therefore set out to identify the major environmental factors governing the macroinvertebrate communities inhabiting wetlands in a region in Ethiopia that is relatively rich in wetlands, but is under severe pressure by rapidly increasing land use intensity.

Macroinvertebrates represent a diverse group of long living sedentary species that react strongly and often predictably to human influences on aquatic systems (Cairns and Prall, 1993). They are considered very appropriate subjects for the assessment of the ecological condition of wetlands, since they are abundant, readily surveyed, and taxonomically rich (Dodson, 2001). Furthermore, they play an important role in the overall functioning of wetland ecosystems as they occupy a central position in the food web of wetlands (Batzer et

al., 1999). Macroinvertebrate community characteristics can reflect primary production and the ability of a wetland to support vertebrate wildlife (e.g. fish) and remove pollutants (Batzer et al., 2006). A better understanding of the factors driving changes in macroinvertebrate community structure along perturbation gradients at several taxonomic levels is therefore important to predict the potential changes in the ecological conditions of wetlands (Trigal-Domínguez, 2009).

In order to predict the habitat requirements of wetland macroinvertebrate communities, there is a clear need for models quantifying species-environment relationships to support decision-making (Broekhoven et al., 2006). Modelling the distribution of taxa as a function of the abiotic environment, often called habitat suitability modelling, has been recognised as a significant component of conservation planning (Guisan and Zimmermann, 2000). Habitat suitability models combine occurrence and/or abundance of species with environmental variables, both biotic and abiotic factors, judging on the habitat quality or predicting the effect on species occurrence of environmental changes within the habitat (Store and Kangas, 2001; Anderson et al., 2003). These models are typically developed by identifying statistical relationships between the occurrence and/or the abundance of the species and the biochemical and physical properties of a given site (Store and Kangas, 2001). In this regard, many approaches including multivariate analysis and modelling techniques such as (Robertson et al., 2001), decision trees (Goethals et al., 2002; Dakou et al., 2007; Boets et al., 2010; Hoang et al., 2010), artificial neural networks (Park et al., 2003; Dedecker et al., 2007; Goethals et al., 2007), fuzzy logic (Broekhoven et al., 2006; Mouton et al., 2009) and Bayesian belief networks (Adriaenssens et al., 2004) have been applied.

The aim of the present study was to analyse the relationship between habitat quality and the occurrence and diversity of macroinvertebrates in Wetlands in Southwest Ethiopia. Therefore, we developed habitat suitability models using decision tree models and used multivariate data analysis in order to analyse the macroinvertebrate community structure in these natural wetlands. The information obtained from this study can be used to inform on environmental factors that are important for community structure of macroinvertebrates and as a guideline for habitat conservation of wetlands.

2.2 Materials and Methods

2.2.1 Study area

The data used for the present study were collected from wetlands located in the Gilgel Gibe watershed, Southwest Ethiopia (Figure 2.1). Six permanent (Koffe, Kitto, Boye, Haro, Bulbul and Balawajo) and two temporary (Haro1 and Haro 2) wetlands located along the Gilgel Gibe river were included. The studied wetlands are varying in size ranging from five hectares to a few hundred hectares. These wetlands serve as a source of drinking water, as breeding grounds for birds and as grazing land. All permanent riverine wetlands are connected upstream and downstream to the rivers flowing into the Gilgel Gibe River and finally to the Gilgel Gibe hydro-power dam. The temporary and Bulbul wetlands are created by a meandering flood plain. These temporary wetlands are characterised by high fish and waterfowl abundance. The major threats from human activities around and in these wetlands included uncontrolled livestock grazing, brick making, vegetation clearance, and land conversion to cropland, drainage, municipal waste discharge and cultivation. Maize (*Zea mays*) cultivation is a common practice in and around these wetlands.

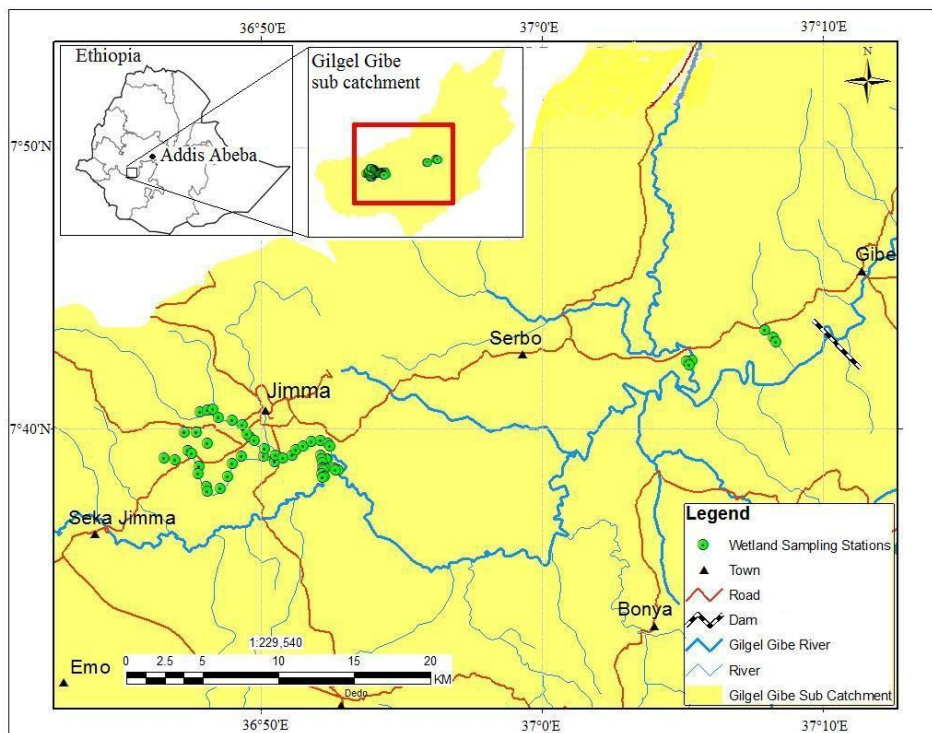


Figure 2.1. Location of the study area and wetland sampling stations in the Gilgel Gibe watershed, Southwest Ethiopia.

2.2.2 Data collection

A total of 57 sampling stations were monitored. Fifty-two permanent sampling sites were sampled both during the dry (March to May 2010), and the wet (August to September 2010) season, whereas five temporary wetland sampling stations were sampled only during the wet season. In this way, 109 samples were available.

2.2.2.1 Habitat and water quality measurements

Habitat characteristics were assessed at each sampling station using the USEPA wetland habitat assessment protocol (Baldwin et al., 2005). The degree of hydrological modifications (drainage, ditching and filling), habitat alteration (tree removal, tree plantation and grazing) and land use patterns such as waste dumping, clay mining, and farming were assessed during sampling (Table 2.1). Physical variables such as sludge depth, water depth, secchi depth and ambient temperature were measured. Dissolved oxygen, conductivity, pH and water temperature were measured in the field when the biological sample was taken using a multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach). Chlorophyll a concentration was measured at each sampling location using a fluorometer (Turner De-sign Aqua fluor). At each site 2 l of water was collected and stored on ice until return to the Laboratory of Environmental Health at Jimma University, where the samples were analysed for total nitrogen (TN), total phosphorus (TP), five day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), orthophosphate, ammonium and nitrate concentration according to the standard methods as prescribed by APHA, AWWA, WPCF (1995).

2.2.2.2 Macroinvertebrate and fish sampling and identification

Macroinvertebrates were collected at each sampling station using a rectangular frame net (20 x 30cm) with a mesh size of 300µm. Each collection entailed a 10-minute kick sampling over a distance of 10 metres (DNRE, 1999). Time was allotted proportionally to the cover of different meso-habitats of the wetland such as open water and emergent vegetation. The bottom sediment was disturbed by foot during sampling in order to also collect the benthic macroinvertebrates. Macroinvertebrates were sorted in the field, stored into vials containing 80% ethanol and labelled. Afterwards, macroinvertebrates were identified to family level using a stereomicroscope (10 x magnifications) and the identification key of Bouchard (2004).

Each site was sampled for fish during both the dry (March to May 2010) and wet season (August to September 2010). Fyke nets were used as well as fish pots. These were positioned in shallow areas (less than one meter depth) at each site. Nets were set during the day and retrieved after about 24 hours. Fish were counted and their length and weight was measured before they were released. *Garra dembecha* and *Oreochromis niloticus* are the dominant fish species in the study wetlands.

Table 2.1. Input variables used for the model development: mean values, standard deviation, and range. TON = total organic nitrogen, NH_4^+ = ammonium, NO_3^- = nitrate, TP = total phosphorus, PO_4^{3-} = orthophosphate, BOD_5 = biological oxygen demand, COD = chemical oxygen demand.

Variables	Unit	Min	25 Percentile	Median	75 Percentile	Max
Ambient temperature	°C	17	23.20	24.90	27.20	34
Water temperature	°C	18	21.10	22.10	23.50	33
pH	-	6	6.76	6.91	7.10	10
Dissolved oxygen	mg/l	0.2	1.92	3.65	5.29	14
Oxygen saturation	%	2	23.50	51.80	74.50	263
Conductivity	µS/cm	41	65.10	80.50	136.60	293
Chlorophyll a	µg/l	11	11.99	12.33	12.99	22
TON	mg/l	0.05	1.26	3.02	6.78	34
NH_4^+	mg/l	0.01	0.03	0.04	0.05	1.6
NO_3^-	mg/l	0.04	0.19	0.48	2.09	12

Chapter 2: Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa

TP	mg/l	0.03	0.06	0.13	0.29	1.2
PO ₄ ³⁻	mg/l	0.01	0.04	0.06	0.10	5.4
BOD ₅	mg/l	1	4.50	9.00	15.60	144
COD	mg/l	3	8.40	17.90	26.80	306
Water depth	cm	5	30.00	48.00	65.00	180
Vegetation cover	%	35	60	80	90	95
Fish	absent(0), present(1)	/	/	/	/	/
Grazing	absent(0), present(1)	/	/	/	/	/
Cultivation/ploughing	absent(0), present(1)	/	/	/	/	/
Clay mining	absent(0), present(1)	/	/	/	/	/
Drainage	absent(0), present(1)	/	/	/	/	/
Waste dumping	absent(0), present(1)	/	/	/	/	/

2.2.3 Data analysis

2.2.3.1 Classification and regression tree models (CART)

Twenty-two environmental variables were used to determine the most important variables for the prediction of the 16 most frequently occurring macroinvertebrate taxa in the wetlands (Table 2.1). Classification and regression tree models (CART) were applied to develop the

models. The classification tree models were built using the J48 algorithm (Quinlan, 1993), a java re-implementation of the C4.5 algorithm, which is a part of machine learning package WEKA (Witten and Frank, 2005). Regression tree models were built using the M5 algorithm in WEKA (Witten and Frank, 2005) in order to relate the abundance of macroinvertebrate taxa to environmental variables. Default parameter settings were used to induce the trees.

Model training and validation was based on a three-fold cross validation procedure (Witten and Frank, 2005). The dataset was stratified into three sub sets, of which two subsets were used as training data and the remaining one subset was used for testing the model. The cross validation process was then repeated three times each with one of the three subsets used once as the validation data set. In this way, three models were built. The results from the three models were averaged to produce a single prediction of the dependent variable.

The percentage of correctly classified instances (CCI) (Witten and Frank, 2005) and Cohen's Kappa statistic (K) (Cohen, 1960) were used to evaluate the predictive performance of the classification tree models. The CCI is the percentage of the true positive and true negative predictions, which is calculated based on a confusion matrix.

CCI is mathematically expressed as follows:

$$CCI = \frac{(TP + TN)}{(TP + FP + TN + FN)}$$

Cohen's Kappa statistic simply measures the proportion of all possible cases of presence or absence that are predicted correctly by a model after accounting for chance predictions. It is mathematically expressed as follows:

$$K = \frac{[(TP + TN) - (((TP + FN)(TP + FP) + (FP + TN)(FN + TN)) / n)]}{[n - (((TP + FN)(TP + FP) + (FP + TN)(FN + TN)) / n)]}$$

Where n is the total number of instances.

TP = True positive, TN = True negative, FP = False positive and FN = False negative instances

Models with *K* higher than or equal to 0.4 were considered reliable (Dakou et al., 2007; Gabriels et al., 2007). CCI is affected by the frequency of occurrence of the taxon being modelled (Manel et al., 2001). Unlike CCI, *K* takes a correction into account for the expected

number of correct predictions due to randomness, which is strongly related to taxon prevalence (Fielding and Bell, 1997; Manel et al., 2001). We used the ranges of K recommended by Landis and Koch (1997) for model performance evaluation: $K \leq 0$ (poor), 0-0.2 (slight), 0.2-0.4 (fair), 0.4-0.6 (moderate), 0.6-0.8 (substantial) and 0.8-1 (almost perfect).

We used the determination coefficient (R^2) value to evaluate the performance of the regression tree models (Déath and Fabricius, 2000). The determination coefficient is a measure of the goodness of fit of the models (Kallimanis et al., 2007). Its value is always between 0 and 1. The closer the value is to 1, the better the model predicts the training data.

Conditional analysis was performed in order to gain insight in the relationship between predictor variables and the abundance of macroinvertebrate taxa. For each of the three models constructed per taxon, the gradient and importance of the predictor variable (e.g. conductivity) on the macroinvertebrate abundance was analysed. This was done by plotting the selected variable between its minimum and maximum value encountered at the sampling sites, while the other parameters that were present in the model were kept constant at their average values. In this way, for each of the three different models a line was plotted showing the relationship between the environmental factors and the abundance of macroinvertebrates as well as the gradient of the different models.

2.2.3.2 Multivariate data analysis

Detrended Correspondence Analysis (DCA) was applied using CANOCO 4.5 (ter Braak and Smilauer, 2002) to examine whether Redundancy Analysis (RDA) or Canonical Correspondence Analysis (CCA) would be appropriate (ter Braak and Jaap, 1994) to analyse the data. The DCA yielded gradient lengths that were higher than three standard deviations, therefore CCA was used. Sixteen macroinvertebrate taxa were selected based on their frequency of occurrence. Macroinvertebrate abundance data were log transformed $\log(x+1)$ prior to analysis to obtain homogeneity of variance. Based on a stepwise forward selection twelve environmental factors were selected as independent variables. All environmental data except pH and presence of fish were $\log(x+1)$ transformed and standardized since the variables were measured in a variety of units. The statistical significance of eigenvalues and

species-environment correlations generated by the CCA were tested using Monte Carlo permutations.

2.3 Results

2.3.1 Variable importance

Twenty-two environmental variables (Table 2.1) were used as predictors to determine the presence/absence of 16 benthic macroinvertebrate taxa. Figure 2.2 shows the frequency of selection of these environmental variables by the classification tree models. The number represents how many times a given variable was selected by the model. Since the training and validation was based on three-fold cross-validation, three models were developed for each taxon.

The most frequently selected variables were vegetation cover (88%), conductivity (81%), water depth (81%), presence/absence of fish (56%), and total phosphorus concentration (56%). Moreover, vegetation cover and conductivity were often selected as root of a tree indicating that these were the most informative attributes to determine the presence/absence of macroinvertebrates taxa. In contrast to the above mentioned variables, ambient and water temperature and chlorophyll a were selected in 6% of the cases and thus were less critical for the taxa presence/absence.

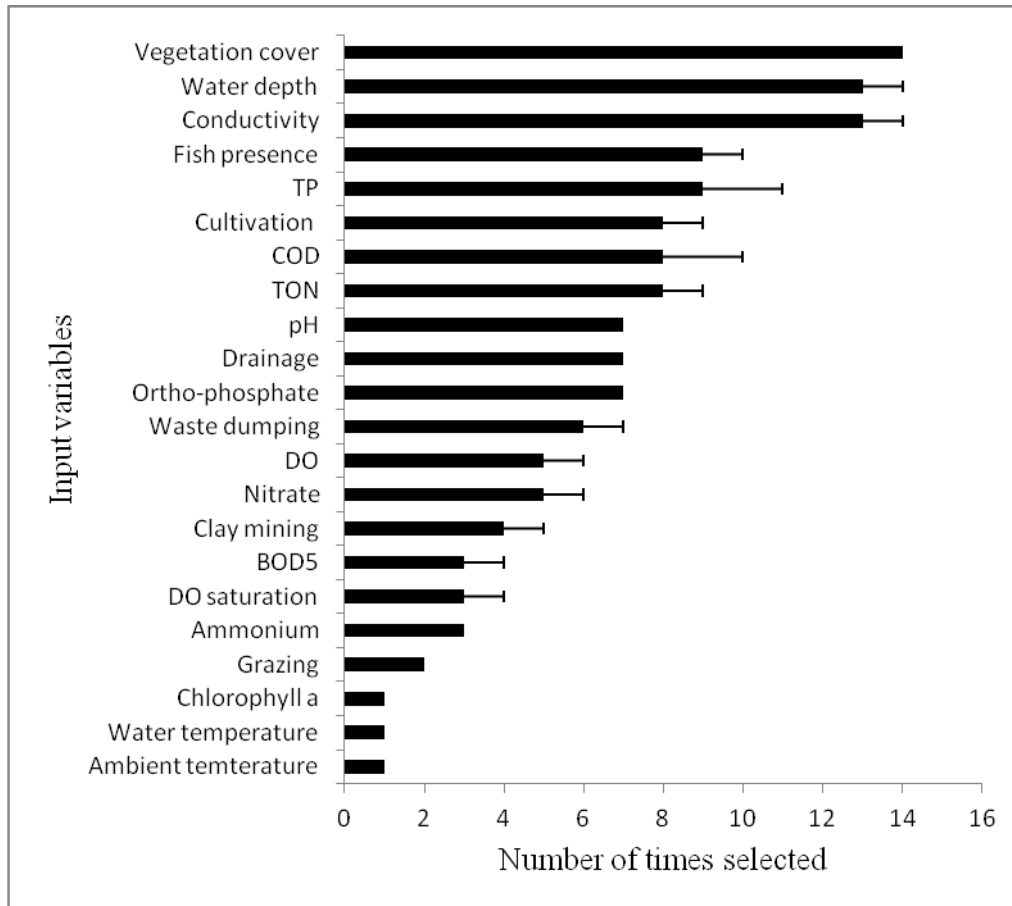


Figure 2.2. Overview of the frequency of selection of the different input variables used in the decision tree models to model the presence or absence of each taxon. Error bars indicate standard deviation of the three subset.

As an example, the classification tree model for Caenidae is depicted in Figure 2.3. This tree has seven leaves and thirteen branches. The classification tree indicates that vegetation cover, given as a root of the tree, is considered as the most informative attribute to predict the occurrence of Caenidae. Caenidae were generally absent when the vegetation cover was less than 55% and pH was higher than 7.23. On the other hand, Caenidae were present in sites where there was no clay mining activity and fish were absent. This classification tree had a good overall predictive performance, with a CCI of 81% and Kappa of 0.47.

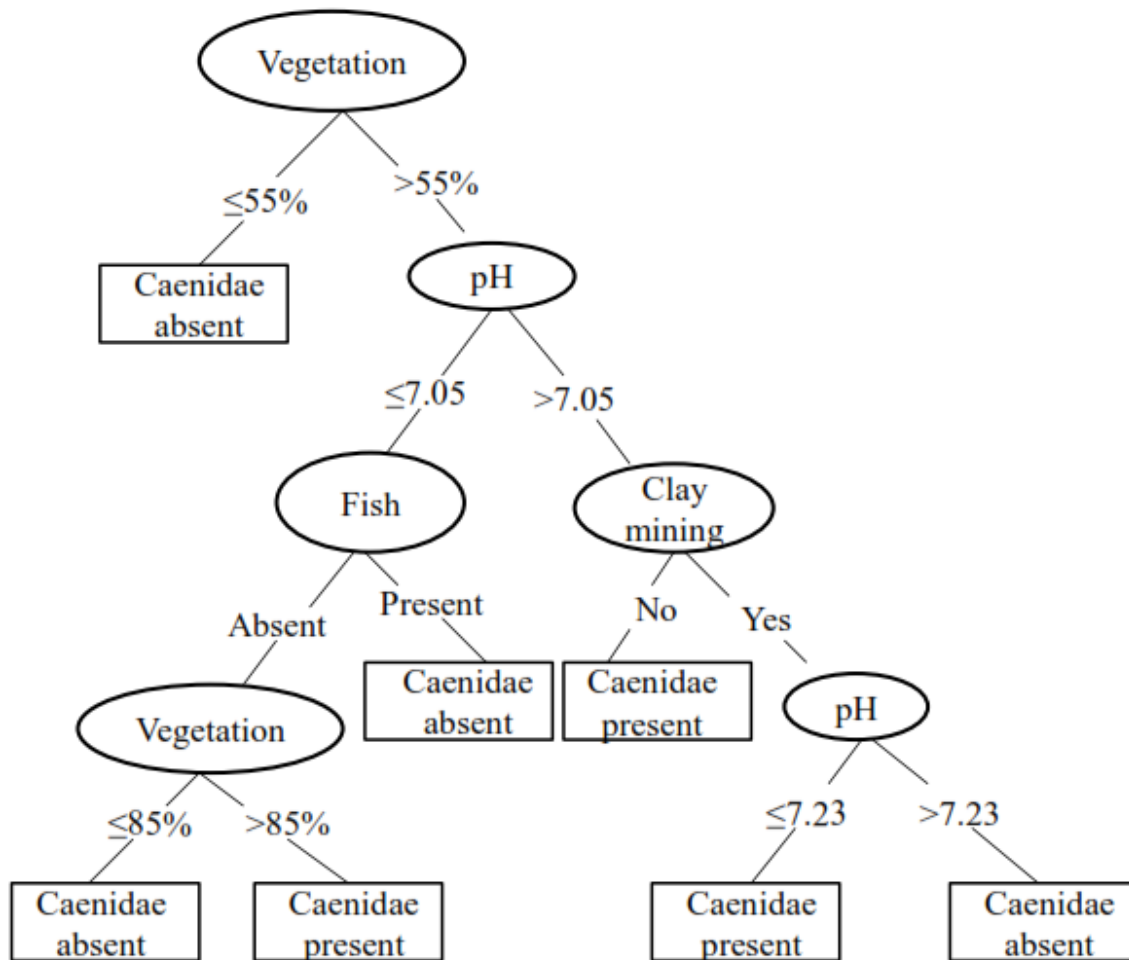


Figure 2.3. Classification tree model predicting the presence or absence of Caenidae (Correctly Classified Instances = 81%, Kappa = 0.47)

2.3.2 Model performance Evaluation

The model performances based on the CCI and Cohen's Kappa statistic of the three-fold cross validation for 16 macroinvertebrate taxa are shown in Figure 2.4a and Figure 2.4b. The CCI varied between $59 \pm 3\%$ to $88 \pm 2\%$. Based on CCI, 13 taxa were predicted with a good reliability by the classification tree models ($CCI \geq 70\%$). Based on CCI, very good predictions were obtained for Chironomidae and Dytiscidae with CCI of $88 \pm 2\%$ and $86 \pm 11\%$, respectively. On the other hand, eight taxa were predicted accurately based on Cohen's Kappa statistic ($K \geq 0.4$). Based on Kappa, the highest model predictive performance was obtained for Corixidae and Baetidae with a K value of 0.58 ± 0.2 , indicating a good model performance. In

contrast, Tipulidae and Belostomatidae had the lowest K value (0.17 ± 0.02), indicating poor model performance.

Although Chironomidae and Dytiscidae had the highest relative CCI, their K values were 0.30 ± 0.27 and 0.38 ± 0.3 , respectively. These high CCI values were related to their high frequency of occurrence: the family Chironomidae was present in 87% and Dytiscidae in 83% of the samples.

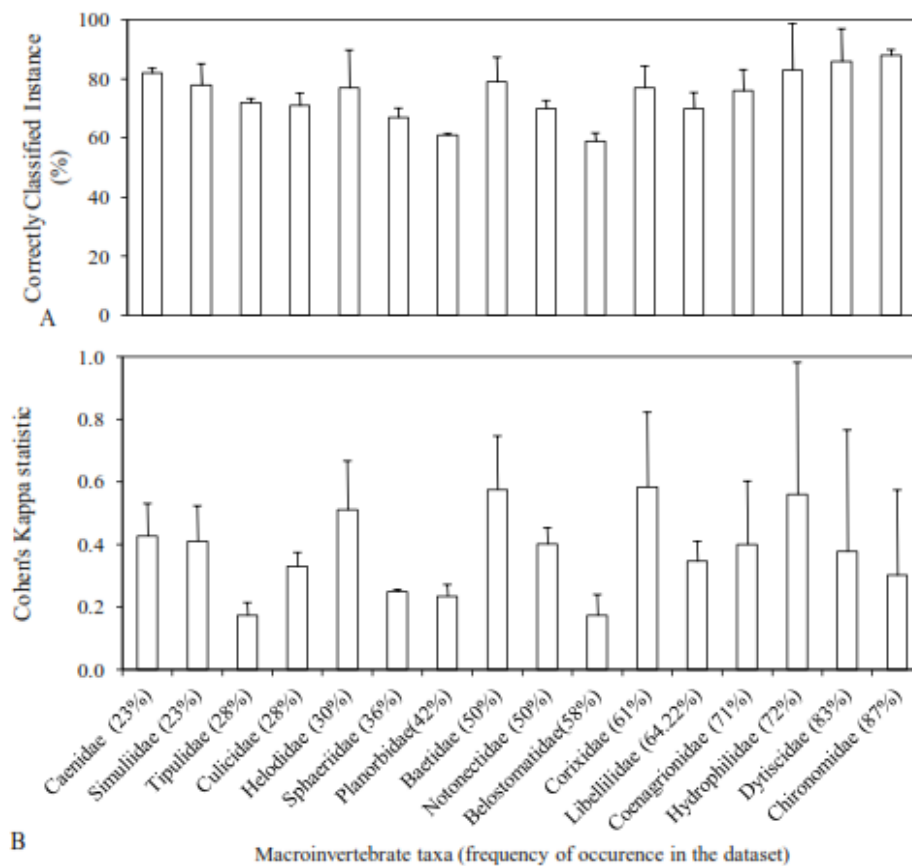


Figure 2.4. Overview of the predictive performance of the models based on (a) Correctly Classified Instances, (b) Cohen's Kappa statistic of all macroinvertebrate taxa that were modelled. The percentage of occurrence at the different sampling sites is given between brackets.

2.3.3 Conditional Analysis

For six macroinvertebrate taxa namely Caenidae, Baetidae, Simuliidae, Dytiscidae, Hydrophilidae and Notonectidae with an acceptable model performance a conditional analysis

was done. The correlation coefficient obtained from the regression tree models for these six taxa varied from 0.29 ± 0.02 to 0.55 ± 0.12 . Vegetation cover and conductivity were used as predictor variables since these were the most important variables selected by the models.

The conditional analysis pointed out that the abundance of Simuliidae (Figure 2.5a) and Baetidae (Figure 2.5b) increased with increasing vegetation cover. For Caenidae their abundance increased up to 80% vegetation cover and became more or less stable afterwards (Figure 2.5c). In contrast to the other taxa, the abundance of Notonectidae decreased with increasing vegetation cover (Figure 2.5d).

A Conditional analysis of the regression tree model analysing the effect of changing conductivity on the abundance of Simuliidae, Dytiscidae and Hydrophilidae is shown in Figure 2.6. The abundance of Simuliidae (Figure 2.6a) decreased with increasing conductivity. In contrast, the abundance of Coleoptera larvae, both Dytiscidae and Hydrophilidae (Figure 2.6b, c), increased with increasing conductivity and remained more or less stable at conductivity levels $> 150 \mu\text{S}/\text{cm}$.

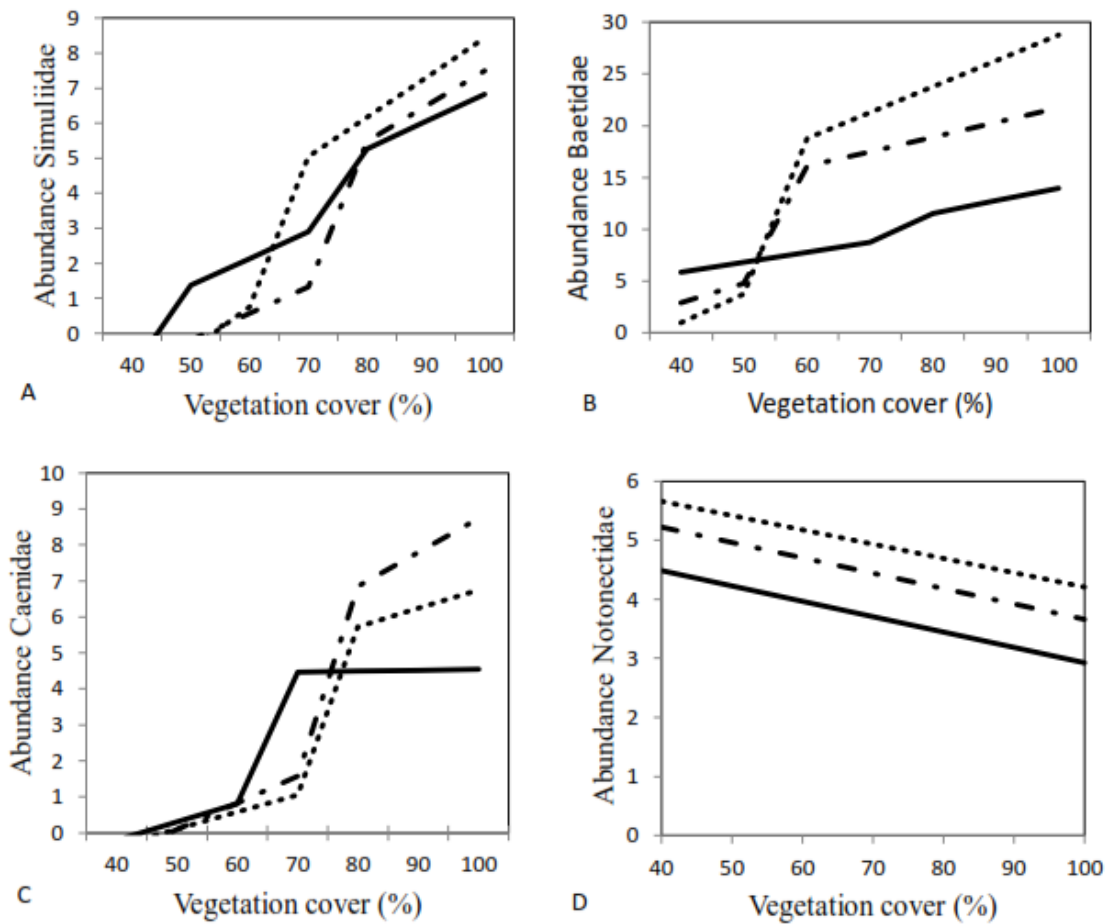


Figure 2.5. Conditional analysis illustrating the abundance (number of individuals per sample) of (A) Simuliidae, (B) Baetidae, (C) Caenidae, (D) Notonectidae in function of vegetation cover (Fold 1 = dotted line, Fold 2 = solid line, Fold 3 = dashed line; for more explanation on folds, see text).

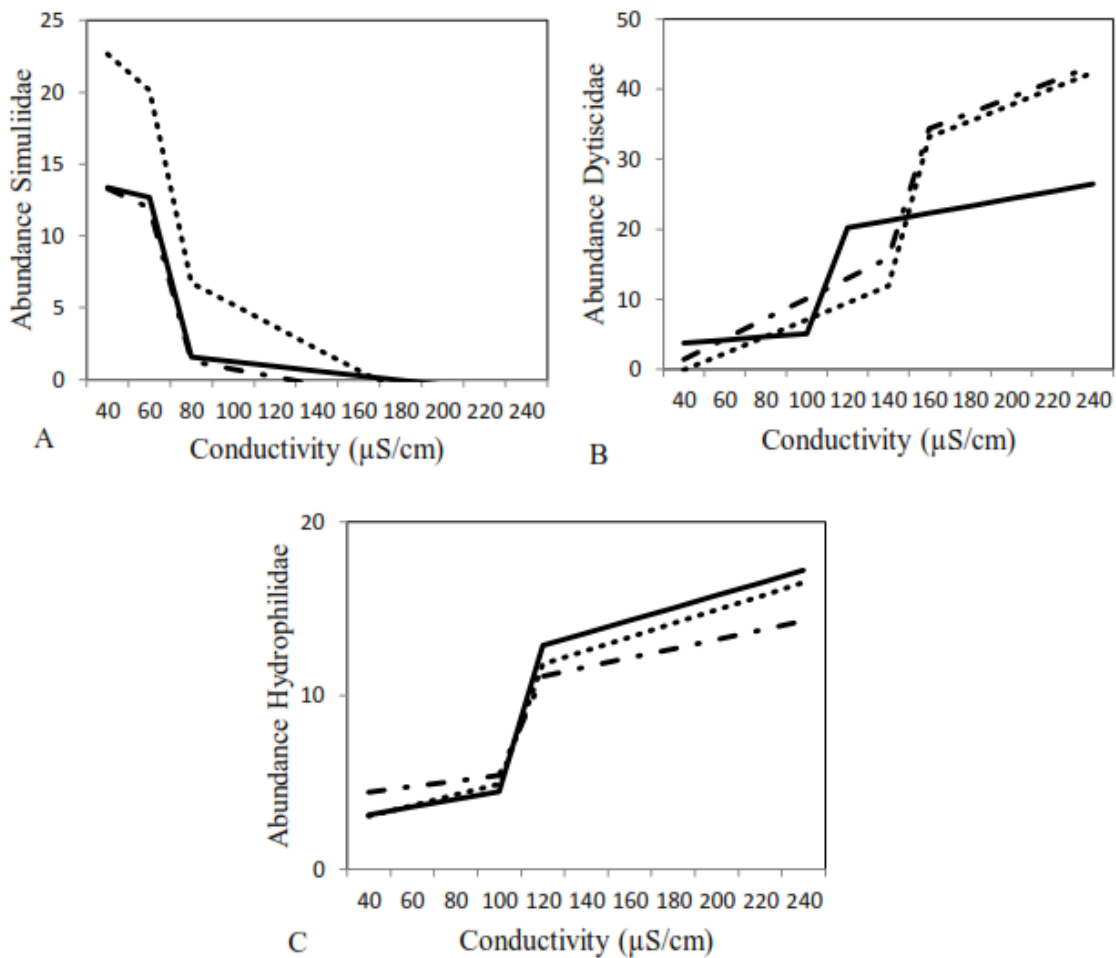


Figure 2.6. Conditional analysis illustrating the abundance (number of individuals per sample) of (A) Simuliidae (B) Dytiscidae and (C) Hydrophilidae in function of conductivity (Fold 1 = dotted line, Fold 2 = solid line, Fold 3 = dashed line; for more explanation on folds, see text).

2.3.4 Multivariate Analysis

The first and the second canonical axes explained 13.7% (eigenvalue of 0.17) and 7.8% (eigenvalue of 0.10) of the variation in the species data, respectively. The species-environment correlation of the first axis was statistically significant in a Monte Carlo permutation test ($P < 0.05$). The first axis was positively correlated with presence/absence of fish ($r = 0.62$), total phosphorus ($r = 0.61$), water depth ($r = 0.54$), dissolved oxygen ($r = 0.53$), and chemical oxygen demand ($r = 0.43$). Vegetation cover and conductivity were negatively correlated with CCA axis 1, with $r = -0.27$ and $r = -0.38$, respectively. CCA axis 2 was positively correlated with conductivity, COD and TP, and negatively with dissolved

oxygen (Figure 2.7). In addition, CCA analysis also revealed that Simuliidae and Caenidae were significantly correlated with vegetation cover ($r = 0.5$ and $r = 0.42$, respectively; $P < 0.05$). Hydrophilidae ($r = 0.45$) and Dytiscidae ($r = 0.47$) were significantly correlated with water conductivity ($P = 0.04$).

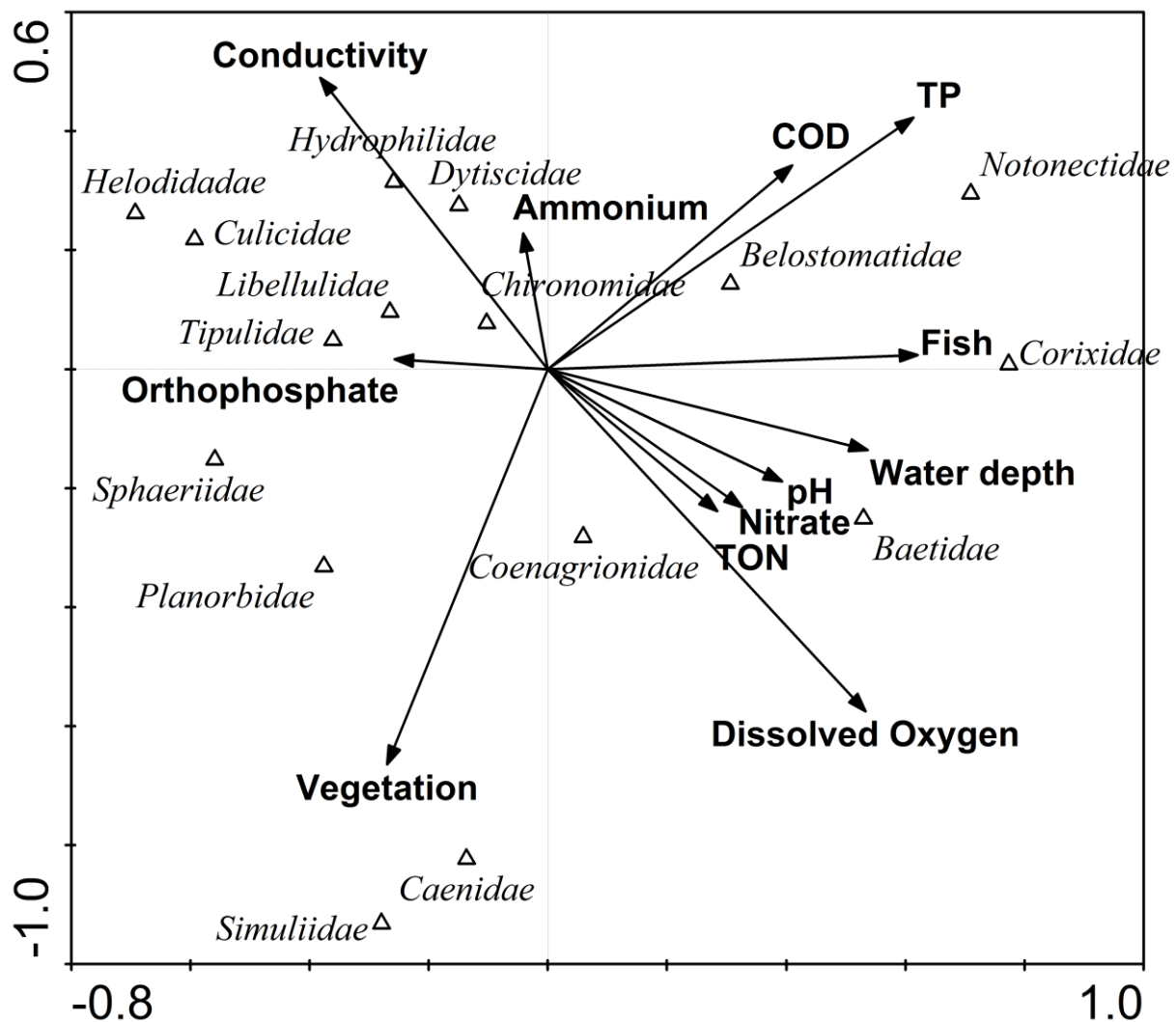


Figure 2.7. Canonical Correspondence Analysis (CCA) of macroinvertebrate taxa and environmental variables in natural wetlands of Southwest Ethiopia (variables are explained in Table 2.1).

A bi-plot of the sampling sites and environmental variables showed that there was a clear distinction between samples taken during the dry or the wet season (Figure 2. 8). Conductivity was strongly positively correlated with dry season, whereas vegetation cover and dissolved

oxygen were more correlated with the wet season. Temporary wetlands clustered together and showed associations with water depth, COD, TP and the presence/absence of fish.

