STUDIES ON SOME BIOLOGICAL AND PHYSICOCHEMICAL PARAMETERS OF ROCK POOL HABITATS OF MOSQUITOES (DIPTERA: CULICIDAE) IN KADUNA STATE, NIGERIA

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APRIL, 2015.

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BY

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DEPARTMENT OF BIOLOGICAL SCIENCES
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AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA.

APRIL, 2015.

DECLARATION

I declare that the work in this Thesis entitled**"STUDIES ON SOME BIOLOGICAL AND PHYSICOCHEMICAL PARAMETERS OF ROCK POOL HABITATS OF MOSQUITOES (DIPTERA:CULICIDAE) IN KADUNA STATE, NORTHERN NIGERIA"**was carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Okechukwu Anthony OBI _____ _____
Signature Date

CERTIFICATION

This thesis entitled **"STUDIES ON SOME BIOLOGICAL AND PHYSICOCHEMICAL PARAMETERS OF ROCK POOL HABITATS OF MOSQUITOES (DIPTERA:CULICIDAE) IN KADUNA STATE, NORTHERN NIGERIA"** by Okechukwu Anthony OBI meets the regulations governing the award of the degree of M.Sc. Zoology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the memory of my late father, Mr. Dominic Obi and to the unparalleled providence of God Almighty.

ABSTRACT

Entomological surveys were conducted to determine the biological and physicochemical parameters influencing mosquito breeding in rock pools on inselbergs in Kaduna State. Available rock pools were searched on the inselbergs fortnightly between June and October, 2013 in 21 settlements distributed in 7 Local Government Areas.This covered theState vegetation from the Guinea Savanna to SudanSavanna.A total of 368 rock pools were sampled for mosquito larvaeusing soup ladle dipper (0.105L) from 269 (69.7%) rock pools harbouring mosquito larvae. Biological (microinvertebrates, macroinvertebrates, macrophytes, algae and vertebrates) and physicochemical (depth, surface area, distances to adjoining pools, temperature, pH, total dissolve solid, electrical conductivity, total suspended solid, turbidity, hardness, dissolve oxygen, biochemical oxygen demand, chemical oxygen demand, phosphate, nitrate and alkalinity) parameters of the pools were determined. Polymerase Chain Reaction was used for the identification of mosquito species of *Anopheles gambiaes.s*. Of the 31,726 mosquito larvae collected, thirteen species in three mosquito genera(*Aedes*, *Anopheles* and *Culex*) including *Ae. vittatus* (95.71%),*An. arabiensis* (0.01%), *An. gambiae s.s*. (0.1%), *An. longipalpis* (0.0%), *An. pretoriensis* (0.0%), *An. rufipes* (0.02%), *Cx. albiventris* (0.84%), *Cx. horridus* (0.33%)*Cx. macfiei* (0.76%), *Cx. perfidiosus* (1.65%), *Cx. pipiens pipiens* (0.44%), *Cx. simpsoni* (0.0%) and *Cx. tigripes* (0.0%) bred in rock pools. *Aedes vittatus*was the most dominant mosquito encountered in all the 21 sampling locations. PCR–based assay revealed 41.6% amplification of the *An. gambiae*complex sample with 38.9% populations belonging to *An.gambiae s.s.* whilst the remaining 2.6% were *An. arabiensis*. Up to 58.4% of the *An. gambiae* complex could not be identified through PCR even after three runs. ANOVA showed that the abundance of mosquito larvaediffered significantlywith pH of the rock pools ($p<0.05$). Highly significant difference existed between the abundance of mosquito

larvae and total dissolve solid, electrical conductivity and alkalinity of the rock pools ($p<0.001$). The abundance of mosquito larvae did not differ significantly with depth, surface area, total suspended solid, hardness and turbidity of the rock pools ($p>0.05$).Principal Component Analysis showed that temperature, electrical conductivity and total dissolve solids were paramount for mosquito breeding in rock pool habitats. Low

positive correlation ($r = 0.394$) existed between dissolve oxygen and abundance of mosquito larvae ($p<0.001$). Strong positive correlation ($r = 1.000$) exist between biochemical oxygen demand and the abundance of mosquito larvae ($p<0.005$). Nitrate ($r=0.047$) and chemical oxygen demand had low positive correlation ($r=0.029$) with mos quito abundance. Strong positive correlation (r) existed between macroinvertebrate and mosquito abundance($p<0.001$) while microinvertebrates correlated negatively (r) with the abundance of mosquito larvae ($p<0.05$) in rock pools.Chlorophytes had widespread occurrence while *Microcystis* spp. had the highest percentage positivity, being the only cyanophyte associated with mosquito larvae ($p<0.05$) in rock pools. *Chlorophytum laxum*was predominant amongst other aquatic macrophytes found with mosquito larvae in rock pools. Epidemiologically, the mosquito species encountered are potential vectors of human and animal diseases, hence rock pools should be inspected to incriminate vectors and be incorporated in mosquito control strategies.

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CHAPTER ONE

1.0 INTRODUCTION

Mosquitoes are probably the most notoriously undesirable arthropods with respect to their ability to transmit pathogens that cause human disesases such as malaria, dengue, yellow fever, filariasis, viral encephalitides and other deadly diseases. In several parts of the world, the indirect effect of malaria and other mosquito-borne diseases accounted for more deaths as well as reduced production following work losses (Rueda, 2008). Emergence of new vector-borne disease entities and the resurgence of old ones are caused by several factors, which are ecological changes that increase vector densities, such as climate, immunity status of humans, human and potential vector population densities and the presence of suitable reservoir amongst others (Adebote *et al*., 2006). The increase in economic activities, tourism and human migration have led to more cases of the movement of both disease vectors and the pathogens they carry thereby increasing the biodiversity of mosquitoes around the world (Manguin and Boete, 2010). Diversity of mosquito breeding environment stems from innate preferences shown by d ifferent taxa to the locations and conditions of various aquatic habitats (Adebote *et al.*,2 008). Oviposition preferences of adult females and the ability of immature stages of mosquitoes to adapt to both biotic and abiotic environmental conditions of a given aquatic habitat determine the abundance and distribution of immature mosquitoes (Dejenie *et al*., 2002). Mosquitoes have diverse habits that allow them to colonize different kinds of environments. The immature stages of mosquito are thus found in a variety of aquatic habitats including ponds, streams, ditches, swamps, marshes, temporary and permanent pools, rock holes, tree holes, crab holes, lake margins, plant containers (leaves, fruits, husks, tree holes, bamboo internodes), artificial containers (tyres, tin cans, flower vases, bird feeders), and other habitats (Rueda, 2008). They can

thrive in a variety of water conditions such as freshwater, brackish water and or any water quality (clear, turbid or polluted), except in marine habitats with high-salt concentration. Part of the problems militating against effective and sustained control of mosquitoes and the diseases transmitted by them is the overt advantages available to mosquitoes to breed in diverse aquatic media that are naturally occurring and or the creation of human activities.

Rock pools are small bodies of water that undergo recurrent, variable wet-dry phases, making them temporary intermittent pools (Levas, 2006). The relatively small nature of rock pools and the few macroscopic biota constituents make it easier to determine their community structure. Rocky outcrops have a profound influence on the distribution and abundance of biodiversity worldwide (Lindenmayer *et al.*, 2008). Such environments a re well documented as biological hotspots which often support unique biotic communities and high levels of endemism. The composition of mosquito fauna of a po ol is influenced by its temporary or permanent nature. The highly variable rock pool environment, with continuously changing abiotic and biotic conditions, therefore continuously tests the tolerance limits of the inhabitants (Jocque *et al.*, 2010). The physicochemical compositions of water bodies are complicated and determine their condition and fauna composition. They include salts, dissolved inorganic and organic matter, degree of eutrophication, turbidity and presence of suspended mud. Other hydrologic factors that affect preimaginal mosquito populations in water are the presence or absence of plants, temperature, light and shade, hydrogen ion concentration, presence of food substances (living or dead), presence of predacious mosquito larvae, fishes, other insects, crustaceans and arachnids (Okogun *et al.*, 2005). Huggettand Griffiths(1986)attempted to relate the communities occurring in rock pools with the extremes of salinity, hydrogen ion concentration, light and temperature

occurring within them. They concluded that temperature was the most important of these parameters. Daniel and Boyden (1975) investigated diurnal variations in water pH, carbon dioxide, salinity, as well as temperature and oxygen concentration within intertidal rock pools and concluded that temperature and oxygen concentrationsshowed the greatest variation that were particularly important in controlling community structure.

The biological and physicochemical attributes of aquatic environments may alter adult mosquito vector competence (Kengluecha *et al.*, 2005). Although aquatic habitats of mosquitoes encompass a broad and complex spectrum, the rock pools comprise a distinct group with unique ecological properties (Vezzani, 2007). Rock pools form a distinct class distinguishable into true rock pools and rock edged pools (Okogun *et al*., 2005). Pool structure is determined by a complex set of biological and physical factors that interact to develop a patchy habitat (Wallenstein *et al*., 2010). Interactions between climate and geology (e.g. limestone, sandstone, granite) generally determine the morphology and hydrology of rock pool habitats, with hydro periods ranging from several days up to the whole year. Pool volume is usually small, resulting in strongly fluctuating environmental conditions, low conductivity and wide variations in pH (from 4.0 to 11.0) and temperature (from freezing point to 40°C) often with well-marked diel cycles(Wallenstein *et al*., 2010). Rock pools usually form in shallow depressions in the rock (Ranta, 1982). Rock pools in general are oligotrophic systems open to nutrient inp uts and outputs (Jocque *et al*., 2010). Even a brief rain shower may, depending on the t opography, fill a rock pool to overflowing. They are mosaically distributed habitats characterized by unpredictable changes in their water contents (Ranta, 1982). Enrichment happens mainly through bacterial degradation of dead aquatic organisms, faeces from large (terrestrial) vertebrates and organic material blown in. Immediately

after filling, dissolved nitrogen and phosphorus concentrations may be quite high, but decline quickly because of nutrient uptake by organisms and a reduced rate of nutrient supply from the sediment (Jocque *et al*., 2010).

The algal composition in rock pools exhibit a marked gradient in many places, with green algae dominating pools that occur higher on the shore, whereas brown and red algae are dominant at lower shore levels, where common species from the adjacent sub-tidal communities occur (Wallenstein *et al*., 2010). In rock pools, Metaxas and Scheibli ng (1993) showed that competitive hierarchies involving 3 species of *Daphnia* can lead to competitive exclusion. Rock pools harbour freshwater communities and are located higher on the shore between the rocky intertidal and terrestrial habitat. Because of the comparatively small spatial dispersal capacity of mosquito larvae, larval control is the principal and most effective tool for mosquito-borne disease management (Siobhan *et al*., 2009). To critically audit the environment for the sources of mosquito that may be involved in disease transmission include searches of aquatic habitats in rock pools amongst several other breeding sites and determination of factors that support habitation (Adebote *et al*., 2008).

The biological and physicochemical factors affecting the breeding of mosquitoes in rock pool habitats have been the subject of rather little ecological interest and research. This could be attributed to the relative inaccessibility of these productive mosquito habitat types that should be given priority in mosquito abatement programmes. Few attempts have also been made to describe the distribution patterns of rock pool biotas in relation to any suspected influence of physicochemical conditions to which the pools are subject and how these in turn affect species composition and relative abundance of preimaginal mosquitoes. Typically in areas where mosquito breed in rock pools, not

every pool is often colonized by juvenile stages to the extent that several apposed pools could be devoid of mosquito while few other neighbouring pools have high density of larvae. Such disjointed larval colonization has not been fully investigated to unravel its ecologic undertone and potential applicability in control.

1.1Statement of Research Problem

The biological and physicochemical factors that influence the behaviour of mosquitoes to colonize and breed in rock pool habitats in Kaduna State have not been fully investigated and therefore largely unknown.

1.2Justification

This studywas designed to unravel key ecological factors that modulate the breeding of mosquito vectors of human and animal diseases in rock pool habitats in Kaduna State, Northern Nigeria, where such habitats are known to be abundant but relatively inaccessible to routine control operations. The knowledge of inherent pool characteristics that make rock pools to be desirable or otherwise for mosquito breeding will enhance knowledge of the biology of the insects and the ability to control them.

1.3Aim of the Study

To evaluate the biological and physicochemical contents of rock pool breeding habitats of mosquitoes in Kaduna State, Northern Nigeria.

1.4 Objectives

i. To determine the species composition and relative abundance of mosquito breeding in rock poolhabitats in Kaduna State.

ii. To determine the biota composition of rock pools link with breeding of mosquitoes.

iii. To determine the physicochemical parameters of rock pools.

1.5 Hypotheses

i. Mosquitoes do not colonize and breed in rock pool habitats.

ii. The colonization and breeding of mosquitoes in rock pools are not influenced by biological factors.

iii. The colonization and breeding of mosquitoes in rock pools are not influenced by physicochemical factors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Mosquito Species

There are some 3300 species of mosquitoes belonging to 41 genera, all belongingin the family Culicidae. This family is divided into three subfamilies: Toxorhynchitinae, Anophelinae (anophelines) and Culicinae (culicines). Mosquitoes have a worldwide distribution. They occur throughout thetropical and temperate regions and extend their range northwards intothe Arctic Circle. The only areas from which they are absent are Antarctica,and a few islands. They are found at elevations of 5500mand down minesat depths of 1250m below sea level.The most important pest and vector species belong to the genera *Anopheles,Culex, Aedes, Mansonia*, *Ochlerotatus*, *Psorophora,Haemagogus* and *Sabethes*.*Anopheles* species, as well as transmitting malaria, are vectors of filariasis(caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) and a fewarboviruses. Certain *Culex* species transmit *W. bancrofti* and a varietyof arboviruses. *Aedes* species are important vectors of yellow fever,dengue, encephalitis viruses and many other arboviruses, and in a fewrestricted areas they are also vectors of *W. bancrofti* and *B. malayi*. Species in the very closely related genus *Ochlerotatus* also transmit filariasis and encephalitis viruses. *Mansonia* species transmit *B. malayi* an d sometimes *W. bancrofti* and a few arboviruses. *Haemagogus* and *Sabethes* mosquitoe s are vectors of yellow fever and a few other arboviruses in Central and South America, while the genus *Psorophora* contains some troublesome pest species in North and Sout h Americaas well as a few transmitting arboviruses (Service, 1997).

2.1.2External morphology of mosquitoes

Mosquitoes possess only one pair of functional wings, the fore-wings.The hind-wings are represented by a pair of small, knob-like halteres.Mosquitoes are distinguished from

other flies of a somewhat similar shapeand size by: (1) the possession of a conspicuous forward projecting proboscis;(2) the presence of numerous appressed scales on the thorax, legs,abdomen and wing veins; and (3) a fringe of scales along the posteriormargin of the wings.Mosquitoes are slender and relatively small insects, usually measuringabout 3–6 mm in length. Some species, however, can be as small as 2 mmwhile others may be as long as 19 mm. The body is distinctly divided intoa head, thorax and abdomen.The head has a conspicuous pair of kidney-shaped compound eyes.Between the eyes arises a pair of filamentous and segmented antennae. Infemales, the antennae have whorls of short hairs (that is pilose antennae),but in males, with a few exceptions in genera of no medical importance,the antennae have many long hairs giving them a feathery or plumoseappearance. Mosquitoes can thus be conveniently sexed by examinationof the antennae: individuals with feathery antennae are males, whereasthose with only short and rather inconspicuous antennal hairs are females (Service, 1997).

Just below the antennae is a pair of palps which maybe longor short and dilated or pointed at their tips, depending on the sex of theadults and whether they are anophelines or culicines. Arisingbetween the palps is the single elogated proboscis, which contains the piercing mouthparts of the mosquito. In mosquitoes the proboscis ch aracteristically projects forwards. The thorax is covered, dorsally and laterally, with scales which may bedull or shiny, white, brown, black or almost any colour. It is the arrangementof black and white, or coloured scales on the dorsal surface of thethorax that gives many species (especially those of the genera *Aedes* and*Ochlerotatus*) distinctive patterns.The wings of mosquitoes are long and relatively narrow, and the number and arrangementof wing veins is virtually the same for all mosquito species. The veins are covered with scales which are usually brown, black, white or creamy yell

ow, but more brightly coloured scales may occasionally be present. The shape of the sc ales and the pattern they form differconsiderably between both genera and species of mosquitoes. Scales alsoproject as a fringe along the posterior border of the wings. In life the wingsof resting mosquitoes are placed across each other over the abdomen inthe fashion of a closed pair of scissors. The legs of mosquitoes are longand slender and are covered with scales which are usually brown, blackor white and may be arranged in patterns, often in the form of rings (Service, 1997).

2.1.3 Life cycle

2.1.3.1Blood-feeding and the gonotrophic cycle

Most mosquitoes mate shortly after emergence from the pupa. After mating,sperm passed by the male into the female enters her spermatheca.This sperm, in the spermatheca, usually serves to fertilize all eggs laidduring her lifetime; thus only one mating and insemination per female isrequired. With a few exceptions, a female mosquito must bite a host andtake a blood-meal to obtain the necessary nutrients for the development ofthe eggs in the ovaries. This is the normal procedure and is referred to asanautogenous development. A few species, however, can develop the firstbatch of eggs without a blood-meal, and more rarely subsequent batches. This process is called autogenous development. The speed of digestion ofthe blood-meal depends on temperature. In most tropical species it takesonly 2–3 days, but in colder, temperate countries blood digestion may takeas long as 7–14 days (Service, 1997).

Each gonotrophic cycle begins with an unfed adult which passes through blood-fed, half-gravid and gravid conditions. After oviposition the female is again unfed and seeks another blood-meal. After a blood-meal the mosquito's abdomen is dilated and bright red incolour, but some hours later the abdomen becomes a much darker red. Asthe

blood is digested and the white eggs in the ovaries enlarge, the abdomenbecomes whitish posteriorly and dark reddish anteriorly. This conditionrepresents a mid-point in blood digestion and ovarian development, andthe mosquito is referred to as being half-gravid. Eventually allblood is digested and the abdomen becomes dilated and whitish due tothe formation of fully developed eggs. The female is now saidto be gravid and she searches for suitable larval habitats in which to layher eggs. After oviposition the female mosquito takes another blood-mealand after 2–3 days (in the tropics) a further batch of eggs is matured. Thisprocess of blood-feeding and egg maturation, followed by oviposition, isrepeated several times throughout the female's life and is referred to as thegonotrophic cycle.Male mosquitoes cannot bite but instead feed on the nectar of flowersand other naturally occurring sugary secretions. Males are consequentlyunable to transmit any disease. Sugar feeding is not, however, restrictedto males; females may also feed on sugary substances to obtain energy forflight and dispersal, but only in a few species (the autogenous ones) is thistype of food sufficient for egg development (Service, 1997).

2.1.3.2 Biology of mosquito eggs

Depending on the species, female mosquitoes lay about 30–300 eggs at anyone oviposition. Eggs are brown or blackish and 1 mmor less long. In manyCulicinae, eggs are elongate or approximately ovoid in shape, but eggs of*Mansonia* are drawn out into a terminal filament. In the Anophelinae,eggs are usually boat-shaped. Many mosquitoes, such asspecies of *Anopheles* and *Culex*, lay their eggs directly on the water surface (Figure 2.1).In *Anopheles* the eggs are laid singly and float on the water, whereas thoseof *Culex* are laid vertically in several rows held together by surface tensionto form an egg raft which floats on the water(Service, 1997).*Mansonia* specieslay their eggs in a sticky mass that is glued to the underside of floatingplants.

None of the eggs of these mosquitoes can survive desiccation andconsequently they die if they become dry (Figure 2.2). In the tropics, eggs hatch within2–3 days, but in cooler temperate countries they may not hatch until after7–14 days, or longer.Other mosquitoes, such as those belonging to the genera *Aedes*, *Ochlerotatus*,*Psorophora* and *Haemagogus*, do not lay eggs on the water surface butdeposit them just above the water line on damp substrates, such as mudand leaf litter, or on the inside walls of tree-holes and clay water-storagepots. Eggs of these genera can withstand desiccation, especially those of*Aedes*, *Ochlerotatus* and *Psorophora* which can remain dry for months oreven years but still remain viable and hatch when soaked in water (Figure 2.3). Becausesuch eggs are laid above the water line of breeding places, it may be manyweeks or months before they become flooded with water, and thus have theopportunity to hatch (Macdonald, 1960).

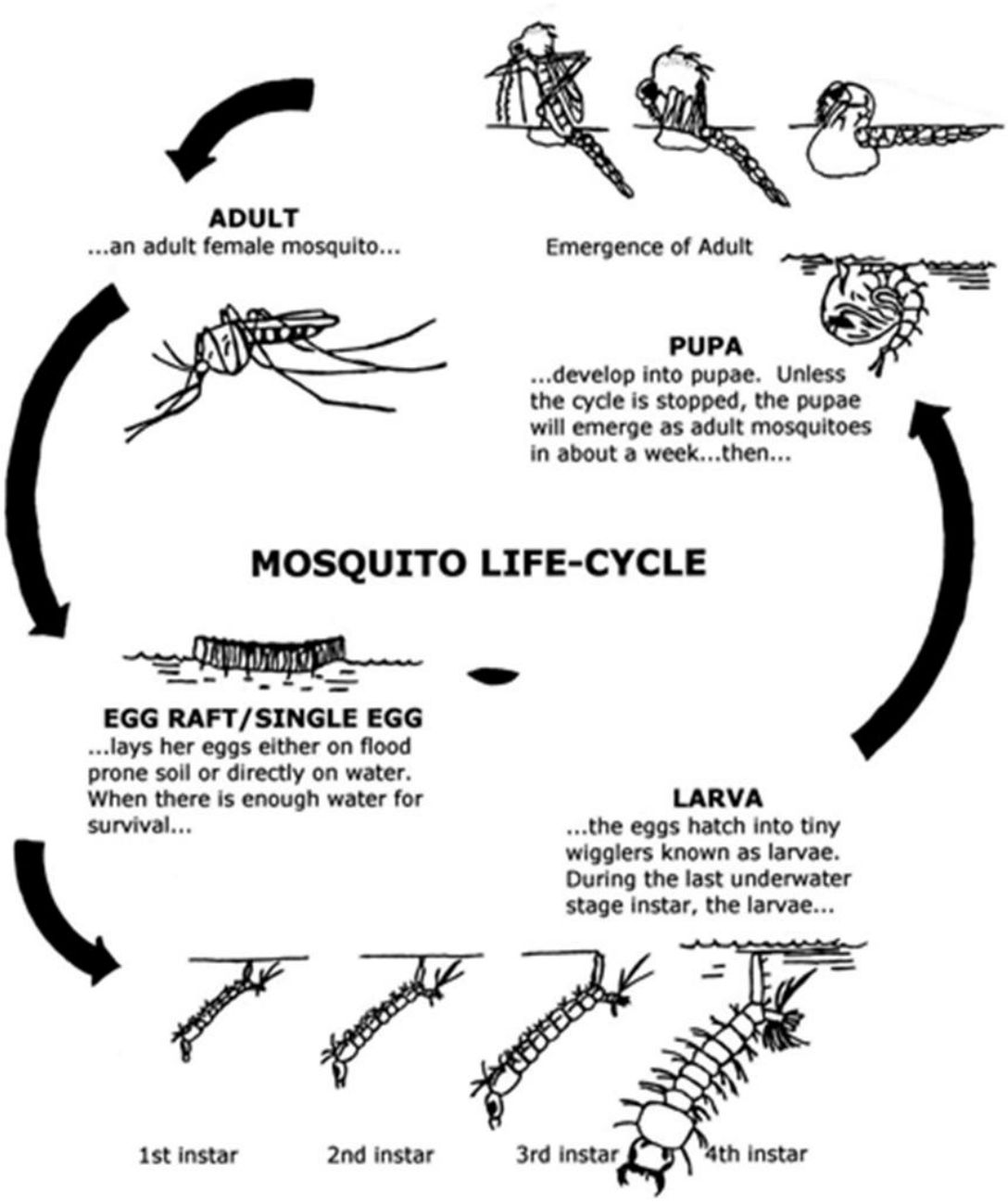


Figure 2.1: Generalized life cycle of mosquitoes (OME, 2008).

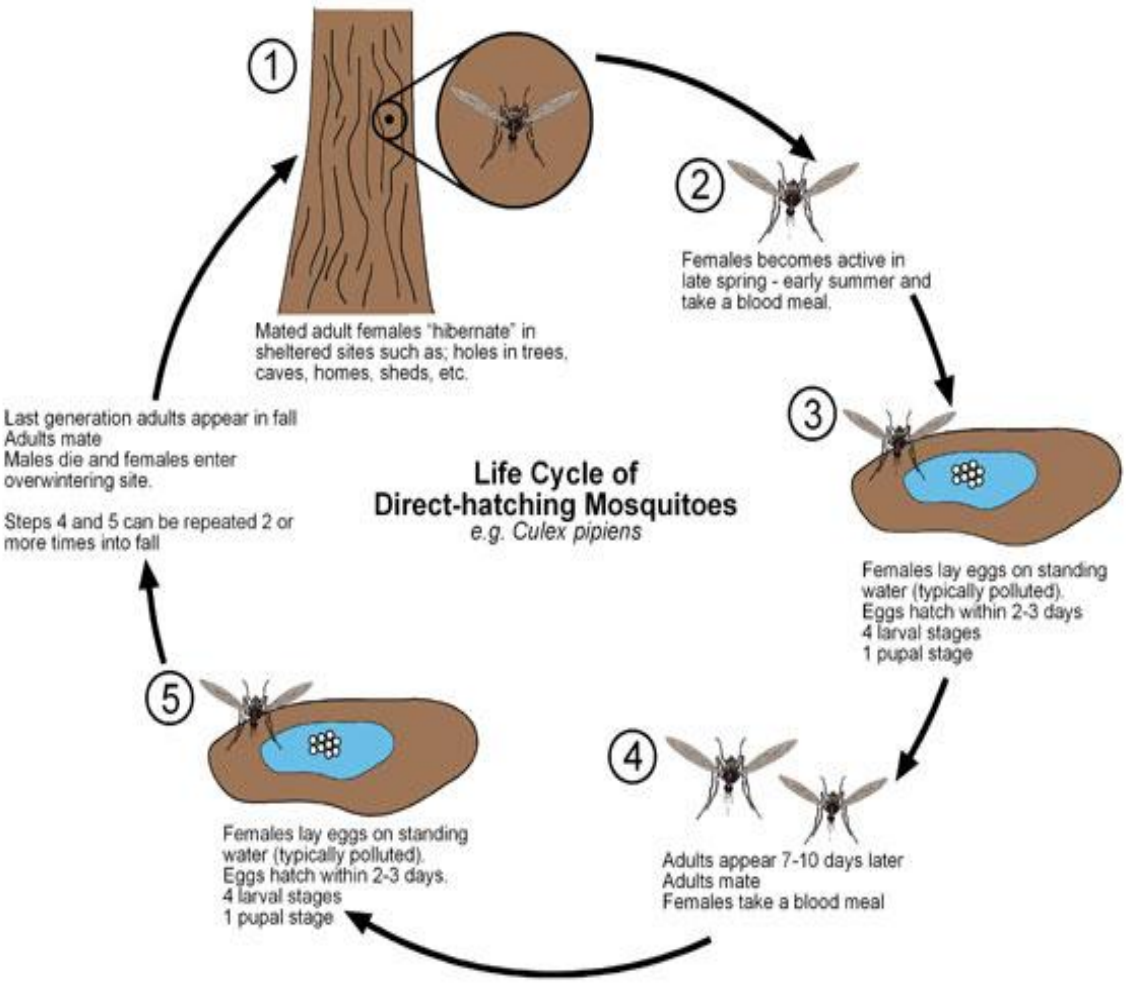


Figure 2.2: Life cycle of direct-hatching mosquitoes whereeggs are laid on top of shallow standing water and hatch within 2-3 days (Darsie and Ward,2005).

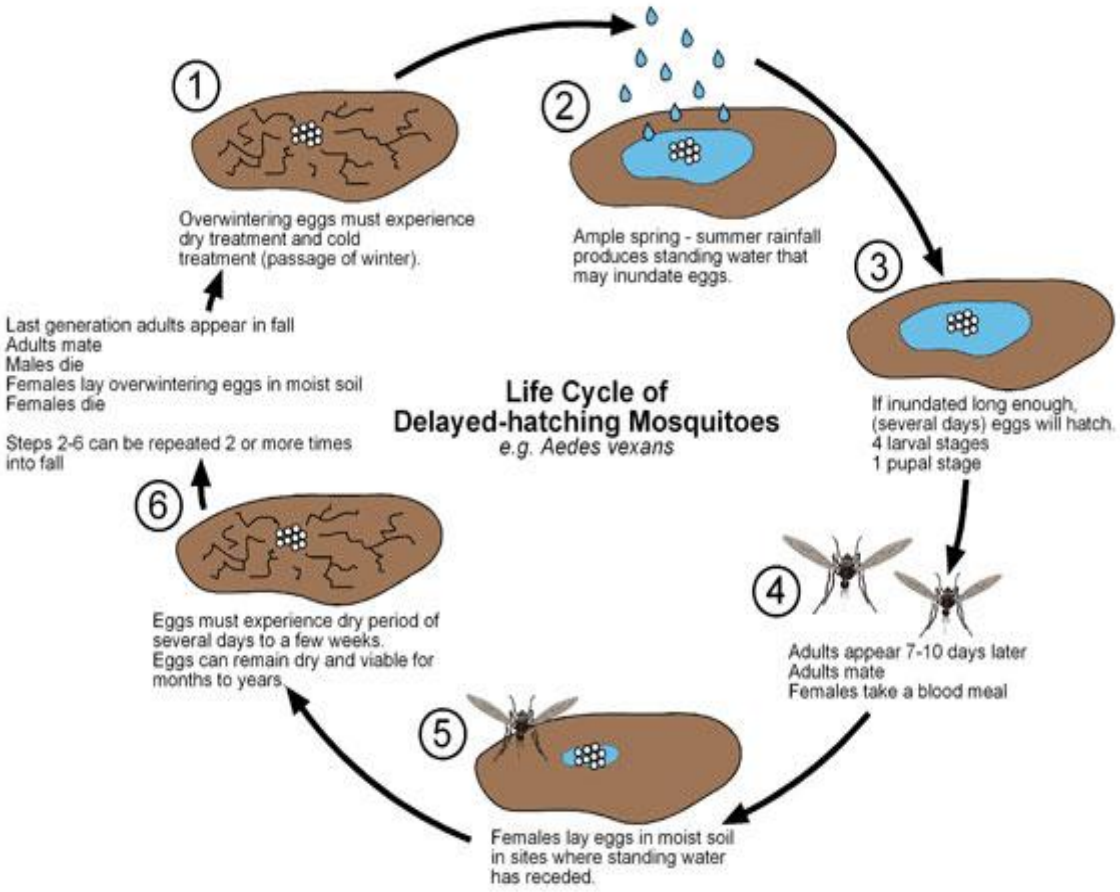


Figure 2.3: Life cycle of delayed-hatching mosquitoes where eggs are laid on moist substrates in sites where standing water existed previously (Darsie and Ward,2005).

2.1.4.Larval biology

Mosquito larvae can be distinguished from other aquatic insects by beinglegless and having a bulbous thorax that is wider than both the head and theabdomen. There are four active larval instars. All mosquito larvae requirewater in which to develop; no mosquito has larvae that can withstand desiccationalthough they may be able to survive short periods, for example,in wet mud.Mosquito larvae have a well-developed head, bearing a pair of antennaeand a pair of compound eyes. Prominent mouthbrushes are presentin most species and serve to sweep water containing minute food particlesinto the mouth. The thorax is roundish in outline and has varioussimple and branched hairs which are usually long and conspicuous. The10-segmented abdomen has nine visible segments, most of which havesimple or branched hairs. The last segment, which differsin shape from the preceding eight segments, has two paired groups of longhairs forming the caudal setae, and a larger fan-like group comprising theventral brush. This last segment ends in two pairs of transparent,sausage-shaped anal papillae, which although often called gills arenot concerned with respiration but with osmoregulation (Service, 1997).Mosquito larvae, with the exception of *Mansonia* and *Coquillettidia*species (and a few other species), must come to the water surface to breathe. Atmospheric air is taken in through a pair of spiracles situated dorsally on the t enth abdominal segment. In the subfamilies Toxorhynchitinae andCulicinae these spiracles are situated at the end of a single dark colouredand heavily sclerotized tube termed the siphon. *Mansonia* and*Coquillettidia* larvae possess a specialized siphon that is more or less conical,pointed at the tip and supplied with prehensile hairs and serrated cutting structures. These enable the siphon to be inserted into the roots orstems of aquatic plants and thus oxygen for larval respiration is obtainedfrom the plants (Service, 1997). In contrast, larvae of the Anophelinae do not have siphon.

