

WEATHER, WATER AND MALARIA MOSQUITO LARVAE

The impact of meteorological factors on water temperature and larvae of the Afro-tropical malaria vector *Anopheles gambiae* Giles

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ABSTRACT

Malaria control using drug treatment and insecticide-impregnated nets or the treatment of mosquito resting sites with long-lasting insecticides are increasingly failing, as drug-resistant parasites emerge and mosquito vectors develop resistance against insecticides. Because malaria is one of the most important infectious diseases in the world, affecting over 350 million and killing over one million people annually, alternative control strategies have been introduced or are under development. Malaria control methods that aim to reduce adult vector populations by targeting their aquatic immature stages are gaining ground. These aquatic stages determine the abundance, dynamics and fitness of mosquito adults and, hence, affect the transmission of malaria. A better fundamental understanding of the biology and ecology of these essential stages could contribute to the implementation of current control methods and to the development of novel strategies. A better understanding of the factors that affect the life-history characteristics of mosquito larvae could furthermore improve current models that assess malaria risk but often do not incorporate the immature stages of the malaria vectors.

The aim of this thesis was to study the effects of physical and biological factors on the biology and ecology of immature stages of the malaria vectors *Anopheles arabiensis* and *An. gambiae*, the most broadly distributed and most efficient vectors of malaria in sub-Saharan Africa. This thesis shows how meteorological variables can affect the life-history characteristics of larvae of *An. arabiensis* and *An. gambiae* and the temperature of their aquatic environment. Meteorological variables and water temperature in differently-sized, semi-natural habitats, which were exposed to the ambient environment, were studied in western Kenya, as well as the growth, development and survival of the immature stages of the malaria vectors in identical habitats.

As temperature is an important determinant in the growth, development and survival of malaria mosquito larvae, more accurate measurements are needed of the temperature fluctuations to which these larvae are exposed on a daily basis. Such measurements in clear, semi-natural water bodies are shown in Chapter 2. The diurnal water temperature dynamics in the smallest habitat differed on both a spatial and a temporal scale from that in larger water bodies. Combining these water temperature data with local meteorological data, a model was developed that accurately predicts the daily fluctuations of the water temperature (Chapter 2). Water temperature dynamics were furthermore affected by habitat turbidity: Turbid water bodies had a higher near-surface water temperature during daytime than clear

water bodies of identical dimensions (Chapter 3).

In addition to the physical experiments, biological experiments were conducted in identical water bodies. Larvae were reared in similar transparent plastic cups that floated in habitats of different sizes, to assess the impact of diurnal water temperature variations on the life-history characteristics of the larvae (Chapter 4, 5). The larval development rate of both species was positively correlated with the size of a habitat: larvae developed more quickly into adults when amplitude of the daily fluctuations of the water temperature decreased (Chapter 4). When the larvae occupied turbid water, their growth and development rates decreased compared to those in clear water. *An. gambiae* furthermore showed a reduction in larval survivorship in turbid water. As both species frequently co-exist in larval habitats, the effects of competition between both species were assessed. When larvae of *An. arabiensis* and *An. gambiae* shared a habitat, their development rate was significantly affected, but in opposite ways: The larval development rate of *An. arabiensis* decreased whereas the larval development rate of *An. gambiae* increased. Moreover, in the smallest water bodies, the mortality of both species seemed to increase when they shared a habitat (Chapter 5). Finally, the direct effect of rainfall on the survival of *An. gambiae* larvae was examined. There were high additional losses of larvae during nights with rainfall compared to dry nights (Chapter 6).

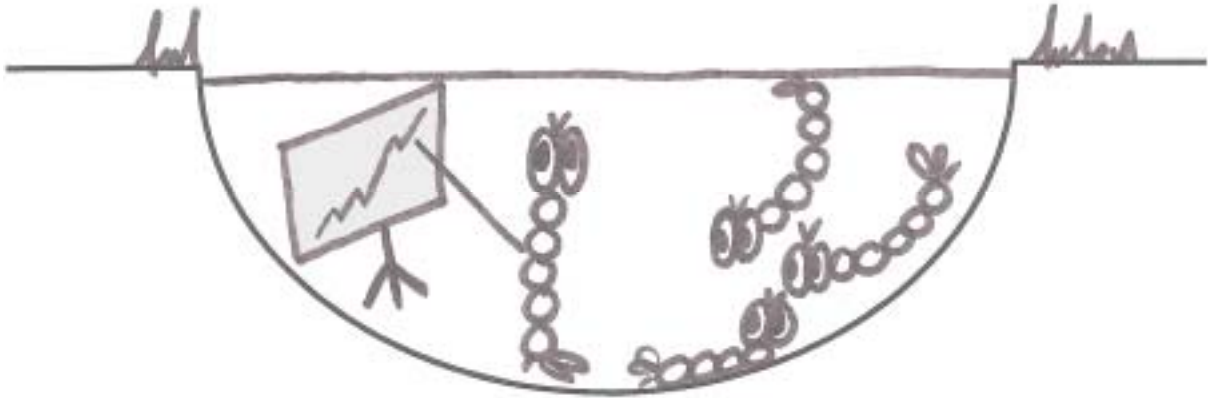
The findings described in this thesis demonstrate that biotic and abiotic factors can significantly affect the growth, development and survival of the aquatic stages of malaria mosquitoes in an important but complex way. Future studies on malaria epidemiology and malaria control should consider these factors in order to improve malaria risk predictions and to successfully incorporate the immature stages of the mosquitoes in malaria intervention strategies. As species-specific interactions occur, which may give cause to differential development, detailed knowledge at species level is required before interventions are implemented.

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Introduction

Krijn Paaijmans



Malaria continues to be one of the most important health problems worldwide. The disease is transmitted by anopheline mosquitoes, which, particularly in the tropics, are well adapted to malaria parasites (*Plasmodium* spp.) affecting humans. Malaria control is achieved mostly by drug treatment and insecticide-impregnated bed nets or treatment of mosquito resting sites with a long-lasting insecticide. However, these tools are increasingly failing because of the development of drug resistance in the parasites and insecticide resistance in the mosquito vectors. For these reasons, new strategies of malaria control are required that target the parasite, the vector, or both. As vector control has been recognised as the most effective way of malaria control, through the interruption of parasite transmission, numerous studies focus on aspects of the vector that would allow to achieve this goal. Most studies focus on the adult stage of malaria vectors, and surprisingly few studies are directed to understanding the biology and ecology of the aquatic immature stages. Yet, these stages determine the abundance, dynamics and fitness of the adults, and, as a consequence, malaria transmission. The research in this thesis was undertaken to get a better fundamental understanding of the effects of abiotic and biotic factors on the biology and ecology of immature stages of Afro-tropical malaria vectors.

1.1 The what, where and how of malaria

1.1.1 Malaria parasites

Malaria remains undisputable one of the most important tropical infectious diseases in humans worldwide, next to HIV/AIDS and tuberculosis. It is caused by protozoan (single-celled) parasites of the genus *Plasmodium*. Four *Plasmodium* species affect human health: *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. A fifth species, the primate malaria *P. knowlesi*, is able to infect humans as well [1]. Infections with *P. falciparum* and *P. vivax* cause most of the clinical malaria cases worldwide [2]. Especially in non-immune individuals, infections with *P. falciparum* are always life-threatening [3].

1.1.2 The burden of malaria

Infections with malaria will result in symptoms such as anaemia, fever and chills, and may eventually lead to a coma or even death. However, the severeness of the symptoms will depend on the level of immunity of an individual. According to the latest World Malaria Report by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), there are between 350 and 500 million clinical cases per year worldwide [2]. However, Snow et al. [4] argued that these numbers are likely to be underestimated.

Concerning human mortality, *P. falciparum* causes over 1 million deaths annually [2]. The estimates of total mortality cases, caused by malaria alone or in combination with other diseases, vary from 1.1 to 2.7 million persons annually [5]. Especially Africa is a continuous malaria centre, from the southern border of the Sahara to South Africa and Namibia [6]. Over 80% of malaria deaths occur here [2] and most of them are children under the age of five years [7].

Besides the morbidity and mortality it engenders, malaria affects the quality of daily life [8]. Examples are the loss of a day's work and therefore the day's income, costs for travelling to and a possible stay in a clinic, expenses for treatment and maybe a burial of a relative, in addition to the pain and grief it can cause. This involves relatively high expenses, as half of the population in sub-Saharan Africa lives on less than one dollar a day [9]. Not surprisingly, therefore, poverty and malaria go hand in hand [10]. Unfortunately, although malaria is so easily treated and prevented, the burden is still increasing due to factors as drug and insecticide resistance, social and environmental changes, travel and population increase [see e.g. 11].

1.1.3 African malaria mosquitoes

Besides a vertebrate host, the life cycle of malaria parasites involves a mosquito. Human malaria is transmitted by female mosquitoes of the genus *Anopheles* from human to human. Approximately 422 species of *Anopheles* are known, of which 68 are indicated as vectors of malaria [12]. The principal vectors of malaria in sub-Saharan Africa include two members of the *Anopheles gambiae* Giles sensu lato complex (hereafter referred to as *An. gambiae* s.l.): *Anopheles gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*) and *Anopheles arabiensis* Patton, and *Anopheles funestus* Giles. As the work presented in this thesis focuses on the vector species *An. arabiensis* and *An. gambiae*, *An. funestus* is not further discussed.

Of the seven currently known sibling species of the *An. gambiae* s.l. complex [13], *An. arabiensis* and *An. gambiae* belong to the most effective vectors of malaria parasites in Africa [14]. Both species are morphologically indistinguishable, but can be identified to species with the Polymerase Chain Reaction or PCR [15]. They furthermore differ in their behaviour: *An. gambiae* is highly anthropophilic [16,17], meaning it almost exclusively feeds on humans, and is, unlike *An. arabiensis*, not likely to be found in the absence of human settlements [18]. *An. arabiensis*, on the other hand, is a more opportunistic mosquito: It may feed on humans as well as on animals, depending on host availability [16,17,19] and can

therefore also be found in areas with scarce or no human population [20,21]. After having taken their blood-meals, females of *An. gambiae* are likely to rest indoors (endophilic), whereas females of *An. arabiensis* are seeking a place to rest outdoors (exophilic) in structures such as in granaries [16,19,22]. However, it has also been shown that females of *An. arabiensis* may feed outdoors and subsequently fly indoors to rest [23] and recently Odiere et al. [24] suggested that females of *An. arabiensis* will divide into indoor and outdoor populations, depending on host availability.

1.2 Ecology of *Anopheles arabiensis* and *Anopheles gambiae*

1.2.1 Spatial and temporal distribution

Although *An. gambiae* is usually the predominant species in wet environments with high humidity and *An. arabiensis* is more common in hotter zones with less rainfall [25,26], both species occur sympatrically across a wide range of tropical Africa [14]. Breeding of *An. gambiae* is mostly restricted to the rainy seasons with larval and adult densities increasing rapidly and the species predominating over *An. arabiensis*, which is the more dominant species during the dry periods [17,22,27-32].

1.2.2 Gonotrophic cycle

The gonotrophic period or gonotrophic cycle is defined as the time period between two ovipositions. This period includes the search for a host, the ingestion and digestion of a blood meal, the maturation of the ovaries and the search for a suitable aquatic breeding site to deposit the mature eggs. Each gonotrophic cycle lasts about 2-4 days for *An. gambiae* s.l. [31,33-35], but its length will depend on factors such as breeding site availability [36], number of previous gonotrophic cycles [37,38] and temperature [37,38].

In the field only a small percentage of females of *An. gambiae* s.l. survive for more than three or four gonotrophic cycles [20,33,35], although a small percentage was found to survive for over ten cycles [35]. *An. arabiensis* and *An. gambiae* can survive for up to approximately one month, whereby *An. gambiae* can survive longer than *An. arabiensis* [20,34,39]. Daily survival estimates of mosquitoes of *An. gambiae* s.l. vary from 0.71-0.95 [31,32,34,35,40-43], but these values largely depend on which methods are used for the estimations. Their lifespan depends on temperature and humidity [44] and survival rates decrease with the age of the females [35,39].

An. gambiae s.l. deposits its eggs throughout the night, approximately two days after a blood meal [45]. In the laboratory, however, it was observed that two-thirds of the females

deposited all their eggs over two consecutive nights [46]. Eggs are laid singly on the water surface or on mud and even on moist sand [47] and are found to be dispersed over several habitats [48,49]. Based on the number of developing eggs in wild-caught females, an average of 150 eggs per female was recorded, with a range from 66 to 275 eggs [33]. Another study showed an average of about 200 eggs per wild-caught female [50].

Why a female chooses some water bodies and not others for oviposition, is poorly understood. It is known that dark substrates receive more eggs than light ones and moist substrates more than dry ones [47,51,52]. Furthermore, turbid water was preferred over clear water [52,53]. By contrast, Munga et al. [54] found that *An. gambiae* preferred clear rainwater over natural water from forests and natural wetlands, which contained more impurities. Other factors that may play an important role in habitat selection are volatile compounds that are produced by microbial populations in the breeding site [55], chlorophyll a content in the breeding site [56] or the presence of conspecific larvae or aquatic predators [57]. Finally, the distance between oviposition site and blood host may affect the oviposition choice. Charlwood & Edoh [58] collected significantly more immatures of *An. arabiensis* than *An. gambiae* from breeding sites near cattle and suggested that species distribution may be explained to a large extent by the presence of suitable hosts instead of breeding site availability. Minakawa et al. [30] showed that immatures of *An. gambiae* would be found in breeding sites closer to houses and further away from cowsheds and a study by Charlwood and Edoh [58] showed that significantly more larvae of *An. arabiensis* than *An. gambiae* were collected in pools close to cattle.

However, despite these findings, the exact mechanisms behind habitat selection are not fully understood and it is not known whether females can choose for habitats that we consider as advantageous for their immatures: Anopheline larvae are frequently absent in suitable breeding sites [59-61].

1.2.3 Larval habitats

There are six immature stages during mosquito development; the egg stage, four larval stages and the pupal stage. After hatching of the eggs, anopheline larvae float horizontally just below the air-water interface (Figure 1.1), making them unique from other mosquito species such as *Aedes* spp. and *Culex* spp., which hang downwards from the air-water interface. The larvae of *An. gambiae* s.l. breathe atmospheric oxygen through two 'spiracular openings' on the eighth segment of their abdomen and feed by moving brushlike structures

on their mouthparts that create a current of water [62]. They filter out microorganisms, particulate organic matter or detritus and biofilm [62,63].

Typical habitats of *An. arabiensis* and *An. gambiae* are puddles, shallow ponds, borrow-pits, brick-pits, tyre tracks, ditches, human foot and animal hoof prints [see 64 for illustrations] and are often created by activities of humans or domestic animals [60,61,65]. These habitats are open, containing no, little or low (grass) aquatic vegetation [29,60,66] and are often of a transient nature, as their availability corresponds to precipitation [29,60,61]. *An. gambiae* s.l. can colonize a breeding habitat within a few days after the site is created [67]. Besides in temporary habitats, *An. arabiensis* is also found in market garden wells [68] and water storage tanks [69 in:70]. Another typical characteristic of breeding sites of *An. gambiae* s.l. is their shallow nature. Gimnig et al. [29] showed that water bodies inhabited by *An. arabiensis* were on average 18.0 (95% CI: 3.5) cm deep, by *An. gambiae* 29.4 (± 10.7) cm and by both species 9.7 (± 4.1) cm. In another field study, average depths of 6.2 (± 5.3 SD) and 10.6 (± 7.2) cm were recorded in dirt tracks and in ditches, respectively [61].



Figure 1.1 An *Anopheles gambiae* larva floating horizontally just below the air-water interface. The larva is reflected in the water.

Despite the dogma that *An. gambiae* s.l. is most often found in turbid water collections, various studies that examined the characteristics of larval habitat or larval population dynamics, failed to give a clear relationship between the presence of *An. gambiae* s.l. immatures and the clarity of breeding sites. Some studies reported no effect of turbidity on the occurrence of *An. gambiae* s.l. [53,71], other studies showed a preference of *An. gambiae* s.l. to breed in rather clear water bodies [68,72], whereas Gimnig et al. [29] observed that *An. gambiae* and *An. arabiensis* were associated with habitats that were high in turbidity and that both species increased in larval densities with increasing water turbidity. Moreover, larvae of *An. gambiae* s.l. were found in habitats that were highly polluted by rotting vegetation, human faeces and even oil in an urban area [72].

An. arabiensis and *An. gambiae* are often found to share larval habitats [29,30,48,53,58,73,74]. A clear difference in requirements for the larval environment of the two species has not been observed, but is subject of discussion. Several studies suggest the requirements are similar [29,58,75], others think they differ, but were unable to show that explicitly [53].

1.2.4 Habitat productivity

How productive a breeding site is in terms of numbers and size of mosquitoes it produces over time, depends, apart from the initial number of eggs that are deposited, on the growth, development rate and survival of the mosquito immatures. Larval development rate, survival and adult size affect the transmission of malaria: The time to develop from an egg into an adult, combined with larval survivorship, determines the numbers of emerging mosquitoes over time. The size of the emerging adults is of importance, as larger females survive longer [76,77] and have a greater fecundity [77,78]. Moreover, smaller and virgin females require a second or third bloodmeal in order to develop mature eggs, prolonging the time to their first oviposition [78]. Adult size furthermore affects host seeking behaviour [76] and parasite infectivity: Intermediate-sized mosquitoes were found to be more infectious to humans [79]. Various biotic and abiotic factors affect the growth, development and survival of the immature mosquitoes and consequently affect habitat productivity (Table 1.1).

Under laboratory conditions, where larvae were exposed to constant temperatures, Bayoh and Lindsay [80] showed that larvae took 9.8 up to 23.3 days to develop into adults, depending on the temperature. Another laboratory study investigated the duration between oviposition and pupation and reported a time period between 7 and 27 days [50]. In the field, it has been shown that the duration of the immature lifetime of *Anopheles gambiae* s.l.

ranges from 8 to 22 days [81] in habitats of different size: Eggs hatch within one day, larvae grow into pupa within 6-19 days and the pupal stage lasts 1-2 days. A field study by Gimnig et al. [82] observed a shorter time range of the development from egg to adult, which was 8.4-11.5 days. However, these experiments were carried out in habitats of the same size. Service [83,84] observed that larvae, which newly hatched from the eggs, took on average 11.8 days to develop into adults, in small ponds and pools, ditches and rice fields.

The mortality observed among the immature stages of *An. gambiae* s.l. in the field is extremely high. Overall only a small fraction (2-8%) of the larvae eventually survives to the adult stage [74,83-89].

It is highly likely that many biotic and abiotic variables, which are mentioned in Table 1.1, interact and that, therefore, a combination of factors affect the productivity of a breeding site [53,68]. In general it is believed that nutrition, larval densities and water temperature are the principal contributing factors that affect growth and development of mosquito immatures [63]. In the remainder of this chapter the focus will be on the latter contributing factor, water temperature.

1.3 Temperature dependency

1.3.1 Insects in general

The distribution and abundance of an insect species depends, besides on its own biological characteristics and the influence of other organisms, on its physical environment. Weather plays a major role [90,91], as insects are poikilothermic or cold-blooded. Metabolic heat, that is generated by most insects themselves, is limited and has little effect on their body temperature [92]. Therefore, their metabolic rate and thus the growth and development rate of insects depend on the temperature of their direct environment.

In general, insects experience an increased growth rate and grow to smaller sizes at higher temperatures [93]. This is due to an increase in enzyme-catalyzed reactions at higher temperatures. There is, however, a certain optimal temperature. When the temperature increases further, the shape of these enzymes will be altered, which destroys their activity. This may eventually lead to the insect's death. Besides this effect on the metabolism of an insect, temperature may affect the nervous and endocrine systems [94].

Insects can respond to unfavourably high temperatures in various ways. They can change their behaviour [95] or their development [96], preventing them from being injured. At the cellular level, insects may increase their thermotolerance by the production of heat shock proteins (hsps) [94,96,97].

Table 1.1 Known biotic and abiotic factors that affect the growth, development and survival of larvae of *An. gambiae* s.l., including references.

Biotic factors	Growth and development	Survival	References
Intraspecific competition			
	x	x	[98]
	x	x	[99]
		x	[100]
	x		[82]
	x		[101]
		x	[102]
	x		[103]
Cannibalism			
		x	[101]
		x	[102]
		x	[89]
Interspecific competition			
		x	[100]
		x	[102]
Predation by sibling species			
		x	[101]
		x	[102]
Predators, parasites and/or pathogens			
		x	[57]
		x	[83-85]
		x	[88]
		x	[86]
		x	[74]
		x	[87]
Nutrition			
	x	x	[99]
	x		[102]
	x	x	[104]
		x	[105]
	x	x	[106]
Abiotic factors			
Desiccation of breeding sites			
		x	[74]
Water depth			
		x	[107]
Soil quality in a habitat			
	x	x	[108]
Water temperature			
	x	x	[98]
	x	x	[80]
		x	[109]

The rate at which mosquito immatures develop is also temperature dependent. Higher temperatures result in a faster development, but in smaller females, as is observed in various mosquito species [see e.g. 110,111,112]. However, there is a limit to larval growth: When larvae develop too fast, many larvae may not accumulate sufficient metabolic reserves for

eclosion and will eventually die [80], as there is a certain critical mass that larvae have to obtain before they pupate [63].

The ability to withstand high temperatures differs between species and even between larval instars, whereby older larvae seem more susceptible to heat than younger ones [113,114]. Apart from the water temperature itself, length of exposure and the rate of temperature change affect larval mortality [114].

The exposure to very high temperatures may damage the larvae irreversibly. This has been shown by Jalil [115] in *Aedes triseriatus* (Say): When larvae were reared at 25 °C, after an exposure to 38 °C, they did not develop into adults. The same study showed that low temperatures can act as inhibitor: Larvae that were reared at low temperatures (6-8 °C) stopped developing, but continued growing when they were placed at 25 °C [115]. Therefore, low temperatures do not necessarily imply an insect's death. However, this effect will again depend on the temperature and the duration of exposure.

1.3.2 *Anopheles gambiae* s.l.

As mentioned previously, breeding sites of *An. gambiae* s.l. are often small, open and sunlit pools. As mosquito larvae are confined to their habitat, they are directly exposed to the temperature of the water that surrounds them. A literature overview of developmental times of *An. gambiae* s.l. immatures at various temperatures found in various laboratory and field studies is given by Depinay et al. [116]. The authors modelled these data and found that the development rate increased up to temperatures of approximately 37 °C, after which it sharply decreased. Water temperatures above 37 °C are, although momentary, observed in natural water collections in tropical areas [53,54,81,117-119]. Bayoh and Lindsay [80,109] further found that no adults were emerging when larvae were reared below 18 °C or above 32 °C. However, in the studies of Bayoh and Lindsay the larvae were reared at constant temperatures, whereas the temperature in natural water collections fluctuates during the day. When larvae are exposed to unfavourable, high temperatures, they will only be exposed to them for a short time period. When the thermal death point of larvae of *An. gambiae* s.l. was determined by exposing larvae of *An. gambiae* s.l. to high temperatures for a few hours only, a tolerance of up to 41 °C was observed [81,120]. Higher temperatures were lethal.

How these diurnal fluctuations in temperature will affect insect development depends on the magnitude and duration of the alternating phases [121]. To our knowledge, experiments in which larvae of *An. gambiae* s.l. are exposed to temperature fluctuations are limited. Lyimo [74] observed that females of *An. gambiae* s.l. (population consisted of 25% *An. arabiensis*

and 75% *An. gambiae*), which emerged from larger water bodies such as rain pools, burrow pits and ditches where the daily temperature fluctuations were more moderate than in smaller water bodies, had on average longer wings than those emerging from small puddles and water-filled footprints. Longer wings are associated with a larger body size [76,122]. However, the study of Lyimo did not control for factors such as pathogens and nutrition.

Both the average water temperature and the daily variations in water temperature, to which mosquito larvae are exposed in the field, are regulated by the natural variation in energy fluxes in the atmospheric as well as water boundary layers.

1.4 Land-water and water-atmosphere interactions

1.4.1 Energy balance

Solar or shortwave radiation is the driving force of the earth's weather and climate and of biological and physical processes. Part of this shortwave radiation reaches the earth surface directly (direct radiation); part is absorbed or scattered by the atmosphere. Some of the scattered radiation will still reach the earth surface (diffuse radiation).

As all objects with a temperature above $-273\text{ }^{\circ}\text{C}$ (absolute zero or 0 Kelvin) emit longwave radiation, the earth's surface receives energy from the atmosphere and emits radiation to the atmosphere as well. The continuous exchange of energy between the earth's surface and the atmosphere determines the net radiation, R_n , which is the net amount of energy that is available at the earth's surface.