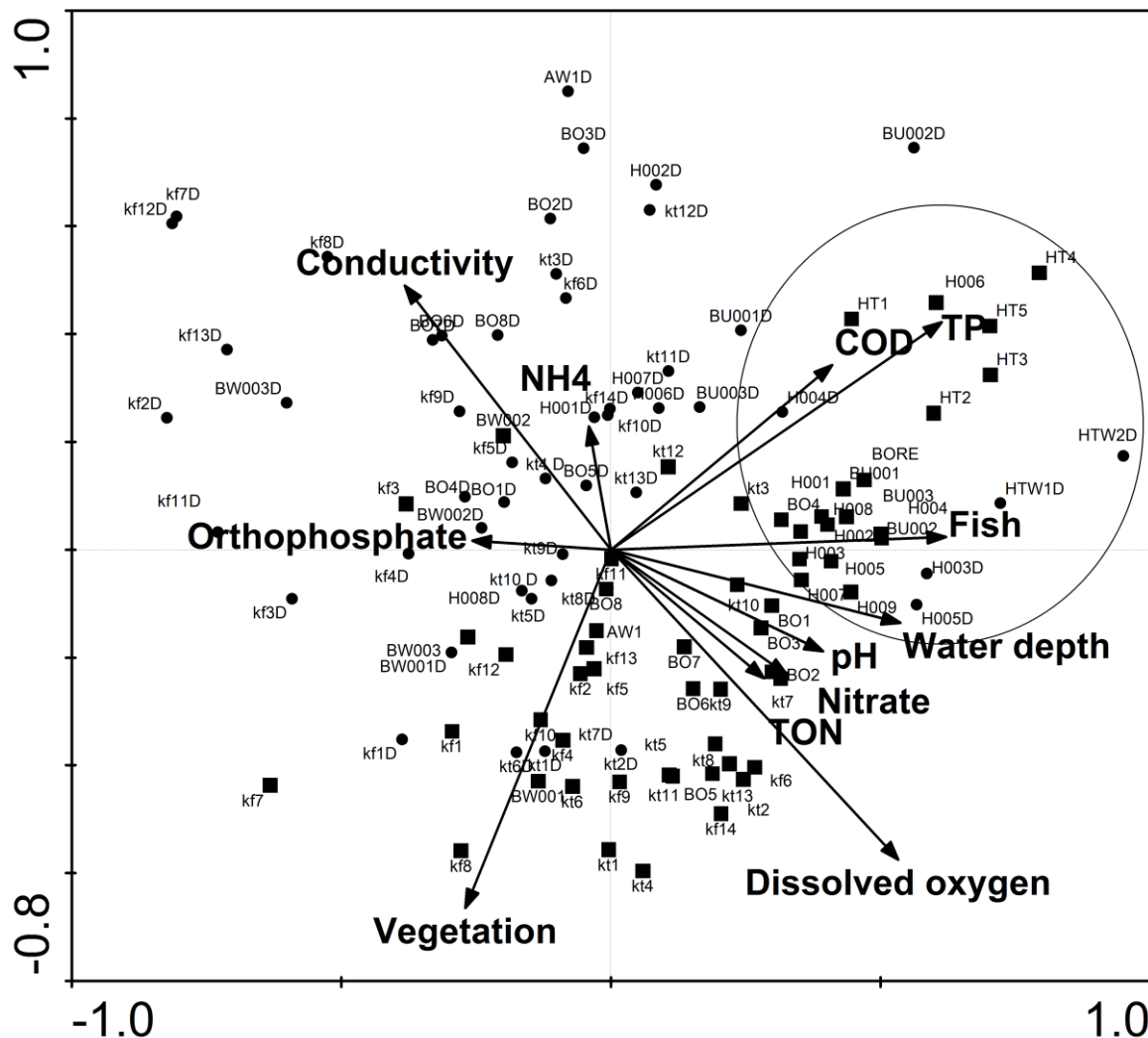


Figure 2.8. Bi-plot of environmental variables and wet (squares) and dry season (circles) sampling sites. The temporary wetlands (wet season samples only) are clustered and indicated by a circle.

2.4 Discussion

Predicting species' distribution has been recognized as a significant component of conservation planning since it helps identifying those regions which yield maximum effect when including restoration efforts (Guisan and Zimmermann, 2000; Loiselle et al., 2003). In the present paper, predictive models allowed identifying important variables structuring the macroinvertebrate community in wetlands in Southwest Ethiopia. Based on Kappa, Tipulidae

and Belostomatidae had the lowest model performance, suggesting that other factors than the ones we quantified determined the distribution of these taxa. On the other hand, the low kappa value and the high CCI of Chironomidae may be related to their high frequency of occurrence, indicating that the prediction was merely based on chance (Fielding and Bell, 1997; Manel et al., 2001). The model tends to learn that the most common taxa are always present and the rarest taxa are always absent (Dedecker et al., 2007). Similarly, in the CCA Chironomidae were plotted in the centre of CCA axis. The weak association between Chironomidae and environmental factors and the fact that this species occurred in 87% of the sites suggest that this taxon is tolerant to disturbance and a resident of impacted environments, as has been reported by many earlier studies (Karr and Rossano, 2001). Corixidae, Baetidae and Hydrophilidae showed high Kappa and CCI values, and their occurrence could be well predicted by our model. This indicates that these taxa have clear requirements regarding their environmental conditions within the habitat gradient we studied.

Vegetation cover, water depth and conductivity were the most important environmental variables determining the presence or absence of macroinvertebrate taxa. These variables were selected in more than 80% of the classification tree models and were also correlated with the axes that explain the largest amount of variation in the ordination analysis. Vegetation is known to be an important parameter in wetlands influencing the diversity of macroinvertebrates (Balcombe et al., 2005; Jurado et al., 2009). Both the Conditional analysis and ordination diagram showed that the abundance of Simuliidae and Caenidae is strongly associated with vegetation cover. Vegetation can provide shelter against water current and predation, can provide more food resources, and is important as oviposition site (Couceiro et al., 2007; Ambelu et al., 2010). Vegetation has been shown to decrease the efficiency of fish predation and provides a refuge for benthic macroinvertebrates against visual predators (Hanson and Butler, 1994; Diehl, 1995). On the other hand, some macrophytes produce dissolved oxygen and in this way create better habitat conditions (Ságová-Marecková and Kvet, 2002). In contrast to the other taxa, the abundance of Hemiptera was negatively correlated with vegetation cover. The crucial factor for the distribution of aquatic Hemiptera species seems to be a high percentage of open water, as also shown in other studies (Bloechl et al., 2010).

Besides vegetation, conductivity is an important parameter affecting the composition of macroinvertebrate communities (Gabriels et al., 2007; Boets et al., 2010). Although the measured conductivity values in our study were generally low ranging between 41-293 $\mu\text{S}/\text{cm}$, several wetlands were strongly influenced by inflow of untreated wastewater from Jimma town, which led to an important input of water with a relatively high conductivity. Several studies have shown that urbanization can contribute to increased levels of conductivity in freshwater ecosystems (Roy et al., 2003). Both the ordination and conditional analysis showed that taxa belonging to the order of the Coleoptera were positively correlated with conductivity. The preference of Coleoptera for relatively high levels of conductivity can be species dependent, as found by Cuppen (1986). Conductivity was lower in water samples taken during the wet season: this likely reflects dilution effects by runoff and precipitation. Culicidae larvae were positively correlated with conductivity and negatively correlated with dissolved oxygen concentration. The latter may be due to the fact that Culicidae have a breathing tube siphon that allows them to obtain oxygen to persist in environments with poor water quality (Chipps et al., 2007). As most other organism groups cannot cope with low oxygen levels, the Culicidae are released from competitive pressure in low oxygen habitats, which increases their likelihood of occurrence. In contrast to conductivity, dissolved oxygen concentrations were higher in wet season samples, suggesting that runoff, precipitation and turbulence increased the dissolved oxygen concentration (Ambelu, 2009).

Temporary wetlands differed from permanent wetlands in their macroinvertebrate composition as well as in their physical and chemical characteristics. Several studies have shown that the hydroperiod plays a critical role in the ecology of wetlands (Steinman et al., 2003). Macroinvertebrate assemblages of temporary wetlands are often characterized by rapidly developing and very active species, or by species that have very high dispersal capacities (Wellborn et al., 1996). The ordination analysis revealed that Hemiptera, Notonectidae, Corixidae and Belostomatidae dominated in temporary wetlands. These taxa are able to re-colonize temporary wetlands within a couple of weeks after flooding (Chase and Knight, 2003). Moreover, in seasonal habitats such as wetlands, community structure can be related to abiotic variables that change in response to seasonal conditions, including water depth, dissolved oxygen and macrophyte coverage (Escalera-Vazquez and Zambrano, 2010).

Fish were mainly found in open water of the temporary wetlands. Several studies demonstrated that the density of fish strongly affects the abundance and distribution of Dytiscidae and other macroinvertebrate taxa (Arnott and Jackson, 2006). In our case, we only quantified the presence or absence of fish, not their abundance. Nevertheless, it is expected that the fish community has an impact on the abundance of macroinvertebrates. This might explain why the abundance and diversity of macroinvertebrates was generally lower in the temporary compared to the permanent wetlands.

The high chemical oxygen demand and high concentration of total phosphorus observed in the temporary wetlands is probably due to agricultural waste products and litter decomposition. Most temporary wetlands were situated in areas with agricultural activity. In the study area, the temporary wetlands are often used as agricultural field or grazing land during the dry season. Cattle can deposit significant amounts of excrements in these fields. When these areas become inundated during the rainy season, the dead organic material from crops and cattle excrements can be decomposed and results in an increase of the concentration of total phosphorus and an increase in chemical oxygen demand (Strand and Merritt 1999; Del Rosario et al., 2002).

In conclusion, both the decision tree models and the canonical correspondence analysis indicated that environmental factors such as vegetation cover, water depth and water conductivity influence the structure of wetland macroinvertebrate communities. These most important variables gave a clear and stable result that was easy to interpret. Therefore, protection of wetlands from human activities such as brick makings and agricultural activities, which entailed destruction of wetland vegetation, is essential to maintain a high diversity in Ethiopia. Further study is recommended to elucidate the ecological implications of these environmental factors on waterfowl and fish, since this could contribute to an improved management of wetlands in Ethiopia.

Chapter 3: Physico-chemical and biological characterization of larval habitats of anopheline mosquito (Diptera: Culicidae): Implications for malaria control

Adapted from:

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Chapter 3: Physico-chemical and biological characterization of larval habitats of anopheline mosquito (Diptera: Culicidae): Implications for malaria control

Abstract

A fundamental understanding of the spatial distribution and ecology of mosquito larvae is essential for effective vector control intervention strategies. In this study, data-driven decision tree models, generalized linear models and ordination analysis were used to identify the most important biotic and abiotic factors that affect the occurrence and abundance of mosquito larvae in Southwest Ethiopia. In total, 220 samples were taken at 180 sampling locations during the years 2010 and 2012. Sampling sites were characterized based on physical, chemical and biological attributes. Decision tree models' predictive performance was evaluated based on correctly classified instances (CCI), Cohen's kappa statistic (κ) and the determination coefficient (R^2). The forward-backward stepwise selection method using Akaike's information criteria (AIC) was used to select the most parsimonious generalized linear model. A conditional analysis was performed on the regression tree models to test the relation between key environmental and biological parameters and the abundance of mosquito larvae. The decision tree model developed for anopheline larvae showed a good model performance (CCI = $84 \pm 2\%$, and $\kappa = 0.66 \pm 0.04$), indicating that the genus has clear habitat requirements. Anopheline mosquito larvae showed a widespread distribution and especially occurred in small human-made aquatic habitats. Water temperature, canopy cover, emergent vegetation cover, and presence of predators and competitors were found to be the main variables determining the abundance and distribution of anopheline larvae. In contrast, anopheline mosquito larvae were found to be less prominently present in permanent larval habitats. This could be attributed to the high abundance and diversity of natural predators and competitors suppressing the mosquito population densities. The findings of this study suggest that targeting smaller human-made aquatic habitats could result in effective larval control of anopheline mosquitoes in the study area. Controlling the occurrence of mosquito larvae via drainage of permanent wetlands may not be a good management strategy as it negatively affects the occurrence and abundance of mosquito predators and competitors and promotes an increase in anopheline population densities.

3.1 Introduction

Mosquitoes are not only a nuisance, but are also responsible for the spread of a wide range of diseases including malaria, yellow fever, dengue, West Nile virus and Rift Valley fever (Maguire et al., 1999; Hay et al., 2002; Shaalan and Canyon, 2009). These mosquito borne diseases, infecting more than 700 million people around the world each year, result in as many as two million deaths annually (Fradin, 1998). One of these diseases, malaria, is transmitted between humans by adult female mosquitoes of the genus *Anopheles*. It is endemic in tropical and sub-tropical regions where it causes over 300 million acute illnesses and at least one million deaths each year (WHO, 2004). In spite of the recent scale-up of control programs, malaria continues to be a major public health problem in most tropical countries and its control is becoming increasingly difficult due to the spread of resistance of the parasite to anti-malarial drugs, resistance of the vector to insecticides and land-use changes (Lambin and Geist, 2006; Antonio-Nkondjio et al., 2009).

Land-use and land-cover changes, such as deforestation, agricultural expansion, infrastructure development, urbanization and human population growth contribute to the proliferation of breeding sites of mosquitoes (WHO, 2004). These environmental or land-use modifications also affect climate processes (Otieno and Anyah, 2012) that are likely to support rapid development of mosquitoes and parasites in regions where there has previously been a low-temperature restriction on transmission. Current episodes of climate variability in Africa are likely to intensify the transmission of malaria in the eastern and southern highlands (Githeko et al., 2012). Moreover, dams and small irrigation projects also contribute to an increase in the mosquito population by, increasing the number of suitable larval habitats, prolonging the breeding season and allowing the expansion of their distribution range. Small dams built for irrigation and mega hydropower dams have been shown to favour malaria transmission in Ethiopia (Ghebreyesus et al., 1999; Yewhalaw et al., 2009).

Several studies have examined the relationship between habitat characteristics and mosquito larval abundance and distribution in Africa (Minakawa et al., 1999; Shililu et al., 2003; Muturi et al., 2008; Kenea et al., 2011; Kweka et al., 2011). *Anopheles arabiensis*, the principal malaria vector in Sub-Saharan Africa, prefers shallow clean water and sunlit temporary habitats such as sand pools, brick pits and rain pools (Shililu et al., 2003; Kenea et

al., 2011). The presence of *An. arabiensis* immature stages in aquatic habitats is mainly influenced by water temperature, emergent plant cover, water current, turbidity, canopy cover, substrate type, and presence of predators and competitors (Shililu et al., 2003, Muturi et al., 2008). Shililu *et al.* (2003) indicated that in low- and highlands in Eritrea, water temperature was positively correlated with larval density. Higher temperatures encourage better development of eggs or allow the development of more microorganisms that are used as food by the larvae (Minakawa et al., 1999). On the other hand, high emergent plant cover of aquatic habitats is likely to reduce mosquito larvae by obstructing gravid females from ovipositing and supporting a high diversity of predators (Muturi et al., 2008). The occurrence of predators and competitors is also a key determinant for the presence of *An. arabiensis* larvae. Muturi et al. (2008) indicated that gravid females of *An. arabiensis* would avoid ovipositing in habitats where members of the family Heptageniidae are present, presumably to avoid direct competition. Furthermore, *An. arabiensis* is virtually absent or present at low abundance in habitats where there are predators such as fish (Tilapia, *Oreochromis sp.*), dragonfly larvae, water bugs and water beetles (Gouagna et al., 2012).

Malaria vector control has been largely dependent on the use of chemical insecticides. Only 12 insecticides belonging to four insecticide classes are recommended for public health use either for indoor residual spraying or to treat mosquito nets (WHO, 2005). Unfortunately, resistance to insecticides has been reported from many malaria vector species. Resistance spreads rapidly, which constitutes a serious threat to malaria control initiatives (WHO, 2005). In Ethiopia, populations of *An. arabiensis*, the major malaria vector in the country, developed resistance to three (organochlorines, organophosphates and pyrethroids) out of the four insecticide families commonly used to combat malaria (Balkew et al., 2010; Yewhalaw et al., 2011). Therefore, alternative malaria vector control tools, targeting mosquito immatures either alone or as part of integrated vector management, should be envisaged to reduce human-vector contact and hence malaria transmission intensity.

Adult mosquitoes are difficult to control since they can fly relatively long distances and survive in a wide range of microhabitats, including the soil and in holes in rocks and trees (Pfaehler et al. 2006). Effective mosquito larval control can be achieved through larval habitat management (Minakawa et al., 1999; Yasuoka et al., 2006). Larval control through environmental management has gained a lot of attention during the last decades (WHO, 1982;

Walker and Lynch, 2007). Environmental management involves changes in potential mosquito breeding areas to prevent, eliminate or reduce the vector's habitat (WHO, 1982). Techniques include draining man-made and natural wetlands, land levelling, filling small ponds or water collecting depressions and changing banks of water impoundments (Walker and Lynch, 2007). However, draining natural water bodies such as wetlands may affect the composition and structure of mosquito predators and species diversity in general more than they do reduce mosquito breeding sites (SWS, 2009). Even after a wetland has been drained, it may often still hold enough water after a rain event to serve as a breeding site for mosquitoes (Berg et al., 2010). In addition, drainage of wetlands often reduces important regulating ecosystem services such as mitigating floods, recharging aquifers, microclimate stabilization and improving water quality (MEA, 2005). So, draining wetlands does not seem to be a good strategy to reduce the habitat of mosquito vectors.

In order to include mosquito larval habitat management as part of an integrated vector management program, detailed knowledge on the ecology of the aquatic immature stages is crucial (Li et al., 2009). To this effect, habitat suitability modelling has been increasingly used to determine the presence of malaria vectors and estimating their population levels. Such information is the basis for risk assessment of mosquito-borne diseases (Ayala et al., 2009; Obsomer et al., 2012). Habitat suitability models take into consideration the occurrence and/or abundance of species in relation to biotic and abiotic environmental factors, evaluating the habitat quality or predicting its effect on species occurrences as a result of environmental changes within the habitat (Anderson et al., 2003). However, species-habitat relationships are influenced by regional conditions and hence, the generality of these models needs to be tested (Li et al., 2012). Therefore, we here developed data-driven models using decision trees and generalized linear models in order to assess the relationship between abiotic and biotic environmental factors and the occurrence and abundance of anopheline mosquito larvae in Southwest Ethiopia. These results could help decision makers to identify priority habitats to be targeted for the control of anopheline mosquito larvae. We specifically addressed the question whether permanent marshlands in the neighbourhood of Jimma (the main city in the Gilgel Gibe catchment), which are bio-diverse areas that are under serious threat by land encroachment and which are perceived as mosquito breeding grounds, are indeed a preferred habitat for anopheline mosquito larvae. These marshlands fulfil many ecosystem services so

their destruction would entail important losses and a good and integrated management is therefore required.

3.2 Materials and methods

3.2.1 Study area

This study was conducted in the Gilgel Gibe I watershed situated in Southwest Ethiopia, lying between latitudes 7°37'N and 7°53'N and longitudes 36°46'E and 37°43'E (Figure 3.1). The elevation of the study area ranges from 1,650 to 1,800 meters above sea level. The mean annual temperature in the area is between 15°C and 22°C, and the mean annual precipitation lies between 1800mm and 2300mm, with maximum rainfall from June till early September and minimum precipitation between December and January (National Meteorological Agency, 2012). The study area is characterized by different land use patterns. The main socio-economic activities of the inhabitants are farming and small stock rearing, with maize (*Zea mays*) and teff (*Eragrostis tef*) being the main crops cultivated in the area. The region is, however, also known for its coffee production. The average population density in this area is approximately 100 to 110 people/km².

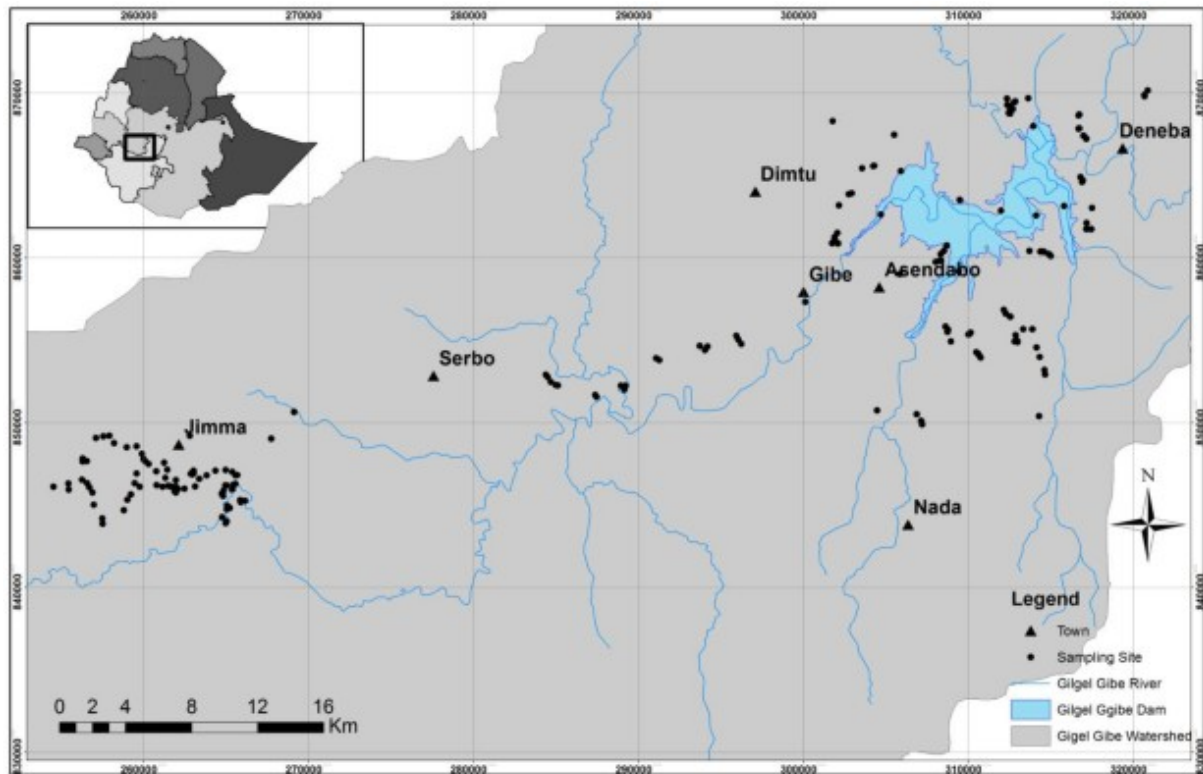


Figure 3.1. Map of the study area and sampling sites in the Gilgel Gibe I watershed, Southwest Ethiopia.

3.2.2 Data collection

3.2.2.1 Characterization of larval habitats

A total of 220 samples were taken at 180 different sampling locations (larval habitats) between August and October 2010 and September to November 2012. Selection of surveyed sites was based on previous reports on surface water quality monitoring (Mereta et al., 2012) and distribution of disease vectors in the region (Yewhalaw et al., 2011). Sampling sites situated in permanent habitats such as natural wetlands, reservoirs and streams were selected along a gradient of visible disturbance including point source pollution, land use patterns, hydrological modification and accessibility. Sampling sites situated in temporary breeding habitats were randomly selected from six villages located up to 8 km from the Gilgel-Gibe hydroelectric dam and from temporary pools located around permanent habitats. Permanent habitats were sampled at exactly the same location during both years, while the sampling location of temporary habitats changed depending on the availability of water. Temporary habitats are those containing water for a short period of time (approximately two weeks after

the end of rainy season). Semi-permanent habitats are those containing water for two to three months after the raining season ends. Permanent habitats are those containing water throughout the year (fed by surface or ground water) and are more stable systems. Surveyed habitats included: natural wetlands (n=60), breeding habitats around the shore of the dam reservoir (n=13), natural ponds (n=10), streamed pools (n=30), farm ditches (n=25), pits for plastering (n=40), rain pools (n=20), vehicle ruts (n=12) and animal hoof prints (n=10) (Figure 3.2). Detailed information on habitat condition, water quality, presence of anopheline larvae and mosquito predators and competitors was collected during the survey.



Figure 3.2. Different habitat types sampled: natural vegetated wetland (A), natural open water wetland (B), stream fringe (C), pond (D), maize field (E), brick pit (F), pool (G), rain pool (H), drainage ditch (I).

Data on the size of the water body (area), substrate type, vegetation cover, canopy cover and land use pattern were collected for each larval habitat. Water depth was measured using a metal ruler at different points of each habitat and average depth was recorded. Substrate was classified into clay, silt, sandy, gravel and artificial substrate (concrete, tire, plastic and mud pot). The emergent, submerged and floating plant cover of a habitat was visually estimated as the percentage cover of these aquatic macrophytes within a 500 meter stretch for large aquatic habitats and the entire area for smaller habitats. Plant cover was categorized as very low (<10%), low (10-35%), moderate (35-65%), high (65-90%) and very high (>90%) (Parsons et al., 2001). Canopy cover was defined as the amount of vegetation covering the water surface. Canopy within or the surrounding of the sampling site was estimated visually based on the percentage of shade (Posa and Sodhi, 2006). The type of land use adjacent to each sampling site was also recorded and checked with the available GIS data on land use. The map templates including land use types were obtained from the Ethiopian Ministry of Water and Energy.

3.2.2.2 Mosquito distribution mapping

Geographic coordinate readings were recorded for all sampling sites using a hand-held global positioning system unit (GPS) (Garmin GPS 60, Garmin international Inc., and Olathe, Kansas, USA). Coordinate readings were integrated into a GIS database using Arc MAP 10 GIS software. All digital data in the GIS were displayed in the World Geodetic System (WGS) 1984 Coordinate system.

3.2.2.3 Mosquito larvae sampling and identification

To collect mosquito larvae, ten dip samples were taken from each habitat using a standard 350 ml dipper (Clarke Mosquito Control Products, Roselle, IL). Mosquito larvae were also sampled using 5 ml graduated pipettes from water bodies, which were too small to use standard dippers. For small habitats such as hoof prints, several hoof prints were pooled to get the required sample volume (350 ml). Quantitative sampling from small habitats may overestimate larval density as compared to large habitats since larvae may not escape in small habitats where whole water can be sampled (Mutuku et al., 2006). The use of different sampling methods may affect the analysis of abundance data, which could be considered as a limitation of the study. Water collected by dippers was emptied into a white enamel sorting tray and mosquito larvae were sorted and identified to genus level as either anopheline or

culicine. The presence of mosquito immature stages was defined by the presence of at least one larva or pupa found in any of the ten dips.

3.2.2.4 Mosquito predator and competitor sampling and identification

Macroinvertebrates sampling and identification was based on Gabriels et al., (2010) and Bouchard (2004), respectively (Chapter 2). Each family was categorized into one of the five functional feeding groups (FFG): gatherer-collector, filterer-collector, predator, scraper, and shredder (Tomanova et al., 2006). When multiple possible FFGs were identified for a particular family, the most commonly occurring classification was used. All identified macroinvertebrates, their frequency of occurrence in the study area and their FFG are presented (Annex 3.1). Filter-collectors such as tadpole, black fly (Simuliidae), bivalve molluscs (Sphaeriidae) caddisfly larvae (Hydropsychidae) and culicine larvae were considered as competitors of anopheline larvae (Barbour et al., 1996). Fish and aquatic invertebrates belonging to the orders Hemiptera (water bugs), Coleoptera (Water beetles) and Odonata (dragonflies and damselflies) were considered as predators (Barbour et al., 1996).

3.2.2.5 Mosquito predator and competitor sampling and identification

Onsite physico-chemical parameters were measured using multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach). Water samples were analysed using standard methods as prescribed by APHA, AWWA, WPCF (1995) (Chapter 2).

3.2.3 Data analysis

We used classification and regression tree (CART) models, generalized linear models (GLMs) and ordination analysis to investigate the relationship between anopheline mosquito larvae occurrence and abundance and different biotic and abiotic variables. CART analysis is a form of binary recursive partitioning that can be used to classify field observations (Breiman et al., 1984). It has a number of advantages over traditional generalized linear models. First, it is well suited for analysis of complex ecological data with high-order interactions (Breiman et al., 1984; De'ath and Fabricius, 2000). Second, it captures non-linear relationships between explanatory and response variables (De'ath and Fabricius, 2000). Third, it does not rely on the assumptions that are required for parametric statistics and the analysis is not restricted by multicollinearity in predictor variables (Lewis, 2000). Fourth, missing values are not dropped from the analysis, instead variables containing information similar to that contained in the

primary splitter are used (Lewis, 2000). CART trees are also relatively simple for non-statisticians to interpret (Lewis, 2000). However, CART may produce different models depending on the selection of input variables (Prasad et al., 2006). GLMs are mathematical extensions of linear models that provide a less restrictive form than classic multiple regressions by providing error distribution for the dependent variable other than normal and non-constant variance functions (Zuur et al., 2009). They are also based on an assumed relationship called a link function between the mean of the response variable and the linear combination of the predictor variables (Guisan and Zimmermann, 2000). Ordination methods are widely used for community analysis (Jongman, 1995), and typically assume that abundance of individual species vary in a linear or unimodal manner along environmental gradients (ter Braak and Prentice, 1988; Guisan and Zimmermann, 2000]. Since the three approaches provide advantages as well as disadvantages with regard to data analysis we opted to draw conclusions based on all three of them.

We made Box-and Whisker plots in STATISTICA 7.0 (Statsoft, Inc, 2004) to visualize the abundance of mosquito predators and competitors in different habitat types. Abundance data were log transformed [$\log(x+1)$] prior to analysis. We used a non-parametric, Kruskal-Wallis test at a significance level of 0.05, to determine whether significant differences in the abundance of invertebrate predators and competitors existed between different habitat types.

3.2.3.1 Classification and regression tree models (CART)

Classification tree (CT) and regression tree (RT) models were used to model the occurrence (presence/absence) of anopheline larvae based on measured environmental factors. The detail description of the CART approach is given in Chapter 2. Conditional analysis was performed in order to see how different values of a predictor variable influence the abundance of anopheline larvae. For each of the three folds, the influence of predictor variables on the abundance of anopheline larvae was analysed. Regression equations obtained from the models were then used to calculate the abundance of anopheline larvae. This was done by taking minimum and maximum values of the predictor variables, while other parameters, which were present in the model, were kept constant at average values. Hence, for each of the three different models (folds) a line was plotted showing the relationship between the predictor variables and the abundance of anopheline larvae.

3.2.3.2 Generalized Linear Models

Generalized linear models were developed in R (version 2.15.1, The R Foundation for Statistical Computing, 2012) to determine which environmental (biotic and abiotic) variables significantly explained the occurrence and abundance of anopheline larvae. Prior to the modeling, we tested for collinearity among all predictor variables using Pearson correlation coefficient. Variables most highly correlated to the dependent variable were retained and any variable highly correlated to it ($r > 0.7$) was removed, until no further multicollinearity existed. Outliers were removed as well based on visual dot plots according to Zuur et al. (2009). We used logistic regression to model the occurrence of anopheline larvae. For the logistic regression, the response variable was transformed by the logit link function, which transforms bound probabilities (between 0 and 1) to unbound values (Ahmadi-Nedushan et al., 2006). Similarly, Poisson regression (log link function) was used to model the abundance of anopheline larvae. We started with a full model including all variables without interactions. The forward–backward stepwise model selection method using Akaike’s information criteria (AIC) was used to select the most parsimonious model (Akaike, 1974). A lower AIC indicates a better model. Models were fitted using a maximum likelihood method (Zuur et al., 2009). Homogeneity was checked by plotting residuals of every model against its respective predictors.

3.2.3.3 Ordination analysis

To determine whether a linear or unimodal type of response was present along environmental gradients, the data-set was first analysed using a detrended correspondence analysis (DCA) in CANOCO for Windows version 4.5 (ter Braak and Šmilauer, 2002). Redundancy analysis (RDA) was then used because all environmental gradients were shorter than 2 standard deviation units. In all RDA analyses, the abundance of anopheline larvae, predators and competitors were considered as response variables, whereas environmental variables were treated as independent variables. A preliminary analysis was performed to test multicollinearity in environmental variables. Variables with a variance inflation factor of 5 were removed from the analysis. Based on a stepwise forward selection, twelve environmental factors were selected as independent variables. Species and environmental data, except for pH, were log transformed [$\log(x+1)$] prior to analysis to stabilize the variance. The statistical significance of eigenvalues and species-environment correlations generated by the RDA were tested using Monte-Carlo permutations.

3.3 Results

3.3.1 Occurrence and distribution of mosquito larvae

Of the 180 sampling sites surveyed, anopheline larvae were more frequently occurring in pits dug for plastering, vehicle ruts and farm ditches and less frequently occurring in natural wetlands and ponds (Table 3.1). Overall, 1220 anopheline larvae individuals were found in 151 samples (69% of the samples). A total of 496 culicine larvae individuals were found in 62 samples (28% of the samples). The distribution of anopheline and culicine larvae is shown in figure 3.3a and 3.3b. The anopheline positive habitats were mainly located in agricultural and agro-pastoral land use types (Figure 3.3a). Anopheline larvae were sparsely distributed in natural wetlands.

Table 3.1. Distribution of anopheline larvae among different larval habitat types, Southwest Ethiopia.

Habitat type	No. of samples N=220	Anopheline positive samples n (%)
Natural wetlands	60	24(40)
Dam	13	7(54)
Farm ditches	25	23(92)
Pond	10	5(50)
Vehicle rut	12	11(92)
Stream pool	30	17(57)
Rain pool	20	17(85)
Pit for plastering	40	38(95)
Hoof print	10	9(90)

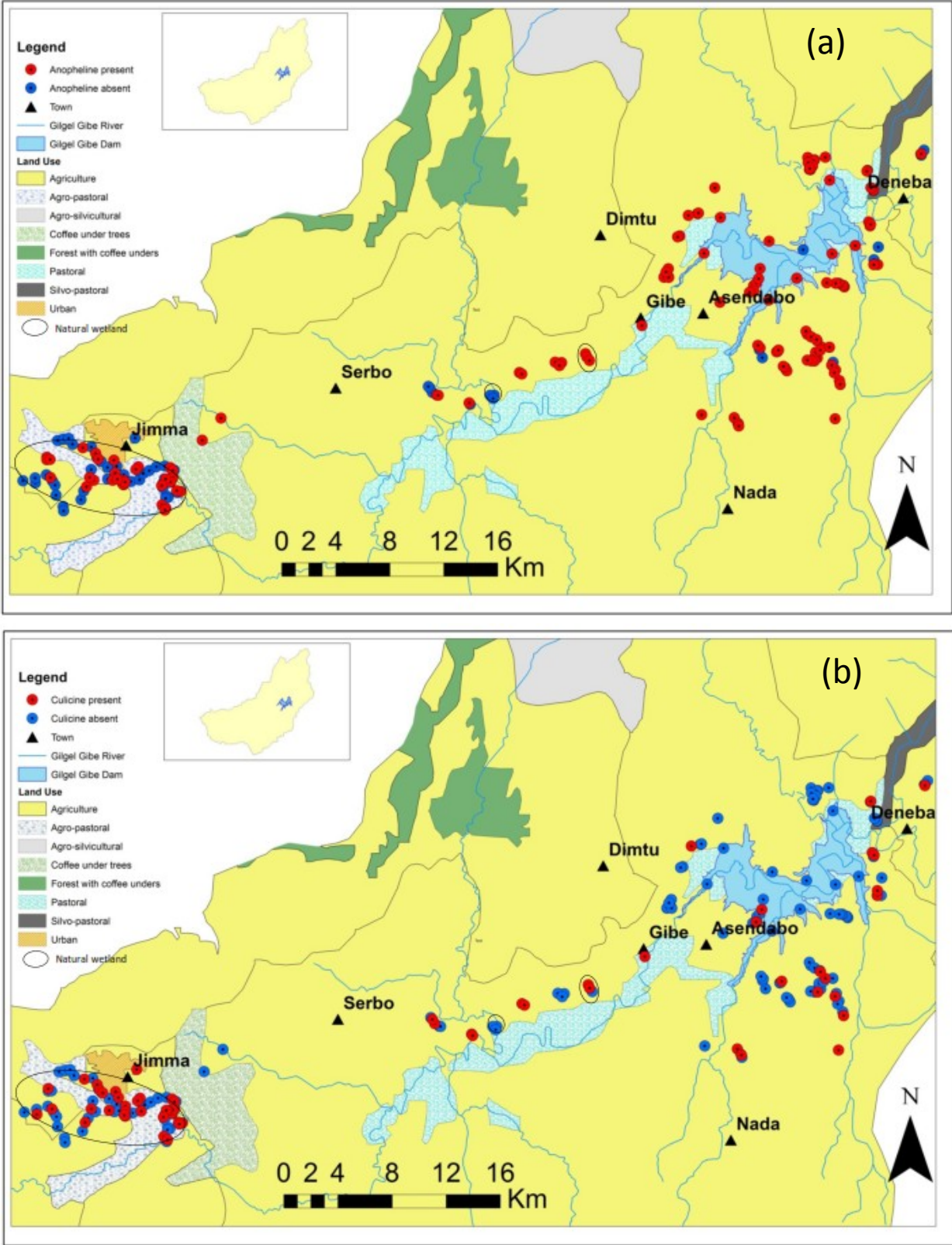


Figure 3.3. Map showing the distribution (presence (blue) and absence (red) of anopheline (a) and culicine (b) larvae in the Gilgel Gibe I sub-catchment, Southwest Ethiopia.

3.3.2 Influence of Environmental factors on the occurrence of anopheline mosquito larvae

Twenty five input variables were used to identify the main predictors of mosquito larvae occurrence and abundance (Table 3.2). Based on the three folds (one model for each fold) developed, the most frequently selected variables were habitat permanency (temporary, semi-permanent or permanent; 100%) and occurrence of predators and competitors (67%) (Figure 4). Moreover, habitat permanency was selected as the root of the tree for all models, indicating that this was the most important variable determining the occurrence of anopheline larvae. On the other hand, most of the water quality parameters such as turbidity, nitrate and orthophosphate were not selected by the models and thus were not considered as important predictors of occurrence of anopheline larvae.

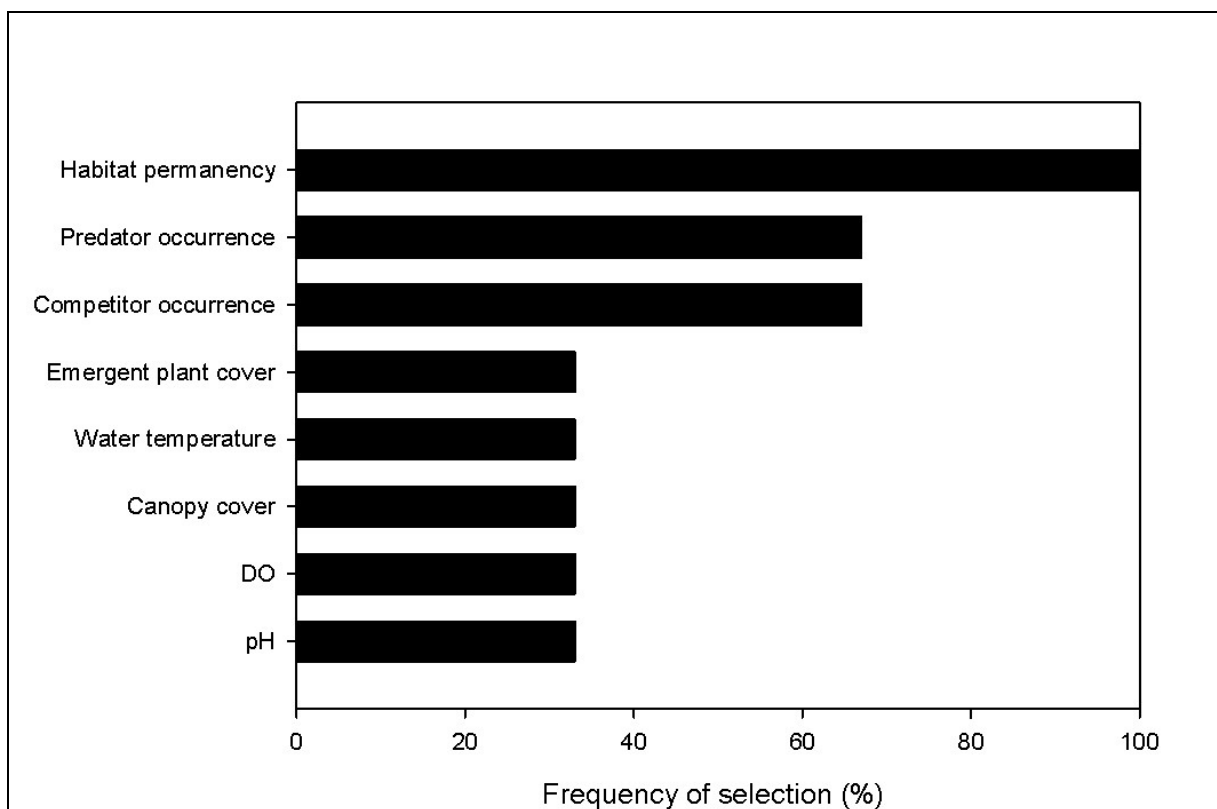


Figure 3.4. Frequency of selection of input variables used to construct the classification tree models.

Table 3.2. Input variables used for model development.

Variables	Unit	Min	25 Percentile	Median	75 Percentile	Max
Altitude	Meter above sea level	1655	1703	1716	1748	1823
Area	Hectare	0.002	0.05	0.3	1	7.8
Water depth	Meter	0.015	0.12	0.29	0.6	1.42
Canopy cover	%	0	0	0	0	100
Air temperature	°C	19	24.5	26.6	28.3	39
Water temperature	°C	16	22	23.5	25.6	34
pH	-	5.4	6.6	6.9	7.2	10
Dissolved oxygen	mg/l	0.47	3.1	4.8	6.2	10
Conductivity	µS/cm	21	68	89	125	513
Total dissolved solid	mg/l	15	52	80	145	425
Turbidity	NTU	4	21	65	176	894
Alkalinity	mg/l	0	40	50	67	250
Hardness	mg/l	0	20	31	48	160
Nitrate	mg/l	0	0.15	0.235	0.44	2.3
Ortho-phosphate	mg/l	0	0.038	0.07	0.11	1.4
Permanency	Temporary(1), semi-permanent(2), Permanent(3)	N/A	N/A	N/A	N/A	N/A

Emergent plant cover	Very low to very high 5 class (0-4)	0	0	2	3	4
Submerged plant	Very low to very high 5 class (0-4)	0	0	0	0	4
Floating plant	Very low to very high 5 class (0-4)	0	0	0	0	4
Habitat type	9 types (see table 3.1)	N	N	N	N	N
Substrate type	Silt(1), sandy(2), gravel(3), artificial substrate(4)	N	N	N	N	N
Land-use	9 types (See Fig. 3.3)	N	N	N	N	N
Fish	Absence(0), presence(1)	N	N	N	N	N
Invertebrate predators	Abundance	0	1	9	44	232
Invertebrate predators	Absence(0), presence(1)	N	N	N	N	N
Competitors	Abundance	0	0	2	8	23
Competitors	Absence(0), presence(1)	N	N	N	N	N

N= not applicable

The classification tree model with a moderate performance representing the occurrence of anopheline larvae, was depicted in Figure 3.5 as an example. The tree has six leaves and ten branches. Habitat permanency was selected as a root of this tree and was considered the most important variable predicting the occurrence of anopheline larvae. Generally, anopheline larvae were present in both temporary and semi-permanent habitats. In contrast, anopheline larvae were absent in permanent habitats, which mostly harboured natural predators and competitors of the mosquito larvae. The classification tree model had a very good predictive

performance, with a CCI of 86% and κ of 0.63. The average predictive performance of all three classification tree models (three folds) was very good (CCI = $84 \pm 2\%$, and $\kappa = 0.66 \pm 0.04$). The classification tree models for the three folds are presented in Annex 3.2.