Mosquito larvae feed on yeasts, bacteria, protozoans and numerousother micro organisms, as well as on decaying plant and animal material found in the water. Some, such as *Anopheles* species, are surfacefeeders,whereas many others browse over the bottoms of habitats. A fewmosquitoes are carnivorous or cannibalistic. There are four larval instarsand in tropical countries larval development, that is the time from egghatching to pupation, can be as short as 5–7 days, but many species requireabout 7–14 days. In temperate areas the larval period may last severalweeks or months, and several species overwinter as larvae (Service, 1997).

2.1.4.1 Larval habitats

Mosquito larval habitats vary from large and usually permanent collectionsof water, such as freshwater swamps, marshes, ricefields and borrowpits, to smaller collections of temporary water such as pools, puddles,water-filled car tracks, ditches, drains and gulleys(Clements, 1963). A variety of 'naturalcontainer-habitats' also provide breeding places, such as water-filled treeholes,rock pools, water-filled bamboo stumps, bromeliads, pitcher plants,leaf axils in banana, pineapple and other plants, water-filled split coconuthusks and snail shells. Larvae also occur in wells and 'man-made containerhabitats',such as clay pots, water storage jars, tin cans, discarded kitchenutensils and motor vehicle tyres (Clements, 1963). Some species prefer shaded larval habitatswhereas others like sunlit habitats. Many species cannot survive in waterpolluted with organic debris whereas others can breed prolifically in watercontaminated with excreta or rotting vegetation.A few mosquitoes breedalmost exclusively in brackish or salt waters, such as saltwater marshes andmangrove swamps, and are consequently restricted to mostly coastal areas.Some species are less specific in their requirements and can tolerate a widerange of different types of breeding places.Almost any collection of permanent or temporary water can constitute

amosquito larval habitat, but larvae are usually absent from large expansesof uninterrupted water such as lakes, especially if they have large numbersof fish and other predators. They are also usually absent from large riversand fast-flowing waters, except that they may occur in marshy areas andisolated pools and puddles formed at the edges of flowing water(Clements, 1963).

2.1.4.2 Larval distribution in nature

A number of ecologist have analysed the water in which mosquito larvae are found and from which they are absent and have concluded that certain solutes are harmful to the larvae, preventing the breeding of mosquitoes where they occur. Harmful properties have been ascribed to ammonium salts, nitrites, nitrates and protein, but these results have generally been contradicted by other workers.The distribution of larvae in natural waters of varying pH shows that many species are able to live under both alkaline and acidic conditions. *Anopheles culicifacies*, for example, has been recorded in the pH range of 5.4-9.8 and the tree-hole species *An. plumbens* and *Ae. geniculatus* from pH 4.4 to pH 9.3. *Aedes flavopictus* larvae develop in the laboratory in media ranging from pH 2 to 9 and *Armigeres subalbatus* larvae in media ranging from pH 2 to 10. In the laboratory acid conditions are sometimes unfavourable to larvae but this may be due to the bacteria which develop under acid conditions or to the acid present, hydrochloric acid, for example, being more toxic than phosphoric acid.Observations such as these and the knowledge that female mosquitoes are highly selective in nature in choice of oviposition site have prompted the suggestion that the distribution of larvae is controlled not by survival in suitable and extinction in unsuitable habitats but by the discrimination of the ovipositing females. This view is now generally accepted but unfortunately the means by which gravid female select the oviposition sites are very incompletely known. Although most mosquitoes are restricted to freshwater, a number

of species can develop in extremely high concentrations of salts. Larvae of *Ae. natronius* have been found in a crater lake in Uganda which had a salinity equivalent to 3.9 percent NaCL (0.67 M), an alkalinity of 0.7 N and a pH exceeding 10.5 (Clement, 1963).The larvae of brackish-water species are able to develop in the concentrated sea water which occurs in littoral pools. *Aedes australis* will develop in sea water containing 7.4 percent salts and *Ae.detritus* has been found in sea water containing 10 per cent salts. Certain fresh-water species can tolerate a fairly high salinity. *Anopheles superpictus*, for example, will develop normally in 30 per cent sea water. Larvae of *Ae. aegypti* reared in fresh water are killed by 0.19 M-NaCL (1.1 per cent NaCL) or by sea water isosmostic with 0.22-0.24 M-NaCL, but by gradually increasing the concentration the larvae can be adapted to 0.19 M-NaCL and to 50 per cent sea water (isosmotic with 0.3 M or 1.75 per cent NaCL). The restriction of species to particular larval habitats is probably due to discrimination by ovipositing females, an activity which will be affected in the long term by selective pressures operating during the larval stage as well as by those effective at oviposition (Clements, 1963).

2.1.4.3 Effect of temperature on mosquito larvae

The rate of postembryonic growth follows the familiar S-shaped curve with temperature. Below a developmental temperature threshold no growth occurs; above the threshold the rate of growth increases with increasing temperature, reaching a maximum at the so-called 'optimum temperature' above which it declines. In *Ae.aegypti*, some growth takes place at 10°C but development is not completed. Above 14°C growth becomes increasingly rapid with rising temperature, reaching the 'optimum' at 32°C. The rate of growth decreases at still higher temperatures and above 36°C development is not completed. The temperature at which development proceeds fastest varies at different stages of development. When *An. quadrimaculatus* was reared

at constant temperatures throughout the life cycle, development of the egg was found to be most rapid at 33.3°C; of first instar larvae at 32.5°C; of fourth instar larvae at 30°C and of pupae at 30.5°C. Development of the larval and pupal stages considered as a whole was most rapid at 31°C. Similar variations were found in *Ae.aegypti*. The apparent drop in optimum temperature was possibly due to the cumulative injurious effects of high temperature (Clements, 1963).

When the immature of mosquito stages were subjected to fluctuating temperatures, development was faster than at constant temperature; equal to the mean of the fluctuating temperatures. When larvae and pupae of *An. quadrimaculatus* were exposed to a raised temperature for 9 hours each day, the velocity of development was accelerated by 13 per cent when the lower temperature was 19°C and by 4 percent when the lower temperature was 23°C, compared to the velocity of development at a constant temperature equal to the mean of the fluctuating temperatures. Variable temperature has been shown to accelerate development in *Ae.aegypti* also, but only over the middle portion of the curve relating developmental rates and temperature. At higher temperatures a fluctuating temperature does not increase the rate of development but at lower temperatures it slows development down. The accelerating effect has been explained by the assumption that the maximum rate of growth at a high temperature can only be sustained for a short time after which it declines, whereas too short a period at the lower temperature does not allow recovery from the deleterious effects of the higher temperature (Clements, 1963).

2.1.5 Pupal biology

All mosquito pupae are aquatic and comma-shaped. The head and thoraxare combined to form the cephalothorax, which has a pair of respiratory trumpets dorsally. The

abdomen is 10-segmented although onlyeight segments are visible. Each segment has numerous short hairs and thelast segment terminates in a pair of oval and flattened structure termed paddles. Some of the developing structures of the adultmosquito can be seen through the integument of the cephalothorax; themost conspicuous features being a pair of dark compound eyes, foldedwings, legs and the proboscis.Pupae do not feed but spend most of their time at the water surfacetaking in air through the respiratory trumpets. If disturbed, they swim upand down in the water in a jerky fashion.Pupae of *Mansonia* and *Coquillettidia* differ in that they have relativelylong breathing trumpets, which are modified to enable them to pierceaquatic vegetation and obtain their oxygen in a fashion similar to the larvae. As a consequence their pupae remain submerged and rarely cometo the water surface (Service, 1997).

2.1.5.1 Moulting, pupation and emergence

Without any known exception, mosquitoes pass through four larval instars. At moulting, the epicuticle can be seen to separate from the cuticle and to secrete new cuticle and bristle beneath it. The tracheal epithelium similarly retracts from the tracheal linings and secretes new cuticular linings around them so that a wide space filled with fluid can be seen between the two.When the larva of *Ae.aegypti* is about to shed its skin, it rest at the surface and swallows water by contractions of the pharynx until the soft body wall becomes extremely taut. From time to time it flexes its head sharply until finally the head capsule splits. The ecdysial line along which the capsule splits follows the epicranial suture, starting in the dorsal midline of the collar and then diverging widely and passing forward on each side of the dorsum of the head to the bases of the antennae. Meanwhile, the old tracheal trunk have broken across just behind their connections to the non-functional spiracles in each segment and as the larva leaves its old skin the fractured pieces of the tracheal system are withdrawn, the fragments in

each case passing out through the pair of spiracle immediately behind them. All the spiracles except the terminal pair are then sealed until the next ecdysis (Clement, 1963).During the process of pupation, the fourth instar larva sheds its skin while the respiratory trumpets and float hairs of the pupa appear as dark bodies beneath the cuticle. The larva comes to lie horizontally at the surface and shortly afterwards air appears in the short tracheae linking the respiratory trumpets to the longitudinal tracheal trunks, subsequently spreading between the larval and pupal cuticles.The larval cuticle and tracheal linings are shed much as at previous ecdyses and during this period the labial and maxillary-palp buds are extruded from their peripodial cavities (Clement, 1963).After pupation, practically the whole cuticle hardens and darkens, in contrast to the small area of cuticle which is sclerotized in the larva. The pupa usuallyrests at the surface, held in position against the surface film by the tips of the trumpets and the float hairs on the first abdominal segment, but it can swim well and when disturbed swims downwards by the beating of the abdomen with its large paddles, later floating to the surface again.Some mosquitoes show a strong tendency to pupate at a particular time of day.

The first sign of emergence is the appearance of a small amount of air beneath the pupal cuticle, at the bases of the respiratory siphons and elsewhere. The abdomen is slowly raised to a horizontal position and shortly after this the pupal cuticle splits along the mid-dorsal line of the thorax. A minute later the thorax of the adult, which is quite dry, protrudes through the split and slowly the whole body of the adult rises into view. It is most probable that the air which appears between the pupal and adult cuticles before the pupal cuticle splits penetrates through the respiratory trumpets when the tracheae from these organs fracture, for pressure on the pupal cuticle causes the bubbles to escape through the trumpets(Clement, 1963).

2.2Global Impact of Mosquito Biodiversity

The efficiency, speed andreach of modern transport networks put indeed people at risk from the emergence of newstrains of familiar diseases or completely new diseases. The globalgrowth of economic activity, tourism, and human migration are leading to ever more cases ofthe movement of both diseases vectors and the pathogens they carry,increasing at the same time the biodiversity of mosquitoes around the world (Table 2.1). In the currentcontext of global warming, the expansion of areas suitable for the major disease vectors isbecoming a threat and even a reality for some regions with several species being classified as invasive. However, this small fraction isresponsible for some of the worst scourges of humankind and the most important vectorsbelong essentially to three genera including *Anopheles*, *Aedes*, and *Culex* among which somespecies have been quite successful of wide-scale invasions(Figure 2.4) (Manguin and Boëte, 2010).

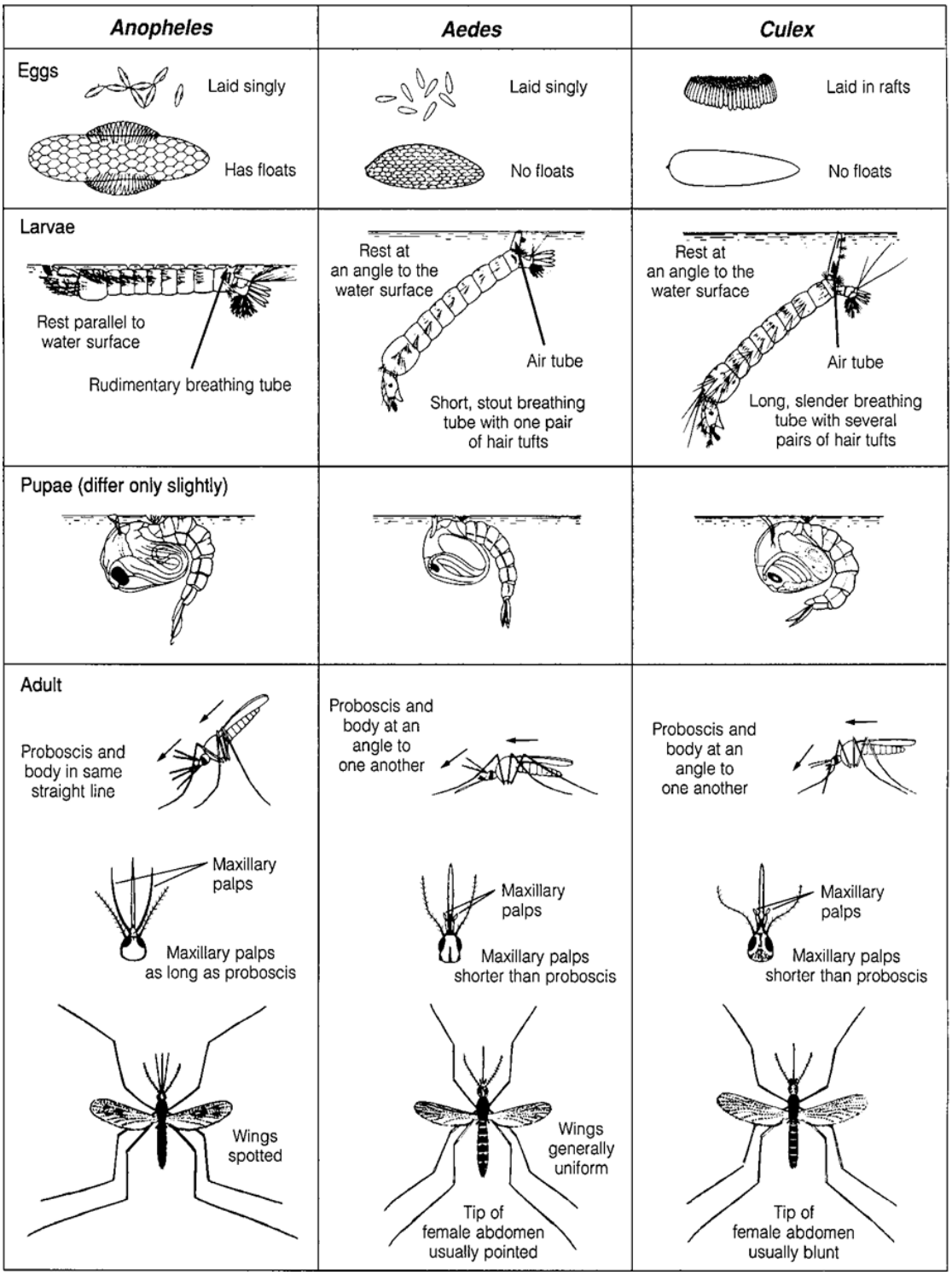


Figure2.4: Main distinguishing characteristics for*Anopheles*, *Aedes* and *Culex* mosquitoes (Rozendaal, 1997).

Table 2.1: Characteristics of major mosquito genera.

PARAMETERS	***ANOPHELES***	***AEDES***	***CULEX***
Sub-family	Anophelinae	Culicinae	Culicinae
Life span	7-10 days (male) 4-5 weeks (female)	Average of 20-30 days. Female deposits ~ 4 batches of eggs.	-
Breeding sites	River margins, river bed pools, canal, rainwater (burrow/p its) Low lying groun ds, hoof marks andw heel ruts, rice field, wells, ponds, brackish water pools.	Containers, discarded tins, empty pots and broken bottle s, coconut shells, artificial collection (of waters) e.g. coolers, tyres (discarded).	Domestic and peridomestic sources such as Cesspools, open ditches, sewage and waste water.
EGGS	Laid singly (has later al floats).	Laid singly, no floats, breed in clean/unpollut ed water (man made/artificial containers).	Clusters/rafts of 100-250 eggs, no floats.
Viability	1-2 days at normal temperature. May be extended up to 1 we ek in winters.	If dried under natural conditions, are viable for 6 months or longer.	~ 2-3 days.
LARVAE	(yellowish, green/ grey) Remain paralle l to surface of water, no siphon (breathing tube).	Remain vertically hanging to water surface, has a siphon (breathing tube).	Remainvertically hanging to water surface, has a siphon.
No. of instar and size			
I Instar	< 1 mm	-	-
II Instar	1-2 mm	-	-
III Instar	4-5 mm	5-10 mm	-
IV Instar	5-7.5 mm	~10 mm	-
To what water depths it can reach	~ upto 1 m	Few meters	-

Manguin and Boëte, 2010.

Table 2.1 Con't: Characteristics of major mosquito genera.

Emmergence	7-10 days normally	7-10 days	-
ADULT SIZE	~ 0.5 cm	0.4-0.5 cm	~ 0.5-0.9 cm
Colour and resting posture	Grey, rest at 45° angle t o surface.	Black with white patches on the legs, rests parallel to surface.	Dark grey, rests parallel to surface.
Sitting posture Resting habits	Body parallel to resting surface. In cattle sheds, under bushes and in tree holes, an indicator of outdoor resting.	-	-
Eating habits: i.) male ii.) female	Nectar/fruit juices Haematophagus.	Nectar/plant juices Haematophagus.	Nectar/plant juices Haematophagus.
Biting habits/time	Both indoor/outdoor Through out night but peak biting occurs between 19 00 and 04 00 hrs. Predominantly zoophilic.	Mostly indoor than out doors. Diurnal, day biters with 2 peaks of biting (1^{st} at dawn after the sunrise and 2^{nd} at dusk before sunset).	Both indoor/outdoor From dawn to dusk.
Flight range	1-3 km	50 -200 m	1-2 km
ADULT DENSITY (needed to maintain transmission).	1 infective bite	No estimate of adult density is known for transmission possibly one infective bite.	Usually very high density is required Needs several infective bites for transmission.
Diseases transmitted	Malaria, Filariasis	Yellow fever, Dengue, Filariasis, Chikungunya fever	Bancroftian filariasis, Japanese encephalitis, West Nile fever, Viral arthrites, Polyarthrites.

Manguin and Boëte, 2010.

An invasive species is defined as a species having a great impact on its new environmentand spreading with measurable growth. Three major biological characteristics found among the most invasive mosquito species are their closeassociation with humans, egg resistance to desiccation (genus *Aedes*) and small larvalhabitats such as a wide variety of water holding containers among which man-made ones are totally suitable (Manguin and Boëte, 2010). Dispersal of mosquitoes can be active or passive. In the former case, mosquitoes are able to extend actively their range by flying from one habitat to another, buttheir flight capacity being fairly reduced (few kilometers for most of them). This naturaldispersal will not allow them to quickly travel long distances. In the latter case, passive dispersal allows long range transportation that can be either due to natural conditions(wind) or human-assisted (population movements), increasing the risk of spread ofmosquitoes and vector-borne diseases. Under exceptional conditions, for instance strongwinds, transport of mosquitoes is occurring on long distances into new areas. The case of thearrival of *An.gambiae s.l.* in Reunion Island (200 km from Mauritius) was attributed to the passageof a cyclone, and led to the first malaria epidemics in 1868(Manguin and Boëte, 2010).However,most of mosquito invasions are due to human-assisted transportation. For centuries, shipbornetransportationallowedmantotravellongdistancesbringingwithhimimmaturestagesofmosquitoesabletocopewiththetransportconstraints.Thisisespeciallytruefor*Ae. aegypti*and*Cx. quinquefasciatus*thathavethecapacitytodevelopin shipwater storage (Manguin and Boëte, 2010).Mosquitolarvaethatoccupysmallwaterbodies,suchasnaturalorartificialcontainers,are easilytransportedandwhen theconditionsarefavourable,mosquitoesestablishandinvadenew territories.

2.3Predator Escape Mechanisms in Mosquitoes

Different oviparous insects have been reportedtoavoidaquaticsiteswherethereisahighpredationriskto their offspring. Mechanisms forpredator detection by insects may involve tactile, visual, or chemical cues (Spencer *et al.*, 2002). The proximate mec hanisms that mediate avoidance behaviour arecertainchemicalexudatesreleasedby predators that are commonly calledkairomones. Chemically mediated avoidance is anadaptation used by prey to detect and evadepredators. Kairomones or semiochemicals emittedfrom predators are normally usedby prey to detect a predator's presence in theenvironment, and the prey can thereby minimizesuch encounters (Kats andDill, 1998). Common responses to chemicals frompredators include an increased use of refuges, marked changes in the intensityof movements (Huryn and Chivers, 1999), reduced foraging, reduced courtshipbehaviour, predator avoidance, and increased growth rates (Kumar and Hwang, 2006).Chivers and Smith (1998) and Kats andDill (1998) reviewed studies showing that fish,amphibians, reptiles, mammals, a bird, and abroad array of invertebrates have evolvedchemosensory mechanisms for detecting predators.Kats and Dill (1998) listed 16 studies whichinvolved larvae of aquatic species that provided evidenceforchemicallymediateddetectionofaquaticpredatorsby insects. Where chemical cues areinvolved, responses to predators are often influencedbythepredator's diet. For example,mayflyand damselfly larvae are more likely to respond tofish chemicals if the fish have recently consumedconspecific prey (Huryn andChivers, 1999). Prey species are more likely toevolve chemically mediated avoidance of predatorswhen visual

detection of predators is limited. Inturn, prey must possess suitable behaviour andchemosensory mechanisms to detect and respondto predators (Kumar and Hwang, 2006).

Mosquitoes appear to meet most ofthese requirements since many species are crepuscularor nocturnal and rely heavily onchemosensory mechanisms when selecting ovipositionsites. Aftermatingandtakingabloodmeal,ahemolymph-borne hormone triggers the femaleto begin searching for a suitable oviposition siteand to become receptive to chemical signals fromthe site. Gravidfemales are known to spend some time flyingaround a water body apparently evaluating it as anoviposition site (Lester and Pike, 2003). The main criteria in such a selection process are the presence of comp etitors, predators, and/or kairomones and container size. Females oviposit on mud or the water's surface (e.g., *Culex* spp.) after sampling the chemical composition of the substrate using receptors on the tarsi, antennae, and tips of the proboscis. Published evi dence convincingly suggests that mosquitoes use chemosensory information toassess several parameters that reflect habitatquality for the offspring, including the availability ofnutrients, the presence of competitors and predators,andtheoverallqualityandpermanenceofthewaterbody.It has been demonstrated that mosquitoes andphantom midges greatly reduced ovipositingratesin experimental pools that contained caged sunfish(*Lepomis*) that were not visible to ovipositingfemales. Grostal and Dicke (1999) demonstrateda similar behaviour in acarine mites, suggesting thatthis phenomenon may occur across a diversearray of arthropods. Recently it has been convincinglyreportedthatadultmosquitoeshavetheabilityto sense the presence of *Gambusia*, and thatmosquitoes reduce egg-laying rates in pools containingtheodour of mosquito fish(Kumar and Hwang, 2006).

In nature, *G. affinis* inhabits temporary habitatssuchasswamps,roadsidepuddles,andornamentalponds,andformsschoolsthatmaycontainhundredsof individuals. Schooling behaviour producesapatchyenvironmentwherepredationrisksmayvarylocally,depending on the location ofschools. Female mosquitoes avoid *Gambusia* atseveral spatial scales. For example, females canuse the strength of fish chemicals to avoid localsites in ponds with dense schools. Alternatively,females might strongly avoid ponds with *Gambusia*and seek out ponds with fewer or no fish.Moreover, recent reviews have not supportedthe effectiveness of the mosquito fish in controllingmosquito populations or mosquito-borne diseases. In some cases the mosquito fish may even indirectly increase the survivalrate of mosquito larvae by feeding on their cladocerancompetitors.One explanation for the ineffectiveness of*Gambusia* in controlling mosquitoes is thatovipositing mosquitoes may seek out fish freehabitats that adjoin large bodies of water containing*Gambusia*. The active avoidance of habitatswith a high predation risk to offspring may ultimately act to sustain high densities of mosquitoes near areas stocked with *Gambusia*. Ritchie and Laidlaw Bell (1994) found that ovipositing *Ae.taeniorhynchus*stronglyavoidedsiteswithhighdensitiesof *G. holbrooki* and shifted to adjoining habitatswith few or no predatory fish. Therefore, female mosquitoes often avoid ovipositingin waters containing predatory fish (Blaustein *et al*., 1995; Blaustein, 1998; Kiflawi *et al*., 2003).

2.4 Biotic Interactions of Larval Mosquitoes with Different Predators and Competitors in Aquatic Ecosystems

Mosquito-borne diseases have been a majorproblem in almost all tropical and subtropical countries,and currently there are no successful vaccinesagainstmostsuchdiseases.For years,mankind has been exploring various

methods tocombat threats from mosquito-borne diseases. Many synthetic insecticides are widely used forcontrolling adult and larval mosquito populations.However, the harmful effects of chemicals on non-targetpopulations and the development of resistancetothesechemicalsinmosquitoes,alongwiththerecent resurgence of different mosquito-bornediseases, have prompted scientists toexplore alternative, simple, sustainable methods ofmosquito control. The eradication of mosquito flyinginsectstocksusingadulticidesisnotaprudentstrategy,as the adult stage occurs alongsidehuman habitation, and they can easily escaperemedial measures (Service, 1992). Until recently, the ecological role of environmentalmanagershasbeenmoreconcentratedonpreventingdamagefrompollutionrat herthanproposingsustainable solutions to different globaland local problems faced by human societies.One of the multiple possibilities of applying ecologicaltheories for human welfare is the use of ourknowledge about the effects and mechanisms ofpredation and competition within various kinds ofpermanent and temporary aquatic habitats. Bymanipulating particular trophic levels, desiredchanges can be achieved in a system. Biotic interactions such as competition andpredation have been reported to be capable of regulatingthe number of mosquito populations byreducing the number of larvae that survive throughlarval development and by increasing the larvaland pupal duration times. It isobvious that different pathogens and disease vectorsarepartoftheecosystem,andanthropogenicalterationsofnaturalconditionsprovidea nadvantagetosomeofthesepathogensanddiseasevectors.The recent resurgence of various mosquito-borne diseases may have been an indirect resultof such altered conditions. Programs to decimatemosquito populations by trying to kill the adultstage frequently fail, because the adults residealongside human populations in their households,and hiding places can often not be detected asrefuges for mosquitoes, thereby allowing

them toescape remedial measures. Even larval mosquitoeslive in places where they are difficult to findand kill: for instance, in old tyres, trash, watertanks, and basically any container that holds water. Precautions also have to be takennot to contaminate drinking water supplies andwater-transport containers with potentially harmfulchemicals. Additionally, mosquitoes have developedresistancetofrequentlyusedpesticidesmakingit even more difficult to control adult populations.Essentially,larval mosquito populationsshould be the first target of all control measures(Briegel, 2003).

The potential for the use of genetics againstmosquito-borne infection has recently been consideredin vector control programs. Recent techniquestomodifygenesofmosquitoesarebelievedtobeanappropriateinterventionalreme dyagainstmalariaanddenguefever.Themainpurposeistoproducea genetically modified strain of mosquitoin the laboratory which does not serve as a carrierof disease and which is competitively superior inthe natural habitat such that wild mosquitoes willeventually be replaced after the release of geneticallyalteredmosquitoesinnature. However,thereareseveral problems with this approach, and it istaking some time to turn the prospects of this technologyintopracticaltools.Therefore, the exploration of more-effectiveand eco-friendly techniques to control mosquitoesseems to be very promising. Various organisms,known as natural biological control agents, can beutilized to control mosquito populations, thusavoiding the use of chemicals and harm to theenvironment in the process. It is desirable to usebiological control agents that can adapt to mosquito breeding habitats, are found naturally, and poseno danger to people. Biological control meansthe use of different kinds of living things, and/ortheir derivatives to eliminate pest populations.Many biological control agents disperse by

themselvesenablingthemtospreadandbuildupviablepopulations.In the field ofapplied ecology, there have been many attemptsto achieve the biological control of pathogens orvectors by introducing new effective natural enemiestotheirnaturalhabitats(Denoth *et al*., 2002). The efficient selection of effective naturalenemies has become increasingly important

for the success of biological control programs. Microbial larvicides such as *Bacillus thu ringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*B. sphaericus*) are gram positiveaer obic (facultative anaerobic entomopathogenic soil bacteria (Bobrowski *et al*., 2002). M osquito larvae ingest themicrobial product which is composed of a dormantspore form of the bacterium and an associatedtoxin (crystalline protein inclusion produced by *Bti*during sporulation). The toxin acts as a stomachpoison by binding to receptor cells present in theinsect but not in mammals. Interestingly, its applicationdestroys larval mosquitoes but spares predators ifpresent in the water (Su and Mulla, 2005). Very recentlynon-larvicidal effects of *Bacillus* formulations havealso been recorded in various mosquitoes. Relatively lower numbers of eggrafts have been recorded in ovitraps treated with*Bti* and *B. sphaericus* than in controls, and furthermore,anadverserelationshipwasfoundbetweenthe*Bacillus* product concentration and oviposition(Zahiri and Mulla, 2005). Contrary to this, a liquidformulation of *Bti* dispersed by ultra-low-volumetechnology in laboratory and field experiments didnot affect the oviposition behaviour of *Ae.albopictus*females. The parasporalproteinaceous toxin produced by *Bti*, however, isphotolabile and destroyed by sunlight. In mosquitobreeding habitats, they are only effective for1-4 weeks after application. In a recent study in Taiwan, Teng*et al*. (2005) recorded that the maximum durationof effectiveness of*Bti* in reducing the larval number was 1 week after application in rice

paddy fields.Essentially, frequent applications to each breedingsite are required, thereby making it more expensiveandrigorous(Amalraj*etal*., 2000).

A fermentation product called spinosad, produced by the actinomycete, *Sacchoropolysp ora spinosa*, isanother naturally derived larvicide. In a recent study in Antalya, Turkey, themosquito species *Cx. pipiens* was eliminatedfrom septic tanks in 7 days after the application ofspinosad at a concentration of 100 active insects(a.i.)/ha (Cetin *et al*., 2005). This has been shownto be less toxic to birds and mammals and no adverse effectswere detected on predatory insects such as ladybirds,lacewings, big-eyed bugs, or minute piratebugs. Its applicability has not been assessed innature. Many mosquito larvae have been reportedto ingest toxic and noxious algae (mostlycyanobacteria) which inhibit larval developmentand decrease survival; however,mosquito breeding sites are usually too dark foralgal growth.

The fungus *Lagenidium giganteum* has beenshown to have the ability to control mosquito populationsthrough oospores that can resist droughtand which can readily be produced in bulk.Oospores survive for many years in the soil, butcan reactivate within only about a month afterflooding. In practice, the spores are activated byremaining in wet for 1-2 weeks before being sprayedon the surface of the site to be treated. The fungus infects and kills onlya portion of larval target mosquitoes, as well. Thelevel might not be sufficient to decrease the risk ofhuman infection. Very recently in rice paddy fieldecosystems in Taiwan, *L. giganteum* has beenfound to be effective for only one week in reducing thenumber of larvae after application (Teng *et al*.,2005), thus necessitating frequent application ofthis product.The use of mosquito parasites, for instance,protozoans and helminths, might be a possibleway to control mosquito populations. Oocystsof *Ascogregarina* (a protozoan parasite) releasesporozoites that

disrupt the gut wall of mosquitolarvae. For instance,*Ascogregarina taiwanensis* and *As. culicis* normallyinfect*Ae.albopictus*and*Ae.aegypti*respectively. Biological control of mosquito larvae with biologicalagents like competitors and predators ismore convenient and alleviates the need for frequent chemical applications. The selection of biological control agentsshould be based on their potential for unintendedimpacts, self-replicating capacity, climatic compatibility, and their capability to maintain very closeinteractions with target prey populations. They eliminate certain preyand sustain such environments (i.e., as when preyis introduced, they eat the prey) for long periodsthereafter. However, this will onlybe possible if the predator possesses extraordinarysearch efficiency irrespective of the illuminationsituation in response to the emergence ofprey.