This net radiation can also be determined over a water surface. A water body receives solar radiation and reflects part of this shortwave radiation (depending on the angle at which the radiation reaches the water surface and properties of the water surface) and receives and emits longwave radiation.

The net radiation, which is available at the air-water interface of shallow and stagnant water bodies, is used for various processes [123]:

$$R_n = H + LE + P + G + \Delta S \quad (1.1)$$

Based on the fundamental law of energy conservation, the net radiation is balanced by the sum of the sensible heat flux or the heating of the air by the underlying water surface (H), the latent heat flux or energy for evapotranspiration (LE), energy supplied or extracted by rainfall (P) and the soil heat flux between the water and surrounding soil (G). The amount of net

energy that is stored in or extracted from the water body (ΔS) determines the magnitude of the change in water temperature.

Due to its physical properties (Box 1.1), water is an important source of storage and transport of energy.

Box 1.1 Important physical properties of water

- With cloudless skies and the sun at least 30° above the horizon, water is one of the most effective solar radiation absorbing surfaces [123]. The albedo (fraction of shortwave radiation reflected back into the atmosphere) depends on solar angle and is low (0.06) near the equator [124].
- 40-50% of the incoming solar radiation (the near infra-red part) is absorbed in the upper water layer [125].
- Most longwave radiation is absorbed by a water body; emissivity (ratio of radiation emitted by the surface to radiation emitted by a blackbody at same temperature) = 0.97 [125].
- Water has a high specific heat capacity. This means a large amount of heat energy is stored before the water begins to warm up.
- Water conducts heat more easily than any liquid, except mercury [126].
- Water is a fluid, so heat transport within water is possible not only by conduction and radiation but also by convection and advection.

1.4.2 Temperature state of a water body

The extinction of the incoming shortwave radiation in stagnant water depends on its wavelength and its incoming angle, and on the turbidity of the water. Components such as clay, silt and suspended particles of organic matter contribute to turbidity [127] and absorb and scatter shortwave radiation. Extinction of solar radiation is of great importance for the temperature of the water in the upper layer, as most of the extinction takes place in the upper layer of a water body. Two temperature situations can be observed in shallow and stagnant collections of water: All layers can be well-mixed (homogeneous water body) or they can differ in temperature over depth (stratified water body).

Stratification in a water body occurs when the extinction of radiation in a water column is high, diminishing the vertical transport of energy and mass. Stratification is driven by the absorption of solar radiation, but other factors such as exposure to wind, turbulence in and turbidity of the water also play an important role. However, a water body has to be sufficiently deep and/or turbid for stratification to occur and shallow water collections may experience

both thermal states within a 24h cycle: At daytime or during periods with low wind conditions, water may be stratified. At night or during periods with high wind speed, a water body may be homogeneous in temperature.

1.4.3 Diurnal temperature variations in shallow water bodies

In general, the behaviour of the water temperature of large, shallow and temperate water collections, such as lakes, differs per season. Stratification occurs during the warmer months, but disappears at the beginning of cooler periods when the thermocline descends rapidly [128,129]. In African lakes near the equator where the maximum depth of the lake is less than about 15-30 meters, complete mixing occurs frequently [130,131 in:132]. In a Kenyan reservoir, only a short-lived stratification was observed in the transition period from the dry to the rainy season [133].

In smaller temperate collections of water, such as aquaculture ponds [125], small pools [134-136] and ditches [137], stratification is most often a diurnal process, which occurs at daytime. At night these water bodies are homothermic. During the winter period these water bodies can be homothermic as well [134]. Diurnal stratification is also observed in smaller tropical collections of water [118,119,132].

To my knowledge, there are no data available that show vertical stratification in the typical, small and shallow breeding sites of Afro-tropical malaria mosquitoes. Differences in water temperature within the same habitat on a horizontal plane have, however, been recorded: In western Kenya, average differences in surface water temperature of 2.9 °C (ranging from 0 to 9.0 °C) and 3.1 °C, (ranging from 0.3 to 8.9 °C) within water-filled dirt tracks and ditches, respectively, were observed [61].

1.4.4 Existing models

Depending on the presence or absence of stratification, two models can be used to estimate the various contributors to the energy balance. Energy budget models that describe the water temperature uniformly [e.g. 138], which are useful in homogeneous water bodies, or models that describe the temperature profile of the water body in detail, by estimating the temperature of various water layers [e.g. 125,137], which can be used for stratified water bodies.

To estimate the daily temperature to which mosquito larvae are exposed, the natural variations in energy fluxes in the atmospheric as well as water boundary layers have to be modelled. There is a variety of data available from studies that modelled the water

temperatures of water bodies, such as lakes [139-141], reservoirs [138], aquaculture ponds [125,142], rivers [143-145] and ditches [137]. These water collections are, however, larger and deeper than the typical, small and shallow breeding sites of malaria mosquitoes, which will require a different approach in micrometeorological measurements and in estimating their water temperatures. Recently, we made a first attempt at modelling the temperature in a small and shallow pool (diameter 1.1 m, water depth 0.25 m) in The Netherlands [136].

1.5 Research questions and outline of the thesis

Anopheles gambiae and *An. arabiensis* are the primary human malaria vectors in sub-Saharan Africa. Despite their importance as vectors, relatively little is known about the biology and ecology of the immature stages, as most field studies focus on the adult mosquito stage or on the malaria parasites.

As the mosquito immatures are confined to their aquatic habitat, they are vulnerably exposed to various biotic and abiotic factors in their short life-span. These factors will have an effect on the life history traits of the immatures, such as their growth, development and survival, which affect habitat productivity and hence the transmission of malaria. Because the larvae spend most of their time horizontally near the air-water interface and thus in the upper water layer, they are directly exposed to meteorological variables such as rainfall, radiation and the temperature of the surrounding water. The direct relations between the life history traits of the larvae and various meteorological variables are not well understood, as Hoshen & Morse [146] wrote: “*Unfortunately, reports giving quantified relationships of temperature and rainfall/humidity dependence of the mosquito dynamics are in short supply, although some data are available. Their further publication would assist the development of models*”.

More studies are required to gain a better insight in the biology and ecology of the larvae of malaria mosquitoes. Gaps in our current understanding of larval ecology combined with the ability of *An. gambiae* s.l. to exploit a wide variety of breeding sites have discouraged efforts to develop and implement larval control strategies [147]. This thesis shows how meteorological variables can affect the life-history characteristics of larvae of *An. arabiensis* and *An. gambiae* and the temperature of their aquatic surrounding, by studying the meteorological variables as well as the immature stages of the malaria vectors in their natural environment in western Kenya.

The central objectives of this thesis are:

- (1) To assess the diurnal fluctuations of water temperature in aquatic habitats of malaria mosquitoes and relate them to meteorological data.
- (2) To develop a physical model that simulates the diurnal fluctuations of the water temperature in malaria mosquito breeding sites using meteorological data and to compare the model with continuous measurements of water temperatures in the field.
- (3) To study the effect of diurnal fluctuations of water temperature on the life-history traits of the immature stages of the Afro-tropical malaria vectors *An. arabiensis* and *An. gambiae* reared in single-species and in mixed-species populations under ambient conditions.
- (4) To assess the direct impact of rainfall on the survival of larvae of *An. gambiae*.

A better understanding of the complexity of interactions (i) between weather and water temperature and (ii) between weather and water temperature on the one hand and habitat productivity on the other hand, has a three-fold advantage. It is likely to improve:

- (a) The currently available models that predict mosquito population dynamics and the risk of malaria transmission.
- (b) The quality of disease management, especially that of larval control, by improving our knowledge on which breeding sites should be targeted and how frequently they should be visited.
- (c) The prediction of how climate change may affect the growth, development and survival of the immature stages of malaria mosquitoes and hence malaria transmission in the future.

This thesis is composed of an introduction (Chapter 1), five experimental chapters (Figure 1.2 gives a schematic overview of these chapters) and a final discussion with future perspectives.

In the first part, the effects of weather on the diurnal water temperature variations are studied. In Chapter 2 the effects of meteorological variables on the diurnal water temperature dynamics in various semi-natural water bodies of different sizes are assessed. Special attention is given to the top water layer, where the mosquito larvae are most frequently found. A model is developed to predict this water temperature behaviour, based on the energy balance of the water bodies. In the next chapter (Chapter 3) the effect of water turbidity on the near-surface water temperature is presented.

In the second part of this thesis, the outcomes of various biological experiments under ambient conditions in combination with meteorological measurements are presented. The effect of habitat size (and thus different daily variations in the water temperature) and water turbidity on the larval development, larval survival and adult size of *An. arabiensis* and *An. gambiae* is shown in Chapter 4. The next chapter (Chapter 5) investigates the effects of competitive interactions between the two species, as both species frequently coexist in breeding sites. In the final experimental chapter (Chapter 6) the effect of rainfall on the survival of larvae of *An. gambiae* is assessed.

In Chapter 7 the overall results are summarized and discussed and suggestions for future work are given.

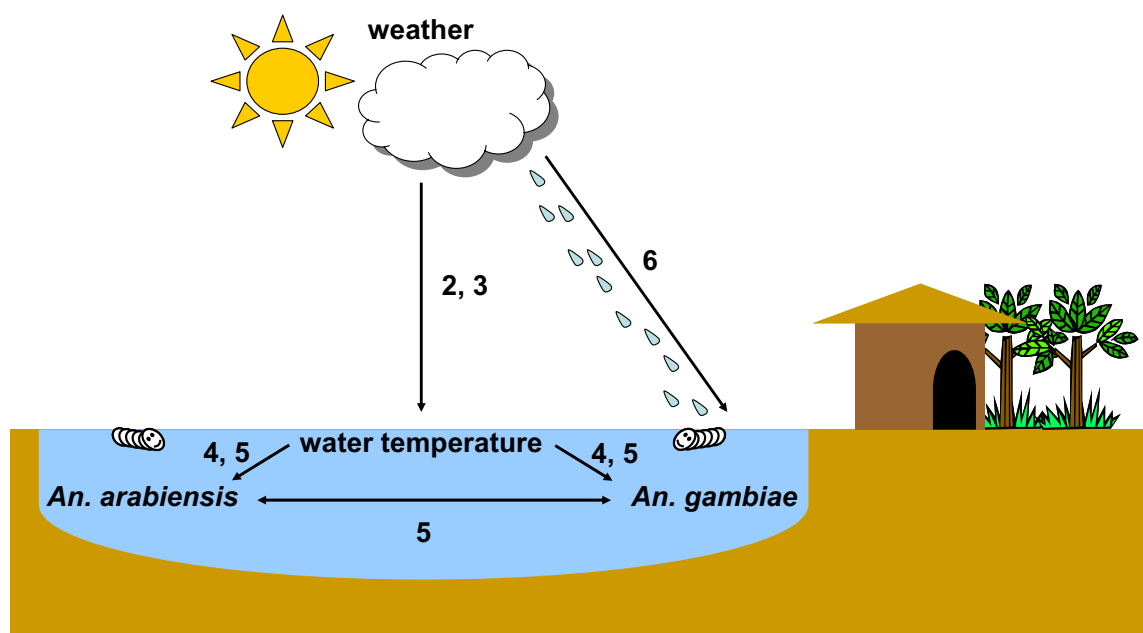


Figure 1.2 Schematic overview of the content of this thesis. The numbers in the figure correspond with chapter numbers.

All experiments were conducted at the Kenya Medical Research Institute, near Kisumu in Kenya, where malaria is still a serious life-threatening disease (Figure 1.3). In the *Daily Nation*, a Kenyan newspaper, of 26 July 2005, UNICEF executive director Venema said: “In Kenya, 34,000 people aged below five die annually. That’s more than 90 daily”. Fortunately, this number has reduced to 16,000 in 2006 according to the World Health Organization. The mass distribution of insecticide-treated bed nets is identified as the biggest contributing factor in the reduction of malaria deaths (*Daily Nation*, 17 August 2007).

Malaria still Kenya's biggest killer disease

Figure 1.3 Heading in the *Daily Nation* (Kenya) on 30 March 2005.

Meteorological data were acquired with an automated weather station (Figure 1.4) and water temperatures in various water bodies of different size were monitored at various depths with a second automated meteorological station nearby (Figure 1.5).



Figure 1.4 Automated weather stations at KEMRI, measuring radiation, air temperature, relative humidity, wind speed and rainfall.



Figure 1.5 Automated micrometeorological stations at KEMRI, measuring water temperatures in various semi-natural water bodies and soil temperatures at various depths.

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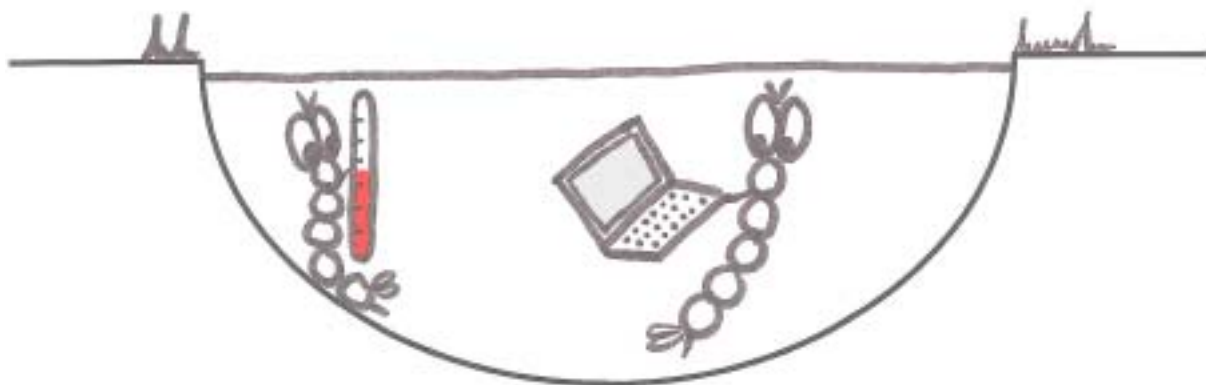
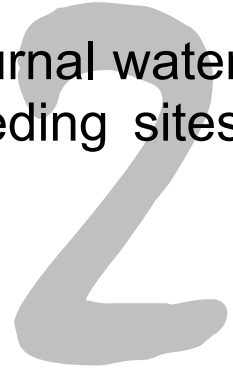
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Observations and model estimates of diurnal water temperature dynamics in mosquito breeding sites in western Kenya

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Abstract

The temperature of water is an important determinant of the growth and development rate of immature malaria mosquitoes. In sub-Saharan Africa, these immatures live in small, shallow and tropical water bodies. To gain a better understanding of the daily temperature dynamics of such mosquito breeding sites and of the relationships between meteorological variables and water temperature, three semi-natural water bodies (diameter×depth: 0.16×0.04, 0.32×0.16 and 0.96×0.32 m) were created in western Kenya. Continuous water temperature measurements at various depths were combined with weather data collections from a meteorological station. A model was developed, which predicts the diurnal water temperature dynamics, based on the estimated energy budget components of these water bodies. No stratification was observed and the daily mean temperature of all water bodies was on average 27.4-28.1 °C. There was, however, a difference at both a temporal and spatial scale. The smallest water body reached its daily minimum and maximum temperature earlier during the day and the magnitude of the fluctuations around the mean temperature decreased with increasing habitat dimensions. The average recorded difference between the daily minimum and maximum water temperature was on average 14.4 °C in the smallest water body versus only 7.1 °C in the largest water body. The average water temperature highly corresponded with the average air temperature. Noteworthy, the temperatures of the water bodies were above the air temperature throughout each 24-hours period. Overall the model estimated the diurnal temperature behaviour of the water bodies accurately. The air-water interface appeared the most important boundary for energy exchange processes and on average 82-89% of the total energy was gained and lost at this boundary. Besides the loss of energy due to longwave radiation, energy loss due to evaporation of water, which continued throughout the day, was largest in all water bodies (average loss of 15-18%). The average estimated daily evaporation ranged from 4.2 mm in the smallest to 3.7 mm in the largest water body. Such evaporation rates have large consequences for the longevity of the smallest larval breeding sites and hence for the chances of mosquito larvae to develop into adults during short periods of drought. The data presented in this chapter improve our knowledge of physical processes in and near the breeding sites of malaria mosquitoes and may improve current predictions of habitat productivity and hence of the transmission of malaria.

2.1 Introduction

Climate plays a major role in distribution and abundance of insect species [1,2]. Being poikilothermic, insects are unable to internally regulate their body temperature and consequently their metabolic rate. Therefore, processes such as activity, growth and development depend on the temperature of the insect's direct environment. In general, within a certain temperature range, insects experience an increased growth rate at higher temperatures [3].

The temperature dependency applies to the immatures of mosquitoes that transmit malaria as well. With over one million deaths and between 350 and 500 million acute cases annually [4], malaria remains one of the most important and widespread tropical infectious diseases in the world. Over 75% of mortality cases relate to children living in sub-Saharan Africa [5], where the two sibling mosquito species *An. arabiensis* Patton and *Anopheles gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*), both belonging to the *An. gambiae* Giles sensu lato complex (hereafter referred to as *An. gambiae* s.l.), are important vectors of the pathogen.

The immature stages of these mosquitoes (an egg stage, four larval stages and a pupal stage) are aquatic and found in transient, sunlit and small water bodies [6-8], the availability of which depends on precipitation [8-10]. Typical examples of breeding sites are burrow pits, road puddles, tire tracks and animal hoof prints and the reader is referred to the paper by Mutuku et al. [11] for illustrations.

Being confined to their habitats, mosquito larvae are exposed to diurnal fluctuations in water temperature [12,13]. How the diurnal fluctuations in water temperature will affect insect development will depend on the magnitude and duration of the alternating phases [14]. In general, higher temperatures will result in a faster larval development, but in smaller females, as is observed in various mosquito species [e.g. 15,16,17]. The development and growth of larvae of *An. gambiae* s.l. is also temperature-dependent [18,19].

Water temperature is governed by various parameters, such as habitat geometry [20], altitude, land cover type or canopy overgrowth [21], water turbidity (Chapter 3), presence of algae or aquatic vegetation, soil properties and local (micro)climate. The relationships between weather and the water temperatures of breeding sites of *An. gambiae* s.l. remain unknown, in spite the fact that water temperature will affect the growth, development and survival of the mosquito immatures, which affects the abundance, dynamics and fitness of the adult vector population and consequently the transmission rate of malaria. To understand and predict water temperature dynamics, fundamental knowledge of the various gains,

losses and transfers of energy involved within water bodies and at its boundaries are required: After all, fluctuations in water temperature are caused by the natural variation in energy fluxes in the atmospheric, water and soil layers.

Energy budgets of water bodies have been studied but the majority focussed on larger systems such as lakes [22-24], reservoirs [25], aquaculture ponds [26,27], rivers [28-30] or ditches [31], all being larger and deeper than typical breeding habitats of *An. gambiae* s.l.

Detailed knowledge of all energy budget components will enable us to predict water temperatures in an area using local meteorological data as input. Water temperatures were studied in great detail by monitoring them at various depths in three shallow, clear and semi-natural water bodies of different size in western Kenya, combined with weather data collections from a meteorological station. Our objectives were (i) to study the diurnal water temperature dynamics in the water bodies, (ii) to examine the effects of meteorological conditions on these dynamics and (iii) to develop and evaluate a model that predicts the diurnal water temperature behaviour by estimating all terms of the heat budget with local meteorological data.

2.2 Material and methods

All measurements were carried out at the Kenya Medical Research Institute (KEMRI), near Kisumu, Kenya between 16 March (Day Of Year 75) and 5 May 2005 (DOY 125), a period that experienced frequent rains. The local soil was black cotton soil, which consists of 12% sand, 28% silt and 60% clay in the top 0.20 m and of 24% sand, 16% silt and 60% clay at a depth of 0.2-1.2 m [32]. Both experimental areas were covered with grass, which was kept short.

2.2.1 Meteorological station (0°04'36.390"S; 34°40'34.770"E; 1126 m above sea-level)

Wind speed at 2 and 10 meters height (cup anemometers; Meteorology an Air Quality, Wageningen University), incoming shortwave and longwave radiation at 1.5 meters above ground (CM11 and CG1 respectively; Kipp & Zonen, The Netherlands), temperature and relative humidity at 2 meters above ground (ventilated probe; Vaisala, Finland) and precipitation (rain gauge; Eijkelkamp, The Netherlands) were measured.

2.2.2 Water station

Nearby this meteorological station different-sized circular water bodies were created by digging holes in the ground: a small-sized water body (hereafter referred to as SWB, with

diameter \varnothing 0.16 m and water depth of 0.04 m), a medium-sized water body (MWB, \varnothing 0.32 m and water depth of 0.16 m) and a large-sized water body (LWB, \varnothing 0.96 m and water depth of 0.32 m). The water bodies were lined with semi-transparent plastic (0.13 mm), pressed tightly against the soil to maximize plastic-soil contact and filled with clear tap water that originated from a well at KEMRI, up to 10 mm under the brim. The plastic was kept in place by a metal ring (diameter 50 mm larger than the water body). A uniform water level was maintained by adding or removing water to starting level every morning to compensate for evaporation or precipitation, respectively. The setup was cleaned and refilled on a weekly basis.

Water temperatures were measured in the centre of the water bodies at various depths (Figure 2.1; SWB: 1 and 30mm; MWB: 1, 30, 60 and 120 mm; LWB: 1, 30, 60, 120 and 240 mm below the air-water interface) with glass bead thermistors (NTC BEAD 4K7, Thermometrics, USA). These thermistors were placed in stainless steel tubes (\varnothing 1.8 mm) which were filled with heat conductivity paste and attached waterproof to the wiring with white heat shrink material. The floating equipment (LWB, Figure 2.1) was made of Teflon tubes (\varnothing 6 mm), Teflon rings (\varnothing 19 mm) and polystyrene balls (\varnothing 40 mm), all connected with Perspex tubes (\varnothing 4 mm). Soil temperatures were measured with similar thermistors at 0.05, 0.17 and 0.33 m depth in the undisturbed soil in the middle of the experimental area.

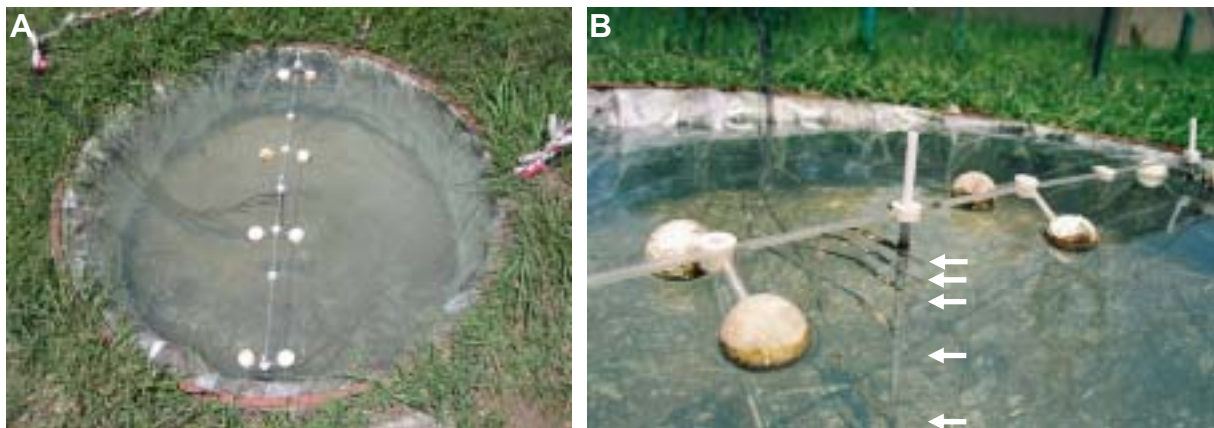


Figure 2.1 (A) Experimental setup: measuring water temperatures at various depths in a large-sized water body. (B) Close-up of the floating equipment. The arrows indicate the thermistors at various depths in the water column.

2.2.3 Data analysis

All data were totalized (precipitation) or averaged (remaining variables) at 5-minutes intervals (meteorological station) or 15-minutes intervals (water station) and stored in a 21x

Micrologger (Campbell Scientific Inc., U.K.). Due to a few days with missing data and the cleaning of our setup on a weekly basis, 40 days are used for analysis. The times mentioned in this chapter are local times (UTC+3).

Nine days were chosen for a detailed energy budget study: three consecutive days with predominantly clear skies (DOY 86-88), three consecutive days with overcast conditions (DOY 95-97) and three consecutive days with heavier overcast and heavy precipitation (DOY 117-119). Table 2.1 shows some meteorological variables for the days that were selected for this chapter.

Correlations between the measured water temperatures and various meteorological variables were analyzed using SPSS software (version 12.0.1, SPSS Inc., Chicago, IL). The statistical significance of the correlations and Spearman's correlation coefficient (ρ) are given. This coefficient indicates the magnitude of the association between two variables.