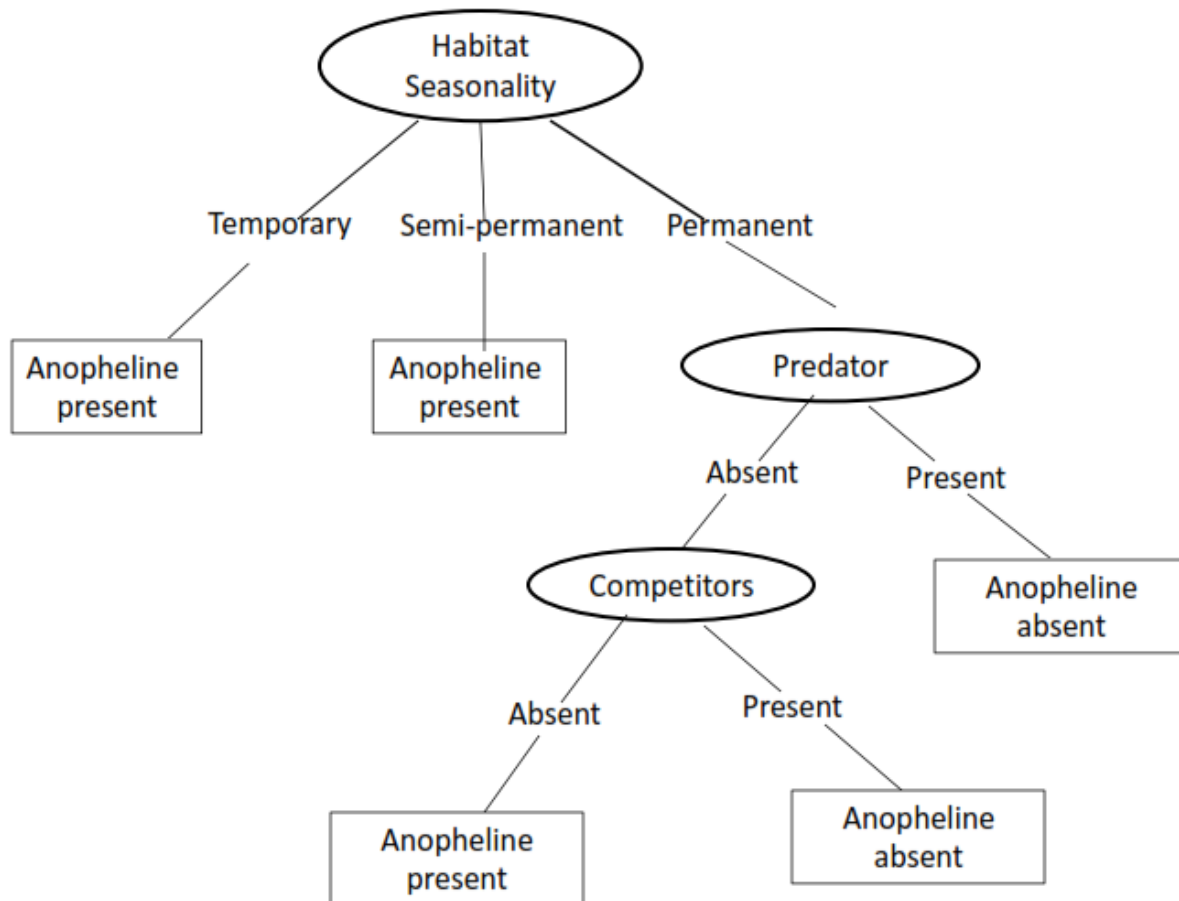


Figure 3.5. Classification tree model assessing the presence or absence of anopheline larvae (Correctly Classified Instances=86%, Cohens kappa statistic=0.63)

According to the logistic regression model, the most important variables that determined the probability of occurrence of anopheline larvae were: habitat type (explains 14% of the variance), permanency, canopy cover, emergent plant cover, occurrence of competitors, invertebrate predators and fish (Annex 3.3). The selected logistic regression model explained 67% of the total deviance ($R^2 = 0.67$, Brier score = 0.09) (Annex3.3). A plot of the logistic regression model shows that the probability of anopheline larvae occurrence was very low in the presence of invertebrate predators and competitors (Figure 3.6).

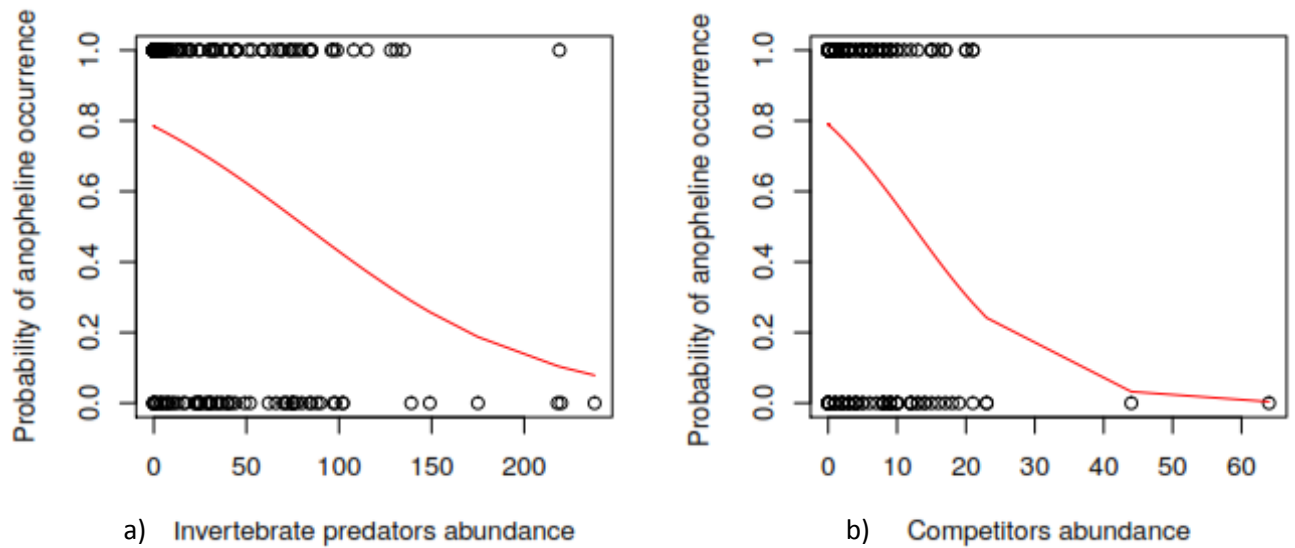


Figure 3.6. Logistic regression model predicting the probability of occurrence of anopheline larvae as a function of the abundance of invertebrate predators (a) and competitors (b).

3.3.3 Influence of Environmental factors on the abundance of anopheline mosquito larvae

The average determination coefficient obtained from the three regression tree models (three folds) analysing the abundance of anopheline larvae was 0.42 ± 0.02 (Annex 3.4). A conditional analysis of the regression tree model showing the effect of water temperature on the abundance of anopheline larvae is shown in figure 3.7a. A slight increase in anopheline larvae abundance was noted at a temperature between 17°C and 28°C , whereas an abrupt increase was observed between 28°C and 34°C . On the other hand, the abundance of anopheline larvae declined with increasing abundance of macroinvertebrate predators (Figure 3.7b). Similarly, the Poisson regression model indicates that the abundance of anopheline larvae was high when water temperature was high and the abundance of invertebrate predators and competitors was low (Annex 3.5).

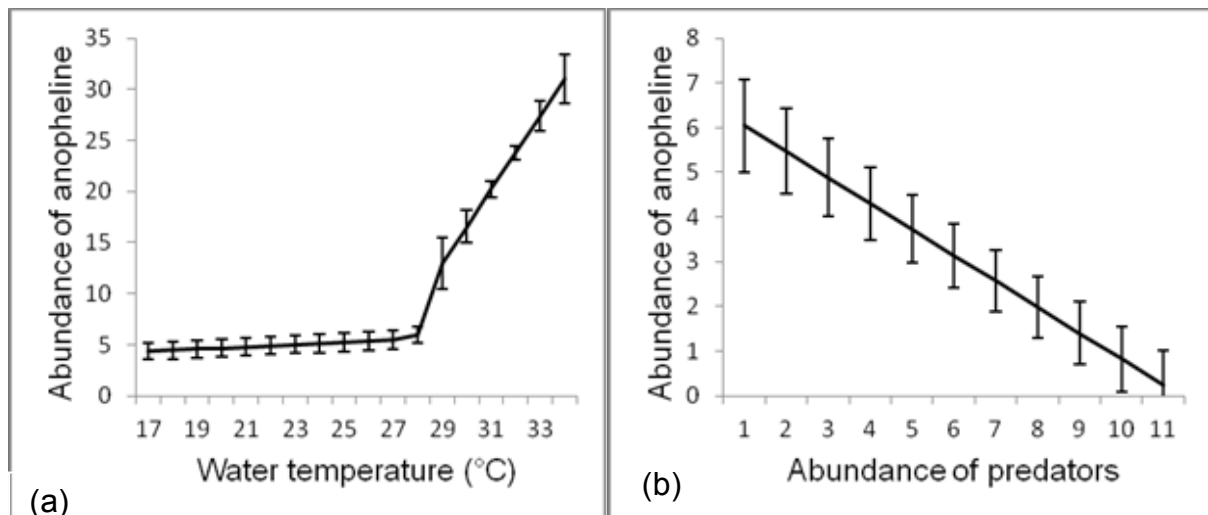


Figure 3.7. Conditional analysis of the abundance (number of individuals per sample) of anopheline larvae in function of (a) water temperature; (b) abundance of macroinvertebrate predators. Error bars indicate standard deviation among the three folds

The detrended correspondence analysis (DCA) gave a length of gradient smaller than 2 standard deviation units, implying that anopheline larvae exhibit a linear response to environmental gradients (ter Braak and Šmilauer, 2002). The association between anopheline larvae and the selected environmental factors was found to be significant ($p < 0.05$) for both the first axis and all canonical axes together (Figure 3.8). The variance of the RDA-biplot of anopheline larvae and environmental variables based on the first two axes explained 33% of the variance in anopheline data and 94% of the variance in the correlated and class means of anopheline larvae with respect to the environmental variables. The eigenvalues of the first two axes were 0.27 and 0.06, respectively. In this ordination, the anopheline larvae-environment correlation for the first two axes was 0.77 and 0.67, respectively. The first axis of the RDA ordination revealed a gradient primarily associated with habitat permanency. This axis was negatively correlated with the occurrence of anopheline larvae ($r = -0.8$, $p < 0.05$). The second canonical axis described the emergent plant and mosquito predators and TDS gradient.

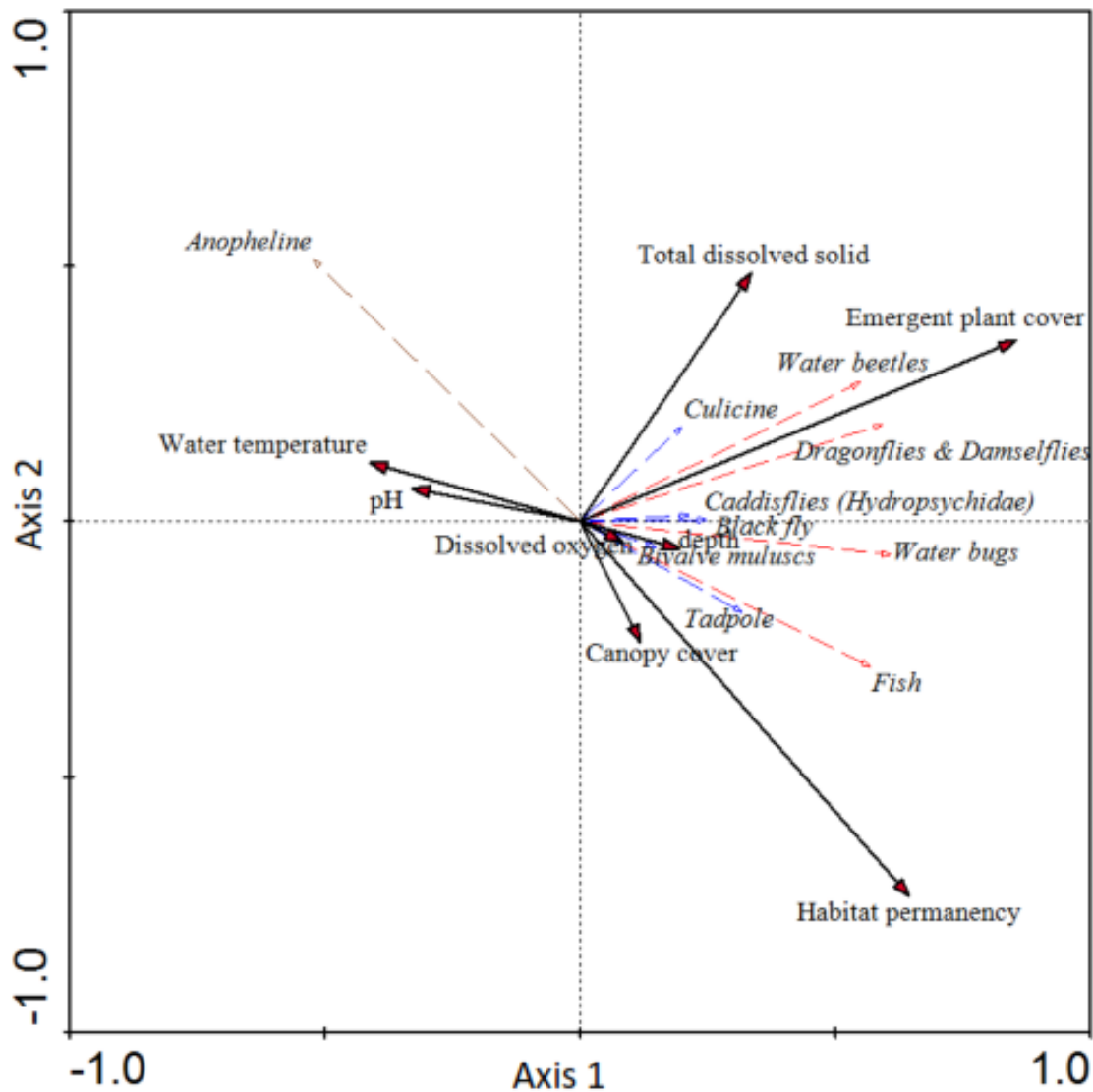


Figure 3.8. Ordination bi-plot of anopheline larvae, predators, competitors and environmental variables based on the redundancy analysis (RDA).

3.3.4 Relationship between the abundance of mosquito predators and competitors and habitat types

The Kruskal- Wallies test indicated that there was a statistically significant difference in the abundance of invertebrate predators ($\chi^2 = 93.2$, $df = 2$, $p < 0.05$) and competitors ($\chi^2 = 15.9$, $df = 2$, $p < 0.05$) among different habitat types (Figure 3.9). Permanent habitats support a significantly higher abundance of macroinvertebrate predators and competitors than temporary habitats ($p < 0.05$).

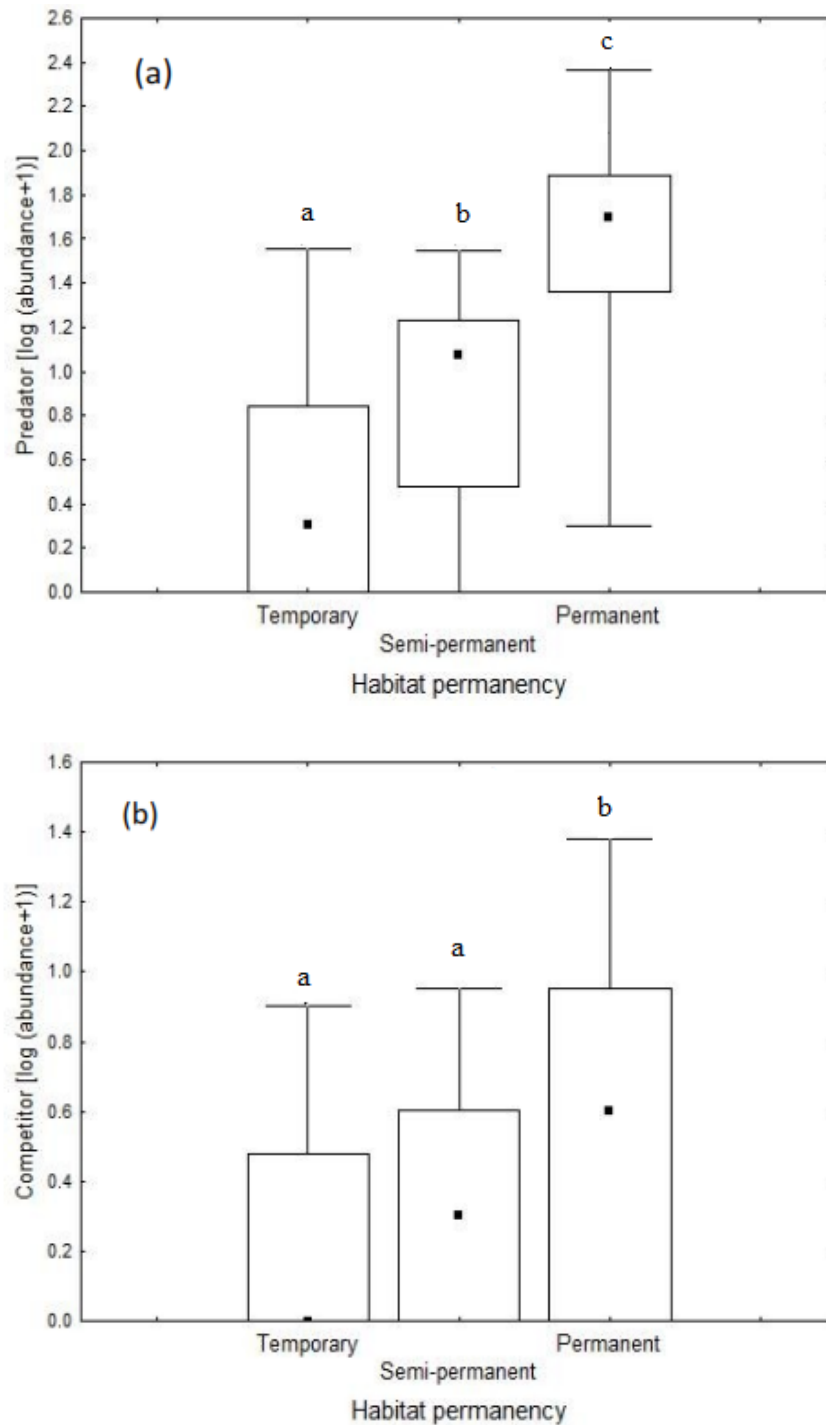


Figure 3.9. Box- and Whisker plots of the $\log(\text{abundance}+1)$ of predators (a) and competitors (b) in relation to habitat permanency. Small black squares represent median numbers, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values. a, b, c indicate statistically significant differences shown by Kruskal-Wallis test ($p < 0.05$).

3.4 Discussion

A fundamental understanding of the ecology of anopheline mosquito larvae is important in order to plan and implement effective malaria vector control intervention strategies (Gouagna et al., 2012). In the present study, habitat permanence, canopy cover, emergent plant cover and occurrence and abundance of predators and competitors were found to be the main variables determining the abundance and distribution of anopheline larvae in aquatic habitats.

This study revealed that the distribution and abundance of anopheline larvae was negatively correlated with emergent plant and canopy cover (Annex 3.3 and Annex 3.5). Anopheline larvae were more abundant in small temporary habitats exposed to sunlight with low emergent plant and canopy cover. Emergent plants and/or canopy cover reduces the amount of sunlight reaching the aquatic habitats, thereby reducing water temperature (Muturi et al., 2008). Low water temperature causes a decline in microbial growth upon which mosquito larvae feed (Muturi et al., 2008). Smaller water bodies are generally characterized by high water temperature, which eventually led to rapid larval development time (Culler and Lamp, 2009). The results of the conditional analysis and Poisson regression indicated that the abundance of anopheline larvae significantly increases when water temperature increases (Figure 3.7a, Annex 3.5).

Temporary water bodies such as agricultural ditches, rain pools, open pits for plastering and clay mining, vehicle ruts and hoof prints were the most preferred habitats (in terms of occurrence and abundance) for anopheline larvae. These habitats were either man-made or associated with anthropogenic activities. It should be noted that although many of these habitats, and especially hoof prints, are very small, they are very abundant in the landscape. Increasing human population in the catchment resulted in enhanced anthropogenic activities including deforestation, agricultural expansion, livestock rearing and brick making which could create more suitable habitats for mosquito larvae (Lambin and Geist, 2006; Norris, 2004). Clearing and drainage, often for agricultural expansion creates favorable habitats for mosquitoes, thereby increasing malaria transmission (Castro et al., 2010; Kamdem et al., 2012). In addition, agriculture can cause increased sedimentation due to erosion, which can slow or block streams and decrease the water depth, creating shallow waters ideal for mosquito breeding (Norris, 2004). Earth excavation for brick making, pot making and pits

dug for wall plastering provide a large number of mosquito larval habitats. In this study area, brick making activities were carried out in natural wetlands, where clay soil was used for brick making. In addition to creating mosquito breeding habitats, brick making is also considered as an important cause of deforestation, as it uses a huge amount of fire wood from wetland riparian forests. Deforestation may in turn alter the local microclimate and biodiversity (Broadbent et al., 2008), which in turn influences the distribution of malaria vectors.

Anopheline larvae were less frequently occurring and found at lower abundance in permanent habitats such as ponds, stream margins and natural wetlands. These habitats are home to a wide diversity of vertebrate and invertebrate predators and competitors and their presence likely suppresses the density of mosquito larvae (Paaijmans et al., 2010). Several studies pointed out that aquatic insects belonging to the orders Coleoptera, Odonata and Hemiptera are responsible for significant reductions in mosquito populations and could be considered in integrated vector management programs (Shaalán and Canyon, 2009). Predators reduce the abundance of mosquito larvae directly via predation, avoidance of oviposition or indirectly via competition for food resources (Knight et al., 2004). Some predators (especially those with chewing mouthparts) eat their prey (Odonata) but others suck the body fluid (hemolymph) of the prey (many beetle larvae and Hemiptera) (Shaalán and Canyon, 2009). Some species of mosquito larvae reduce the chance of predator detection by reducing their activities (Bond et al., 2005; Ferrari et al., 2010). However, this has the disadvantage of reducing feeding efficiency, which in turn prolongs larval development and is also likely to result in smaller adults with probably a reduced longevity and fecundity (Bond et al., 2005).

Previous studies have reported that the occurrence and abundance of mosquito larvae reduced in response to predator cues (Blaustein et al., 2004). For example, backswimmers (Notonectidae) released predator cues (kairomone) that have a potency to repel ovipositing female mosquito over a week (Shaalán and Canyon, 2009). The predator's cues are not only affecting mosquito's oviposition, but also cause a decrease in mosquito survival, delayed immature development and reduction in body size of emerged mosquitoes (Blaustein et al., 1995; Blaustein et al., 2004; Shaalán and Canyon, 2009). The abundance of anopheline larvae can be limited by the presence of competitors in permanent habitats (e.g. natural wetlands). Molluscs and anurans are the most common competitors, which feed on the same type of food as mosquito larvae. Several studies have shown that competitors decrease mosquito longevity

and increase the developmental time of mosquito larvae (Shaalán and Canyon, 2009). In this study, Box- and Whisker-plots showed that permanent habitats support a significantly higher abundance of macroinvertebrate predators and competitors than semi-permanent and temporary habitats (Figure 3.9). In addition, the conditional analysis, ordination diagram and GLMs demonstrated that the abundance of anopheline larvae was negatively related to invertebrate predators.

Permanent habitats such as natural wetlands in the vicinity of Jimma town were, opposite to what was expected, less suitable as breeding sites for anopheline larvae. This may be due to the high abundance and diversity of non-mosquito invertebrates and fish in these habitats, which could suppress mosquito population by predation and competition. This suggests that conservation of permanent habitats such as natural wetlands could be one strategy in the integrated malaria control program. The use of predaceous insects to control mosquito larvae is not only ecological friendly but also a means by which more effective and sustainable control can be achieved (Shaalán and Canyon, 2009). However, detailed knowledge on the interaction between mosquito larvae and their predators is very crucial for implementing successful vector control interventions. Contrarily, environmental modifications (e.g. drainage) of permanent habitats such as natural wetlands for malaria control could reduce the natural predator and competitor population densities, and thus be counter-productive and enhancing the occurrence and abundance of mosquito larvae.

The findings of this study suggest that malaria vector control intervention strategies in the study area should target (man-made) temporary water bodies. In view of the presence of insecticide resistant anopheline mosquito populations in the study area, targeting these temporary water bodies for anopheline mosquito larval control should be considered as an alternative next to traditional methods to reduce vector density and hence prevalence and/or incidence of malaria at local scale. The use of microbial insecticides such as *Bacillus thuringiensis* can be more environmental friendly in natural systems (Charbonneau et al., 1994). However, the use of chemical insecticides in natural systems may pose deleterious effect on non-target organisms such as predators and competitors. Since different anopheline mosquito species can have different micro-habitat preferences, it might be useful to identify habitat preferences of each anopheline mosquito species separately.

The findings of this study revealed that anopheline larvae were frequently occurring and more abundant in shallow temporary habitats. Their abundance is positively influenced by high water temperature and the absence of natural predators and competitors. Malaria vector control intervention strategies should target these temporary water bodies in order to optimize the efficacy of malaria control. The drainage or conversion of natural marshlands for larval control may not be an efficient vector control strategy as wetlands were not found to be the most prolific mosquito breeding sites in the study area. Moreover, degradation and conversion of these natural wetlands may have negative effects on the ecosystem services provided by these wetlands.

Chapter 4: Sediment and nutrient retention efficiencies of riverine wetlands in Southwest Ethiopia

Adapted from:

Mereta, S.T., Boets P., Endale, H., Yitbarek, M., De Meester L., Goethals, P.L.M., in preparation. Sediment and nutrient retention efficiencies of riverine wetlands in southwest Ethiopia.

Chapter 4: Sediment and nutrient retention efficiencies of riverine wetlands in Southwest Ethiopia

Abstract

Although wetlands in temperate regions have been extensively studied and generally are considered as natural systems of nutrient and sediment retention, the retention efficiency of natural wetlands in tropical environments is largely unknown. In this study, total suspended solid (TSS) and nutrients retention capacities of natural wetlands in Southwest Ethiopia were investigated. A mass balance approach was used to estimate the amount of TSS and nutrients retained at 40 different study sites located in four natural wetlands situated around (Jimma). A stepwise multiple regression analysis was used to investigate the relationship between retention and habitat disturbances. The mean net TSS retention of Awetu and Boye wetland was estimated to be 1691 and 77 tons/ha/year, respectively. In contrast, Kito and Kofe wetlands had a mean net release of 57 and 21 tons/ha/year, respectively. On the other hand, net nutrient retention varied between 0.2 to 14.8 tons/ha/year in Awetu and Boye wetlands for several nutrients measured, whereas Kito and Kofe wetlands had a net release between 0.02 to 2.9 tons/ha/year for different nutrients. This study indicates that TSS and nutrients retention efficiency of wetlands was influenced by the concentration of the inflow and the intensity of habitat disturbances and aquatic vegetation cover. Therefore, proper management of liquid and solid wastes generated from Jimma town, which could reduce the concentration of pollutants in the receiving tributaries and protection of wetlands from habitat alterations could improve their TSS and nutrient retention efficiency.

4.1 Introduction

Riverine wetlands are hydrologically connected to the river and surrounding catchment and are often important to regional hydrology (Ceballos et al., 2001; McJannet, 2007). These wetlands provide many ecosystem services such as providing habitat for many plants and animals including endangered species, providing products (such as fish, reed, timber, fuel, wood and medicines), mitigating floods, recharging aquifers, providing micro climate stabilization and improving water quality (Craft and Casey, 2000; Millennium Ecosystem Assessment, 2005; Mateos et al., 2009).

Water quality functions of wetlands are a composite of many different biogeochemical processes, which act collectively to alter and usually improve the quality of surface water (Hemond and Benoit, 1988; Mateos et al., 2009). Biogeochemical processes that occur within wetlands can effectively remove a variety of pollutants from the water column (Knox et al., 2008). These processes include microbial transformation to gaseous forms, plant uptake of nutrients, microbial degradation of pesticides and other organic compounds and sedimentation, (Blahnik and Day, 2000; Woltemade, 2000; Jordan et al., 2003; Fisher and Acreman, 2004; Knox et al., 2008).

Wetland ecosystems are effective sediment traps, generally intercepting and retaining more suspended sediments than they export (Fennessy et al., 1994; Christopher and David, 2004). Sediment deposition in wetlands is an important mechanism in improving water quality as sediments retained many nutrient and toxic substances through sorption processes (Clausen and Johnson, 1990; Cooper et al., 2000; Noe and Hupp, 2009). The retention of suspended solids in wetlands is controlled by particle size, hydrologic regime, flow velocity, wetland morphometry and residence time (Schubel and Carter, 1984; Reinelt and Homer, 1995; Verstraeten et al., 2006). Hydraulic resistance from the vegetation and soil decreases the velocity of water entering a wetland and enhances the settling and deposition of suspended solids (Reinelt and Homer, 1995; D'Arcy et al., 2007).

Wetlands can act as sinks and transformers of nitrogenous compounds by various mechanisms (Keenan and Lowe, 2001; Jordan et al., 2003; Day et al., 2004). These mechanisms include denitrification, assimilation, and retention by vegetation, and transformation to ammonia and organic nitrogen (Correll, 1994). Vegetation may influence denitrification and nitrification by influencing oxygen concentration of wetland substrate

within the rhizosphere (Mitchell et al., 1995). On the other hand phosphorous retention and removal from wetlands is driven by a combination of chemical, biological and physical processes (Reddy et al., 1999). These processes are regulated by vegetation, periphyton, plant litter and detritus accumulation, water flow velocity, water depth, hydraulic retention time and hydrologic fluctuations (Jordan et al., 2003, McJannet, 2007). Aquatic plants are an essential component in these processes since they increase nutrient retention through vegetative uptake and obstruction of flow, create root channels, thereby increasing the infiltration capacity, provide a large surface area for microbial growth and transport oxygen to anaerobic layers (Vought et al., 1994; Schoonover et al., 2005). Furthermore, aquatic plants create an ideal environment for denitrification by increasing the supply of potentially limiting organic carbon and nitrate to denitrifying bacteria (Weisner et al., 1994; Dhote, 2007).

The loss and degradation of wetlands however, has been reducing its pollutant mitigation potential (Hemond and Benoit, 1988; Mironga, 2005). The primary direct drives of degradation and loss include agriculture, discharging of untreated wastewater, overgrazing and deforestation (Millennium Ecosystem Assessment, 2005). Use of fertilizers to improve agricultural production leads to eutrophication of surface waters (Carpenter et al., 1998; Crumpton, 2001; Zhenlou et al., 2002). Moreover, in intense agricultural areas, riparian transport has been shown to contribute to the deposition of large amounts of sediment to riparian wetlands, which contributes to the degradation of water quality downstream (Heimann and Roell, 2000). These sediment loads result in sedimentation problems to reservoirs and dams as it reduces water storage capacity (Devi et al., 2008; Adwubi et al., 2009). For example, preliminary studies estimated that the volume of the reservoir of the Gilgel Gibe hydroelectric dam, situated in Southwest Ethiopia, will reduce by half within 12 years and would be completely filled with sediments and characterized by eutrophic conditions within 24 years, although it was expected to serve for at least 70 years (Devi et al., 2008).

Although wetlands in temperate regions have been extensively studied and generally are considered as natural systems of nutrient and sediment retention, the retention efficiency of natural wetlands in tropical environments is largely unknown because of a lack of research (McJannet, 2007). The objective of this study was to determine the TSS and nutrients retention efficiencies and to identify the effect of anthropogenic factors influencing the

retention of these pollutants in riverine wetlands. This information provides valuable input to develop wetland management practices in Ethiopia, where wetlands are important resources for food security and rural livelihood.

4.2 Methods and Materials

4.2.1 Study area

This study was conducted in the Awetu sub-catchment, part of the Gilgel Gibe I watershed, situated in Southwest Ethiopia and lying between latitudes 7°37'N and 7°53'N and longitudes 36°46'E and 37°43'E (Figure 4.1). The total area of the sub-catchment is about 500 km². Elevation of this sub-catchment area ranges between 1,700 and 2610 m a.s.l (Figure 4.2). The mean annual temperature is between 15°C and 22°C, and the mean annual precipitation is between 1500 mm and 2300 mm, with maximum rainfall from June till early September and minimum precipitation between December and January (National Meteorological Agency, 2012). Four riverine wetlands namely Awetu, Boye, Kito and Kofe were included in this study. These wetlands are varying in size ranging from 12 hectares to 111 hectares. Streams flowing through these wetlands contribute for about 25% to the flow of the Gilgel Gibe river (Mereta et al., unpubl. data), a major contributor to the Gilgel Gibe reservoir. The reservoir is situated 60 km downstream of the study wetlands.

These riverine wetlands have been extensively modified by human activities (Mereta et al., 2013). The major threats from human activities around and in these wetlands include disposal of domestic sewage, drainage, farming, clay mining, removal of riparian vegetation and uncontrolled livestock grazing (Mereta et al., 2013). Untreated wastewater and solid wastes generated by more than 200,000 inhabitants of Jimma town are directly dumped into the tributaries of Awetu and Boye wetlands (Mereta et al., 2013). In addition, river incisions and back erosions as a result of heavy rainfall, steep slopes and deforestation have been contributed to landslides in the catchment (Broothaerts et al., 2012). This may lead to extensive erosion from the upland areas and increase the sediment load in the receiving rivers and siltation problem to the Gilgel Gibe hydroelectric reservoir. The erosion rate in the Gilgel Gibe catchment is estimated to be 22 ton/ha/year (Devi et al., 2008).

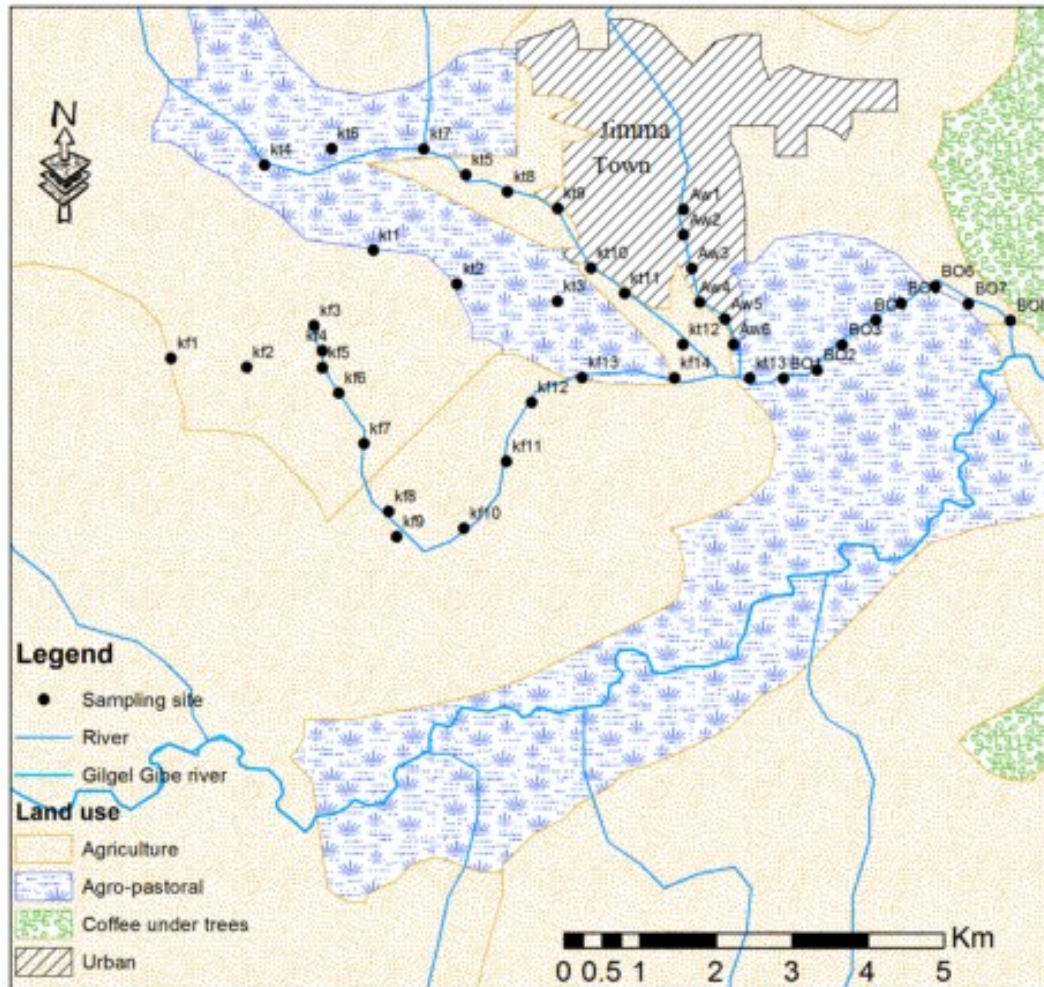


Figure 4.1. Location of wetland sampling sites and land use pattern, Awetu subwatershed, Southwest Ethiopia. Wetland name is represented by letters (Aw = Awetu; Kf = Kofe; K t = Kito and Bo = Boye).

A total of 40 sites were selected in these wetlands along a gradient of visible disturbance including both nearly non-impacted and heavily disturbed sites (e.g. presence of point source pollution, eutrophication, hydrological modification, etc.) and based on a previous study conducted by Mereta et al. (2013). The number of sampling sites was evenly distributed among the wetlands depending on their size, with the smallest wetlands having a lower number of sampling sites. Sampling was also performed before and after the confluence when one or more rivers joined since this might give an idea of the impact on the receiving wetland or stream.

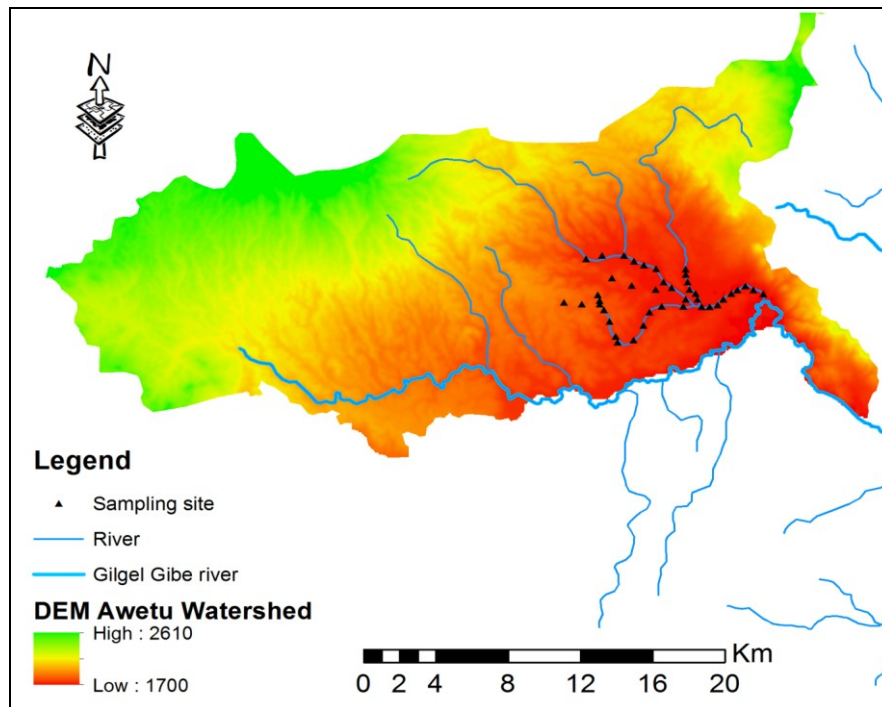


Figure 4.2. Digital Elevation Model (DEM) of Awetu watershed and wetland sampling sites, Southwest Ethiopia.

4.2.2 Data collection

4. 2.2.1. Habitat Sampling

Forty wetland sampling sites were monitored twice a year: once during the dry (February and March) and once during the wet season (i.e. after the end of rainy season) (October to November) in 2011. Awetu wetland was sampled only during wet season of 2011. In total, 75 samples were collected. Habitat characteristics were assessed at each sampling station using the USEPA wetland habitat assessment protocol (Baldwin et al., 2005). The degree of hydrological modifications (drainage, ditching and filling), habitat alteration (tree removal, tree plantation and grazing) and land use patterns such as waste dumping, clay mining, and farming were assessed during sampling. Land use, habitat alteration and hydrological modifications were quantified based on their intensity in the studied wetlands according to Hruby (2004). A score of 1 was awarded for no or minimal disturbance, 2 for moderate and 3 for high disturbance (Table 4.1). The final disturbance score was then computed by summing nine disturbance types. The final disturbance score ranged from 9 to 27 and was divided into five classes: 9-11= very low, 12-15 = low, 16-19 = moderate, 20-23 = high and 24-27 = very high.