2.4.1Amphibian tadpoles

Omnivorous tadpoles are potential predatorsof mosquito larvae and exert significant impacts on freshwaterecosystems (Blustein*et al.*, 1996). Larvae of the giant Cuban tree frog(*Hyla septentrionalis*) have been reported todestroy aquatic insects and algae growing in watercontainers. However, the dietary nichebreadth of larvivorous tadpoles includes otherpredators of mosquito larvae as well, hence theycan efficiently predate on other co-occurring biocontrolagentsofmosquito. Their efficiency in utilizingmosquitolarvaeinthepresenceofalternatepreyhasnotbeenproperlyelucidated. Itha sbeenreportedthat tadpoles of *Rana tigrina* are moreefficientmosquito pupal predators than others. However, in nature, tadpolesare preyed upon by many predators, for example, mosquito fish, catfish, and dr agonfly larvae. Thesepredators prey on amphibian eggs and tadpoles,and contribute to substantial anuran declines.Eklöv (2000) recordedthat larval dragonfly, *Anax junius*, can utilize bullfrog(*Rana catesbeiana*) larvae more efficiently thanthe bluegill sunfish

(*Lepomis macrohirus*). Ofcourse any introduction of amphibian tadpolesbeyond their natural range is difficult and requiresutmost caution. Tadpoles can rarely be accommodatedinsmallcontainersthatholdlessthanseveralliters of water and seem to have small impactson larval populations of mosquitoes (Kumar and Hwang, 2006).

2.4.2Aquatic insects

Some aquatic insects play important roles inmosquito control. In general, almost allaquatic insect predators prey on mosquito larvaeand pupae, the aquatic coleoptera (especially notonectidsanddysticids)andodonateshavebeenobservedto ingest mosquito larvae as apart of their natural food assemblages. Thebackswimmer, *Notonecta undulata* (Hemiptera,Notonectidae), has been shown to efficiently utilizethe second instar of mosquito larvae as prey (Blaustein *et al*., 1995; Blaustein, 19 98). The role of the backswimmer, *Anisops assimilis*, in controlling mosquitoes wasnoted

when some stock troughs with *Anisops assimilis* were free of mosquitoes, whereas pud dles in depressions surrounding the troughs contained energetic mosquito activity. How ever, thepredation efficiency ofbackswimmersonmosquitolarvaewas found to be container-specific (Lesterand Pike, 2003).

2.4.3 Larvivorous psorophora

Toxorhynchites, a kind of mosquito with cannibalisticlarvae, has attracted much attention asbiological control agents. Certain kinds of*Toxorhynchites* mosquitoes are good for the controlof*Ae.aegypti*becausetheybreedinthesamekindsofcontainers. However, it proved to beunsuccessful in the field. Repeated release of*Toxorhynchites* 1st instar larvae in waterloggedplaces between bamboo trees had no effect onmosquito populations in Indonesia (Annis *et al*.,1990). Ironically it was recognized that

itincreased prey density. This type of interferingeffect became evident when the prey was presentin excess. Subsequent testsand trials have obtained similar results (Annis *et al*., 1990).

2.4.4Odonate larvae

The order Odonata includes insects like thedragonfly and damselfly. They enjoy a wide distributionandareparticularlyprominentaroundaquaticecosystems in tropical countries.Invariably the adults mate near water bodies, andthe females lay eggs in water soon thereafter.Aquatic larval instars are predators of mosquitolarvae. Dragonfly larvae, in particular, may be usefulfor controlling mosquito larval populations (Brendonck *et al*., 2002). In an experiment in Myanmar (Burma), 2 dragonf ly larvae were introduced in domestic containers accommodating *Ae*. *aegypti*. The mosquito larvaedisappeared quite rapidly after dragonfly larvaintroduction to each container, and the density oftarget adults declined about six weeks later. The dragonflyand damselfly are true enemies of mosquitoesasthelarvaeoftheseinsectsareabletoutilizemosquito larvae as food, and the adults areefficient predators of airborne adult mosquitoes. However, they not only consume largenumbers of mosquitoes, in both larval and adultforms, but also predate on different zooplankton, beetles, and other insects (Brendonck *et al*., 2002). Further more, Stav *et al*. (2000) eval uated the overall consumptive and non-consumptive(conditionedwater)effectofthenymphof*Anax imperator* (Aeshnidae: Odonata)on populations of larval mosquitoes. They foundno difference in the oviposition of the mosquito,*Cs. longiareolata*. Still, the quantitative evaluationofconsumptionofmosquitolarvaebyodonatelarvaein the presence of other prey iswarranted. Their analysis revealed thatthe larvae preyed upon chironomid larvae, and

also found corixids, cladocerans, ostracods, and aquatic mites. No remains of mosquito larvae were detected in any of th e specimens, even though mosquito larvae (*Aedes*, *Culex*, *Culiseta*, *Mansonia*, and *Psor ophora*) were observed in the pond where the damselfly larvae were collected. Certain water bugs, e.g., *Sphaerodema annulatum* and *Sphaerodema rusticum* (Heteroptera: Belostomatidae),areknown to utilize mosquito larvae.Recently it was found, however, that the consumptionof larvae by these bugs is dependent on thedensity of larval prey in the medium, so a considerableamount of mosquito larvae is likely to be leftunconsumed (Aditya *etal*., 2005). None of these agents has shownany promise for mosquito control, as they havebeen proven difficult to rear and store, as well asbeing unstable or inefficient in the field. Their abilityto aerially distribute themselves can be anadvantage, as the adults have access to most,even tiny, water bodies where they lay their eggs.Therefore, their larvae can be found in small watertubs and tanks as well, ideal places where mosquitoesmaybreed.Further in-depth studies need tobe done to assess the role of aquatic insects andtheir contribution to the control of mosquitoes.

2.4.5Larvivorous organisms in temporary water bodies

Temporary or ephemeral aquatic ecosystems(pools, puddles, floodplains, etc.) are natural bodies of water, which experience arecurrent dry phase of varying duration. In otherwords, temporary aquatic systems are those inwhich the entire habitat shifts from being availableto unavailable to aquatic organisms, for a durationand/or frequency sufficient to affect the entire biota. Generally, the hydroperiodin ephemeral water bodies corresponds to thebreeding periods of mosquitoes, and such bodiesbecome ideal breeding sites for mosquitoes, particularlyforthe genera *Aedes* and *Anopheles*. In intermittent puddles, the even shorter permanence of the habitat mak es it favourable to breed immature culicids as a consequence of the rare exposure to pre

dators. The composition and density of larval mosquito communities are stronglyinfluenced by the ephemeral or permanent natureof the pools (Okogun *et al*., 2005). James (1966) reported that immaturemosquitoes are less exposed to natural enemies intemporary than in permanent ponds. Themostimportant suspected invertebrate predators in temporarypoolsareturbellarians, notonectids, divingbeetles (Coleoptera), dragonfly larvae (Odonata)(Blaustein *et al*., 1995), and crustaceans, e.g., tadpoleshrimp.Turbellariansassume a particularly important position inephemeral ponds, as they have the ability to produceresting eggs which can survive dry periods. They are present and becomeeffective within the first few days after rains beginto fall, while most other invertebrate predatorsbecome effective only later in the hydro-period ofindividual pools or even at a later stage during therainy season. The most important flatworm predatorsarespeciesof*Mesostoma*thatoccurinawiderangeof habitats. These species display a wide variety of predationmechanisms. Some species produce a kind ofmucus that functions as a toxic web to trap and killprey organisms. Otherspecies just wait to attack the approaching prey(sit and wait or ambush predation). Somespecies may also actively search for suitable preyor reveal prey selectivity. Freshwatercrustaceans, tadpole shrimp, and some copepodsare adapted to temporary bodies particularly in aridzones (Brendonck *et al*., 2002).

2.4.6Cyclopoid copepods

Cyclopoid copepods are abundant in eutrophicwater bodies and play important roles in their trophodynamics. Like many predators in aquatic environments, cyclopoid copepo ds are known to strongly influence the structural and functional organization of prey co mmunities on which theyfeed. Predominantly carnivorous but with an abilitytoutilize plants as well (Kumar and Rao, 1999), cyclopids have a wide spectrum of potential food items available in their habitat, including algae, ciliates, rotifers, clado

cerans, and copepods. The patterns of prey selection and feeding rates in this group of invertebrate predators have been the focus of considerable research. Some cyclopoids have long been known to have the ability to utilize mosquito larvae as food (Marten *et al.*, 1994). Kumar and Rao (2003) conducted a series of behavioural observa tions of thecyclopoid *Mesocyclopsthermocyclopoides* handling and predating onmosquito larvae.

Cyclopoids now appear to offer high promise asbiological control agents for *Ae. aegypti* (Marten *et al*., 1994), *An. stephensi* (Kumar and Rao, 2003) and *Ae. albopictus* mosquitoes. Malarial mosquito larvae were found to be absent from aquatic habitats in Latin America that contained natural populations of *M. longisetus* (Marten *et al*., 1994) and virtually disappearedafter *M. longisetus* and other species of*Mesocyclops* were introduced to rice fields andsmall marsh areas in Louisiana (Marten *et al*., 1994). The technology using predatory cyclopiodsfor controlling mosquitoes is "appropriate" atmodest costs. However, biological control usingcyclopoids as predators against mosquito larvaehas not received serious consideration in countriesaffected by mosquito-borne diseases. An additional advantage of cyclopoids overother aquatic predators is that they are wastefulkillers with the capability to kill more mosquito larvaethantheyactuallyingest.Iflarvaearenumerous,they eat a small part of each larva, whichmeans that each copepod has the capacity to kill30-40 larvae/d, far more than they actually eat(Kumar and Rao, 2003). In natural water bodiesthey are found in large numbers.However, only about 10% of places with waterwhere mosquitoes might breed contain naturalpopulations of cyclopoids which can drasticallyreduce the survival of mosquito larvae (Kumar and Rao, 2003).However, not all copepods destroy all mosquitoes.Differential selection by copepods is relatedto mosquito larval species and instar stage. Cyclopids show distinct prey selectivity

behavioural patternsandtheirpreyselectionpatternsareinfluencedbymanyattributesof the prey such asmorphology, behaviour, and taste (Kumar and Rao,2003). These are known to differ from species tospecies (Kumar, 2005).Some species of cyclopoids usually select smallerprey items from the available prey size spectrum(Kumar, 2003),while others may actively select the largest preythey can capture. The extentof carnivory in omnivorous cyclopoids appears tobe directly related to their body size. Animal prey selectivity ofomnivorous cyclopoids has been demonstrated tobe a function of their extent of dependence on analgal diet; for instance, some animals (e.g.,*M. thermocyclopoides*) can completetheir entire life cycle exclusively on an animal diet,whereas others (e.g., *Cyclops vicinus*) have a foodbottleneck in their life cycle. The selectivity foranimal prey in those species that include algae intheir diet is also influenced by the extent of theirdependence on algae. In the laboratory, Kumarand Rao (2003) studied the prey consumptionrates of the most abundant cyclopoid copepod intropical and subtropical water bodies, *M. thermocyclopoides*onfirstandfourthinstarlarvaeoftwospeciesof mosquito (*An. stephensi* and *Cx. quinquefasciatus*)in relation to their densities. Sinceprey vulnerability is a product of prey encounterrates with and ease of capture by the predator(Kumar, 2005), certain morphologicalandbehaviouralattributesofdifferentpreyspeciesthat determine capturability, and their relative proportions (whichaffect encounter rates) in the medium may be ableto explain observed differential larval selectivitypatterns in copepods.Considering this point,Kumar and Rao (2003) also studied the preyselectivity of *M. thermocyclopoides* towards mosquitolarvaeinthe presence of alternate prey (thecladocerans, *Moina macrocopa* and *Ceriodaphniacornuta*) in different proportions. That laboratorystudy demonstrated that *M. thermocyclopoides*had biocontrol potential against *An. stephensi*even in the presence of alternative prey. However,against *Cx.*

quinquefasciatus, there was a significantreduction in larval consumption in the presenceof*Moinamacrocopa*, but not in the presence ofeither *D. similoides* or *Ceriodaphniacornuta*. Itshould be noted that the surface area of the containerisone of the determinants of the predatorefficiency of controlling larval mosquitoes(Lester and Pike, 2003).Therefore, further studies are required to assessthe role of *M. thermocylopoides* against mosquitolarvae breeding in containers and other smallwater-collecting coolers, tyres, etc.

It is further advantageous that mosquitoes donot show habitat avoidance on the basis of info-chemicalssecretedbythecopepod. Recently, those authorsdemonstrated that gravid *Ae. aegypti*females weresignificantly more attracted to ovitraps containingcopepods or to ovitraps containing copepod-conditionedwater than the controls. They concludedthat the copepod infochemicals may be responsiblefor attracting gravid *Ae. aegypti*females andmay increase the number of potential prey for thecopepod. Such attractants may play an importantrole in the surveillance and control of mosquitopopulations. Additionally, the cyclopoid can further be used in combination w ith *Bti* to obtain better results(Su and Mulla, 2005). Although *M. thermocyclopoides* is dominant indifferent lakes and ponds, its population is limitedat places where mosquitoes may breed.Therefore, mass culture of cyclopoids should beestablished, and active adult cyclopoids should beintroduced to different mosquito breeding habitats.The life cycle of cyclopoid copepods and their abilityto thrive on a wide spectrum of food organismsmake mass production easy and inexpensive; furthermore,theyarehighlyresilientandcanfunctioninopencontainersofanysizeorshape.Fe malesareinseminated when entering adolescence andno further contact with males is required to produce150-250 eggs/week for their lifespan that canlast for several months (Kumar and Rao, 1999).Cyclopoid copepods can survive in and colonizesuch habitats

whereas other predators are morelikely to find larger habitat patches. Among non-copepod crustaceans, notostracantadpoleshrimp and malacostracan prawn arepredators of mosquito larvae. They constitute animportant group on many floodplains.Different malacostracan species show differential potentials for predation on larval mosquitoes. F or example, *Paratelphusa spinigera* and *Varuna litterata,* are more aggressive predator s than *Macrobrachium borelli* and *Macrobrachium lamarrei.* Malacostracan imposed mortality on larval mosquitoes recorded by Collins (1998)was insufficient to consider these malacostrcans tobe potential biocontrol agents of mosquitoes innature.Notostracan tadpole shrimphave been considered to be potential predatorsadapted to ephemeral aquatic habitats in aridregions and rice paddy fields (Su andMulla, 2002). It was demonstrated that*Triops granarius* had the ability to decimate *An.gambiae s.l.*larvae in temporary breeding sourcesaround huts in a village in Somalia.The larvicidal ability of tadpole shrimp combinedwith their life history strategy (formation of restingeggs in sediments, egg hatching success on dehydration,fasterdevelopmentalrates,andhighfecundity;indicates that theymay be a suitable candidate for mosquito larvalcontrol in ephemeral habitats. Furthermore,tadpole shrimp were demonstrated to have the potential to enhance the efficacy of microbial control agents such as *Bti* for mosquit o control as their digging activity and vertical foraging process in the water column faci litate the availability of *Bti* toxin particles to mosquito larval feeding. Su and Mulla (20 01) collected useful information with respect to colonization, stocking, introduction in mosquito breeding habitat, and establishment of *Triopsnewberryi* for the biocontrol of immature larvae inephemeral habitats.

2.5 Aquatic Insect Predators and their use in Mosquito Control

Many aquatic insects in the ordersColeoptera, Diptera, Hemiptera and Odonataare known to prey upon mosquito larvae. Predators may be polyphagous: feeding on a broad range of prey species,oligophagous: with a restricted range of prey;or monophagous, with a very limited range of prey (specialist predators). Most insect predators of mosquitoes tend to be from thegeneralist type. Some predators (especially those withchewing mouthparts) eat their prey(Odonata), but others suck the body fluid(haemolymph) of the prey (many beetle larvaeand Hemiptera). Although predation mayoccur during any life stage, most researchesfocused on mosquito larval and pupal stagesbecause egg predation appears to be a minorcomponent of mosquito mortality and predation on the adult stage seems unlikelyto provide reliable levels of control in mostcases. Also surplusor 'wasteful' killing of uneaten prey ischaracteristic to the fourth larval instar of various species of the predatory mosquitogenus*Toxorhynchites*; it is recently documented in fourth larval instar of the predatory midge genus *Corethrell a* (Lounibos *et al*., 2008). According to hunting strategies, predators are classified into neustons that float on the top of the water (Hemiptera: Vellidae), free swimming (some microcrustaceans), climbing stalkers (Zygoptera: Odonata), sprawling ambushers(Odon ata:Anisoptera), and cursorialsearchers (Coleoptera: Dytiscidae and Hydrophyllidae). Predaceous insects are alsocategorized into surface predators andaquatic predators. The first group comprisedinsects that forage near or below the watersurface to catch their prey and all belongs toOrder Hemiptera. Predators in the lattergroup are good swimmers and are able toforage beneath water or/and on sub-surfaceterrain beneath vegetation such as OrdersOdonata and Coleoptera and somehemipterans (Shaalan *et al*., 2007).

2.5.1 Coleopteran predators

Although aquatic coleopterans arecommonly associated with mosquito larvaein different habitats, they have been lessexplored compared to other insect predators(Chandra *et al*., 2008). Among coleopterans, families Dytiscidae and Hydroph yllidae have received attention as mosquito larvae predators. Adults and larvae of Dytiscidaeand Hydrophilidae are common predatorsin ground pools, permanent and temporary ponds, and artificial mosquito breeding sitesand were reported from phytotelmata aswell. Although they can reduce mosquitoesdensities in some pools (Lundkvist*et al*., 2003), their mosquito controlefficacy perhaps is perhap limited by incompletehabitat overlap, alternative prey preference,emigration and cannibalism (Lundkvist*etal*., 2003).

Likely, species of the genera *Laccophilus*, *Agabus*and*Rhantus*have also been reported as potential agents of biological control of mosquitoes(Aditya*et al*., 2006). A field study showed that *Acilius sulcatus* (Family:Dytiscidae) larvae have significant im pact on mosquito larvae (*Cx. quinquefasciatus*, *Cx. bitaeniorhynchus*, *Cx. tritaeniorhyn chus*, *Cx. vishnui*, *Cx. gelidus*, *An. subpictus*, *An.vagus*, *An. aconitus*, *An. barbirostris*, *An. annularis* and *Armigeres subalbatus*) that prevail in cement tanks in Sainthia in the district of Birbhum, West Bengal, India (Chandra *et al*., 2008). A significant decrease i n larval density of different mosquito species after 30 days from the introduction of *Aci lius sulcatus* larvae was noted. On withdrawal, a significant increase in larval density w as noted indicating the efficacy of *Acilius sulcatus* inregulating mosquito immatures. In thecontrol tanks, mean larval density did notdiffer throughout the study period(Chandra *et al*., 2008).

2.5.2 Dipteran predators

The most common and famous dipteranmosquito predator is*Toxorhynchites* mosquito that has been introduced as abiological control agent of container-breeding mosquitoes in many differentecological habitats. A preliminary field trialon the Caribbean island of St. Maarten demonstrated the feasibility of using the predaceous mosquito larva, *Tx. brevipalpis* to control *Ae. aegypti* larvae. Sixteen days after the introduction of *Tx.brevi palpis*eggs into*Ae.aegypti*breeding containers,the 21 houses sampled were negative for*Ae.aegypti*and*Cx.quinquefasciatus*and the house index (the percentage of examined houses that are positive for*Ae.aegypti*larvae) dropped to zero. Focks*et al*. (1982) used*Tx. rutilus rutilus*to control*Ae.aegypti*and*Cx.quinquefasciatus*mosquitoes in residentialblocks within a substandard urban area of New Orleans, Lousiana. Mosquitoesemergence from automobile tyres, bucketsand paint cans, treated with 1 or 2 first instarlarvae of *Tx.rutilus rutilus*decreased by 65 and72% respectively, while overall control forboth treatment levels was 74%. Weeklyreleases of another*Toxorhynchites*larval predator,*Tx.amboinensis*, intoa 16-block neighbourhood with sub-standardhousing in New Orleans, Louisiana, during 1982 reduced*Ae.aegypti*densities by 45%when compared with similar but untreatedareas. Increasing thenumber of adults released per week from 100to 300 females per block did not improve thedegree of control achieved, indications thatlower release numbers may be adequate toachieve this level of control, whilst releasing100 female predators per block resulted in a40% reduction in*Cx. quinquefasciatus*(Focks *et al*., 1982). Collins and Blackwell (2000) reported thatother attempts to control vector mosquitoesusing*Toxorhynchites* spp. mosquitoes havebeen made in many regions of the worldincluding the Caribbeans, Asia and Africa.In one example, the larval density of *Ae. aegypti*were reduced by more than 90%after the release of *Tx.splendens*in water tanks in sub-urbanBangkok, Thailand (Focks *et al*., 1982). These results suggest that it

may be possibleto develop a practical method to control*Ae. aegypti*mosquitoes in urban areas using*Tx.amboinensis*(Focks *et al*., 1982).

The application of *Toxorhynchites* mosquitoes to control *Ae. aegypti* larvae in developi ng countries has two additionalbenefits. Firstly, mosquito has an unusual lifecycle in that they are not capable of bloodfeeding and therefore are not pests or vectors. Secondly, these mosquitoes couldbe reared locally instead of importinginsecticides. Although the aforementioneddata are examples of successful mosquitosuppression with*Toxorhynchites* mosquitoes, Annis*et al*. (1990)reported that this predator is unsuccessfulin field application in Indonesia. Repeatedrelease of first instar larvae of *Toxorhynchites* in waterlogged places had no effect onmosquito population in Indonesia due totheir inability to withstand periods of starvation and to their accidental removalfrom containers during the act of waterconsumption. However, a study conducted in Zikaforest, Uganda, on the breeding interactionsbetween*Ae.africanus*and two mosquito predators,*Tx. brevipalpis conradti*and*Tx. kaimosi*, revealed a significant reduction in the numbers of *Ae. africanus* larvae and pup ae in the tree holes that were also inhabited by predator larvae.

2.5.3 Hemipteran predators

Belostomatidae, Nepidae and Notonectidae are the most important families of predaceo us hemipteran bug. Thebackswimmers (Family: Notonectidae) arethe most common bugs preying uponmosquito larvae and are important inreducing immature mosquito population and considered promising in mosquito control. The role of hemipt eran predators incontrolling mosquito larvae wasrecognized as far back as 1939 in New Zealand, whenstock troughs with *Anisops assimilis*werefound to be free of mosquitoes whereas puddles in depressions surrounding thetroughs contained mosquitoes (Kumar

and Hwang, 2006). It was found that almost 100% of mosquito emergence was prevented in field-situated, screened, 100 gallonfibreglass tubs with one square meter of water surface containing *Notonecta unifasciata*compared to more than 12,000 adultmosquitoes that emerged from the control tubs.In another field experiment, in stock tanks (troughs holding drinking water for cattleand horses) in Santa Barbara County,California, *Notonecta hoffmani* wasshown to strongly influence mosquito larvae populations. The striking effects of these predaceous bugs,*Notonecta* and*Anisops* were probablydue to the physical simplicity of these troughs, tanks and tubs, and particularly tothe lack of prey refuges. For instance,emergent vegetation in natural ponds and otherwater bodies provide partial protection formosquito immatures. This effect wasexperimentally investigated and confirmedby Shaalan (2005) and Shaalan *et al*. (2007) whereby predation potentials of *Anisops* and *Diplonyc hus* bugs were significantly reducedby the presence of vegetation. Although the costs of colonization andmass production, coupled with the logisticsof distribution, handling and timing of release at the appropriate breeding sites,impede the use of notonectids in mosquitocontrol. Results of a recentstudy for mass rearing and egg release of the predatory backswimmer,*Buenoa scimitar* for the biological control of *Cx. quinquefasciatus*were impressive (Rodriguez-Castro *et al*., 2006).

2.5.4 Odonatan predators

Odonata nymphs are voracious and important predators of mosquito larvae in freshwaterecosystems. They detect their preys using theircompound eyes and mechanicalreceptorsand capture them with their labium.The dragonfly nymphs of *Trithemisannulata scorteci*were intense and active predators when used to control mosquitolarvae, especiallyof *An. pharoensis*, inirrigation channels in the Gezira Province, Sudan.Bay (1974) reported thatdragonfly nymphs preyed heavilyon bottom

feeder mosquitoes like *Aedes*larvae. Sebastian*et al*. (1980) found acomplete elimination of all*Ae. aegypti*larvaeand pupae between day 4 and 9 depending on the density of aquatic stagesof mosquitoes present per container whenthe nymph of the*Libellula* sp., were used. Thenymphal stages were found to last 2-3 monthsin the containers. These long lives, coupled with high predation rate are likely to makedragonfly nymphs highly successful predatorsthat could be used in biological control of *Aedes*mosquitoes. Again, Sebastian*et al*.(1990) conducted a pilot field study,involving periodic augmentative release of predaceous nymphs of the dragonfly,*Crocothemis servilia*, to suppress*Ae.aegypti*during the rainy season in Yangon, Myanmar. Four laboratory reared, three week old *Crocothermis servilia* larvae were placed in each maj or source of *Ae. aegypti* larvae immediately after the 3rd collection and then monthly fo r 3 successive months. The larval population of *Ae. aegypti*reduced to verylow levels in 2 to 3 weeks and suppressed itprogressively until the trial ended. The adultmosquito population was greatly reducedafter about 6 weeks and progressivelydiminished thereafter until the trial ended.Chatterjee*et al*. (2007) found that significantdecrease in*An. subpictus*larval density indipper samples was observed 15 days afterthe introduction of *Brachytron pratense* dragonfly larvae in concrete tanks under fi eld conditions in India.

2.6Predator-Mosquito Association by Habitat Types

Several ecological studies of predator-preyassociations involving mosquito larvae in different aquatic habitats have beendocumented. The following sections arereviewing this association besides factorsinfluencing it in different habitats(Shaalan *et al*., 2007).

2.6.1Temporary water bodies (Habitats)associations

Many predaceous insects were foundassociated with both nuisance and mosquito vectors in temporary habitats such as man-made ponds, snow melt pools, rain pools,flood water pools and other different pools.Larson and House (1991) studied the arthropodfauna of small, acidic pool over an ice-free season.Taxa varied in abundance between pools of various classes and two principlecommunities were identified. Oligochaetes(segmented worms with few setae), beetlesand mosquitoes dominated small, astatic pools and odonates, chironomids and severalother taxa predominated in large, stable, vegetated pools. Within the large pools,odonate larvae were the dominant predators.In a similar study, Nilsson and Svensson (1994)compared assemblages of dytiscid water-beetles and immature mosquitoes in twoboreal snowmelt pools that differed chieflyin temperature owing to difference inshading and duration. The total abundanceof dytiscids (including larvae) was similar inthe two pools, whereas species richness wasmore than twice as high in the warmer, lessephemeral pools. The mosquito fauna of both pools were strongly dominated by*Ae.communis*, whose initial numbers weresimilar in the two pools, however, 1stinstarlarvae suffered much higher mortality in thewarmer pools (Lozano*et al*. 1997).

Mogi*et al*. (1999) studied the mosquitolarvae and larvivorous predator communitieson lands deforested for rice fielddevelopmentin dry and wet area in centralSulawesi, Indonesia. Collected predatorswere from 3 insect orders (Odonata,Hemiptera and Coleoptera). In the dry area, Anisoptera larvae, notonectids and dytiscids were the dominant predators while in the wet area; dytiscids, zygopterans andanisopterans were the dominant predators. Surface predators all belonged to order Hemiptera and they wer e less abundant than aquatic predators. Munga *et al*. (2007) identified seven families (H ydrophilidae, Dytiscidae, Corixidae, Nepidae, Notonectidae, Belostomatidae and Corduliidae) of larvalmosquito predators from the larval habitats(drainage ditches, cow

hoofprints and disused goldmines) of the malaria vector*An.gambiaes.l.* in natural habitats in WesternKenya Highlands. Predator density in disusedgoldmines was significantly higher than that of other habitat types. Invertebrate predators were fou nd to associate larvae of the malaria vector *An. albimanus* in 78.6% of the body types h arbouring immature mosquitoes in a low lying area of Haiti. Fischer and Schweigmann (2008)found six mosquito species and 23 predatoryinsect taxa in temporary rain pools duringthe summer and fall season in Buenos Airescity. Both mosquito immature and predatorswere disproportionally more abundant in pools with high flooded surface, depth andduration.

2.6.2 Tree-hole associations

A variety of invertebrates utilize tree holesas breeding sites. Because they are primarybreeding sites for many disease vectors, particularly mosquitoes and biting midges,tree holes are an economically importanthabitat. Although*Toxorhynchites*mosquitoes are well knownas tree-hole mosquito predators, severalother predaceous insects are important tree hole occupants Collins and Blackwell (2000). Commonly recorded preda ceous insects in tree holes are dragon flies, damselflies and a new genus of water bug in the family Vellidae. Tree holes are not the primaryhabitat for odonates. Out of approximately 6000 species, only 47 have been reportedfrom this habitat, at least 64% being Zygoptera. Anisopteran and zygopteran larvae inhabiting tree holes were recorde d in forests of different geographical regions. Orr (1994) reported that *Pericnemis trian gularis* (Coenagrionidae) and *Indaeschna grubaueri* (Aeshnidae) were breeding in phytotelmata in the understorey of lowlandmixed dipterocarp rainforest in Borneo.Larvaeoccurred more often in tree holes of largersurface area and gape size. These attributescorrelated positively with median water volume (0.1 - 42L) and height

above theforest floor (up to 22.45m) (Symondson, 2002). Larvae ofchironomidae and culicidae predominatednumerically among prey of odonate larvae,with smaller larvae preying more on theformer and larger ones on the latter. Twoother insect predators were encountered intree holes:*Toxorhynchites*sp. and a newgenus of the water bugs of family Veliidae(order: Hemiptera). Veliids were found in11.2% and*Toxorhynchites*sp. in 41% of treehole samples for which their presenceor absence was noted. Neither taxon wasassociated negatively or positively withthe occurrence of odonates. Louton *et al*. (1996) surveyed the aquatic macrofauna of water filled internod es of Guadua bamboo in a lowland tropical forest in Peru. They found a community of 29 species dominated by Diptera and Odonata. The predaceous insects comprised 4 da mselflies (*Mecistogaster jocaste, Mecistogaster linearis,* unknown *Mecistogaster* speci es and *Microstigma rotundatum*) and Dipterous larvae includingfamily Ceratopogonidae (subfamily: Ceratopogoninae) and family Culicidae(*Toxorhynchites*sp. and the facultative predators*Sabethes*spp. A and B, and*Trichoprosopon pallidiventer* and *Trichoprosopon* sp.). Besides the predaceous mos quito, *Toxorhynchites* theobaldi, larvae of 5 common species of Odonata (*Gynacantha membranalis, Triacanthagyna dentate, Microcystin linearis,Mecistogaster ornata*and*Megaloprepuscoerulatus*) were collected from water-filledtree holes in a lowland forest in Panama(Service,1973).

2.7Predators' Influence on Mosquito Oviposition, Development, Survival, Abundance and Fitness

Research findings indicating that thedevelopment, survival and abundance of larval mosquito populations in the field arelimited by predaceous insects which are primarily responsible for mortality inimmature stages of mosquitoes. This effecthas been reported in many different aquatichabitats and is responsible for restraining thedensity of such

prey populations below thecritical threshold where transmission of diseases could not occur(Shaalan *et al*., 2007).

2.7.1Predators' influence on mosquito oviposition

Animals take risk of predation into accountwhen making decisions about how to behavein particular situations. Chemosensory cuesare important and are used to detect the presence of predators or even their presencein the immediate past, and may also provideinformation on predator activity level anddiet (Kats and Dill, 1998). In their review articlefor chemical detection of natural enemies byarthropods, Dicke and Grostal (2001) reportedthat of all chemical information gathered byanimals, cues about predation risk usuallyhas important and immediate consequencesfor the future fitness of animals and theyresult in various responses strategiestowards such predators including avoidance.For all mosquito species, location andselection of an oviposition site is essentiallife-cycle behaviour and involves visual,olfactory and tactile responses. Oviposition is an importantcomponent of most mosquito borne diseasesbecause pathogen acquisition by mosquito vectors usually requires taking of at least oneblood meal and disease transmission usuallyrequires the completion of at least oneoviposition cycle before pathogen transfercan occur with subsequent blood meal.