Table 2.1 Total daily incoming short and long wave radiation load, daily average air temperature, daily average water temperatures of the three different sized water bodies, the difference between daily maximum air and daily maximum water temperatures, the difference between daily minimum air and daily minimum water temperatures and daily rainfall quantity measured during a period with clear days, a period with overcast days and a period that experienced heavy and long overcast and heavy precipitation.

DOY	Clear days			Overcast days			Extreme overcast days		
	86	87	88	95	96	97	117	118	119
R_s^\downarrow (MJ m ⁻²)	26.3	26.2	26.8	20.4	20.3	18.5	21.8	07.9	10.5
R_l^\downarrow (MJ m ⁻²)	33.3	32.6	32.2	33.9	34.7	34.0	34.8	35.5	34.8
T_a (°C)	23.9	23.0	23.8	23.1	21.7	22.4	25.0	21.4	21.4
r_a (mm)	05.8	02.0	00.2	00.0	08.8	04.6	05.4	40.6	03.4
$T_{w,s}$ (°C)	27.9	28.0	28.3	26.7	26.4	26.4	28.7	24.6	25.0
$T_{w,m}$ (°C)	28.5	28.6	28.6	27.0	26.8	26.7	29.0	25.2	25.3
$T_{w,l}$ (°C)	28.8	29.1	28.9	27.5	27.1	27.0	29.1	25.9	25.3

R_s^\downarrow incoming short wave radiation flux density, R_l^\downarrow incoming long wave radiation flux density, T_a air temperature at two meters, r_a rainfall quantity, $T_{w,s}$ water temperature of SWB, $T_{w,m}$ water temperature of MWB, $T_{w,l}$ water temperature of LWB.

2.2.4 Energy budgets

It appeared that the water temperatures measured at the various depths were similar and behaved homogeneously throughout each diurnal cycle, making it possible to treat each water body as a homogeneous system.

2.2.4.1 Radiative heat exchange at a water body

The total absorbed radiation in a water body, R_a (W m^{-2}), is calculated by:

$$R_a = R_s^* + \varepsilon_w (R_l^\downarrow - \sigma T_w^4) \quad (2.1)$$

where R_s^* (W m^{-2}) is the available net shortwave radiation, ε_w (0.97) the emissivity value for water [26], R_l^\downarrow (W m^{-2}) the incoming longwave atmospheric radiation, σ ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$) the Stefan-Boltzmann constant and T_w (K) the temperature of the water body. R_s^* is parameterized as follows (Figure 2.2A):

$$R_s^* = (1 - a_w) [\beta + (1 - \beta)(1 - a_s)] R_s^\downarrow \quad (2.2)$$

where a_w (0.06) is the albedo of the water body [33], β the proportion of total incoming solar radiation at the water surface that is absorbed in the upper water layer which is approximately 0.4-0.5 [26]. In this study we used a value of 0.45 for β as all the near-infrared radiation (constituting approximately 45% of all incoming shortwave radiation) is absorbed in the upper layer of each water body. In addition, a_s (0.20) is the albedo of the soil at the soil-water interface [34] and R_s^\downarrow (W m^{-2}) is the incoming shortwave radiation. Because the plastic that was used in this study was not completely transparent, it will reflect part of the incoming solar radiation at the bottom. To compensate for this effect, the albedo of the soil was increased with 0.1.

2.2.4.2 Non-radiative heat exchange at a water body

The total absorbed radiation by a water body is partitioned (Figure 2.2B) as follows:

$$R_a = H + LE + P + G + \Delta S \quad (2.3)$$

where H (W m^{-2}) is the sensible heat flux, LE (W m^{-2}) the evaporation or latent heat flux, P (W m^{-2}) the amount of energy stored in or extracted from a water body due to precipitation and G (W m^{-2}) the heat flux between water and soil. ΔS (W m^{-2}) is the amount of remaining energy that is stored in or extracted from the water and determines the magnitude of the change in water temperature.

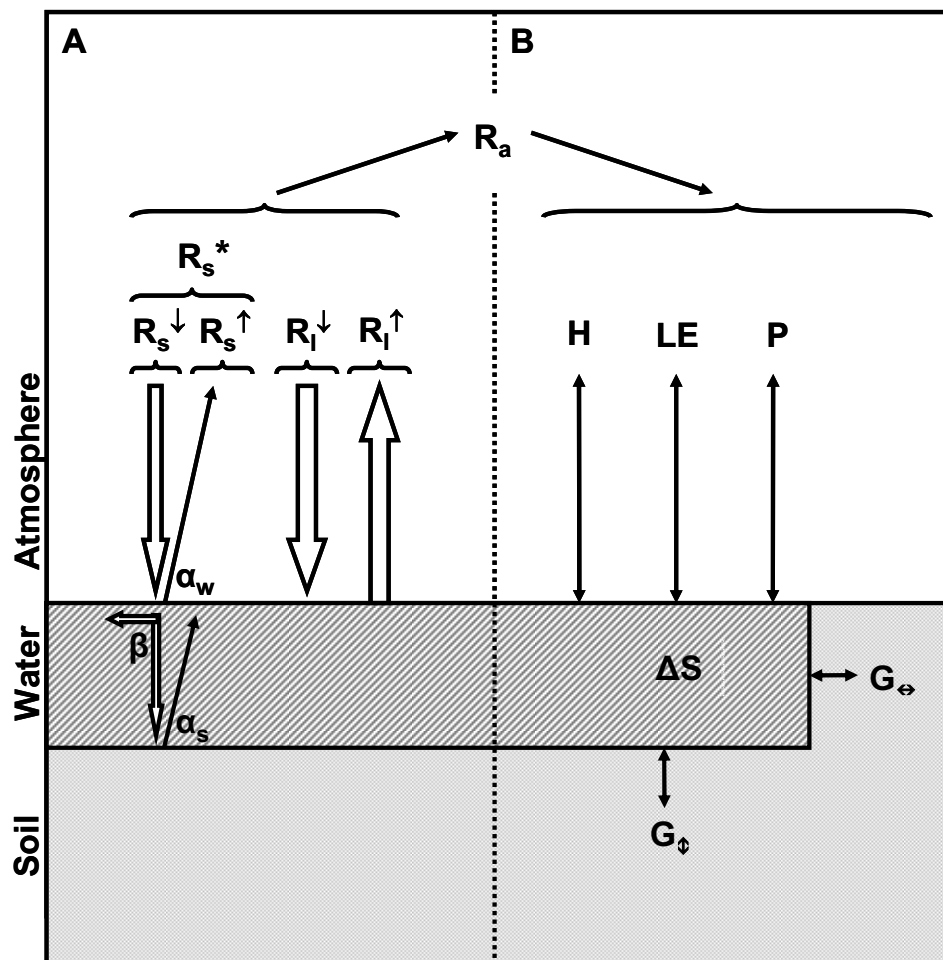


Figure 2.2 Conceptual model of radiation and energy fluxes at the air-water and soil-water interfaces of small, shallow and clear water bodies **(A)** radiative heat exchange and **(B)** non-radiative heat exchange. R_a total absorbed radiation in a water body, R_s^* available net shortwave radiation, R_s^\downarrow incoming short wave radiation, R_s^\uparrow outgoing short wave radiation, R_l^\downarrow incoming long wave radiation, R_l^\uparrow outgoing long wave radiation, a_w the albedo of the water body, β the proportion of total incoming solar radiation at the water surface that is absorbed in the upper water layer, a_s the albedo of the soil at water-soil interface, H sensible heat flux density and LE latent heat flux density, P amount of energy stored in or extracted from a water body due to precipitation, G_\downarrow soil heat flux density vertically, G_e soil heat flux density sideways, ΔS energy storage.

The movement of heat energy from the water surface to the atmosphere by conduction and convection, the sensible heat flux H , is parameterized by [34]:

$$H = \rho_a c_p C_h u (T_w - T_a) \quad (2.4)$$

where ρ_a (kg m^{-3}) is the density of air, c_p ($\text{J kg}^{-1} \text{K}^{-1}$) the specific heat capacity of air at constant pressure, C_h the sensible heat transfer coefficient at 2 m height, u (m s^{-1}) the wind speed at 2 m, T_a (K) the air temperature at 2 m.

The movement of heat energy from the water surface to the atmosphere due to evaporation of water, the latent heat flux LE , is parameterized by [34]:

$$LE = \rho_a L C_w u (q_s - q_a) \quad (2.5)$$

where L (J kg^{-1}) is the latent heat of evaporation, C_w the water vapour transfer coefficient at 2 meters height, q_s (kg kg^{-1}) and q_a (kg kg^{-1}) the saturation-specific humidity and specific humidity at two meters, respectively. The latent heat of evaporation is temperature dependent and calculated according to Fritschen and Gay [35]. The saturation specific humidity for water-vapour and air are estimated using the Clausius-Clapeyron equation.

Due to the similarity of the transfer mechanisms of heat and water vapour (Reynolds analogy), we may assume that $C_h \approx C_w$. Both transfer coefficients were initially approximated over the surrounding grassland with the Monin-Obukhov Similarity Theory [34] using a roughness length for momentum (z_{0M}) of 1 mm above short grass [36] and a roughness length for heat (z_{0H}) of $z_{0H} = z_{0M}/10$ [37]. We arrived at $C_h = 0.003$.

Although there was a difference between the temperature of the water bodies and the temperature of the rain droplets and precipitation clearly affected the water temperature, it appeared from our calculations that the energy transferred advectively by precipitation, P , could be ignored in our model.

The movement of heat energy by conduction through the soil, the soil heat flux G , is described by the slightly modified Fourier's Law. The energy exchange between soil and water is calculated in both a vertical and sideward direction and is parameterized by:

$$G = -\lambda_s \frac{\delta T}{fD} \quad (2.6)$$

where λ_s ($\text{W m}^{-1} \text{K}^{-1}$) is the thermal conductivity of the soil, which is 1.2 for the saturated soil [36]. We assumed that soil temperatures were similar and behaved homogeneous over a horizontal plane.

Regarding the vertical soil heat flux, δT (K) was the temperature difference between the temperature of the water body and the soil temperature at depth d (m), the depth of the water

body. Regarding the horizontal soil heat flux, δT was the temperature difference between water temperature and average soil temperature over depth d . The damping depth D (m) of the diurnal temperature wave in the soil was multiplied by f (0.3), a factor that depends on d and had to be applied at the SWB only, since the surrounding soil will influence the temperature of the small water volume, whereas this water volume will have relatively less impact on the temperature of the surrounding soil. This factor was determined by trial and error. D is parameterized by [38]:

$$D = \frac{z_1 - z_2}{\ln(A_{z_2}) - \ln(A_{z_1})} \quad (2.7)$$

where z (m) is the measurement depth in the soil and A_z the amplitude of the soil temperature at depth z .

Finally the water temperature is given by:

$$T_{w,n} = T_{w,n-1} + \frac{\Delta S \Delta t}{\rho_w c_w d} \quad (2.8)$$

where n is the time step (s). An initial value needs to be given to $T_{w,0}$, which was done by using the observed initial temperature.

2.3 Results

2.3.1 Meteorological variables

The average daily incoming radiation load over our study period was 21.9 (± 0.6 SEM; range 7.9-27.1) MJ m⁻² day⁻¹ for shortwave and 33.7 (± 0.1 ; range 30.6-35.6) MJ m⁻² day⁻¹ for longwave radiation. The average daily air temperature was 23.4 (± 0.2 ; range 21.4-25.5) °C, with an average daily minimum temperature of 18.5 (± 0.1 ; range 14.7-21.2) °C and an average daily maximum temperature of 29.4 (± 0.2 ; range 24.4-32.9) °C. A total of 32 days experienced precipitation ranging from 0.2 (lower rain-gauge threshold level) up to 40.6 mm day⁻¹, the total precipitation being 270.3 mm in this period and the highest recorded intensity 9.5 mm in 5 minutes.

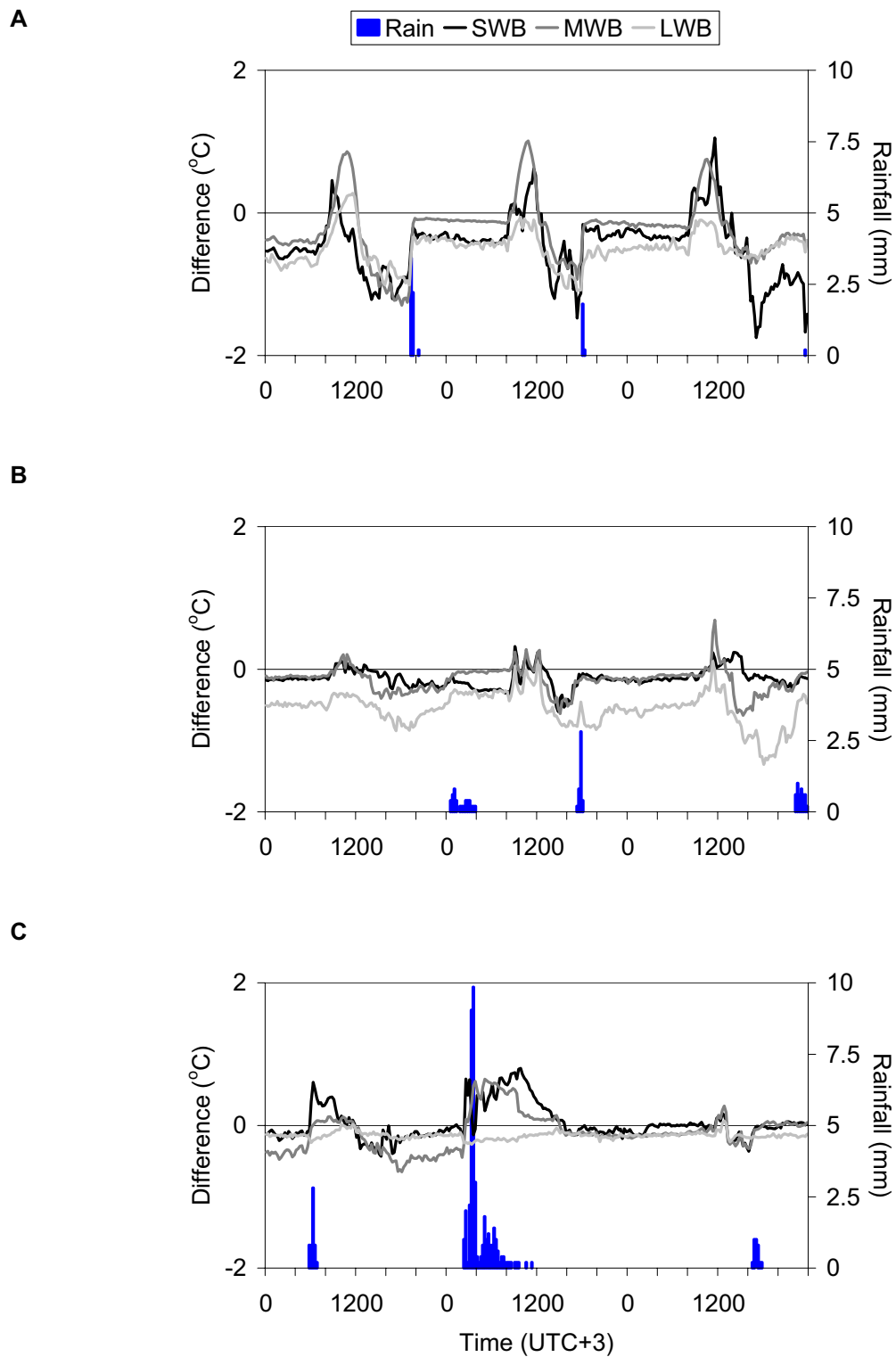


Figure 2.3 Temperature difference between top water layer (thickness of 2 mm) and the deeper water layer(s) in the SWB (black line), the MWB (dark grey line) and the LWB (light grey line) and rainfall (vertical bars) on **(A)** DOY 86-88 **(B)** DOY 95-97 and **(C)** DOY 117-119. Values above 0 °C indicate a warmer top layer.

2.3.2 Observations of diurnal water temperature dynamics

2.3.2.1 Mean water temperatures and diurnal fluctuations

Apart from the upper water layer, the water temperatures measured in the deeper layers were similar and behaved homogeneously throughout each diurnal temperature cycle. The top water layer (thickness of 2 mm) differed in temperature and in its diurnal temperature dynamics (Figure 2.3) compared to the deeper layers. In general, the upper water layer of the SWB and MWB was cooler (up to approximately 2 °C) during most of the afternoon and during night-time, but became warmer (up to about 1 °C) during the first hours after sunrise. This difference in water temperature was larger on clear days compared to overcast days. The difference in temperature between the top water layer and the layers beneath of the LWB showed similar dynamics, whereby the top layer remained cooler during larger parts of the day or during the whole day compared with the smaller water bodies.

Rainfall clearly affected the difference between the temperature of the top water layer and the layers beneath, but the magnitude of this difference and whether it results in a increase or decrease in difference will depend on parameters such as habitat dimensions, the existing water temperature, the temperature of rainwater, the duration and amount of precipitation and the time of day.

Considering the whole water body, the SWB reached its daily minimum (night-time) and maximum (day-time) temperature earlier than the MWB and LWB did (Figure 2.4-2.6). The times at which these minimum and maximum values were recorded differed at various weather conditions.

On a spatial scale, the SWB showed the largest diurnal temperature fluctuations and this water body heated (day-time) and cooled (night-time) more than the larger water bodies (Figure 2.4-2.6). The daily average minimum water temperature recorded during the study period was 22.4 (± 0.1 ; range 20.8-23.9) °C in the SWB, 23.9 (± 0.1 ; range 22.3-25.2) °C in the MWB and 25.1 (± 0.1 ; range 23.1-26.2) °C in the LWB. The daily average maximum water temperature was 36.8 (± 0.3 ; range 29.4-39.0) °C in SWB, 33.9 (± 0.3 ; range 27.8-35.8) °C in MWB and 32.1 (± 0.2 ; range 27.5-33.8) °C in LWB. This resulted in average differences between daily minimum and maximum water temperature of 14.4 (± 0.3 range 7.8-16.8) °C, 10.1 (± 0.3 range 4.7-12.2) °C and 7.1 (± 0.2 range 3.8-8.5) °C in the SWB, MWB and LWB, respectively.

The daily minimum water temperature significantly ($\chi^2=87.2$; $P<0.001$) increased when habitat size increased, whereas the daily maximum water temperature and the daily difference between minimum and maximum water temperature significantly ($\chi^2=76.3$ and

$\chi^2=90.5$, respectively; $P<0.001$) decreased when habitat size increased. Besides the difference in temperature between the water bodies, the temperature increase and decrease per unit time during daytime was higher in smaller water bodies.

Despite these differences on a temporal and spatial scale, the average daily water temperature in the different water bodies was similar: 27.4 (± 0.2 ; range 24.6-29.1) °C in the SWB, 27.8 (± 0.2 ; range 25.2-29.0) °C in the MWB and 28.1 (± 0.2 ; range 25.3-29.3) °C in the LWB.

Noteworthy, the water temperatures in all our water bodies were higher than the air temperature at two meters above ground every day at any given time (Figure 2.4-2.6), which has important consequences for the behaviour of the sensible and latent heat fluxes, as will be shown later. The average differences between daily minimum air and daily minimum water temperatures during the study period were 3.9 (± 0.1 ; range 2.2-5.2), 5.3 (± 0.1 ; range 3.7-6.8) and 6.5 (± 0.1 ; range 4.4-8.1) at the SWB, MWB and LWB, respectively. The average differences between the daily maximum air and daily maximum water temperatures was 7.6 (± 0.2 ; range 4.8-10.2), 4.7 (± 0.2 ; range 2.2-7.5) and 2.9 (± 0.2 ; range 0.3-5.6) at the SWB, MWB and LWB, respectively.

2.3.2.2 Relations between water temperatures and meteorological variables

In general, the small-sized water body reacted more dynamically to sudden changing meteorological variables. Its temperature corresponded better to incoming solar radiation. This effect was most distinct on clear days where a change in incoming solar radiation load (ΔR_s^\downarrow) and a change in water temperature (ΔT_w) were highly and significantly (15-minute intervals; $P<0.001$) correlated (DOY 86-88; SWB $\rho=0.78$, MWB $\rho=0.57$, LWB $\rho=0.49$).

Precipitation affected the water temperature directly by increasing or decreasing the water temperature to a certain extent, which depends on various parameters as mentioned earlier. A decrease in water temperature was recorded during 75% (SWB), 94.5% (MWB) and 96.5% (LWB) of the 15 minutes periods that experienced rainfall, with an average decrease of 0.45 (± 0.1) in the SWB, 0.25 (± 0.0) in the MWB and 0.17 (± 0.0) °C in the LWB. The maximum recorded decrease in water temperature during rainfall was 5, 2.6 and 1.4 °C in 15-minutes in the SWB, MWB and LWB, respectively, on DOY 124 (17h45) when the highest rainfall intensity was recorded (19.3 mm in 15 minutes). There was a significant ($P<0.001$) but moderate correlation between the decrease in water temperature and the amount of rainfall (SWB $\rho=-0.59$; MWB $\rho=-0.54$; LWB $\rho=-0.57$).

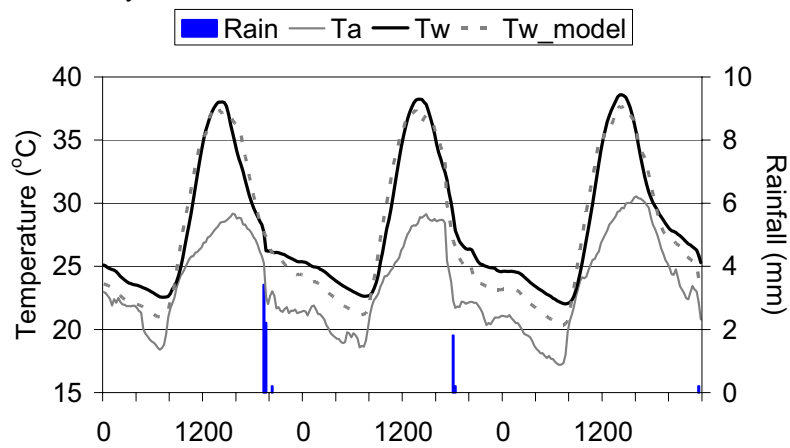
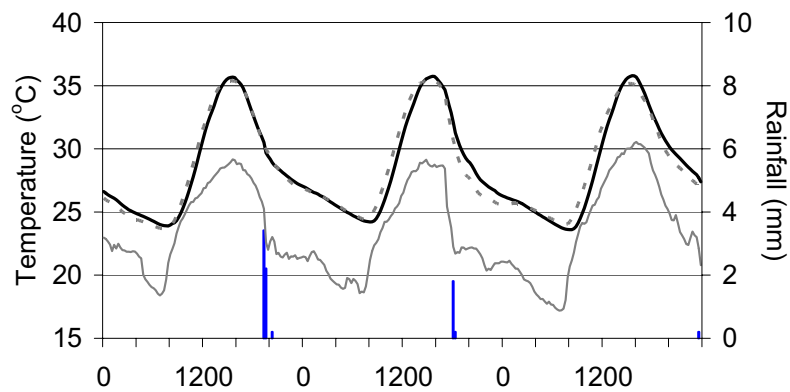
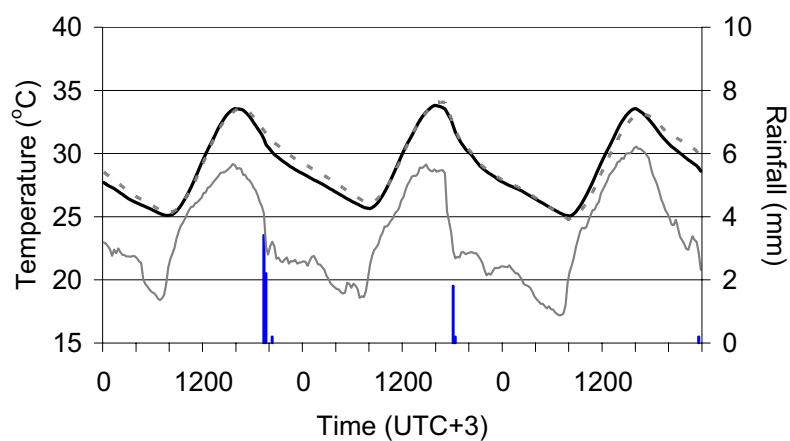
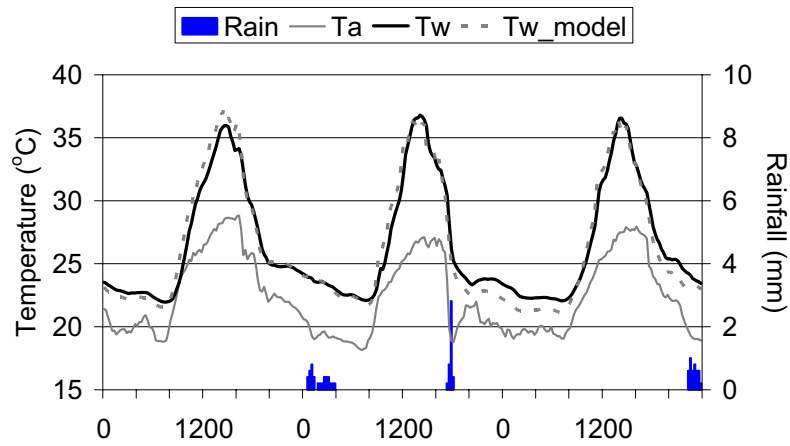
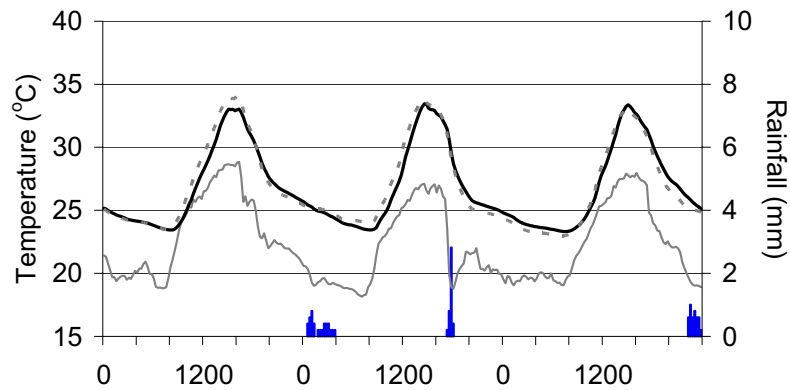
A Small-sized water body**B** Medium-sized water body**C** Large-sized water body

Figure 2.4 Measured air temperature (T_a) and measured precipitation and the measured (T_w) and modelled (T_w_{model}) diurnal temperature behaviour of **(A)** the small-sized water body **(B)** the medium-sized water body and **(C)** the large-sized water body, during DOY 86-88.