Table 4.1. Criteria used for scoring habitat disturbances (Modified from Hruby, 2004). A score of 1 was awarded for no or minimal disturbance, 2 for moderate disturbance and 3 for high disturbance

Disturbance		Score = 1	Score = 2	Score =3
Habitat alteration	Grazing	Minimal grazing	Moderate grazing	High density grazing
	Vegetation removal	< 10% vegetation removal	10-50% of vegetation removal	> 50% vegetation removal
	Tree plantation	No tree plantation or plantation at > 50 m	Tree plantation at < 50 m but not in the wetland	Tree plantation in the wetland
Land use	Farming	No farming or farming at > 50 m from the wetland	Farming in a distance of < 50 m from the wetland	Farming in the wetland it self
	Clay mining	No clay mining at > 50 m from the wetland	Clay mining < 50 m	Clay mining in the wetland
	Waste dumping	No waste dumping	Waste dumping near the wetland	Active sign of waste dumping in the wetlands
Hydrological modification	Draining and ditching	No draining, nor ditching	Draining nearby < 50 m	Draining in the wetlands
	Filling	No filling	Filling near the wetland	Filling in the wetland
	Water abstraction	No dewatering	Dewatering near wetland	Dewatering in the wetland

4.2.2.2. Meteorological data collection

Climate data were collected for the year 2011. Daily weather data of the surrounding area was collected from Jimma meteorological station situated near the study wetlands, about 6 km from the farthest wetland site. The precipitation volume was measured with standard rain gauges. Water loss from evapotranspiration was measured by Piche evaporimeter. Ambient air temperature and relative humidity were measured by thermo-hygrometer.

4.2.2.3. Land use mapping

Geographic coordinate readings were recorded for all sampling sites using a hand-held global positioning system unit (GPS) (Garmin GPS 60, Garmin international Inc., and Olathe, Kansas, USA). Coordinate readings were integrated into a GIS database using Arc MAP 10 GIS software. All digital data in the GIS were displayed in the World Geodetic System (WGS) 1984 Coordinate system. The map templates including land cover types were obtained from the Ethiopian Ministry of Water and Energy. A digital elevation model (DEM) of the study area was created by digitizing the contour lines of the 1:50,000 scale topographic map (Ethiopian Mapping Agency, 1980). The contour lines have a vertical interval of 20 m. The created DEM has a spatial resolution of 20 m (Figure 4.2).

4.2.2.4. Water sampling and analysis

Onsite physico-chemical parameters were measured using multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach). Water samples were analysed using standard methods as prescribed by APHA, AWWA, WPCF (1995) (Chapter 2). For total suspended solid determination, a known volume of water was filtered through pre-dried (105°C; 12 h) and pre-weighed GF/F-filters. After sampling, the filters were dried in an oven at 103 to 105°C for 12 hours, cooled in a desiccator to balance temperature, and weighed. The amount of suspended matter was calculated as:

$$\text{mg total suspended solids} / L = \frac{(A - B) * 1000}{\text{Sample volume (ml)}} \quad (4.1)$$

Where:

A = weight of filter + dried residue (mg)

B = weight of filter (mg).

4.2.2.5. Measuring flow velocity and discharge

The volume of water inflow and outflow of the wetland was calculated by multiplying the area of water in the channel cross section by the average velocity of the water in that cross section. First, the channel cross section was divided into 5 to 10 subsections depending on the channel size. In each subsection, the area was obtained by measuring the width and depth of the subsection and by multiplying these values. The velocity was determined using a Valeport BFM001 channel flow meter. Measurements were repeated three times and the average value was used to calculate the discharge. The discharge in each subsection was calculated by multiplying the subsection area by the measured velocity. The total discharge was then calculated by summing the discharge of each subsection.

$$\text{Total discharge} = ((\text{Area}_1 * \text{Velocity}_1) + (\text{Area}_2 * \text{Velocity}_2) + \dots (\text{Area}_{10} * \text{Velocity}_{10})) \quad (4.2)$$

4.2.3 Loading rate and flux calculation

A nutrient and sediment load of the wetland was calculated by multiplying the measured concentrations by instantaneous discharge. Fluxes (surface loadings) were then calculated by dividing loading rate by wetland surface area (hectare). Rate of nutrient and sediment retention or release of each wetland site was calculated as the difference between the flux at the inflow and the flux measured at the outflow. The loading rates were corrected for water loss through evaporation and water input from precipitation.

$$\text{Retention} = (I+P) - (O+E) \quad (4.3)$$

Where I = Surface inflow, O = surface outflow, P = Precipitation, E = Evapotranspiration

$$\text{Loading (ton/year)} = \text{Concentration (ton/m}^3\text{)} * \text{Discharge (m}^3\text{/year)} \quad (4.4)$$

$$\text{Flux (ton / ha / year)} = \frac{\text{Loading (ton / year)}}{\text{Area (ha)}} \quad (4.5)$$

4.2.4 Data analysis

Nutrients and TSS retention data were log transformed [$\log(x+1)$] prior to analysis to meet normality assumptions. Principal Component Analysis (PCA) was used to reduce the dimensionality of the data and to address the problem of multicollinearity. We used direct

oblimin rotation with Kaiser Normalization to simplify the factor loading structure and to achieve more meaningful and interpretable solutions (Davis, 1986). We then removed variables from the analysis with communalities less than 0.5. A stepwise multiple regression was used to investigate the relationships between TSS and nutrient retention and disturbance types. In the preliminary analysis disturbance factors with an inflation factor greater than 5 were removed from the analysis to ensure that none of the models exhibited multicollinearity (Marquardt, 1970). Models were compared using ANOVA to determine whether there was a difference in the amount of variance explained by the independent variables. R^2 values of each model were also compared to gain the relative importance of each model. We used Wilcoxon rank-sum to compare TSS and nutrient retentions between dry and wet season samples. Statistical analysis was performed using SPSS version 16 statistical software (SPSS Inc., Chicago, IL).

Box- and Whisker plots were made in STATISTICA 7.0 (Statsoft, Inc.) to visualize the retention of TSS and nutrients at different levels of disturbance. A non-parametric, Kruskal-Wallis test was used at a significance level of 0.05, to determine whether significant differences in the retention of TSS and nutrient concentrations existed between different levels of disturbance.

4.3 Results

4.3.1 Patterns of precipitation and evapotranspiration in the study area

The wetlands water balance calculations considered precipitation and evapo-transpiration (ET) data of the catchment. Precipitation rate has high inter-monthly variations with an increase in the amount of rainfall in wet season (June to September) (Figure 4.3). The total annual precipitation at the study sites was calculated to be 1560 mm, two-third of which precipitates during rainy season (June to September). The highest monthly precipitation value measured in 2011 was recorded in June (a total of 311 mm). The total annual and mean monthly ET was 1065 mm and 89 mm, respectively. The rate of ET was higher than the rate of precipitation during dry season. The ratio of ET/precipitation in dry season was 1.6. In contrast, evaporation was lower than precipitation in wet season (ET/precipitation ratio was 0.2). The dry season discharges for all wetlands were about 25% lower than the wet season discharge.

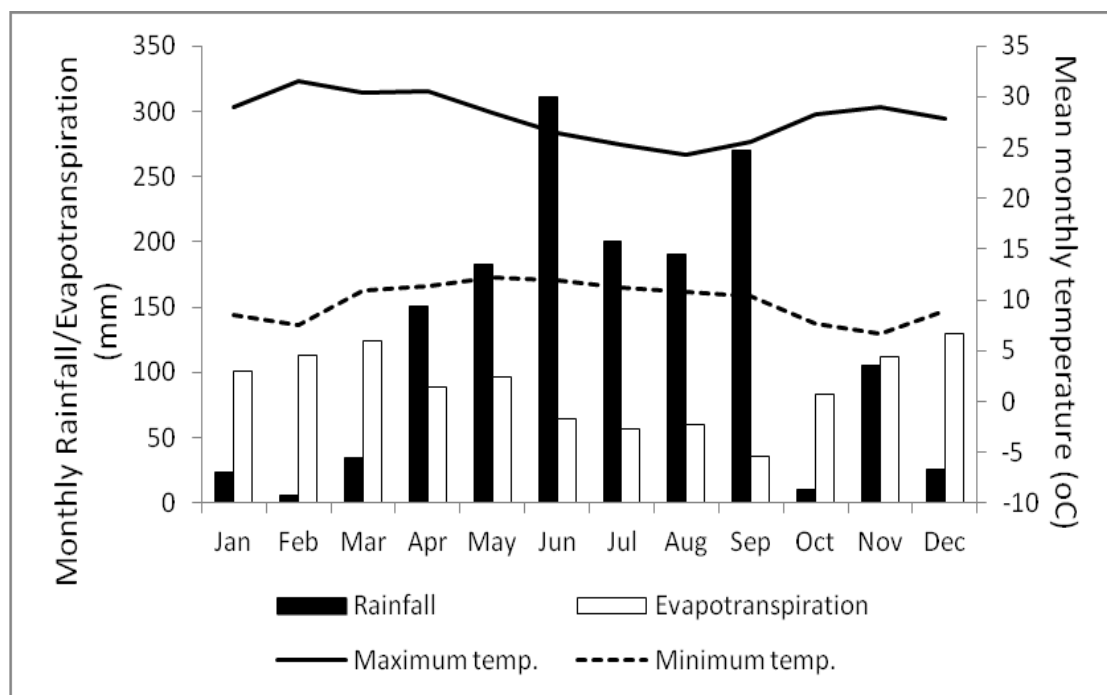


Figure 4.3. Observed monthly climate parameters (temperature, precipitation and evapotranspiration) in Jimma area, 2011.

4.3.2 TSS concentration, retention and release

4.3.2.1. Awetu-Boye wetland

Figure 4.4 shows the total suspended solid (TSS) concentration and retention in Awetu-Boye wetland at different sampling locations. In Awetu's first upstream sampling location (AW1), 547 tons of TSS per hectare per year was released, which could be considered as an input. The concentration of TSS decreased from 198 mg/l (first sampling location, AW1) to 67 mg/l (last sampling location, AW5). The net TSS retention in Awetu wetland was 1691 tons/ha/year, which means that 83% of the input is retained. Kito stream, having an average flow rate of 3.75 m³/s and a TSS concentration of 10 mg/l, joins the outflow of Awetu below AW5 and flows through Boye wetland. The first upstream sampling point of Boye wetland (Bo1) had a measured TSS concentration of 10 mg/l. The highest concentration of TSS (41 mg/l) was measured at Bo4, after the entrance of Becho-Bore stream having a flow rate of 0.5 m³/s and a TSS concentration of 88 mg/l. The net TSS retention in Boye wetland was 20 tons/ha/year (Table 4.2).

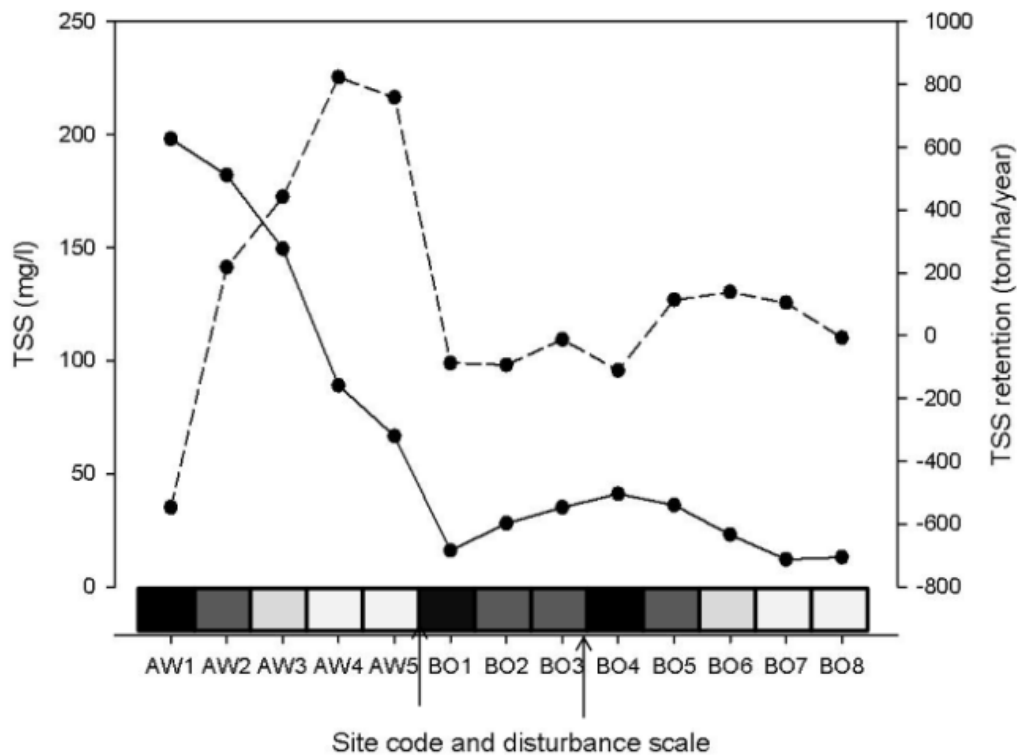


Figure 4.4. TSS concentration (solid line) and retention (dotted line) in Awetu-Boye wetland (Wet season). Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

4.3.2.2. Kito wetland

Figure 4.5 shows the total suspended solid (TSS) concentration and retention in Kito wetland. A reduction of the TSS concentration was observed in the first three upstream sites (Kt1 to Kt3). The highest release of TSS was measured at Kt5, in which 1330 ton/ha/year and 626 ton/ha/year was released during wet and dry season, respectively. The net TSS release in Kito wetland was 33 and 13 tons/ha/year for wet and dry season, respectively (Table 4.2). The net TSS release during wet season was significantly higher than during the dry season ($p = 0.009$).

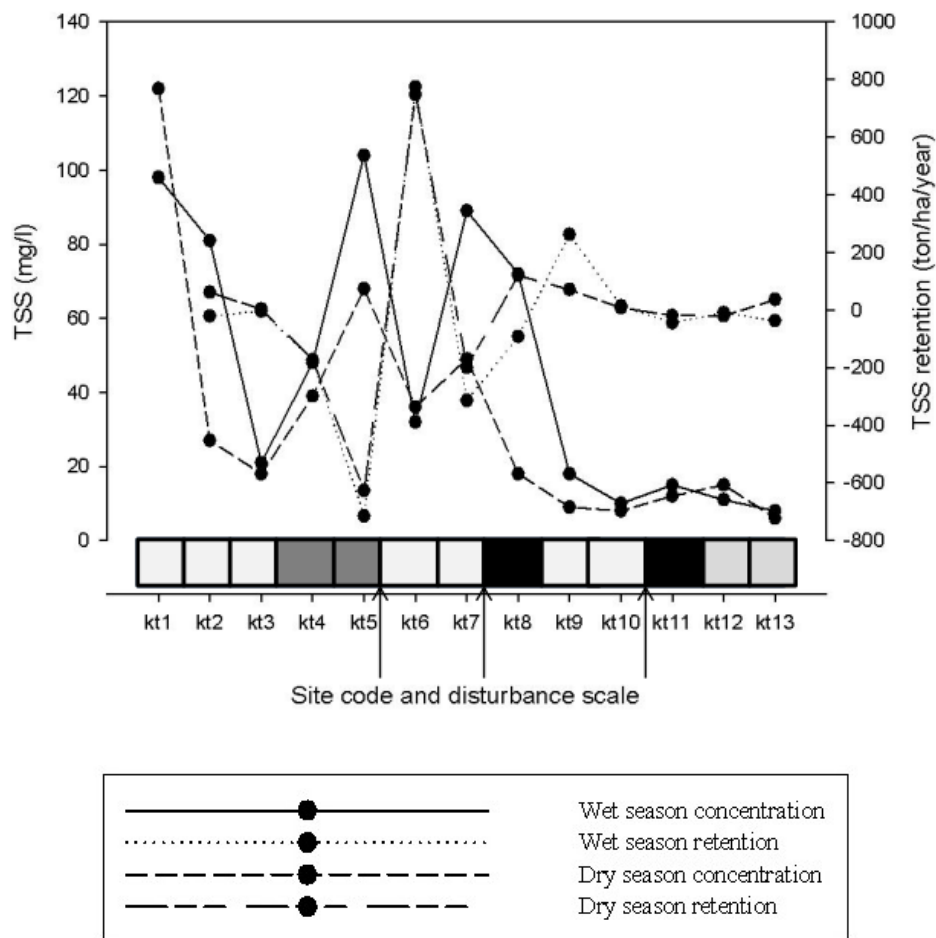


Figure 4.5. TSS concentration and retention in Kito wetland. Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

4.3.2.3. Kofe wetland

Figure 4.6 shows the total suspended solid (TSS) concentration and retention in Kofe wetland. TSS concentration reduced from 15 mg/l to 5 mg/l during wet season and from 37 mg/l to 16 mg/l during dry season, as water flew from Kf1 (upstream) to Kf4 (downstream). The highest release of TSS was measured at the more downstream location (Kf14), in which 40 mg/l and 17 mg/l was released during wet and dry season, respectively. The net TSS release was 80 and 34 tons/ha/year for wet and dry season, respectively (Table 4.2). The net TSS release during the wet season was significantly higher than during the dry season ($p = 0.002$).

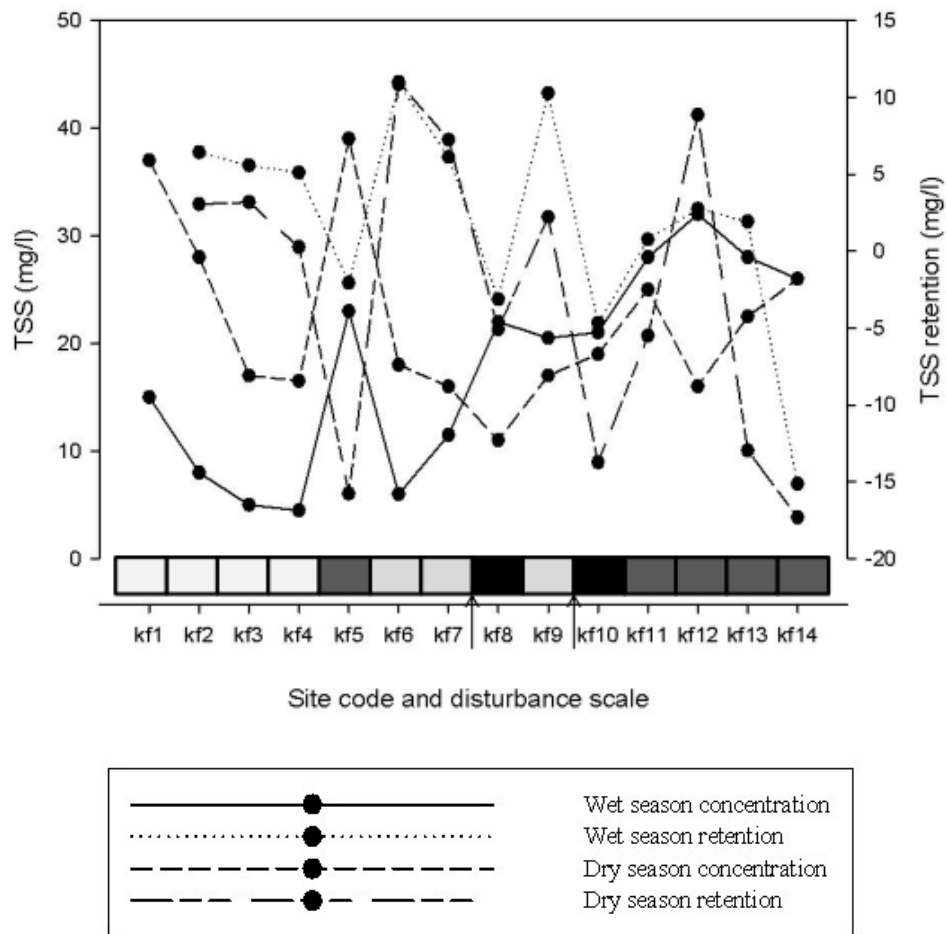


Figure 4.6. TSS concentration and retention in Kofe wetland. Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

Table 4.2. Net retention/release of sediment and nutrients in wet season and dry season of the studied wetlands and an indication of the differences between both.

Wetland	Parameter	Wet season		Dry season		p-value
		Net retention (ton/ha/year)	% retention	Net retention (ton/ha/year)	% retention	
Awetu	TSS	1691	83	Na	—	—
	COD	196	90	Na	—	—
	TON	3.76	56	Na	—	—
	NH ₄	2.65	69	Na	—	—
	NO ₃	0.97	27	Na	—	—
	TP	14.85	88	Na	—	—
	PO ₄ ³⁻	1.31	90	Na	—	—
Boye	TSS	20.24	77	39.47	62	0.08
	COD	71.57	74	66.62	79	0.72
	TON	10.08	64	8.59	78	0.62
	NH ₄ ⁻	0.94	85	0.19	88	0.06
	NO ₃ ⁻	7.23	63	6.83	48	0.93
	TP	1.5	97	1.41	98	0.97
	PO ₄ ³⁻	0.29	72	0.19	64	0.93
Kito	TSS	-33	-28	-12.9	-10	0.009*
	COD	-57.48	-40	-46.70	-75	0.45
	TON	-1.73	-83	-1.40	75	0.01
	NH ₄ ⁻	-0.28	-24	-0.27	-50	0.86
	NO ₃ ⁻	-5.6	-66	-11.9	-74	0.52
	TP	-1.47	-70	-1.37	-63	0.68
	PO ₄ ³⁻	-0.26	-54	-0.28	-60	0.95
Kofe	TSS	-80	-58	-34	-61	0.002*
	COD	-112	-59	-93	-54	0.34
	TON	-0.68	-31	0.8	-24	0.06
	NH ₄ ⁻	-0.19	-48	0.82	-54	0.81
	NO ₃ ⁻	-1.11	-46	-2.9	-64	0.52
	TP	-0.09	-43	-0.02	-51	0.88
	PO ₄ ³⁻	-1.17	-72	0.02	-70	0.52

*Significant at p<0.05

Na: no data available; a negative retention is considered the same as release

4.3.3 Nutrients concentration, retention and release

4.3.3.1. Awetu -Boye wetland

Nutrient concentration and retention in Awetu-Boye wetland is shown in Figure 4.7. Generally, measured nutrient concentrations decreased and retention increased in the Awetu downstream sampling locations, except for nitrate. Unlike other nutrients, nitrate concentration and retention was higher in the more downstream site (Aw 5). The percentage retention of these nutrients ranges from 27 to 90% (Table 4.2). In Boye wetland the measured nutrient concentration decreased from Bo1 to Bo3 except for TON. The highest concentration and lowest retention for all the nutrients was measured at Bo4. The percentage nutrient retention in Boye wetland ranged from 48 to 98% (Table 4.2). Overall, nutrient concentrations decreased as water flew from Bo4 (upstream) to Bo8 (downstream). The mean net retention of nutrients in Boye ranged from 0.19 to 10.1 tons/ha/year.

4.3.3.2. Kito wetland

Figure 4.8 shows the nutrient concentrations and retentions in Kito wetland. A reduction in nutrient concentrations was observed in the first three upstream sites (Kt1 to Kt3). The highest concentration and lowest retention was observed at Kt8. The percentage nutrient retention in Kito wetland ranged from 24 to 83% (Table 4.2). The net release of these nutrients ranged from 0.27 to 11.9 tons/ha/year. There was no statistically significant difference in nutrient retention between dry and wet season ($p>0.05$) (Table 4.2).

4.3.3.3. Kofe wetland

Figure 4.9 shows the nutrient concentrations and retentions in Kofe wetland. In general, the concentration of nutrients increased towards the downstream locations. The highest concentration was measured at Kt8. The nutrient retention ranged from 24 to 72% (Table 4.2). The net nutrient release ranged from 0.02 to 11.9 tons/ha/year. There was no statistically significant difference in retention between dry and wet season ($p>0.05$).

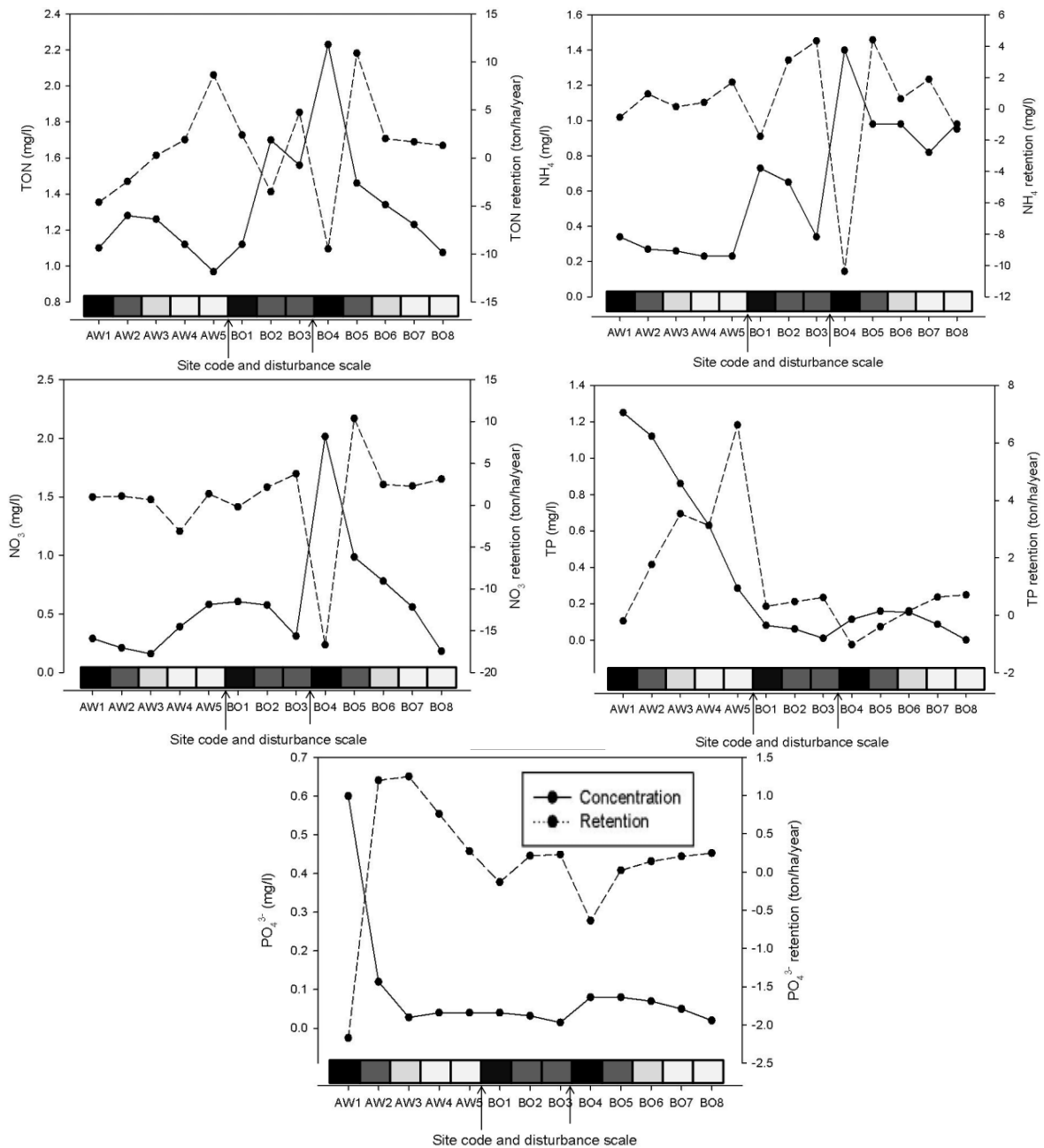


Figure 4.7. Nutrient concentration and retention in Awetu-Boye wetland (Wet season). Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

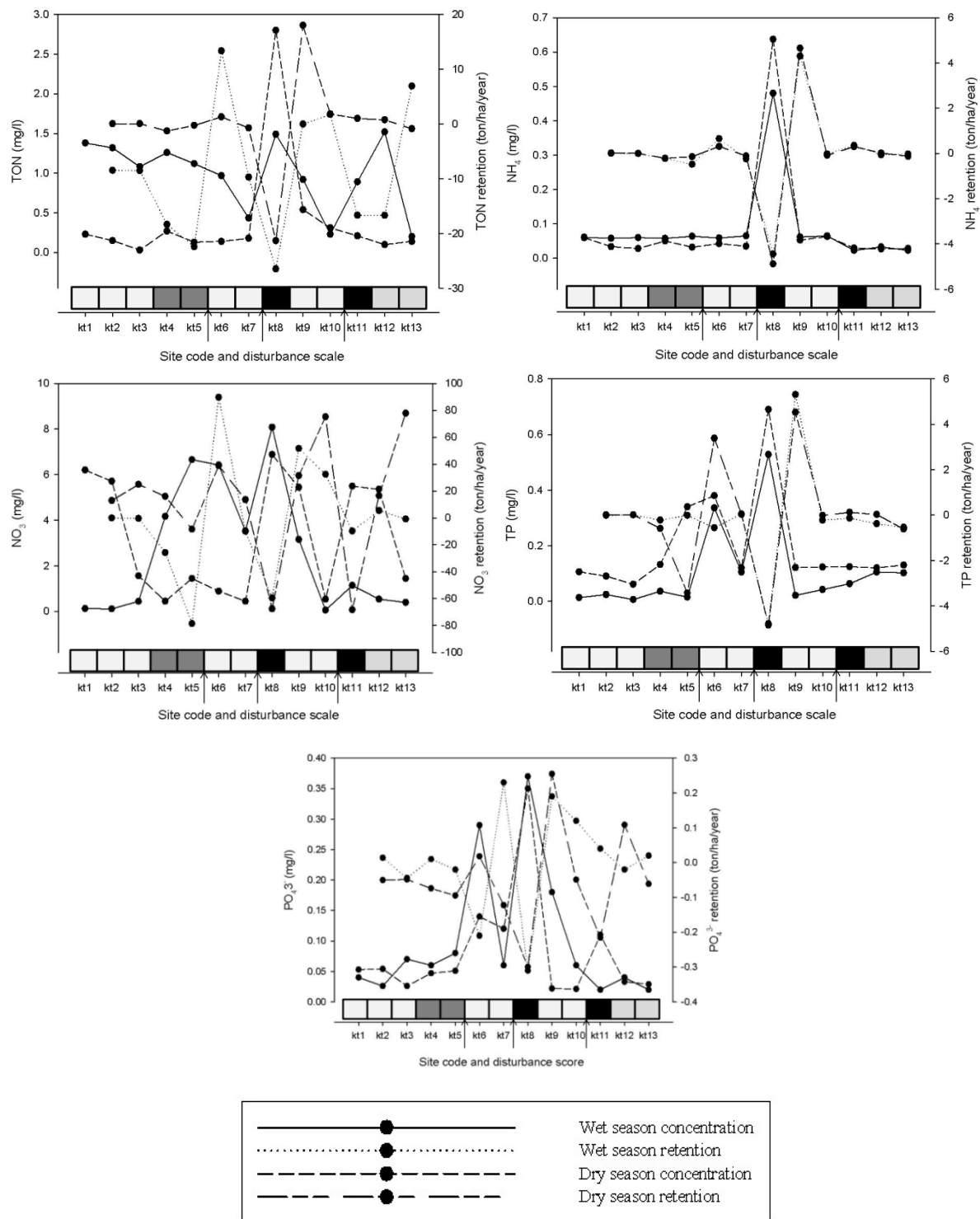


Figure 4.8. Nutrient concentration and retention in Kito wetland. Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

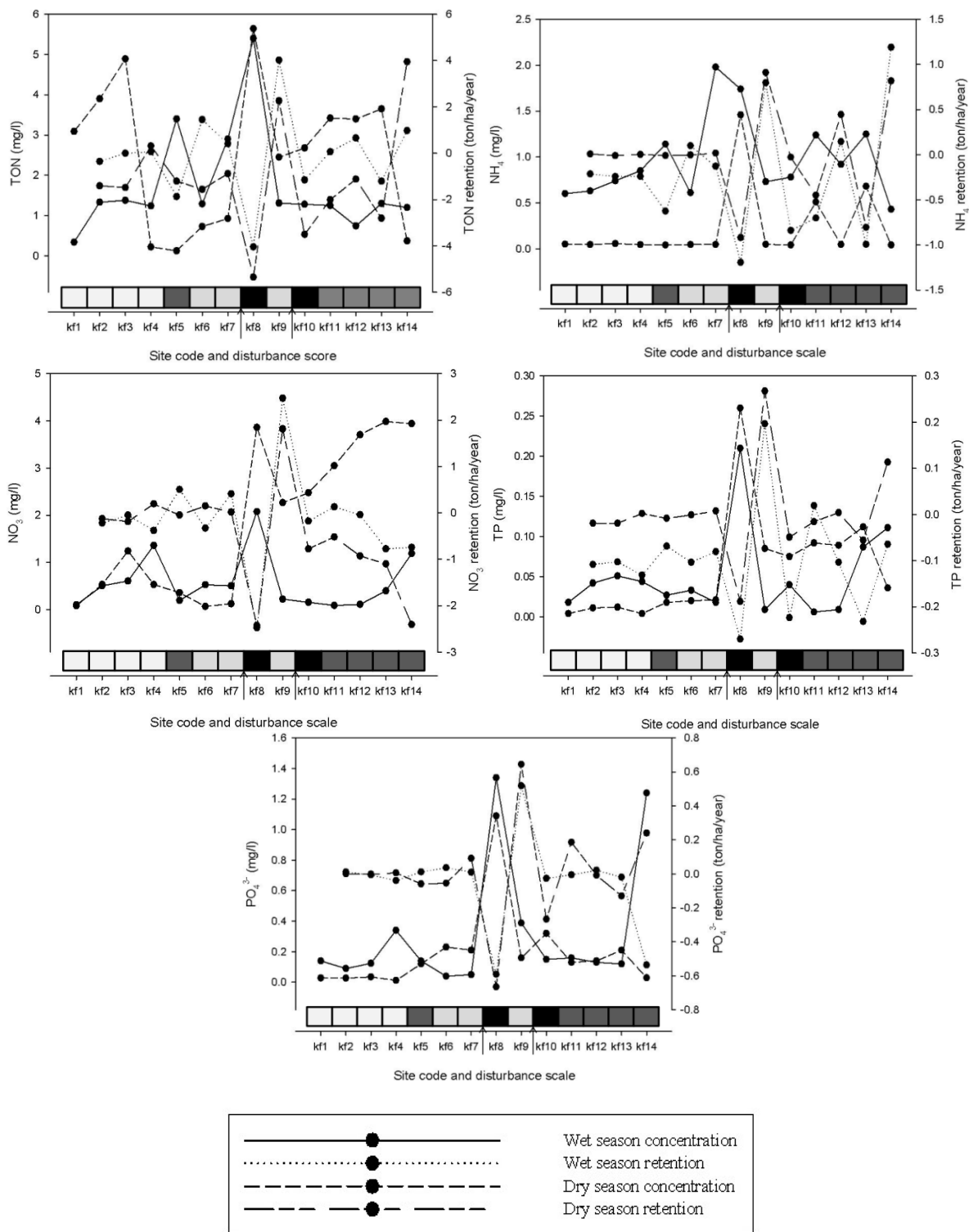


Figure 4.9. Nutrient concentration and retention in Kofe wetland. Disturbance scale ranges from very low (white) to very high (black). An arrow indicates a site where a tributary enters into the main stream.

4.3.4 Factor loading for TSS and nutrient retention data

The PCA yielded two components for both TSS and nutrient retention data. These components explained 73% of the total variation in the data. The factor loadings for the components are shown in Table 4.3. In all cases, the communalities for the variables were greater than 0.62, which indicates that the components extracted could explain more than 62% of the variation in the data. The disturbance types that contributed most to the variation in the PC1 were: draining, farming, vegetation clearance, clay mining, grazing, filling and waste dumping. The second axis was mainly related to plantation and water abstraction.

Table 4.3. Factor loadings for TSS and nutrient retention. Higher factor loadings are marked in bold

Disturbance	TSS retention Component (Axis)		Nutrient retention Component (Axis)	
	1	2	1	2
Draining	0.961	0.079	0.967	0.064
Farming	0.933	0.005	0.934	-0.003
Clearance	0.912	0.101	0.916	0.088
Mining	0.887	-0.113	0.894	-0.133
Grazing	0.874	-0.082	0.870	-0.083
Filling	0.825	0.149	0.829	0.137
Dumping	0.621	0.343	0.624	0.334
Plantation	-0.004	0.735	-0.002	0.748
Abstraction	0.412	-0.659	0.408	-0.659

4.3.5 Relationship between TSS retention and habitat disturbance

Among the nine disturbance factors, draining and vegetation clearance were positively and highly correlated with farming and had variance inflation factor of 11 and 6 respectively. Accordingly, these two variables were excluded from the final analysis. Out of the seven remaining variables, only four disturbance types contributed to the final linear regression model. This model explains 73% of the variation in TSS retention ($N = 75$; $R^2 = 0.73$; $p < 0.001$). The retained variables for the final model were: farming, waste dumping, clay mining and grazing. Farming alone explains 58% of the variation ($R^2 = 0.58$) (Annex 4.1). The four variables were used in the following regression equation:

$$Y = 4.220 - 1.382X_1 - 0.830X_2 - 0.736X_3 - 0.716X_4$$

Where Y = TSS retention; X₁ = Farming; X₂ = Waste dumping; X₃ = Clay mining; X₄ = Grazing

The Kruskal-Wallis test indicated that there was a statistically significant difference in TSS retention ($\chi^2 = 31$, $df = 4$, $p < 0.05$) among different classes of disturbance (Figure 4.10a). Very low disturbed sites had significantly higher TSS retention than moderately to very highly disturbed sites ($p < 0.05$). However, there was no statistically significant difference in retention among moderately to very highly and between low and very low disturbed sites ($p > 0.05$).

4.3.6 Relationship between nutrient retention and habitat disturbance

The developed stepwise multiple regression model was able to explain 28% of the variation in nutrient retention data ($N = 355$, $R^2 = 0.28$, $p < 0.001$). The retained variables for the final model were: grazing, waste dumping and farming. Grazing alone explained 25% of the variation ($R^2 = 0.25$) (Annex 4.1). Three variables were included in the regression equation:

$$Y = 3.319 - 1.009X_1 - 0.445X_2 - 0.385X_3$$

Where Y = Nutrient retention; X₁ = Grazing; X₂ = Dumping; X₃ = Farming

The Kruskal-Wallis test indicated that there was a statistically significant difference in nutrient retention ($\chi^2 = 98$, $df = 4$, $p < 0.05$) among different classes of disturbance (Figure 4.10b). Very low and low disturbed sites had a significantly higher retention than moderately to very highly disturbed sites ($p < 0.05$). However, there was no statistically significant difference among moderately to very highly and between very low and low disturbed sites ($P > 0.05$).

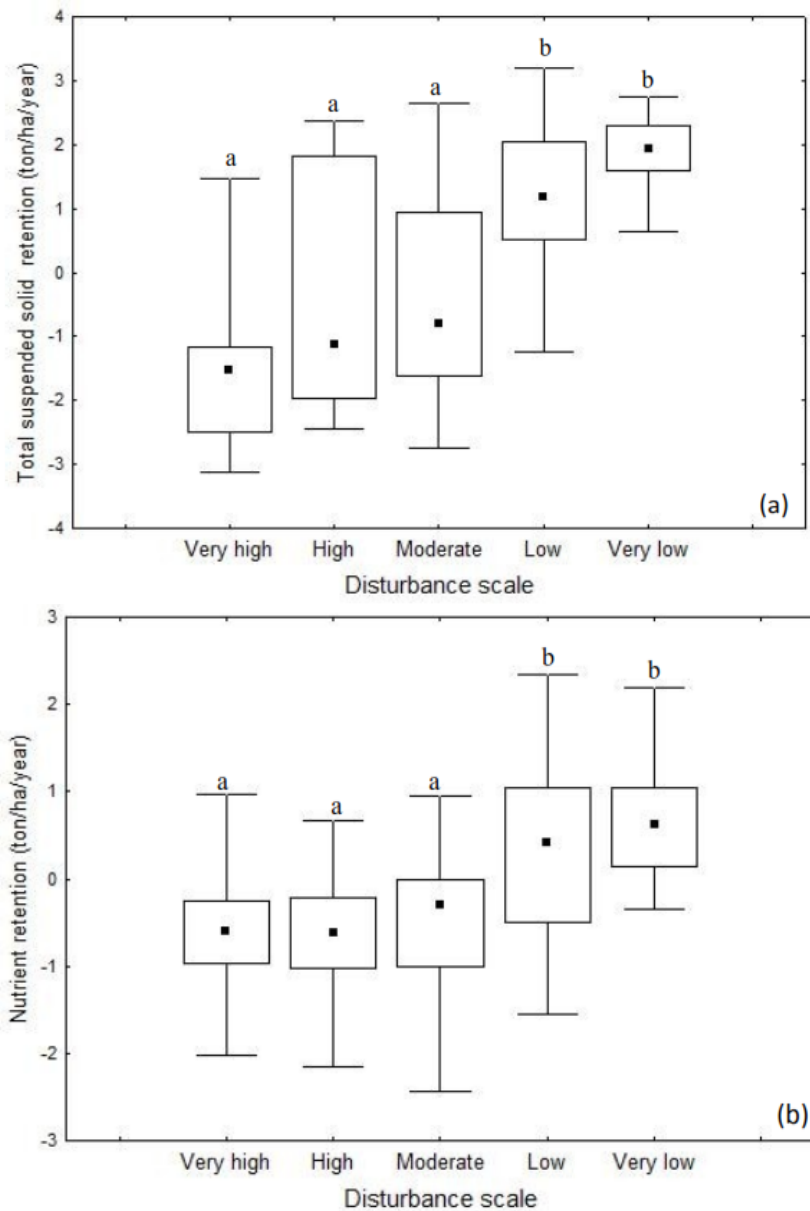


Figure 4.10. Box- and Whisker plots of the TSS (a) and nutrient (b) retention $\log(x + 1)$ in relation to the disturbance classes. Small black squares represent median numbers, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values. a, b, c indicate statistically significant differences shown by Kruskal-Wallis test ($p < 0.05$). Nutrient retention refers to the retention of total organic nitrogen, ammonia, nitrate, total phosphorous and ortho phosphate.