It was believed for a long period that predator-prey interaction was largelyattributed to predation mechanisms untilChesson (1984) showed that the effect of notonectid bugs on mosquito larvae is mainlydue to selective oviposition by gravidmosquitoes. He manipulated the density of aquatic predaceous bugs, (*N. hoffmani*and*N.kirbyi*) in stock troughs to assess the predator's effect on mosquito larvae. Over athree-month sampling period very few largemosquito larvae or pupae were collectedfrom the side of the trough wherenotonectids were located, whereas largedensities were collected from the side freefrom notonectids. To ensure that this was notan artifact of the side of the

trough chosenfor notonectid addition or removal, the predators were moved from one side to theother and same results were obtained. It wasthought that these experimental resultscould be explained by selective mosquitooviposition. This hypothesis was supportedby laboratory experiments in which femalemosquitoes laid the fewest egg rafts in tubescontaining the predaceous notonectids.Moreover, laboratory and field experimentsalso demonstrated that notonectids maydisrupt mosquito egg rafts, but no evidenceof a reduction in subsequent hatchingsuccess was obtained. This means that the predator does not feed on the mosquito egg-rafts and confirms the selective ovipositionhypothesis. Other predators such asdragonfly larvae, however, consume eggrafts. Likely, later studies have investigatedovipositional responses of the mosquito*Cs. longiareolata*to some insect predators. Stav*et al*. (1999) reported that the predaceous dragonfly larvae of *Anax imperator* produced 52% reduction in *Cs. longiareolata* oviposition in outdoor artificial pools. The reduced num ber of *Cs.longiareolata*egg rafts found in the presenceof *Anax imperator* was largely due tooviposition habitat selection by*Cs. longiareolata*females. Larvae of *Cs. longiareolata*were highly vulnerable topredation compared to*Cx. laticinctus*andwere also the only dipteran species thatavoided*Anisops*pools when ovipositing. Stav*et al*. (2000) foundthat the egg rafts of the mosquito*Cs. longiareolata*deposited in the free*Anax*treatment were fewer than deposited incaged*Anax*and control treatments. Therewas no statistically significant difference in the number of egg rafts between control andcaged*Anax*pools which means that, while*Culiseta* females oviposit fewer egg rafts inthe presence of *Anax*, they did not respondto predation risk from the caged*Anax*. Ingeneral, this individual response could have population-level consequences. Forinstance, it may increase the equilibrium sizeof the*Cs. longiareolata*population relativeto the population in which oviposition isdiscriminative

with respect to *Notonecta maculata*(Spencer*et al*., 2002). Furthermore, females of the malariamosquito vector*An. gambiae s.l*laidsignificantly fewer eggs in rainwaterconditioned with the predatorybackswimmer, *Notonecta sp*. than inconditioned rainwater, indicating that predators influence selection of ovipositionsite by this malaria mosquito vector. More interestingly, females of the malaria mosquito vector*An.gambiaes.l.* tend to avoid oviposition sites containingolder instar larvae of the*An.gambiae s.l.* The reason was discoveredlater on to be avoidance of offspring predation by older instar larvae.

These previously mentioned studiesshowed that notonectid bugs and dragonflylarva, *Anax imperator* affect oviposition habitatselection in some mosquito species at astable density of these predators. However,the relationship between predator densityand mosquito oviposition response was notstudied until Eitam and Blaustein (2004) tested the oviposition response of 2 mosquitospecies,*Cs. longiareolata*and*Cx.laticinctus*, to a range of the predator*N.maculata*in artificial pools. Both mosquitospecies oviposited less in predator pools butthe response was not related to the predatordensity, whereas vulnerability of *Culiseta*immatures to predation was densitydependent. So, although mosquitoes candetect the predator at any density, they maybe unable to discriminate predator density.The vulnerability of *Culiseta*to predationcould thus be due to mitigating effects of thebiotic community inside the pools. Similarly,effects of pool depth combined with risk of predation on oviposition habitat selection by*Cs. longiareolata*were studied(Arav and Blaustein, 2006). Results indicatedthat although*N. maculata*affectedoviposition pattern of this mosquito, pooldepth did not affect oviposition habitatselection for this mosquito.All these studies have not assessed themode of detection of predators untilBlaustein*et al*. (1996) demonstrated andconfirmed that the cue for ovipositionavoidance of *Cs.*

*longiareolata*to *N. maculata*was a predator-released chemical(kairomone). Consequently, thismode of detection is an advantage for predators and it is very important from themosquito control point of view whereas suchkairomones could be producedcommercially for mosquito control.Furthermore, oviposition habitat selection in*Cs.longiareolata*is an adaptive response tothe trade-off between the risk of predationand negative density-dependent effects(Spencer*et al*., 2002) whilst findings of Kiflawi*et al*. (2003) suggest that it is drivenby a mixed strategy, played by all females,whereas all females follow a single, simplebehavioural 'decision rule' that is responsiblefor the lack of complete predator avoidance.Mosquitoes may detect predators cues eitherfrom the air, when the cues possessessufficient volatility, or by a gustatorymechanism involving direct contact with thewater, when the cues possess low volatility. Silberbush and Blaustein(2008) tested whether*Cs.longiareolata*candetect the chemical cues from*N. maculata*,without touching the water.*Cs.longiareolata*oviposited significantly morein the central pools surrounded by channelscontaining control water than in poolssurrounded by*Notonecta*conditioned waterchannel (56 of 81 egg rafts (69%) wereoviposited in the control pools) indicatingthat gravid*Cs.longiareolata*femalesdetected predators' cues from the air whichmeans that predator-released cues(kairomones) are air-borne cues. The predators' cues not only affectingmosquitos' oviposition but also alter their lifecycle traits. Resultsof their experiments showed that chemicalcues from the predator*N. glauca*fed with prey's (*Cx. pipiens*) conspecifics caused adecrease survival, delayed immaturesdevelopment and reduction in body size of emerged mosquitoes while chemical cuesfrom predators fed with *Daphnia magna*(acrustacean invertebrate animal) producedonly delayed development. In summary, selection of oviposition siteby female mosquitoes depends more on the presence of predators and less on predatordensity. Furthermore, predator

density, asindicated by the concentration of theirkairomones, could affect the ovipositiondeterrent potential and would be animportant consideration in utilizing either predators or their kairomones for biologicalcontrol of mosquitoes.

2.7.2Predators' influence on mosquito's development, survival, abundance

Unlike*Culex*, information about roleof predators on development, survival andabundance of *Aedes*and*Anopheles*mosquitoes is limited.In some cases, variations in predationeffects are due to difference in predatorspecies breeding with the mosquito species.For instance, role of predators on thedevelopment and survival of immaturestages of *Cx. annulirostris* in different regions in Australia was variable.Mottr am and Kettle (1997) found that predatordensities in three surveyed sites weresignificantly different, being lowest in thetemporary pools (0.32 %) and highest in theflooded grassland (1.76 %).The mortalitycalculations suggested that predators killed69.1%, 68.7% and 43.2% of immature*Cx. annulirostris*in the flooded grassland, semi- permanent pool and temporary pool,respectively. For the same mosquito speciesat the Ross River dam in Townsville, North Queensland, Australia, the invertebrate predators, dominated by dytiscids and dragonfly larvae, reduced larval survival by 58%. In other cases this variation is attributed to both mosquito species and different predators. The most obvious example is thefield experiment that has been conducted by Lundkvist*et al*. (2003) in artificial ponds overtwo successive years to determine how population levels of mosquito larvae are affected by predaceous diving beetles (Dytiscidae). Mosq uitoes that colonized the ponds were predominantly species of the genus*Culex*. In 2000, most of the dytiscids that had colonized the ponds were small(*Hydroporus*spp.) and had no impact on the size of larval mosquito populations. Conversely, in 2001, larger beetles (*Ilybius*,*Rhantus* and*Agabus*spp.) were more common and mosquito larvae

were significantly fewer in ponds with high numbers of dytiscids.A recent study conducted by Das*et al*. (2006) mentioned that breakdown of predator populations was responsible for the sudden increases in vector populations above the threshold for disease transmission during heavy rainy periods. In rice fields,notonectid predators exhibited a significant positive correlation with *Cx. vishnui* larvae. Predation by predaceous midge larvae, *Pentaneura* sp., produced low densities of mosquito larva e found in the water field bracts of *Heliconia imbricate*. This predation affected 2 mosq uito species*Wyeomyia pseudopecten*, a resident species, and *Trichoprosopon digitatum*, a non resident species. Predation kept resident mosquito densities low while completel y excluded the non-resident mosquito from the bracts.

2.7.3 Predators influence on mosquitos' fitness

The influence of predators on mosquito fitness was first reported by Louniboset *al*.(1993) when they investigated the influences of food type and predation on fitness of thetree-hole mosquito*Ae.triseriatus*. Results indicated that the presence of *Tx.rutilus*significantly affected the fitness of *Ae.triseriatus*to a greater degree than foodtype. Survivorship of immature stages in cohorts with predator access was very lowwhile the mean of P50(time to 50% pupation)was significantly greater than cohorts with detritus. The female size in cohorts with the predaceous mosquito, particularly wing size,was unexpectedly smaller than other cohorts with or without food access. It was suggestedto be due to the fact that the presence of *Tx. rutilus*may reduce movement of larval *Ae.triseriatus*, thereby decreasing food intake and size at metamorphosis. Additionally,Dicke and Grostal (2001), in their review for chemical detection of natural enemies by arthropods, reported that predation risk usually has important and immediate consequences on prey fitness. Lundkvist*etal*. (2003) found a negative correlation between the number of diving beetles in artificial ponds and the mean body

length of mosquito larvae that has seriousconsequences on the fitness of emergingfemales. More interestingly, recent study (Diabaté*et al.*, 2008) indicated that predaceous insects influencing the divergent selection amongst the molecular forms of the malaria vector*An. gambiaes.s.* Prediction increased the developmental success of larvae of M form over the S form in both puddles and rice fields. Higher density of predators belonging to Notonectidae (*Anisops*sp. and *Anithares*sp.) and Dytiscidae (*Laccophilus*sp.)families increased the relative success of theM form whilst higher density of Libellulidae (*Tramea* sp.) and Hydrophilidae (*Berosus*sp.) specimens appeared to decrease the relative success of the M form, but their effects were not significant.

2.7.4Factors influencing capacity of predaceous insects

These factors are classified into biological and physical. Unfortunately, the literatures have shown that investigations for factors influencing predation capacity of aquatic beetles are limited compared to the other predaceous insects (Diptera, Hemipter a and Odonata). Increased predation of one mosquito prey species over another by a pre daceous insect does not always mean a real preference since it could be due to differences in their means of evaluating predation risk. The behaviour of *Ae. aegypti*larvae towards the predator*N.undulata*wasa response to disturbance per se, whilst the*Cx. pipiens*response was mediated by chemical cues that may have involved a combination of notonectid digestive enzymes and partially digested mosquito materials, associated with the actual predation act. So, because*Cx.pipiens*hasan evolutionary history of contact with notonectids, it suffered a lower predation rate from*Notonecta*than did the*Ae. aegypti*that lacking this evolutionary behaviour. Consequently, the reduced predation ratecould be explained as*Cx.pipiens*showedboth stronger and more precise antipredatorresponses

than*Ae.aegypti*. Further to this,the findings of Husbands (1978) imply that prey behaviour could influence its persistence in the mosquito larvae-notonectid system. Hefound that notonectid predators quicklydestroyed*Ae. nigromaculis*compared to*Cx. tarsalis*due to the former showinglittle reduction in their movement or shift intheir habitat use but, in contrast, the lattershifting to feed quickly among emergent vegetation. Grill and Juliano (1996) alsosuggested that in some systems, preybehaviour patterns are more related to vulnerability to predation. In furtherconfirmation of this hypothesis,*Ae. albopictus*did not respond to cues producedby*Tx.rutilus*and was more vulnerable to predation than*O.triseriatus*. Collins and Resh (1985) stated otherfactors influencing the capacity of damselflies at Coyote Hills Marsh, Fremont, CA. The dams elfly micro-distribution, age-specific feeding habits, phenology, and thearchitecture of the habitat that supports thelarvae were anticipated to reduce the predaceous capacity of *Enallagma civile, E. carunculatum* and*Ischnura cervula*against *An. occidentalis*.Lee (1967) found that mosquito larvae are consumed more than pupae by predatorsand assumed that this was due to theinclination of pupae to exhibit rapid tumblingaction when startled. Contrarily, both bugsof family belostomatidae (Order: Hemiptera)and*Toxorhynchites* mosquito larvae have anadvantage over the other aquatic predaceousinsects that restrict their prey selection tothe larval instar only (Shaalan *et al*., 2007).

2.8 Interactions between Mosquito Larvae and Species that Share the same TrophicLevels

Ecologists strive to understand how variousfactors, including abiotic conditions and interspecificinteractions, contribute to distributionand abundance of a particular speciesof interest (hereafter called target species).Decades of modeling, observational

studiesand experimental approaches have led ecologiststo conclude that species sharing the sametrophic level (hereafter called controphicspecies) often play important but highly diverseroles in affecting each other. Traditionally, interactions amongcontrophic species were viewed as a simple,mutually negative interaction either directlythrough interference or indirectly throughexploitation of shared resources.Understanding such mutually negative interactionsamong competing species remains acornerstone of community ecology.More recently, community ecologists have recognized the way in which controphicspecies can indirectly interact with one anotherwhen embedded in complex communities.Apparent competition occurs when two (or more) prey species share a commonpredator, and the predator numbers are limitedby prey availability. By presenting itself asan additional food resource, the second preyspecies allows the abundance of the predatorto increase and, as a result, reduces thedensity of the target species. A similar mechanismis the case of competition for enemy-freespace, in which prey species are confinedto using certain resources (e.g., refuges, foodtypes) in the presence of predators. If morethan one prey species is present, and both utilizethose enemy-free resources, the speciescan limit each others' numbers.An apparent mutualism between preyspecies that share a common predator canarise if predator populations are limited byfactors other than prey availability (as is thecase for apparent competition). In thiscase, the presence of a controphic prey speciessatiates the predator, thus allowing a relief ofpredation pressure on the target species; thatis, the controphic species indirectly benefitsthe target species.When the enemy of the target species is a parasite or pathogen, a mechanism similarto apparent competition arises if thecontrophic species, which are also hosts tothe parasite, are equally susceptible to thatpathogen/parasite. In this case, the presenceof the second controphic species cancause the target species to have higher infectionprevalence(Blaustein *et al*., 1996).

The controphic species can share bothpredators and resources. Here, the impact ofthe controphic species on the target speciesdepends on a balance between competition forresources and apparent competition throughshared predators. The presence ofa controphic species and predator can benefitthe target species if the former is moresusceptible to predation, but harm the targetspecies if the former is less susceptible topredation.The above six mechanisms, as well as many other more specificcases, illustrate the rich diversity of effects,other than interspecific competition,that controphic species can have on one anotherwhen they are embedded in a complexfood web. The question of how controphicspecies affect a target species enters the realmof applied ecology when the target species inquestion is considered a nuisance or pest or a rare endangered species.Numerous studies document that species sharing the same trophic level of agriculturalpest species indirectly affect pests via sharedpredators, parasitoids, and/or parasites. In fact, traditional ideas of preferable traits for natural or introducedbiological control agents in agriculturalsystems included generalist predators whosepopulation sizes would increase when the pestspecies was not abundant by feeding on alternativeprey.Surprisingly, the role of controphic species as an important component affecting anotherimportant group of pests, mosquitoes(Diptera: Culicidae), remains largely unexplored,even though controphic species ofmosquitoes likely have a strong impact onmosquito populations and may more likelybe an effective management tool for controllingmosquito populations than for controllingagricultural pests for at least two reasons.First, controphic species of agriculturalpests are generally pests themselves (or at best,weaker pests, whereas a wide array ofcontrophic species of mosquito larvae are notpests. Second, controphic species of mosquitolarvae are often orders of magnitude moreabundant than mosquito larvae, andthus they should have a greater effect via competitionand mediating predator effects.Traditionally, scientists

with backgrounds in medicine, and not community ecology,studied mosquito biology, and they understoodthat controlling mosquito populationswas necessary for human health (particularlyin tropical regions). As a result, eventhough conceptual community ecology hasbeen used in various applied fields for decades(e.g., biological control); the applicationof basic community ecological principlesto mosquito ecology and control has lagged.However, the basic principles of communityecology should apply to mosquitoes, particularlyto their larval habitats, where interactionswith resources, predators, pathogens,and controphic species can be important.Mosquito larvae develop in lentic habitats ranging in size from small, water-filledaxils of plants to vast wetlands, and fromhighly ephemeral to permanent. Becausesmall, lentic habitats are convenient systemsfor testing ecological theory,considerable information is known generallyabout how different components of these systemsaffect populations and community structure.This knowledge can be applied to predictinghow controphic species might affectmosquitoes under varying conditions and how controphic species might be manipulated to reduce the success of mosquitoes (Blaustein *et al*., 1996).

2.8.1 Interference and exploitation competition

Interference competition (a direct physical orchemical negative interaction) and exploitativecompetition (indirect negative interactionby depletion of resources) are importantforces affecting abundance and distributionof herbivorous and detritivorous species inlentic habitats(Wilbur,1997). It has been argued thatthe role of competition in ephemeral aquatichabitats is a function of hydroperiod length. This scenario suggeststhat competition (particularly exploitativecompetition) is less likely to be importantin habitats of short hydroperiod or in the earlyperiods of longer-lived ephemeral habitats becausedensities of competitors are unlikely tobe high and

resources are unlikely to be reduced.Competition should increase duringthe middle period of long-lived habitats becausedensities of competitors are likely to bemuch higher at the end period of moderatehydroperiod habitats. Moreover, risk of desiccationshould not supplant the importance ofcompetition but exacerbate it, because competitionworks to increase the time it takesfor an individual to reach metamorphosis andemerge from the aquatic habitat into adulthood, enhancing the risk of desiccationfrom habitat drying.Depending on the species, mosquito larvaemay filter feed particulate matter such asphytoplankton, microorganisms, and detritus;they may graze on periphyton; or, in fewercases, they themselves may be facultative orobligate predators.

2.8.2 Apparent competition and apparent mutualism with shared predators

Strict apparent competition and apparent mutualismscenarios require that the target andcontrophic species share predators but not food. Although we are unawareof any studies on mosquitoes and their controphicspecies that show strict apparent competitiveor mutualistic scenarios, because theylikely also share food, there are some possiblecases in which such indirect effects might exist.For example, mobile generalist predators,such as fish, may forage in different habitattypes, such that a controphic species from onehabitat type could influence the density of fishin another habitat type by serving as preyand, as a result, influence the predation rateon mosquitoes in the second habitat. If themobile generalist predator increases its density,this could result in an apparent competitionscenario, whereas if the mobile generalistpredator species spends more time consumingthe controphic species, this could result in anapparent mutualism scenario(Blaustein *et al.*, 1996).

2.8.3 Intraguild predation

Mosquitoes and controphic species may alsobe intraguild predators. In rock pools in theNegev Desert (Israel), in addition to consumingthe same resources and competing withone another, *Cx. longiareolata* larvae and toad(*B. viridis*) tadpoles mutually prey upon eachother; which species is predator and whichis prey depends primarily on the relative size of the individuals. Individual size, inturn, is determined primarily by which speciescolonizes the pool first. Thus, the presence ofthe controphic species (toad tadpoles in thiscase) can either benefit or inhibit the mosquitospecies.Some mosquito larvae such as *An.quadrimaculatus*may prey upon the early stages ofcladocerans. Mosquito larvae commonlyprey on protozoans, often having a large impact, but can also compete withthem for bacterial resources. Thus, itis possible that protozoans could either benefitor harm mosquito populations, dependingon the balance between their effects asintraguild prey and as competitors. *Ae.sierrensis*,a treehole mosquito, and the protozoan*Lambornella clarki* represent a uniqueintraguild predation/parasitism relationship. The mosquito preys on the free-livingciliate. However, the presence of the mosquitoinduces the transformation of the protozoainto an obligate parasite of the mosquito.A complex scenario that is not strict intraguild predation, but shares some similarities,is when entomopathic bacteria such as*Bacillus thuringiensis var. israelensis* (*Bti*) areapplied for mosquito control. Mosquitolarvae are killed when they feed on sufficient*Bti* spores. *Bti* does not replicate insidethe gut of the mosquito larva, but the sporecontains protein crystals that are toxic to themosquito larvae. *Bti* spores do not stay in suspensionfor long periods, but they quickly adsorbto the sediment. Consequently, althoughthe spores are still potentially pathogenic tomosquito larvae if consumed, filter-feedingmosquito larvae no longer can efficiently consumethe spores adhering to the substrate.However, some protozoa, such as *Tetrahymena*,feed in both the sediment and watercolumn. Laboratory studies suggest that the*Tetrahymena*

consume the *Bti* cells in the sedimentand accumulate the toxic protein crystals.They themselves are unaffected by the*Bti* protein crystals. The *Tetrahymena* thencan deliver the *Bti*when they enter the watercolumn and are ingested by the mosquito larvae. How effective such protozoansmay be in increasing efficacy of *Bti* underfield conditions remains to be tested.Another similar but more complicated scenario is the case in which controphic speciesindirectly influence mosquitoes by consumingpossibly harmful bacterial pathogens such as*Bti*. Whereas *Bti* is toxic to mosquito larvaewhen consumed, most other controphicspecies, such as crustaceans, that ingest thebacteria do not incur mortality. Itis then possible that those controphic speciesthat are not intraguild prey of the mosquitolarvae, through their consumption, reducethe amount of the pathogenic bacteria availablefor consumption by mosquito larvae. Inthis case, the net effect of the controphicspecies on mosquitoes depends on the balancebetween the positive respite they receivefrom the pathogenic bacteria and thenegative impact of the controphic species onthe mosquito's other food resources (Blaustein *et al*., 1996).

2.8.4 Indirect mutualism

Indirect mutualisms arise when controphicspecies benefit each other by consuming differentparts of the resource base, which themselvescompete for limiting resources. For example,in pond ecosystems, periphyton andphytoplankton compete for nutrients, andblooms of phytoplankton can reduce UV radiationnecessary for periphyton photosynthesis. An herbivore that consumes one group ofalgae (periphyton or phytoplankton) is likelyto confer an advantage to the second group ofalgae both by reducing the competitive abilityof the algae and by recycling nutrients. Thus,we might expect efficient periphyton grazers such as tadpoles and snails to indirectly positivelyaffect filter-feeding mosquitoes such asmost *Anopheles* and *Culex* species. Likewise,we might expect that efficient phytoplanktonfilter-feeders such as *Daphnia*

would positivelyaffect mosquito larvae that are primarilyperiphyton grazers such as some *Aedes* and*Culiseta*. Wemight expect that *Cx. longiareolata*, thought tobe primarily a periphyton grazer, mightdo better in the presence of this zooplanktonassemblage than in its absence; this was not thecase, and instead, there was a small reductionin survival. However, although *Culiseta* isprimarily a grazer, it can also filter-feed onphytoplankton. Moreover, increases inperiphyton may not necessarily translate intoincreases of highly edible periphyton.This indirect mutualism outlined above, although an interesting indirect effect thatcould arise between controphic species, maynot be commonly important for mosquitoesin nature. This is because many mosquitospecies both graze and filter-feed and manynatural controphic species assemblages haveboth filter-feeders and grazers. One exampleof an exception may be rock pools in intermittentriver beds of the Negev Desert. Here,filter-feeding zooplanktons are not common,and grazing anuran tadpoles can be abundant.High densities of *B. viridis* tadpoles stronglydepress the periphyton-grazing *Culiseta* larvae, whereas filter-feeding mosquitoessuch as *Culex* and *Anopheles* may instead benefitfrom the presence of controphic toad tadpoles (Blaustein *et al*., 1996).

2.8.5 Competition for resources and shared predators

This more complex module includes fourplayers, the mosquito and the controphicspecies, both of which share common resources and common predators. Ifthe predator's negative effect on mosquito larvaeis outweighed by its reducing competitionby controphic species, then the predator canbe thought of as a keystone predator,allowing mosquitoes with controphic speciesto do better in the presence of predators. Conversely,if the controphic species serve as alternativeprey as well as potential competitorsand thus reduce the negative effect of predationon mosquito larvae, then the net effect ofthe controphic species on mosquito larvae ispositive.

Blaustein and Karban (1990)performed a study comparing the developmentrates of *Cx. tarsalis* mosquito larvaein predator-free cages placed withinflooded rice plots that either did or did not have predatory mosquitofish (*Gambusia affinis*). They found that mosquitofish a ctually benefited the mosquito larvae. In the presenceof mosquitofish, the mosquito larvaedeveloped faster and more survived to pupation,most likely because mosquitofish drasticallyreduced filter feeding zooplankton densities,which in turn were likely stronglarval mosquito competitors.

No keystone predator effect was observedfor the survival or growth rates of *An. quadrimaculatus*larvae in experimental mesocosmswith zooplankton competitors and predatorybackswimmers (*Notonecta undulata*). Thisresult occurred presumably because the overallnet effect of predators on mosquitoes wasso strong that the concurrent reduction ofcontrophic zooplankton could not provide arespite from this intense predation. Finally,both competitors(a zooplankton assemblage) and predators(larvae of the dragonfly *Anax imperator*),when alone, reduced the success of *Cx. pipiens*,whereas when both predator and competingcontrophic species were together, therewas neither net advantage nor disadvantageconferred to the mosquito. In this case, thepredator did not reduce zooplankton densities,and thus it appears as if the negativeeffect of competition and the positive effectof reduced predation intensity affordedby the alternative prey cancelled each otherout.

2.8.6 Effect of productivity on mosquito interactions in food webs

From theoretical arguments, the impact of predatorson mosquitoes and controphic species maybe expected to vary along a gradient of primaryproductivity (e.g., nutrient inputs). Ina simple keystone predator module, in whichcompetitive ability and predator

vulnerabilityare inversely related, increasing productivitywill change the outcomes of competitiveinteractions among the controphic species.If the larval mosquito is the superior competitor,but is more susceptible to predators,controphic species will be outcompetedat low productivity, can coexist and negativelyimpact mosquitoes at intermediate productivity,and eliminate mosquitoes throughapparent competition at high productivity.Alternatively, if mosquitoes are inferior competitorsbut less susceptible to predators, theopposite results are expected along a productivitygradient. This scenario would be predictedprovided that predators show a strongnumerical response to variation in productivity.Moreover, the predicted outcomes of theseinteractions along the productivity gradientassume a positive linkage between mosquitosurvival/emergence in a given water body andoviposition rates. Although such effects havenot been examined in mosquito controphicspecies systems, they have been observed inother parts of the aquatic food web(Blaustein *et al*., 1996).

2.8.7Role of oviposition in habitat selection

Much of what we have considered thus far hasbeen the effect, or predicted effect, of controphicspecies at the level of a single pool.However, a mosquito population is not confinedto a single pool. Instead, a mosquitopopulation exists in the adult stage over awider terrestrial area and includes a number of larval patches (i.e., water bodies). Ther e are increasing numbers of studies that showthat mosquito females consider controphicdensities or larval food resource levels when ovipositing. Thus, by choosingthe best quality sites (here, low conspecificdensities), oviposition habitat selection inresponse to conspecific densities should mitigateregionally the overall effect of controphicspecies. This needs to be considered with allother factors that might influence ovipositionhabitat selection such as risk of predation. Further study may show that the ovipositionresponse to controphic densities may notbe linear, as observed in a

study of ovipositionresponse to conspecific density, orthat high food resource levels supersede highconspecific densities. Mosquitoes may preferto oviposit where controphic densities arepresent if food resource levels are adequateto reduce predation intensity on mosquitolarvae (Blaustein *et al*., 1996).

2.9 Ecological Relevance of Physico-chemical Parameters of Water

Mosquitoes exploit almost all types of aquatic habitats for breeding. Prevailing physicochemical parameters in these habitats are important factors for survival and development of mosquito (Oyewole *et al*., 2009).Many species of mosquitoes are very specific in their requirement ofphysico-chemical characteristic of their breeding waters.Mosquitoes use chemical and biological cues to detect the presence of larval competitors and avoid ovipositing in such habitats.Larvae of mosquitoes in clear water of suitable pH, temperature and nutrientconditions have been found to thrive in aquatic bodies such as fresh composition (Tiimub*et al*., 2012). Various chemical properties of the larval habitat observed in gutters,peri domestic runoff are related to vegetation and a wide range of heavy metal, nutrients andphysicochemical characteristics of the water, ranging from pH, optimum temperature, total suspended solids, totaldissolved solids, electrical conductivity etc. have been found to affect larval development and survival (Mutero *et al.,* 2004).Diagnostic and scientific research have shown that many mosquito species prefer habitats without oxygen tensioncausing physical harm to the larvae with vegetation whilst some breed in open, sunlight pools.Some important physical andchemical parameters influencing the aquatic environment include temperature, rainfall, pH, salinity, dissolved oxygenand carbon dioxide. Others are total suspended anddissolved solids, total alkalinity and acidity, biological oxygen demand, chemical oxygen demand, nitrate, phosphate etc. Mosquito species differin the type of

aquatic habitats they prefer for oviposition based on location, physicochemicalcondition of the water body and the presence of potential predators.

2.9.1 Water temperature

Temperature is a limiting factor in the aquaticenvironment.Water temperature is probably themost important environmental variable. It affectsmetabolic activities, growth, feeding, reproduction,distribution and migratory behaviours of aquaticorganisms. It affects solubility of gasses in water,which decreases with increased temperature.Temperature is affected by time of the day; hightemperatures may be recorded in daytime and become low at night (Lawson, 2011).Aquatic organisms, from microscopic microbes to fish, are dependent on specific temperature ranges for their optimum health and survival. If temperatures are outside the optimal range for a prolonged period, organisms are stressed and can die.Macroinvertibrates sp (for example, insects, crayfish, worms, clams, and snails) will move about in the stream bed to find their optimal temperature. The water temperatureis of enormous significance as it regulates various abiotic characteristics and biotic activities of an aquatic ecosystem. Temperature is measured in degrees Fahrenheit (180° between the freezing and boiling point of water) or degrees Celsius (100° between the freezing and boiling point of water). Many of the physical, biological, and chemical characteristics of surface water are dependent on temperature(optimum temperature most rapid for larval and pupal development is 31°C) (Bellingham, 2013).

2.9.2pH

Hydrogen ion concentration or pH as one of the vitalenvironmental characteristics decides the survival,metabolism, physiology and growth of aquatic organisms.pH describes the acidity or alkalinity of water and represents the balance between hydrogen

ions (H^+) and hydroxide ions (OH^-) in water. The pH is also a useful indicator of the chemical balance in water. A high or low pH will adversely affect the availability of certain chemicals or nutrients in the water for use by plants. Ramanathan *et al*. (2005) recommended optimum range of pH6.8-8.7 for maximum growth and production of shrimp andcarp. pH is influenced by acidity of the bottom sedimentand biological activities. High pH may result from highrate of photosynthesis by dense phytoplankton blooms.pH higher than 7 but lower than 8.5 according to Abowei(2010) is ideal for biological productivity, but pH at <4 isdetrimental to aquatic life. pH may be affected by totalalkalinity and acidity, run off from surrounding rocks and water discharges (Lawson, 2011). Lower values in pH are indicative of high acidity, which can be caused by the deposition of acid forming substances in precipitation. A high organic content will tend to decrease the pH because of the carbonate chemistry. As microorganisms break down organic material, the by product will be CO_2 that will dissolve and equilibrate with the water forming carbonic acid (H_2CO_3). Other organic acids such as humic and fluvic acid can also result from organic decomposition (Bellingham, 2013).The pH of a water body is a diurnally variable property according to temperature variation in the system. When pH is within the range of 5.5 to 8.5 a balance in an ecosystem is maintained. Increased surface pH in water bodies is due to increased metabolic activities of autotrophs, because in general they utilize the CO_2 and liberate O_2 thus reducing H^+ ion concentration. Moreover, in the bottom of water body's liberation of acids from decomposing organic matter under low O_2 concentration results in low pH. In natural waters pH also changes diurnally and seasonally due to variation in photosynthetic activity which increases the pH due to consumption of carbon dioxide in the process. The pH of water also depends upon relative contents of free CO_2, carbonates, bicarbonates and calcium. The water tends to

be more alkaline when it possesses carbonates, but less alkaline when it supports more bicarbonates, free CO_2 and calcium(Bellingham, 2013).

2.9.3Dissolved oxygen

Dissolved oxygen (DO) refers to the amount of oxygen (O_2) dissolved in water.Dissolved oxygen affects the solubility of andavailability of nutrients. Its low levels can result indamages to oxidation state of substances from theoxidized to the reduced form thereby increasing the levelsof toxic metabolites (Bellingham, 2013).Dissolved carbon dioxide in aquaticenvironment increases with decreased dissolved oxygen. It is important parameter in primary production andphytoplankton biomass and representsa critical indicator of a water body's ability to support healthy ecosystems. The DO reflects the water quality status and physical and biological processes in waters and shows the metabolic balance of a water body. Water acidity increases withincreased dissolved carbon dioxide. High rate ofdissolved carbon dioxide is detrimental to survival, physiology and metabolic activities of aquatic animals (Lawson, 2011). Level s above 5 mg/L are considered optimal, and most fish cannot survive for prolonged periods at levels below 3 mg/L. Microbial communities in water use oxygen to breakdown organic materials, such as manure, sewage and decomposing algae. Low levels of dissolved oxygen can be a sign that too much organic material is in a water body. In nature, oxygen from the atmosphere can be mixed into (diffused into) a body of water (Bellingham, 2013). Oxygen tends to be less soluble as temperature increases (Bellingham, 2013).