A Small-sized water body



B Medium-sized water body



C Large-sized water body

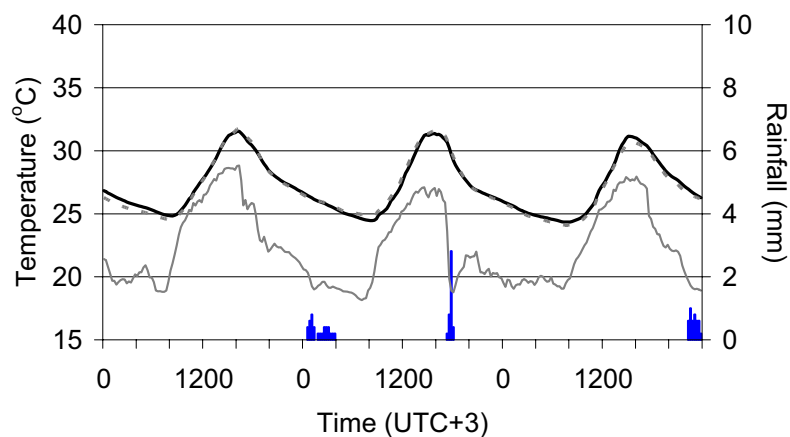
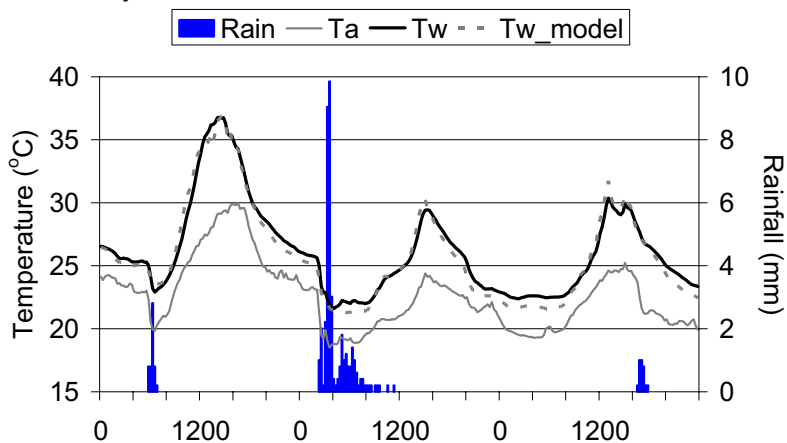
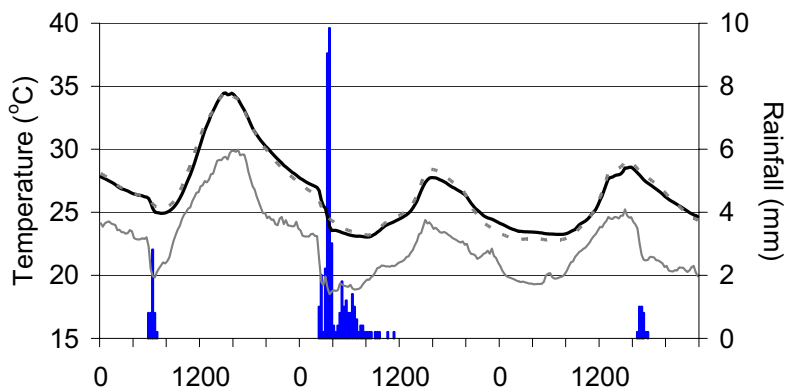


Figure 2.5 Measured air temperature (T_a) and measured precipitation and the measured (T_w) and modelled (T_{w_model}) diurnal temperature behaviour of **(A)** the small-sized water body **(B)** the medium-sized water body and **(C)** the large-sized water body, during DOY 95-97.

A Small-sized water body



B Medium-sized water body



C Large-sized water body

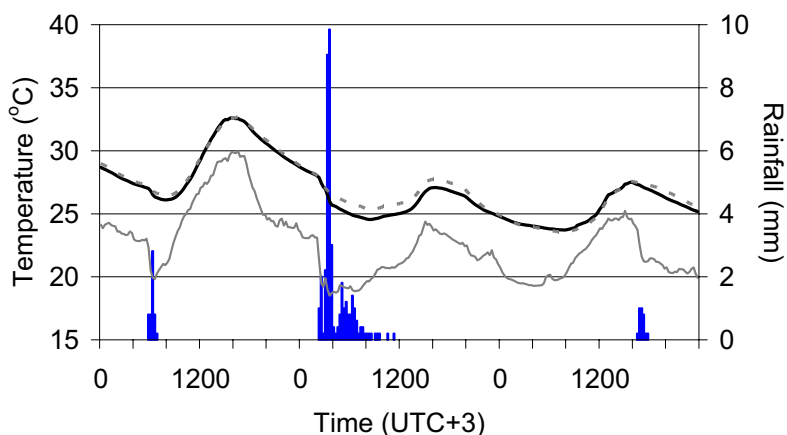


Figure 2.6 Measured air temperature (T_a) and measured precipitation and the measured (T_w) and modelled (T_{w_model}) diurnal temperature behaviour of **(A)** the small-sized water body **(B)** the medium-sized water body and **(C)** the large-sized water body, during DOY 117-119.

The average increase in water temperature during rainfall was 0.11 (± 0.02), 0.07 (± 0.02) and 0.04 (± 0.01) °C in the SWB, MWB and LWB, respectively. The maximum recorded increase in water temperature during rainfall was 0.6, 0.2 and 0.1 °C in the SWB, MWB and LWB, respectively.

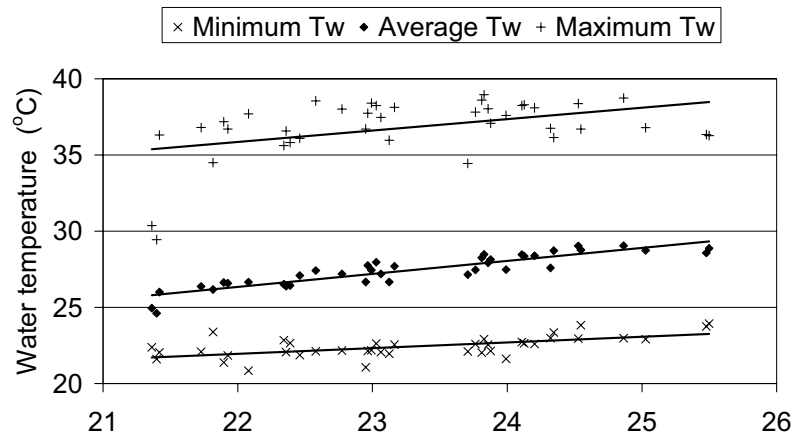
The daily average water temperature corresponded highly and significantly with various meteorological variables (Table 2.2), especially with the average air temperature at 0.1 and 2 m height. Such simple linear relationships make these meteorological variables good predictors of the daily average water temperature in water bodies. The relations between water temperatures (average, minimum and maximum) and (i) average air temperature and (ii) total daily incoming solar radiation load are shown in Figures 2.7 and 2.8, respectively. The daily maximum water temperature was highly and significantly ($P < 0.001$) correlated with the daily total incoming solar radiation load and this correlation was best for the larger water bodies (Figure 2.8; SWB $\rho = 0.67$; MWB $\rho = 0.86$; LWB $\rho = 0.90$). The daily minimum water temperature and the difference between daily minimum and maximum temperature corresponded highly and significantly ($P < 0.001$) with the daily average water temperature (SWB $\rho = 0.61$; MWB 0.74; LWB 0.81) and the daily maximum water temperature (SWB $\rho = 0.86$; MWB $\rho = 0.82$; LWB $\rho = 0.74$), respectively, and corresponded less well with the measured meteorological variables.

Table 2.2 Correlations (Spearman's rho > 0.75 for at least one water body) between the daily average water temperature and the daily average or daily total of various meteorological variables. All correlations were significant ($P < 0.001$).

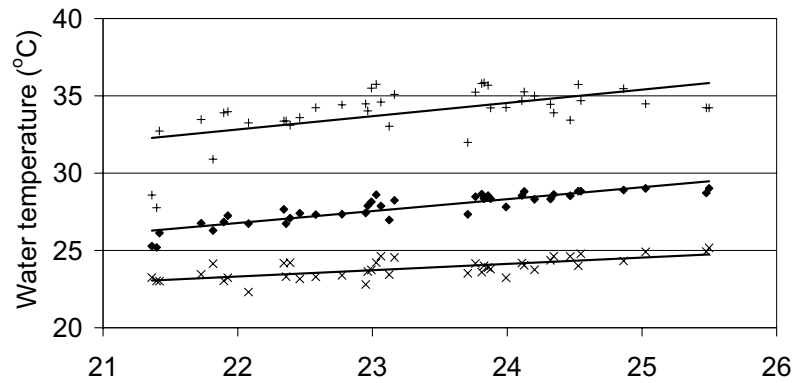
Average T_w vs.	SWB	MWB	LWB
Average T_{10}	0.94	0.93	0.90
Average T_a	0.93	0.91	0.86
Average $T_{.5}$	0.89	0.89	0.86
Average max T_a	0.85	0.80	0.73
Average T_{-17}	0.80	0.84	0.82
Total R_s^\downarrow	0.74	0.76	0.79

SWB Small water body; MWB Medium water body; LWB Large water body, T_w water temperature, T_{10} air temperature 10 cm above grass, T_a air temperature at 2 meters above ground, $T_{.5}$ soil temperature at 5 cm depth, max maximum, T_{-17} soil temperature at 17 cm depth, R_s^\downarrow incoming short wave radiation load.

A Small-sized water body



B Medium-sized water body



C Large-sized water body

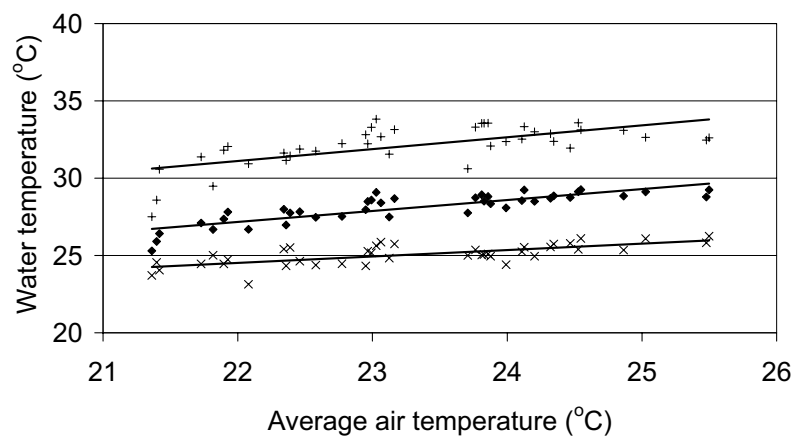
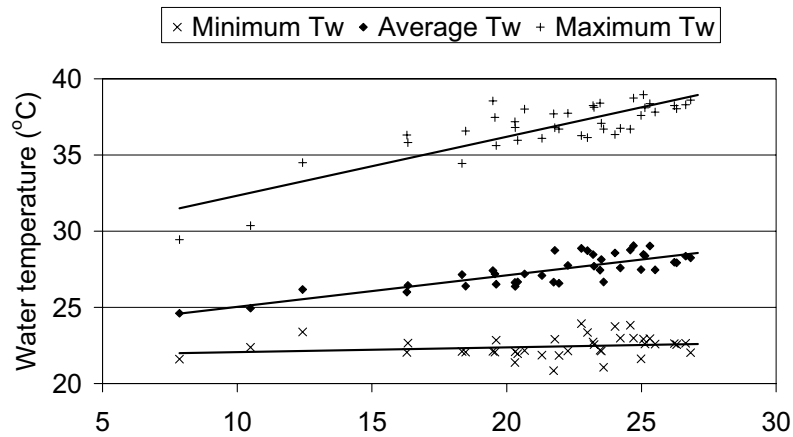
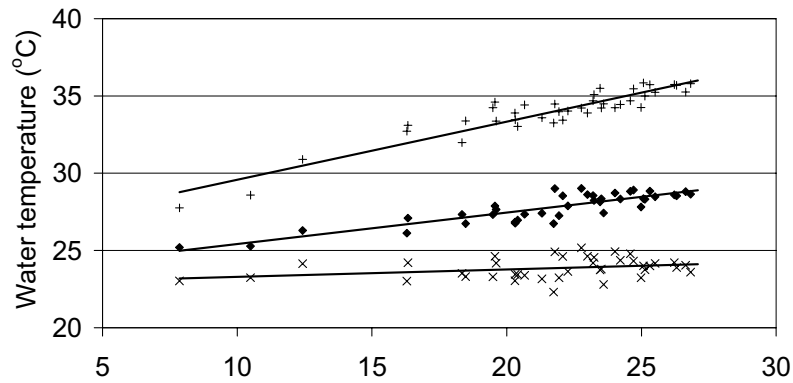


Figure 2.7 Minimum, average and maximum daily water temperatures (T_w) of **(A)** the small-sized water body **(B)** the medium-sized water body and **(C)** the large-sized water body, plotted against the average daily air temperatures. The black lines represent regression lines.

A Small-sized water body



B Medium-sized water body



C Large-sized water body

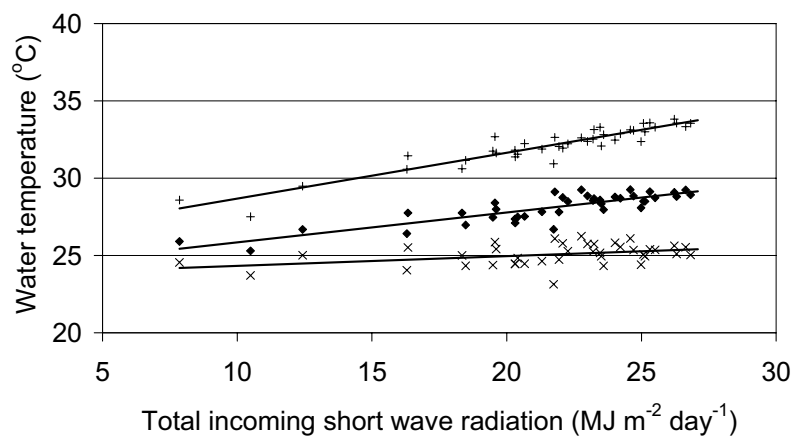


Figure 2.8 Minimum, average and maximum daily water temperatures (T_w) of **(A)** the small-sized water body **(B)** the medium-sized water body and **(C)** the large-sized water body, plotted against the daily total incoming shortwave radiation. The black lines represent regression lines.

2.3.3 Model estimates of diurnal water temperature variations

Model simulations for the three selected periods have been executed and the results are shown in Figure 2.4-2.6. Generally, it can be observed that the modelled water temperature dynamics were similar to the measured water temperatures. Quantitatively, the maximum values, minimum values and changes of temperature by rainfall occurred more or less at the same time and were of the same magnitude.

A small dissimilarity between the modelled and measured water temperatures was obtained during nocturnal cooling of the SWB, mainly during the period with clear days, whereby the modelled temperature was up to 2.0 °C lower than the actual water temperature. The largest dissimilarities (up to 2.5 °C in the SWB) were obtained during the first hours after sunrise up to around local noon. This is due to the rapid increase in water temperatures whereby the modelled temperatures increased slightly earlier in time. However, as mentioned before, the diurnal course was similar and similar minimum and maximum temperatures were reached during the day and, therefore, such dissimilarities can be neglected.

2.3.4 Model estimates of the energy budget components

Table 2.3 shows the estimated energy gains and losses through the various terms of the energy budget during the second day of each selected period. The percentages given hereafter are averages over the entire study period.

Energy gained at the air-water interface through radiative processes contributed most to the total energy gained at the water bodies: on average 88.1% (± 0.2 ; range 85.1-91.6%), 84.3% (± 0.2 ; range 81.6-89.7%) and 82.9% (± 0.3 ; range 79.3-91.1%) was gained by shortwave and longwave radiation at the SWB, MWB and LWB, respectively. The relative importance of energy that was stored in the water increased with increasing water body volume (the average percentage was 5.0 (± 0.1 ; range 3.4-5.6) at the SWB, 11.9 (± 0.2 ; range 7.7-13.4) at the MWB and 15.3 (± 0.3 ; range 7.2-17.3) at the LWB, whereas the contribution of the soil heat flux to energy gain (both vertical and sideways) decreased with increasing water body volume, with the average percentage of the total soil heat flux being 6.9 (± 0.2 ; range 3.5-9.4) at the SBW, 3.8 (± 0.2 ; range 1.8-5.6) at the MWB and 1.8 (± 0.1 ; range 0.3-3.8) at the LWB.

Similar to the energy gains, the largest percentage of energy loss occurred at the air-water boundary: 88.6% (± 0.3 ; range 85.8-92.2%), 83.5% (± 0.3 ; range 80.6-89.2%) and 81.8% (± 0.4 ; range 77.7-89.6%) was lost at the SWB, MWB and LWB, respectively, through

longwave radiation, latent heat and sensible heat. The contribution of longwave radiation to the energy loss was largest: On average this percentage was 68.6 (± 0.6 ; range 63.9-80.5%) in the SWB, 65.9 (± 0.5 ; range 60.5-79.2%) at the MWB and 65.2 (± 0.6 ; range 60.0-79.8%) at the LWB.

Because the air temperature was lower than the water temperatures of all water bodies at any given time of the day, there was a continuous heating of air above water bodies (positive sensible heat flux) and a continuous evaporation of water (positive latent heat flux). The percentage of energy loss through sensible heat was relatively small (an average contribution of 2-2.4% to the total loss at all water bodies with a maximum of 3.2%), whereas the latent heat flux accounted for a large part of the energy lost; on average 17.6% (± 0.6 ; range 8.6-23.4%) at the SWB; 15.5% (± 0.4 ; range 8.3-19.7%) at the MWB and 14.7% (± 0.4 ; range 8.2-18.5%) at the LWB. The contribution of evaporation to the total energy loss decreased during the (heavy) overcast days.

Table 2.3 Measured or modelled daily percentages of energy gains and losses at the studied water bodies on the second day of each selected period is shown whereby DOY 87 was a clear, DOY 96 an overcast and DOY 118 a heavy overcast and rainy day.

	DOY 87			DOY 96			DOY 118		
	SWB	MWB	LWB	SWB	MWB	LWB	SWB	MWB	LWB
Gains									
R_s^* (%)	34.5	32.8	32.9	28.5	27.1	26.6	13.7	13.7	14.0
R_l^\downarrow (%)	52.4	49.8	50.0	59.6	56.7	55.6	75.9	75.9	77.2
G_t (%)	5.8	4.7	0.1	4.9	3.9	2.1	5.3	2.1	1.6
G_w (%)	2.5	0.9	0.2	2.2	1.1	0.7	1.6	0.5	0.1
ΔS (%)	4.8	11.8	16.9	4.9	11.2	15.1	3.4	7.7	7.2
Losses									
R_l^\uparrow (%)	63.9	60.5	60.0	66.9	63.5	62.1	80.1	74.5	69.4
H (%)	2.9	3.0	3.0	3.1	3.2	3.1	1.8	2.3	2.8
LE (%)	19.6	18.7	18.5	17.8	17.0	16.2	9.1	10.4	11.6
G_t (%)	4.8	2.5	0.1	4.0	2.3	1.1	2.2	0.3	0.0
G_w (%)	3.7	2.5	0.0	2.7	1.9	1.4	2.3	0.5	0.8
ΔS (%)	5.0	12.8	18.4	5.4	12.1	16.1	4.5	12.1	15.4

R_s^* available net shortwave radiation, R_l^\downarrow incoming long wave radiation, R_l^\uparrow outgoing long wave radiation, G_t soil heat flux density vertically, G_w soil heat flux density sideways, ΔS energy storage, H sensible heat flux density and LE latent heat flux density.

Similar to the energy gains, the percentage of energy lost to the soil decreased with increasing water body dimensions (6.4% (± 0.3 ; range 3.0-8.8%) at the SWB, 4.5% (± 0.2 ; range 0.8-6.8%) at the MWB and 2.8% (± 0.2 ; range 0.1-5.5%) at the LWB), whereas the energy that was extracted from the water was more important for the larger water bodies (5.0% (± 0.1 ; range 3.9-6.1%) at the SWB, 12.0% (± 0.2 ; range 7.6-13.4%) at the MWB and 15.4% (± 0.2 ; range 9.7-18.4%) at the LWB).

With respect to the terms that contributed to the gains as well as the losses of energy over the study period, it seemed that the gains and losses through the storage term (overall all water bodies lost 0.1%) and through the soil heat (the SWB gained on average 0.5%, the MWB lost 0.6% and the LWB lost 0.9%) were approximately of similar quantity. Contrarily, more longwave radiation was emitted from than received at the air-water interface of the water bodies, resulting in an average loss of 10.4% at all water bodies through this heat flux.

Taking a closer look at the evaporation rates, the small-sized water body showed a higher evaporation (mm day^{-1}) during daytime compared to the large-sized water body, but this situation was reversed during the night. During the study period, the average daily estimated evaporation was 4.2 (± 0.2 ; range 1.6-5.9) mm in the SWB, 3.8 (± 0.1 ; range 1.6-5.0) mm in the MWB and 3.7 (± 0.1 ; range 1.6-5.1) mm in the LWB.

2.4 Discussion

2.4.1 Observations of diurnal water temperature dynamics

2.4.1.1 Mean water temperatures and diurnal fluctuations

The upper millimetres of a water body are important in the larval population dynamics of *An. gambiae* s.l., as malaria mosquito larvae generally live horizontally near the air-water interface of aquatic habitats [39]. In this study we demonstrated that the top layer (upper 2 mm) of each water body differed in temperature from the layers underneath. During the afternoon and at night, the temperature of the top layer was up to 2 °C lower than the layers below, and during the first hours after sunrise, the temperature of this layer was up to about 1 °C higher, depending on the weather conditions. The observed higher temperature of the top water layer during the first hours after sunrise is caused by the incoming radiation load combined with a relatively small difference between water and air temperature. Around local noon, the difference between air and water temperature becomes larger and more energy will be extracted from the top water layer due to evaporation (latent heat flux) and the heating of the air above the water surface (sensible heat flux), resulting in a lower temperature of the top water layer.

No stratification was observed in the small and clear water bodies, meaning that the water bodies were well mixed. The diurnal water temperature behaviour of the three different-sized water bodies differed on both a spatial and a temporal scale. The small-sized water body reached its minimum and maximum temperature at an earlier stage of the day than the larger ones. Spatially, higher daily maximum and lower daily minimum water temperatures were recorded when habitat dimension decreased. Water temperatures below 18 °C were not recorded (20.8 °C was the lowest recorded temperature in the SWB). When larvae were reared at 18 °C in the laboratory, no adults emerged [19,40].