4.3.7 Influence of aquatic vegetation on TSS and nutrient retention

The linear regression analysis revealed that both the TSS and nutrient retention are predicted best by the aquatic vegetation cover ($P < 0.001$). The retention of both TSS and nutrients significantly increased as vegetation cover increased (Figure 4.11). Net release (negative retention) of TSS and nutrients was observed at low aquatic vegetation cover.

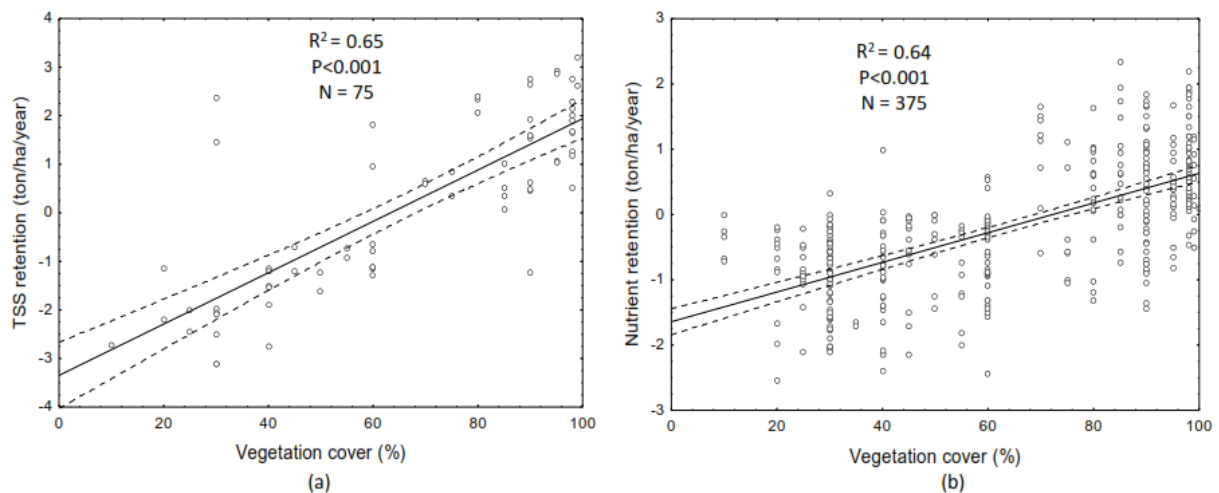


Figure 4.11. The effects of percentage vegetation cover on Total Suspended Solids (TSS) (a) and on nutrients (b) retention in riverine wetlands. Retention values are log transformed. Nutrient retention refers to the retention of total organic nitrogen, ammonia, nitrate, total phosphorous and ortho phosphate.

4.4 Discussion

Natural wetlands are key landscape elements and provide a wide range of ecological and socio-economic functions including sediment and nutrient retention (Bondar et al., 2007). In this study natural wetlands showed a variable retention of TSS and nutrients due to the differences in input concentration and degree of habitat disturbance. Awetu and Boye wetlands, receiving untreated wastewater from Jimma town, retained a substantial amount of total suspended solids (TSS) and nutrients. In contrast, Kofe and Kito wetlands, which are considered more natural, particularly in the upstream sites, were characterized by a release of TSS and nutrients. Overall, the retention and/or release capacity of these wetlands was largely governed by the mass loading rate (i.e. quality of the input water), intensity of habitat disturbances and percentage vegetation cover.

The mass balance analysis generally showed that the net TSS retention in Awetu wetland was nearly 80 times higher than in Boye wetland. This may be due to the indiscriminate discharge of untreated wastewater and dumping of solid wastes into Awetu river, which flows through Awetu wetland and then to Boye wetland. This high input of liquid and solid wastes may contribute to the very high measured TSS concentration in Awetu 1, which was 198 mg/l (547 ton/ha/year releases). Nutrient retention in Awetu wetland varied from 27 to 90%. Studies have shown that higher mass loading rates resulted in higher sediment removal rates in constructed wetlands (Tanner et al., 1995; Redmound, 2012). Although Awetu wetland retained 83% of the incoming TSS, the outflow concentration (67 mg/l) was still higher compared to the measured concentration in the other wetlands.

Boye wetland can be considered very important for the retention of TSS and nutrients. The average retention was 70% and 76% for TSS and nutrients, respectively. There was no significant difference in retention capacity between dry and wet season. The highest concentration and lowest retention for TSS and all the nutrients was observed at Bo4. This is due to the entrance of Becho-Bore stream at Bo4 containing a high concentration of TSS and nutrients. However, the retention capacity was higher in the downstream sites, compared to the upstream sites. Distance from the inflow and the resultant reduction of water velocity are known to have an effect on the sedimentation rate and nutrient retention (Kadlec, 2009). Moreover, the downstream sites were highly vegetated, which reduced flow velocity and increased sedimentation rate. This is also supported by the regression analysis which indicated that percentage vegetation cover was strongly related to TSS and nutrient retention capacity of wetlands.

Aquatic vegetation increases nutrient retention through vegetative uptake, reduced flow velocities and thus facilitates sedimentation (Stevfnson, 1988). In addition, the vegetation creates root channels thereby increasing the infiltration capacity and provides a large surface area for microbial growth and transport of oxygen to anaerobic layers (Vought et al., 1994; Schoonover et al., 2005), which favors nutrient degradation. Rooted macrophytes reduce the amount of deposited sediment that can be resuspended by wave and current action and exported from a wetland system (Madson et al., 2001). Furthermore, aquatic plants create an ideal environment for denitrification by increasing the supply of potentially limiting organic carbon and nitrate to denitrifying bacteria (Weisner et al., 1994; Dhote, 2007).

Kito and Kofe wetlands were characterized by a net release of TSS and nutrients both in dry and wet seasons. TSS releases were significantly higher in wet season than in dry season. The increased rainfall during wet season can be attributed to an increase in hydraulic loading and decrease in residence time, which in turn affects the sedimentation of TSS in wetlands (Wilson et al., 2011). Sampling sites located in the upstream part of Kito and Kofe wetlands are more natural and were characterized by better water quality compared to the downstream sampling sites. Although Kito and kofe wetlands had a net release of TSS and nutrients, the outflow concentrations are much lower than that of Awetu and Boye wetland that were characterized by net retentions.

Habitat disturbances, particularly the conversion of riparian wetlands into cultivated land, livestock grazing, clay mining and waste dumping may contribute to the release of TSS and nutrients in these wetlands. The stepwise regression analysis revealed that farming explains 58% of the variation in TSS retention. It has been estimated that about 48% of the catchment is agricultural land (Bizuayehu, 2002). Drainage and vegetation clearing of wetlands for agricultural production results in enhanced degradation of the soil organic matter and leads to accelerated rates of soil erosion within agricultural landscapes, which might increase the sediment load in surface waters and cause major modifications to terrestrial carbon, nitrogen and phosphorous cycling (Oenema and Roest, 1998; Quinton et al., 2010). Knox et al. (2008) reported that wetlands drained for agricultural use were characterized by lower retention rates and higher export of nutrients and sediments compared to natural reference wetlands. Vaithyanathan and Correll (1992) also indicated that the flux of phosphorous associated with runoff from an agricultural watershed was found to be 8 to 10 times higher than that from a similar, but forested landscape.

Uncontrolled livestock grazing in wetlands can contribute to the release of TSS and nutrients. This study indicated that grazing explains 25% and 3% of the variation in nutrients and TSS retention, respectively. Grazing alters the hydrology and the drainage pathways at a site by compacting the topsoil, which in turn decreases the infiltration capacity of the soil (Gathumbi et al., 2004; Pietola et al., 2005) and, consequently, leads to an increase in the release of nutrients and sediments by erosion (Kurz et al., 2005). In addition, grazing may lead to alteration in wetland plant community composition and structure which are able to intercept sediments and nutrients (Gathumbi et al., 2004). The deposition of dung and urine during

grazing are one of the sources of nitrogen and phosphorous to surface water (Edwards et al, 2000). Line et al. (2000) indicated that exclusion of grazing animals from streams reduced the TSS by 82%, TON by 55% and TP by 78%.

This study shows that waste dumping explains 9 % and 2% of the variation in TSS and nutrient retention, respectively. Awetu, Boye and some sites of Kito wetlands are receiving untreated wastewater and solid wastes. Wastewaters generated by more than 200,000 inhabitants of Jimma town, mostly derived from domestic, industrial and institutional sources are directly discharged into the major tributaries without any form of treatment. Moreover, 88 tons of solid wastes generated each day from the town, of which 25% are collected by the municipality, are dumped in fallow and farm lands (Getahun et al., 2012). The remaining 75% are either dumped in the backyard or in water ways. This improper waste management practice may contribute to the high TSS and nutrient loads measured in the wetlands.

High levels of suspended solids potentially cause sedimentation problems and reduce the life span of reservoirs. For example, the life span of Gilgel Gibe hydroelectric dam reservoir, located 60 km downstream of the studied wetlands is expected to reduce by one third due to high sediment transport from the catchment (Devi et al., 2008). On the other hand, suspended solid concentration in wetlands reduces the depth of the photic zone and hence reduce the light available for primary production (Llames et al., 2009). This in turn, alters aquatic food webs as well as basic wetland functions related to water quality improvement, nutrient cycling, and other biogenic processes that transform and sequester pollutants (Havens et al., 1999).

Nutrient enrichment in surface water can produce algal blooms and increase aquatic weed growth, which reduces water clarity and dissolved oxygen concentration (Gabor et al., 1994, Chen et al., 2002). Nutrient enrichment has been shown to significantly influence wetland community structure and composition (Guntenspergen et al., 2002). As nutrient loads to wetlands increase, fast growing species flourish and outcompete native species adapted to low fertility conditions (Grevilliot et al. 1998). Nutrient enrichment also threatens aquatic life through low dissolved oxygen and ammonia toxicity. Therefore, retention of nutrients and sediments in wetlands could play a vital role in improving river water quality and reducing siltation and eutrophication problems to the surface water waters situated downstream (e.g. reservoir).

In conclusion, riverine wetlands located in Awetu sub-watershed have the potential to retain TSS and nutrients. However, their retention efficiency is largely influenced by the quality of inflow and magnitude of habitat disturbances. Since the volume and quality of inflow (input concentration) varies with time, the calculated pollutant retention may not give the actual yearly surface loading rate. Awetu and Boye wetlands that are receiving untreated wastewater and solid wastes from Jimma town were characterized by a net retention. However, the outflow concentration of TSS and nutrients was higher compared to the concentrations measured in the incoming streams of Kito and Kofe wetlands. Kito and Kofe wetlands had a better water quality in the more upstream location and progressively deteriorated towards the downstream sites as a result of anthropogenic activities. Therefore, TSS and nutrient retention efficiencies of these riverine wetlands can be maximized through proper management of liquid and solid wastes generated from Jimma town and by minimizing anthropogenic pressures such as farming, uncontrolled grazing and clay mining in these wetlands. A good management could further help to sustain other ecological, social and economic benefits that these wetlands provide to humans.

Chapter 5: Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia

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Chapter 5: Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia

Abstract

Biotic indices are widely applied for conservation and management of aquatic resources since they allow water resources monitoring agencies to get insight in complex biological data and yield policy relevant information. Despite the worldwide popularity of biotic indices, little information on their use and applicability in Eastern Africa is available. Here, we develop a multimetric index based on macroinvertebrates to assess the ecological condition of natural wetlands in Southwest Ethiopia. Index development was based on a dataset of 222 samples collected during two consecutive years from 63 sites located at eight different wetlands. We used physico-chemical and hydro-morphological variables (land use pattern, habitat alteration, hydrological modification and chemical water quality) to classify sites as reference or degraded. We tested a total of 58 potential metrics representing various aspects of macroinvertebrate assemblages including family richness, composition, tolerance measures and presence and abundance of functional feeding groups. Metrics were selected for the development of a final index based on their sensitivity in discriminating reference from impaired sites, strength of correlation with the anthropogenic disturbance gradient, chemical measurements, and the degree of redundancy. Metrics retained for the final index were overall family richness, family richness of Ephemeroptera, Odonata and Trichoptera (EOT), and percentage of filterer-collectors. The final index, derived from the sum of three metric scores, was divided into five water quality classes (very bad, bad, moderate, good and very good). Our final multimetric macroinvertebrate index (MMI) distinguished well between reference and impaired wetland sites and showed a significant negative response to a gradient of disturbances ($R^2 = 0.86$, $p < 0.05$). Moreover, it classified a validation dataset accurately with a correctly classified instance of 80% and a Cohen's Kappa value of 0.6. This MMI can be considered as a robust and sensitive tool that can be applied to evaluate the ecological condition of natural wetlands in Ethiopia, where wetland resources are under high pressure as a result of agricultural activities such as grazing and urbanization.

5.1 Introduction

In many parts of sub-Saharan Africa, wetlands are under high pressure due to land use changes, while they are increasingly being recognized as vital resources for achieving food security and rural livelihoods (Millennium Ecosystem Assessment, 2005; Schuyt, 2005). Wetlands are used for a wide range of services including food production, cultivation, collection of drinking water, harvesting of wood, forage and craft materials, and extraction of clay for pottery and brick making (Adams, 1993; Acreman and Hollis, 1996; Dixon and Wood, 2003b).

Despite the benefits and services that they provide for humans, wetlands all over the world are threatened (Schuyt, 2005). The main causes for wetland loss and degradation are human activities (e.g. sewage influx and waste dumping, uncontrolled grazing, overharvesting, drainage for agriculture) and climate change (Millennium Ecosystem Assessment, 2005). High population growth rate and expansion of urban and suburban areas has increased the need for more fertile agricultural land, thereby increasing the loss of wetland resources (Schuyt, 2005). Therefore, understanding the adverse impact of human activities on aquatic ecosystems has resulted in growing worldwide calls for the sustainable management of this fragile resource (Kangalawe and Liwenga, 2005). A first step in this management is to develop assessment tools for water resources.

Biotic and saprobic indices proven to be useful to determine the status of aquatic ecosystems (e.g. Armitage et al., 1983; Gabriels et al., 2010; Junqueira et al., 2010; Lock et al., 2011; Raburu and Masese, 2012). A Biotic Index represents the quality of the environment by characterizing the type of organisms present in it. Several biotic indices (e.g. Biological Monitoring Working Party, Belgian Biotic Index etc) have been established and are widely used for biological assessment of streams and lakes. The Saprobic approach is mainly used to assess the water quality of rivers. The Saprobic Index is based on the presence of indicator species, which assigned saprobic values based on their pollution tolerance. According to the Saprobic Index several water quality classes ranging from a good water quality (oligosaprobic) to a bad water quality (polysaprobic) can be distinguished.

A wide range of organisms (bioindicators) is employed to assess aquatic ecosystems. Among the biological communities, macroinvertebrates have proven to be useful indicators to determine the status of rivers, since differences in environmental requirements among taxa

produce community characteristics that reflect ecological conditions (Gabriels et al., 2010). Macroinvertebrates such as snails, crustaceans and the larvae of many insects that have an aquatic life stage respond to a broad range of environmental conditions are relatively immobile and live in close contact with both bottom sediments and the water column, thereby having the potential for exposure to stressors via both sediment and aqueous pathways (Feio et al., 2007). Furthermore, macroinvertebrate communities can respond to nutrient enrichment (Lücke and Johnson, 2009), oxygen availability (Saloom and Duncan 2005), food quantity and quality (Cross et al., 2006), and changes in habitat structure (Steinman et al., 2003). In this study, we therefore focussed on benthic macroinvertebrates to assess the ecological water quality of wetlands.

Commonly used methods for analysing macroinvertebrate data include multimetric and multivariate approaches (Lücke and Johnson, 2009). A multimetric technique describes the state of an ecosystem by means of a combination of several individual metrics (Karr and Chu, 1999; Ofenböck et al., 2004; Applegate et al., 2007), whereas a multivariate approach describes patterns and relationships between macroinvertebrate communities and the environment (Hawkins et al., 2000; Clarke et al., 2003). Besides these, clustering macroinvertebrates into ecologically meaningful species groups has been proven useful to discriminate between polluted and non-polluted sites based on species composition and their sensitivity to pollution (Learner et al., 1983).

A multimetric index integrates different individual biological measures into a single value that can potentially reflect multiple effects of human impact on the structure and function of aquatic ecosystems (Barbour et al., 1995; Menetrey et al., 2011). The development of such a multimetric index is based on comparing biological metrics in impaired to reference or at least less impaired sites, the latter representing best attainable conditions for a watershed within a region (Stoddard et al., 2006; Whittier et al., 2007). Multimetric indices are increasingly applied for the purpose of conservation actions, since they allow water resources monitoring agencies to get insight in complex biological data and yield policy relevant information for regulatory agencies and decision makers (Karr and Chu, 1999). Therefore, they have become a popular tool for regional assessment of aquatic resources in Europe (Hering et al., 2006) and the United States (Stoddard et al., 2008).

Although multimetric indices have several advantages, there are also some pitfalls related to aggregated indices because any indicator remains a proxy for the natural environment and an

estimation of the reliability of an indicator is often unknown or not considered (Feest et al., 2010). A good index should provide a good representation of the various aspects of biodiversity such as species richness, evenness/dominance, biomass and rarity Feest (2006). Several authors have therefore advised the use of metrics assessing the various aspects of biodiversity (Hooper et al., 2005; Feest et al., 2010).

Within the context of the implementation of the European Union water framework directive, several European countries currently employed multimetric techniques for evaluating the ecological condition of their water bodies to achieve a Good Ecological Status (both a good biological and a good chemical status as determined by the European Commission (Directive 2000/60/EC)) for all water bodies in the member states of the European Community by 2015 (Hering et al., 2006). Recently, several metrics, including Saprobic Indices, were combined in a study on the development of a multimetric index for the assessment of Dutch streams using benthic macroinvertebrates (Vlek et al., 2004). A similar suggestion was made by Junqueira et al. (2010), who proposed to use their Saprobic Index for Brazilian Rivers as a foundation for the development of a more complex multimetric index. A drawback of the Saprobic Index is, however, the difficulty to determine values for each species regarding 'pollution sensitivity' and the fact that identification to species level is needed.

In this study, we opted for a multimetric approach based on a combination of macroinvertebrate metrics. The strength of this approach lies in its ability to integrate information from the various features of a community to give an overall classification of degradation. However, one of the problems related to these aggregated indices is the loss of information. To avoid this, it is important to report the values of the different submetrics. Another advantage is that these multimetric techniques that classify sites into categories of water quality do not rely on empirical measures or limits, but on comparisons with unimpaired reference sites within the same ecoregion (Reynoldson et al., 1997; Thorne and Williams, 1997).

Even though there are some clear generalities across indices developed for macroinvertebrates in different regions (Chessman and McEvoy, 1998), it remains important to develop and validate indices for different regions in the world separately, because of local peculiarities in reference conditions, anthropogenic pressures and regional species pools. Little information is available on biological assessment and monitoring tools in Eastern Africa (but see for example Beyene et al., 2009; Raburu et al., 2009a, b; Ambelu et al., 2010;

Yimer and Mengistu, 2009; Atnafu et al., 2011; Getachew et al., 2012; Raburu and Masese, 2012). In addition, only a limited number of studies have attempted to use multimetric indices for the assessment of river-associated wetland conditions in South Africa (Bird, 2010) and so far none in East Africa. The current study provides a well-funded basis for the development of a multimetric macroinvertebrate index (MMI), based on an extensive dataset, that can be practically implemented in water quality management.

Since many Important Bird Areas and their associated wetlands are currently not protected in Ethiopia, it is important to develop a multimetric index specifically designed to assess the water quality of these natural wetlands and to protect hot spots for biodiversity. The objective of this study was to develop and test a multimetric index based on macroinvertebrates using a dataset comprising four seasons (two wet and two dry seasons) collected from 63 wetland sampling sites located in Gilgel Gibe watershed, Southwest Ethiopia. We tested to what extent the multimetric macroinvertebrate index (MMI) is capable of discriminating reference from impaired wetland sites and validated its performance on a separate subset of the data. In this way, we wanted to develop an index, which can be used by decision makers in order to assess the ecological conditions of wetlands in Ethiopia.

5.2 Materials and Methods

5.2.1 Study Area

This study was conducted in five riverine wetlands (Awetu, Boye, Balawajo, Kofe and Kito), and three floodplain wetlands (two permanent: Bulbul, Haro; one temporary: Haro) located in the Gilgel Gibe watershed, lying between latitudes 7°37'N and 7°43'N and longitudes 36°46'E and 37°43'E (Figure 2.1, Chapter 2). The studied wetlands are varying in size ranging from five ha to a few hundred hectares. The mean annual temperature in the area is between 15°C and 22°C, and the mean annual precipitation is between 1800mm and 2300mm, with maximum rainfall between June and September and minimum precipitation between December and January (National Meteorological Agency, 2012).

The study wetlands harbour a high biodiversity with more than 140 bird species (Mereta et al., unpubl. data). The Wattled crane (*Bugeranus carunculatus* Gmelin, 1789), which is included in the IUCN red list as vulnerable, and two endemic species (Wattled ibis, *Bostrychia carunculata* Rüppel, 1837 and Rouget's Rail, *Rougetius rougetii* Guérin-Méneville, 1843) have breeding grounds in these wetlands. Kofe swamp is classified as an

Important Bird Area (IBA) for mainly two target species: the above-mentioned Wattled crane and the Abyssinian longclaw (*Macronyx flavicollis*). Small numbers of various Palearctic and Afrotropical ducks and geese have been recorded as well. Ethiopia is also a member of the African-Eurasian Waterbird Agreement (AEWA) that is concerned with conservation actions, management of human activities, research and monitoring, etc. Despite these efforts and agreements, Kofe and the surrounding wetlands are currently still totally unprotected (Birdlife International, 2012).

5.2.2 Data collection

The main threats for the wetlands around Jimma are disposal of domestic sewage, drainage, farming, clay mining, removal of riparian vegetation and uncontrolled livestock grazing. Among the riverine wetlands, Awetu and Boye receive untreated wastewater generated by the more than 200,000 inhabitants of Jimma town. Clay mining is a common practice in Kofe, Kito and some parts of Boye wetland. Parts of the floodplains of the temporary wetlands are intensively used for maize cultivation during the dry season (Mereta et al., 2012). Site selection was based on a preliminary study conducted during August and September 2009 (Vande Walle, 2010) in combination with previous research focussing on environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands located in the Gilgel Gibe watershed (Mereta et al., 2012). Sites were selected within each wetland along a gradient of visible disturbance including both nearly non-impacted and heavily disturbed sites (e.g. presence of point source pollution, eutrophication, hydrological modification, etc.). The number of sampling sites was evenly distributed among the wetlands depending on their size, with the smallest wetlands having a lower number of sampling sites. Sampling was also performed before and after a confluence when one or more rivers joined the main channel since this might give an idea of the impact of the tributary on the receiving wetland or stream.

Sixty three wetland sampling stations were monitored twice a year during two years: once during the dry (January to March) and once during the wet season (September to October) in both 2010 and 2011. In total, 222 samples were available. The dataset was split in two subsets using random number technique: two-thirds (148 samples) were used for the development of the multimetric macroinvertebrate index (MMI) and the remaining one-third (74 samples) was used for validation.

Habitat characteristics were assessed at each sampling station using the USEPA wetland habitat assessment protocol (Baldwin et al., 2005) (Chapter 4). Onsite physico-chemical parameters were measured using multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach). Water samples were analysed using standard methods as prescribed by APHA, AWWA, WPCF (1995) (Chapter 2).

Macroinvertebrates sampling and identification was based on Gabriels et al., (2010) and Bouchard (2004), respectively (Chapter 2). In total, 73 different macroinvertebrate families were encountered in this study (Annex 5.1). Based on literature, all families were assigned a tolerance score, which was used for the development of the multimetric macroinvertebrate index. Although some macroinvertebrate taxa have a range of tolerances for pollution it is generally accepted to assign one tolerance score to each taxon to assess the water quality in order to be able to calculate biotic indices (see e.g. Armitage et al., 1983; Gabriels et al., 2010).

5.2.3 Selection of reference and impaired sites

The development of a multimetric macroinvertebrate index as part of a bioassessment program requires establishing reference conditions (Barbour et al., 1996). The designation of sites as reference and impaired may be based on a prior knowledge of pressures acting over different locations (e.g. presence of point source pollution, eutrophication, hydrological modification, etc.) (Barbour et al., 1996) or may involve a post classification based on measured/recorded abiotic and biotic variables. The latter approach was applied in this study. We designated wetland sites as reference and impaired based on land use patterns, the degree of habitat degradation as quantified by the USEPA protocol (USEPA, 2002d), variables characterizing hydrological modification, and the Prati index as a measure of chemical water quality. The basic Prati index is calculated based on the concentration of ammonium, chemical oxygen demand and oxygen saturation (Prati et al., 1971). A Basic Prati index value of two or less was considered as good water quality and an index greater than two was considered as poor water quality. Land use, habitat alteration and hydrological modifications were quantified based on their intensity in the studied wetlands according to Hruby (2004). A score of 1 was awarded for no or minimal disturbance, 2 for moderate and 3 for high disturbance (Table 4.1, Chapter 4). Based on these criteria, of the 148 samples used for the development of the index, 57 (39%) samples were categorized as reference and the remaining 91 (61%) samples as impaired.

Table 5.1. Water quality parameters and environmental conditions of the reference and impaired sites used to develop the Multimetric Macroinvertebrate Index (MMI). P-values associated with the Mann-Whitney U test for testing differences of the given parameters among reference and impaired sites. P-values lower than 0.05 are considered significant (TON = total organic nitrogen, TP = total phosphorous, COD = chemical oxygen demand, NH₄ = ammonium) (SD=standard deviation).

Environmental or water quality parameter	Reference samples	Impaired samples	p-value
	Mean ± SD (Range)	Mean ± SD (Range)	
	N=57	N=91	
Oxygen saturation (%)	64±27 (15-143)	60±47 (4-263)	0.006
Chlorophyl a (µg/l)	13±2.5 (11-27)	15±5.9 (12-66)	0.005
TON (mg/l)	3.62±5.6 (0.05-16.7)	3.8±4.6 (0.09-34.1)	0.6
TP (mg/l)	0.13±0.14 (0.03-0.5)	0.26±0.28 (0.04-1.23)	0.003
NH ₄ (mg/l)	0.20±0.28 (0.01-1.0)	0.25±0.56 (0.01-2)	0.017
COD (mg/l)	17±11 (3-52)	67±102 (4-488)	0.000
Basic Prati index	2±0.99 (1-3)	5±3.4 (1-25)	0.0002
Land use	3±0.42 (3-4)	5±1.59 (3-9)	0.001
Habitat alteration	3±0 (3-3)	4±0.86 (3-8)	0.000
Hydrological modification	3±0.62 (3-4)	4±1.99 (3-9)	0.001

5.2.4 Metric selection and scoring

A total of 58 candidate metrics (Table 5.2) representing various aspects of the macroinvertebrate community were selected based on literature (Resh and Jackson, 1993;

Bode and Novak, 1995; Barbour et al., 1996; Seaby and Henderson, 2007). These metrics were related to family richness, taxonomic composition, tolerance measures, biotic indices and the composition of functional feeding group. We tested the normality of the candidate metrics with normal probability plots and with a Kolmogorov-Smirnov test of normality. All metrics had a non-normal distribution and hence we used non-parametric tests. Core metrics were selected based on their capacity to discriminate reference from impaired sites. This was tested by a non-parametric Mann-Whitney U test ($p < 0.05$) and the degree of inter-quartile (IQ) overlap in Box- and Whisker plots (Barbour et al., 1996). A metric with a p-value < 0.05 in a Mann-Whitney U test and a sensitivity score of 3 was considered to be a strong discriminator between reference and impaired conditions and was considered as a metric for the final MMI development, as recommended by Barbour et al. (1996) and Baptista et al. (2007). We tested for redundancy among metrics using Spearman rank order correlation analysis in STATISTICA 7.0. Metrics were considered redundant if the spearman correlation coefficient was higher than 0.75 and the p-value was smaller than 0.05 (Whittier et al., 2007). From the metrics considered as redundant, the one with the highest correlation coefficient with environmental variables, the highest sensitivity score and the most user-friendly to implement for monitoring purposes was selected.

The range of metric values obtained from the Box- and Whisker plot was divided into three possible scores (Barbour et al., 1996). A score of 5 indicates that the sample meets the reference condition, a score of 3 represents an intermediate condition and a score of 1 indicates the highest deviation from the reference condition (Barbour et al., 1996; Baptista et al., 2007; Ferreira et al., 2011). For decreasing metric values in response to increasing impairment, values above the lower quartile (25%) of the reference condition were scored 5. On the other hand, metrics whose value expected to increase in response to increasing impairment were assigned a score of 5 if the value was situated below the upper quartile (75%). We performed Spearman rank order correlation using STATISTICA 7.0 to relate the candidate metrics to environmental and water quality variables. Based on these relationships we selected the core metrics.

5.2.5 MMI development and validation

The values of each metric value were combined into a Multimetric Macroinvertebrate Index (MMI) by summing up the score of each individual metric. In addition, the score of each individual metric was reported between brackets in order to increase clarity. The final MMI

score was divided into five quality classes: very bad, bad, moderate, good and very good quality. Linear regression was used to test the relationship between the final multimetric macroinvertebrate index and the disturbance index and the water quality index in the SPSS package version 16.

The assessment performance of the multimetric macroinvertebrate index was tested on a validation dataset. We used the percentage of correctly classified instances (CCI) (Witten and Frank, 2005) and Cohen's Kappa statistic (K) (Cohen, 1960) for the performance evaluation. Both the CCI and Cohen's Kappa statistic (K) were computed from the confusion matrix. Kappa is simply a derived statistic that measures the proportion of all possible cases that are predicted correctly after accounting for chance predictions (Cohen, 1960) (Chapter 2).

5.2.6 Multivariate data analysis

Detrended Correspondence Analysis (DCA) was applied using CANOCO 4.5 (ter Braak and Šmilauer, 2002) to determine the appropriate response model (linear or unimodal) for both the macroinvertebrate metrics and the environmental data. The DCA yielded gradient lengths less than two standard deviations. Therefore, Redundancy Analysis (RDA) was used to investigate the relationship between macroinvertebrate metrics and environmental variables. For the RDA analysis, data were $\log(x+1)$ transformed and divided by the standard deviation to standardize the ordination diagram (ter Braak and Šmilauer, 2002). Significance was tested using Monte Carlo tests with 999 permutations (ter Braak and Šmilauer, 2002).

5.3 Results

5.3.1 Selection of core metrics

Out of the 58 candidate metrics, 39 (67%) had a significant discriminating power to separate the reference from impaired sites according to the Mann-Whitney U test ($p < 0.05$) (Table 5.2). However, the sensitivity test from the Box- and Whisker plots showed that only six metrics were highly sensitive (score = 3) and consequently these were retained for the redundancy test and final metric selection (Table 5.3, Figure 5.1): total family richness, family richness of Ephemeroptera, Odonata and Trichoptera (EOT), family richness of Ephemeroptera and Trichoptera (ET), percentage filterer-collectors, Margalef's index, and the Biological Monitoring Working Party (BMWP) score.

The redundancy test showed that the BMWP score was highly correlated with total family richness, EOT family richness and Margalef's index ($R^2 = 0.9$, $R^2 = 0.78$ and $R^2 = 0.89$, respectively; all $p < 0.05$). Margalef's index was also highly correlated with total family richness ($R^2 = 0.89$, $p < 0.05$). ET family richness was highly correlated with EOT family richness ($R^2 = 0.79$, $p < 0.05$). Therefore, BMWP score, Margalef's index and ET family richness were considered as redundant and excluded from the final MMI (Table 5.3).

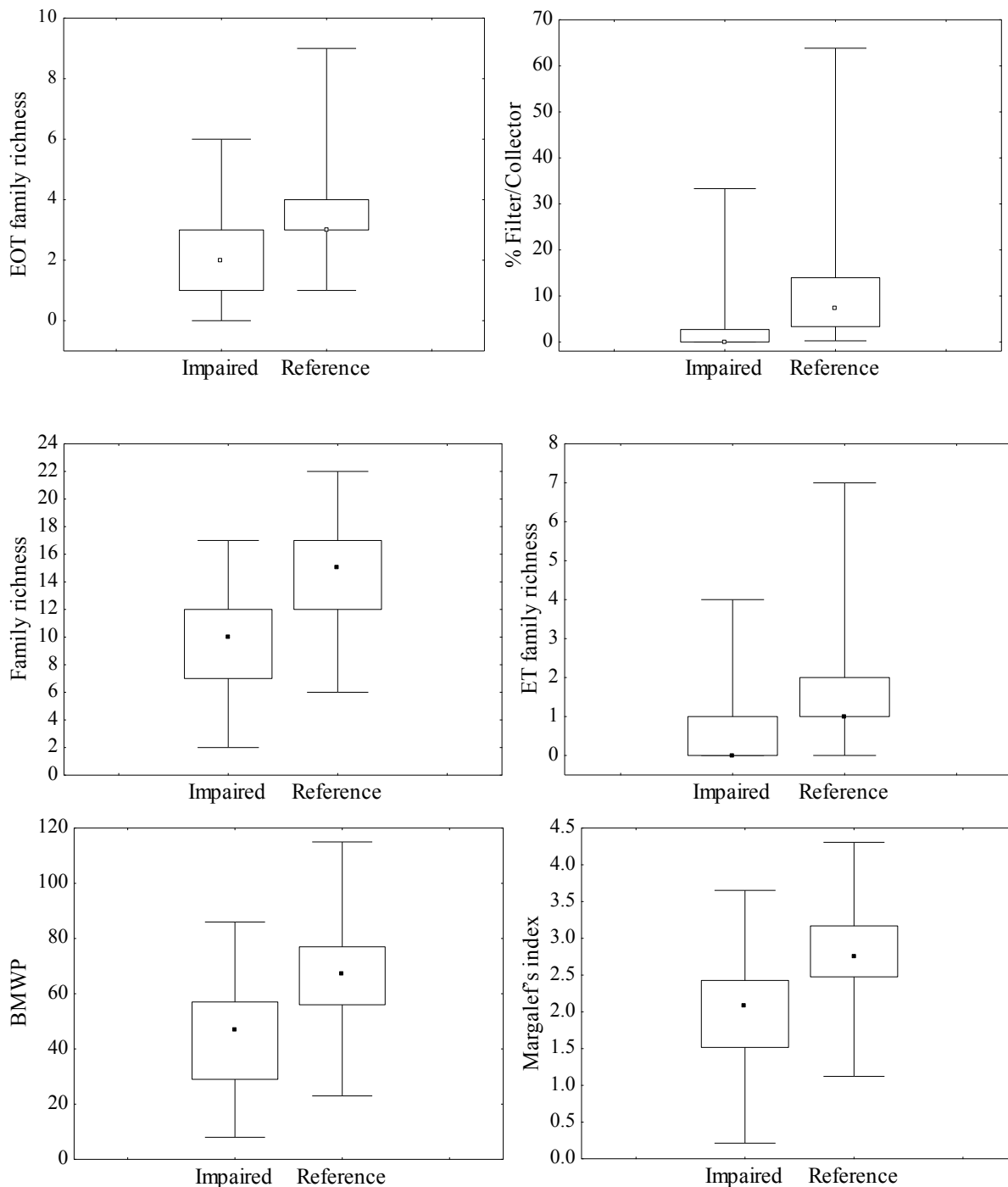


Figure 5.1. Box- and Whisker plots of each of the six selected metrics used to discriminate between reference and impaired sites. Small black squares represent median numbers, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values (EOT = Ephemeroptera, Odonata, Trichoptera; BMWP = Biological monitoring working party).

Table 5.2. Metrics selection criteria and response to impairment ^a. FBI = Family Biotic Index; ASPT = Average Score per Taxon; BMWP = Biological Monitoring Working Party; EOT = Ephemeroptera, Odonata, Trichoptera; # = abundance.

Metric	Response to impairment	U-test	p-value	Sensitivity score	Meets the test criteria
Family richness	Decrease	764	0.0001*	3	Yes
Abundance	Decrease	1927	0.009*	0b	No
Shannon index	Decrease	1436	0.0005*	2	No
Margalef's index	Decrease	893	0.0001*	3	Yes
Menhinick Index	Decrease	1464	0.0009*	2	No
McIntosh D	Decrease	1744	0.0008*	1	No
Simpson (1-D)	Decrease	1653	0.0002*	1	No
Simpson (1/D)	Decrease	1653	0.0002*	1	No
Shannon Evenness	Decrease	2201	0.12	0b	No
Simpsons Evenness	Decrease	2394	0.43	0b	No
Smith and Wilson 1-D	Decrease	2005	0.02*	0b	No
Smith and Wilson – lnD,	Decrease	2265	0.20	0b	No
Heip Evenness	Decrease	2551	0.87	0b	No
McIntosh Evenness	Decrease	2072	0.04*	0b	No
FBI	Increase	1896	0.006*	0b	No
ASPT-BMWP	Decrease	2149	0.08	0b	No
BMWP	Decrease	975	0.0001*	3	Yes
%Chironomidae	Increase	2156	0.08	0b	No
%Trichoptera	Decrease	2110	0.006*	0b	No
%Ephemeroptera	Decrease	1972	0.01*	1	No
%ET	Decrease	1915	0.005*	1	No
%Odonata	Decrease	1711	0.0004*	1	No
%EOT	Decrease	1830	0.002*	1	No
%Coleoptera	Decrease	2536	0.82	0b	No
%Diptera without	Increase	2341	0.32	0b	No

Chironomidae					
%Diptera	Increase	2280	0.223	0b	No
%Hemiptera	Increase	1708	0.0005*	2	No
%Non-insect larvae	Increase	1839	0.003*	0b	No
%Gastropoda	Increase	1881	0.004*	0b	No
%Oligochaeta	Variable	2531	0.69	0b	No
%Bivalvia	Increase	1849	0.0003*	0b	No
% Hirudinae	Increase	2269	0.18	0b	No
%Predators	Variable	2501	0.72	0b	No
%Gather/Collector	Variable	2374	0.39	0b	No
%Filterer/Collector	Decrease	984	0.0001*	3	Yes
%Shredder	Decrease	2553	0.87	0b	No
%Scraper	Variable	1805	0.002*	1	No
Baetidae/	Increase	2366	0.004*	0b	No
Ephemeroptera					
Scraper/Filterer- collector	Decrease	1633	0.0004*	1	No
EOT/Chironomidae	Decrease	1754	0.0009*	1	No
EOT/Diptera	Decrease	2104	0.053	0b	No
E family	Decrease	1656	0.0007*	2	No
T family	Decrease	2072	0.0003*	0b	No
ET family	Decrease	1522	0.0006*	3	Yes
Odonata Family	Decrease	1515	0.0008*	2	No
EOT family	Decrease	1153	0.0000*	3	Yes
Coleoptera family	Decrease	1668	0.0002*	1	No
Hemiptera family	Decrease	2274	0.19	0b	No
Diptera family	Decrease	1729	0.0004*	1	No
#E	Decrease	2226	0.12	0b	No
#T	Decrease	2112	0.0006*	0b	No
#ET	Decrease	2103	0.04*	0b	No
#Odonata	Decrease	1512	0.0002*	1	No
#EOT	Decrease	1593	0.0008*	1	No
# Coleoptera	Decrease	2193	0.11	0b	No

# Hemiptera	Variable	1928	0.008*	0b	No
#Diptera individuals	Increase	1976	0.01*	0b	No
#Hirudinae	Increase	2165	0.07	0b	No

*Significant at $p < 0.05$. ^a A sensitivity score of 3 was given if there was no overlap in the interquartile range (IQ) of Box-Whisker plots of the metric values; a score of 2 reflects if there was some overlap but both medians were outside the IQ range overlap; a score of 1 was given if there was moderate overlap of IQ range but one median was outside the IQ range overlap; a score of 0a reflects that one range was completely overlapping the other IQ range but one median was outside the IQ range overlap; and a score of 0b reflects that both medians were inside IQ range overlap.

Table 5.3. Values of Spearman rank order correlation coefficients verifying redundancy ($R^2 > 0.75$) in the metrics selected as valid for inclusion in the final Multimetric Macroinvertebrate Index (BMWP = biological monitoring working party, EOT = Ephemeroptera, Odonata and Trichoptera, ET = Ephemeroptera and Trichoptera).

	BMWP	Family richness	% Filter-collector	EOT family richness	Margalef's index	ET family richness
BMWP	–					
Family richness	0.9*	–				
%Filter-collector	0.5	0.56	–			
EOT family richness	0.8*	0.67	0.42	–		
Margalef's index	0.9*	0.89*	0.50	0.64	–	
ET family richness	0.6	0.47	0.32	0.78*	0.41	–

*Significant at $p < 0.05$

5.3.2 Environmental stressor gradient and relationship with core metrics

Spearman rank order correlations indicated that most of the environmental variables were significantly correlated with the three core metrics ($p < 0.05$). Land use and habitat alteration appeared to be the most significant and were negatively related to the core metrics. The presence of hydrological modifications was also negatively correlated with all three core

metrics. Among the water quality variables COD and chlorophyll a were negatively correlated, whereas dissolved oxygen concentration was positively correlated with the EOT family richness (Table 5.4).

5.3.3 MMI development and validation

Each metric value was rescaled to comparable values before it was aggregated into a single MMI value (Table 5.5). The final MMI score ranged from 3 to 15. The lower threshold (minimum score of 3) represents the most undesirable or impaired condition, whereas the upper threshold (maximum score of 15) represents the most desirable or reference condition. Finally the MMI range was divided into five quality classes: 3-5 = very bad, 6-8 = bad, 9-11 = moderate, 12-13 = good and 14-15 = very good.

The results of linear regression confirmed that there was a strong negative inter-correlation between the MMI and the human disturbance gradient ($R^2 = 0.85$, $p < 0.05$). Similarly, the MMI score was also negatively inter-related with the chemical water quality index ($R^2 = 0.34$, $p < 0.05$) (Figure 5.2).

Table 5.4. Core metrics correlation with environmental variables using Spearman rank order correlation (EOT = Ephemeroptera, Odonata, Trichoptera, DO = dissolved oxygen, COD = chemical oxygen demand, TON = total organic nitrogen, TP = total phosphorous).