2.9.4Total alkalinity (TA)

Alkalinity of a water body is a measure of its capacityto neutralize acids to a designated pH. Alkalinity isan indirect measure of the concentration of anions inwater. The dissolved anionsmay be sourced from bicarbonates, carbonates,hydroxides, phosphates borates or silicates which may bederived from industrial wastes, dissolved rocks, salts,soils or bottom sediments (Lawson, 2011). Alkalinity between 30 and500 mg L^{-1} is generally acceptable to fish and shrimpproduction, between 20 and 50 mg L^{-1}, will permit plankton production for fishculture. High alkalinity results in physiological stress onaquatic organisms and may lead to loss of biodiversity (Lawson, 2011). Low alkalinity value of freshwater may be attributed to increased uptake or release of carbon dioxide by organisms there by changing the proportion of carbonate and bicarbonate ions in water (Bellingham, 2013).

2.9.5 Total suspended solids (TSS)

Total solids (TS)is a direct measure of all the dissolved and suspended matters in water. It comprises dissolved salts, suspended particles, soil particles, discharged effluents and decomposed organic matter(Bellingham, 2013). Total suspended and dissolved solids affectmetabolism and physiology of fish and other aquaticorganisms. They are products of run offs. They increasewith increased rainfall and have adverse effects ondissolved oxygen and carbon dioxide. Suspended solidsin water are directly proportional to dissolved solids.Dissolved solids could directly influence waterconductivity, the higher the dissolved solids the higher the conductivity (Lawson, 2011). TSS is typically composed of fine clay or silt particles, plankton, organic compounds, inorganic compounds or other microorganisms. These suspended particles range in size from 10 nm to 0.1 mm although in standardized laboratory tests, TSS is defined as the material that cannot pass through a 45µm diameter filter (Bellingham, 2013).

2.9.6 Electrical Conductivity

The electrical conductivity of water is directly related to the concentration of dissolved solids in the water. Ions from the dissolved solids in water influence the ability of that water to conduct an electrical current. Electrical conductivity (EC) in natural waters is the normalized measure of the water's ability to conduct electric current. This is mostly influenced by dissolved salts such as sodium chloride and potassium chloride. The common unit for electrical conductivity is Siemens per meter (S/m). Most freshwater sources will range between 0.001 to 0.1S/m. The source of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from rain water run off, or other discharges. EC is also the measure of the water quality parameter "Total Dissolved Solids" (TDS) or salinity. The more total dissolved solids in water, particularly salts, the greater is its electrical conductivity. At about 0.3 S/m is the point at which the health of some crops and fresh water aquatic organisms will to be affected by the salinity (Bellingham, 2013). The relationship between TDS and EC can be described by the equation;

$$\text{TDS (mg/L)} \approx \text{EC (mS/cm)} \times 640$$

2.9.7 Nitrate

Nitrate ion (NO_3^-) is the common form of nitrogen in natural waters. Nitrite (NO_2^-) will oxidize into nitrate after entering an aerobic regime(Bellingham, 2013).

$$NO_2^- + H_2O \rightleftharpoons NO_3^- + 2\,H^+$$

Similarly, plants and microorganisms will reduce nitrate into nitrite but nitrite ion will quickly oxidize back into nitrate once it re-enters the water. Natural sources of nitrate are igneous rock, plant decay and animal debris. Nitrate levels over 5 mg/L in natural waters normally indicates man made pollution, 200 mg/L is an extreme level. Man made sources of include, fertilizers, livestock, urban runoff, septic tanks, and waste

water discharges. In general, nitrates are less toxic to people than ammonia or nitrite however at high levels nitrate will become toxic especially to infants. Eutrophication is usually the result of nitrate and phosphate contamination and is a significant reduction of water quality. Nitrate can exist naturally in groundwater but can increase dramatically on irrigated lands if the irrigation operation is not managed properly. Groundwater contaminated with nitrate can contaminate sources of drinking water in wells, and will contaminate the surface water as the ground water recharges streams and lakes (Bellingham, 2013).

2.9.8Phosphate

Phosphate (orthophosphate or total reactive phosphorus [TRP]) is the ionized form of Orthophosphoric acid.

$$H_3PO_4H^+ + H_2PO_4^-$$

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

$$HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$$

The higher the pH, the more PO_4^{3-} will form from the deprotination of HPO_4^{2-}. Unlike total ammonia, phosphates are less soluble and less volatile, therefore, phosphates will form salts with sodium and calcium and fall out of solution to accumulate in the sediment. Phosphates ions in natural waters will exist in solution in its ionized form, as salts, in organic form or as a particulate species. Higher concentrations rarely occur, because after it enters a water system, it will be rapidly up taken by plants(Bellingham, 2013).In general, phosphorous is an essential nutrient to living organisms. In unpolluted waters, phosphorous can enter a water system from the weathering of phosphorous baring rocks and minerals.

2.9.9Turbidity

Turbidity, like transparency, is a measure of water clarity (how far light can travel through water). The more particles suspended in a sample of water, the more difficult it is for light to travel through it and the higher the water's turbidity, or murkiness. Although the suspended particles that reduce clarity can include organic particles (microbes, algae and plant particles, and animal detritus) as well as inorganic particles (silt and clay particles),turbidityprovides an estimate of the muddiness or cloudiness of water caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds, plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.When turbidity is high, water loses its ability to support a diversity of aquatic organisms (Bellingham, 2013).

2.10The Ephemeral Rock Pool Environment

There are many definitions, which describe an ephemeral rain-pool. McLachlan (1983) described an ephemeral pool as a temporary water body supported mainly by rainfall, which collects in a depression. Dodson (1987) described rain-filled rock pools as ephemeral aquatic habitats found wherever flat areas of rock are exposed. Where the rain collects in depressions on rock, the pools may persist for weeks or months. They may then develop a particular community, which illustrates features of a simple standing water system. Ephemeral pools create an environment that is a host to a unique assemblage of organisms when they fill up. Protozoa, Rotifers and Cladocera usually occur in these pools. They survive by feeding on algae and bacteria. Amphibians lay eggs in these pools. The tadpoles feed on the algal populations in the pools. As the pools begin to dry, tree frogs migrate away from the pools and aestivate for the long dry seasons in burrows, crevices and old logs. Plants, invertebrates and

amphibians have to complete their life cycle before the pool dries up (McLachlan, 1983). The pools provide a habitat for many organisms such as toads, which rely on the pool's warm waters for breeding. The warmer the water, the faster the tadpoles develop, an adaptation to the short life that many of the pools have. The ephemeral rock pools are filled mainly by rainfall (McLachlan, 1983) though, in some cases water draining from the catchment may fill them up as well. The pool environment is unstable with large unpredictable hydro cycles that vary in space and in time. Shallow pools can fill with water, dry up and then refill again several times during a rain season. Chemical and biological changes, both cyclic and irreversible occur very rapidly in these pools. Large diurnal fluctuations occur in temperature and dissolved oxygen concentration while pH and conductivity values exhibit a high degree of variation. The communities may be exposed to extremes in temperature, and dissolved oxygen concentration and pH values at the beginning, mid and end of the rain season (Anusa, 2007).

2.10.1 Factors influencing macroinvertebrates diversity in rock pools

Identifying the factors important in shaping patterns of species among habitats is a central goal of community ecology. Across many systems, species diversity can be regulated by local biotic factors (such as competition, predation and disease), local abiotic factors (such as pH, disturbance, temperature, light, and soil composition) and regional factors (such as dispersal of individuals between habitats). Local factors have been shown to regulate species diversity mainly by setting constraints on colonization success and the replacement of taxa over time. In contrast, regional factors regulate species diversity by regulating which taxa and in what quantity may arrive in a

community(Jackson, 2010).Freshwater rock pools provide an excellent system for empirically testing questions related to diversity and community composition. These habitats have many advantages for studying factors driving diversity, because they are often locally numerous, vary in both frequency and degree of factors known to alter diversity such as disturbance, and are easy to manipulate due to their small size. Temporary rock pools can present an obvious challenge to any aquatic organisms that reside in them. Because rock pools are temporary they typically pass through three distinct phases: filling, aquatic and drying. Each phase may contain different fauna and flora groups. During these phases rock pools may experience extreme fluctuation in temperature and wide variations in pH values from 4.0 to 11.0. Species diversity and community composition in rock pools can be highly dependent on rock pool hydroperiod. Rock pools that have relatively long hydroperiods tend to have higher species diversity, due to reduced local extinction, increased effects of predation, and longer time for colonists to accrue. Habitat size has been shown to influence species composition. Habitats that are larger in area and are nearer to source populations tend to have higher immigration and often offer a wider range of macrohabitats/niches and decreased local extinction, which result in higher species diversity. In contrast, habitats that have smaller area and are more isolated are known to have lower species diversity, due to a lower rate of immigration and higher rate of local extinction. Disturbance has been identified to play an important role in shaping patterns of diversity across a wide range of systems. Disturbance can be defined as any relatively discrete event in time or space that removes and opens up space or resources that can be used by the same or different species. For a system to have the greatest diversity these systems must contain the following three components; first, some form of disturbance must occur; second,

competition must be operating within the community and third, disturbance must result in a resetting of the system(Jackson, 2010).

2.10.2Effects of pool flooding and drying

Rock pools are generally shallow; hence they are very prone to flooding and drying throughout the duration of the rain season. The flood effects can vary with depth, time and flood water quality. Floods are associated with a pulse of nutrients imported from the catchment by flood water. The pulse of nutrients immediately after flooding drives the increases in primary production and a proliferation of the phytoplankton community. Diatoms are associated with flooding and become dominant in the phytoplankton community at this time. Most literature on the drying of rock pools states evaporation as the dominant process. As the pools dry up, a number of changes occur, which are; reduced nitrogen inputs, higher pH, greater deoxygenating rates and a proliferation of large motile flagellates. The absence of flushing out results in increase in algal abundance and changes in algal species composition. Another feature is that biotic interactions such as competition and predation increase, because of declining habitat and increase in the proportion of predatory taxa respectively. When the pool dries up, drought resistant propagules of micro invertebrates and plants remain within the sediments (Anusa, 2007).

2.10.3Biological communities in ephemeral rock pools

Presence or absence of some phytoplankton species in a pool depends on random dispersal of propagules through wind or animals. Physicochemical conditions and biotic interactions during each wet cycle determine establishment and maintenance of viable populations. In Malawi mostly algae, insect larvae and tadpoles colonize small temporary rain–filled rock pools. Community composition varies spatially among pools

and temporarily in the same pools (Anusa, 2007). Four major groups of organisms (protozoa, rotifera, cladocera and copepoda) dominate the freshwater zooplankton. Temporary pools give shelter to large branchiopod crustaceans (Brendonck *et al*., 2002). Many branchiopod species can withstand oxygen concentration of less than $5mgl^{-1}$. Nearly all cladocera species are found at a pH ranging from 6.5 to 8.5. The ephemeral nature of the pools excludes fish, except *Gnathobranchius* species. The most important invertebrate predators in temporal pools on rock substrate are turbellarians. When the turbellarians are present in large densities, they reduce the zooplankton densities drastically, sometimes with total extinction of the active population. Predation of zooplankton by both amphibians and various invertebrates may be an important biotic mechanism regulating temporary pool communities. Overflows that occur between shallow rock pools disperse floating eggs. This is an effective means of short range dispersal. Passive transfer by waterfowl, aquatic insects and in wind blown dust explains the extensive distribution of some of the species. Some species are restricted to very small geographic areas, sometimes a single pond. It is difficult to account for their distribution since few direct measurements have been made in any zooplankton groups. Freshwater cyclopoid copepods bridge unfavourable dry periods by means of resting eggs (Brendonck *et al*., 2002). The resistant resting eggs synchronize their life cycle with favourable habitat phases. They form egg banks to buffer against catastrophic population crashes due to the early drying of pools. They enter diapause in the late copepodid stage, and can survive for several years in the dry sediments of temporary ponds. The cyclopoid produce resting eggs, but resistant copepodid cysts are the most common. Passive dispersal is brought about in this stage, where first cyclopoids to appear in dried mud cultures are in the copepodid stage. Many copepods are found every year in temporary pools, which are completely dried up during the

greater part of the year. Resting eggs and cysts in the dry mud carry the species over. The larvae of flies dominate the invertebrate community of ephemeral water bodies. They feed on the sediment, particularly its contained bacteria. *Chironomus imicola* and *Polypedilum vanderplanki*, larvae of two midge species (Chironomidae) occurs in rainwater pools on rock surfaces at high densities. A reduction in the duration of aquatic stages increases the chances of an individual emerging as a fly before the pool it inhabits dries. *Chironomus imicola* completes its life cycle from egg to adult in twelve days. It favours larger pools which allow time for development of larger larvae and adults. *Polypedilum vanderplanki* survive interrupted conditions as its pool dries between rainstorms and refills. Phytoplankton quickly develops in temporary rock pools. This comes from inocula, which are readily dispersed as dry cysts or spores by wind. If the rainfall is heavy and storms follow each other quickly, the pools may be flushed out so rapidly that algal populations do not have time to form. However, motile algal species such as *Euglena* and *Chlamydomonas* species, which can use their flagella to remain suspended in the water, can withstand being flushed out. Major inorganic nutrients that are required by phytoplankton are nitrogen and phosphorus. Algal growth is limited by phosphorus such that presence of additional phosphorus compounds can s timulate algal productivity and enhance eutrophication processes. In surface water, nitrate is the nutrient taken up by plants and assimilated into cell protein. It is relatively a soluble ion and is easily leached so it usually available in water, though it may limit algal growth at times. The productivity of any aquatic environment depends on the primary producers (phytoplankton) and the environmental conditions that affect them. Each species of phytoplankton has a particular response to different concentrations of limiting nutrients and each has a maximum growth rate. Phytoplankton growth in ephemeral rock pools is influenced by variations in water chemistry, irradiance,

nutrient supply, and presence of tadpoles, temperature and the washout rate (McLachlan, 1983). The variation in the chemical composition of natural waters might be important in regulating the abundance, composition and the geographical and periodic distribution of phytoplankton.

2.11 Freshwater Rock Pools: Habitat Characteristics, Faunal Diversity and Conservation Value

The name 'freshwater rock pool' groups all types of depressions that occur on rocky substrata and (periodically) hold fresh water. A distinction should be made, however, between pools filled by precipitation and those fed by rivers and ground water. Ground-water-fed rock pools (e.g. quarry ponds) and potholes scoured out in bedrock in river floodplains have biological communities largely composed of organisms brought in with the water. Animals in these communities are not particularly adapted to temporary pool conditions, whereas those in precipitation-dependent rock pools contain communities of organisms able to survive dynamic and unpredictable flooding cycles. Rain-fed rock pools are the more typical freshwater rock pool habitats. Freshwater rock pools are found worldwide in all major biomes and are known under different names:'oricangas' in South America, 'rock pools' in South Africa, 'gnammas' in Australia, 'lithothelms' in Bul-garia, 'potholes' in USA, 'tinajas' in the Western USA and Mexico and 'Opferkessel' in Germany. 'Pans' are terms often used for flat-floored, mostly shallow rock pools and 'pitgnammas' are deep rock pools in Australia that may contain water throughout the year, even in very dry regions (Jocque *et al.*, 2010).

2.11.1 Geological formation of rock pools

Pools form in eroded depressions on outcropping rock layers and depend on precipitation for filling. The shape and dimensions of rock pools depend on weathering rates and patterns, dictated by local climate and the composition and resistance to

erosion of the rock. The pan is probably the most common rock pool type characterised by almost vertical edges and a horizontal floor. The archetypal topology of freshwater rock pool habitats consists of clusters of close-set pools on top of granite outcrops or 'inselbergs'. The term inselberg is German for 'Island Mountain' and refers to the remarkable differences in fauna and flora on these outcrops compared with the surrounding environment. The differential erosion rate between solid masses of resistant granite and the softer surrounding geology results in remnant rock layers lying scattered in the landscape as humps in a flat matrix. Based on the conservative estimate that 2–6 million years of weathering are needed to lower a plain by 100 m, an inselberg 300–400 m high would be at least 20–30 million years old, with the rock pools on top presumably of a similar age (Jocque *et al*., 2010).Rock pools also occur on sandstone and limestone formations. Rock pools on sandstone are typically shallow and pan-shaped and occur in large numbers on relatively young exposed flats. Occasionally, however, pool basins may be several metres deep and wide, particularly on older sandstone ridges. Sandstone is more readily erodible than granite, and sandstone slabs are often younger than granite layers. The age of the rock pools will be much younger still, as they are formed only when the sandstone becomes exposed. Limestone is even more erodible than sandstone, and rock pool basins rapidly lose their water holding capacity. Once formed, most rock pool basins will persist for a long time but may lose their water holding capacity by filling up with sediment and the overgrowth of terrestrial plants. A potential succession sequence would start with the generation of a sediment layer by erosion and aeolian activity. This thin layer of sediment can then be colonised by (water) plants (for instance *Isoetes* sp.). The (aquatic) vegetation adds dead vegetative material to the sediment layer, and via a 'windscreen' effect, this may further facilitate the accumulation of aeolian transported material. Eventually, the

sediment layer will allow terrestrial plants to establish and over time the pool may be completely filled with sediment and vegetation (Jocque *et al*., 2010).

2.11.2 Hydrology

Frequency and duration of inundations depend on basin morphometry, catchment area, presence of vegetation and local climate. Maximum depth of the basin often gives a reasonable indication of the maximum length of the inundation period. A more exact filling pattern for individual pools can be modelled with information on rainfall, evapotranspiration and basin geometry. Rock pools occurring in semi-arid regions with low rainfall and high evaporation rates, such as in south eastern Botswana, are highly unpredictable in both timing and length of the inundation period. The length of the inundation period (hydroperiod) averages from several days to little more than a month. Rock pools in more temperate regions may remain inundated for several seasons (Jocque *et al*., 2010).

2.11.3 Chemical and physical characteristics

Rock pools around the world have a very similargeomorphological structure and abiotic environment.They all originate through weathering and erosion, resulting in a similar geomorphology ofthe basin, only varying in surface and depth.Rock pools are usually pan or bucket shaped witha cylindrical or ellipsoid surface and variable dimensions.Weathering and erosion may lead tofusion of neighbouring pools resulting in morecomplex shape.In general, basins are filled withrain water, resulting in a highly diluted environmentat the start of the inundation with conductivitiesbelow 10 μS cm^{-1}, approaching those ofdistilled water. The generally shallow rock pools,with water depth usually varying between 5 and30 cm, have poor buffering capacity to

environmentalchanges, closely follow air temperature, and also show large diurnal fluctuations in pHand dissolved oxygen(Jocque *et al*., 2010).

Rock pools are characterised by low conductivity immediately after filling, typically fluctuating between 10 and 30 µS cm^{-1}. As the water evaporates, conductivity increases mainly because of the concentration of metabolites and can reach values up to1400 µS cm^{-1} in pools with the longest hydroperiod. Water temperature in rock pools depends on climate and maxima have been shown to vary from 32°C in Finland, 35°C in Utah to over 40°C in Botswana. Water temperature seldom exceeds 40°C because of the balance between cooling through evaporation and heating by insolation. Dissolved oxygen also follows a strong diel and seasonal cycle, with concentrations in one study fluctuating between 5.8 and 7.9 mg L^{-1}.De Vries (1996) averaged over eight pools measured during 8 days in South Africa. Variation between pools in oxygen concentration over a complete inundation cycle seems to be small, however, ranging in one study from 3.5 to9.6 mg L^{-1} (De Vries, 1996). It is likely that variability is greater in other systems. Where measurements have been made, water in rock pools ranges from acid (pH = 4.3) to basic (pH = 11.3). At night, respiration from the phytoplankton and macrophytes produces carbon dioxide, thus decreasing pH early in the morning; pH values can vary by 1.5 units in a diurnal cycle (Jocque *et al*., 2010).Rock pools in general are oligotrophic systems open to nutrient inputs and outputs. Enrichment happens mainly through bacterial degradation of dead aquatic organisms, faeces from large (terrestrial) vertebrates and organic material blown in. Immediately after filling, dissolved nitrogen and phosphorus concentrations may be quite high, but decline quickly because of nutrient uptake by organisms and a reduced rate of nutrient supply from the sediment. Nutrients in the sediment get into the water via bioturbation by tadpoles and some crustaceans (e.g. Notostraca and Spinicaudata). Removal of

nutrients from the system is largely by flushing by intense rain, sediment erosion by wind and, to a lesser extent, by terrestrial predators and scavengers removing organisms from the pool basins, and possibly also by the emergence of adult insects (Jocque *et al*., 2010).

2.11.4 Succession and community assembly

Jocque *et al*. (2007) studied invertebrate communityassembly and dynamics in 16 ephemeralrock pools at two rock pool sites in Botswana. Succession or species replacements only occurred in a smallfraction of pools, especially in those with thelongest hydroperiods. Concurrent with a decreasein the densities of the anostracan *B. wolfi*, populationsizes of *Leberis* sp. and Culicidae (*Aedes*sp. and *Anopheles* sp.) increased in these pools.Although it was possible to distinguish two successionalphases at one rock pool site, communityassembly was generally a gradual processdetermined by dispersal strategies of the inhabitants. Additional rains after initial filling triggereddispersal by active dispersers and positively influenced colonization success. A reducedhydroperiod shortened the community developmentdown to a critical point below which lackof time eliminated the possibility of species replacements. Based on these findings, ephemeral waters were defined as aquatic habitats lackingspecies replacements.

2.11.5 Rock pool inhabitants and comments on selected taxa: General

Rock pool inhabitants can be divided in two groups based on life cycle and dispersal strategy: active and passive dispersers. Passive dispersers survive the dry periods in situ via drought resistant resting stages such as resting eggs (Crustacea, Turbellaria, monogonont, Rotifera), encapsulation with water (Oribatidae) or ‘tun’ formation (Tardigrada). Encapsulation is the formation of a vesicle surrounding the animal that contains water from the pool. Tun formation is the contraction of limbs and the body

after which a waxy extrusion covers the surface and protects the organism against desiccation. Passive dispersers need vectors such as water, wind or other animals to disperse between pools. Active dispersers, such as insects, are usually only present in the rock pools during the wet phase and the adults can migrate before pools dry up. A few exceptions to this rule have been documented, with larvae of active dispersersable to survive droughts. *Polypedilum vanderplanki* Hinton (Chironomidae) has the ability to survive in a diapause state. *Dasyhelea sublettei* Wirth (Ceratopogonidae) burrows into the sediment and encysts in capsules filled with fluid and fixed tothe rock surface. *Cloeodes hydration* Nolte, Tietbohl and McCafferty (Ephemeroptera) can survive brief (up to 9 hours) droughts in the larval phase. In addition, some active dispersers have desiccation-resistant eggs, such as *Ae.vittatus* Bigot (Jocque *et al*., 2010). The most important variable explaining distributional patterns and community structure in rock pools, and in temporary pools in general, is the length of inundations (hydroperiod). Community stability (composition over several inundation cycles) in rock pools is known to decrease with increasing frequency of pool desiccation and short-lived pools contain fewer species (Jocque *et al*., 2010).

The highly variable rock pool environment, with continuously changing abiotic and biotic conditions, therefore continuously tests the tolerance limits of the inhabitants. Average values of environmental variables may therefore be of little use in explaining the community structure in specific pools, and extremes of temperatures, conductivity and pH may sometimes be more important than mean values. Species-specific requirements may also account for community structure. This idea is nicely illustrated by a detailed study of the comparative distribution of three larval Diptera (*Polypedilum vanderplanki*, *Chironomus imicola* Kieffer,and *Dasyhelea thompsoni* de Meillon), in rock pools in central Africa. The distribution of these Diptera was primarily

explained by pool permanence. As *Chironomus imicola* has the longest larval lifespan of the three, and has no physiological adaptations against desiccation, it needed pools of long duration. *Dasyhelea thompsoni* and *Polypedilum vanderplanki* both occurred in pools with a shorter hydroperiod than the pools inhabited by *C. imicola*. Further research showed that the characteristics of the substratum, which is relevant for tube construction in *P. vanderplanki*, and the presence of dung of civet and genet cats (Viverridae) for *D. thompsoni*, further explained the differential occurrence of these last two species (Jocque *et al*., 2010). As for all temporary water bodies, appropriate timing of colonisation (for active dispersers) and hatching (for passive dispersers) is essential for inhabitants to maximise the chances of maturation before the habitat disappears. In general, the best time for colonization is immediately after the rains that fill the pool basins. Increased presence of active dispersers after additional rains was observed in a study of the colonisation sequence of freshwater rock pools in Botswana (Jocque *et al*., 2007). Passive dispersers in freshwater rock pools, on the other hand, frequently use low conductivity and temperature, which is lowest immediately after rain, as a trigger to hatch. Besides timing, there is also a level of uncertainty associated with the length of the inundation that depends on additional rains and evaporation rate after filling. Passive dispersers often exhibit partial hatching of the resting bank, a specific sort of risk-spreading strategy, with a smaller fraction of the resting egg bank hatching in more ephemeral pools, hereby compensating for the uncertainty of the length of the hydroperiod. Some active dispersers also use risk-spreading strategies to maximise their reproductive output and are often combined with different degrees of habitat selection. Different species of odonates in Namibian rock pools have different strategies. Some dragonflies e.g. *Pantala flavescens* (Fabricius) distribute their eggs stochastically over different pools, clearly spreading the risk of selecting an

unfavourable pool for the offspring. Other species select a single pool e.g. *Trithemis kirbyi* (Selys), favouring large and deep pools with a long hydroperiod (Jocque *et al*., 2010).

2.11.6 Biotic interactions

Predation is one of the major community structuring forces in permanent water bodies, and ephemeral rock pools also house numerous predatory taxa. Some studies found that predator–prey interactions in rock pools can explain the distribution of specific species or entire assemblages. Notonectidae can strongly reduce *Daphnia* sp. populations, while *Culiseta* sp. and Corixidae, in turn, predate on Chironomidae. Turbellaria (*Mesostoma* sp.) species actively prey on fairy shrimps (Anostraca) and other branchiopod crustaceans and probably have a significant impact on prey population dynamics. Competitive interactions, both inter- and intra-specific, are common in rock pool habitats as reported, for instance, for gyrinid beetles and tadpole. Ranta (1982), Vepsalainen (1978) and Pajunen (1990) reported competition between the rock pool corixids *Arctocorisa carinata* Sahlberg and *Callicorixa producta* Reuter based on the sharing of a similar habitat and reproductive phenology. The impact of competitive interactions on local community structure and distributional patterns within a metacommunity is often less clear than the impact of strong agents such as predation. Still, competition has been shown to increase the local extinction rates and limit species richness in rock pool systems. This was demonstrated in a long-term study of three species of non-rock pool specialist Cladocera (*Daphnia magna*, *D. pulex* and *D. longispina*) with common food resources. In an experimental setup of small (4 L) to large (500 L) artificial rock pools, Bengtsson (1989) found that coexistence of three cladoceran species led to depletion of food and intense interspecific competition, negatively affecting reproduction rate. Small population sizes in conjunction with

interspecific competition made populations more vulnerable to extinction. In addition to predation and competition, parasite communities are an important component of rock pool communities. For *Daphnia* species inhabiting coastal rock pools in Finland, for instance, parasites were important drivers of population dynamics and extinction (Jocque *et al*., 2010). As the diversity of communities decreases with decreasing hydroperiod, biotic interactions become less important. Towards the more ephemeral side of the hydroperiod gradient, the outcome of competitive and predatory interactions seems to depend more and more on a temporal advantage obtained by early colonization. This stochastic aspect, which may also be important early during community development, was also observed in a long-term dataset on invertebrate communities in rock pools in Jamaica (Romanuk and Kolasa, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Sites

This study was carried out on theexotic plateaux and rocky hills ofKaduna State located between (Lat 8°30'0"N and 11°30'0'N) and (Long 6°0'0'E and 8°30'0"E), Northern Nigeria. Vegetation type comprises tropical grassland in the southernGuinea Savannah to Sudan Savannah (NACD, 2013). Rainfall is heavy in the southern part and around

Zaria in the northern part; with average rainfall of about 1016mm (NACD, 2013). The State enjoys a rainy season of about five (5) months. Available rock pools sampled for mosquito breeding were spatially distributed in 21 settlements endowed with inselbergs. Sampling locations is made up of seven (7) Local Government Areas out of the23 in the state. The southern Guinea Savannaharea comprise: Jaba, Jema'a, Kachia, Kagarko, Kaura, Sanga and Zango-Kataf,Birnin-Gwari (partly Guinea Savanna and partly Sudan Savannah), Chikun, Igabi, Lere, Kaduna- North, Kaduna-South, Kajuru, Kauru, whilst the Sudan Savannah areas are: Kubau, Kudan, Makarfi, Ikara, Giwa,Zaria, Sabon-gari and Soba. Jere, Kagarko (Kagarko L.G.A.), Kwoi, Nok, Chori, Samban-Gida (Jaba L.G.A.) and Kagoma (Jema'a L.G.A.), Kangimi (Igabi L.G.A.), Malali (Kaduna-North L.G.A.), Baban-Sora, Kujama (Kajuru L.G.A.), Kajuru, Tudun-Mare and Kufana (Kajuru L.G.A.) settlements were sampled in Guinea Savannah. Hanwa (Sabon-Gari L.G.A.), Danmagaji, Wusasa, Kufena, Dutsen-Abba, Dumbi and Zango-Aya (all in Zaria L.G.A) settlements were sampled in Sudan Savannah.

Parts of the State like Zaria, Kagoro and Kwoi areas have protruding hard resistant granite rocks result out due to weathering through the ages of previous existing pre-Cambrian rocks. The erosive activities resulted in the outgrowth of rocks forming inselbergs and large rocky upland regions(NACD, 2013).The undulating plateaux possess several depressions in which rainwater collects to form discrete pools during the rainy season. These collections of water serve as effective breeding habitats for mosquitoes. The State occupies part of the central position of the northern part of Nigeria (with Kaduna as its capital) and shares common borders with Zamfara, Katsina, Niger, Kano, Bauchi and Plateau states. To the South-West, the state shares a border with the Federal Capital Territory, Abuja. Kaduna State is the fourth most populous state in the federation and represents a major focus and centre of political and economic

activities in the nation.The State occupies an area of approximately 48,473.2 square kilometers and has a population of 6,113,503 and a population density of 130 people per square kilometre. It accounts for 4.3% of Nigeria's total population(NPC, 2006). There are two marked seasons in the State, a dry windy season and a rainy (wet) season. The wet season is usually from April through October with great variations as onemove northwards.The two climatic conditions (wet and dry) in the state greatly influence activities of the people, who are predominantly engaged in agriculture.

3.2 Study Duration and Determination of Geographic Attributes

The study was conducted for five (5) months between June and October, 2013. Available true rock pools (as opposed to edged rock pools) were searched fortnightly on the inselbergs as much as access permitted, assisted by the local community members. A GARMIN e Trex Venture HC hand held global positioning system (GPS) was used to determine the geographic coordinates, altitude above sea level and altitude above the surroundings of the rock pools in the settlements across the State.