The same laboratory studies showed that no adults were emerging when larvae were reared above 32 °C. The larvae were, however, continuously exposed to constant temperatures. Apparently, as this study showed that 32 °C was regularly exceeded on both clear and overcast days in the water bodies, larvae are able to withstand these higher temperatures, which are reached for a relatively short period during the day. The maximum temperature that larvae of *An. gambiae* s.l. can endure for a short period is believed to be 41 °C [13,41]. Higher temperatures were lethal, but such extreme water temperatures were not recorded during this study; the average maximum water temperature was 36.8 (± 0.3) °C in the smallest water body. This temperature is near the temperature at which mosquito larvae have the highest development rate [42].

Our data show that mosquito immatures in small and shallow pools can be exposed to a wide temperature range under natural conditions, as the average recorded difference between the daily minimum and maximum water temperature was 14.4 °C in the SWB with a maximum recorded difference of 16.8 °C. Therefore, they may experience many of the constant temperatures at which they are reared in laboratory experiments in one single diurnal temperature cycle.

Notwithstanding the spatial and temporal differences between the water bodies of different size, the average daily water temperature over the whole period was quite similar (27.4-28.1 °C) for all water bodies. Similar mean water temperatures were found in The Gambia by Bayoh [43] in puddles (27.1 °C) and in habitats in rice fields (27.4 °C). Laboratory studies revealed that 27 °C was the optimal temperature for the survival of *An. gambiae* [18] and that 28 °C was the optimal temperature for their rate of development [19]. Since these optimal temperatures are approaching the average water temperatures in the field we suggest that the immature mosquitoes are evolutionarily adapted to their direct environment. It is likely that any differences in larval development and survival between the differently sized breeding sites should be sought in the magnitude and duration of a) the deviations from this mean or

b) the temperature change per unit time. The relationship between different daily fluctuations in water temperature and the life-history characteristics of mosquito immatures should be the subject of future studies.

Besides affecting the mosquito immatures directly, water temperature will have an effect on the diversity, densities and activity of other aquatic organisms, including possible predators and pathogens and food resources, such as algal matter. Moreover, water temperature affects hydrological parameters, such as dissolved oxygen, pH and conductivity [26]. Subsequently different temperatures may affect competition between larvae of the sibling species (Chapter 5).

Comparing our temperature data with temperature data of natural mosquito larval habitats described in other studies [e.g. 12,44] remains difficult, as important parameters such as habitat dimensions [this chapter; 20], water turbidity (Chapter 3) and factors affecting local microclimate such as land cover type and canopy overgrowth [21] and altitude may each affect the water temperature. In the highlands of western Kenya, comparable maximum water temperatures are reached in farmland pools as in our small-sized water body [44]. The minimum and average water temperatures are, however, lower in the highlands. More detailed studies are required to show the effect of altitude on the local microclimate in such elevated areas in order to understand and predict water temperatures of mosquito larval habitats. These areas are affected by malaria [45] and higher altitudes within malaria areas will possibly see an increase in malaria transmission due to the creation of a more suitable environment [46] under a changing global climate.

Remarkably, even within one single larval habitat large temperature differences may occur: Average differences in water temperature of 2.9 (± 2.6 s.d.) and 3.1 (± 2.4) °C have been recorded in dirt tracks and ditches, respectively, with a maximum difference within a single water body (dirt track) of 9 °C [10]. This is possible in very large water bodies, such as extended tire-tracks on a dirt road or extended ditches, whereby some parts may even be shaded and others sunlit, enlarging the difference.

2.4.1.1 Relations between water temperatures and meteorological variables

The change in water temperature corresponded better to a change in incoming solar radiation at the small-sized water body. This will be due to its small volume which results in a small heat capacity. Larger water bodies have a larger buffer capacity and, therefore, sudden changes in the local microclimate, such as a change in incoming solar radiation, will have less effect on the water temperature. The larger buffering capacity of the large-sized water

body was probably the reason that this water body reached a lower minimum temperature on DOY 119 compared to DOY 118. During the latter day the lowest value in our study period of the total incoming solar radiation load was recorded and all water bodies were subjected to excessive rainfall, whereby the small-sized and medium-sized water body reached a lower minimum temperature compared to DOY 119.

Rainfall caused a decrease in water temperature and the maximum recorded decrease during precipitation was 5 °C (in 15 minutes) in the small-sized water body. The water level in this water body was generally more susceptible to rainfall due to its smaller depth, which results in a higher precipitation-depth: water-depth ratio. Therefore a relatively large percentage of the water volume consists of the cooler rainwater during rainfall. However, the magnitude and direction of the change of the water temperature depends on parameters such as habitat dimensions, water temperature of the habitat, temperature of the rainwater, duration and quantity of the precipitation and the time of the day when rainfall occurs.

Rainfall may also affect the daily average water temperature and the magnitude of the fluctuations around this mean. Bayoh [43] observed a significant decrease in daily mean water temperature of both a puddle and a rice field after six days of rainfall. After the rains, mean hourly water temperatures decreased up to 6 °C compared with water temperatures before the rains and the amplitude of the diurnal temperature was reduced. Young [47] found similar results in Kenya: Even in a large dammed pond (11552 m²) the daily mean water temperature dropped by almost 2-3 °C and diurnal fluctuations decreased by more than half after one day of rainfall.

Remarkably, the air temperature at two meters above ground was below the water temperatures of all water bodies throughout each day, even during the rapid increase of temperatures during the first hours after sunrise. Young [47] observed that the average weekly air temperature was always below the average minimum weekly water temperature of the large dammed pond in Kenya mentioned above. Our data showed that organisms that live in small and shallow waters, such as larvae of *Anopheles gambiae* s.l., are exposed to temperatures which can differ considerably from the air temperature. The largest differences between the air and water temperature during daytime were observed in the small-sized water body, with a difference up to 10.2 °C, whereas the largest differences during nighttime occurred in the large-sized water body, with a difference up to 8.1 °C. The observed differences between air and water temperature have important consequences for ecological models that use the air temperature as an input parameter for larval development and survival [48]. Air temperature can be used as an input parameter when the exact relation between water temperature and

air temperature is known. This study showed that there were strong and significant correlations between the average water temperature and various meteorological variables, including the average air temperature. This gives us the opportunity to predict the average water temperature quite accurately using the average air temperature.

2.4.2 Model estimates of diurnal water temperature variation and the energy budget components

The exchange of heat across the air-water interface is considered one of the more important factors that govern the temperature of a water body [49]. The air-water interface was the most important boundary for energy exchange with 83-88% of the energy being gained from shortwave and longwave radiation. Similar quantitative data have been reported by other studies [28,30]. The same interface was furthermore the most important boundary for energy losses; 82-89% of the losses occurred at this boundary, whereby longwave radiation (65-69%) accounted for the largest percentage of heat loss. The estimated average energy loss due to evaporation (15-18%) and the sensible heat exchange (<2.5%) was lower than the reported 24-30% and 5-11%, respectively, in other studies [28,30]. However, these studies are carried out in a different non-stagnant water system (a river) on a different geographical latitude, where climate is different and distinct seasons are present.

Although the contribution of the soil heat exchange to energy gains and losses is relatively small, both the exchange horizontally and sideways needs to be incorporated in models that try to estimate water temperatures of very small water systems. These terms are not negligible due to the relatively small volume of our water bodies compared to that of the rivers, ponds and lakes in other studies.

Moreover, when modelling small and shallow water bodies, it is necessary to have accurate estimations of soil properties. Soil properties, such as heat conductivity, will depend largely on soil moisture, which will vary between days: In Kenya it can be extremely hot and dry one day and extremely wet the next. Therefore, by measuring the soil heat flux and soil moisture directly, our understanding of soil processes would improve. Hoshen and Morse [48] suggested that the inclusion of hydrology and soil type in models of malaria transmission may improve the understanding of the connection between precipitation and the larval habitats, but it may also provide us with useful information in order to estimate the diurnal water temperature behaviour of such habitats.

The use of the transfer coefficients for heat and water vapour, which were derived from measurement above a grass land surface and by using the typical values for the roughness

length for heat and momentum above this type of surface cover, proved possible. As the grass was kept short, the areas with the meteorological stations were both open grasslands and the water bodies were small, the microclimate above the water surfaces was similar to that above the surrounding grass surface. Changes in the surroundings, such as the presence of tall grass vegetation, or changes within a habitat, such as the presence of aquatic vegetation, may result in a reduction of wind (wind shielding) and radiation (a reduced view factor). This will require the introduction of correction-factors, as a standard weather station will not sense these changes in microclimate.

Evaporation is an important factor that determines the longevity of a water body to a large extent. During clear days, small water bodies will evaporate more during daytime than larger ones, as the difference between the water temperature of smaller water bodies and the surrounding air temperature is larger. In the evening the situation is reversed: Larger breeding sites will vaporize more water due to larger difference between their water temperature and the air temperature. When water body dimensions decreased, more water vaporized cumulatively on clear and overcast days. On the extreme overcast day the situation was reversed.

The longevity of a breeding site depends on a range of other factors, such as soil structure, soil moisture, incoming water by precipitation and human disturbances, but this study illustrates the transient nature of natural mosquito breeding sites. The estimates imply that, with an average evaporation rate of 4.8 mm during clear days, the small-sized water body would be desiccated within 8.3 days when no water is added. Therefore mosquito larvae that inhabit such small water bodies only have a short time to develop into adults in the absence of rains, while it is known that larvae of *An. gambiae* s.l. may take between one and three weeks to develop into adult mosquitoes under ambient condition in the field [13,50]. However, desiccation of a habitat does not directly affect the mortality of the immatures, since eggs of *An. gambiae* s.l. can remain viable for 12 days [51] and larvae are capable of surviving on moist mud from 64 hours for the youngest larvae up to an estimated 113 hours for the oldest larvae [52].

We showed the diurnal temperature dynamics of typical *An. arabiensis* and *An. gambiae* breeding sites and assessed the importance of the various energy fluxes that contribute to the energy balance of such water bodies, including the amount of heat that is stored in the water volume, which eventually determines the diurnal water temperature behaviour. Being capable of predicting the water temperature, we may be able to estimate the magnitude of

the changes in water temperatures under a changing global climate. Furthermore, our findings may be translated to other continents where similar or other mosquito species are transmitters of important vector-borne diseases.

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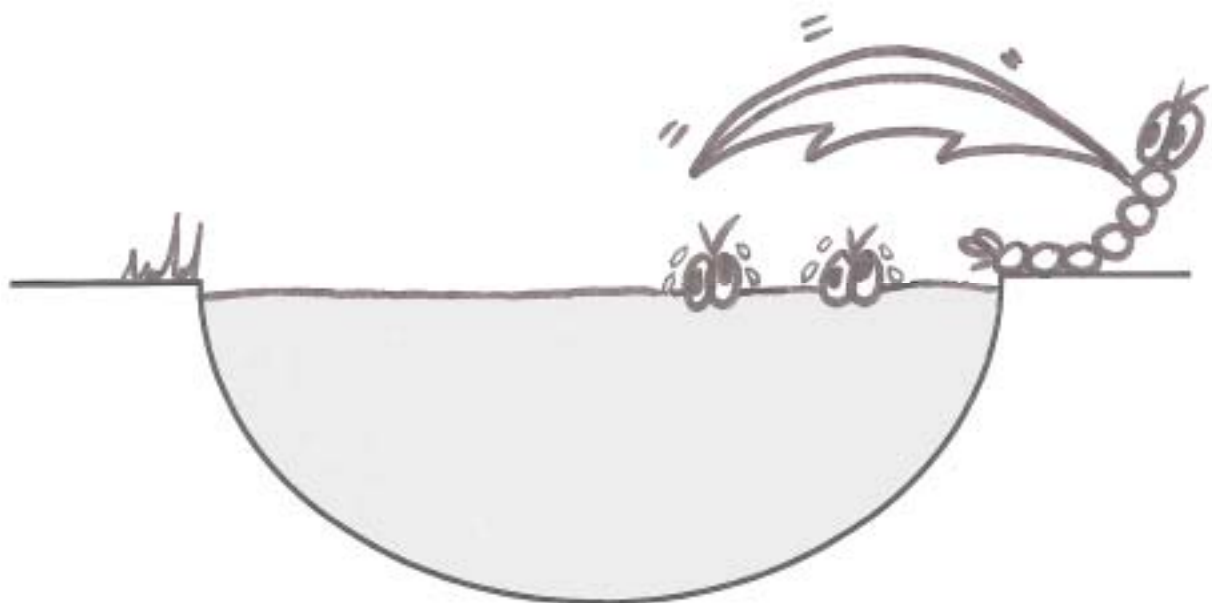
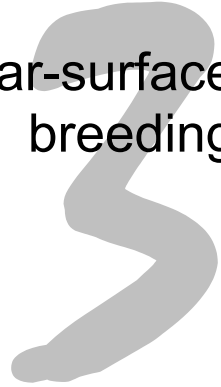
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The effect of water turbidity on the near-surface water temperature of malaria mosquito breeding sites

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Abstract

Extinction of solar radiation determines the temperature of a water body to a large extent. It is a function of the absorption and scattering of sunlight by suspended particles in a water column. To get a better understanding on the relationship between water turbidity and water temperature, which is one of the major determinants in the growth and development rate of malaria mosquito larvae, a series of semi-natural breeding sites (diameter 0.32 m, water depth 0.16 m) with increasing water turbidity was created. Here we show that at midday the upper water layer (thickness of 10 mm) of the most turbid water body was on average 2.8 °C warmer than the same layer of the clearest water body. Suspended soil particles increase the water temperature during daytime and affect the diurnal temperature behaviour of small water bodies, which results in a longer exposure of mosquito larvae to higher temperatures, compared to clear water collections. These spatial and temporal differences in water temperature of small and shallow water collections may be an explanatory factor for the observed differences between larval growth and development in clear and in turbid water collections.

3.1 Introduction

The two sibling mosquito species *Anopheles arabiensis* Patton and *An. gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*), both belonging to the *An. gambiae* Giles sensu lato complex (hereafter referred to as *An. gambiae* s.l.), occur sympatrically across a wide range of tropical Africa, where they are the most efficient vectors of malaria parasites [1].

Both species breed in small, shallow, open and sunlit water collections [2-4], such as burrow pits, brick pits, tire tracks, small puddles, human foot and animal hoof prints. These water bodies are often of a transient nature, as their availability corresponds to rainfall [3,5,6].

Although females of *An. gambiae* s.l. seem to prefer darker substrates [7-9] or substrates turbid with soil over clear water [7,10] to deposit their eggs on, this preference is not reflected in the field: Various studies, examining larval habitat characteristics or larval population dynamics, failed to give a clear relationship between the presence of *An. gambiae* s.l. immatures and the turbidity of breeding sites. Studies observed no effect of turbidity on the occurrence of *An. gambiae* s.l. [10,11], a preference of *An. gambiae* s.l. to breed in rather clear water bodies [12,13], whereas Gimnig et al. [3] observed that *An. gambiae* and *An. arabiensis* were associated with habitats that were high in turbidity and that both species increased in larval densities with increasing water turbidity.

Turbidity is defined as 'an expression of the optical properties of a sample that causes light rays to be scattered and absorbed rather than transmitted in straight lines through the sample' and particles such as clay and silt, finely divided organic matter, plankton and micro-organisms contribute to turbidity [14].

Water that flows down from the higher surrounding areas into the water body and mixing of water with soil or sediment particles that are present at or near the soil-water interface, both due to rainfall, will affect water turbidity. Moreover, turbidity can be increased by biological activities, such as increasing algal matter and phytoplankton abundance, or by the disturbance of water pools by humans (e.g. in road puddles or water collection sites) or animals (e.g. in puddles near cattle).

There are several direct and indirect ways in which water turbidity can affect the mosquito immatures. It may affect their distribution when water turbidity does in fact influence the oviposition behaviour of the female mosquitoes, as mentioned previously. Subsequently, a preference for turbid water can be advantageous as, due to a lower visibility, a higher turbidity may decrease the chance of being preyed upon, which increases the chance of

survival for the mosquito larvae. Predation is often considered to be an important factor explaining the high mortality rates that are observed in natural breeding sites [15-18].

Moreover, turbidity will alter the efficiency of light penetration through a water body and affect the water temperature, as the suspended particles reflect and absorb the incoming solar radiation. Light exposure and temperatures will affect the presence of algae, important food sources for *An. gambiae* s.l. larvae and possible regulators of larval populations [19,20], as algal growth is favoured by higher temperatures and more light [21].

Indirectly, the light regime in water bodies can affect algal biomass by affecting the submerged aquatic plants: Light is a major factor limiting submerged macrophytes [21]. These macrophytes can suppress algal growth by a reduction in available nutrients [22] and by allelopathy, the secretion of substances that are toxic to algae [reviewed in 23]. This might explain the observations by Gimnig et al. [3] that larvae of *An. gambiae* s.l. were associated with habitats that contained algae but little or no vegetation.

As the mosquito immatures are poikilothermic and therefore unable to internally regulate their body temperature, their metabolic rate will depend on the temperature of the water they inhabit. In general, within a certain temperature range, an increase in water temperature will result in a faster development of the aquatic stages [24,25], but will decrease the size of the emerging adults [25] and at higher temperatures fewer adults are produced due to increased mortality [26].

Besides, higher water temperatures in turbid water pools will result in a faster evaporation and hence affect the longevity of the water body. In very small and turbid water collections this means that the mosquito immatures will have a relative shorter time span to reach the adult stage. For example, larvae of *An. gambiae* s.l. may take as soon as one week, but as long as three weeks to develop into adult mosquitoes under ambient condition in the field [20,27], which necessitates the continuous presence of water in the breeding site for this time period, although larvae are known to survive for a few days on damp soil [28].

Finally, as the mosquito larvae seem to filter particles indiscriminately (*An. quadrimaculatus* Say) [29], they will ingest particles that are not edible. Therefore, in more turbid water collections, it is plausible that more time and energy is required to obtain the same quantity of nutrition compared to larvae that feed in clear water bodies. Ye-Ebiyo et al. [30] suggested that turbidity may interfere with larval feeding. It was observed that sites with relatively clear water produced more *An. arabiensis* pupae and larger adults than habitats with turbid water. However, this effect was nullified when food was available at the water surface.

The biotic and abiotic factors that affect the growth, development and survival of the immature stages of *An. gambiae* s.l. require more attention. They will determine the abundance and the fitness of the resultant adult mosquito populations, which will consequently affect the transmission of malaria. Here, we assessed the effect of water turbidity on the near-surface water temperature of small, semi-natural mosquito breeding sites in western Kenya. Understanding factors that affect the water temperature is of major importance, not only because the water temperature is an important determinant in the development and survival of malaria mosquito immatures, but it may also affect other aquatic species, including the species that parasitize or prey upon the mosquito immatures.

3.2 Material and methods

The experiment was carried out in May 2006, at the grounds of the Kenya Medical Research Institute (KEMRI) near Kisumu, Kenya. The experimental area consisted of black cotton soil (soil composition is given in Chapter 2) and was covered with grass, which was kept short. Semi-natural water bodies were created by digging holes (\varnothing 0.32 m, depth 0.17 m) in the soil, at least 0.3 m apart. The holes were lined with transparent plastic (0.13 mm), which was pressed tightly against the soil to maximize plastic-soil contact and kept in place by a small metal ring (diameter 50 mm larger than the water body).

One pit was filled with clear tap water up to 10 mm under the rim (site no. 1). The water originated from a well at the KEMRI grounds. Eight other pits were filled with water of different turbidity. Red clay soil was collected in the vicinity of Maseno (25 km northwest of Kisumu), sun-dried and fine clay particles of up to 1 mm in diameter were selected by sieving the soil through a sieve with mesh size of 1 mm. The highest turbidity in the gradient series was created by adding 5.8 kg of friable clay soil to 85 litres of tap water. The mixture of water and soil was stirred for 30 seconds, after which the surplus soil was allowed to settle at the bottom of the basin for 10 minutes. Part of the supernatant was used to fill one pit (site no. 9) and the remainder was mixed with clear tap water in a 3:1 ratio, stirred again for 30 seconds, after which another pit was filled (site no. 8). The dilution and mixing of the solution and the subsequent filling of a hole was continued until all semi-natural water bodies were filled. The water bodies of different turbidity were randomly distributed over the experimental area.

Daily at 9h00, 13h00 and 17h00, the water temperature of the upper 10 mm of each water body was measured in the centre with a handheld digital thermometer (GTH 175/Pt; Greisinger Electronic GmbH, Germany). The part of the probe above the air-water interface was shielded from direct sunlight by a white polystyrene cover. Subsequently, the water

turbidity (i) of the top 10 mm, (ii) halfway the water body and (iii) of the bottom 10 mm was measured in the centre of each water body with a portable Turbidimeter (TN100; Eutech Instruments Pte Ltd., Singapore) in Nephelometric Turbidity Units (NTU).

Each morning before 8h00 clear tap water was added to the water bodies to compensate for evaporation and to maintain a uniform water level, after which all water bodies were stirred thoroughly for 15 seconds to create a homogeneous turbidity. The experimental setup was covered during the evening and night to prevent rainfall from disturbing the experiment. Each week, all water bodies were emptied, the plastic lining cleaned and, subsequently, the pits were refilled with clear or turbid water.

Eleven days were selected for the study. The relation between water turbidity and the near-surface water temperature is discussed in more detail for two separate but consecutive days, which differed in the daily incoming solar radiation load. This approach was chosen as the water turbidity and water temperature of the water bodies in the morning, as well as the climatic conditions throughout the day, varied per experimental day, as the experiments were carried out under natural weather conditions outdoors. The first day (25 May 2006) was a clear day and the second day (26 May 2006) an overcast day.

Data were analyzed using SPSS software (version 12.0.1, SPSS Inc., Chicago, IL). A GLM was used to test for the effect of “measurement depth”, “water body” or for an interaction between “measurement depth × water body” on the water turbidity. Experimental series was included in the GLM as a random factor and a double square root transformation was used for water turbidity to meet normality assumptions. The strength of relationship between water turbidity and water temperature is calculated with Spearman’s rank correlation test, using a Bonferroni correction for multiple tests. The time mentioned in this chapter is the local Kenyan time (UTC+3).

3.3 Results

3.3.1 Water turbidity over water depth

Considering the data collected at 13h00, water turbidity varied significantly between the experimental water bodies (Figure 3.1; $F_{8,260}=613.8$, $P<0.001$). In general, the water layer near the bottom was more turbid than the top water layer ($F_{2,260}=69.9$, $P<0.001$) and, as there was a strong interaction between water layer and experimental water body ($F_{16,260}=5.8$, $P<0.001$), this difference seemed to increase when the average turbidity of a water body increased. This difference was not observed in the clear and near-clear water bodies.

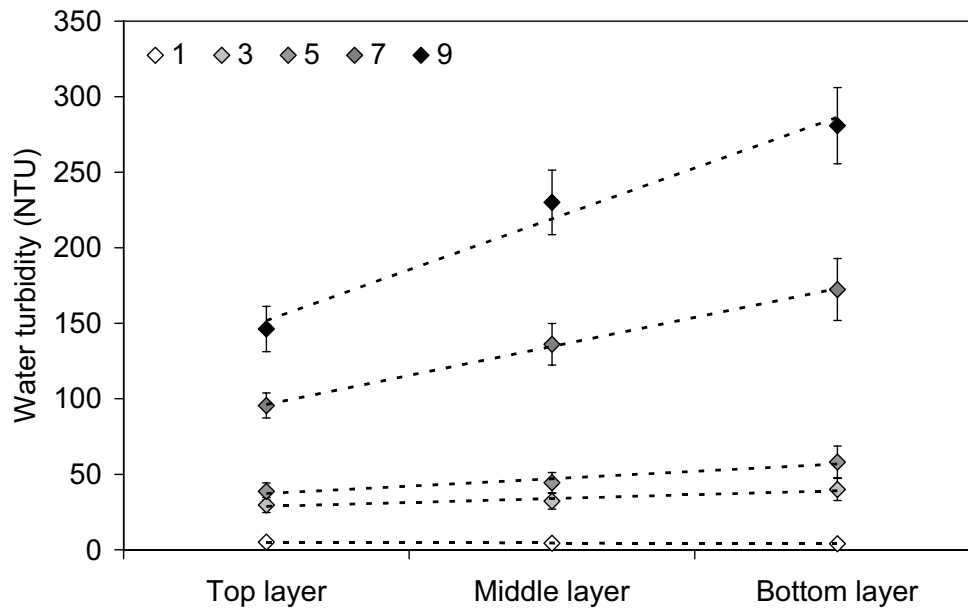


Figure 3.1 Average water turbidity (Nephelometric Turbidity Units or NTU) of the upper 10 mm (top layer), in the middle (middle layer) and of the lowest 10 mm (bottom layer) of five selected experimental water bodies at 13h00. Numbers shown in the legend correspond with the level of turbidity of each water body, whereby 1 indicates the clear water body and 9 the most turbid water body. The error bars indicate the standard error of the mean ($n=11$ days) and for each water body the regression line is shown (dotted lines).