	Family richness	% Filter-Collector	EOT family richness	Hydrological modification	Habitat alteration	Land use	DO (%)	Chlorophyll a ($\mu\text{g/l}$)	COD (mg/l)	TON (mg/l)	TP (mg/l)
Family richness	-										
% Filter/Collector	0.56*	-									
EOT family richness	0.67*	0.42*	-								
Hydrological modification	-0.45*	-0.22*	-0.37*	-							
Habitat alteration	-0.75*	-0.71*	-0.67*	0.35*	-						
Land use	-0.66*	-0.69*	-0.61*	0.35*	0.70*	-					

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DO_ (%)	-0.04	0.06	0.19*	-0.007	0.003	-0.03	-				
Chlorophyll a (µg/l)	- 0.34*	- 0.43*	-0.23*	0.05	0.36*	0.28*	0.33*	-			
COD (mg/l)	- 0.60*	- 0.46*	-0.44*	0.12	0.55*	0.50*	0.10	0.30*	-		
TON (mg/l)	- 0.16*	-0.07	-0.19*	0.20*	0.20*	0.14	0.20*	0.16*	0.09	-	
TP (mg/l)	-0.05	- 0.21*	-0.06	-0.08	0.20*	0.16	0.17*	0.36*	0.07	0.20*	-

*Significant at $p < 0.05$

Table 5.5. Scores of the three selected metrics, according to the trisection scoring method from the appropriate percentile of the data distribution (USEPA, 1998b). A score of 5 indicates that the sample meets the reference condition, a score of 3 represents an intermediate condition and a score of 1 indicates the highest deviation from the reference condition (Barbour et al., 1996). %FC = percentage filter-collector, EOT = Ephemeroptera, Odonata and Trichoptera, Min = minimum, Max = maximum.

Metrics	Box plot value					Scores		
	Min	25%	50%	75%	Max	5	3	1
Family richness	6	12	15	17	23	≥ 12	11-6	< 6
EOT family richness	1	3	3	4	9	≥ 3	2-1	0
%FC	0	4	8	14	64	≥ 4	$< 4 > 0$	0

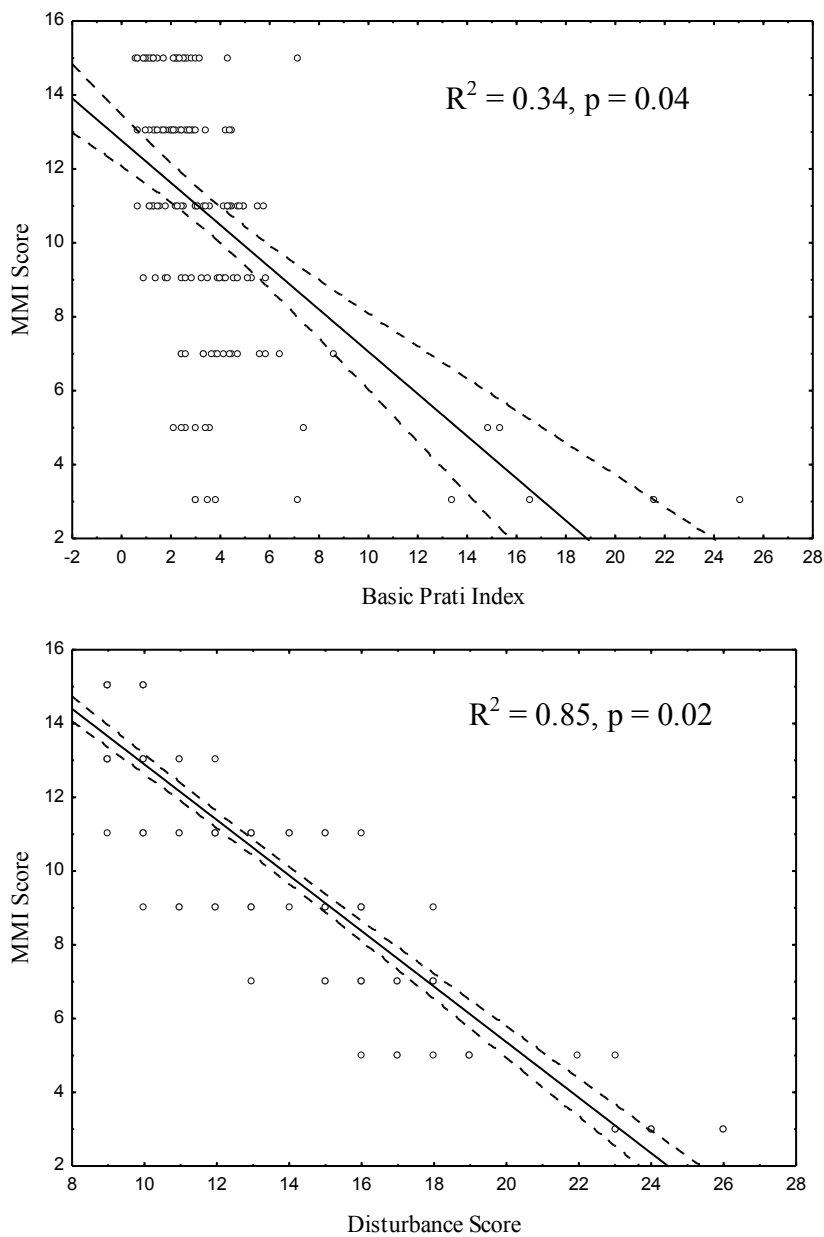


Figure 5.2. Relationship (95% CI) between the multimetric macroinvertebrate index (MMI) and water and habitat quality based on Basic Prati index (Prati et al., 1971) and USEPA disturbance score (all values are log transformed).

Of the 74 samples used as validation dataset, 35 were designated as reference and the remaining 39 were as impaired based on habitat and water quality. Among the 74 samples 59 were correctly classified by the multimetric macroinvertebrate index (CCI = 80%). The assessment performance of the multimetric macroinvertebrate index based on Cohen's Kappa was also reliable ($K = 0.6$, see Gabriels et al., 2007).

5.3.4 Multivariate analysis

The first two axes of the RDA biplots explained 56.8% of the total variance in macroinvertebrate metrics and 99.6% of the variation in environmental data (Table 5.6). The RDA ordination showed a strong relationship between macroinvertebrate metrics and environmental variables (Figure 5.3), with correlation coefficients of 0.84 and 0.39 for the first and second axis respectively (Annex 5.3). Vegetation cover was positively correlated with both the core metrics and the final MMI (Figure 5.3). Hydrological modifications and physico-chemical variables such as chemical oxygen demand (COD) and chlorophyll a were negatively correlated with the core metrics and the final MMI (Figure 5.3)

Table 5.6. Detailed results of the Redundancy Analysis relating the core metrics to the environmental variables.

Axes	1	2	3	4	Total Variance
Eigenvalues	0.54	0.029	0.002	0.000	1.0
Species environmental correlations	0.84	0.385	0.243	0.209	
Cumulative percentage variance					
Species data	54.0	56.8	57.1	57.1	
Species-environmental relation	94.6	99.6	99.9	100	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.571

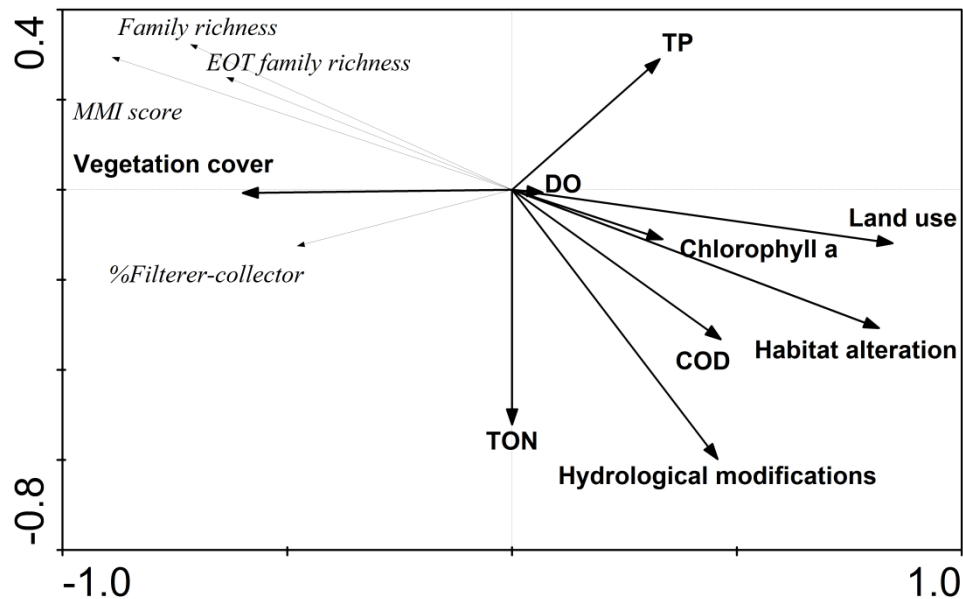


Figure 5.3. Redundancy analysis of macroinvertebrate metrics and environmental variables of natural wetlands in Southwest Ethiopia (COD = chemical oxygen demand, TON = total organic nitrogen, DO = dissolved oxygen, TP = total phosphorous).

5.4 Discussion

In this study, 58 candidate metrics were tested for inclusion in a multimetric macroinvertebrate index. Most of them are widely recognized as being sensitive to a range of anthropogenic stressors (Vlek et al., 2004; Ferreira et al., 2011; Couceiro et al., 2012; Verdonschot et al., 2012), and they are used in the bioassessment of wetlands (Kashian and Burton, 2000). However, most of these candidate metrics were eliminated because they did not discriminate well among reference and impaired sites. Three core metrics were selected to develop a multimetric macroinvertebrate index. These three metrics reflect different features of macroinvertebrate assemblages. Total family richness directly relates to biodiversity, while family richness of Ephemeroptera, Odonata and Trichoptera targets diversity of sensitive taxa. Finally, one metric reflects the relative abundance of functional feeding groups (percentage filterer-collectors). All three metrics proved useful for characterizing the ecological condition of river-associated wetlands. They were not strongly correlated amongst themselves and all showed a negative response to increasing habitat degradation as assessed by traditional land use and water quality parameters. The RDA analysis revealed that all core metrics (e.g. EOT family richness) were negatively correlated with environmental

disturbance (e.g. habitat alteration) and positively correlated with vegetation cover. High richness generally reflects physical habitat diversity, good water quality and a high availability of food resources (Barbour et al., 1996).

Many studies indicate that Ephemeroptera, Plecoptera and Trichoptera show a strong negative response to anthropogenic disturbances in aquatic ecosystems (e.g. Ode et al., 2005). However, the frequency of occurrence of the order Plecoptera in this study was very low due to their absence in stagnant water conditions. Hence, Odonata were included together with Ephemeroptera and Trichoptera because of their sensitivity to human disturbance (Samways and Steytler, 1996; Hornung and Rice, 2003) and their relatively frequent occurrence at our sampling sites. Family richness and EOT family richness are metrics that reflect the diversity of aquatic organisms and are related to the health of aquatic ecosystems (Baptista et al., 2007).

Ephemeroptera is an order of aquatic insects commonly used in bioassessment and biomonitoring of freshwater ecosystems all over the world (e.g. Arimoro and Muller, 2010). They are considered an ecologically important group that are of high importance for assessing biodiversity in aquatic ecosystems. In case of a good water quality, they are often the most abundant insects encountered in submerged vegetation and in the littoral zone, especially at sites with a sufficiently high dissolved oxygen concentration (Barber-James et al., 2008; Sharma and Rawat, 2009; Arimoro and Muller, 2010; Shelly et al. 2011). A previous study investigating the relationship between macroinvertebrates and environmental factors in Southwest Ethiopia found that Caenidae were highly correlated with vegetation and mainly found at sites with a good water quality (Mereta et al., 2012). Trichoptera are often less consistent in detecting impacts, but the inclusion of this taxon is recommended for detecting short-term impacts (Kashian and Burton, 2000). Trichopterans include valuable taxa for water quality biomonitoring due to their high taxa richness, ecological diversity and abundance in virtually all types of aquatic habitats with a sufficiently good water quality (Houghton, 2004). Odonates are considered as vital components of a wetland ecosystem (Hornung and Rice, 2003). Odonates are strongly related to the vegetation present in wetlands as they are carnivores that mainly look for food around roots and leaves of plants (Shelly et al., 2011). Destruction and degradation of this critical habitat poses the greatest threat to odonate populations (Moore, 1997). In addition to contributing to the biodiversity of wetlands, odonates are ecologically important as both dominant predators and as prey for a

considerable range of organisms (e.g. fish). Their trophic position and sensitivity to environmental degradation allow odonates to function as indicators of ecosystem quality with strong relevance for conservation and management efforts (Samways and Steytler, 1996; Hornung and Rice, 2003). Although we found that odonates are relatively sensitive to pollution and can be used as a good indicator of water quality there is some variation in tolerance to pollution of the taxa belonging to this group. For example the family Gomphidae is classified among the most sensitive taxa whereas Coenagrionidae are far less sensitive to pollution. Nevertheless, the larval odonate community has been successfully used as an indicator of habitat and water quality in both lentic and lotic systems (Foote and Hornung, 2005).

The usefulness of assessing the relative abundance of different functional feeding guilds in benthic macroinvertebrates has been debated (Barbour et al., 1999). Difficulties with the proper assignment of taxa to functional feeding groups (Karr and Chu, 1997) and changes in feeding mode with life stage (Allan, 1995) have contributed to the reluctance to use feeding mode as a reliable metric. Several studies have indicated that metrics based on functional feeding modes yield variable responses to perturbation (Barbour et al., 1999; Tomanova et al., 2006; Moya et al., 2011). In contrast, the results of the present study show a consistent decrease in the relative abundance of filterer-collectors with increased impairment. This is in agreement with the study conducted in coastal wetlands by Kashian and Burton (2000). Many of the filterer-collectors in this study belonged to the Trichoptera and Simuliidae which are common in diverse communities (USEPA, 2007).

All three core metrics were strongly correlated with most of the habitat and water quality parameters. Land use pattern including clay mining, sewage dumping and farming was strongly and negatively correlated with the core metrics. Studies indicated that wetlands structure and function can be impacted by agricultural activities, due to increased sedimentation associated with tillage practices, increased pesticide runoff and altered hydrological regimes (Steinman and Rosen, 2000). Spackman and Hughes (1995) found that agricultural cropping occurring within or adjacent to a riparian corridor may jeopardize the integrity, continuity and persistence of riparian corridors as a shifting mosaic of habitat types and associated biota (Mensing et al., 1998). Likewise, habitat degradation (e.g. grazing, eucalyptus plantation) was also strongly and negatively correlated with the core metrics. Cattle grazing can have a strong impact on wetlands by increasing nutrient inputs via urine

and faecal deposition or via trampling of sediments, which in turn can affect the organisms that rely on this habitat (Steinman and Rosen, 2000; Steinman et al., 2003).

Among the water quality variables, chlorophyll a and chemical oxygen demand (COD) concentration were negatively related to all the core metrics ($p < 0.05$). The decrease of macroinvertebrate species along a trophic gradient has repeatedly been reported (Carpenter et al., 1998). A consistently high concentration of chlorophyll a in a system is an indicator of potential eutrophication, which is harmful to many aquatic organisms and reduces biodiversity (Carpenter et al., 1998; Smith et al., 1999; Declerck et al. 2005). Oxygen saturation was positively correlated with family richness of Ephemeroptera, Odonata and Trichoptera. These orders are known to contain many taxa that are sensitive to changes in water quality and require a moderate to high concentration of dissolved oxygen (Hofmann and Mason, 2005; Hughes, 2006; Sharma and Rawat, 2009).

Although, numerous studies have demonstrated that the relative abundance of tolerant taxa such as Chironomidae increases with increasing disturbance (e.g. Moya et al., 2007), a variable response for this parameter in relation to habitat degradation was observed in the present study. A possible reason for this may be that this taxon was generally very abundant in our sampling stations (Mereta et al., 2012) and the relatively low resolution at which we identified them. Kerans and Karr (1994) suggested that chironomids are a very diverse group that includes species with different pollution sensitivities, and must be identified to genus or species level in order to use them as water quality indicators. The same conclusions were drawn by Learner et al. (1983), who found, based on a clustering of ecological meaningful species groups that several chironomid species showed a different sensitivity towards pollution in the River Ely in South Wales (UK).

The multimetric macroinvertebrate index we propose here is a summation of three metrics reflecting different aspects of the structure and functioning of macroinvertebrate assemblages. Regression analysis revealed that this MMI was negatively related to both disturbance as scored by the USEPA wetland habitat assessment protocol ($r = 0.86$, $p < 0.05$) and to water quality as estimated by the Prati index ($r = 0.34$, $p < 0.05$) (Figure 5.3). This successful validation indicates that the MMI responds appropriately to generalized measurements of disturbance and represents a suitable tool to detect environmental degradation.

Due to the high pressures that wetlands face worldwide, international agreements that take into consideration the protection of wetlands have led to the establishment of the Convention on Wetlands of International Importance or the Ramsar Convention. One of the commitments is the requirement to designate at least one wetland site of international importance upon accession and also make the effort to maintain its ecological integrity. To date Ethiopia has not yet ratified the Ramsar Convention. According to the Important Bird Area Programme carried out by the Ethiopian Wildlife and Natural History Society there are 73 hotspots for birds in Ethiopia. Out of these 73 sites, 41% are wetlands (Mengistu, 2003). Birdlife International (2012) identified 31 sites that could be qualified as Ramsar sites. According to Abebe (2004) designating Ramsar sites would be a great step forward to conserve wetland fauna and flora and to use the functions and resources related to the wetlands in a sustainable way. It is especially with respect to this point that the MMI developed here could be of importance to decision makers or monitoring agencies to assess the quality of the wetlands in Ethiopia since it is a robust method that has a relatively low cost and is easy to apply on routine monitoring data of macroinvertebrates. Our MMI performed well in the assessment of pollution status of the studied wetland sites and the description of the pollution gradient. The provided method and protocol for water quality monitoring (Annex 5.2 and 5.3)) can be a step forward in the assessment and protection of natural resources since it is an easy tool that can be used to report in an objective way on the status of natural wetlands in Ethiopia. In this way, hot spots of biodiversity indicated by a high biological index (high MMI) can be targeted for conservation.

By combining three measures of habitat quality, the multimetric macroinvertebrate index developed in the present study was effective in discriminating reference from impaired sites in river-associated wetlands in Southwest Ethiopia. The MMI showed a strong relationship to a broad range of water quality measures and human disturbances. The observed relationships indicate that macroinvertebrate communities are good candidates for assessing ecological integrity of wetlands. The resulting multimetric macroinvertebrate index is a robust and sensitive tool that can be easily applied to assess the ecological condition of natural wetlands in Ethiopia, where wetland resources are under high pressure because of agricultural activities and urbanization. Our multimetric macroinvertebrate index would be easily applicable on a wider geographic scale, but it may be necessary to first carry out a calibration study if considerably different types of habitats or landscapes are studied.

Chapter 6: General discussion and future prospect

6.1 The use of macroinvertebrates to assess ecological status of wetlands

In this study, decision tree models and multivariate analyses were used to identify the most important factors that are influencing macroinvertebrate community structure in natural wetlands. Vegetation cover, water depth and conductivity were the most important environmental factors determining the occurrence of macroinvertebrate taxa. Both the decision tree models and the ordination analysis indicated that aquatic vegetation favours the occurrence of intolerant taxa. Harrison et al. (2000) suggest that riparian vegetation provides shelter from predators and water current, provide more food resources, and is important as oviposition site. In this study, Simuliidae, Baetidae and Caenidae were found at high abundance in highly vegetated sites and absent in sites where the percent vegetation cover was very low. On the other hand, the frequency of occurrence of tolerant taxa (e.g. Chironomidae) was relatively higher in unvegetated sites. Agriculture, clay mining and uncontrolled livestock grazing are the major human activities that reduce aquatic and riparian vegetation cover in the study area and consequently reduce the diversity of macroinvertebrate taxa (Chapter 2 and 3). Allan (2004) found that agricultural activities leads to the decline of benthic aquatic insects, which are sensitive to mud on their integument and gills and to the filling of interstitial habitats due to accumulations of silt.

Although, numerous studies have demonstrated that the relative abundance of tolerant taxa such as Chironomidae increases with increasing disturbance (e.g. Moya et al., 2007), a weak association to environmental factors was observed in this study. This taxon was widely distributed in the wetlands and found in 87% of the sites. The low level of resolution i.e. family level identification may be the reason that this taxon found in both impaired and less impaired sites. Raposeiro et al. (2009) suggested that Chironomids exhibit high diversities and abundances that includes species with different pollution sensitivities, and must be identified to species level in order to use them as water quality indicators. Chronomids have been proposed as relevant water quality indicators, and some species are likely to be as sensitive as other biological indicators such as the well-known (Ephemeroptera Plecoptera, Trichoptera (EPT) taxa (Arimoro et al., 2007; Carew et al., 2007).

Water conductivity was also one of the most important factors that affect the occurrence of macroinvertebrates. Awetu and Boye wetlands, which are strongly influenced by the inflow

of untreated wastewater and solid wastes from Jimma town, had a high concentration of electric conductivity. Several studies have shown that urbanization can contribute to increased levels of conductivity in freshwater ecosystems (Roy et al., 2003). Studies have shown that macroinvertebrate taxa have variable responses to water conductivity depending on their osmo-regulator adaptations (Olson, 2012). Although Chironomidae is cosmopolitan in its distribution, it was found at high abundance in Awetu and Boye wetlands, which had high concentration of conductivity from sewage discharge.

Temporary floodplain wetlands differed from permanent wetlands in their macroinvertebrate composition as well as in their physico-chemical characteristics (Chapter 2). These habitats are often considered harsh environments because of their short hydroperiod separated by long periods when the wetland is dry. The remineralisation of nutrients during dry season increases their productivity (Collinson et al., 1995; Euliss and Mushet, 2004). During the dry season, these areas are predominantly used for grazing and the cultivation of maize and vegetables. In addition, dry season grazing contributes to the deposition of significant amounts of excrements (and thus nutrients) in these fields. When these areas become inundated during the rainy season, the dead organic material from crops and cattle excrements can be decomposed and results in an increase of the concentration of total phosphorus and chemical oxygen demand (Chapter 2).

In addition, temporary floodplain wetlands are highly vulnerable to human disturbances and other stressors. However, this harsh environment provides unique opportunities for specially adapted species (Collinson et al., 1995). The ordination analysis revealed that Hemipterans (Notonectidae, Corixidae and Belostomatidae) were dominated the macroinvertebrate community in temporary wetlands. These taxa are able to re-colonize temporary wetlands within a couple of weeks after flooding (Chase and Knight, 2003). In contrast, invertebrates with long life cycles such as odonates are unable to exist in the shortest hydro-period. Odonates such as Coenagrionidae prefer permanent habitats with high vegetation cover. Muller et al. (2003) indicated that removal of vegetation from littoral zone resulted in the decline of odonate taxa richness. The occurrence of odonates, particularly Coenagrionidae showed strong association with wetland vegetation cover. Odonates use vegetation as oviposition sites. Muller et al. (2003) indicated that removal of vegetation from littoral zone resulted in the decline of odonate taxa richness. Based on the multivariate analysis and the

developed models it is clear that certain macroinvertebrate species show a clear response to environmental conditions.

In this study, macroinvertebrate taxa showed a clear response to environmental conditions. Hence, understanding the relationship between environmental factors and the occurrence of wetland macroinvertebrates may be useful to develop assessment tools. In this regard, we opted to develop macroinvertebrates multimetric index to assess ecological condition of wetlands in the region. Multimetric indices are increasingly applied for the purpose of conservation actions, since they allow water resources monitoring agencies to get insight in complex biological data and yield policy relevant information for regulatory agencies and decision makers (Karr and Chu, 1999). A multimetric index integrates different individual biological measures into a single value that can potentially reflect multiple effects of human impact on the structure and function of aquatic ecosystems (Barbour et al., 1995; Menetrey et al., 2011). The first step in developing a multimetric index is the classification of sites as reference quality and another set of sites classified a priori as impaired, and that these classifications are based on information independent of biological data (Karr and Chu, 1999). The second step is to identify those biological attributes that respond reliably to human activities, are minimally affected by natural variability (Boesh, 2000). The third step is to combine different metrics into an index system from the assumption that these metrics represent different structural and functional aspects of communities and ecosystems (Karr, 1981).

In this study, wetland sites were designated as reference and impaired based on land use patterns, the degree of habitat degradation as quantified by the USEPA protocol (USEPA, 2002d), variables characterizing hydrological modification, and the Prati index as a measure of chemical water quality. Therefore, the reference sites represent the sites with a minimal disturbance and an acceptable water quality based on the Prati index that can be achieved within a classification category for the catchment. The most appropriate way to define reference conditions for this study was the use of minimally impaired reference sites, since no wetland site was completely unaffected by human influence. Sites with a disturbance score greater than 9 and a basic Prati index of greater than 2 were classified as impaired sites. Based on these criteria, of the 148 samples used for the development of the index, the majority were classified as impaired 91 (61%), and 57 (39%) samples were categorized as

reference. This indicates that habitat and water quality degradation is intensified in the study area.

In comparison to streams, wetland assessment techniques are poorly developed and the field of wetland assessment and monitoring is in its infancy (Brooks et al., 2004). Hence, we used several metrics, which were originally developed for stream bioassessment (Karr and Chu, 1999). However, these metrics may not be responsive to wetland habitat disturbances, since wetland faunal assemblages and ecological processes that occur within wetland are unique and specific data from those assemblages are required to develop metrics for wetlands. Among, the 58 candidate metrics tested, three metric namely total family richness, family richness of Ephemeroptera, Odonata and Trichoptera, and percentage of filterer-collectors were responsive to habitat and water quality degradation, and metric score decreases with increasing in disturbances.

Taxa richness is one of the most reliable indicators in most multimetric indices, and shows good responsiveness to human disturbance (Barbour et al., 1995). Family richness of the order Ephemeroptera, Odonata and Trichoptera (EOT) was also responsive to human disturbances. This metric is well known for its sensitivity to environmental degradation. However, the use of EOT related metrics requires a cautious use because of different tolerance levels among families (Thorne and Williams, 1997). For example, families belonging to order odonata have a wide range of tolerance scores. Gomphidae, a family under order odonata is classified among the most sensitive taxa (tolerance score of one), whereas Coenagriionidae are far less sensitive to pollution (tolerance score of nine) (Bode et al., 1996, Hauer and Lamberti, 1996).

The percentage of filterer-collectors was also responsive to habitat disturbances, and was included as a core component of the MMI. This metric provides information on the available food sources in wetland ecosystems. However, the usefulness of assessing the relative abundance of different functional feeding guilds in benthic macroinvertebrates has been debated (Barbour et al., 1999). Difficulties with the proper assignment of taxa to functional feeding groups (Karr and Chu, 1997) and changes in feeding mode with life stage (Allan, 1995) have contributed to the reluctance to use feeding mode as a reliable metric. The results of the present study show a consistent decrease in the relative abundance of filterer-collectors was observed with increased human disturbance. Many of the filterer-collectors in this study

belonged to the Simuliidae and some to the Trichoptera. These macroinvertebrates are mainly found in river associated wetlands, particularly in wetland receiving water from low order streams, due to their high affinity to dissolved oxygen concentration.

All the three core metrics decreased with increasing human disturbance. The MMI developed in this study has high classification accuracy 80 percent of the validation data set. The MMI showed a strong relationship to a broad range of water quality measures and human disturbances. The observed relationships indicate that macroinvertebrate communities are good candidates for assessing ecological integrity of wetlands. However, the application of this MMI in regional or national bioassessment requires field validation because factors other than disturbances can affect this index, depending on the geographical location and type of wetland. Our MMI can be considered as a robust and sensitive tool that can discriminate less impaired and impaired conditions. The advantage of this MMI is that family level identification was found to be sufficient taxonomic resolution. Hence, this MMI is a rapid and relatively cheap tool for bioassessment of wetlands in the Southwest Ethiopia.

6.2 The role of wetlands on the occurrence and abundance of mosquito

Wetlands are recognized as natural breeding grounds of mosquitoes and are often included in management programs designed to reduce mosquito population (SWS, 2009). There is a long history of draining wetlands for the reduction of mosquito nuisance and disease transmission (Perry and Vanderklein, 1996). The malaria prevention and control program in Ethiopia for example, encourages mosquito larval control through wetland drainage and aquatic vegetation clearance (MOH, 2002). However, knowledge on anopheline larval ecology is insufficient to achieve effective vector control through environmental management. Therefore, a fundamental understanding of the ecology of anopheline mosquito larvae is important in order to plan and implement effective malaria vector control intervention strategies. This study specifically helps to address the question whether permanent wetlands in the neighbourhood of Jimma, which are biodiverse areas that are under serious threat by, land encroachment and is by the public perceived as mosquito breeding grounds, are indeed a preferred habitat for mosquito larvae.

Our results indicate that preferred anopheline breeding sites were pits for plastering and clay mining, agricultural trenches, rain pools, vehicle ruts and animal hoof prints. These habitats are mainly created by human activities. In developing countries, rapid population growth

triggers expansion of agricultural areas, resettlement of landless people, and over exploitation of natural resources (Shewaye, 2008). In our study area, about 48% of the catchment is agricultural land (Bizuayehu, 2002). Agricultural practices such as the use of irrigation for crop cultivation and drainage farming provide suitable breeding grounds for mosquitoes. Agriculture is also considered as the biggest direct cause of deforestation in tropical countries, mostly for subsistence, which is growing crops or raising livestock (Rowe et al., 1992). Deforestation on the other hand leads to changes in micro-climatic conditions and favours the survival and distribution of mosquitoes (Patz and Olson, 2006). Several studies have reported that human disturbance of the natural environment through the action deforestation can favour the spread and colonization of new areas by malaria vectors, increasing the risk of transmission (Guerra et al., 2006; Vittor et al., 2009).

Land cover changes, largely due to deforestation release of CO₂, reduce its uptake by plants, and result in regional climate change (Vitousek, 1991). Model results suggest that the combined effects of past tropical deforestation may have exerted a regional warming of approximately 0.2°C (Chase et al., 2000; Pielke et al., 2002). Changes in temperature due to climate change are expected to influence the behaviour and geographic distribution of the malaria vector (Alemu, et al., 2011). Evidence shows that changes in ambient air temperature and precipitation have already changed the distribution and behaviour of the vector and the number of malaria cases in East African highlands (Zhou et al., 2004). In Ethiopia, malaria was known to occur in areas below 2000 meters above sea level, but currently it has been documented to occur in areas above 2400 meters above sea level (Weyessa et al., 2004), may be due to climatic changes.

Climate change may also increase the amount of energy striking the earth and consequently increasing the water temperature (Stevens, 2012). Higher water temperature favours larval development and allows more microorganisms to grow, which provide food sources for mosquito larvae (Paaajmans, 2008). Our study revealed that small man-made temporary habitats generally had higher water temperature as compared to permanent habitats. Small temporary habitats are characterized by low vegetation cover and are highly exposed to solar radiation. Furthermore, smaller habitats are influenced by ambient air temperature more quickly than large water bodies (Osmond et al., 1995). In this study, an abrupt increase in the abundance of anopheline larvae was observed when the water temperature was between 28°C and 34°C (Chapter 3).

On the other hand, environmental alteration because of deforestation, swamp drainage mainly for agriculture and clay mining for pottery and brick making may ultimately cause a decrease in mosquito predators and competitors abundance and diversity. This in turn, increases the occurrence and abundance of mosquitoes. Studies have shown that wetland drainage for mosquito control and crop production likely reduces the abundance and diversity of mosquito predators and competitors (SWS, 2009). However, this modification creates patches of isolated habitats, which may still hold enough water after a rain event to act as a breeding site for mosquitoes. Mosquito larvae are better dispersers, easily colonized isolated habitats and thus are less influenced by the direct effects of habitat isolation as compared to its competitors and predators (Chase and Shulman, 2009).

This study revealed that permanent water bodies such as ponds, stream margins and natural wetlands were not the preferred habitats for mosquito larvae. These habitats support a wide variety of flora and fauna (Chapter 3). Wetland flora can provide shelter against water current and predation by fish, can provide more food resources, and is important as oviposition site (Couceiro et al., 2007; Ambelu et al., 2010). The decision tree models and ordination diagram demonstrated that the occurrence and abundance of some macroinvertebrate taxa were positively related to wetland vegetation cover (Chapter 2). The occurrence and abundance of invertebrate predators and competitors in less impaired permanent wetlands were significantly higher than semi-permanent and temporary habitats (Chapter 2 and Chapter 3). These predators and competitors are likely to suppress mosquito population.

Environmental modifications (e.g. drainage) of permanent habitats such as natural wetlands for malaria control could reduce the natural predator and competitor population densities, and thus be counter-productive and enhancing the occurrence and abundance of mosquito larvae (Chapter 3). Wetland drainage for mosquito control has entailed a tremendous loss of native ecosystems and reducing the ability of wetland to provide critical ecosystem services (e.g. water supply, water purification, climate regulation, sedges, medicinal plants, fish etc). Therefore, targeting smaller human-made aquatic habitats could result in effective larval control of anopheline mosquitoes in the study area.

6.3 Sediment and nutrient retention efficiency of riverine wetlands

In this study, natural wetlands showed a variable retention of TSS and nutrients due to the differences in input concentration, vegetation cover and the degree of habitat disturbances. Kofe and Kito wetlands, which are considered as more natural, particularly in the upstream sites, are characterized by a release of TSS and nutrients. However, Awetu and Boye wetlands, receiving untreated wastewater and solid wastes from Jimma town had a net retention. Although Awetu and Boye showed a higher retention of TSS and nutrients, the outflow concentration was higher than Kito and Kofe wetlands. The high retention in these wetlands may be due to the high input concentration (mass loading rate). Studies have shown that higher mass loading rates resulted in higher sediment removal rates in constructed wetlands (Tanner et al., 1995; Redmound, 2012).

Awetu, Boye and downstream of Kito wetlands are receiving wastewaters and solid wastes generated by more than 200,000 inhabitants of Jimma town, mostly derived from domestic, and institutional sources, which are directly and indirectly discharged into the major tributaries (Kito river, Awetu river and Becho Bore stream) without any form of primary treatment (Mereta, 2013). Untreated wastewater from municipal slaughterhouse also directly discharged in to Awetu river. Slaughterhouse wastewaters are characterized by high concentration of organic matter, nutrients, solids, fats, oil and greases as well as high microbial load (Kobyia et al., 2006). In addition to increasing the TSS and nutrient loads, this high waste input were increasing the chemical oxygen demand and electric conductivity. However, the concentration of dissolved oxygen and the occurrence and abundance of intolerant macroinvertebrate taxa were very low in Awetu and Boye wetlands (Chapter 2). Therefore, proper management of wastewaters and solid wastes generated by the inhabitants of Jimma town is very essential to reduce the undesirable effect of these pollutants on the receiving rivers/streams and on the wetlands.

Anthropogenic activities such as farming, crazing and clay mining were the main predictors of TSS and nutrients retention in Kofe and Kito wetlands. The rapid population growth and the decline in the soil fertility of the upland trigger expansion of agricultural areas and resettlement of land-less people in wetlands (Shewaye, 2008). In addition, several small-scale activities have been established to drain wetlands for crop cultivation (personal observation). Furthermore, river incisions and back erosions because of heavy rainfall, steep slopes and deforestation have been contributed to landslides in the catchment (Broothaerts et al., 2012).

This may lead to extensive erosion from the upland areas and increase the sediment load in the receiving rivers and siltation problem to the Gilgel Gibe reservoir (Devi et al., 2008). The erosion rate in the Gilgel Gibe catchment is estimated to be 22 ton/ha/year (Devi et al., 2008). This study indicated that, Kofe and Kito wetlands, which are situated in agricultural area, had a net release of TSS and nutrients both in dry and wet seasons. The upstream locations of these wetlands are more natural and progressive deterioration in water quality was observed as water flew downstream. Knox et al (2008) reported that wetlands drained for agricultural use were characterised by lower retention rates and higher export of nutrients and sediments compared to natural reference wetlands.

Wetlands in the region are communal grazing lands. The uncontrolled and free grazing system has caused severe degradation of these resources. This study indicated that grazing explains 25% of the variation in nutrient retention. The deposition of urine and faeces lead to a very high nutrient input and physical changes in the topsoil may decrease the infiltration capacity of the soil (Gathumbi et al., 2004; Pietola et al., 2005) and, consequently, lead to an increase in the release of nutrients and sediments by erosion (Kurz et al., 2005). Angassa and Obo (2010) confirmed that uncontrolled grazing in Southern Ethiopia had a significant effect on the floristic composition of rangelands. Vegetation removal through grazing may influence the nutrient and sediment retention capacity, since vegetation increases nutrient retention through vegetative uptake and facilitates sedimentation (Stevfnson, 1988). In addition, vegetation creates an ideal environment for denitrification by increasing the supply of potentially limiting organic carbon and nitrate to denitrifying bacteria (Weisner et al., 1994; Dhote, 2007).

Brick making is also one of the most serious threats to wetlands in the study area. The construction boom in Jimma and the neighbouring towns triggers the high production of bricks by small-scale enterprises. Brick making is an important source of livelihoods for the local community. Clay bricks are mainly produced by traditional methods where biomass fuels are used for brick burning. Therefore, brick making enterprises act as serious agents of vegetation clearance and removal of the top soil. This facilitates erosion and increases in the sediment loading in rivers and wetlands.

If the current land use patterns now prevailing are allowed to continue further, high quantity of sediment and nutrients will be released. This may lead to siltation and eutrophication problem to the downstream Gilgel Gibe reservoir. Furthermore, alteration of wetlands may also contribute to the loss of biodiversity and other services for local people, who depend on wetland resources not only for drinking water, but also for food and income generating activities. Therefore, sustainable management systems are very essential to maintain their ecological integrity and sustain the ecosystem services. In this regard, devolving rights to the local communities to manage wetland resources by establishing rules and regulations, and enforcing these rules can be necessary for successful community based management.

6.4 Management of the wetlands in southwest Ethiopia

Since Ethiopia is subjected to desertification and recurring drought, the effects of wetland loss could be more visible in complicating the situation locally. Therefore, the rapid loss and degradation of wetlands and its resources and associated ecological and socio-economic impact call for an urgent need for the conservation and wise use. In this regard, the government of Ethiopia has treated wetlands in the water resources, agricultural and environmental policies. However, implementation of wetland management and conservation in the context of the above policies is influenced by a more pressing wetland task force, extension package and food security policies that may seek to convert wetlands for agricultural purposes (Hailu, 2001).

In Ethiopia, different stakeholders view wetlands from their own perspective and institutional objective. The agricultural sector considers wetlands as the most productive land to be drained for crop cultivation, the water sector as a source of water supply and the health sector considers it as a breeding ground for mosquito larvae, which need to be drained or filled for malaria control. The absence of wetland focused institutions, which coordinates management efforts and the weak relation to wetland affiliated global institutions such as the Ramsar Secretariat has hampered its capacity building opportunities and sustainable management of wetlands in the country.

The involvement of local communities in wetland management is of paramount importance, since they are the immediate beneficiaries of wetland resources and are the first victims of wetland loss. Evidence suggested that local communities have managed wetlands in a sustainable manner for generations, and that this management does not necessarily lead to

degradation (Dixon, 2002). An interesting success story of community-based wetland management has been reported from Southwest Ethiopia (Hailu, 2001). In Illubabour zone, most communities have local policies concerning wetland resources utilization, often in the form of 'unwritten' rules based on tradition and the fact that wetlands are communal property (Wood, 2000; Hailu, 2001). Hence, wetland management and conservation plans take into account community management strategies that have evolved over time through the development of local knowledge via the passing down of ancestral knowledge. Wood (2000) pointed out that, local communities have a wealth of knowledge built up over generations on hydrology and soil dynamics and they have developed management practices accordingly, which seem to permit the long-term use of these wetlands without degradation. Therefore, a good communication with the different stakeholders is very important to build up a sustainable wetland management plan.

In conclusion, wetlands of Ethiopia provide various ecological and socioeconomic functions. However, they are losing their vigour at alarming rate due to unwise management. Poor watershed management practices in the uplands such as deforestation, poor farming methods, overgrazing by domestic livestock, clay mining for brick making and effluent discharge from domestic and industrial plants particularly to wetlands adjacent to urban centres are the major threats to wetlands. These alterations contribute to the degradation of water quality, decrease in the abundance and diversity of wetland's fauna and flora, create mosquito breeding grounds, and consequently increase the transmission of malaria. Furthermore, these alterations also reduce the availability of wetland products (sedges, craft materials and medicinal plants) and the related ecosystem services. This in turn has an adverse effect on food security and poverty alleviation with considerable impact on communities who heavily depend on wetland products for their livelihood.

The absence of accountable institutions, which coordinates management efforts and the weak relation of the country to wetland affiliated global institutions such as the Ramsar Secretariat has complicated the problem of conservation and management. Therefore, it becomes necessary that there should be a wetland policy for achieving wise use goals and necessary legal and institutional back up for sustainable wetland management. It is also essential to establish institutions with a mandate to implement policies, provide alternatives to actions that cause wetland degradation and to formulate modalities for a national wetland

management program. Nationwide inventory of wetlands is very essential to develop national policy and management strategies.

6.5 Future prospect

Our results demonstrated that macroinvertebrates have proven to be useful indicators to determine the status of wetlands. However, it is still important to test the response of other indicator taxa particularly macrophytes and birds to compare with the macroinvertebrate based biotic index. For example, investigating water fowl may help to detect aspects of wetland landscape condition that cannot be detected by other indicator groups particularly the connectivity between wetlands at large spatial scales (DWAF, 2004). Macrophytes on the other hand are regarded as the most popular biotic assemblage used for wetland bioassessment, since they are stationary and therefore can integrate seasonal or disturbance factors (Carbiener et al., 1990). In this sense, future research needs to investigate the response of macrophytes and waterfowl to habitat and water quality degradation in the study area.