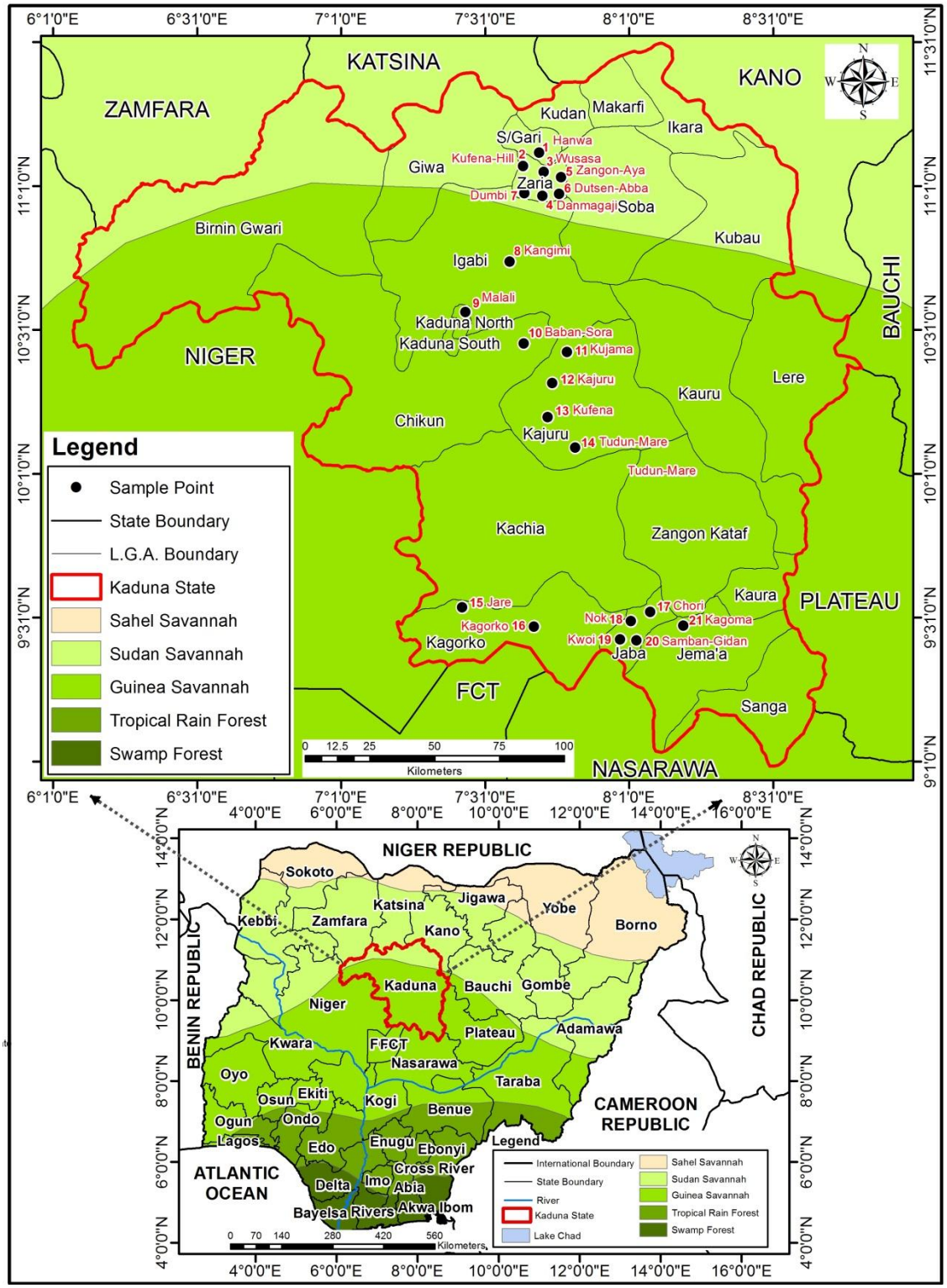


Fig 3.1: Kaduna State showing sampling points.

3.3Determination of Physicochemical Parameters of Rock Pools

Mean depth of water in each rock pool was determined by dipping a metal rod to touch the bottom of the pool at three locations and the wet regionof the rod measured against a transparent ruler. The mean of the three measurements were taken to determine the mean depth of each rock pool. The surface areas of rock pools were determined with a metre rule to measure the length and width of each pool after making the necessary adjustment for irregular surfaces according to the method Adebote *et al*., 2008.The area of each pool was then calculated. Point distances between sampled pools were determined with a tape measure. The pH, electrical conductivity, total dissolved solids and temperature of water in each rock pool were determined by means of a HANNA HI 98129 pH/ EC/TDS/Temp metre. Other parameters, which included turbidity, total alkalinity, total suspended solids, total hardness, dissolved oxygen, biological oxygen demand, chemical oxygen demand, phosphate and nitrate,were determined according to standard protocols described by APHA (1998).

3.4Sampling and Specimen Preservation Techniques

Samplings were carried out forthnightly in the three senatorial zones of the state for a period of five (5) months. Ten dips of water in every other rock pool were obtained with a plastic soup ladle dipper (0.105 L capacity) (Service, 1976). Water from each pool was collected in a white plastic bowl and carefully observed for the presence of preimaginal mosquitoes. Anopheline larvae were collected alive into plastic bottles, transported to the laboratory and reared to adults in small plastic bowls (11x 5.5 cm) on a diet of bakers' yeast (Service, 1993). Adult anopheline mosquitoes were preserved dried on silica gel in labeled specimen bottles. Culicine larvae, in their fourth instar, were concentrated in a sieve and carefully picked with dropping pipette into labeled specimen bottles and preserved in 70% ethanol. Culicine larvae in their lower instars

were nurtured to the fourth instar in labeled plastic bowls on a diet of bakers yeast and subsequently preserved in 70% ethanol in appropriately labeled specimen bottles. The otherbiota within each pool was sampled using a fine mesh invertebrate net. All the macro-invertebrates isolated were sorted and preserved in 70% ethanol in appropriately labeled specimen bottles. Plankton net was used to obtain samples of microalgae and zooplankton and these were preserved in 4% formaldehyde in appropriately labeled specimen bottles. Aquatic macrophytes in the pools were uprooted with hand and taken fresh to the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria for identification.

3.5Species Identification

3.5.1 Mosquitoes

All the mosquitoes collected were morphologically identified to the species taxon and counted under the x50 magnification of a stereo-microscope. Anopheline mosquitoes were identified based on the pictorial keys of Gillies and DeMeillon (1968) and Gillies and Coetzee (1987). For specimens of *Anopheles gambiae s.l.*, genomic DNA were extracted using the method of Collins *et al.* (1987) and Polymerase Chain Reaction (PCR)-based methods was used to identify members of thecomplex (Scott *et al.*, 1993). Amplified fragments were analyzed by electrophoresis in a 1.5% agarose gel with 14µl of ethidium bromide and visualized under ultraviolet light (illuminator). Culicine mosquitoes were identified based on the pictorial keys of Hopkins (1952).

3.5.2 Micro and macroinvertebrates

Micro and macro invertebrate fauna isolated from the rock pools were identified microscopically to species using the identification keys in Pennak (1953).

3.5.3 Algae

Algae encountered in the rock pools were quantified and identified to species by the methods and keys of Bellinger and Sigee (2010).

3.5.4 Aquatic macrophytes

Aquatic macrophytes of the rock pools were identified at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

3.6Data Analysis

Pearson's correlation analysis was used to establish relationships between the physicochemical parameters of water, biota abundance in rock pools and abundance of species of mosquito breeding therein. Principal component analysis (PCA) was used to examine the relationships (interaction effects) amongst physicochemical parameters of water and toselect water parameters that were most useful in distinguishing mosquito species habitats in rock pools. One-way analysis of variance (ANOVA) was employed to test for significant differences of physicochemical factorsin relation to mosquito abundance, using least significant difference to separate means that differed significantly. All levels of statistical significance weredetermined at $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

A total of 386 rock pools distributed in 21 settlements on several inselbergs at heights ranging from 597m to 811m above sea levelwere examined for larval stages of mosquito (Table 4.1). A total of 31,726 mosquito larvae were collected from 269(69.7%) rock pools.These ranged from 6 pools on Kufana inselbergs to 37 pools on Zango-Aya inselbergs. The numbers of pools examined were reflective of their relative availability on the inselbergs during the rainy season. Malali inselberg had the highest (100%) percentage larval positivity and was among the inselbergs encounterd with least number of pools. The highest numbers (37) of pools was observed on Zango-Aya inselberg, with(67.6%) larval positivity. The least number of pools (6) was observed on Kufana inselberg, without larval mosquito (0%). The least (31.8%)larval positivitywas observed on Dutsen-Abba inselbergs. The highest number of larvae (5,042) was collected from Hanwa,inselberg while the least (242) was collected from Nok inselberg. The relative abundance of larvae was highest (701.7) on Wusasa inselberg which also had the highest larval density(526.3) per pool while the least (29.5)larval abundance was observed on Tudun-Mare inselberg. Kujama inselberg was the tallest at (76m)while Kangimi inselberg had the least 4m height amongst the inselbergs studied.

Eight species of mosquito larvae distributed in three genera (*Aedes, Anopheles, Culex*) which included*Ae. vittatus, An. arabiensis, An. gambiaes.l., An. gambiae s.s., An. longipalpis, Culex perfidiosus, Cx. pipiens pipiens* and *Cx. simpsoni* bred in rock pools in northern part of the State (Table 4.2).

Table 4.1: Occurrence and abundance of mosquito larvae in rock pools on inselbergs, in Kaduna State, Nigeria

Inselbergs (Coordinates)	Altitude above sea Level (m)	Height above surrounding Area (m)	No. of pools examined	No. of pools positive (%)	No. of larvae collected	Relative abundance of larvae (%)	Larval No. per pool (density)
SUDAN SAVANNA							
Danmagaji (11°05' 17.9"N) (07° 41' 17.2"E)	676	6	16	8(50.0)	2196(6.9)	274.5	137.3
Dumbi (10° 57' 33.3"N) (07° 39' 16.6"E)	711	9	17	14(82.4)	1202(3.8)	85.9	70.7
Dutsen-Abba (10° 59' 26.5"N) (07° 38' 52.7"E)	699	15	22	7(31.8)	314(1.0)	44.9	14.3
Hanwa (11° 07' 14.2"N) (07° 42' 13.4"E)	677	18	29	25(86.2)	5042(15.9)	201.7	173.9
Kufena (11° 05' 08.5"N) (07° 39' 35.4"E)	718	36	29	18(62.1)	1086(3.4)	60.3	37.4
Wusasa (11° 04' 36.1"N) (07° 40' 28.5"E)	699	18	8	6(75.0)	4210(13.3)	701.7	526.3
Zango-Aya (10° 53' 14.6"N) (07° 38' 43.9"E)	750	49	37	25(67.6)	1200(3.8)	48.0	32.4
GUINEA SAVANNA							
Baban-Sora (10° 30' 23.6"N) (07° 27' 51.7"E)	602	20	10	8 (80.0)	1636(5.2)	204.5	163.6
Chori (09° 30' 58.1"N) (08° 01' 54.4"E)	760	17	15	13(86.7)	656(2.1)	50.5	43.7
Jere (09° 34' 03.5"N) (07° 25' 48.0"E)	597	35	21	20(95.2)	2664(8.4)	133.2	126.9
Kagarko (09° 29' 08.3"N) (07° 41' 50.1"E)	680	16	10	8(80.0)	869(2.7)	108.6	86.9

Table 4.1 Cont'd: Occurrence and abundance of mosquito larvae in rock pools on inselbergs, in Kaduna State, Nigeria

Inselbergs (Coordinates)	Altitude above sea Level (m)	Height above surrounding Area (m)	No. of pools examined (%)	No. of pools positive (%)	No. of larvae collected	Relative abundance of larvae (density)	Larval No. per pool
Kagoma (09° 27' 46.1"N) (08° 05' 24.0"E)	804	16	22	18(81.8)	812(2.6)	45.1	36.9
Kajuru (10° 19' 14.9"N) (07° 41' 02.4"E)	732	47	21	14(66.7)	1309(4.1)	93.5	62.3
Kangimi (10° 43' 13.9"N) (07° 34' 17.4"E)	656	4	24	21(87.5)	1442(4.5)	68.7	60.1
Kufana (10° 19' 18.6"N) (07° 44' 54.5"E)	765	48	6	0 (0.0)	0(0.0)	0.00.0	
Kujama (10° 28' 23.7"N) (07° 37' 29.3"E)	736	76	16	11(68.8)	1429(4.5)	129.9	89.3
Kwoi (09° 27' 12.9"N) (08° 00' 53.4"E)	794	14	16	11(68.8)	911(2.9)	82.8	56.9
Malali (10° 32' 41.8"N) (07° 28' 15.9"E)	616	18	10	10(100.0)	3476(11.0)	347.6	347.6
Nok (09° 28' 46.3"N) (08° 01' 19.3"E)	811	67	14	8(57.1)	242(0.8)	30.3	17.3
Samban-Gida (09° 27' 06.4"N) (08° 03' 10.7"E)	798	24	20	13(65.0)	706(2.2)	54.3	35.3
Tudun-Mare (10° 19' 15.0"N) (07° 42' 57.4"E)	722	22	23	11(47.8)	324(1.0)	29.5	14.1
Total			386	269(69.7)	31726	117.9	82.2

Table 4.2: Species composition and abundance of mosquito larvae in Sudan Savanna of Kaduna State, Nigeria

Inselbergs	Mosquito species and abundance								Total
	Ae. vittatus	*An. arabiensis*	*An. gambiae s.l.*	*An. gambiae s.s.*	*An. longipalpis*	*Cx. perfidiosus*	*Cx. p.pipiens*	*Cx. simpsoni*	
Danma-gaji	2196(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2196
Dumbi	1002(83.4)	1(0.1)	23(1.9)	7(0.6)	0(0.0)	146(12.1)	21(0.2)	1(0.1)	1201
Dutsen-Abba	306(97.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	8(2.5)	0(0.0)	0(0.0)	314
Hanwa	5042(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5042
Kufena	1085(99.9)	0(0.0)	2(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1087
Wusasa	4210(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4210
Zango-Aya	1096(91.3)	0(0.0)	3(0.3)	3(0.3)	1(0.1)	6(0.5)	91(7.8)	0(0.0)	1200
Total	14937(97.9)	1(0.01)	28(0.2)	10(0.1)	1(0.07)	160(1.05)	112(0.73)	1(0.01)	15250

Figure in brackets represent abundance percentage (%)

Table 1: Species composition and abundance of mosquito larvae in Guinea Savanna of Kaduna State, Nigeria

Inselbergs	Mosquito species and abundance											Total
	Ae vittatus	*An. arabiensis*	*An.gambiae s.l.*	*An.gambiae s.s.*	*An. pretoriensis*	*An. rufipes*	*Cx. albiventris*	*Cx.. horridus*	*Cx. macfiei*	*Cx. perfidiosus*	*Cx. p. pipiens*	*Cx. tigripes*	
Baban-Sora	1623(99.3)	1(0.1)	3(0.2)	1(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(0.4)	0(0.0)	2(0.1)	0(0.0)	1636
Kajuru	1019(77.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.1)	0(0.0)	0(0.0)	30(2.3)	245(18.7)	14(1.1)	0(0.0)	1309
Kangimi	1427(99.0)	0(0.0)	6(0.4)	7(0.5)	1(0.1)	1(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1442
Kufana	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0
Kujama	1389(97.2)	0(0.0)	0(0.0)	1(0.1)	0(0.0)	3(0.2)	1(0.1)	0(0.0)	35(2.4)	0(0.0)	0(0.0)	0(0.0)	1429
Malali	3476(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3476
Tudun-Mare	320(98.8)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	0(0.0)	324
Chori	656(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	656
Jere	2657(99.8)	0(0.0)	3(0.1)	3(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2663
Kagarko	494(56.8)	0(0.0)	2(0.2)	5(0.6)	0(0.0)	0(0.0)	264(30.4)	105(12.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	870
Kagoma	785(96.7)	0(0.0)	1(0.1)	2(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	24(3.0)	0(0.0)	0(0.0)	0(0.0)	812
Kwoi	764(83.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	22(2.4)	118(13.0)	7(0.8)	0(0.0)	911
Nok	236(97.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.1)	0(0.0)	4(1.7)	0(0.0)	0(0.0)	1(0.4)	242
Samban-Gida	583(82.6)	0(0.0)	1(0.1)	0(0.0)	0(0.0)	0.(0.0)	0(0.0)	0(0.0)	120(17.0)	0(0.0)	2(0.3)	0(0.0)	706
Total	15338(93.09)	1(0.01)	17(0.10)	20(0.12)	1(0.01)	5(0.03)	266(1.61)	105(0.64)	241(1.46)	363(2.20)	27(0.16)	1(0.01)	16476

Figure in brackets represent abundance percentage (%)

There was preponderance (14,937) of *Aedes vittatus* larvae, and this accounted for 97.9% of mosquito larvae caught from the Northern part of the State (Table 4.2). *Aedes vittatus* also occurred at high proportion (above 80%) on inselbergs in all seven settlements in this part of the state. *Anopheles arabiensis, An. longipalpis* and *Cx. simpsoni* had the least occurrence of only 0.006% of all encountered species and were each found on only one inselberg. The other four species (*An. gambiae s.l., An.gambiae s.s., Cx. perfidiosus*. and *Cx. p. pipiens*) of mosquito occurred on two to three inselbergs in Kaduna north. Hanwa inselberg had the highest (5,042) larval abundance of *Ae. vittatus* and on Danmagaji and Wusasa inselbergs where the species bred prolifically as single species. Dumbi and Zango-Aya inselbergs supported the highest mosquito diversity of seven and six species respectively; whilst Danmagaji, Hanwa and Wusasa inselbergs supported the breeding of only *Ae. vittatus*.

Ten species of mosquito larvae distributed in three genera (*Aedes*, *Anopheles* and *Culex*) were encountered breeding in rock pools on six of the seven inselbergs in the central part of the State (Table 4.3). These included one species of *Aedes*, five species of *Anopheles* and four species of *Culex* mosquitoes. By far the most abundance species (92.2%) was *Ae. vittatus* which ranged from (77.8%-100.0%) in six of the seven inselbergs, with the exception of Kufana inselberg. The highest larval abundance of *Ae. vittatus* (3,476) was encountered on Malali inselberg, where it bred as single species. *Anopheles arabiensis, An. pretoriensis* and *Cx. albiventris* had the least abundance of 0.01% each of all encountered species and were each found on only one inselberg (Table 4.3). Baban-Sora inselberg had the highest diversity of six mosquito species breeding in its rock pools; other inselbergs including Kajuru, Kangimi and Kujama supported the breeding of five mosquito species each while Tudun-Mare and Malali had four and one species respectively and Kufana had none.

Four species of mosquito which included *An. pretoriensis*, *An. rufipes*, *Cx. albiventris* and *Cx. macfiei* which were not encountered in northern part were encountered in the central part of the State. The pair of *An. longipalpis* and *Cx. simpsoni* which occurred in Kaduna north was not encountered in Kaduna central.

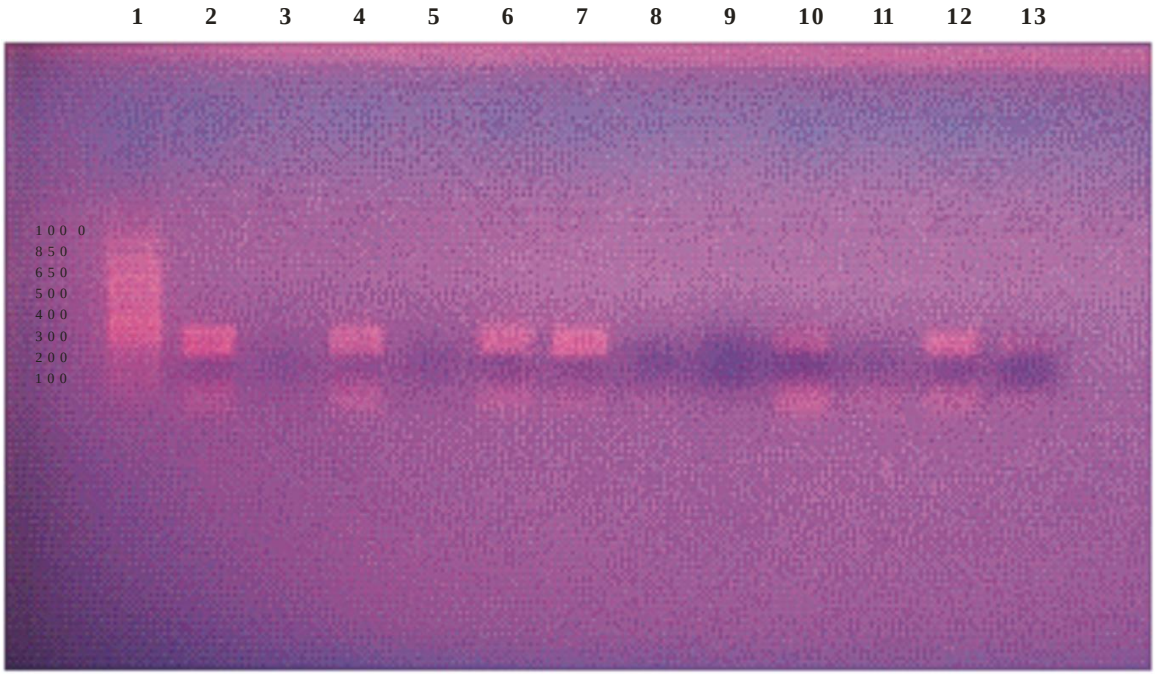
Nine species of mosquito, distributed in three genera (*Aedes*, *Anopheles* and *Culex*) were encountered breeding in rock pools on seven inselbergs in part of Guinea Savannah of the State. These included one species of *Aedes*, two species of *Anopheles* and six species of *Culex*(Table 4.4). This part of the State had the highest species diversity of *Culex* mosquitoes, dominated by *Cx. albiventris* with *Cx. macfiei* being the most widespread in terms of inselberg colonisation. As in northern and central part of the State, *Ae.vittatus* was dominant and accounted for 6175 (90.0%) of all larval collections in the rock pools. *Aedes vittatus* occurred at high percentages (56.8%-100.0%) on all the seven inselbergs in the southern Guinea savannah. The highest larval abundance of *Ae.vittatus* (2,657) was encountered on the Jere inselberg. *Culex tigripes*had the least abundance 1(0.01%) of larvae, found on the Nok inselberg. Kagarko inselberg supported the breeding of the highest diversity of five mosquito species on its rock pools; Chori inselberg had the least diversity of only one mosquito species breeding in its rock pools. The other inselbergs supported the breeding of four species of mosquito, with the exception of Jere inselberg where three species of mosquito bred. In the southern part was encountered both *Cx. horridus* and *Cx. tigripes* not found in the other two parts of the State. *Anopheles arabiensis* which was found in both the northern and central part was not encountered in the southern part of the State.

A total of 77 *Anopheles gambiae* complex species of mosquito were isolated from rock pools on inselbergs (Table 4.5).The number of larvae encountered decreased in magnitude from northern (39 larvae), central (21 larvae) and southern (17 larvae) part of the state. The Polymerase Chain Reaction (PCR)–based testconducted revealed 41.6% amplification of the *An. gambiae* complex.Up to 38.9% of the population belonged to *An.gambiae s.s.* whilst 2.7% were *An. arabiensis*. *Anopheles gambiae s.s.* was predominant in all the collections within the northern Guinea savanna. Up to 58.4% of the *An. gambiae* complex could not be identified using PCR even after three runs. Only two specimens of the *An. arabiensis* were recorded in rock pools in the central and northern parts of the State. Plate I showed the amplification bands for the members of *An. gambiae s.s.* and *An. arabiensis*. Lane 1is the DNA molecular ladder, Lane 2 is the positive control, Lane 3 is the negative control, Lane 4 is *An. gambiae s.s.*, Lane 5 is unamplified sample, Lane 6 and 7 are *An. gambiae s.s.*, Lane 8 and 9 are unamplified samples, Lane 10 is *An. gambiae s.s.*, Lane 11 is unamplified sample, Lane 12 is *An. gambiae s.s.* and Lane 13 is *An. arabiensis*.

Rock pools were found to habour different species of mosquitoes, ranging from one to four species (Table 4.6). Seven species of mosquito bred as single species in the rock pools examined.Two hundred and thirteen (79.2%) of 269 positive rock pools had single species of mosquitoes, 34(12.6%) double species, 5(1.9%) triple species and 3(1.1) *Aedes vittatus*, amongst the mosquitoes breeding as single species predominated in 213(79.2%) rock pool on nineteen inselbergs. Nine combinations of two species of mosquito breeding per pool were observed in 34(12.6%) rock pools. The heterogeneric pairs of *Ae. vittatus* and *Anopheles gambiaes.l.* predominated amongst the mosquitoes breeding as double species per pool in 13(4.8%) rock pools on five inselbergs.

Table 4.4: Agarose gel identification for *Anopheles gambiae s.s.*

Lane	No. of PCR tube	Name on tube	Species i.d.
1	DNA Ladder		
2	*An. gambiae* +	KISUMU	
3	Negative cont.		
4	Tube 1	KANGIMI	*An.gambiae s.s.*
5	Tube 2	TUDU-MARE	-
6	Tube 3	TUDU-MARE	*An. gambiae s.s.*
7	Tube 4	DUMBI	*An.gambiae s.s.*
8	Tube 5	DUMBI	-
9	Tube 6	DUMBI	-
10	Tube 7	DUMBI	*An. gambiae s.s.*
11	Tube 8	DUMBI	-
12	Tube 9	KAGARKO	*An. gambiae s.s.*
13	Tube 10	KAGARKO	*An. gambiae s.s.*



PlateI: *Anopheles gambiae s.s*. and *Anopheles arabiensis* ID.

Lane 1- DNA molecular ladder, Lane 2 – Kisumu(Positive control), Lane 3 –Negative control, Lane 4 – *An. gambiae s.s.*, Lane 5 – Unamplified sample, Lane 6 – *An. gambiae s.s.*, Lane 7 – *An. gambiae s.s.*, Lane 8 – Unamplified sample, Lane 9 – Unamplified sample, Lane 10 – *An. gambiae s.s.*, Lane 11 – Unamplified sample, Lane 12 – *An. gambiae s.s.*, Lane 13 – *An. arabiensis*.

Table 4.5: Mosquito species in different rock pools on inselbergs in Kaduna State, Nigeria

Species composition per rock pool	No. (%) of positive rock pools	Inselberg and number of rock pools in brackets(n = 269)
(a) Single species		
*Aedes vittatus*213(79.2)	Baban-Sora (7), Chori (13), Danmagaji (8), Dutsen-Abba (5), Hanwa (25), Jere (19), Kagarko (6), Kagoma (16), Kajuru (9), Kangimi (16), Kufena(17), Kujama (8), Kwoi (10), Malali (10), Nok (5), Tudun-Mare (8), Samban-Gida (10), Wusasa (6), Zango-Aya (15)	
Anopheles gambiae s.l.	9(3.3)	Dumbi (2), Kufena (1), Tudun-Mare (1), Zango-Aya (5)
Anopheles longipalpis 1(0.4)		Zango-Aya (1)
*Anopheles rufipes*1(0.4)		Kajuru (1)
*Culex macfiei*1(0.4)		Nok (1)
*Culex pipiens pipiens*1(0.4)		Zango-Aya (1)
*Culex tigripes*1(0.4)		Nok (1)
Total	227(84.4)	
(b) Double species		
Aedes vittatus + *Anopheles gambiae s.l.*13(4.8)		Dumbi (6), Jere (1), Kagarko (1), Kagoma (1), Kangimi (4)
Aedes vittatus + *Culex albiventris*	2(0.7)	Kujama (1), Nok (1)
Aedes vittatus + *Culex macfiei*	1(0.4)	Kujama (1)
Aedes vittatus + *Culex perfidiosus*5(1.9)		Dumbi (1), Dutsen-Abba (2), Kajuru (1), Zango-Aya (1)
Aedes vittatus + *Culex pipiens pipiens*	8(3.0)	Dumbi (1), Kajuru (2), Samban-Gida (2), Tudun-Mare (2), Zango-Aya (1)
Anopheles gambiae s.l. + *Anopheles rufipes* 1(0.4)		Kujama (1)
Anopheles gambiae s.l. + *Culex macfiei*1(0.4)		Kagoma (1)
Anopheles gambiae s.l. + *Culex perfidiosus*	2(0.7)	Dumbi (1), Zango-Aya (1)
Culex albiventris + *Culex horridus*1(0.4)		Kagarko (1)
Total	34(12.6)	

Table 4.5 Cont'd: Mosquito species in different rock pools on inselbergs in Kaduna State, Nigeria

Species composition per rock pool (n = 269)	No. (%) of positive rock pools	Inselberg and number of rock pools in brackets
(c) Triple species		
Aedes vittatus + *Anopheles gambiae s.l.* + *Culex macfiei*	1(0.4)	Samban-Gidan (1)
Aedes vittatus + *Anopheles gambiae s.l.* + *Culex perfidiosus*	1(0.4)	Dumbi (1)
Aedes vittatus + *Anopheles gambiae s.l.* +*Culex pipiens pipiens*	1(0.4)	Dumbi (1)
Aedes vittatus + *Culex macfiei* + *Culex perfidiosus*	1(0.4)	Kajuru (1)
Anopheles gambiae s.l. + *Anopheles rufipes* + *Anopheles pretoriensis*	1(0.4)	Kangimi (1)
Total	5(1.9)	
(d) Quadruple species		
Aedes vittatus + *Anopheles gambiae s.l.* + *Culex macfiei* + *Culex pipiens pipiens*	1(0.4)	Baban-Sora (1)
Aedes vittatus + *Culex macfiei* + *Culex perfidiosus* +*Culex pipiens pipiens*	1(0.4)	Kwoi (1)
Anopheles gambiae s.l. + *Culex perfidiosus* *Culex pipiens pipiens* + *Culex simpsoni*	1(0.4)	Dumbi (1)
Total	3(1.1)	

Congeneric pairs of *An. gambiae s.l.* and *An. rufipes* as well as *Cx. albiventris* and *Cx. horridus* were also encountered breeding per pool in one pool each (Table 4.6). Five combinations of triple species of mosquito breeding per pool were encountered in this study. These were in four combinations of heterogeneric and heterospecific trio in one pool each on four inselbergs. Three combinations of quadruple species of mosquito breeding per pool were observed in this study; each as heterogeneric and heterospecific quads in one pool each on a total of 3(1.1%) pools on three inselbergs.

The physical parameters of the rock pools utilized for breeding by thirteen species of mosquitoes encountered in this study are shown in Table 4.7. *Aedes vittatus* bred in rock pools with the shallowest (0.4cm) depth while *An. gambiae s.l.* and *Cx. perfidiosus* bred in the deepest pools (65cm). Three mosquito species which included *Ae. vittatus, An. gambiae s.l.* and *Cx. macfiei* bred in rock pools with the largest surface areas (1970m^2). The surface areas of the rock pools supportive of the breeding of the various species of mosquito differed significantly ($p<0.05$). *Culex macfiei* bred in rock pools with the largest mean surface area (340.1m^2) while *Cx. tigripes* bred in pools with the least (10.1m^2) surface area (Table 4.7). *Anopheles gambiae s.l.* bred in rock pools closed to other pools with conspecific species while *Ae. vittatus* bred in pools more distant to other pools with conspecific species. *Culex perfidiosus* bred in rock pools at closer range to other rock pools with heterogeneric species while *Cx. pipiens pipiens* bred in pools distant to other pools with heterogeneric species.

Table 4.6: Mosquito species andrange (Mean±SE) of physical parameters of rock pool on inselbergs in Kaduna State, Nigeria

Species	Physical parameters					
	Depth (cm)	Surface Area (m^2)	Distance to adjoining conspecific positive pool (cm)	Distance to adjoining heterospecific positive pool (cm)	Distance to adjoining negative pool (cm)	Temperature (°C)
Aedes vittatus	0.4-41 (5.85±0.35)[a]	1.5-197 (60.7±8.6)[a]	05-110	00-354	08-178	23.3-36.5
Anopheles gambiae s.l	2.0-65.0 (13.52±2.31)[a]	5.5-197 (130.9±65.1)[a]	012-153	512-1535	30-1600	22.8-36.3
Anopheles longipalpis	2.0[a]	100[a]	ND	ND	ND	29.8
Anopheles pretoriensis	4.2-6.2 (5.2±1.0)[a]	27.9-150 (88.95±61.1)[a]	ND	110-550	ND	28.1-28.5
Anopheles rufipes	6.2-15.0 (9.73±2.67)[a]	4-372 (175.3±107)[a]	ND	110-1365	ND	27.4-30.3
Culex albiventris	1.1-41 (15.8±12.67)[a]	45-88 (67±12.42)[a]	ND	ND	ND	26.6-32
Culex horridus	5.2[a]	88[a]	ND	ND	ND	26.8
Culex macfiei	3.2-14 (7.14±1.61)[a]	10.6-1970 (340.1±272.7)[a]	ND	65-650	ND	22.8-33.4
Culex perfidiosus	2-65 (17.01±6.11)[a]	10.9-120 (57.46±12.74)[a]	ND	12-360	ND	24.7-35
Culex p. pipiens	1-28 (7.7±2.4)[a]	6-345 (69.9±28.11)[a]	ND	65-204	040-400	26.6- 36.1
Culex simpsoni	20.5[a]	23.5[a]	ND	ND	ND	31.8
Culex tigripes	15[a]	10.1[a]	ND	ND	ND	33.7
P value	0.156ns		0.522ns			

Means followed by the same superscript letter along column are not significantly different (p>0.05).ND - Not Determined. ns -not significant. * - significant. ** - highly significant.