3.3.2 Water turbidity vs. water temperature

Figure 3.2 shows all recorded data points during the selected eleven days. Although accumulating all data results in deviations in water temperature in relation to water turbidity, due to different daily experimental conditions as mentioned in the 'material and methods' section, there were significant correlations between water turbidity and the near-surface water temperature of a water body. During midday (13h00), water temperature increased with increasing water turbidity ($\rho=0.58$, $P<0.001$), whereby the difference between the clearest and most turbid water body was on average $2.8 (\pm 0.2 \text{ SEM}; \text{range } 1.0\text{-}3.3) ^\circ\text{C}$. At 17h00, a decrease in water temperature with increasing water turbidity was observed ($\rho=-0.36$, $P=0.002$) and the difference between the clearest and most turbid water body was on average $0.3 (\pm 0.1; \text{range } -1.2\text{-}0.0) ^\circ\text{C}$. No significant correlation was recorded at 9h00.

Subsequently, Figure 3.2 shows that daily turbidity of the water bodies decreased over time: The turbidity readings at 17h00 were lower than the turbidity readings in the morning at 9h00 (Mann-Whitney U test, $P<0.001$).

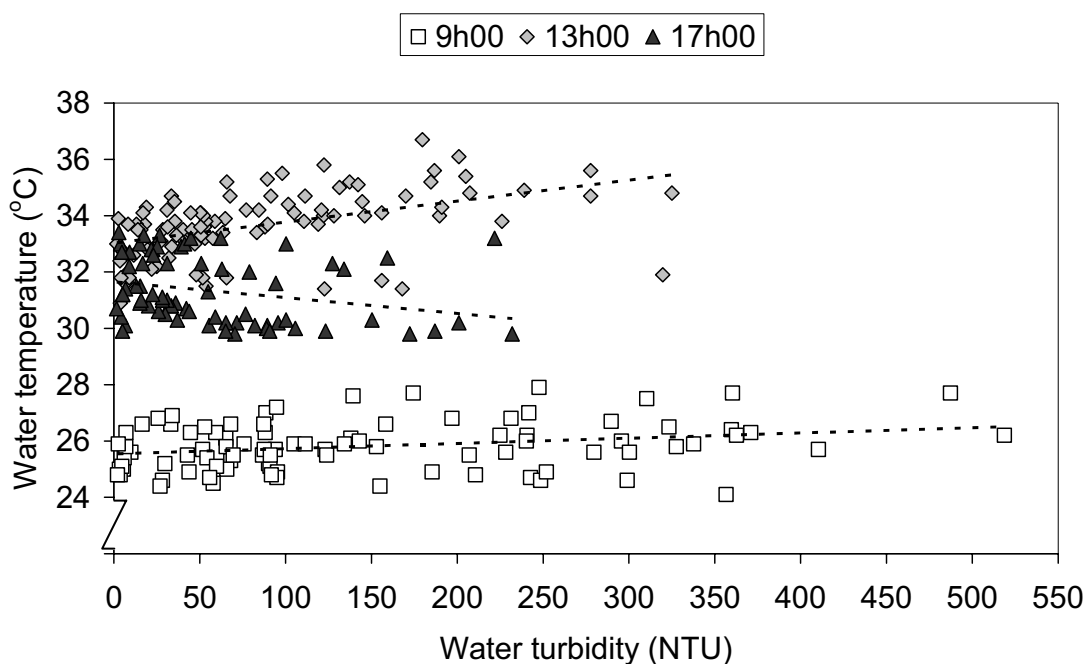


Figure 3.2 Relationship between the near-surface water temperature ($^{\circ}\text{C}$) and the average water turbidity (NTU) of all experimental water bodies over the whole study period at 9h00 ($n=10$ days), 13h00 ($n=11$ days) and 17h00 ($n=8$ days). For each time the regression line is shown (dotted lines).

The incoming solar radiation load on the two selected consecutive days is shown in Figure 3.3. The total incoming shortwave radiation load was 23.8 MJ m^{-2} on the clear day (25 May 2006) and 18.9 MJ m^{-2} on the overcast day (26 May 2006). The total received incoming solar radiation load at 13h00 was 12.7 and 10.1 MJ m^{-2} on the clear and overcast day, respectively. At 17h00 these quantities were 22.8 and 18.6 MJ m^{-2} , respectively. The maximum recorded incoming solar radiation is higher on the overcast day, as extra solar radiation reached the earth's surface which was reflected by clouds.

The relationship between the near-surface water temperature and the average water turbidity on both selected days is shown in Figure 3.4. In the morning, at 9h00, no large differences were observed between the measured water temperatures of the various water bodies. However, at 13h00 there was a clear positive correlation between water temperature and water turbidity, on both the clear day ($\rho=1.00$; $P<0.001$) and the overcast day ($\rho=0.97$, $P<0.001$): Water bodies of a higher turbidity were warmer. The difference between the water temperature of the clearest and of the most turbid water body was 3.3 and $2.9 \text{ }^{\circ}\text{C}$ on the clear and the overcast day, respectively.

In the late afternoon, at 17h00, the water temperature of the most turbid water body was 0.2 °C lower than that of the clearest water body on the clear day. On the overcast day, when the incoming solar radiation showed a sudden decrease of 488 W m⁻² in 30 minutes, 1.25 hours before 17h00, this difference was 1.2 °C and there was a clear negative correlation between water temperature and water turbidity ($\rho=-0.80$; $P=0.010$).

Finally, to obtain a better insight in the difference in the diurnal water temperature behaviour between clear and turbid water bodies, Figure 3.5 shows the measured diurnal temperature behaviour of a clear water body of similar dimensions during a clear day in the previous year (20 May 2005), as well as the possible temperature behaviour of a turbid water body during daytime, based on measurements in the most turbid water body during the present study on a clear day (25 May 2006). This illustrates that, apart from reaching a higher maximum temperature, turbid water collections are likely to be warmer than clear water bodies for a substantial part of the day.

3.4 Discussion

In small tropical pools, suspended soil particles caused enhanced temperatures of the water at midday. This effect, caused by the absorption of incoming energy from the sun by the particles, was observed during clear and overcast days.

As the daily temperature and turbidity of each water body at the start of each experimental day, as well as the daily weather conditions, varied between the days, a comparison of the temperature-turbidity relationship under various weather conditions is not reliable. However, there was a significant overall trend: around midday (13h00), the time when normally the maximum incoming solar radiation load reaches the earth's surface, waters that were more turbid had a higher near-surface water temperature. Due to the apparent linear correlation between water turbidity and water temperature, we assume that similar water bodies, which are more turbid than the water bodies in this experiment, will be even warmer.

This study only measured water turbidity and water temperature at three time points during the day. Permanent monitoring of the temperature in turbid water collections, as carried out in various different-sized clear water bodies (Chapter 2), will provide us with useful information, such as the maximum water temperature during daytime and the temperature behaviour at night. Our data suggest that the water temperature of turbid water bodies will be lower than that of clear water during at least a certain part of the late afternoon, as there was an overall negative correlation between the water temperature and

water turbidity at 17h00. However, it remains to be studied whether this difference is temporary or maintained throughout the night.

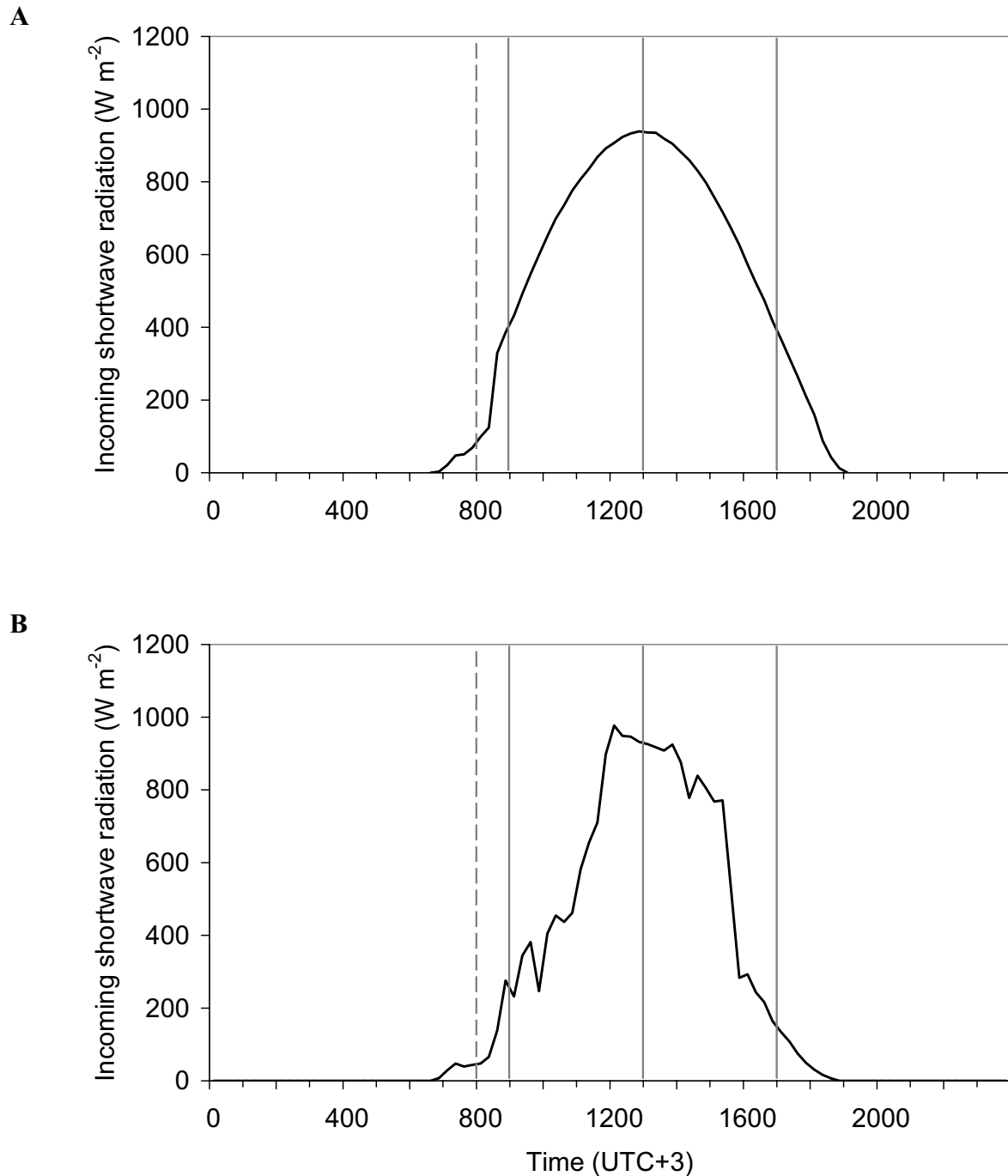


Figure 3.3 Incoming shortwave radiation (W m^{-2}) on **(A)** 25 May 2006, a clear day and **(B)** 26 May 2006, an overcast day. The discontinuous vertical lines indicate the time at which the water of each experimental site was mixed thoroughly (8h00), the solid vertical lines when the near-surface water temperature and water turbidity were measured (9h00, 13h00 and 17h00).

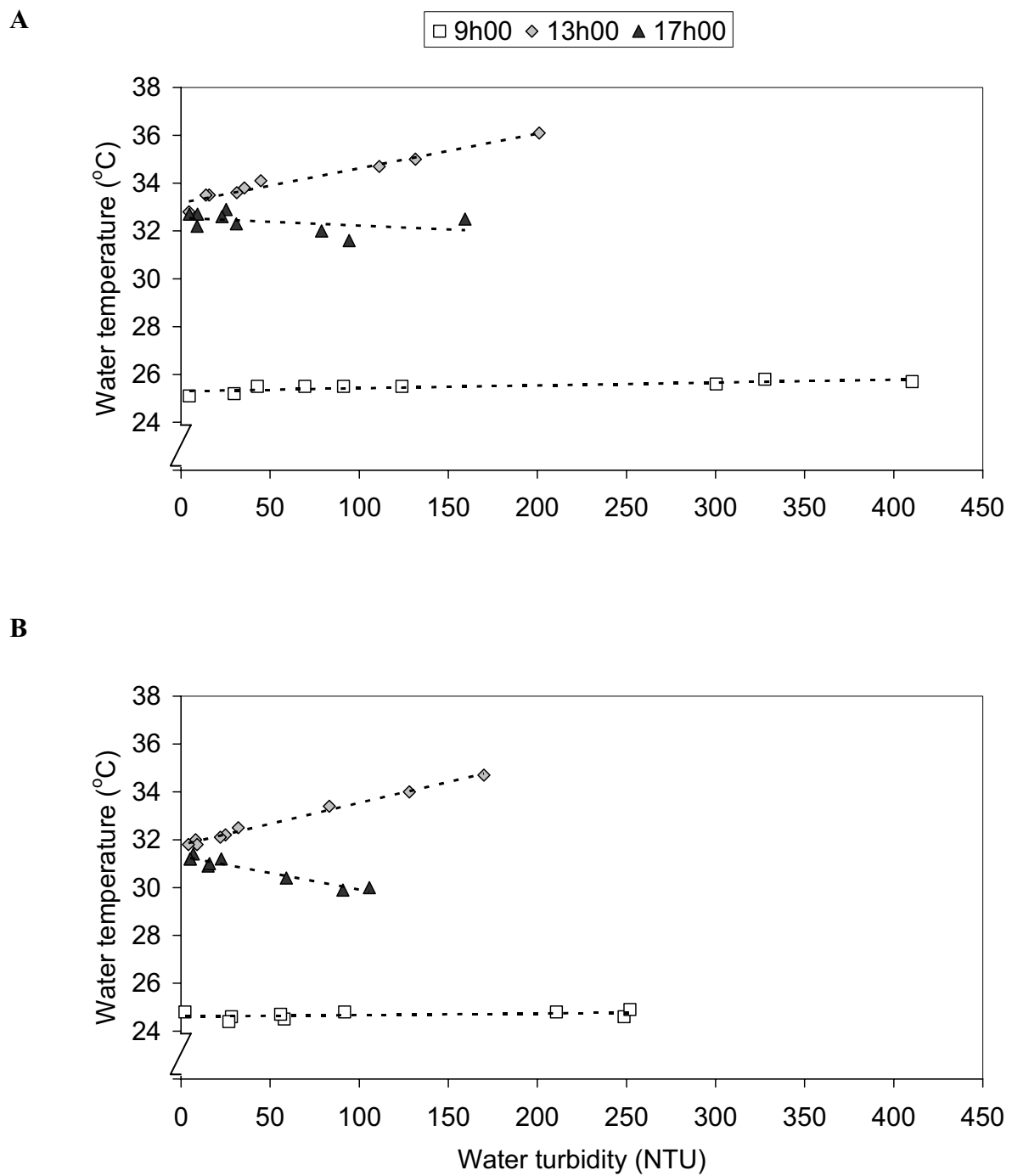


Figure 3.4 Relationship between the near-surface water temperature (°C) and the average water turbidity (NTU) of each water body at 9h00, 13h00 and 17h00 on **(A)** 25 May 2006, a clear day and **(B)** 26 May 2006, an overcast day. For each time the regression line is shown (dotted lines).

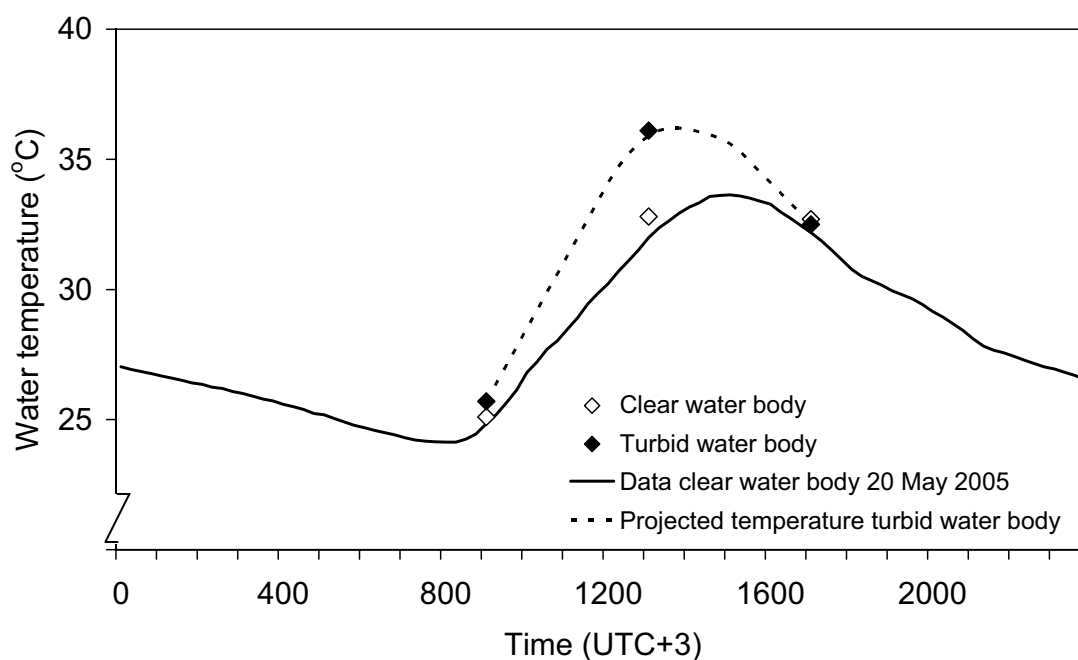


Figure 3.5 Water temperature ($^{\circ}\text{C}$) of a clear water body (with similar dimensions as the water bodies in this experiment) during a clear day in the previous year (20 May 2005), with therein plotted the temperature data collected from the clearest and most turbid water body during this experiment on a clear day (DOY 145). The dashed line shows the plausible temperature behaviour of the most turbid water body during daytime.

Besides an effect on the near-surface temperature, preliminary data showed that a high turbidity of small water collections will result in thermal stratification during daytime (KPP, unpublished data), whereby the lower layers will be cooler. As larvae of *An. gambiae* s.l., which normally live horizontally near the air-water interface, dive and can stay submerged for longer periods [31,32], they will experience different temperatures in turbid waters while diving, compared to clear water bodies that are homothermic (Chapter 2). In very turbid water collections, this diving behaviour may be a mechanism to escape unfavourably high temperatures near the air-water interface during daytime.

The present study shows the results for a water body of one particular size only. In sub-Saharan Africa, immatures of *An. gambiae* s.l. can be found in a wide range of different water pools, which can be smaller, such as human foot-prints and animal hoof-prints, or larger, such as tyre-tracks, brick-pits and even rice fields. As light can penetrate a turbid water column only to a certain depth (depending on the water turbidity and the quantity and angle of incoming light), deeper water bodies will have a relatively larger part of this column

that is not reached by the light. This part will, as a result, be lower in temperature. When the water column becomes well-mixed, the temperature of a large water body will be lower than the temperature of a very shallow pool of similar turbidity, where cooler lower layers are not present, as the light could still reach the bottom. We know that the temperature in small puddles behaves more dynamically than in larger water bodies (Chapter 2) and can reach temperatures near 40 °C [10,27,33-36]. In small and clear water bodies (diameter 0.16 m, water depth 0.04 m) the daily maximum water temperature often reached 38-39 °C (Chapter 2). When such water pools are very turbid, even higher daytime temperatures may be recorded. As a consequence, these temperatures may reach or even exceed the thermal death point of the larvae of *An. gambiae* s.l., which is 42 °C [27,37].

Ye-Ebiyo et al. [30] observed that the water turbidity in natural puddles increased as the rainy season ended and the diameter of the puddles diminished. We observed that the turbidity of the water bodies decreased over one day. In natural breeding sites this may not be observed, as turbidity is caused by other particles than only soil particles. However, the water turbidity will be affected by rainfall, human or animal disturbance and biological activities in the water body. This may result in a dynamic turbidity over time and should be studied further, together with the course of water turbidity over depth.

In summary, this study demonstrates that suspended soil particles affect the temperature of breeding sites of the malaria mosquitoes *An. gambiae* s.l. Apart from reaching higher daytime temperatures, the diurnal behaviour of the water temperature of turbid water bodies will differ from that of clear water bodies. This will result in a longer exposure of the mosquito immatures to higher temperatures. As these immatures are cold-blooded, an increase in temperature will affect their development rate and survival [24-26].

A better understanding of the relation between water turbidity and water temperature can improve current models that estimate the water temperature of mosquito breeding sites (Chapter 2). Moreover, a better knowledge of the factors that affect the life-history traits of the immature malaria mosquitoes is needed, as they will determine the productivity of breeding sites and, therefore, affect malaria transmission.

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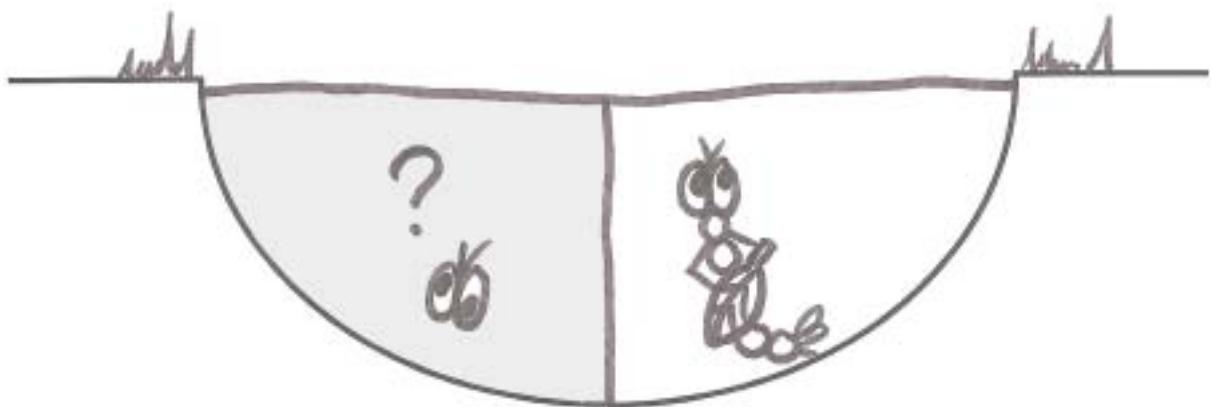
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Water temperature fluctuations and water turbidity affect life-history traits of *Anopheles arabiensis* and *Anopheles gambiae* under ambient conditions in western Kenya

Krijn Paaijmans, Andrew Githeko & Willem Takken



Abstract

Malaria mosquitoes breed in a variety of shallow aquatic habitats. A fundamental understanding of the biotic and abiotic factors that affect the growth, development and survival of the immature stages is important in order to predict the abundance, dynamics and fitness of the resultant adult mosquito populations. In this study we assessed the impact of natural diurnal temperature fluctuations in water bodies of different size and of water turbidity on the life-history traits of larvae of *Anopheles arabiensis* and *An. gambiae*, two main malaria vectors in sub-Saharan Africa. Both species were reared at the same densities and at similar food conditions in identical cups that floated in small-sized (\varnothing 0.16 m; depth 0.05 m), medium-sized (\varnothing 0.32 m; depth 0.17 m) and large-sized water bodies (\varnothing 0.96 m; depth 0.33 m). The water used in the cups and water bodies was either clear or turbid. In turbid water, which was a combination of clear water with suspended autoclaved, fine clay particles, larvae of *An. arabiensis* and *An. gambiae* had a longer development time and emerged as smaller female mosquitoes, compared to larvae that were reared in clear water. In larger habitats larvae of both species developed faster into pupae than in smaller habitats, regardless of water turbidity. Mortality of the larvae was not affected by habitat size, but turbidity did affect the survival of *An. gambiae*: Rearing of larvae in clear water resulted in a larger proportion of larvae reaching the pupal stage. The observations that habitat size and habitat turbidity will affect life-history characteristics of *An. gambiae* s.l. will contribute to better predictions of mosquito habitat productivity and consequently of the risk of malaria transmission.

4.1 Introduction

Anopheles gambiae Giles sensu stricto (hereafter referred to as *An. gambiae*) and *An. arabiensis* Patton, two of the seven sibling species of the *Anopheles gambiae* Giles sensu lato complex (hereafter referred to as *An. gambiae* s.l.), are highly efficient vectors of malaria in sub-Saharan Africa, where they often occur sympatrically [1,2]. The aquatic immature stages of the mosquitoes are found in small, shallow, open and sunlit water collections [3,4], such as burrow pits, tire tracks and rain puddles. These habitats are often of a transient nature, as their availability corresponds to rainfall [4-6].