The MMI developed in this study was able to accurately discriminate the reference and impaired sites. However, the application of this MMI at regional or national bioassessment requires field validation because factors other than disturbances can affect this index, depending on the geographical location and type of wetland. Therefore, validation needs to be carried out before a countrywide application is possible.

A thorough understanding of ecological and socio-economic dimensions can enhance sustainable wetland management. However, economic and livelihood values were not included in this study. Therefore, future research needs to investigate on socio-economic aspects such as the willingness to pay for wetland conservation, best management practices for agriculture and forest management and feasibility study for ecotourism for some important wetlands. For example wetlands in the vicinity of Jimma town harbour a high biodiversity of waterfowl, with more than 140 species including endemic species (Wattled ibis, *Bostrychia carunculata* Rüppel, 1837 and Rouget's Rail, *Rougetius rougetii* Guérin-Méneville, 1843) and IUCN red list species (Wattled crane (*Buggeranus carunculatus* Gmelin, 1789), which makes the area an ideal eco-tourism site.

Appendices

Annex 2.1 Model fitting evaluation

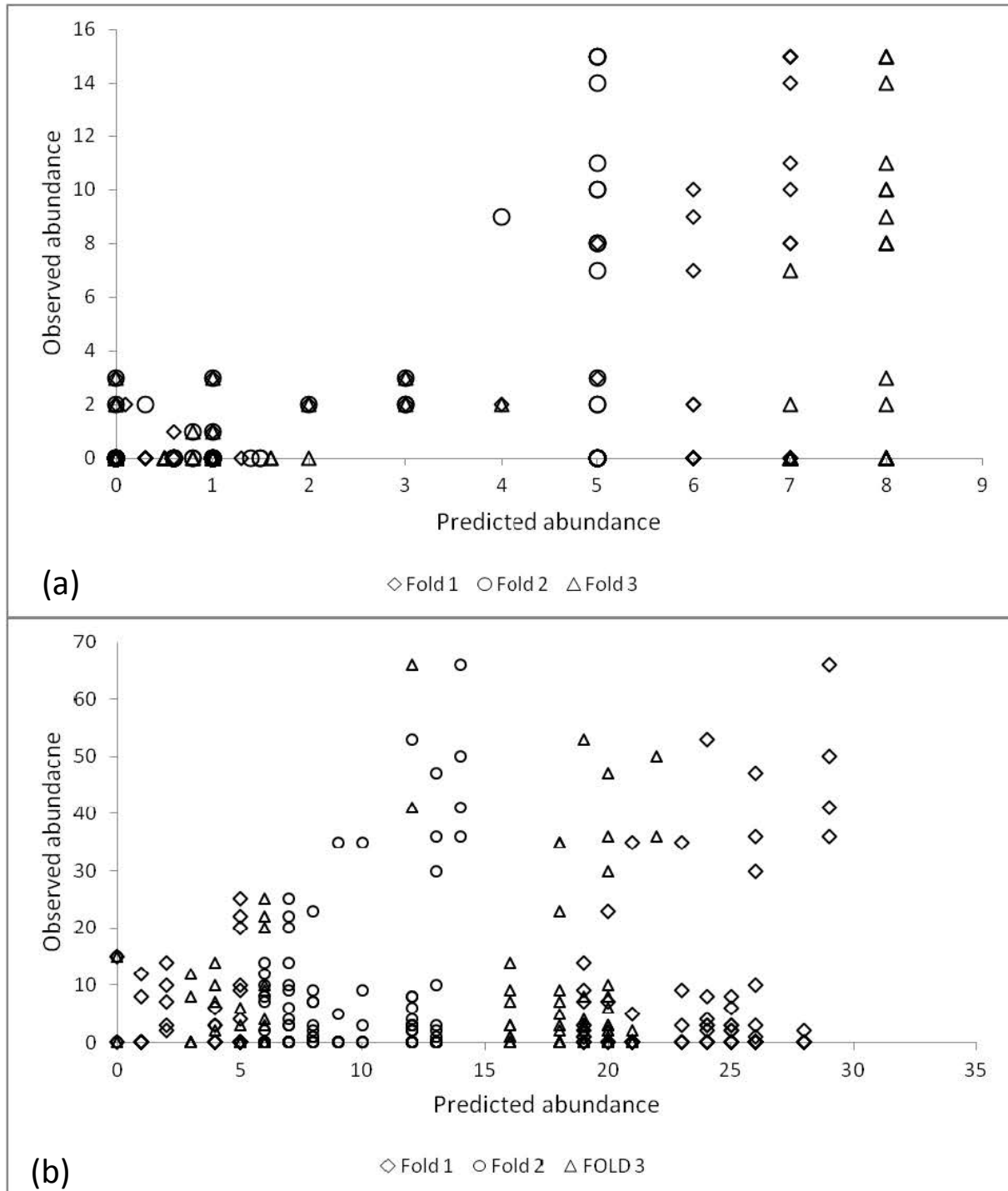


Figure A2.1. The predicted vs observed abundance of Caenidae (a) and Baetidae (b). Predicted abundance was calculated based on aquatic vegetation cover. Fold 2 under predicts the abundance of both Caenidae and Baetidae.

Annex 3.1. Frequency of Macroinvertebrate families collected in the surveyed sites with indication of their functional feeding group.

Family	Frequency of occurrence (%)	Functional feeding group	Reference
Aeshnidae	12	Predator	Bode et al., 1996
Baetidae	27	Gatherer collector	Hauer and Lamberti, 1996
Belostomatidae	24	Predator	Lunde and Resh, 2010
Brachycentridae	1	Gatherer collector & Shredder	Bode et al., 1996
Caenidae	15	Gatherer collector	Hauer and Lamberti, 1996
Ceratopogonidae	7	Predator	Hauer and Lamberti, 1996
Chaoboridae	1	Predator	Bode et al., 1996
Chironomidae	37	Gatherer collector	Bode et al., 1996
Chrysomelidae	4	Shredder	Hauer and Lamberti, 1996
Coenagrionidae	37	Predator	Hauer and Lamberti, 1996
Corduliidae	8	Predator	Bode et al., 1996
Corixidae	45	Predator	Barbour et al., 1999
Culicine	28	Filterer-collector	Bode et al., 1996
Dixidae	5	Gatherer collector	Bode et al., 1996
Dytiscidae	37	Predator	Bode et al., 1996
Elmidae	8	Shredder & Scraper	Hauer and Lamberti, 1996
Ephermerllidae	1	Gatherer collector	Bode et al., 1996

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Erpobdelidae	9	Predator/parasite	Lunde and Resh, 2010
Gerridae	6	Predator	Lunde and Resh, 2010
Glossosomatidae	1	Scraper	Hauer and Lamberti, 1996
Glossiphoniidae	21	Predator	Bode et al., 1996
Gomphidae	1	Predator	Bode et al., 1996
Gyrinidae	16	Predator	Bode et al., 1996
Helodidae	10	Scraper	Bode et al., 1996
Heptageniidae	3	Scraper & Gatherer collector	Hauer and Lamberti, 1996
Hirudinidae	1	Predator	Hauer and Lamberti, 1996
Hydrobilidae	1	Scraper	Barbour et al., 1996
Hydrometridae	5	Predator	
Hydrophilidae	38	Gatherer collector	Bode et al., 1996
Hydropsychidae	4	Filterer-collector	Hauer and Lamberti, 1996
Lepidostomatidae	1	Shredder	Hauer and Lamberti, 1996
Leptoceridae	3	Shredder	Hauer and Lamberti, 1996
Libellulidae	30	Predator	Hauer and Lamberti, 1996
limnephilidae	1	Shredder	Bode et al., 1996
Lymnaeidae	16	Scraper	Barbour et al., 1996
Mesoveliidae	1	Predator	Lunde and Resh, 2010
Naididae	4	Gatherer collector /predator	Bode et al., 1996

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Naucoridae	7	Predator	Hauer and Lamberti, 1996
Nepidae	28	Predator	Hauer and Lamberti, 1996
Notonectidae	13	Predator	Lunde and Resh, 2010
Physidae	3	Scraper	Barbour et al., 1996
Piscicolidae	10	Unkown	
Planorbidae	2	Scraper	Barbour et al., 1996
Psychodidae	8	Gatherer collector	Hauer and Lamberti, 1996
Simuliidae	7	Filterer-collector	Bode et al., 1996
Sphaeriidae	1	Filterer-collector	Barbour et al., 1996
Stratiomyidae	1	Gatherer collector	Bode et al., 1996
Syrphidae	6	Gatherer collector	Hauer and Lamberti, 1996
Tipulidae	1	Shredder, Gatherer collector & predator	Hauer and Lamberti, 1996
Tubificidae	2	Gatherer collector	Barbour et al., 1999
Veliidae	4	Predator	Bode et al., 1996
Tadpole	45	Filterer-collector and predator	Alting et al., 2007

Annex 3.2: Classification trees for the three different subsets (folds)

Classification tree anopheline, subset 1 (*0 =anopheline absent; 1 = anopheline present*; values between brackets indicate instances in which rules are true/false).

Habitat permanency = 1: 1 (65.0/1.0)

Habitat permanency = 2: 1 (11.0/1.0)

Habitat permanency = 3

| Predator = 0

| | Competitors = 0: 1 (16.0/4.0)

| | Competitors = 1: 0 (33.0/9.0)

| Predator = 1: 0 (21.0/1.0)

Number of Leaves: 5; Size of the tree: 8

=== Evaluation on test set ===

Correctly Classified Instances 64 86.5 %

Incorrectly Classified Instances 10 13.5 %

Kappa statistic 0.63

Mean absolute error 0.18

Root mean squared error 0.32

Relative absolute error 43 %

Root relative squared error 71 %

Total Number of Instances 74

=== Confusion Matrix ===

a b <-- classified as

13 6 | a = 0

4 51 | b = 1

Classification tree anopheline, subset 2 (*0 =anopheline absent; 1 = anopheline present*; values between brackets indicate instances in which rules are true/false)

Habitat permanency = 1: 1 (71.0/1.0)

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Habitat permanency = 2: 1 (16.0/1.0)

Habitat permanency = 3

| Predator = 0: 1 (6.0/2.0)

| Predator = 1

| | Water temperature <= 20.7: 0 (15.0)

| | Water temperature > 20.7

| | | Water temperature <= 21.1: 0 (4.0)

| | | Water temperature > 21.1: 1 (34.0/8.0)

Number of Leaves: 6; Size of the tree: 10

=== Evaluation on test set ===

Correctly Classified Instances	61	82.4 %
Incorrectly Classified Instances	13	17.6 %
Kappa statistic	0.63	
Mean absolute error	0.21	
Root mean squared error	0.36	
Relative absolute error	49 %	
Root relative squared error	77 %	

=== Confusion Matrix ===

a b <-- classified as

21 3 | a = 0

10 40 | b = 1

Classification tree anopheline, subset 3 (*0 = anopheline absent; 1 = anopheline present*;
values between brackets indicate instances in which rules are true/false).

Habitat permanency = 1: 1 (72.0)

Habitat permanency = 2

| pH <= 6.55: 0 (3.0/1.0)

| pH > 6.55: 1 (8.0)

Habitat permanency = 3

| Competitor = 0

| | Emergent plant cover = 0

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| | | DO <= 6.11: 0 (6.0/1.0)
| | | DO > 6.11: 1 (4.0/1.0)
| | Emergent plant cover = 1: 1 (0.0)
| | Emergent plant cover = 2: 1 (4.0)
| | Emergent plant cover = 3: 0 (2.0)
| | Emergent plant cover = 4
| | | pH <= 6.43: 1 (2.0)
| | | pH > 6.43
| | | | Canopy cover <= 10: 1 (3.0/1.0)
| | | | Canopy cover > 10: 0 (2.0)
| Competitor = 1: 0 (40.0/10.0)

Number of Leaves: 12, Size of the tree: 19

=== Evaluation on test set ===

Correctly Classified Instances	64	86.5%
Incorrectly Classified Instances	10	13.5 %
Kappa statistic	0.71	
Mean absolute error	0.19	
Root mean squared error	0.32	
Relative absolute error	42 %	
Root relative squared error	66 %	

=== Confusion Matrix ===

a b <-- classified as

23 3 | a = 0

7 41 | b = 1

Annex 3.3. Output GLM presence–absence data/Logistic regression model

Call:

glm(formula = Anopheline presence ~ Habitat type + Permanency + Canopy cover (%) + Emerged plant cover + Invertebrate predator + Fish + Competitors, family = binomial, data = dataset mosquito)

Table A3.3.1. Results of logistic regression analysis

	Parameter estimate	Std. Error	z value	Pr(> z)
Intercept	2.376e+00	1.043e+00	2.279	0.02267 *
Habitat Type				
Marshland				
Reservoir	3.396e+00	1.226e+00	2.770	0.00561 **
Stream margin	6.252e-01	8.528e-01	0.733	0.46349
Pond	2.036e-02	1.002e+00	0.020	0.98378
Farm ditch	1.547e+00	1.419e+00	1.090	0.27575
Pits	1.211e+00	1.190e+00	1.018	0.30887
Road puddle	-1.224e+00	1.391e+00	-0.880	0.37870
Hoof print	6.517e-01	1.419e+00	0.459	0.64602
Rain pool	1.759e+01	1.865e+03	0.009	0.99248
Permanency				
Temporary				
Semi-permanent	1.762e+00	1.284e+00	1.372	0.17018
Permanent	-2.873e+00	7.134e-01	-4.027	5.6e-05 ***
Canopy cover	-4.151e-02	.539e-02	-2.697	0.00699 **
Emergent plant cover				
<10%				
10-35%	1.814e+01	1.499e+03	0.012	0.99034
35-65%	-0.536e-01	8.956e-01	-0.506	0.61256
65-90%	-2.879e+00	1.042e+00	-2.762	0.00575 **
>90%	-2.932e+00	1.012e+00	-2.896	0.00378 **
Invertebrate predator	-1.640e-02	7.461e-03	-2.199	0.02790 *
Fish	-1.421e+00	6.031e-01	-2.356	0.01849 *
Competitor	-1.292e-01	4.689e-02	-2.756	0.00586 **

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 270.63 on 215 degrees of freedom

Residual deviance: 129.02 on 197 degrees of freedom

AIC: 167.02

Number of Fisher Scoring iterations: 17

Table A3.3.2. Logistic Regression model performance

		Model Likelihood ratio test		Discrimination index		Rank discrimination index	
Observation	216	LR chi2	141.60	R ²	0.673	c	0.938
0	69	df	18	G	4.215	Dxy	0.877
1	147	Pr(> chi2	<0.0001	Gr	67.689	gamma	0.877
max deriv	0.1			Gp	0.38	tau-a	0.383
				Brier	0.090		

Annex 3.4: Regression trees for the three different subsets (folds)

Regression tree with regression equations predicting the abundance of anopheline larvae:
subset 1

Instances: 146

Attributes: 8

Number of rules: 2

Predator abundance <= 12.5: LM1 (77/94.857%)

Predator abundance >12.5: LM2 (69/83.233%)

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(LM 1): Anopheline abundance = $0.8052 * \text{Water temperature} - 0.8995 * \text{Dissolved oxygen (DO)} + 0.0024 * \text{Total dissolved solid (TDS)} + 9.7576 * \text{Nitrate} - 0.0088 * \text{Predator abundance} - 11.4928$

(LM 2): Anopheline abundance = $0.2232 * \text{Water temperature} + 0.0237 * \text{Alkalinity} + 2.9972 * \text{Nitrate} - 0.0138 * \text{Predator abundance} - 5.0098$

=== Evaluation on test set ===

Correlation coefficient	0.39
Mean absolute error	5.6
Root mean squared error	10.4
Relative absolute error	105 %
Root relative squared error	98 %
Total Number of Instances	74

Regression tree with regression equations predicting the abundance of anopheline larvae:
subset 2

Instances: 146

Attributes: 3

Number of rules: 3

Predator abundance ≤ 1.5 :

| Water temperature ≤ 28.25 : LM1 (39/89.184%)

| Water temperature > 28.25 : LM2 (10/197.755%)

Predator abundance > 1.5 : LM3 (97/42.449%)

(LM 1): Anopheline abundance = $0.4845 * \text{Water temperature} - 0.0086 * \text{Predator abundance} - 2.933$

(LM 2): Anopheline abundance = $2.92 * \text{Water temperature} - 0.0086 * \text{Predator abundance} - 72.7887$

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(LM 3): Anopheline abundance = $0.6446 * \text{Water temperature} - 0.0186 * \text{Predator abundance} - 10.5555$

=== Evaluation on test set ===

Correlation coefficient	0.44
Mean absolute error	4.4
Root mean squared error	7.2
Relative absolute error	80 %
Root relative squared error	81%
Total Number of Instances	74

Regression tree with regression equations predicting the abundance of anopheline larvae:
subset 3

Instances: 146

Attributes: 13

Number of rules: 3

Water temperature ≤ 26.95 : LM1 (115/50.628%)

Water temperature > 26.95 :

| Water temperature ≤ 29.15 : LM2 (20/46.294%)

| Water temperature > 29.15 : LM3 (11/171.559%)

(LM 1): Anopheline abundance = $0.0862 * \text{Water temperature} + 0.0143 * \text{TDS} + 0.0068 * \text{Turbidity} - 0.0211 * \text{Predator abundance} - 0.271 * \text{Competitor abundance} + 1.402$

(LM 2): Anopheline abundance = $1.9378 * \text{Water temperature} + 0.438 * \text{DO} + 0.0062 * \text{TDS} + 0.0018 * \text{Turbidity} + 3.9375 * \text{Nitrate} - 0.0779 * \text{Predator abundance} - 0.1059 * \text{Competitor abundance} - 52.3147$

(LM 3): Anopheline abundance = 4.984 * Water temperature + 0.5896 * DO + 0.0062 * TDS + 0.0018 * Turbidity + 12.7011 * Nitrate - 0.1016 * Predator abundance- 0.1059 * Competitor abundance - 144.5709

=== Evaluation on test set ===

Correlation coefficient	0.42
Mean absolute error	3.80
Root mean squared error	6.03
Relative absolute error	77 %
Root relative squared error	70%
Total Number of Instances	74

Annex 3.5. GLM output count data/Poisson regression model

Call:

```
glm(formula = Anopheline ~ Habitat type + Permanency + Canopy cover +
    Emerged plantcover + Submerged plant cover + Substrate type + Water temperature +
    DO + pH + Invertebrate predator + Fish + Competitors, family = Poisson,
    data = dataset_mosquito)
```

Table A3.5.1. Results of Poisson regression analysis

	Parameter estimate	Std. Error	z value	Pr(> z)
Intercept	-0.343001	0.511487	-0.671	0.502478
Reservoir	0.845047	0.193685	4.363	1.3e-05 ***
Stream margin	-0.016435	0.182070	-0.090	0.928073
Habitat Type				
Pond	0.065095	0.180986	0.360	0.719095
Farm ditch	0.334415	0.181829	2.019	0.043498 *
Pits	0.074204	0.187612	0.615	0.538572
Road puddle	0.644388	0.223464	2.884	0.003931 **

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	Hoof print	0.433696	0.201117	2.156	0.031049 *
	Rain pool	0.003626	0.215831	0.017	0.986595
Permanency	Semi-permanent	0.092696	0.195469	0.474	0.635340
	Permanent	-0.876157	0.144182	-6.077	1.2e-09 ***
Canopy cover		-0.019400	0.004287	-4.526	6.0e-06 ***
Emergent plant cover		-0.116150	0.030566	-3.972	7.1e-05 ***
	10-35%	-0.265812	0.167552	-1.586	0.112638
	35-65%	-15.243596	631.586632	-0.024	0.980745
Submerged plant cover	>90%	0.924783	0.385020	2.402	0.980745
	Sand	0.307249	0.222254	1.382	0.166842
Substrate type	Gravel	0.305550	0.203512	1.501	0.133255
	Artificial substrate	-1.434391	0.723136	-1.984	0.047304 *
Water temperature		0.052713	0.011732	4.493	7.1e-06 ***
DO		-0.035606	0.015993	-2.226	0.025988 *
pH		0.168407	0.052501	3.117	0.001338 **
Invertebrate predator		-0.005012	0.001316	-3.807	0.00014 ***
Fish		-0.504069	0.107376	-4.694	2.7e-06 ***
Competitors		-0.034006	0.008007	-4.247	2.2e-05 ***

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Poisson family taken to be 1)

Null deviance: 1410.67 on 215 degrees of freedom

Residual deviance: 716.12 on 191 degrees of freedom

AIC: 1292.6

Number of Fisher Scoring iterations: 13

Pseudo R squared = 0.96

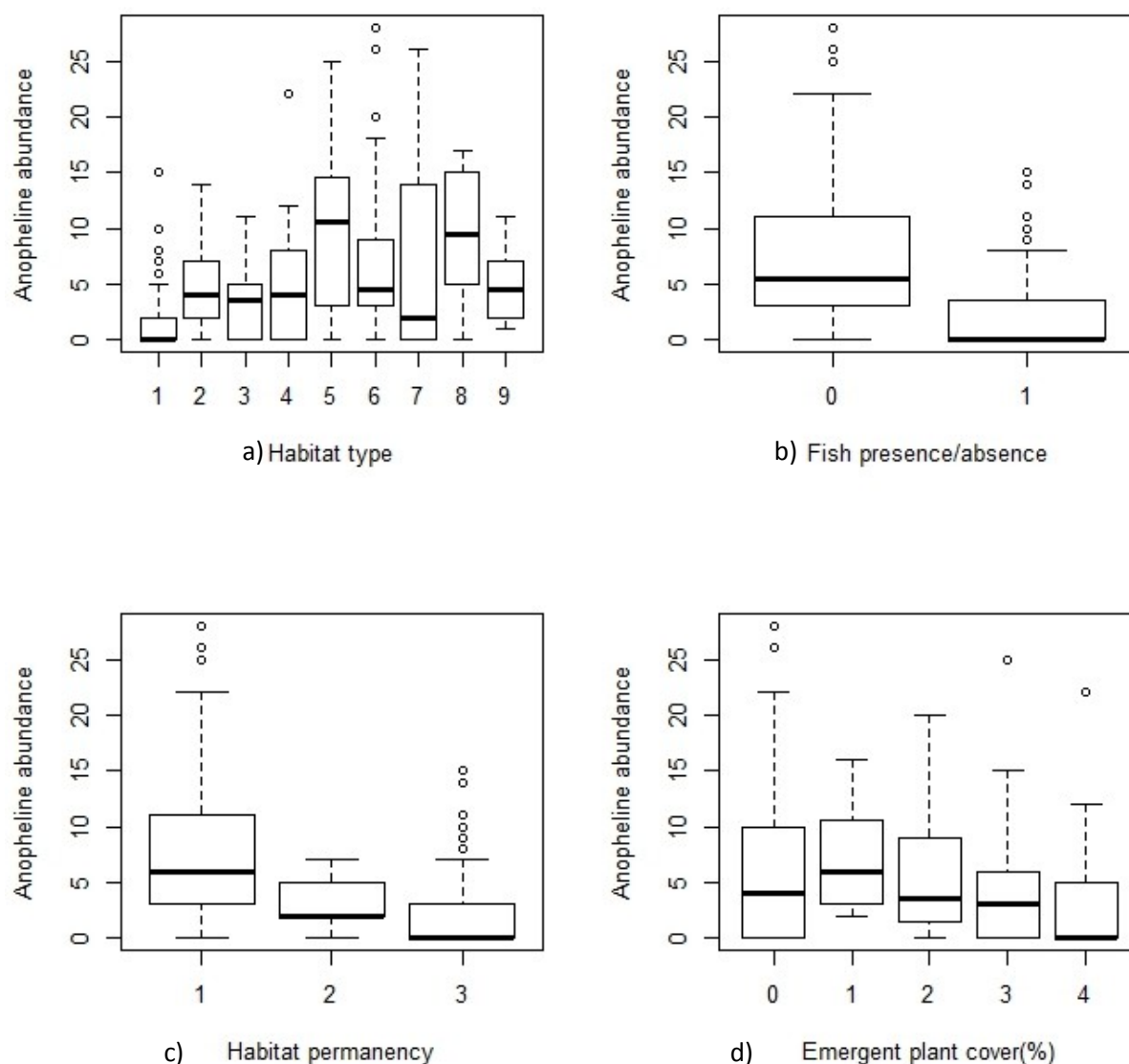


Figure A3.5.1. Box plots showing the effect of habitat type (a), occurrence of fish (b), habitat permanency (c) and vegetation cover (d) on the abundance of anopheline larvae. Small black squares represent median numbers, boxes represent inter-quartile ranges (25–75% percentiles) and range bars show maximum and minimum values, circles are used to denote outliers. Habitat type (1= Marshland, 2 = Reservoir, 3 = Stream margin, 4 = Pond, 5 = Farm ditch, 6 = Pits, 7 = Road puddle, 8 = Hoof print, 9 = rain pool); Fish (0 = absence, 1 = present); Habitat permanency (1 = temporary, 2 = Semi-permanent, 3 = permanent); Emergent plant cover (0 = <10%, 1 = 10-35%, 2 = 35-65%, 3 = 65-90% , 4 = >90%).

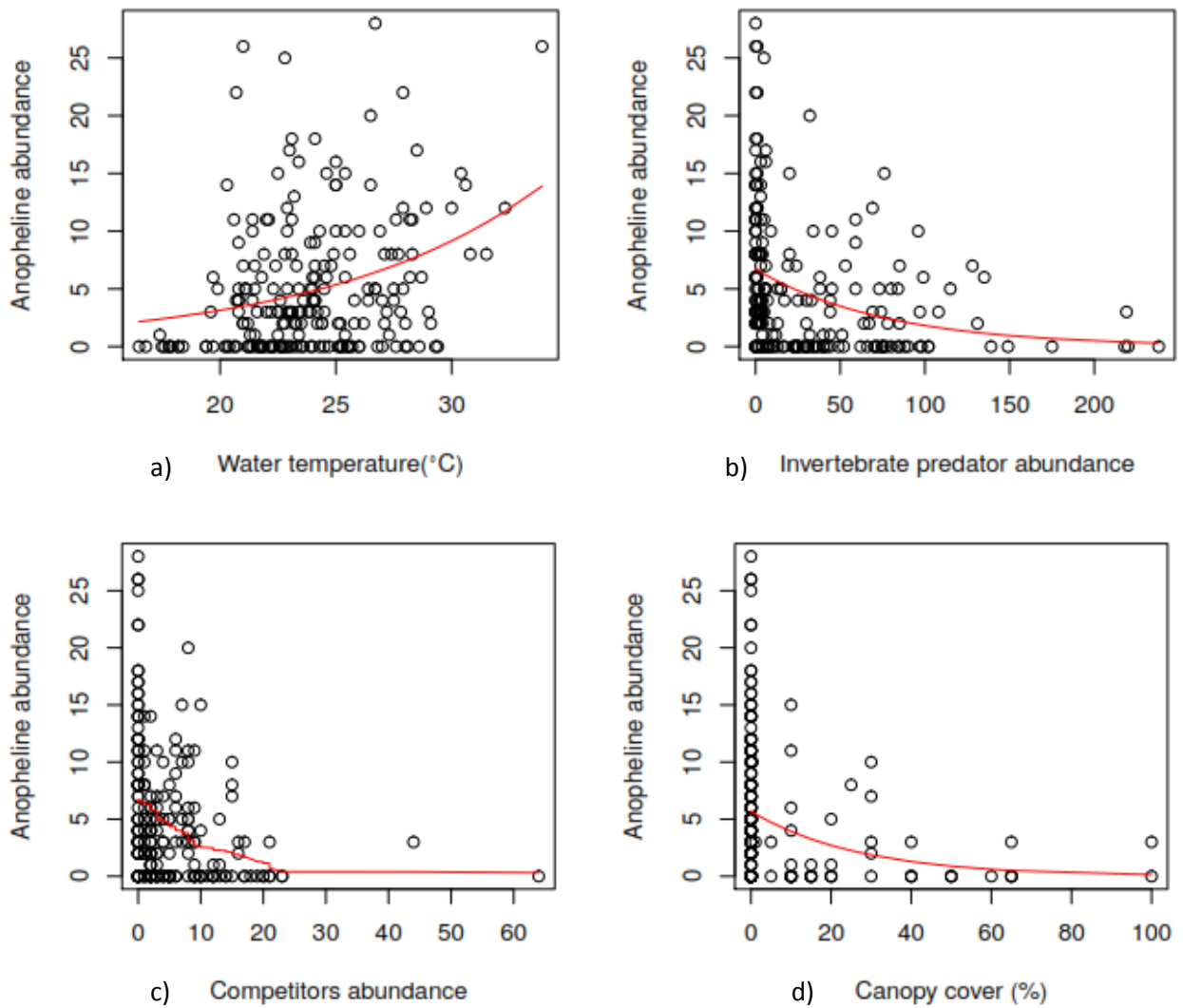


Figure A3.5.2. Poisson regression models predicting the abundance of anopheline larvae in function of water temperature (a), Invertebrate predators abundance (b), competitors abundance (c) and canopy cover (d).

Annex 4. Results of stepwise multiple regression analysis

Table A4.1: Stepwise multiple regression model of TSS retention/release in relation to habitat disturbances

Model		Coefficient	Standard error	Beta	t-value	Significance value	Variance inflation factor
1	Constant	2.848	0.307		9.290	p<0.001	
	Farming	-1.388	0.144	-0.758	-9.656	p<0.001	1.00
2	Constant	3.275	0.287		11.407	p<0.001	
	Farming	-0.957	0.159	-0.523	-6.020	p<0.001	1.56
	Dumping	-0.763	0.169	-0.391	-4.505	p=0.001	1.56
3	Constant	3.178	0.277		8.348	p<0.001	
	Farming	-1.432	0.234	-0.782	-6.109	p<0.001	3.70
	Dumping	-0.827	0.164	-0.424	-5.044	p<0.001	1.60
	Mining	-0.654	0.245	-0.332	-2.665	p=0.010	3.51
4	Constant	4.220	0.505		8.348	p<0.001	
	Farming	-1.382	0.227	-0.755	-6.082	p<0.001	3.75
	Dumping	-0.830	0.158	-0.426	-5.246	p<0.001	1.60
	Mining	-0.736	0.239	-0.373	-3.076	p=0.030	3.58
	Grazing	-0.716	0.295	-0.169	-2.403	p=0.018	1.18

Model Summary

Model	R	R ²	Adjusted R ²	Standard error of the estimate
1	0.76	0.58	0.57	1.17
2	0.82	0.67	0.66	1.03
3	0.84	0.70	0.69	0.99
4	0.85	0.73	0.71	0.96

Table A4.2. Stepwise multiple regression model of nutrient retention /release in relation to habitat disturbances

Model		Coefficient	Standard error	Beta	t-value	Significance value	Variance inflation factor
1	Constant	2.712	0.260		10.448	p<0.001	
	Grazing	-1.512	0.139	-0.500	-10.839	p<0.001	1.00
2	Constant	3.190	0.291		10.945	p<0.001	
	Grazing	-1.210	0.163	-0.400	-7.403	p<0.001	1.41
	Dumping	-0.568	0.166	-0.185	-3.417	p=0.001	1.41
3	Constant	3.319	0.297		11.169	p<0.001	
	Grazing	-1.009	0.191	-0.333	-5.288	p<0.001	1.95
	Dumping	-0.445	0.176	-0.145	-2.524	p=0.012	1.61
	Farming	-0.385	0.191	-0.131	-2.017	p=0.044	2.07

Model Summary

Model	R	R ²	Adjusted R ²	Standard error of the estimate
1	0.50	0.25	0.25	2.34
2	0.52	0.27	0.27	2.31
3	0.53	0.28	0.28	2.30

Annex 5.1. List of macroinvertebrate families encountered in this study with indication of their frequency of occurrence, abundance, tolerance score (TS) and functional feeding group. Tolerance values range from 0 for organisms very intolerant of organic wastes to 10 for organisms very tolerant of organic wastes.

Family	Frequency of occurrence (%)	Abundance	TS	Functional feeding group	Reference
Aeshnidae	32	362	5	Predator	Bode et al., 1996
Baetidae	52	1690	4	Gatherer-collector and Scraper	Hauer and Lamberti, 1996
Belostomatidae	55	833	10	Predator	Lunde and Resh, 2011
Beraidae	1	7	4	Gatherer-collector	Hauer and Lamberti, 1996
Brachycentridae	2.3	22	1	Gatherer-collector and Shredder	Bode et al., 1996
Caenidae	27	936	7	Gatherer-collector	Hauer and Lamberti, 1996
Calopterygidae	1.4	22	8	Predator	Hauer and Lamberti, 1996
Ceratopogonidae	29	481	6	Predator	Hauer and Lamberti, 1996
Chaoboridae	5	19	8	Predator	Bode et al., 1996
Chironomidae	83	2885	8	Predator, Gatherer-collector	Bode et al., 1996
Chrysomelidae	8	33	2	Shredder	Hauer and Lamberti, 1996
Coenagrionidae	2685	72	9	Predator	Hauer and Lamberti, 1996
Corbiculidae	1.4	15	4	Filterer-collector	Bode et al., 1996

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Cordulgastridae	2.7	17	3	Predator	Hauer and Lamberti, 1996
Corduliidae	9.5	148	5	Predator	Bode et al., 1996
Corixidae	66.2	3087	5	Predator	Barbour et al., 1999
Culicidae	24.3	275	8	Filterer-collector	Bode et al., 1996
Dixidae	5.9	46	1	Gatherer-collector	Bode et al., 1996
Dolichopodidae	1	2	4	Predator	Hauer and Lamberti, 1996
Dryopidae	0.45	2	5	Scraper	Bode et al., 1996
Dytiscidae	76.1	1928	5	Predator	Bode et al., 1996
Elmidae	21	279	4	Shredder /Scraper	Hauer and Lamberti, 1996
Empididae	1	3	6	Predator	Hauer and Lamberti, 1996
Enchytraeidae	0.45	1	10	Gatherer collector	Bode et al., 1996
Ephermerllidae	5.5	49	1	Gatherer-collector	Bode et al., 1996
Erpobdelidae	17.6	135	8	Predator/Parasite	Lunde and Resh, 2011
Gerridae	5.4	17	5	Predator	Lunde and Resh, 2011
Glossosomatidae	1.4	23	0	Scraper	Hauer and Lamberti, 1996
Glossiphoniidae	45.5	561	8	Predator	Bode et al., 1996
Gomphidae	6.3	52	1	Predator	Bode et al., 1996
Gyrinidae	24	212	4	Predator	Bode et al., 1996
Haliplidae	1	3	5	Shredder	Bode et al., 1996
Haplotaxidae	2.7	10	5	Predator	Bode et al., 1996
Helodidae	22.5	419	5	Scraper	Bode et al., 1996

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Heptageniidae	2.7	9	4	Scraper/Gatherer-collector	Hauer and Lamberti, 1996
Hirudinidae	5.4	51	10	Predator	Hauer and Lamberti, 1996
Hydrobiidae	2.7	11	7	Scraper	Barbour et al., 1996
Hydrophilidae	73	1653	5	Gatherer-collector	Bode et al., 1996
Hydrometridae	16.2	63	6	Predator	Bode et al., 1996
Hydropsychidae	10	244	4	Filterer-collector	Hauer and Lamberti, 1996
Hygrobiidae	2.7	30	5	Predator	Bode et al., 1996
Lepidostomatidae	1.8	31	1	Shredder	Hauer and Lamberti, 1996
Leptoceridae	1.8	10	4	Shredder	Hauer and Lamberti, 1996
Lestidae	1	4	9	Predator	Hauer and Lamberti, 1996
Libellulidae	56	1029	9	Predator	Hauer and Lamberti, 1996
Limnephilidae	1	4	4	Shredder	Bode et al., 1996
Lumbricidae	1	3	6	Gatherer-collector	Bode et al., 1996
Lumbriculidae	10	55	5	Gatherer-collector	Bode et al., 1996
Lymnaeidae	55	748	6	Scraper	Barbour et al., 1996
Mesoveliidae	1.8	4	5	Predator	Lunde and Resh, 2011
Naididae	2.7	51	8	Gatherer-collector /predator	Bode et al., 1996
Naucoridae	17.2	122	5	Predator	Hauer and Lamberti, 1996
Nepidae	15	64	5	Predator	Hauer and Lamberti, 1996
Noteridae	15.3	167	5	Predator	Shah et al. 2011
Notonectidae	48	2009	10	Predator	Lunde and Resh, 2011

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Perlodidae	1	3	2	Predator	Hauer and Lamberti, 1996
Philopotamidae	1	6	3	Filterer-collector	Hauer and Lamberti, 1996
Physidae	15.3	221	8	Scraper	Barbour et al., 1996
Planorbidae	34	426	7	Scraper	Barbour et al., 1996
Psychodidae	3.2	8	10	Gatherer-collector	Hauer and Lamberti, 1996
Scatophagidae	1.4	3	6	Shredder	Bode et al., 1996
Sciomyzidae	4	40	6	Predator/Parasite	Bode et al., 1996
Sericostomatidae	1	4	3	Shredder	Hauer and Lamberti, 1996
Simuliidae	19.4	602	6	Filterer-collector	Bode et al., 1996
Sphaeriidae	28	499	8	Filterer-collector	Barbour et al., 1996
Stratiomyidae	8.2	72	7	Gatherer-collector	Bode et al., 1996
Syrphidae	3.2	9	10	Gatherer-collector	Hauer and Lamberti, 1996
Tabanidae	1	4	6	Predator	Hauer and Lamberti, 1996
Tipulidae	18	97	3	Shredder/Gatherer	Hauer and Lamberti, 1996
Tubificidae	2.7	39	10	Gatherer-collector	Barbour et al., 1999
Uniodidae	1.8	12	8	Filterer-collector	Barbour et al., 1996
Veliidae	3.6	14	6	Predator	Bode et al., 1996

Annex 5.2. List of sampling sites, sampling season, score of the core metrics and the final Multimetric Macroinvertebrate Index (MMI) and site quality class. A score of 5 indicates that the sample meets the reference condition, a score of 3 represents an intermediate condition and a score of 1 indicates the highest deviation from the reference condition (Barbour et al., 1996). Finally the MMI range was divided into five quality classes: 3-5 = very bad, 6-8 = bad, 9-11= moderate, 12-13 = good and 14-15 = very good. Kt: Kito, Kf: Kofe, Bo: Boye, Aw: Awetu, H: Haro, Bu: Bulbul, Bw: Balawajo, HT: Haro temporary.