Aedes vittatus, *An. gambiae s.l.* and *Cx. pipienspipiens* bred in pools distant to adjoining negative pools. Temperature of water in the rock pools at the time of sampling ranged from 22.8 to 36.5°C; the high temperature extremes were associated with *Ae. vittatus* and *An. gambiae s.l* (Table 4.7).

pH of the rock pools varied from neutral (pH 7.05) to strong alkalinity (pH 12.69). The four anopheline species (*An.gambiae s.l.*, *An. longipalpis*, *An. pretoriensis* and *An. rufipes*) were associatedwith alkaline pools (pH 8.89–12.12) (Table 4.8).The culicines occurred partly in neutral pools (pH 7.05–7.54) and partly in strong alkaline pools (pH 8.89–12.69).*Culex macfiei* amongst other culicines bred in pools with strongest range of alkalinity (pH 10.32– 12.19).*Anopheles gambiae s.l.* was associated consistently amongst other anopheline with total dissolved solid (4-272 ppm)and electrical conductivity (11-572 µs/cm)in the rock pools. *Aedes vittatus*, *Cx. perfidiosus* and *Cx. pipiens pipiens* was associated consistently amongst other culicine with the total dissolve solid and electrical conductivity in the rock pools. *Anopheles longipalpis*, *Cx. albiventris*, *Cx. horridus* and *Cx. simpsoni*occurred once in pools with total dissolve solid (0, 122,122and99 ppm respectively)and electrical conductivity (93, 210,210 and 198µs/cm respectively)(Table 4.8).Analysis of Variance (ANOVA) shows that the abundance of mosquito larvae significantly differed with pH of the rock pools ($p<0.05$). Highly significant difference existed in the abundance of mosquito larvae to total dissolved solid, electrical conductivity and alkalinity of the rock pools ($p<0.001$). The abundance of mosquito larvae did not differ significantly with depth, surface area, total dissolved solid, hardness and turbidity of the rock pools ($p>0.05$). Weak positive correlation existed between dissolved oxygen ($r = 0.394$) and abundance of mosquito

larvae while strong positive correlation exist between biochemical oxygen demand (r = 1.000) and the abundance of mosquito larvae.

Table 4.7: Mosquito species and range (Mean±SE) of chemical parameters of rock pools on inselbergs in Kaduna State, Nigeria

Species	Chemical parameters					
	pH	TDS	EC	TSS (ppm)	TURB (µs/cm)	HARD (mg/l) (NTU) (mg/l)
Aedes vittatus	7.05-12.6	90.0-276	0.0-572	10-130	2.3-92.4	30.3-545.4
	(10.91±0.05)^{ab}	(57.6±10.9)^{ab}	(117.7±21.5)^{ab}	(51.9±9.2)^{a}	(24.9±5.4)^{a}	(176.5±26.9)^{a}
An. gambiae s.l.	8.89-12.12	4-272	11-572	10-150	2.3-178	70-404
	(10.71±0.17)^{abc}	(59.9±17.82)^{ab}	(123.2±37.2)^{ab}	(76.7±21.1)^{a}	(45.02±28.04)^{a}	(212±51.96)^{a}
Anopheles longipalpis	10.79^{abc}	0^{b}	93^{ab}	ND	ND	ND
Anopheles pretoriensis	11.45-11.71	0^{b}	0^{b}	ND	ND	ND
	(11.58±0.13)^{a}					
Anopheles rufipes	9.65-11.45	0^{b}	0^{b}	20^{a}	23.9^{a}	141.4^{a}
	(10.81±0.58)^{abc}					
Culex albiventris	9.87-11.42	122^{ab}	210^{ab}	ND	ND	ND
	(10.69±0.45)^{abc}					
Culex horridus	9.87^{c}	122^{a}	210^{a}	ND	ND	ND
Culex macfiei	10.32-12.19	0^{b}	0^{b}	20^{a}	1.97^{a}	70.7^{a}
	(11.21±0.26)^{ab}					
Culex perfidiosus	7.54-11.88	41-99	84- 198	60-150	17-178	70-404
	(10.26±0.36)^{bc}	(68.67±16.8)^{ab}	(136.3±33.24)^{a}	(105±45)^{a}	(97.5±80.5)^{a}	(237±167)^{a}
Culex p. pipiens	7.05-12.01	23-113	51- 198	10-150	22.5-178	373.7- 404
	(10.44±0.42)^{bc}	(78.33±27.96)^{a}	(121.7±42.53)^{ab}	(80±70)^{a}	(100.3±77.75)^{a}	(388.9±15.15)^{a}
Culex simpsoni	10.07^{c}	99^{a}	198^{a}	150^{a}	178^{a}	404^{a}
Culex tigripes	11.43^{a}	0^{b}	0^{b}	ND	ND	ND
P value		0.018*	0.008**	0 .008**	0 .268ns	0.181ns 0 .099ns

Means followed by the same superscript letter along the same column are not significantly different (p>0.05).ND = Not Determined. * - significant. ** - highly significant.
TDS – Total Dissolved Solid, EC – Electrical Conductivity, TSS – Total suspended solid, TURB – Turbidity, HARD – Hardness.

Table 4.7 Cont'd: Mosquito species and range (Mean±SE) of chemical parameters of rock pools of on inselbergs in Kaduna State, Nigeria

Species	Chemical parameters					
	DO (mg/l)	BOD (mg/l)	COD (mg/l)	PO_4^{3-} (mg/l)	NO_3^- (mg/l)	ALKA (mg/l)
Aedes vittatus	1.4-1.9 (1.63±0.15)	1.0-1.4 (1.27±0.13)	80-480 (257.6±23.22)^a	0.16-12.50 (2.63±0.79)^a	8.5-74.70 (23.79±3.88)^a	2.0-111.0 (27.43±5.57)^{ab}
An. gambiae s.l.	ND	ND	80-430 (340±54.16)^a	0.3-11.10 (5.31±1.91)^a	2.9-109.4 (40.47±15.72)^a	14-83 (44±9.28)^a
Anopheles longipalpis	ND	ND	ND	ND	ND	ND
Anopheles pretoriensis	ND	ND	ND	ND	ND	ND
Anopheles rufipes	ND	ND	120^a	0.33^a	22.6^a	19^b
Culex albiventris	ND	ND	ND	ND	ND	ND
Culex horridus	ND	ND	ND	ND	ND	ND
Culex macfiei	ND	ND	300^a	0.39^a	9.5^a	12^b
Culex perfidiosus	ND	ND	330-420 (375±45)^a	6.2-11.10 (8.65±2.45)^a	52.70-109.4 (81.05±28.35)^a	33.3-50 (41.50±8.50)^a
Culex p. pipiens	ND	ND	100-420 (260±160)^a	0.66- 11.10 (5.88±5.22)^a	16.90-109.4 (63.15±46.25)^a	15-50 (32.50±17.50)^a
Culex simpsoni	ND	ND	420^a	11.1^a	109.4^a	50^a
Culex tigripes	ND	ND	ND	ND	ND	ND
P value			0 .196ns	0 .238ns	0 .082ns	0.008**

Means followed by the same superscript in the same column are not significantly different (p>0.05).ND = Not Determined. ns - not significant. * - significant. ** - highly significant.

DO – Dissolved oxygen, BOD - Biochemical oxygen demand, COD - Chemical oxygen demand, PO_4^{3-} Phosphate, NO_3^- - Nitrate, ALKA – Alkalinity.

Principal component analysis (PCA) shows that interaction effect of physicochemical parameters of breeding microhabitats of mosquitoes are largely similar and negatively correlated with the study locations(Figure 4.1).Temperature, Electrical conductivity and toal dissolved solid strongly correlated ($p<0.05$) withthe abundance of mosquito larvae in rock pools on Dumbi inselberg while nitrate and turbidity were vital in influencing mosquito breeding on Hanwa inselberg. Water hardness also correlated strongly ($p<0.05$) with the abundance of mosquito larvae on Kufena inselberg. The colonization of mosquitoes in rock pools on inselbergs did not significantly correlate with dissolved oxygen, biochemical oxygen demand, chemical oxygen demand and pH ($p>0.05$).

Twenty-one different aquatic macrophytes were found with mosquito larvae in patchy rock pool habitats. *Chlorophytum laxum* (Liliaceae) had widespread abundance, occurring in almost all the pools (Table 4.9). *Dopatrium longidens* (Schrophulariaceae), *Oplismenus burmanni* (Poaceae) and *Xyris straminea* (Xyridaceae) were second in abundance in rock pools. They were associated with abundance of the three main gener a (*Aedes*, *Anopheles* and *Culex*) of mosquito vectors of human diseases in their rock po ol microhabitats. *Ageratum conyzoides* (Asteraceae), *Caperonia palustris* (Euphorbiace ae), *Cyperus podocarpus* (Cyperaceae), *Ficus abutilifolia* (Moraceae), *Rottboellia exaltata* (Poaceae) and *Tephrosia bracteolata* (Fabaceae) were identified in rock pools negative for mosquito larvae.

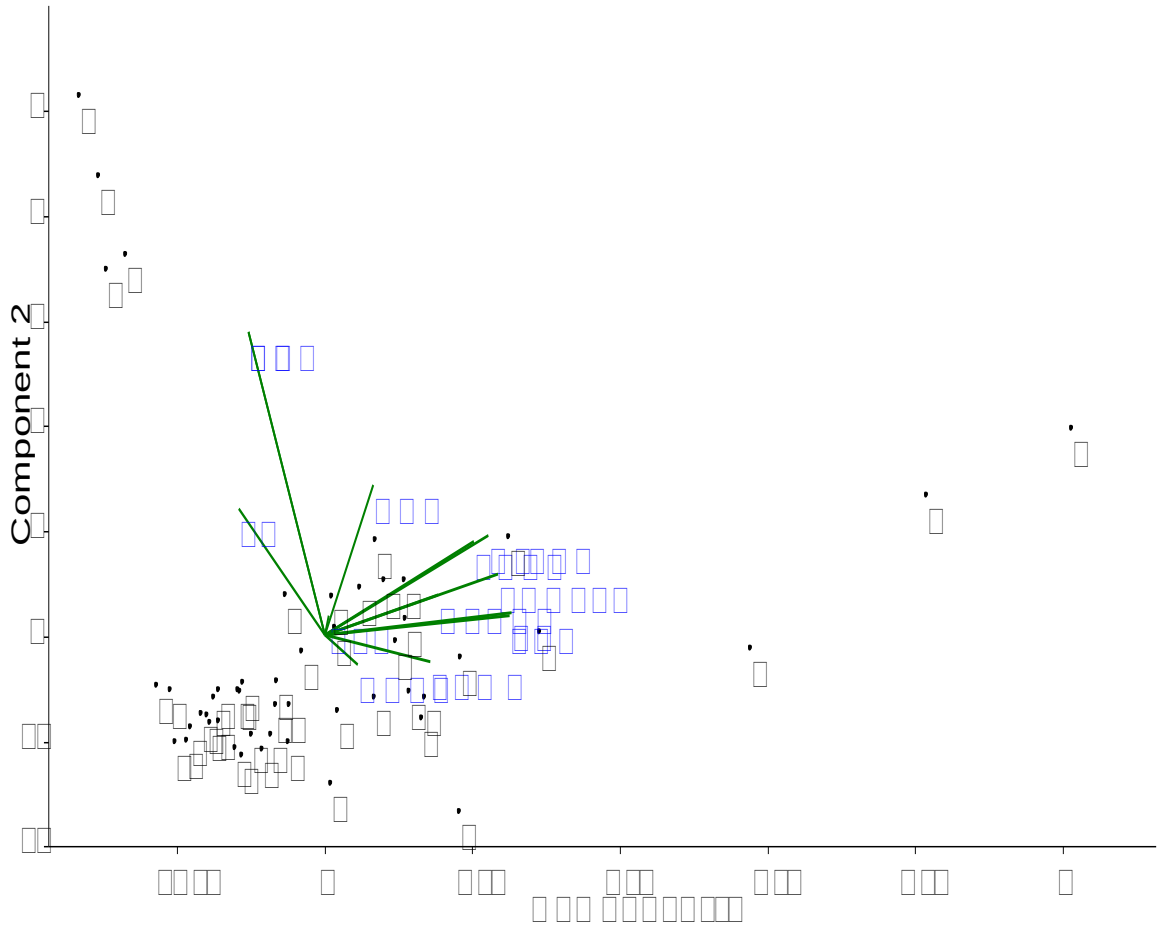


Figure 4.1: Interaction effect of the physicochemical parameters of the rock pools amongst the inselbergs.

Key: a – Dumbi, b – Danmagaji, c – Hanwa, d – Dutsen-Abba, e – Kufena, f – Wusasa, g – Zango-Aya, i – Kujama, k – Tudu-Mare, m – Kagoma, r – Kajuru, t – Jere, v – Kwoi, x –Nok, y – Chori, z – Samban-Gida

Table 4.8: Mosquito larvaeand associated aquatic macrophytes in rock pools on inselbergs in Kaduna State, Nigeria

Macrophyte species	Family	No. (%) Collected: mosquito +ve pools	No. (%) Collected: mosquito -ve pools	Macrophytes in mosquito species pools	Total inselbergs Macrophytes where mosquito (abbreviated)	No. of associated
Aeschynomene unifloraAn. preto., *An. rufi*.)	Papilionaceae	2(100.0)	0(0.0)	2	1	4(*Ae. vitt*., *An. gamb*.,
Ageratum conyzoides	Asteraceae	0(0.0)	1(100.0)	1	1	0
Caperonia palustris	Euphorbiaceae	0(0.0)	1(100.0)	1	1	0
Chlorophytum laxumAn. long., *Cx. macf*., *Cx. perf*., *Cx. p.p.*)	Liliaceae	57(66.3)	29(33.7)	86166(*Ae. vitt*., *An. gamb*.,		
Commelina macrospatha	Commelinaceae	1(50.0)	1(50.0)	2	2	1(*Ae. vitt*.)
Corrigiola russelliana	Illecebraceae	1(100.0)	0(0.0)	1	1	2(*An.gamb*., *An. rufi*.)
Cyperus difformis	Cyperaceae	5(62.5)	3(37.5)	8	5	2(*Ae.vitt*.,*An. gamb.*)
Cyperus iria	Cyperaceae	1(100.0)	0(0.0)	1	1	1(*Ae.vitt*.)
Cyperus podocarpus	Cyperaceae	0(0.0)	1(100.0)	1	1	0
Cynodon dactylon	Poaceae	1(100.0)	0(0.0)	1	1	1(*Ae.vitt*.)
Dopatrium longidens Cx. macf., Cx. p. p.)	Schrophulariaceae	6(40.0)	9(60.0)	15	5	4(*Ae.vitt., An. gamb*.,
Eleusine indica	Poaceae	1(50.0)	1(50.0)	2	2	1(*Ae.vitt*.)
Ficus abutilifolia	Moraceae	0(0.0)	1(100.0)	1	1	0
Ludwigia decurrens	Onagraceae	1(33.3)	2(66.7)	3	2	2(*Ae. vitt.,Cx. p. p.*)
Mariscus ligularis	Cyperaceae	1(100.0)	0(0.0)	1	1	2(*Ae.vitt., An. gamb.*)

Key: *Ae. vitt. – Aedes vittatus, An. gamb.-Anopheles gambiae, An. long. –Anopheles longipalpis, An. preto. - Anopheles pretoriensis, An. rufi.-Anopheles rufipes, Cx. macf. – Culex macfiei, Cx. p.p. – Culexpipiens pipiens*

Table 4.8 Cont'd: Mosquito larvae and associated aquatic macrophytes in rock pools on inselbergs in Kaduna State, Nigeria.

Macrophyte species	Family	No. (%) Collected: Macrophytes in mosquito +ve pools	No. (%) Collected: Macrophytes in mosquito -ve pools	Total No. of inselbergs where species found	Macrophytes in mosquito +ve pools	No. of associated (abbreviated)
Oplismenus burmanni	Poaceae	13(81.3)	3(18.8)	16	11	5(*Ae. vitt., An. gamb., An. rufi., Cx. macf., Cx. preto.*)
Physalis angulata	Solanaceae	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Pycreus flavescens	Cyperaceae	1(100.0)	0(0.0)	1	1	3(*An. gamb., An. preto., An. rufi.*)
Rottboellia exaltata	Poaceae	0(0.0)	1(100.0)	1	1	0
Stylosanthes sp.	Papilionaceae	1(50.0)	1(50.0)	2	1	3(*An. gamb., An. preto.,*
Tephrosia bracteolata	Fabaceae	0(0.0)	1(100.0)	1	1	0
Xyris straminea	Xyridaceae	12(54.5)	10(45.5)	22	8	5(*Ae. vitt.,An. gamb., An. preto., An. rufi., Cx. macf.*)
Total		110(64.7)	65(38.2)	170	65	

Key:*Ae. vitt. – Aedes vittatus, An. gamb. – Anopheles gambiae, An. rufi. – Anopheles rufipes, An. preto. – Anopheles pretoriensis, Cx. macf. – Culex macfiei*.

Eight species of algae belonging to Bacillariophyta, Chlorophyta and Cyanophytawere found with mosquito larvae in rock pool microhabitats. *Microcystis* sp. had the highest percentage positivity (83.3%), occurring as the sole Cyanophytain mosquito positive pools (Table 4.10). Chlorophyta was heterogenerically dominant amongst the rock pools, occurring on thirteen inselbergs, more than any other algal division encountered in this study. *Spirogyra* occured in pools on eight inselbergs with the least percentage positivity (63.6%) while*Microcystis* sp (83.3%) identified on seven inselbergs.*Microcystis* sp.was the highest occuring algal flora.*Rhizoclonium* sp. and *Ulothrix zonata* (Chlorophyta) were found in rock pools negative for mosquito breeding.

Thirty different species of microinvertebrates were found in rock pools with the three main genera (*Aedes*, *Anopheles* and *Culex*) of mosquito larvae predominated by cladocerans. *Monhystera* sp (roundworm), *Daphnia pulex* (water fleas) *and Moina macrocopa* (water fleas) were also found to a greater extent with mosquito larvae in the their breeding water pockets on inselbergs (Table 4.11). *Candona parallela* although not in abundance but was associated with highest number of heterogeneric species of mosquito larvae. *Allocrangonyx pellucidus* (sideswimmers), *Branchionus plicatilis* (rotifers) *Candona intermedia* (seed shrimps), *Diaphnosoma birgei* (water fleas), *Diaph nosoma brachyurum* (water fleas), *Heteromeyenia tenosperma* (sponges), *Macrothrix p ulex* (water fleas), *Manodaphnia macleayi* (water fleas), *Pleurotricha* sp. (protozoan) and *Rhabdolaimus* sp. (roundworm) were least found with mosquito larvae in rock pools. *Diplogaster* sp. (roundworm) and *Chydorus barroisi*(water flea) were observed only in mosquito negative pools. Although microinvertebrate presence correlated negatively with mosquito abundance, malaria vectors were found largely in rock pools dominated by these organisms.

Table 4.9: Mosquito larvae and associated algae in rock pools on inselbergs in Kaduna State, Nigeria

Algal species	Algae Division	No. (%) Collected: mosquito +ve	No. (%) Collected: mosquito -ve	Total No. of mosquitowhere found	No. of mosquito in pools	No. of inselbergs in pools	associated species (abbreviated)
Desmidium sp.	Chlorophyta	3(75.0)	1(25.0)	4	1		1(*Ae. vitt.*)
Micropora floccusa	Chlorophyta	1(100.0)	0(0.0)	1	1		1(*Ae. vitt*.)
Microcystis sp.	Cyanophyta	15(83.3)	3(16.7)	18	7		5(*Ae. vitt.,An. gamb*., *Cx.perf., Cx. p. p., Cx. simp.*)
Rhizoclonium sp.	Chlorophyta	0(0.0)	1(100.0)	1	1		0
Spirogyra	Chlorophyta	7(63.6)	4(36.4)	11	8		3(*Ae. vitt.,An. gamb*., *Cx. macf*.)
Stephanodiscus rotula	Bacillariophyta	1(100.0)	0(0.0)	1	1		1(*Ae. vitt*.)
Tabellaria fenestrata	Bacillariophyta	1(100.0)	0(0.0)	1	1		1(*Ae. vitt*.)
Ulothrix zonata	Chlorophyta	0(0.0)	2(100.0)	2	2		0
Total		28(71.8)	11(28.2)	39	22		

Key: *Ae.vitt. – Aedes vittatus, An. gamb. - Anopheles gambiae, Cx. macf. - Culex macfiei, Cx. perf. – Culex perfidiosus, Cx. p.p. – Culex pipien pipiens, Cx. simp. – Culex simpsoni.*

Table 4.10: Mosquito larvae and associated microinvertebrates in rock pools on inselbergs in Kaduna State, Nigeria

Microinvertebrate speciesin mosquito +ve-ve poolspools	Class/Order in mosquito found	No. (%) Collected Microinverts. in mosquito species (abbreviated)	Microinverts. inselbergs where mosquito	Total associated	No. of	No. of
Heteromeyenia tenosperma	Porifera	1(50.0)	1(50.0)	2	2	1(*Ae. vitt*.)
Chlamydomonas reinhardtii	Protozoa	5(83.3)	1(16.7)	6	2	2(*Ae. vitt.,Cx. perf*.)
Paramecium caudatum	Protozoa	2(100.0)	0(0.0)	2	1	1(*Ae. vitt*.)
Pleurotricha sp.	Protozoa	1(100.0)	0(0.0)	1	1	1(*Ae. vitt*.)
Allocrangonyx pellucidus	Amphipoda	1(50.0)	1(50.0)	2	2	1(*Ae. vitt*.)
Branchionus plicatilis	Rotatoria	1(100.0)	0(0.0)	1	1	3(*An. gamb.,Cx. perf., Cx. simp.*)
Philodina sp.	Rotatoria	3(100.0)	0(0.0)	3	2	1(*Ae. vitt.*)
Chydorus barroisi	Cladocera	0(0.0)	1(100.0)	1	1	0
Bosmina longirostris	Cladocera	2(66.7)	1(33.30)	3	2	2(*Ae. vitt.,An. gamb*.)
Daphnia pulex	Cladocera	18(69.2)	8(30.8)	269	4	(*Ae. vitt.,An. gamb., Cx. perf., Cx. p. p.*)
Diaphnosoma birgei	Cladocera	1(100.0)	0(0.0)	1	1	2(*Ae. vitt.,Cx. p. p*.)
Diaphnosoma brachyurum	Cladocera	1(100.0)	0(0.0)	1	1	2(*Ae. vitt.,An. gamb*.)
Laptonopsis Occidentalis	Cladocera	3(60.0)	2(40.0)	5	5	2(*Ae. vitt.,Cx. p. p*.)
Macrothrix pulex	Cladocera	1(100.0)	0(0.0)	1	1	2(*An. gamb., Cx. perf*.)
Macrothrix rosea	Cladocera	2(50.0)	2(50.0)	4	4	2(*Ae. vitt., .Cx. p. p*.)

Key: *Ae. vitt*. – *Aedes vittatus, An. gamb. –Anopheles gambiae, Cx. perf. – Culex perfidiosus, Cx. p.p. – Culex pipiens pipiens, Cx. simp*. – *Culex simpsoni*.

Table 4.10 Cont'd: Mosquito larvae and associated microinvertebrates in rock pools on inselbergs in Kaduna State, Nigeria

Microinvertebrate species	Class/Order	No. (%) Collected: Microinverts. in mosquito +ve pools	Microinverts. in mosquito -ve pools	Total Microinverts. where found	No. of inselbergs mosquito species (abbreviated)	No. of associated
Manodaphnia macleayi	Cladocera	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Moina macrocopa	Cladocera	12(75.0)	4(25.0)	16	7	4(*Ae. vitt.*,*An. gamb.*, *Cx. perf., Cx. p. p.*)
Sida crystallina	Cladocera	2(100.0)	0(0.0)	2	2	3(*Ae. viit.*,*An. gamb.*, *Cx. perf.*)
Candona intermedia	Ostracoda	1(100.0)	0(0.0)	1	1	2(*Ae. vitt.*,*Cx. p. p.*)
Candona parallela	Ostracoda	2(66.7)	1(33.3)	3	2	5(*Ae. vitt.*,*An. gamb.*,) *Cx perf., Cx. p. p., Cx. simp.*)
Cypria obesa	Ostracoda	3(100.0)	0(0.0)	3	2	3(*Ae. vitt.*,*An. gamb.*, *Cx. perf.*)
Cypricercus reticulatus	Ostracoda	7(77.8)	2(22.2)	9	4	4(*Ae. vitt., An. gamb.*, *Cx macf., Cx. perf.*)
Cyprinotus incongruens	Ostracoda	5(83.3)	1(16.7)	6	3	3(*Ae. vitt. An. gamb.*, *Cx. p. p.*)
Potamocypris hyboforma	Ostracoda	5(100.0)	0(0.0)	5	2	3(*Ae. vitt.*,*An. gamb.*, *Cx. macf.*)
Cyclops sp.	Copepoda	3(100.0)	0(0.0)	3	1	4(*Ae. vitt.*,*An. gamb.*,
Macrocyclops sp.	Copepoda	2(100.0)	0(0.0)	2	1	2(*Ae. vitt.*,*An. gamb.*)
Alaimus sp.	Nematoda/Enoplida	4(57.1)	3(42.9)	7	6	2(*Ae. vitt., Cx. p .p*)
Diplogaster sp.	Nematoda	0(0.0)	1(100.0)	1	1	0
Monhystera sp.	Nematoda	27(67.5)	13(32.5)	40	11	4(*Ae. vitt.*,*An. gamb.*, *Cx. perf., Cx. simp.*)
Rhabdolaimus sp.	Nematoda	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Total		70(74.5)	24(25.5)	94	45	

Key: *Ae. vitt. – Aedes vittatus, An. gamb. – Anopheles gambiae, Cx. macf. – Culex macfiei, Cx. perf. – Culex perfidiosus, Cx. p.p. – Culex pipiens pipiens, Cx. simp. – Culex simpsoni*

Amongst macroinvertebrates observed were four categories of insect orders: Coleoptera, Diptera, Hemiptera and Odonata. *Libellula* sp. (Odonata)more than any other macroinvertebrates had widespread occurrence with heterogeneric species of mosquito (Table 4.12). *Chironomus* sp. (non-biting midges) and *Tendipestentans* (true midges) predominated in mosquito positive rock pools, occurring on nine and eleven inselbergs respectively. Dragonfly nymphs (*Aeschna* sp.) recorded relatively higher abundance, occurring on fifteen inselbergs same as *Libellula* sp.*Belostoma fluminea*, (giant water bug) *Hydrophilus ater* (water scavenger beetle), *Isotomurus palustris* (springtails) and *Ramphocorixa acuminata* (water boatmen) occurred in pools with least number of mosquito species. Microinvertebrates correlated negatively with the abundance of mosquito larvae ($p<0.05$) while macroinvertebrates had strong positive correlation ($p<0.001$) with the abundance of mosquito larvae in rock pool habitats.

In this study, vertebrates observed in rock pools were largely tadpoles of frogs and toads belonging to the family Pyxicephalidae and Bufonidae respectively. The representatives of these families were found in abundance in mosquito negative pools. To a lesser extent, water turtles (*Trachemys gaigeae*) were observed more in mosquito negative pools than in mosquito positive pools (Table 4.13). *Cacosternum* sp. and *Poyntonophrynus beiranus* (tadpoles of frogs and toads) predominated in mosquito positive rock pools, occuring on nine inselbergs and in mosquito negative rock pool, occurring in six inselbergs respectively. *Amietophrynus* sp. and *Natalobatrachus bonebergi* (toad and frog respectively) were found in rock pools negative for mosquito larvae.

Table 4.11: Mosquito larvaeand associated macroinvertebrates in rock pools on inselbergs in Kaduna State, Nigeria

Macroinvertebrates species +v -vefound	Class/Order	No. (%) Collected Macroinverts. in mosquito pools	Macroinverts. in inselbergs mosquitowherepools	Total Macroinverts. associated mosquito species (abbreviated)	No. of	No. of
Aeschna sp.*Cx. macf., Cx. perf., Cx. p. p.*)	Insecta/Odonata	12(40.0)	18(60.0)	30	15	5(*Ae. vitt.,An. gamb.,*
Libellula sp. *Cx. perf., Cx. p. p.*)	Insecta/Odonata	19(59.4)	13(40.6)	32	15	6(*Ae. vitt.,An.gamb., An. rufi., Cx. macf.,*
Belostoma fluminea	Insecta/Hemiptera	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Buenoa margaritacea	Insecta/Hemiptera	4(40.0)	6(60.0)	10	6	4(*An. gamb., An. rufi., Cx. macf., Cx. p. p*)
Nepa cinereaAn. gamb.)	Insecta/Hemiptera	6(66.7)	3(33.3)	9	5	3(*Ae. vitt., An. albi.,*
Plea striola	Insecta/Hemiptera	3(50.0)	3(50.0)	6	5	1(*Ae.vitt.*)
Ramphocorixa acuminata	Insecta/Hemiptera	1(20.0)	4(80.0)	5	5	2(*An. gamb., An. rufi.*)
Hydrophilus ater	Insecta/Coleoptera	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Psephenus herricki	Insecta/Coleoptera	0(0.0)	1(100.0)	1	1	0
Chironomus sp.*Cx. p. p.*)	Insecta/Diptera	18(85.7)	3(14.3)	21	9	3(*Ae. vitt.,An. albi.,*

Key: *Ae. vitt. – Aedes vittatus, An. gamb. – Anopheles gambiae, An. albi. -Anopheles albiventris, An. rufi. - Anopheles rufipes, Cx. macf. – Culex macfiei, Cx. perf. – Culex perfidiosus, Cx. p.p. – Culex pipiens pipiens*.

Table 4.11 Cont'd: Mosquito larvae and associated macroinvertebrates in rock pools on inselbergs in Kaduna State, Nigeria

Macroinvertebrate species	Class/Order	No. (%) Collected: Macroinverts. in mosquito +ve-ve pools	Macroinverts in mosquito where mosquito pools found species	Total pools (abbreviated)	No. of inselbergs	No. of associated species
Dasyhelea sp.	Insecta/Diptera	10(90.9)	1(9.1)	11	4	1(*Ae. vitt.*)
Helocordulia whleri	Insecta/Diptera	2(100.0)	0(0.0)	2	2	2(*An. gamb., Cx. perf.*)
Isogenus modesta	Insecta/Diptera	0(0.0)	1(100)	1	1	0
Isotomurus palustris	Insect/Diptera	1(100.0)	0(0.0)	1	1	2(*An. gamb., Cx. perf.*)
Palpomyia tibialis	Insecta/Diptera	5(62.5)	3(37.5)	8	6	1(*Ae.vitt.*)
Tendipes tentans	Insecta/Diptera	15(68.2)	7(31.8)	22	11	4(*Ae. vitt., An. gamb., Cx. perf., Cx. p. p.*)
Total		92(57.2)	63(37.6)	161	94	

Key: *Ae. vitt. – Aedes vittatus, An. gamb. – Anopheles gambiae, An. rufi - Anophelesrufipes, Cx. perf. – Culex perfidiosus, Cx. p. p. – Culex pipiens pipiens*.