Malaria mosquitoes are *r*-strategists, which have a high reproductive rate and are unsuccessful competitors with other organisms for the limited resources in temporary habitats. The mortality that is observed among the immature stages of *An. gambiae* s.l. in the field is high and overall only a small fraction (2-8%) of the larvae survives to the adult stage, which is attributed to predators, parasites, pathogens [7-13] or to cannibalism [14]. Other known factors that affect larval survivorship are given in Table 1.1.

Besides the survivorship, also the larval development rate and adult size are important life-history traits of *An. gambiae* s.l. All three life-history characteristics affect the transmission of malaria: The time to develop from an egg into an adult, combined with larval survivorship, determines the numbers of emerging mosquitoes over time. The size of the emerging adults is important, as larger females survive longer [15,16] and have a greater fecundity [16,17]. Adult size furthermore affects host seeking behaviour [15] and parasite infectivity: Intermediate-sized mosquitoes were found to be more infectious to humans [18]. Factors that affect larval growth and development rate are shown in Table 1.1.

In this study we will focus on (i) habitat size and, consequently, on different diurnal temperature variations, and (ii) habitat turbidity as regulators of larval development, survivorship and adult size of *An. arabiensis* and *An. gambiae*.

4.1.1 Temperature

Mosquito immatures are poikilothermic and, therefore, their activity depends to a large extent on the temperature of their direct environment. Besides nutrition and density, temperature is a principal factor that affects the growth and development of mosquito larvae [19]. This has been shown for larvae of *An. gambiae*: Within most of the temperature range in which they are able to develop and survive, an increase in water temperature will result in a faster development [20,21], but will decrease the size of the emerging adults [21]. Bayoh and Lindsay [22] furthermore observed that at the upper temperature range at which larvae

developed into adults (30–32 °C), mortality was high (approximately 70%).

However, the larvae in these experiments were exposed to constant temperatures, whereas the water temperature in natural breeding sites of *An. gambiae* s.l. varies considerably throughout the day [Chapter 2; 23,24]. Small water collections will differ in their diurnal temperature dynamics on both a spatial and temporal scale, compared to larger water pools (Chapter 2). The effects of variations in water temperature on the various life-history traits of the immatures of *An. gambiae* s.l. are poorly understood, as experiments in which *An. gambiae* s.l. is reared at different daily temperature variations have been rarely reported: Lyimo [11] observed that females of *An. gambiae* s.l. (population consisted of 25% *An. arabiensis* and 75% *An. gambiae*), which emerged from larger water bodies such as rain pools, burrow pits and ditches where the daily temperature fluctuations were more moderate than in smaller water bodies, had on average longer wings than those emerging from small puddles and footprints. However, other factors such as pathogens or nutrition were not controlled for.

4.1.2 Turbidity

Larvae of *An. gambiae* s.l. are found in both clear and turbid water collections and, although females seem to prefer turbid or dark substrates over clear or lighter ones to deposit their eggs on [25-28], a clear relation between habitat turbidity and larval occurrence in the field has not been observed. A higher abundance in both clear [29,30] and turbid water collections [4] has been reported, as well as no effect of turbidity on the occurrence of larvae [28,31].

To our knowledge, the only study that looked at the productivity of clear and turbid water bodies containing *An. gambiae* s.l. is that of Ye-Ebiyo et al. [32]. They showed that the survivorship of *An. arabiensis* larvae in water pools with relatively clear water was higher and that these sites produced larger adults compared to habitats with turbid water, unless food was present at the water surface in the form of maize pollen. It was suggested that turbid water may interfere with larval feeding. Apart from micro-organisms such as green algae and phytoplankton, which act as food for the mosquito larvae [33,34], inedible particles such as clay and silt contribute to turbidity [35] and will be ingested by the larvae, as they feed indiscriminately (*An. quadrimaculatus* Say) [36].

From a previous study (Chapter 3) we know that turbid water collections have higher temperatures during daytime, which will have an effect on the larval development time, as mentioned earlier. Other possible effects of water turbidity may include changes in the abundance of algal biomass or a more rapid desiccation of a water body over time compared

to clear water (see Chapter 3 for further details).

Larval survival, age at pupation and adult size will reflect the environmental conditions to which the aquatic stages of *An. gambiae* s.l. were exposed during their life time. Although many physiochemical and biological variables will interact [28,30], a better understanding of how individual biotic and abiotic variables affect the various life-history traits of malaria mosquito immatures is needed to improve our knowledge on the productivity of breeding sites and hence on the transmission of malaria. Moreover, such information may be useful when malaria control strategies are applied, which aim at reducing the number of mosquito immatures.

Only few studies investigated the effects of microclimatic conditions in natural breeding sites of *An. arabiensis* and *An. gambiae* mosquitoes on the life-history traits of their immatures. In this study, we assessed the effect of different water temperature fluctuations throughout the day (using habitats of different size) as well as habitat turbidity on larval survival, larval development rate and adult size of *An. arabiensis* and *An. gambiae* during outdoor experiments in western Kenya.

4.2 Material and methods

4.2.1 *Anopheles gambiae* mosquitoes

Experiments were carried out between March and May 2006 on the grounds of the Kenya Medical Research Institute (KEMRI) in Kisumu, Kenya. Larvae of *Anopheles gambiae*, maintained at the Centre for Vector Biology and Control Research (CVBCR) at KEMRI or larvae of *Anopheles arabiensis* were used in the experiments. Only one species was studied at a time and experiments with both species alternated throughout the study period. The *An. gambiae* colony was frequently replenished with field-caught mosquitoes. As *An. arabiensis* was not available in culture, wild-caught gravid females were collected from houses near the Ahero rice fields, approximately 30 km from Kisumu, prior to each experimental series. These females were allowed to oviposit in an insectary. A subset of the offspring was identified to species, using the polymerase chain reaction (PCR) for species differentiation within the *An. gambiae* s.l. complex [37] and was confirmed to be *An. arabiensis*.

4.2.2 Habitat size and turbidity

Different-sized circular water bodies were created by digging holes in the ground; six small-sized water bodies (\varnothing 0.16 m; depth 0.05 m), six medium-sized water bodies (\varnothing 0.32 m;

depth 0.17 m) and six large-sized water bodies (\varnothing 0.96 m; depth 0.33 m). The holes, which were randomly distributed over a 10×10 meters plot, were lined with semi-transparent plastic (0.13 mm), pressed tightly against the soil to maximize plastic-soil contact. The plastic was kept in place by a thin metal ring (diameter 50 mm larger than the water body).

Subsequently, all holes were filled with water up to 10 mm under the brim. Three replicate holes of each size were filled with clear tap water that originated from a well at KEMRI and three holes were filled with the same tap water but were made turbid by adding 68.4 grams of red clay soil per litre water. This red clay soil was collected in the vicinity of Maseno (25 km northwest of Kisumu) and sun dried and fine clay particles of up to 1 mm in diameter were selected by sieving the soil through a sieve with mesh size of 1 mm.

A uniform water level was maintained by adding or removing water to starting level every morning, compensating for evaporation or precipitation, respectively. The clear water bodies were cleaned and refilled on a weekly basis; the water bodies with turbid water were cleaned and refilled on a monthly basis. To obtain a homogeneous turbidity in the water bodies every day, all water bodies were stirred thoroughly for 15 seconds every morning at 8h00.

No water temperature data were recorded at the time of the experiments, due to failure of the equipment. The diurnal temperature variation of identical clear water bodies have been monitored for 40 days in the previous year (Chapter 2; March-May 2005). Over that period, the average water temperature in the small-sized water body was 27.4 (± 0.2 SEM; average minimum temperature 22.4, average maximum temperature 36.8) °C, 27.8 (± 0.2 ; 23.9-33.9) °C in the medium-sized water body and 28.1 (± 0.2 ; 25.1-32.1) °C in the large-sized water body. The daily average difference between the minimum and maximum water temperature was 14.4 (± 0.3) °C, 10.1 (± 0.3) °C and 7.1 (± 0.2) °C in the small-sized, medium-sized and large-sized water body, respectively.

In Chapter 3, using water bodies with similar dimension as the medium-sized water bodies in this study, we showed that the temperature of turbid water can differ considerably in water temperature from that in clear water during midday. The turbidity of the water bodies in this experiment is similar to the turbidity of the most turbid water body in Chapter 3, as the same ratio of soil to water was applied.

4.2.3 Assessing life-history characteristics of mosquito larvae

To examine the effects of daily temperature fluctuations on the life-history traits of larvae of *An. arabiensis* and *An. gambiae*, larvae were reared in identical transparent plastic cups, in the absence of predators and with a constant density-dependent and food-resource factor.

Each cup (Figure 4.1A, B) was provided with three polystyrene floats and three screened (0.20 mm mesh size) openings (\varnothing 6 mm) in the cup wall to drain excess rain water. They were covered with netting (1 mm mesh size) throughout the experiment and floated in the small-sized, medium-sized or large-sized water bodies (Figure 4.1C), one per water body.

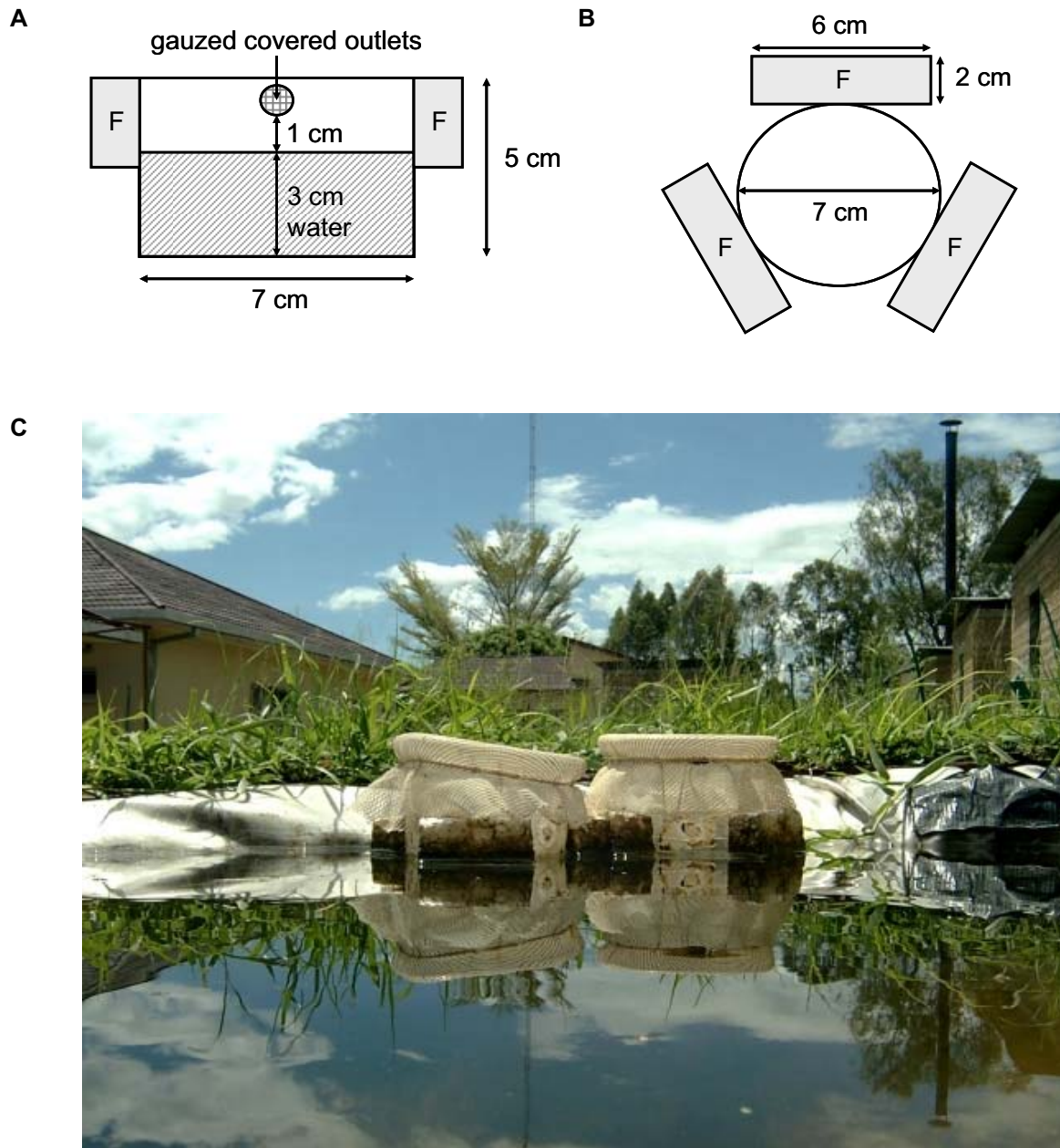


Figure 4.1 (A) Schematic drawing of the side view of an experimental cup; (B) Schematic drawing of the top view of an experimental cup; (C) Experimental cups floating in a large-sized semi-natural water body. F indicates a polystyrene float.

The cups were filled with 3 cm water (originating from the well at KEMRI) that had been stored in large reservoirs for a few days prior to the experiments, to allow for the sediments and other dissolved inorganic particles to settle at the bottom. The cups that floated in the clear water bodies received clear water; the cups that floated in the turbid water bodies were filled with the same water to which fine soil particles were added: The same sieved red clay soil and the same soil to water ratio were used as described previously for the turbid water bodies. However, the soil that was used in the experimental cups was autoclaved at 120 °C for 30 minutes to eliminate microbial activity. After mixing the soil with water, the surplus soil was allowed to settle at the bottom of a basin for 5 minutes prior to adding the supernatant to the cups. This was to prevent a layer of soil from forming at the bottom of the cups.

Each cup contained thirty newly hatched (~1 hour old) first instar (L1) larvae (1.3 larvae/cm²). Each morning, larvae were counted, categorized to larval stage and fed 0.3 mg Tetramin[®] *Baby* fish-food (TetraWerke, Melle, Germany) per larva per day. Dead larvae and pupae were discarded. To obtain a homogeneous turbidity in the experimental cups, the water in each cup was stirred daily for at least five seconds before larvae were placed back. Every two days all cups (both containing clear and turbid water) were cleaned and water was replaced to prevent scum formation and accumulation of metabolites that may be toxic to the larvae [20]. Pupae were collected and reared to adults in paper cups, containing 2 cm of clear tap water and covered with netting. When female mosquitoes emerged, they were stored on silica gel. The lengths of the wings of at least five females per experimental cup were measured, as described by Lyimo et al. [21], using a dissecting microscope. Longer wings are associated with a larger body size [15,38].

4.2.4 Meteorological data

An automated meteorological station at KEMRI measured the air temperature at 2 meters above ground (ventilated probe; Vaisala, Finland). Averaged data were stored every 5 minutes on a 21x Microdatalogger (Campbell Scientific Inc., UK). The average air temperature during the study was 22.7 (±0.1 SEM; range 20.9-24.6) °C, with an average minimum of 18.3 (±0.1; range 16.5-20.4) °C and an average maximum of 28.7 (±0.2; range 26.3-31.7) °C.

4.2.5 Data analysis

Per experimental cup, the average development time (from a newly hatched L1 larva to a pupa, in days), mortality (percentage of larvae that did not reach pupal stage) and mean

female wing length (in mm) were determined. Three replicates for each habitat size (small, medium or large) and for each habitat turbidity (clear or turbid water) were used per series, with two separate series for each species.

Data were analyzed using the SPSS software (version 12.0.1, SPSS Inc., Chicago, IL). A General Linear Model (GLM) was used to test for the effect of “habitat size” and “habitat turbidity” or for an interaction “habitat size × habitat turbidity” on the development time, mortality and female wing length. To compare the development time, mortality and female body size of *An. arabiensis* and *An. gambiae*, a second GLM was used to test for the effect of “habitat size”, “species” or for an interaction “habitat size × species” on the same response variables. The different experimental periods were included in the GLMs as a random factor and subsequently removed when these were statistically insignificant.

4.3 Results

4.3.1 Development time

The mean time to pupation was negatively correlated with habitat size (Figure 4.2) for larvae of both *An. arabiensis* ($F_{2,31}=5.9$, $P=0.007$) and *An. gambiae* ($F_{2,31}=6.0$, $P=0.006$). The time to develop from L1 larva to a pupa (Figure 4.2) decreased by one day for *An. arabiensis* in the clear water bodies (from 9.9 ± 0.2 days in the small-sized water bodies to 8.9 ± 0.1 days in the large-sized water bodies) and by one and a half day in the turbid water bodies (from 12.0 ± 0.4 days in small-sized water bodies to 10.5 ± 0.4 days in large-sized water bodies). The time to pupation for *An. gambiae* (Figure 4.2) decreased from 8.8 ± 0.2 days in the small-sized clear water bodies to 8.0 ± 0.1 days in the large-sized clear water bodies. Age at pupation of *An. gambiae* was 9.5 ± 0.6 days in the small-sized, 8.8 ± 0.3 in the medium-sized and 9.0 ± 0.3 in the large sized turbid water bodies.

Development time was significantly affected by water turbidity (Figure 4.2), which resulted in a longer time to pupation in turbid water compared to clear water, for both *An. arabiensis* ($F_{1,31}=35.8$, $P<0.001$) and *An. gambiae* ($F_{1,31}=15.9$, $P<0.001$).

4.3.2 Mortality

The survival of the larvae of *An. arabiensis* was not affected by habitat size ($F_{2,31}=2.0$, $P=0.150$) or by habitat turbidity ($F_{1,31}=0.36$, $P=0.556$). The average mortality of the larvae was $36.2 (\pm 2.2)\%$ (Figure 4.3).

Larvae of *An. gambiae* showed no difference in mortality when habitat size increased ($F_{2,32}=3.0$, $P=0.067$), but a significantly higher number of larvae survived to the pupal stage in

clear water bodies, compared to turbid water bodies (Figure 4.3; $F_{1,32}=11.0$, $P=0.002$). The average mortality in clear water bodies ($16.7 \pm 2.1\%$) was almost 10% lower than the average mortality in turbid water bodies ($26.1 \pm 2.3\%$).

The daily survival rate, S , was estimated with $S = p^{1/t}$, where p is the proportion of larvae that survived to pupation and t is mean time to pupation in days. The survival rate was 0.957 day^{-1} for *An. arabiensis* and 0.978 and 0.967 day^{-1} for *An. gambiae* in clear and turbid water, respectively.

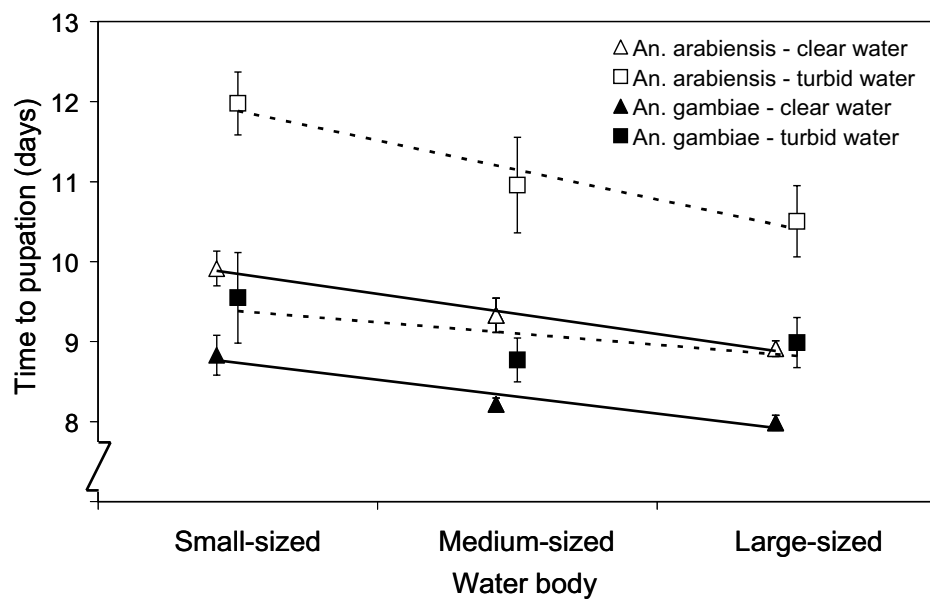


Figure 4.2 Mean time to pupation of larvae of *An. arabiensis* and *An. gambiae* under ambient conditions in small-sized, medium-sized and large-sized water bodies that were clear (regression: solid line) or turbid (regression: dotted line). Error bars indicate the standard error of the mean. The number of replicates per data point is six, except for larvae of *An. arabiensis* reared in the small-sized and turbid water body, where $n=5$.

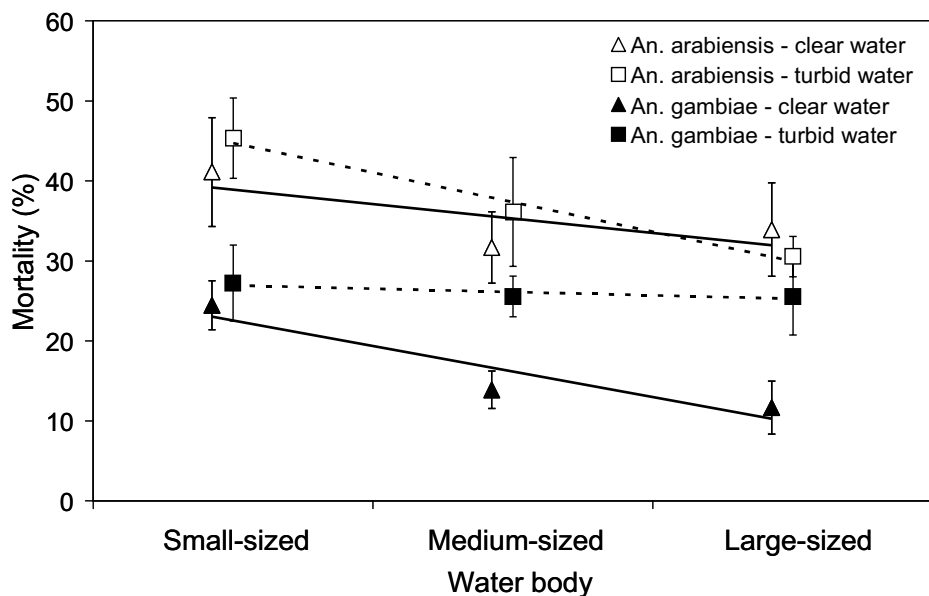


Figure 4.3 Average mortality of larvae of *An. arabiensis* and *An. gambiae* under ambient conditions in small-sized, medium-sized or large-sized water bodies that were clear (regression: solid line) or turbid (regression: dotted line). Error bars indicate the standard error of the mean. The number of replicates per data point is six, except for larvae of *An. arabiensis* reared in the small-sized and turbid water body, where n=5.

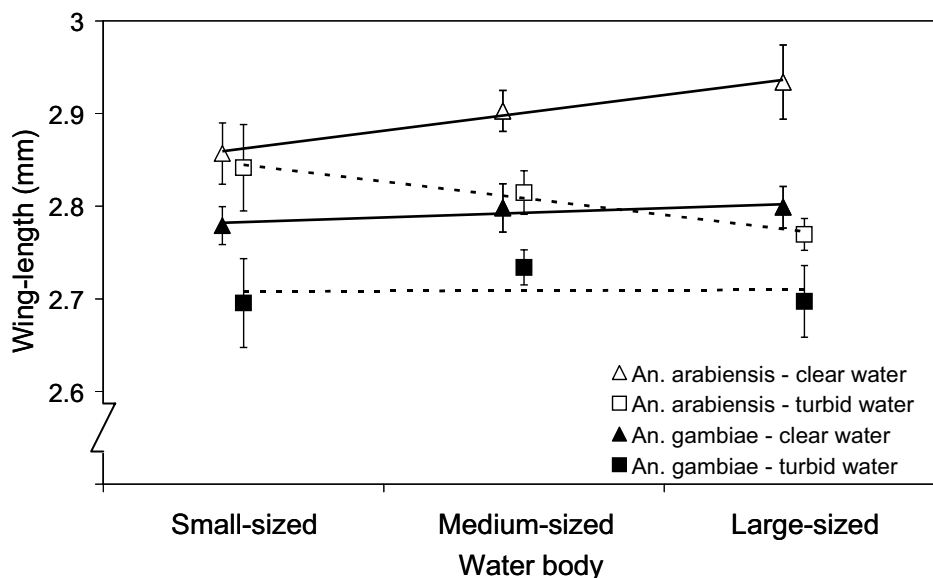


Figure 4.4 Average female wing length of *An. arabiensis* and *An. gambiae* reared under ambient conditions in small-sized, medium-sized or large-sized water bodies that were clear (regression: dotted line) or turbid (regression: solid line). Error bars indicate the standard error of the mean. The wing lengths of at least five females per replicate were measured. The number of replicates per data point is six, except for larvae of *An. arabiensis* reared in the small-sized and turbid water body (n=5).