Site code	Sampling period	Season	Family richness score	EOT family richness score	% Filterer-Collector score	MMI score	Quality class
kt1	Apr-10	dry	5	5	5	15	Very good
kt2	Apr-10	dry	5	5	5	15	Very good
kt3	Apr-10	dry	5	5	3	13	Good
kt4	Apr-10	dry	5	5	3	13	Good
kt5	Apr-10	dry	5	5	5	15	Very good
kt6	Apr-10	dry	5	5	5	15	Very good
kt7	Apr-10	dry	5	5	5	15	Very good
kt8	Apr-10	dry	5	5	5	15	Very good
kt9	Apr-10	dry	5	5	5	15	Very good
kt10	Apr-10	dry	5	5	5	15	Very good

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kt11	Apr-10	dry	3	3	3	9	Moderate
kt12	Apr-10	dry	3	3	1	7	Bad
kt13	Apr-10	dry	3	3	1	7	Bad
kf1	Feb-10	dry	5	5	5	15	Very good
kf2	Feb-10	dry	3	3	1	7	Bad
kf3	Feb-10	dry	5	5	5	15	Very good
kf4	Feb-10	dry	3	3	3	9	Moderate
kf5	Feb-10	dry	5	3	5	13	Good
kf6	Feb-10	dry	5	5	3	13	Good
kf7	Feb-10	dry	3	3	1	7	Bad
kf8	Feb-10	dry	3	3	3	9	Moderate
kf9	Feb-10	dry	3	3	3	9	Moderate
kf10	Feb-10	dry	5	5	5	15	Very good
kf11	Feb-10	dry	5	5	5	15	Very good
kf12	Feb-10	dry	3	3	3	9	Moderate
kf13	Feb-10	dry	3	3	5	11	Moderate
kf14	Apr-10	dry	3	1	1	5	Very bad

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Bo1	Apr-10	dry	3	5	3	11	Moderate
Bo2	Apr-10	dry	3	3	3	9	Moderate
Bo3	Apr-10	dry	3	1	3	7	Bad
Bo4	Apr-10	dry	5	3	5	13	Good
Bo5	Apr-10	dry	5	5	3	13	Good
Bo6	Apr-10	dry	3	1	3	7	Bad
Bo7	Apr-10	dry	3	1	3	7	Bad
Bo8	Apr-10	dry	3	1	3	7	Bad
Aw1	Apr-10	dry	3	3	3	9	Moderate
H001	Apr-10	dry	3	5	1	9	Moderate
H002	Apr-10	dry	5	5	3	13	Good
H003	Apr-10	dry	3	3	3	9	Moderate
H004	Apr-10	dry	5	5	1	11	Moderate
H005	Apr-10	dry	5	5	3	13	Good
H006	Apr-10	dry	3	5	1	9	Moderate
H007	Apr-10	dry	3	5	1	9	Moderate
H008	Apr-10	dry	5	5	3	13	Good

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Bu1	May-10	dry	1	1	1	3	Very bad
Bu2	May-10	dry	3	1	1	5	Very bad
Bu3	May-10	dry	3	5	1	9	Moderate
Bw1	Apr-10	dry	5	5	5	15	Very good
Bw2	Apr-10	dry	5	3	5	13	Good
Bw3	Apr-10	dry	3	3	3	9	Moderate
HT3	Apr-10	dry	3	1	1	5	Very bad
HT4	Apr-10	dry	3	1	1	5	Very bad
kt1	Sep-10	wet	5	5	5	15	Very good
kt2	Sep-10	wet	5	5	5	15	Very good
kt3	Sep-10	wet	1	3	1	5	Very bad
kt4	Sep-10	wet	5	5	3	13	Good
kt5	Sep-10	wet	5	5	5	15	Very good
kt6	Sep-10	wet	5	5	3	13	Good
kt7	Sep-10	wet	5	5	3	13	Good
kt8	Sep-10	wet	5	5	3	13	Good
kt9	Sep-10	wet	3	5	1	9	Moderate

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kt10	Sep-10	wet	3	5	1	9	Moderate
kt11	Sep-10	wet	3	3	3	9	Moderate
kt12	Sep-10	wet	3	5	1	9	Moderate
kt13	Sep-10	wet	3	5	1	9	Moderate
kf1	Sep-10	wet	5	5	5	15	Very good
kf2	Sep-10	wet	3	3	5	11	Moderate
kf3	Sep-10	wet	3	5	1	9	Moderate
kf4	Sep-10	wet	3	3	1	7	Bad
kf5	Sep-10	wet	5	3	5	13	Good
kf6	Sep-10	wet	3	3	3	9	Moderate
kf7	Sep-10	wet	5	3	5	13	Good
kf8	Sep-10	wet	5	5	5	15	Very good
kf9	Sep-10	wet	3	3	3	9	Moderate
kf10	Sep-10	wet	5	5	5	15	Very good
kf11	Sep-10	wet	5	5	5	15	Very good
kf12	Sep-10	wet	5	5	5	15	Very good
kf13	Sep-10	wet	5	5	3	13	Good

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kf14	Sep-10	wet	5	5	5	15	Very good
Bo1	Sep-10	wet	3	3	1	7	Bad
Bo2	Sep-10	wet	3	3	1	7	Bad
Bo3	Sep-10	wet	3	3	1	7	Bad
Bo4	Sep-10	wet	5	3	1	9	Moderate
Bo5	Sep-10	wet	5	3	1	9	Moderate
Bo6	Sep-10	wet	5	5	1	11	Moderate
Bo7	Sep-10	wet	3	1	1	5	Very bad
Bo8	Sep-10	wet	3	3	1	7	Bad
Aw1	Sep-10	wet	3	3	3	9	Moderate
H001	Aug-10	wet	3	3	3	9	Moderate
H002	Aug-10	wet	3	3	1	7	Bad
H003	Aug-10	wet	3	1	1	5	Very bad
H004	Aug-10	wet	3	5	1	9	Moderate
H005	Aug-10	wet	3	3	1	7	Bad
H006	Aug-10	wet	3	5	1	9	Moderate
H007	Aug-10	wet	3	3	3	9	Moderate

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H008	Aug-10	wet	3	5	1	9	Moderate
H009	Aug-10	wet	3	5	3	11	Moderate
Bu1	Aug-10	wet	3	1	1	5	Very bad
Bu2	Aug-10	wet	3	5	1	9	Moderate
Bu3	Aug-10	wet	5	5	1	11	Moderate
Bw1	Aug-10	wet	5	5	5	15	Very good
Bw2	Aug-10	wet	5	5	3	13	Good
B3	Aug-10	wet	5	5	5	15	Very good
HT1	Sep-10	wet	3	5	1	9	Moderate
HT2	Sep-10	wet	3	3	1	7	Bad
HT3	Sep-10	wet	5	5	3	13	Good
HT4	Sep-10	wet	3	1	1	5	Very bad
HT5	Sep-10	wet	3	5	1	9	Moderate
Bore	Aug-10	wet	1	3	1	5	Very bad
kt1	Mar-11	dry	5	5	5	15	Very good
kt2	Mar-11	dry	3	5	5	13	Good
kt3	Mar-11	dry	5	5	5	15	Very good

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kt4	Mar-11	dry	5	5	3	13	Good
kt5	Mar-11	dry	5	5	3	13	Good
kt6	Mar-11	dry	3	5	3	11	Moderate
kt7	Mar-11	dry	5	5	5	15	Very good
kt8	Mar-11	dry	3	3	3	9	Moderate
kt9	Mar-11	dry	5	5	5	15	Very good
kt10	Mar-11	dry	5	5	5	15	Very good
kt11	Mar-11	dry	3	3	3	9	Moderate
kt12	Mar-11	dry	5	5	3	13	Good
kt13	Mar-11	dry	3	3	1	7	Bad
kf1	Mar-11	dry	5	5	5	15	Very good
kf2	Mar-11	dry	3	5	1	9	Moderate
kf3	Mar-11	dry	5	5	5	15	Very good
kf4	Mar-11	dry	3	3	5	11	Moderate
kf5	Apr-11	dry	3	3	5	11	Moderate
kf6	Apr-11	dry	3	3	1	7	Bad
kf7	Apr-11	dry	1	1	1	3	Very bad

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kf8	Apr-11	dry	1	1	1	3	Very bad
kf9	Apr-11	dry	1	1	1	3	Very bad
kf10	Apr-11	dry	1	1	1	3	Very bad
kf11	Apr-11	dry	1	1	1	3	Very bad
kf12	Apr-11	dry	3	1	1	5	Very bad
kf13	Apr-11	dry	1	1	1	3	Very bad
kf14	Mar-11	dry	3	5	1	9	Moderate
Bo1	Mar-11	dry	3	3	3	9	Moderate
Bo2	Mar-11	dry	3	3	5	11	Moderate
Bo3	Mar-11	dry	3	5	1	9	Moderate
Bo4	Mar-11	dry	3	3	5	11	Moderate
Bo5	Mar-11	dry	1	1	1	3	Very bad
Bo6	Mar-11	dry	3	3	1	7	Bad
Bo7	Mar-11	dry	3	3	1	7	Bad
Bo8	Mar-11	dry	3	3	5	11	Moderate
Aw1	Mar-11	dry	3	3	5	11	Moderate
H001	Mar-11	dry	5	5	1	11	Moderate

Appendices

H003	Mar-11	dry	3	3	1	7	Bad
H004	Mar-11	dry	3	1	5	9	Moderate
H005	Mar-11	dry	3	1	3	7	Bad
H006	Mar-11	dry	3	3	1	7	Bad
H007	Mar-11	dry	5	5	1	11	Moderate
H008	Mar-11	dry	3	5	5	13	Good
H009	Mar-11	dry	3	5	1	9	Moderate
HT3	Mar-11	dry	1	1	1	3	Very bad
HT4	Mar-11	dry	1	1	1	3	Very bad
Bu1	Mar-11	dry	1	1	1	3	Very bad
Bu2	Mar-11	dry	3	1	1	5	Very bad
Bu3	Mar-11	dry	1	1	1	3	Very bad
kt1	Oct-11	wet	5	5	5	15	Very good
kt2	Oct-11	wet	5	5	3	13	Good
kt3	Oct-11	wet	5	3	5	13	Good
kt4	Oct-11	wet	3	5	3	11	Moderate
kt5	Oct-11	wet	5	5	5	15	Very good

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kt6	Oct-11	wet	5	5	5	15	Very good
kt7	Oct-11	wet	5	5	5	15	Very good
kt8	Oct-11	wet	3	5	1	9	Moderate
kt9	Oct-11	wet	5	5	3	13	Good
kt10	Oct-11	wet	5	5	3	13	Good
kt11	Oct-11	wet	3	5	1	9	Moderate
kt12	Oct-11	wet	3	3	1	7	Bad
kt13	Oct-11	wet	3	3	3	9	Moderate
kf1	Oct-11	wet	5	5	5	15	Very good
kf2	Oct-11	wet	3	3	5	11	Moderate
kf3	Oct-11	wet	3	5	1	9	Moderate
kf4	Oct-11	wet	3	3	1	7	Bad
kf5	Oct-11	wet	3	1	5	9	Moderate
kf6	Oct-11	wet	3	3	1	7	Bad
kf7	Oct-11	wet	3	3	1	7	Bad
kf8	Oct-11	wet	3	3	3	9	Moderate
kf9	Oct-11	wet	5	5	5	15	Very good

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kf10	Oct-11	wet	5	5	5	15	Very good
kf11	Oct-11	wet	5	3	5	13	Good
kf12	Oct-11	wet	3	5	1	9	Moderate
kf13	Oct-11	wet	3	3	3	9	Moderate
kf14	Oct-11	wet	3	5	1	9	Moderate
Bo1	Oct-11	wet	3	3	1	7	Bad
Bo2	Oct-11	wet	5	5	3	13	Good
Bo3	Oct-11	wet	3	5	1	9	Moderate
Bo4	Oct-11	wet	3	5	1	9	Moderate
Bo5	Oct-11	wet	1	1	1	3	Very bad
Bo6	Oct-11	wet	3	3	1	7	Bad
Bo7	Oct-11	wet	3	3	1	7	Bad
Bo8	Oct-11	wet	5	3	5	13	Good
H001	Oct-11	wet	3	5	1	9	Moderate
H002	Oct-11	wet	5	5	5	15	Very good
H003	Oct-11	wet	5	5	3	13	Good
H004	Oct-11	wet	3	5	1	9	Moderate

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H005	Oct-11	wet	3	3	1	7	Bad
H006	Oct-11	wet	3	3	1	7	Bad
H007	Oct-11	wet	3	5	1	9	Moderate
H008	Oct-11	wet	3	5	1	9	Moderate
H009	Oct-11	wet	5	5	1	11	Moderate
Bu1	Oct-11	wet	5	5	3	13	Good
Bu2	Oct-11	wet	3	3	3	9	Moderate
Bu3	Oct-11	wet	3	5	5	13	Good
Bw1	Oct-11	wet	3	5	3	11	Moderate
Bw2	Oct-11	wet	5	5	1	11	Moderate
Bw3	Oct-11	wet	3	5	5	13	Good
Aw1	Nov-11	wet	5	5	5	15	Very good
Aw2	Nov-11	wet	1	3	1	5	Very bad
Aw3	Nov-11	wet	3	5	3	11	Moderate
Aw4	Nov-11	wet	3	3	1	7	Bad
Aw5	Nov-11	wet	3	5	1	9	Moderate
Aw6	Nov-11	wet	3	5	1	9	Moderate

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Bore	Oct-11	wet	5	5	3	13	Good
Bore1	Nov-11	wet	3	3	1	7	Bad
HT1	Oct-11	wet	5	5	3	13	Good
HT2	Oct-11	wet	3	5	1	9	Moderate
HT3	Oct-11	wet	5	3	3	11	Moderate
HT4	Oct-11	wet	3	5	3	11	Moderate
HT5	Oct-11	wet	5	5	1	11	Moderate
Aw4	Oct-11	wet	5	5	5	15	Very good

Annex 5.3. Protocol for biological and physico-chemical wetland monitoring in Ethiopia

Applicability: This protocol is applicable to freshwater wetlands located in Ethiopia. It involves a detailed description of the collection of biological (macroinvertebrates) samples, chemical water quality and habitat data.

Steps to determine the ecological water quality of wetlands

Step1. Site selection

Selection of sites for biomonitoring is an important process and adequate time and effort should be assigned to this task to ensure that sites are representative. Sites can be selected within each wetland along a gradient of visible disturbance including both nearly non-impacted and heavily disturbed sites (e.g. presence of point source pollution, eutrophication, hydrological modification, etc.). The number of sites is generally determined by the homogeneity of the area being monitored and the variety of potential anthropogenic impacts on wetlands health. Financial and logistical constraints also influence the number of sites selected.

Step 2. Determination of the sampling period/frequency

Permanent wetlands should be sampled during wet season, immediately after the end of the rainy season and during dry season to ensure that seasonal differences in macroinvertebrate assemblages are considered. Temporary wetlands are and can only be sampled during wet season.

Step 3. Data collection/sampling

i. Habitat assessment

- Take a GPS point from each sampling station
- Make a sketch of the sampling site and fill out the sampling site field protocol (S2)
- Estimate the proportion of each habitat type present
- Take digital photos of the site and record the photo number

ii. Water quality assessment

a. Field measurement

- Measure sludge depth, water depth, secchi depth and ambient air temperature

- Measure dissolved oxygen, conductivity, pH and water temperature using a multi-probe meter.
- Measure chlorophyll a concentration using a fluorometer
- Fill out all values on the field protocol (S2)

b. Laboratory analysis

- Collect 2 liters of water from each site and store it on ice until return to the Laboratory.
- Analyze total organic nitrogen (TON), total phosphorus (TP), five day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), orthophosphate, ammonium and nitrate concentration according to the standard methods as prescribed by APHA, AWWA, WPCF (1995).

iii. Macroinvertebrate monitoring

- Allocate the time of sampling proportionally to different mesohabitats of the wetland such as open water and emergent vegetation.
- Collect macroinvertebrates at each sampling station using a rectangular frame net (20 × 30 cm) with a mesh size of 300µm.
- Each collection entails a 10-minute kick sampling with a hand net over a distance of approximately 10 meter.
- Disturb the bottom sediment by kicking with the feet during sampling in order to effectively collect benthic macroinvertebrates.
- Sort macroinvertebrates in the field, store them into well labeled vials containing 80% ethanol.
- Provide each sample with detailed information such as site code, date and transport the samples to the laboratory for identification afterwards.
- Identify macroinvertebrates to family level in the laboratory using a stereomicroscope (10 × magnifications) and the identification key of Clifford (1991) and Bouchard (2004).
- Count and write down the number of taxa and their respective abundance for each sample.
- Samples should be properly labeled, preserved and stored safely in the laboratory for future reference (store the data in a database).

Note: See list of sampling equipment and supplies in S3

Step 4. Designation of reference and impaired sites

Reference sites are selected to represent the natural or almost natural condition i.e. least impaired. A reference site acts as a bench mark with which a monitoring site is compared. Sites may range from those showing little impact to those experiencing a large impact with respect to water quality or habitat degradation. Designate wetland sites as reference and impaired based on land use patterns, the degree of habitat degradation as quantified by the USEPA protocol (USEPA, 2002b), variables characterizing hydrological modification, and the Prati index as a measure of the chemical water quality (Prati et al., 1971).

- a. Calculation of the basic Prati index (Prati et al., 1971).

Variables in the formulas:

X: Index according to Prati

Y: Measured variables

Data transformation is conducted by means of given formulas

Dissolved oxygen transformation for Prati index calculation

DO saturation <50%	$X=4.2-0.43*(100-Y)/5+0.042*((100-Y)/5)^2$
DO saturation 50 -100 %	$X= 0.08*(100-Y)$
DO saturation >100%	$X=0.08*(Y-100)$

- COD (mg/l): $X=Y/10$
- NH₄-N (mg N/L): $X=2^{2.1*\log(12*Y)}$

Water quality assessment according to Prati et al. (1971)

Description of Prati index	Very Pure <1	Acceptable <2	Slightly polluted <4	Polluted <8	Heavily polluted >8
DO (%)	88-112	75-125	50-150	20-200	<20 or >200
COD (mg/L)	10	20	40	80	>80
NH ₄ -N (mg/L)	0.1	0.3	0.9	2.7	>2.7

Basic Prati Index = Average of DO, COD and NH₄-N scores

b. Habitat quality

Quantify the degree habitat alteration, hydrological modification and land use pattern based on Table 4.2 (Chapter 4).

Step 5. Compiling and calculating the core metrics

- Total family richness: Family richness reflects health of the community through measurements of the diversity of families present. This metric generally increases with increasing water and habitat quality. Count the total number of families collected in the sample.

- b. EOT family richness: The EOT family richness is the total number of families within the groups of order Ephemeroptera (mayflies), Odonata (dragonflies) and Trichoptera (caddisflies). They generally increase with an increase in water and habitat quality. Count the total number of EOT families.
- c. Percentage of Filterer-collector: Abundance of detritivores, which feed on fine particulate organic matter typically, decreases with increased disturbances. Record the value obtained by dividing the number of individuals belonging to these families by the total number of individuals in the sample. (See Appendix 5.1 for the functional feeding guilds of each family).

Step 6. Metric scoring

Convert the calculated metric value into a standardized score via transformation. This standardization allows that each metric has the same value and importance. A trisection of the scoring range can be used as recommended by Barbour et al. (1996) (Table 4.1, Chapter 4).

Scores of the three selected metrics, according to the trisection scoring method from the appropriate percentile of the data distribution (USEPA, 1998a). A score of 5 indicates that the sample meets the reference condition, a score of 3 represents an intermediate condition and a score of 1 indicates the highest deviation from the reference condition (see Barbour et al., 1996 for details).

Metric	Score		
	5	3	1
Family richness	≥ 12	11-6	< 6
EOT family richness	≥ 3	2-1	0
%Filterer-collector	≥ 4	< 4 > 0	0

Step 7. Combine metrics into a Multimetric Macroinvertebrate Index (MMI) score

- a. The MMI is formed by combining the three metric into one final index.
- b. The final MMI score ranges from 3 to 15.

c. The MMI score can be divided into five quality classes:

- 3-5 = very bad,
- 6-8 = bad,
- 9-11 = moderate,
- 12-13 = good
- 14-15 = very good.

Field protocol for wetland assessment

A. General Information

1. DD/MM/YYYY-----Time (h)-----
2. Name of Wetland -----Sampling station -----
3. Weather condition -----
4. Previous day weather conditions-----
5. Photo number -----
6. Size of site under assessment (ha)-----
7. Size of total wetland complex (ha)-----

Notes and/or sketch of the site

B1. Physico-chemical parameters (Field)

8. Ambient Temperature (°C)-----pH -----
9. Water temperature (°C) -----DO (mg/l)-----%-----EC (µS/cm)-----

10. Turbidity(NTU)-----Transparency (cm)-----

11. Chlorophyll a (ABS)----- $(0.1309 \cdot \text{ABS} + 11.274)$ -----($\mu\text{g/l}$)

12. Color-----Odor-----

B2. Physical-chemical parameters (laboratory)

13. COD -----BOD₅-----

14. Chloride-----NH₄-----

15. TSS-----TON-----

16. TP -----PO₄³-----

17. NO₃-----

C. Hydro-morphological assessment

18. Wetland geomorphic settings

a. Riverine-----

b. Digressional -----

c. Meandering flood plain -----

d. Other -----

19. Site setting /degree of isolation from other wetlands

a. The site is connected upstream and downstream with other wetlands

b. The site is only connected upstream with other wetlands

c. The site is only connected downstream with other wetlands

d. Other wetlands are nearby (within 250m) but not connected

e. The wetland site is isolated

20. Water depth (cm)

a. Minimum -----b. Maximum-----Average -----

21. Sludge depth (cm)

a. Minimum -----b. Maximum-----Average -----

22. Apparent hydroperiod

a. Permanently flooded

b. Seasonally flooded

c. Saturated (Surface water seldom present)

d. Artificially flooded

e. Artificially drained

23. Hydrophytic vegetation coverage (%)

- a. Woody plants----- e. Floating macrophytes-----
- b. Water grasses----- f. Periphyton -----
- c. Emerged macrophytes----- g. Filamentous algae-----
- d. Submerged macrophytes----- h. Other specify-----

24. Name of dominant macrophytes

- a. -----
- b. -----
- c. -----
- d. -----
- e. -----

25. Wetland Fauna (indicate presence and list most important ones)

- a. Birds-----d. Anurans -----
- b. Fish -----e. Hippopotami -----
- c. Invertebrates-----f. Others-----

26. Hydrological modifications

- a. Ditching in the wetland -----e. Ditching <50 meter-----
- b. Draining in the wetland -----f. Draining <50 meter -----
- c. No draining -----g. No ditching -----
- d. Other (Specify) -----

27. Adjacent land use pattern

Activity	In the wetland	<50 meter distance	>50 meter distance
Farming			
Clay mining			
Waste dumping			

28. Habitat alteration

- a. Grazing:
 - Minimal -----
 - Moderate-----
 - High-----
- b. Vegetation removal:
 - less than 10% of vegetation removed-----
 - 10-50% removed-----

- >50% removed-----

c. Tree plantation:

- No tree plantation/or plantation >50 meter-----
- Tree plantation at <50 meter, but not in the wetland-----
- Tree plantation in the wetland itself-----

29. Others threats (indicate if present or not)

- a. Washing-----
- b. Sand mining -----
- c. Road construction -----
- d. Swimming -----
- e. Fishing -----
- f. Use of fertilizers -----
- g. Pesticide use-----

30. Ecological state of the wetland under study

- a. Unmodified, natural-----
- b. Largely natural with few modifications-----
- c. Moderately modified-----
- d. Largely modified-----
- e. Seriously modified-----
- f. Critically / Extremely modified-----

31. Any additional comments-----

Field equipment and supplies needed for wetland sampling

- A. Biological survey
 - 1. Standard D-frame net 300 μ m mesh size
 - 2. Sieve and bucket
 - 3. Ethanol 80%
 - 4. Forceps
 - 5. Vials
 - 6. Labelling material
 - 7. Wading suit
 - 8. Permanent marker
 - 9. Sorting tray
 - 10. Pencil
 - 11. Clipboard
 - 12. Pipettes
- B. Water quality survey
 - 1. Bottle for water sample
 - 2. Multi-parameter probe
 - 3. Deionized water
 - 4. Spare batteries for meters
 - 5. Thermometer
 - 6. Fluorometer for chlorophyll a
 - 7. Cool box
- C. Habitat survey
 - 1. Tape measure
 - 2. Field protocol
 - 3. Portable GPS
 - 4. Camera

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Summary

The growing awareness about the adverse ecological, social and economic impacts of the unwise use of natural wetlands has fostered the interest in studies on the diversity and distribution of wetlands. Consequently, several monitoring and assessment tools are being developed and used by developed nations for the management and conservation of these resources. However, there is a lack of information on the use of these tools in developing countries and eventually a lack of management decisions. Thus, this study aimed to develop assessment tools to provide scientific support to the management and conservation of wetlands in southwest Ethiopia. To this end, abiotic and biotic data were collected from 63 wetland sampling sites located in eight wetlands from 2010 to 2011. During this period, 220 samples were collected both in wet and dry season. Additionally 220 samples were collected from temporary and permanent water bodies for habitat characterization of mosquito larvae, vector of malaria.

This study indicates that macroinvertebrates are very good indicators to assess wetlands' ecological condition. Their abundance and distribution were affected by habitat disturbances and water quality deterioration. The decision tree models and the multivariate analyses indicated that vegetation cover, water depth and water conductivity were the main factors influencing the structure of wetland macroinvertebrate communities.

In order to develop a tool for the assessment of the biological integrity and health of wetland ecosystems, a multimetric index based on benthic macroinvertebrate communities has been developed. Fifty eight candidate metrics were initially evaluated for the multimetric index in terms of their sensitivity. Most of these did not discriminate well between reference and impaired sites. Three metrics, which reflect different features of macroinvertebrate assemblages, were included in the final multimetric macroinvertebrate index (MMI) as being applicable to wetlands in Southwest Ethiopia. Total family richness directly relates to biodiversity, while family richness of Ephemeroptera, Odonata and Trichoptera targets diversity of sensitive taxa and finally, one metric which reflects the relative abundance of functional feeding groups (percentage filterer-collectors). All three metrics proved useful for characterizing the ecological condition of wetlands. They were not strongly correlated amongst themselves and all showed a negative response to increasing habitat degradation as assessed by traditional land use and water quality parameters. Moreover, the final MMI classified a validation dataset accurately. This MMI can be considered as a robust and

sensitive tool that can be applied to evaluate the ecological condition of natural wetlands in Southwest Ethiopia

The general public and policy makers perceive wetlands as mosquito breeding grounds. Environmental modification (wetland drainage and aquatic vegetation clearance) is one of the strategies of malaria control in Ethiopia. However, detailed knowledge on the ecology of aquatic immature stages is very crucial to include larval habitat management into integrated malaria control program. To this end, we developed habitat suitability models to address the question whether permanent wetlands are preferred habitats for anopheline mosquito larvae, the main vector of malaria. This study pointed out that in permanent water bodies such as ponds, stream margins and natural wetlands mosquito larvae were absent or occurred at very low abundances. These permanent habitats harbour a high diversity of macroinvertebrate predators and competitors and their presence likely suppresses the density of mosquito larvae.

Temporary water bodies such as agricultural ditches, rain pools, open pits for plastering and clay mining, vehicle ruts and hoof prints were the most preferred habitats (in terms of occurrence and abundance) for anopheline larvae. These habitats were either man-made or associated with anthropogenic activities. Increasing human population in the catchment resulted in enhanced anthropogenic activities including deforestation, agricultural expansion, livestock rearing and brick making which could create more suitable habitats for mosquito larvae. The findings of this study suggest that malaria vector control intervention strategies should target temporary water bodies. The drainage or conversion of natural marshlands for larval control may not be an efficient vector control strategy, as wetlands were not found to be the most prolific mosquito breeding sites in the study area. Moreover, degradation and conversion of these natural wetlands can be counter-productive and enhancing the occurrence and abundance of mosquito larvae.

In this study, natural wetlands showed a variable retention of TSS and nutrients due to the differences in input concentration, vegetation cover and the degree of habitat disturbances. Kofe and Kito wetlands, which are considered as more natural, particularly in the upstream sites, were characterized by a release of TSS and nutrients. Awetu and Boye wetlands, receiving untreated wastewater from Jimma town, retained a substantial amount of total suspended solids (TSS) and nutrients. However, the out flow concentration in Awetu and Boye wetlands were higher than in Kito and Kofe wetlands. Human activities such as farming, grazing, waste dumping and clay mining were the main predictors for release of TSS

and nutrient in these wetlands. If the current land use patterns now prevailing are allowed to continue further, high quantity of sediment and nutrients will be released from these wetlands. This may lead to siltation and eutrophication problem to the downstream Gilgel Gibe reservoir. Furthermore, alteration of wetlands may also contribute to the loss of biodiversity and other ecosystem services.

In conclusion, wetlands of Ethiopia provide various ecological and socioeconomic functions. However, they are losing their vigour at alarming rate due to unwise management. Poor watershed management practices in the uplands such as deforestation, poor farming methods, overgrazing by domestic livestock, clay mining for brick making and effluent discharge from domestic and industrial plants particularly to wetlands adjacent to urban centres are the major threats to wetlands. These alterations contribute to the degradation of water quality, decrease in the abundance and diversity of wetland's fauna and flora and create mosquito breeding grounds and consequently increase the transmission of malaria. Furthermore, these alterations also reduce the availability of wetland products (sedges, craft materials and medicinal plants) and the related ecosystem services. This in turn has an adverse effect on food security and poverty alleviation with considerable impact on communities who heavily depend on wetland products for their livelihood.

The absence of wetland conservation policy and accountable institution, which coordinates management efforts and the weak relation of the country to wetland affiliated global institutions such as the Ramsar Secretariat has complicated the problem of conservation and management. Therefore, it becomes necessary that there should be a wetland policy for achieving wise use goals and necessary legal and institutional back up for sustainable wetland management. It is also essential to establish institutions with a mandate to implement policies, provide alternatives to actions that cause wetland degradation and to formulate modalities for a national wetland management program. Nationwide inventory of wetlands is very essential to develop national policy and management strategies.

Samenvatting

Het toenemende bewustzijn over de negatieve ecologische, sociale en economische impact met betrekking tot het niet duurzaam gebruik van natuurlijke wetlands heeft voor een grote belangstelling gezorgd voor onderzoek naar de diversiteit en de verspreiding van wetlands. Als gevolg hiervan zijn er verscheidene monitoring- en beoordelingsmethoden ontwikkeld in ontwikkelde landen om deze natuurlijke rijkdommen te gaan beheren en behouden. Echter is er een gebrek aan informatie over hoe deze methoden kunnen aangewend worden in ontwikkelingslanden om een duurzaam beleid met betrekking tot wetlands op te stellen. In deze studie werd een methode ontwikkeld, op basis van wetenschappelijk onderzoek, om het beheer en het behoud van wetlands in zuidwest Ethiopië te ondersteunen. Zowel biologische als fysico-chemische data van 63 verschillende staalnameplaatsen in de wetlands werd gedurende 2010 en 2011 verzameld. Gedurende deze periode werden in totaal 220 stalen verzameld zowel in het droge als in het regenseizoen. Daarenboven werden 220 tijdelijke en permanente waterlichamen bemonsterd in het kader van een onderzoek naar de verspreiding van muggenlarven, de vector van malaria in de regio.

Deze studie toont aan dat macroinvertebraten kunnen gebruikt worden als goede indicatoren bij de beoordeling van de ecologische conditie van een wetland. De abundantie en het voorkomen van de macroinvertebraten werd bepaald door de mate van habitat verstoring en de waterkwaliteit. De beslissingsbomen en de multivariate analyse toonden aan dat vegetatie, water diepte en geleidbaarheid de structuur en samenstelling van de macroinvertebraten gemeenschap sterk beïnvloedt.

Om de ecologische kwaliteit en de status van de wetlands te beoordelen werd er een multimetrische macroinvertebraten index (MMI) ontwikkeld voor wetlands. Initieel werden 58 verschillende kandidaat metrieken geëvalueerd op basis van hun sensitiviteit en hun capaciteit om goede van slechte sites te onderscheiden. De meeste van deze metrieken scoorden hierop echter matig tot slecht. Finaal werden er drie metrieken behouden die de verschillende karakteristieken van de macroinvertebraten gemeenschap weerspiegelden en welke toepasbaar waren in Ethiopië. Deze drie metrieken waren: het totale aantal families welke gerelateerd is aan de biodiversiteit, het aantal families behorende tot de Ephemeroptera, Odonata en Trichoptera, welke de diversiteit van de gevoelige taxa weerspiegelt en finaal het percentage filter-verzamelaars, welke de relatieve abundantie van

de functionele voedselgroepen weergeeft. De drie metrieken gaven een goede karakterisatie van de status van een wetland. Deze waren niet gecorreleerd met elkaar en toonden allen een negatieve respons met toenemende habitat degradatie welke bepaald was op basis van landgebruik en waterkwaliteit. De finale MMI classificeerde een validatiedataset op accurate wijze. De MMI kan dan ook beschouwd worden als een robuuste en gevoelige methode voor het beoordelen van de status van natuurlijke wetlands in zuidwest Ethiopië.

Het grote publiek en de beleidsmakers zien wetlands als broedplaatsen voor muggenlarven. Eén van de belangrijkste controle strategieën van malaria in Ethiopië is het veranderen van het habitat waaronder het draineren van wetlands en het verwijderen van de aquatische vegetatie. Echter een goede kennis in verband met de ecologie van de muggenlarven is van cruciaal belang om een goed beleid te kunnen opstellen als deel van een geïntegreerde malaria controle strategie. Daarom werden er in deze studie habitat geschiktheidsmodellen ontwikkeld die een antwoord op de vraag of permanente wetlands de broedhaarden van muggenlarven van het genus *Anopheles*, de vector van malaria, zijn, tracht te geven. De resultaten toonden aan dat in permanente waterlichamen zoals vijvers en natuurlijke wetlands, muggenlarven slechts sporadisch voorkwamen en meestal bij zeer lage abundantie. Deze permanente wetlands huisvesten een hoge diversiteit aan predatore macroinvertebraten en andere competitieve soorten die de densiteit van muggenlarven onder controle houden.

Tijdelijke waterlichamen zoals grachten, regenplassen, putten gegraven voor het winnen van klei, bandensporen en hoefafdrukken waren de belangrijkste habitats van de muggenlarven. Deze habitats zijn door de mens gemaakt of gerelateerd aan menselijke activiteiten. Een toename van de populatie in het gebied leidde tot een toename van de menselijke activiteiten zoals ontbossing, uitbreiding van het landbouwgebied en het houden van vee wat voor een toename in het aantal beschikbare habitats heeft gezorgd. Deze resultaten van deze studie geven weer dat de anti-malaria campagnes moeten focussen op de bestrijding in tijdelijke waterlichamen. Het draineren van de natuurlijke wetlands is geen goed en efficiënte controle strategie aangezien deze natuurlijke wetlands geen broedhaarden vormen voor muggenlarven. De degradatie en conversie van deze wetlands kan net het tegenovergestelde in de hand werken en dus zorgen voor een uitbreiding van het beschikbare habitat van de muggen.

Deze studie toonde aan dat natuurlijke wetlands een zeer variabele retentie vertoonden van totaal opgeloste stoffen (TOS) en nutriënten. Deze variabiliteit is voornamelijk te wijten aan de kwaliteit en kwantiteit aan water dat deze wetlands instroomt, de verstoring door de mens

en de vegetatie en het landgebruik in het bekken. Kofe en Kito, welke beschouwd worden als meer natuurlijke wetlands vooral dan in het stroomopwaarts gelegen gebied werden gekenmerkt door het vrijgeven van TOS en nutriënten. Awetu en Boye, welke ongezuiverd water van Jimma stad ontvangen weerhielden een groot deel aan TOS en nutriënten. De concentratie gemeten aan de uitstroom was echter nog steeds hoger dan deze gemeten in Kofe en Kito. Habitat verstoring als gevolg van landbouwactiviteiten, veeteelt, drainage en ontginning van klei kunnen een belangrijke bijdrage leveren aan de toename in nutriënten en sedimentatie van de wetlands. Indien de huidige verstoring en landgebruik gewoon wordt verder gezet, zal een hoge concentratie aan sediment en nutriënten vrijgesteld worden uit deze wetlands. Dit kan leiden tot een verzilting en eutrofiëring problemen in het stroomafwaarts gelegen Gilgel Gibe reservoir. De conversie van deze wetlands zorgt daarenboven voor een verlies aan biodiversiteit en de ecosystemendiensten gerelateerd aan deze wetlands.

Wetlands in Ethiopië voorzien de bevolking van verschillende ecologische en sociaal-economische functies. Echter worden zij sterk bedreigd door het ongecontroleerde gebruik van deze rijkdommen. Gebrekkig beheer van de natuurlijke rijkdommen in het stroomopwaarts gelegen gedeelte en daarbij aansluitende activiteiten zoals ontbossing, overbegrazing, kleiontginning en het rechtsreeks lozen van afvalwater vormen de belangrijkste bedreigingen voor de wetlands. Deze veranderingen dragen bij tot een daling van de waterkwaliteit, een daling in de biodiversiteit en bijgevolg een stijging in het voorkomen van malaria. Daarenboven zorgen deze veranderingen ook voor een afname in de wetland gerelateerde producten en diensten, welke op zijn beurt een effect heeft op voedselvoorziening en de gemeenschappen die sterk afhankelijk zijn van deze wetlands.

Het ontbreken van een beleid aangaande het behoud van wetlands, welke de beheersmaatregelen coördineert en overlegt met het Ramsar secretariaat bemoeilijkt het duurzaam beheer van deze wetlands. Daarom is het belangrijk om een beleid op te stellen en een officiële instantie te voorzien die het duurzaam beheer en behoud van de wetlands coördineert. Het is ook noodzakelijk om dat er een instantie wordt opgericht die de beleidslijnen daadwerkelijk kan implementeren en alternatieven voorziet om de verdere degradatie tegen te gaan. Een nationale inventarisatie van de wetlands is een goede eerste stap richting beleids- en beheeracties.

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Certificates and short courses

- 2011 Qualitative research and application using MAXQDA software. 26-28 December 2011, Jimma University, Ethiopia.
- 2010 Advanced academic english writing skills, language center, Gent University.
- 2006 Permanent low country studies, Gent University.
- 2005 Ambient air quality monitoring, Addis Ababa, Ethiopia.
- 2003 Physico-chemical and bacteriological examination of water, wastewater, and food, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.
- 2003 Physico-chemical analysis of water and wastewater, Addis Ababa water and sewerage authority, Addis Ababa, Ethiopia.

Work experience

- 2007-present Lecturer at School of Environmental Health Sciences, Jimma University, Ethiopia.
- 2003-2004 Assistant lecturer at School of Environmental Health Sciences, Jimma University, Ethiopia.
- 2002-2003 Graduate assistant II at School of Environmental Health Sciences Jimma University, Ethiopia.
- 1994-1999 Tehuledere Woreda Environmental Health and Health Education expert, Wello, Ethiopia.

1993-1994 Worehimeno Awuraja Environmental Health and Health Education expert, Wello, Ethiopia.

Peer reviewed journal

Mereta, S.T., Legesse, W., Endale, H., Faris, K., 2003. Factors affecting drinking water quality from source to home in Tehuledere woreda Northeast Ethiopia. *Ethiopian Journal of Health Science* 13(2): 95-106.

Abegaz, T., Legesse, W., Mereta,S.T., 2005. Determination of critical concentration and diurnal variation of dissolved oxygen (DO) in relation to physicochemical variables in Boye pond, southwest Ethiopia. *African Journal of Environmental Assessment and Management*, Volume 10, ISSN 1438-7890.

Peer reviewed journal science citation index

Getachew, M., Ambelu, A., Mereta, S.T., Legesse, W., Adugna, A., Kloos, H., 2012. Ecological assessment of Cheffa Wetland in the Borkena Valley, Northeast Ethiopia: Macroinvertebrate and bird communities. *Ecolo Indi* 15, 63–71.

Mereta, S.T., Boets, P., Ambelu, A.B., Malu, A., Ephrem, Z., Sisay, A., Endale, H., Yitbarek, M., Jemal, A., De Meester, L., Goethals, P.L.M., 2012. Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia. *Ecol Inform* 7(1): 52-61.

Mereta, S.T., Boets P., De Meester L., Goethals, P.L.M., 2013. Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia. *Ecolo Indi* 29: 510-521.

Mereta, S.T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S.O., Legesse, W., De Meester, L., Goethals, P.L.M., submitted. Physico-chemical and biological characterization of larval habitats of anopheline mosquito (Diptera: Culicidae): Implications for malaria control strategies. *Parasite and Vector*

Lecture notes

Mereta, ST, 2004. Water Supply and Safety Measures: Lecture note for health extension trainees in Ethiopia. The Carter Center Ethiopian Public Health Training Initiatives.

Mereta, ST, 2004. Food Hygiene and Safety Measures. Lecture note for health extension trainees in Ethiopia. The Carter Center Ethiopian Public Health Training Initiatives

Mereta, ST., and Hailu E., 2009. Environmental pollution and control. text book. Lecture Note Series, Jimma, Ethiopia.

Mereta, ST, Malu, A., Dadi, D., 2009. Air pollution and control text book. Lecture Note Series, Jimma, Ethiopia

Hailu E., Mereta, ST., and Amana J., 2009. Physicochemical quality control of water and wastewater. Text Book. Lecture Note Series, Jimma, Ethiopia.

Mereta, ST., Haddis, A., 2009. Wastewater treatment text book. Lecture note series, Jimma, Ethiopia

Abstracts of oral presentations

Mereta S.T., Ambelu Bayih A., Boets P., De Meester L., Goethals P.L.M., 2010. Classification and regression trees for habitat analysis of macroinvertebrate taxa in the natural wetlands of southwestern Ethiopia. Seventh International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Mereta S.T., Yehualaw, D., Boets P., De Meester L., Goethals P.L.M. (2012). Application of decision tree models for the prediction of two mosquito genera (anopheline and culicine) in Southwest Ethiopia. Eighth International Conference of the Ecological Informatics Society, Brasilia, Brazil, 3 to 6 December 2012.

Poster presentations

Mereta S.T., Yehualaw, D., Boets P., De Meester L., Goethals P.L.M. (2012). Ecological assessment of wetlands in southwestern Ethiopia. Institutional University Cooperation September, 2010, Jimma, Ethiopia.

Mereta S.T., Yehualaw, D., Boets P., De Meester L., Goethals P.L.M., 2012. Quantifying sediment retention capacity of Boye wetland, Southwest Ethiopia. Institutional University Cooperation, March, 2012, Jimma, Ethiopia.

First promoter of MSc thesis

Melaku Getachew, 2010. Ecological assessment of Cheffa wetland in Borkena valley, Northeast Ethiopia. Jimma University, Master of Sciences in Environmental Science and Technology.

Alemu Tolcha, 2011. Removal of Excess fluoride ion from water using seeds of cabbage tree (*Moringa stenopetala*). Jimma University, Master of Sciences in Environmental Science and Technology.

Selamawit Negassa 2012. Phosphate sorption capacity of solid residue of Awash Melkassa Aluminum Sulphate and Sulphuric Acid Factory. Jimma University, Master of Sciences in Environmental Science and Technology.

Yifru Waktole, 2013. Simultaneous removal of nitrate and phosphate ions from aqueous solution using solid waste from alum and sulphuric acid manufacturing process. Jimma University, Master of Sciences in Environmental Science and Technology.

Delelegn Giemiso, 2013. The role of wetland in the population dynamics of anopheles mosquito immature stages in Jimma, Southwest Ethiopia. Jimma University, Master of Sciences in Environmental Science and Technology.

Co-promoter MSc thesis

Mekonnen Birhane, 2009. A comparative study on the efficiencies of wastewater treatment technologies among ISO certified and non-certified breweries in Ethiopia. Jimma University, Master of Sciences in Environmental Science and Technology.

Kasahun Kefyalew, 2010. Assessment on the trophic state of Gilgel Gibe I reservoir. Jimma University, Master of Sciences in Environmental Science and Technology.

Temesgen Eshetu, 2011. Pollution effects of wastewater generated from Hawassa textile factory on Tikur Wuha river water quality, Southern Ethiopia. Jimma University, Master of Sciences in Environmental Science and Technology.

Jawar kassim, 2012. Experimental evaluation of *Moringa stenopetala* and chlorine combination for removal of turbidity and microbial load for household water treatment. Jimma University, Master of Sciences in Environmental Science and Technology.

Esayas Embaye, 2013. Composition and Abundance of Rotiferan and Crustacean Zooplankton in Relation to Primary Productivity, Fish Predation and Physico-chemical Factors in Gilgel Gibe Reservoir, Jimma, Southwest Ethiopia. Jimma University, Master of Science in Biology (Ecological and Systematic Zoology).

Course offered

Introduction to Environmental health

Food Hygiene Safety Measures

Air pollution and Control

Environmental Quality Control I

Environmental Quality Control II

Advanced Ecology

Wastewater Treatment

Environmental Pollution and Control

Awards

Best BSc thesis award in the annual research symposium, Jimma University, July 2002.

Fellow of the VLIR scholarship, 2005-2007.