Table 4.12: Mosquito larvae and associated vertebrates in rock pools on inselbergs in Kaduna state, Nigeria

Vertebrate species +ve-veinselbergs pools found (abbreviated)	Family	No. (%) Collected: Vertebrates in mosquito pools	Vertebrates in mosquito associated pools	Total number of where species	Number of mosquito species	Number
Amietia angolensis	Pyxicephalidae	1(100.0)	0(0.0)	1	1	1(*Ae.vitt.*)
Amietophrynus sp.	Bufonidae	0(0.0)	1(100.0)	1	1	0
Bufo terrestris	Bufonidae	1(100.0)	0(0.0)	1	1	1(*Ae. vitt.*)
Cacosternum sp.	Pyxicephalidae	4(40.0)	6(60.0)	10	9	2(*Ae. vitt.,An. gamb.*)
Natalobatrachus bonebergi	Pyxicephalidae	0(0.0)	3(100.0)	3	1	0
Poyntonophrynus beiranus	Bufonidae	3(33.3)	6(66.7)	9	6	3(*Ae. vitt.,An. gamb., An. rufi.*)
Trachemys gaigeae	Emydidae	1(33.3)	2(66.7)	3	1	1(*An. gamb.*)
Total		10(35.5)	18(64.3)	28	20	

Key:*Ae. vitt. – Aedes vittatus, An. gamb. – Anopheles gambiae, An. rufi. – Anophelesrufipes*

CHAPTER FIVE

5.0 DISCUSSION

In this study, rock pools constitute breeding microhabitats to the three main genera (*Anopheles, Aedes* and *Culex*) of mosquito vectors responsible for the worst scourges of humankind. The physicochemical and biological factors determining theproliferation of mosquitoes in rock pool habitats were elucidated in this study. This study therefore provides information on the spatial distribution and ecology of preimaginal stages of mosquitoes in the relatively accessible patchy rock pool habitats as influenced by species assemblages of freshwater invertebrates, vertebrates and aquatic macrophytes. The abundance of the organisms was reflective of the durational instability (disappearance and appearance) of the pools during the rainy season. Rock pools are maintained exclusively by rain which within 3 to 4 days becomes effective breeding habitats for mosquitoes. However, rain seizure during the preliminary stage of this work left some of the pools on the rocks with freshly filled rainwater without mosquito larva. This was evident on Danmagaji and Kufena inselbergs which recorded relatively low abundance of mosquito larvae amongst other inselbergs sampled for mosquito breeding in parts of Kaduna North. Similarly,rainless (break) period towards June ending may have accounted for the low volume or absence of waters in majority of rock pools and invariably the low population or absence of mosquito larvae particularly. This could be the cause of the negativity in the abundance of mosquito larvae on the Kufana inselberg in the central part of Kaduna state. Since rock pools communities are completely dependent on length and frequency of inundations, the active community reflects the prevailing weather conditions (Jocque *et al*., 2010). Ranta (1982) also observed that rock pool organisms can either have to escape from the

habitatwhen conditions deteriorate or spend the unfavourable period in some kind of dormant forms.

Results of this study revealed a high diversity in species compositions of *Culex* mosquitoes in the southern part of the state (Table 4.4), yet with minimal larval abundance compared to the abundance of *Ae. vittatus*.This could be due to preference shown by *Ae. vittatus* for breeding in rock pool habitats over *Culex* mosquitoes. This is supported by Adebote *et al*. (2008) whoreported that the more diversifiedthe availability of rock pools on inselbergs, the lessare the relative abundance and mean number of larval mosquitoes per pool. This is very evident on some of the inselbergs located at Baban Sora, Kagarko, Malali and Wusasa where the relative abundance and mean number of mosquito larvae found in rock pools exceeded by far those of their counterparts with relatively more number of rock pools (Table 4.1). There was marked variation in the relative abundance of larval mosquitoes amongst the different vegetation zones of the state. The population of *Ae.vittatus* in the rock pools far outweighed that of all other mosquito species observed inhabiting the same microhabitats both in relative abundance and mean larval number per pool.The peculiarity of *Ae.vittatus*is its amenability for rock pools (Robinson, 1948; Hopkins, 1952). Hopkins (1952) identified true rock pool as the most preferred and characteristics breeding habitats of *Ae. vittatus*, and seldom breed elsewhere. This has been reported by Robinson (1948) who showed that stoney riverbeds and rock pools favour the breeding of *Ae. vittatus* in Livingstone, Northern Rhodesia (Now Zambia). In contrast, Idowu *et al*. (2013) identified bamboo as breeding receptacle for *Ae. vittatus* in Abeokuta, Southwestern Nigeria. Rajesh *et al*. (2013) identified *Ae. aegypti* and *Ae. vittatus* as container breeding mosquitoes and principal vectors of dengue fever in India.

The results of this study also show that *Ae. vittatus* bred in shallow pools (0.4-41 cm shallow than most pools where other species of mosquito larvae were found)and this probably underline the reason why they were more abundant in Sudan savannah compared to the central and southern Guinea Savannah. Seasonal instability in the Sudan savannah may have restricted oviposition, successful breeding and possible emergence of adult mosquitoes in the few available pools while prolonged rainfall in the southern Guinea Savannah could have largely washed away most of the larvae from their characteristics shallow pools. Eggs of *Aedes, Ochlerotatus* and *Psorophora*species of mosquitoes can withstand desiccation and remain dry for months or even years but still remain viable and hatch when soaked in water (Service, 1997).This could explain the reason for prolific breeding of *Ae. vittatus*compared to other species in the Sudan Savannah(central part of Kaduna State) (Table 4.3) compared to the other parts especially in the face of seasonal instability with respect to rainfall.The ability of gravid female mosquitoes to distinguishamong potential oviposition sites that will or will not support thegrowth, development and survival of their offspring are critical tothe maintenance of the mosquito populations(Rajesh *et al*., 2013). Despite the higher diversity of *Culex* mosquitoes observed in this study, they contributed minimally to larval population (Table 4.2-4.4).There are wide ranges of factors used by different species of mosquitoes in discerning their oviposition sites. The presence of organic matter in breeding sites, whether sterile or putrid, renders water highly attractive for oviposition to*Culex* mosquitoes, which prefer such water to freshwater. The degree of the preference reflecting the extent of contamination normally found in the natural larval habitats (Clements, 1963). The overall reduced larval population of *Culex* species in this study could therefore be due to gravid *Culex* mosquitoes being highly selective in their choice of oviposition site. *Culex* species favour slightly to very eutrophic or

polluted water or water highly contaminated with organic material as breeding habitats; even in latrines and septic tanks. Rain-filled rock pools are largely clean freshwater and are therefore not the preffered *Culex* habitats hence the least number of *Culex* mosquitoes encountered in the rock pools. It is plausible that the relatively few *Culex* encountered were associated with few pools on the rocks that were rich in organic matter. *Culex* mosquitoes also breed in cleaner water but in artificial containers (Macdonald, 1960).The restriction of species to particular larval habitats is probably due to discrimination by ovipositing females, an activity which will be affected in the long term by selective pressures operating during the larval stage as well as by those effective at oviposition.

One common feature of all the inselbergs sampled in this study is the unavoidable presence of humans on the inselbergs and close proximity of the inselbergs to human habitation. Children and adolescent utilized the inselbergs for adventure. Adults are either consistently quarrying on the rock or utilizing the rocks as platform for drying their harvested farm produce.Churches of variousdenominations have acquired some of the inselbergs as outstations and camping grounds for special prayers throughout the seasons. Dutsen-Abba inselberg, though not far from human habitation, was observed with patas monkeys gallivanting on top of the inselberg. Therefore considering the anthropophilic nature of *Ae. vittatus* and its possible zoophilic as well as its implications as one of the principal vectors of yellow fever, it is therefore very pertinent to conclude that the humans around the inselbergs are greatly exposed to high risk of contracting yellow fever and as such could play key role in rural yellow fever epidemics. Religious camping on the rocks for several days could contribute to disseminating urban yellow fever. The report agree with that of Adebote *et al*. (2006)

who observed high yellow fever epidemic risk posture around Zaria due to elevated *Ae. aegypti* larval indices in peridomestic containers.

The existence of the most important members of *An. gambiaesensu lato*, vectors of malaria (*An. gambiae s.s.* and *An. arabiensis*) in rock pools is unquestionably of great importance in maintaining high level of malaria transmission especially among the residents surrounding these inselbergs. Members of*Anopheles gambiae sensu lato* (*s.l.*) are the mostimportant vector of malaria in Africa and comprises ofseven morphologically indistinguishable sibling species (Ebenezer*et al*., 2012). The results of this studycontradicts the findings of Adebote *et al*. (2008) that rock pools on inselbergs around Zaria do not constitute natural breeding habitats for malaria vectors. It was made known that the particular inselberg examined by Adebote *et al.* (2008) amongst the group of inselbergs available at Dumbi was different and farther away from the one examined in this study (Adebote personal communication). The inselberg examined at Dumbi in this study, was at very close proximity to the Dumbi village and *An. gambiae s.s.* was known to breed close to human habitations (Ebenezer*et al*., 2012).In this study, Dumbi inselberg recorded overall dominance of *An. gambiae s.l.* in rock pools (Table 4.2).Quarrying activities, observed on majority of these inselbergs could create environmental disturbances that favour the colonization of rock pools by the most efficient vectorsof malaria. This agrees with Collins and Besansky (1994) that *An. gambiaes.l.*has marked preferencefor human environmentsand humans as hosts andadapt so rapidlyto changes in the environmentinduced byhuman habitation and agriculture.Rock pools on the Dumbi, Kufena, Jere inselbergs etc. have been excavatedthereby increasing their water holding capacity which was used by the residents to manually irrigate their crops due to rain resure.The abundance of rice fields around these inselbergs could also support colonization of rock pools by these vectors

since rice fields constitute the most productive habitat for *An. gambiaes.l.* (Gillies and De Meillon, 1968; Mwangangi, 2006).The moderately high proportion(38.9%) of*An. gambiae s.s.*(Plate 4.1) in northern Guinea Savanna, contrasted other reports that highlighted widespreadoccurrence of *An. gambiae s.s.*, the major malaria vectorin Nigeria (Oyewole *et al.*, 2005). The low proportion(2.6%) of *An. arabiensis*(Plate 4.1)reported in this study, agrees with that of Samdi *et al.*(2006) that *An. arabiensis*constituted 95% of the *An. gambiae s.l.* population in the Sahelsavannah region.In Nigeria, highpopulations of *An. arabiensis* were recorded in the aridnortheast region, while high populations of *An.gambiae s.s.* were collected in the southern tropical rainforestareas (Awolola *et al*., 2003; Oyewole *et al*., 2005).Previous study by Kenea *et al*. (2011) on reported the abundance of larvae of the principal malaria vector (*An. arabiensis*) in sand poolsalong the edge of the Meki River during the dry and small rainy season in Ethiopia.The identification, to species level, of morphologicallyidentical taxa within the complex is an essential componentof the epidemiological study of malaria and the subsequentformulation of control strategies in malaria endemicareas (Mbogo *et al*., 2003).

Some of the mosquito species observed in this study corroborates with Robinson (1948) that of the *Anopheles, An. pretoriensis* prefer to breed in rock pools in Livingstone while*An. rufipes* favours stony pools in drying riverbeds, in Northern Rhodesia (Now Zambia). The three anophelines (*An. longipalpis, An. pretoriensis* and *An. rufipes*) are generally man-bitters and are regarded as incidental vectors of human malaria, but their exact public health importance remains doubtful (Gillies and De Meillon, 1968). The common house mosquito, *Cx. pipiens pipiens* feeds principally on birds, but willfeed on humans, particularly when inside a house. *Cx. pipiens pipiens* females may travel up to 1.2 km in a single night in search of a blood meal, but

generally are found within 0.8 km of their breeding habitat. They are the most important primary vector (feed on birds and maintain West Nile Virus in bird population) as well as bridge vector of WNV. *Culex albiventris, Cx. horridus, Cx. macfiei, Cx. perfidiosus* and*Cx. simpsoni* are zoophilic and bridge vectors (feed on numerousanimal species including horses, humans and birds and move the virus frombird to mammal) of WNV. *Culex tigripes*is predaceous on other mosquito larvae and as such serves as natural biological agent (Gillies and De Meillon, 1968).

The prevailing physicochemical parametersof rock pools are important factors for survival and development of mosquito. This study observed an overall wide temperature range slightly above the optimum temperature (31°C) considered for rapid larval and pupal development (Oyewole *et al*., 2009).This apparent temperature increase over the optimumwithin the rock pools could be attributed height and exposure of the inselberg above the ground positioning the pools to direct incident radiation from the sun. In addition, the granitic nature of the inselbergs allows for rapid absorption and retention of heat from the sun. Jocque *et al*.(2010) observed that water temperature in rock pools depends on climate and seldom exceeds 40°C because of the balance between cooling through evaporation and heating by insolation. The depth of the rock pool habitats has been shown to influence the species abundance of mosquito breeding therein. In this study, *Ae.vittatus* was observed to exhibit prolific breeding in shallow pools. This could be a natural selective pressure that controls the immigration of its potential predators into such shallow pools as they appeared to be somewhat constrained to deeper pools. Habitat size also influences the species composition of mosquitoes in rock pools.Rock pool habitats with large surface areas(1.5-1970 m^2) were observed to be densely inhabited by tadpoles of both frogs and toads which are natural enemies of mosquito larvae. Habitats with large surface areastend to have

higher immigration and often offer a wider range of microhabitats/niches and decreased local extinction, which result in higher species diversity (Kumar and Hwang, 2006). Mosquitoes use chemosensory information to assess several parameters that reflect habitat quality for the offspring, including the availability of nutrients, the presence of competitors and predators, and the overall quality and permanence of the water body (Kumar and Hwang, 2006). Semiochemicals emitted from predators are normally used by mosquitoes to detect a predator's presence in the environment, and then mosquitoes can thereby minimize such encounters (Kats and Dill, 1998). Therefore, the active avoidance of habitats with a high predation risk byoviposition adult female mosquitoes may ultimately act to sustain high densities of mosquitos' larvae in the rock pools.Since rock pools are mosaically distributed habitats, the restriction of the congeneric or heterogeneric species of mosquitoes to varying distances of the rock pools is presumed to be due to discrimination by ovipositing females, an activity which will be affected in the long term by selective pressures operating during the larval stage as well as by those effective at oviposition.The fact that female mosquitoes are highly selective in nature in their choice of oviposition site has prompted the suggestion that the distribution of larvae is controlled not by survival in suitable and extinction in unsuitable habitats but by the discrimination of the ovipositing female (Clements, 1963).

This study shows that neutral to strong alkalinity (2.0-111.0 mg/L for *Ae. vittatus*, 14-83 mg/L for *An. gambiae*, 33-50 mg/L for *Cx. perfidiosus*)were associated with the abundance of mosquito larvae, and thus play pivotal role for their prolific breeding in rock pool habitats. When pH was within the range of 5.5 to 8.5, a balance in an ecosystem was maintained. Increased surface pH in water bodies could be due to increased metabolic activities of autotrophs, since they generally utilize the

carbondioxide and liberate oxygen thus reducing H^+ ion concentration (Clement, 1963). Since *Ae.vittatus* accounted for more than 90% of mosquito larvae observed in this study, it can therefore be added that strong alkalinity may be a strong indicator for their choice of breeding in rock pool habitats.Rock pools sampled in this study were largely devoid of organic nutrients which tend todecrease the pH of the environment due to carbonate chemistry (Bellingham, 2013).According to CDC (2004), water of a near neutral pH of 6.8-7.2 is preferable for breeding of many species of mosquitoes. Water pH higher than 7 but lower than 8.5 according to Abowei (2010) is ideal for biological productivity, but pH less than 4 is detrimental to aquatic life.Lower values of pH are indicative of high acidity, which can be caused by the deposition of acid forming substances in precipitation (Lawson, 2011).

Electrical conductivity (EC) and Total dissolved solid (TDS) obtained in this study were largely below detectable limit with the HANNA instrument. This corroborates the report of Ranta (1982) that the small nature of thevolume of water in rock pools results in strong fluctuation in electrical conductivity.Jocque *et al*.(2010) also observed that rock pools were characterized by low conductivity immediately after filling, typically fluctuating between 10 and 30 µS cm^{-1}. As the water evaporates, conductivity increases mainly because of the concentration of metabolites and can reach values up to1400 µS cm^{-1} in pools with the longest hydroperiod (Jocque *et al*., 2010).The amount of oxygen dissolved(1.4-1.9 mg/L) in water of the pools significantly supported the community structure of the rock pools.The results are in agreement with Huggett and Griffiths (1986) and Daniel and Boyden (1975) who related the communities occurring in rock pools with the extremes of salinity, hydrogen ion concentration, light and temperature occurring within them. They identified temperature and oxygen concentration were the

most important of these parameters that were particularly important in controlling community structure of the rock pools. It is pertinent to state like Jocque *et al*. (2010) thatoxygen could also be introduced into water of the rock pools by green aquatic plants and algae during photosynthesis.

Inorganic nutrients such as nitrate, phosphorus, hardness, total suspended solid, alkalinity, chemical oxygen demand and turbidity had no significant effect on the abundance of mosquito larvae breeding in rock pools. This is in agreement with the observation of Jocque *et al*. (2010) that nutrients such as nitrate, phosphorus, alkalinity etc.declined quickly because of nutrient uptake by organisms and a reduced rate of nutrient supply from the sediment.Immediately after filling, dissolved nitrogen and phosphorus concentrations may be quite high but nutrients in the sediment get into the water via bioturbation by tadpoles and some crustaceans (e.g. Notostraca and Spinicaudata) (Jocque *et al*., 2010).Published data on nutrient availability on inselbergs indicated that phosphorus and nitrogen were severely limited (Dörrstock *et al*., 1996).Removal of nutrients from the system was largely by flushing by intense rain, sediment erosion by wind and, to a lesser extent, by terrestrial predators and scavengers removing organisms from the pool basins, and possibly also by the emergence of adult insects (Jocque *et al*., 2010). Nitrate and phosphate are major inorganic nutrients that are required by phytoplanktons. Algal growth is limited by phosphorus such that presence of additional phosphorus compounds can stimulate algal productivity and enhance eutrophication processes (Kenea *et al*., 2011).

The algal compositions of the rock pools examined in this study were dominantly chlorophytes. Previous studieshave shown that algal growth was a key factor for the growth of some anopheline species (Kenea *et al*., 2011). Observation of the rock pools

showed that most pools with algal bloom were found without mosquito larvae. The mosquito larvae must come to the surface of the water for oxygen intake or otherwise suffocate. Accordingly, algal bloom that form mat on the surface of the water could consequently deprive mosquito larvae of oxygen. Results revealed that associations of these algae with mosquito larvae in rock pools were at a very low detectable limit. The preponderance of green algae in rock pools is in agreement with Wallenstein *et al*. (2010) who recorded a marked gradient in the algal composition of rock pools in many places, with green algae dominating the rock pools. The high densities and distribution of the often toxic and noxious *Microcystis* species (cyanophyta) in mosquito positive poolsas observed in this study seems to be the only toxic and noxious species of algae that does not inhibit to a greater extent, larval development and survival. Microinvertebrates such as Cladocera, Copepoda, Ostracoda, Rotifers etc. survive in rock pools by feeding on algae and some microinvertebrates like Protozoans(Noël *et al*., 2010).Algae are the major primary producers of temperate rocky shores. More importantly, they contribute to the functioning of ecosystems by providing food, dissolved organic matter, habitats and nurseries for many invertebrates (Noël *et al*., 2010). Mosquito larvae presumably directly feed opportunistically on these microinvertebrates. Therefore, the abundance of microinvertebrates in examined rock pools in this study could have provided adequate food supply for mosquito larvae and therefore supportive of their breeding in these microhabitats. Macroinvertebrates observed in this study belonged to four orders of insects: Coleoptera, Diptera, Hemiptera and Odonata and are known predators of mosquito fauna of rock pools (Shaalan *et al*., 2007). There existed mutually negative interactions among mosquito larvae and *Libellula* and *Aeschna*species of odonatan nymphsthat are important voracious predators of mosquito larvae in freshwater ecosystems.

The composition and density of larval mosquito communities are strongly influenced by the ephemeral nature of the pools (Okogun *et al*., 2005). James (1966) reported that immature mosquitoes are less exposed to natural enemies in temporary than in permanent ponds. This study revealed coinhabitationof macroinvertebrates and breeding mosquito larvae in rock pools. Despite this, mosquito abundance still remained high. This could be due to the polyphagous nature of some of the predators.When two (or more) prey species share common predator, the predator's number will be limited by prey availability. This could have offered larval mosquitoes the opportunity to develop to pupal and adult stages.Lee (1967) observed that mosquito larvae were often consumed more than pupae by predators and assumed that this could be due to the inclination of pupae to exhibit rapid escape tumbling actions when startled.Sebastian *et al*. (1980) found a complete elimination of all *Ae. aegypti* larvae and pupae between days 4 and 9 by the nymphal dragonfly, *Libellula* species, depending on the density of aquatic stages of mosquitoes present per container. Hemipteran predators are good swimmers andthey forage beneath water(Shaalan *et al*., 2007). This implies that they share different trophic level with mosquito larvae in rock pools and may not constitute feeding competitors with mosquito larvae. With regard to bottom foraging of Hemipteran predators, turbidity could reduce the predator's efficiency to detect and catch mosquito larvae and other preys, as was demonstrated with predatory fishes (Shaalan *et al*., 2007). A more likely common mechanism is that the controphic species could serve as an alternative prey source for the predator, thus diluting predation intensity on mosquito larvae in the absence of numerical predator responses. The most common and famous dipteran mosquito predator, *Toxorhynchites*species (Shaalan *et al*., 2007) was not encountered in this study. But another predator mosquito, *Culex tigripes*, was encountered at very low density on the

Nok inselberg. Since prey vulnerability is a product of prey encounter rates with and ease of capture by the predator (Kumar, 2005), certain behavioural attributes of mosquito species that determine capturability, and their relative proportions in the habitats could explain the observed mutually negative interaction amongst the predators and mosquito larvae in the rock pool habitats.

Usually plant covers are very sparse in rock pools due to severe environmental conditions (high insulation and evaporation rates) on inselbergsand because of the lack of a continuous soil cover (Porembski, 2007).These could probably explain the overt absence of macrophytes in majority of the pools sampled for mosquito breeding. Despite the harsh growth conditions on inselbergs, plants belonging to the families Liliaceae and Schrophulariaceae (i.e. *Chlorophytum laxum* and *Dopatrium longidens* respectively) must have develop specific adaptive traits that led to their colonization and preponderancewithin the hydroregime of the rock pools. According to Porembski (2007), both species of macrophyte arefrequent colonizers of marshy grounds and ponds.Macrophytes could permit the fixation of *Mansonia* species ofmosquito (not encountered in this study) to their roots, thus representing a potentially favourable breeding ground for such species of mosquito.They live generally attachedto the macrophyte roots and stems by means of their siphon (forlarvae) or trumpet (for pupae), which are modifiedrespiratory apparatuses through which they are ableto obtain oxygen directly from the lacunal spaces in theplant tissues(Kengne *et al.*, 2003).Associationsof mosquito larvae with these aquatic macrophytescould also be for the derivation of the oxygen released by the plants and possible provision of hide outs from sympatric predators.

CHAPTER SIX

6.0 CONCLUSIONAND RECOMMENDATIONS

6.1 Conclusion

This study has documented the occurrence and abundance of thirteen species of mosquito larvae (*Ae. vittatus, An. arabiensis, An. gambiae s.s., An. longipalpis, An. pretoriensis, An. rufipes, Cx. albiventris, Cx. horridus, Cx. macfiei, Cx. perfidious, Cx. pipiens pipiens,Cx. simpsoni* and *Cx. tigripes*)distributed in three genera (*Aedes, Anopheles* and *Culex*). *Aedes vittatus* was by far the most dominant mosquito encountered in all the twenty one (21) sampling locations. Epidemiologically, encountered mosquito species were potential vectors of malaria, yellow fever, dengue,filariasis and the encephalides. Two important malaria vectors (*An. gambiae s.s.* and *An. arabiensis*) were genomically identified breeding in sympatry in rock pool habitats within the state. The first hypothesis is therefore rejected.The anthropogenic activities (quarrying) observed on the inselbergs along with weather could be responsible for sustaining theriskof disease vectors andthespreadofvector-bornediseases, thus extension of their geographic distribution in the study area. Diverse invertebrate and vertebrate taxa from protozoans to reptilian as well as microalgae and macrophytes occurred in rock pools within the study locations.Encountered biota were partly potential food organisms while others were potential mosquito predators. The second hypothesis is also rejected.The abundance and distribution of preimaginal stage of mosquitoes in rock pools is determine by the physicochemical condition of the rock pool habitats.Water pH and temperature appeared to play significant determinant roles in the occurrence and abundance of mosquito species in rock habitats, hence third hypothesis is rejected.Knowledge on mosquito ecology in relation to biological and physicochemical parameters facilitating mosquito breeding in rock pools have bearing

on vector population and distribution and consequent diseases transmission in the study locations.Therefore, rock pools should be inspected to incriminate vectors and need to be incorporated in mosquito control equations.

6.2 Recommendations

There is need to educate residents in the study locations and religious groupson the inselbergs concerning the vectorial roles of the encountered mosquito species in rock pools to prevent the spread of disease in surrounding locations. Introduction of biological competitors and predators of mosquito larvae that are desiccation tolerant into rock pools may also be a more convenient and reduce the need for frequent chemical applications and consistent involvement of residents in antilarval measures on the inselbergs.Therefore, use of mosquito control tools that will not ruin or harm biological control prospect but which incorporate indigenous community participation should be contemplated in rock pools to stem vectorial roles of identified species. Mosquito predatory roles of the potential predators encountered need to be ascertained in order to explore their real biological control potentials against noxious species. Succession pattern of rock pool communities need to be further exposed. Future lines of research should unravel food chain and food web nature of rock pool communities in the study locations and elsewhere. A further study to test the biological and physicochemical variables on mosquito breeding in the laboratory is also recommended.

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APPENDICES

Appendix I: Correlation analysis of some physicochemical parameters on biological communities of rock pool habitats

		TEM	PH	EC	TDS	LAR	PLA	MIC	MAC	VER	ALG
TEM	Pearson Correlation	1	-.127*	.259**	.250**	.006	-.043	.139**	-.014	.058	.208**
	Sig. (2-tailed)		.012	.000	.000	.903	.401	.006	.789	.256	.000
PH	Pearson Correlation	-.127*	1	-.072	-.076	.025	.091	-.027	.009	-.013	.070
	Sig. (2-tailed)	.012		.160	.136	.626	.075	.592	.863	.794	.170
EC	Pearson Correlation	.259**	-.072	1	.996**	.072	-.080	.078	-.023	-.018	.129*
	Sig. (2-tailed)	.000	.160		.000	.160	.118	.124	.653	.730	.011
TDS	Pearson Correlation	.250**	-.076	.996**	1	.074	-.070	.073	-.024	-.018	.129*
	Sig. (2-tailed)	.000	.136	.000		.149	.167	.152	.636	.719	.011
LAR	Pearson Correlation	.006	.025	.072	.074	1	-.043	-.049	.134**	-.046	.092
	Sig. (2-tailed)	.903	.626	.160	.149		.398	.333	.008	.368	.071
PLA	Pearson Correlation	-.043	.091	-.080	-.070	-.043	1	.024	-.031	.138**	.049
	Sig. (2-tailed)	.401	.075	.118	.167	.398		.632	.548	.007	.341
MIC	Pearson Correlation	.139**	-.027	.078	.073	-.049	.024	1	.065	.016	.000
	Sig. (2-tailed)	.006	.592	.124	.152	.333	.632		.203	.754	.993
MAC	Pearson Correlation	-.014	.009	-.023	-.024	.134**	-.031	.065	1	.045	.047
	Sig. (2-tailed)	.789	.863	.653	.636	.008	.548	.203		.377	.357
VER	Pearson Correlation	.058	-.013	-.018	-.018	-.046	.138**	.016	.045	1	.205**
	Sig. (2-tailed)	.256	.794	.730	.719	.368	.007	.754	.377		.000
ALG	Pearson Correlation	.208**	.070	.129*	.129*	.092	.049	.000	.047	.205**	1
	Sig. (2-tailed)	.000	.170	.011	.011	.071	.341	.993	.357	.000	

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

AppendixII: Pearson Correlation Analysis of Some Biological and Physicochemical Parameters of Rock Pool Habitats on Mosquito Larvae.

		HARD	TURB	NITRA	PHOSPH	COD	ALKAL	TSS	DO	BOD	LARVAE	PLANTS	MICRO	MACRO	VERTEB	ALGAE
HARD	Pearson Correlation	1	.112	-.057	-.032	-.222	.349*	-.056	-.021	-.030	.107	-.275	-.142	-.050	.072	.044
	Sig. (2-tailed)		.439	.695	.828	.121	.013	.698	.886	.836	.458	.054	.326	.731	.620	.763
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
TURB	Pearson Correlation	.112	1	.676**	.436**	.255	.386**	-.043	-.082	-.080	-.064	-.096	.104	.026	-.088	.375**
	Sig. (2-tailed)	.439		.000	.002	.074	.006	.768	.571	.581	.659	.508	.471	.859	.546	.007
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
NITRA	Pearson Correlation	-.057	.676**	1	.720**	.465**	.335*	.212	-.155	-.156	-.025	-.139	.297*	.045	-.091	.263
	Sig. (2-tailed)	.695	.000		.000	.001	.017	.140	.283	.278	.862	.335	.036	.758	.528	.065
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
PHOSPH	Pearson Correlation	-.032	.436**	.720**	1	.258	.292*	-.049	-.148	-.152	.047	-.255	.091	-.039	-.103	.305*
	Sig. (2-tailed)	.828	.002	.000		.070	.040	.734	.305	.290	.744	.074	.531	.785	.478	.031
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
COD	Pearson Correlation	-.222	.255	.465**	.258	1	.025	.064	.117	.124	-.005	-.215	.091	.111	-.037	.162
	Sig. (2-tailed)	.121	.074	.001	.070		.864	.661	.420	.389	.974	.133	.528	.444	.800	.260
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
ALKAL	Pearson Correlation	.349*	.386**	.335*	.292*	.025	1	.117	-.077	-.085	.029	-.236	-.066	-.044	-.044	.313*
	Sig. (2-tailed)	.013	.006	.017	.040	.864		.420	.597	.556	.841	.099	.648	.763	.764	.027
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

AppendixII Cont'd: Pearson Correlation Analysis of Some Biological and Physicochemical Parameters of Rock Pool Habitats on Mosquito Larvae.

		HARD	TURB	NITRA	PHOSPH	COD	ALKAL	TSS	DO	BOD	LARVAE	PLANTS	MICRO	MACRO	VERTEB	ALGAE
TSS	Pearson Correlation	-.056	-.043	.212	-.049	.064	.117	1	-.030	-.030	-.031	.097	.096	-.056	-.030	-.043
	Sig. (2-tailed)	.698	.768	.140	.734	.661	.420		.836	.837	.828	.502	.508	.700	.839	.767
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
DO	Pearson Correlation	-.021	-.082	-.155	-.148	.117	-.077	-.030	1	.995**	.441**	.059	-.108	.343*	-.064	.055
	Sig. (2-tailed)	.886	.571	.283	.305	.420	.597	.836		.000	.001	.686	.457	.015	.657	.705
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
BOD	Pearson Correlation	-.030	-.080	-.156	-.152	.124	-.085	-.030	.995**	1	.394**	.042	-.112	.327*	-.065	.039
	Sig. (2-tailed)	.836	.581	.278	.290	.389	.556	.837	.000		.005	.772	.440	.020	.655	.789
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
LARVAE	Pearson Correlation	.107	-.064	-.025	.047	-.005	.029	-.031	.441**	.394**	1	-.109	-.048	.590**	-.055	.253
	Sig. (2-tailed)	.458	.659	.862	.744	.974	.841	.828	.001	.005		.451	.741	.000	.704	.077
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
PLANTS	Pearson Correlation	-.275	-.096	-.139	-.255	-.215	-.236	.097	.059	.042	-.109	1	.251	.063	-.099	-.088
	Sig. (2-tailed)	.054	.508	.335	.074	.133	.099	.502	.686	.772	.451		.079	.662	.496	.543
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
MICRO	Pearson Correlation	-.142	.104	.297*	.091	.091	-.066	.096	-.108	-.112	-.048	.251	1	.470**	-.023	-.083
	Sig. (2-tailed)	.326	.471	.036	.531	.528	.648	.508	.457	.440	.741	.079		.001	.873	.568
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

AppendixII Cont'd: Pearson Correlation Analysis of Some Biological and Physicochemical Parameters of Rock Pool Habitats on Mosquito Larvae.

		HARD	TURB	NITRA	PHOSPH	COD	ALKAL	TSS	DO	BOD	LARVAE	PLANTS	MICRO	MACRO	VERTEB	ALGAE
MACRO	Pearson Correlation	-.050	.026	.045	-.039	.111	-.044	-.056	.343*	.327*	.590**	.063	.470**	1	-.041	.129
	Sig. (2-tailed)	.731	.859	.758	.785	.444	.763	.700	.015	.020	.000	.662	.001		.778	.372
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
VERTEB	Pearson Correlation	.072	-.088	-.091	-.103	-.037	-.044	-.030	-.064	-.065	-.055	-.099	-.023	-.041	1	.283*
	Sig. (2-tailed)	.620	.546	.528	.478	.800	.764	.839	.657	.655	.704	.496	.873	.778		.047
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
ALGAE	Pearson Correlation	.044	.375**	.263	.305*	.162	.313*	-.043	.055	.039	.253	-.088	-.083	.129	.283*	1
	Sig. (2-tailed)	.763	.007	.065	.031	.260	.027	.767	.705	.789	.077	.543	.568	.372	.047	
	N	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)