4.3.3 Female wing length

The wing length of the females of neither species was affected by habitat size (Figure 4.4; *An. arabiensis*: $F_{2,31}=0.04$, $P=0.959$; *An. gambiae*: $F_{2,30}=0.86$, $P=0.435$), but the wing length of females that emerged from turbid water was significantly smaller (Figure 4.4; *An. arabiensis*: $F_{1,31}=10.6$, $P=0.003$; *An. gambiae*: $F_{1,30}=17.3$, $P<0.001$), compared to that of females that eclosed from clear water. The average wing length of females of *An. arabiensis* was 2.90 (± 0.02) mm from clear and 2.81 (± 0.02) mm from turbid water bodies; for females of *An. gambiae* these values were 2.79 (± 0.01) mm and 2.70 (± 0.02) mm, respectively.

4.3.4 Comparison between *An. arabiensis* and *An. gambiae*

In both clear and turbid water bodies, *An. arabiensis* took longer to pupate (clear water: $F_{1,32}=57.8$, $P<0.001$; turbid water: $F_{1,31}=31.2$, $P<0.001$), produced fewer pupae (clear water: $F_{1,32}=25.9$, $P<0.001$; turbid water: $F_{1,31}=8.8$, $P=0.006$) and produced larger females (clear water: $F_{1,32}=22.0$, $P<0.001$; turbid water: $F_{1,31}=19.7$, $P<0.001$).

4.4 Discussion

In turbid water, larvae of both *An. arabiensis* and *An. gambiae* had a longer development time and eclosed as smaller adult insects, compared to larvae that were reared in clear water. Habitat turbidity furthermore affected the survival of *An. gambiae*: Rearing of larvae in clear water resulted in a lower larval mortality. Habitat size was positively correlated with the development rate of both species in both clear and turbid water. Larval mortality and female wing length of *An. arabiensis* and *An. gambiae* were not affected by habitat size.

When larvae of *An. gambiae* were exposed continuously to constant temperatures in the laboratory, water temperature affected their growth, development and survival [20-22]. How the same life-history characteristics are affected by the diurnal fluctuations of the water temperature, which occur in natural water pools, is poorly understood. A study by Lyimo [11] showed that females of *An. gambiae* s.l. that emerged from larger water bodies, where the temperature fluctuations were more moderate, were larger than those emerging from smaller collections of water. However, Lyimo's study did not assess the effects of daily temperature variations per se, as the experiments in natural water collections were uncontrolled for other factors such as pathogens, nutrition or water turbidity. Therefore, the effects on adult body size may not have been affected by the fluctuations in water temperature exclusively, but also by other factors.

The present data suggest that the smaller temperature fluctuations in larger breeding sites

affect the growth of mosquito populations positively, as larvae of *An. arabiensis* and *An. gambiae* will develop faster into pupae in larger water bodies, without consequences for their survival or adult size. As food quantity and larval densities were kept constant in our experiments and the average water temperature in the different sized water bodies is comparable, 27.4-28.1 °C (see material and methods), which is believed to be the optimal temperature for larval survival [21] and larval development rate [20], the difference in larval development time between the different-sized water bodies may be explained by a different magnitude and duration of the alternating phases. The difference between the daily minimum and maximum water temperature was on average 14.4 (± 0.3) °C in the small-sized, 10.1 (± 0.3) °C in the medium-sized to 7.1 (± 0.2) °C in the large-sized water bodies. Depinay et al. [39] showed the relation between larval development rate of *An. gambiae* s.l. and water temperature, based on the outcome of various studies in the laboratory and in the field and estimated that the temperature, at which larval development rate is highest, is around 37 °C. The development rate decreases sharply at higher temperatures. Small-sized water bodies experience higher temperatures, resulting in higher development rates of the larvae, compared to larger water bodies (Chapter 2). These higher temperatures are, however, reached for a relative short time during the day and, as the larval development rate in the small-sized water bodies was lower than that in the larger water bodies, we suggest that the longer exposure to lower temperatures during the evening and at night contributed to the longer larval development time in the small-sized water bodies. As the larger water bodies experience less variation in water temperature, larvae will have more equivalent hourly growth rates throughout the day, which seems advantageous.

Larval survival and female size were not affected by habitat size, meaning that larvae in both small and larger water bodies were able to withstand the temperature fluctuations and were able to grow to similar sizes under different thermal regimes.

Compared to turbid water, both *An. arabiensis* and *An. gambiae* clearly thrived in clear water, which was expressed in a faster development rate and in larger adult insects. Moreover the survivorship of larvae of *An. gambiae* was higher in clear water bodies. Our finding that inhabiting turbid waters is not advantageous for larvae of both mosquito species, contrasts with the observed preference of *An. gambiae* s.l to oviposit on turbid or dark substrates [25-28].

Ye-Ebiyo et al. [32] showed that larval survival of *An. arabiensis* in sites with relatively clear water was higher and that, similar to our study, larger adults were produced than in habitats with turbid water. The disadvantageous effect of turbidity was explained by feeding

efficiency. As larvae ingest particles indiscriminately [36], they will ingest inedible particles such as clay and silt. In the proximity to flowering maize, which released pollen that can act a larval food source directly at the air-water interface where the larvae feed, water turbidity did not affect mosquito numbers and adult size [32].

Besides an effect on larval feeding, water turbidity will affect the diurnal temperature behaviour in a water body. Higher temperatures are reached during daytime in turbid water compared to clear water (Chapter 3); in the medium-sized water bodies, the average difference at midday between the upper water layer (thickness of 10 mm) of the most turbid water body and the same layer of the most clear water body was on average 2.8 °C over a period of 11 days. It is likely that this effect will be larger in the small-sized water body and smaller in the large-sized water body, as is discussed in Chapter 3.

We suggest that the differences in larval development rate between clear and turbid water are caused by a changed feeding efficiency rather than by the difference in water temperature. If water temperature would be the main explanatory factor, the differences in the larval development rate between the clear and turbid large-sized water body would have been smaller than the difference in development rate between the clear and turbid small-sized water bodies: Mosquito larvae will be longer exposed to higher temperatures in the turbid small-sized water bodies, compared to identical but clear water bodies, whereas in the large-sized water bodies this difference in temperature between clear and turbid water will be smaller. As we believe that the given amount of food, in combination with the cleaning of the experimental cups every two days, provided sufficient nutrition and minimalized the accumulation of metabolites that may be toxic to the larvae, we suggest that not the quantity of food, but the large amount of non-nutritious soil and clay particles caused the observed differences between clear and turbid water bodies. This agrees with the explanation given by Ye-Ebiyo et al. [32]. We argue that the results would be similar when non-autoclaved soil would have been used, which will increase food resources [40]: A recent study by Okech et al. [40] showed that the use of autoclaved soil did not affect larval growth, development and survival, compared to the use of soil that was not autoclaved. However, further studies are needed to examine the exact cause behind the observed effects of water turbidity.

Finally, the lower survival of *An. gambiae* in turbid water may be explained by the particles as well, as they may have damaged the intestines of the larvae. The effect of water temperature is excluded, as larval mortality was higher in the medium-sized and large-sized turbid water bodies as well. The temperature in these water bodies will not exceed the lethal upper temperature of larvae of *An. gambiae* s.l., which is considered to be 42 °C [24,41].

Compared to *An. gambiae*, *An. arabiensis* had a longer development time and a higher mortality but emerged as larger adults in both clear and turbid water bodies. Similar differences in adult size between *An. arabiensis* and *An. gambiae* have previously been reported in the field [42,43]. The larger size of females of *An. arabiensis* will be related to the longer larval development, as they will take longer to obtain enough mass prior to pupation [19], as was also suggested by Schneider et al. [44]. The observation that turbid water prolongs the time to pupation of larvae of *An. arabiensis* more than that of *An. gambiae*, compared to clear water, suggests that *An. gambiae* is a more efficient feeder, which is also suggested in Chapter 5. The higher mortality among larvae of *An. arabiensis*, compared to *An. gambiae*, has also been observed by Schneider et al. in the laboratory [44].

Our results on the development time may further attribute to the debate whether both species have different larval requirements. Minakawa et al. [28] reasoned that *An. arabiensis* must have a different habitat preference, as it also utilizes more permanent habitats, such as rice fields, where, in western and central Kenya, it is the sole or predominant vector despite the presence of *An. gambiae* in surrounding rural areas [5,9,45,46]. Our data suggest that it would be advantageous for *An. arabiensis* to breed in even larger water collections, such as rice fields, as they may even develop faster, when the plotted regression line in Figure 4.2 will continue downwards when habitat size increases further.

This study shows that increased habitat size and therefore smaller fluctuations around the mean temperature, was positively correlated with the development rate of larvae of both *An. arabiensis* and *An. gambiae*. Moreover, larval development rate in turbid water was lower and the mosquitoes emerged as smaller adults. In addition, larvae of *An. gambiae* survived better in clear water.

Although larger and clear breeding sites appear more suitable for larval development, mosquitoes are *r*-strategist that will deposit large numbers of eggs frequently. They are likely to distribute their eggs over several breeding sites [47,48] and oviposit in smaller and/or turbid habitats, such as rain puddles, [4,28,31].

Finally, there are more known physiochemical and biological factors that affect larval development, survivorship and adult size (see Table 1.1) and it is likely that many factors interact [28,30]. This may lead to the observation that apparent important regulating factors are cancelled out by other factors. This was shown for larvae of *Aedes aegypti* (L.), which developed faster in shaded habitats, which had a higher nutrition availability but were on average 2.6 °C cooler, compared to similar sunlit habitats [49].

As long as the factors that affect the growth, development and survival of larvae of *An. arabiensis* and *An. gambiae* are not fully understood, obtaining more information remains a necessity to improve the predictions of the abundance, dynamics and fitness of the adult vector populations and consequently of malaria transmission.

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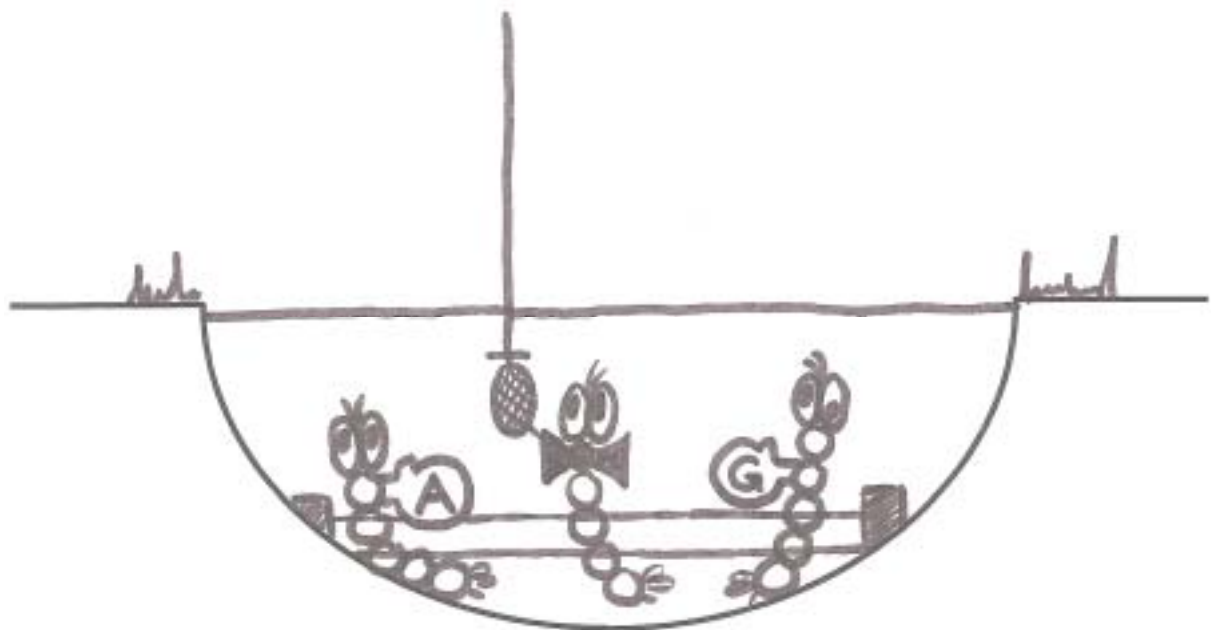
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Competitive interactions between larvae of *Anopheles arabiensis* and *Anopheles gambiae* under ambient conditions in western Kenya

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Abstract

The present chapter reports the occurrence of interspecific competition between larvae of the malaria mosquito sibling species *Anopheles arabiensis* and *An. gambiae* under ambient conditions in western Kenya. Larvae of both species were reared at the same density outdoors in single-species and mixed-species populations (species ratio 1:1) in transparent cups that floated in small and large semi-natural water bodies, which experienced different diurnal variations in water temperature. In a second experiment, both species were reared at the same density in trays in either single-species or in mixed-species populations at different proportions (species ratio 1:1, 1:3 or 3:1). In both experiments larvae were reared under the same food conditions. Habitat sharing significantly affected the larval development time of both species in both experiments, but in an opposite way: the development time of larvae of *An. arabiensis* increased whereas the development time of larvae of *An. gambiae* decreased. Moreover, in the small water bodies of the first experiment it seemed that the mortality of both species increased when they shared one habitat. The occurrence of competitive interactions between larvae of *An. arabiensis* and *An. gambiae* needs to be considered in the design and implementation of programmes that aim to reduce malaria transmission and may alter the species composition in the field. In addition, we discuss the possible mechanisms behind the competitive interactions.

5.1 Introduction

The two sibling malaria mosquito species *Anopheles arabiensis* Patton and *An. gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*), both belonging to the *An. gambiae* sensu lato complex (hereafter referred to as *An. gambiae* s.l.), are widespread throughout sub-Saharan Africa. Although *An. gambiae* is usually the predominant species in environments with high humidity and *An. arabiensis* is more common in zones with less rainfall, both species occur sympatrically across a wide range of tropical Africa [1,2].

Their immature stages require an aquatic environment to develop and are found in transient, sunlit and small pools [3,4]. A clear difference in breeding site preference of the two species has not been observed, but the requirements of both species for their larval habitats are subject of discussion. Several studies suggest these preferences are similar [4,5], others think these differ but were unable to show that explicitly [6]. As both species are often found to share larval habitats [4-8] and *An. gambiae* s.l. larvae tend to aggregate [9,10], there will be frequent contact between individuals. However, the effects of interspecific competition between larvae of *An. arabiensis* and *An. gambiae* have rarely been studied. A laboratory study by Schneider et al. [11] showed a competitive advantage of *An. gambiae* over *An. arabiensis*, which was expressed in a higher survival rate in mixed-species populations. In a separate study, Koenraadt and Takken [12] demonstrated the occurrence of interspecific competition within the *An. gambiae* s.l. complex, whereby older larvae preyed on younger ones of the sibling species.

The existence of interspecific competition between immatures of certain culicine mosquito species (mainly *Aedes* species) has been well established and a wide range of effects has been observed. Interspecific competition may affect larval development rate [13-15], larval survivorship [14-17], the resistance to larval starvation [14], sex ratios [18] and adult mosquito size [13]. Moreover, it may alter mosquito-virus interactions as shown by Alto et al. [19] for *Aedes albopictus* Skuse and therefore affect pathogen transmission.

These studies further showed that the extent of interspecific competition may depend on a variety of factors, including temperature [13,20], larval densities [18,20,21] and food quality and quantity [14,20,22,23]. Furthermore, interspecific differences in larval foraging behaviour may result in a difference in resource acquisition [24] and one species might obtain more energy from the substrate than the other [14]. This may be explained by morphological differences of the mouth brushes [25], different speed of movement of the mouth brushes and therefore different food quantity consumed per unit time [13,25,26], a difference in feeding activity [27] or by a different efficiency of the digestive system [26].

Various mosquito control methods, such as the use of insecticide-treated bednets (ITNs) and the larvicide *Bacillus thuringiensis israeliensis* (*Bti*), are currently being applied and technologies like genetically modified mosquitoes and the sterile-male technique are under development. These methods may not only result in decreasing mosquito abundance but also in a shift in the current species composition. This has recently been shown in an ITN study by Lindblade et al. [28], which overall reduced the number of vectors in the area, but also changed the *An. arabiensis* to *An. gambiae* ratio from 1:1 to proportionally more *An. arabiensis*.

As both species are frequently found to co-exist in the same larval habitats, a better understanding of the competitive interactions between *An. arabiensis* and *An. gambiae* is necessary. These interactions may alter the distribution and abundance of adult mosquitoes and hence the risk of malaria. Therefore we studied whether competitive interactions occur between larvae of *An. arabiensis* and *An. gambiae* under ambient conditions in western Kenya. In a first experiment we examined the effects of habitat sharing in habitats of different sizes, which experienced different diurnal temperature variations. In a second experiment the effect of various species ratios within a population was assessed. We analyzed the effect of habitat sharing and habitat size on the development time and mortality of the immature stages and on the adult sex-ratio and female wing length.

5.2 Material and methods

5.2.1 Mosquitoes

Experiments were carried out at the Kenya Medical Research Institute (KEMRI) in Kisian, Western Kenya. We used larvae of *An. gambiae*, maintained at the Centre for Vector Biology and Control Research (CVBCR) at KEMRI and larvae of *An. arabiensis*. As there was no *An. arabiensis* available in culture, wild-caught gravid females were collected from houses near the Ahero rice fields, approximately 30 km from Kisumu, prior to each experimental series. These females were allowed to oviposit in the insectaries and a large subset of the offspring was identified to species, as described below, which confirmed a homogeneous *An. arabiensis* population. The *An. gambiae* colony at the CVBCR was frequently replenished with field-caught mosquitoes.

In both experiments, larvae were counted, categorized to larval stage and fed 0.3 mg Tetramin[®]*Baby* fish-food (TetraWerke, Melle, Germany) per larva per day. Every two days the cups or basins that were used for rearing were cleaned and water was replaced to prevent scum formation and accumulation of metabolites that may be toxic to the larvae [29].

Dead larvae and pupae were discarded daily. Living pupae were collected and reared to adults in paper cups that contained 2 cm of tap water and were covered with netting. The tap water that was used for larval and pupal rearing originated from a well at KEMRI. Before use, water was stored in closed basins (100 L) for several days to allow sediments and other inorganic particles to settle.

Gender of the emerged mosquitoes was recorded and females were stored in vials with some silica gel, whereby females from the mixed-species treatments were stored in separate vials to prevent contamination. Wing length of each female adult (one wing per female) was measured with a dissecting microscope as described by Lyimo et al. [30].

All females from the mixed-species populations as well as a large subset (approximately 80%) of the *An. arabiensis* progeny that was reared in the single-species populations, were identified using the polymerase chain reaction (PCR) for species differentiation within the *An. gambiae* complex [31].

5.2.2 Experiment 1: Effects of interactions in different sized semi-natural water bodies

The experiment was carried out in April and May 2006 in two experimental series with, per series, three replicates for each treatment (single-species *An. arabiensis* or *An. gambiae* and mixed-species populations) in each habitat size (small-sized or large-sized water body). In a third series, two replicates for each treatment were used in the small-sized water body only, as two cups with mixed-species populations were lost in the second series.

Fifteen newly hatched (~1 hour old) first instar larvae (hereafter referred to as L1 larvae) of *An. arabiensis* and 15 newly hatched L1 larvae of *An. gambiae* were combined in a transparent plastic cup (0.78 larvae/cm²). The control groups consisted of 30 newly hatched L1 larvae of either *An. arabiensis* or *An. gambiae* in identical cups. For a description of the experimental cups, the reader is referred to Chapter 4 (section 4.2.3 and Figure 4.1)

The cups floated in small (Ø 16 cm, water depth 4 cm) or large (Ø 96 cm, water depth 32 cm) semi-natural water bodies (Figure 4.1C). The water bodies were created by digging holes in the ground and lined with transparent plastic (0.13 mm). The plastic was pressed tightly against the soil to optimize plastic-soil contact and filled with clear tap water up to 10 mm under the brim. The plastic of the large water bodies was kept in place by metal rings (50 mm wider in diameter than the water body). Water was added or removed daily in the early morning to compensate for evaporation or precipitation, respectively, and to maintain a uniform water level. The setup was cleaned and refilled on a weekly basis.

By using cups that floated in water bodies of different size, larvae were reared in the absence of predators and with a constant density-dependent and food-resource factor. Therefore, we were able to study the effects of different daily water temperature dynamics in the different water bodies on the competitive interactions between larvae of *An. arabiensis* and *An. gambiae*.

5.2.3 Experiment 2: Effects of interactions at disproportionate species ratios

This second experiment was carried out in May 2006 in an open greenhouse with a semitransparent roof. Circular plastic trays (\varnothing 12 cm, 5 cm deep) were filled with 3 cm of tap water and 60 newly hatched (~1 hour old) L1 larvae were placed in each tray (0.53 larvae/cm²). The following *An. arabiensis* to *An. gambiae* ratios were tested: 1:0, 3:1, 1:1, 1:3 and 0:1, with three replicates for each ratio. All trays were covered with netting (1 mm mesh size) throughout the experiment. Water was added or removed daily in the early morning to compensate for evaporation or precipitation, respectively, and to maintain a uniform water level.

5.2.4 Meteorological data

An automated meteorological station at KEMRI measured the incoming short wave radiation at 1.5 meters above ground (pyranometer; Kipp & Zonen, The Netherlands), temperature and relative humidity at 2 meters above ground (ventilated probe; Vaisala, Finland), wind speed at 2 meters above ground (cup anemometers; homemade) and rainfall (rain gauge; Eijkelkamp, The Netherlands). Totalized (rainfall) or averaged (other meteorological variables) data were stored every 15 minutes on a 21x Micrologger (Campbell Scientific Inc., UK). Table 5.1 shows the meteorological conditions during both experiments. There was no significant difference between the climatic conditions of experiment 1 and experiment 2.

5.2.5 Statistical analysis

Per experimental unit (cup or basin) and per treatment (rearing of single-species or mixed-species populations), the development time (mean time to pupation), mortality (percentage of larvae that did not reach adult stage), sex ratio (number of male mosquitoes divided by number of females) and wing length (mean female wing length) were determined per species. To establish the development time, only pupae that developed into adults were taken into account.

Data were analyzed using SPSS software (version 12.0.1, SPSS Inc., Chicago, IL). In both experiments, a GLM was used to test for the effect of “habitat sharing”, “species” and for an interaction between “habitat sharing × species” on the development time and mortality. Tests were done separately for small and large water bodies. Similarly, a GLM was used to test for the effect of “habitat size”, “species” and for an interaction between “habitat size × species” on the same response variables. Experimental series was included in the GLM as a random factor and subsequently removed when it appeared to be statistically insignificant. Comparison of sex ratios and wing lengths between treatments and between small and large water bodies was done by Mann-Whitney *U* tests, using a Bonferroni correction for multiple tests.

Table 5.1 Summed (rainfall only) or mean (\pm SEM) meteorological variables during experiment 1 (n=45 days) and 2 (n=19 days).

	Experiment 1 9 April - 25 May	Experiment 2 8 - 26 May
Air temperature		
Daily average ($^{\circ}\text{C}$)	22.3 \pm 0.1	22.2 \pm 0.1
Daily average minimum ($^{\circ}\text{C}$)	18.1 \pm 0.1	18.0 \pm 0.2
Daily average maximum ($^{\circ}\text{C}$)	28.1 \pm 0.1	27.8 \pm 0.2
Precipitation		
Total during experiment (mm)	167.8	72.6
Maximum recorded (mm day $^{-1}$)	18.9	17.7
Incoming solar radiation		
Average daily total (MJ m $^{-2}$ day $^{-1}$)	21.0 \pm 0.4	20.0 \pm 0.6
Relative humidity		
Daily average (%)	77.4 \pm 0.6	78.0 \pm 1.0
Daily average minimum (%)	51.1 \pm 1.0	51.6 \pm 1.9
Daily average maximum (%)	95.6 \pm 0.2	95.5 \pm 0.4
Wind speed		
Daily average (m s $^{-1}$)	0.9 \pm 0.0	0.9 \pm 0.0
Daily average minimum (m s $^{-1}$)	0.3 \pm 0.0	0.3 \pm 0.0
Daily average maximum (m s $^{-1}$)	2.5 \pm 0.5	2.4 \pm 1.2

5.3 Results

5.3.1 Experiment 1: Effects of interactions in different sized semi-natural water bodies

5.3.1.1 Effect on development time

The effect of habitat sharing on the development time of the larvae differed for both species. In small water bodies, the development time of *An. arabiensis* increased (from 9.3 \pm 0.1 to 10.8 \pm 0.3 days) whereas the development time of *An. gambiae* decreased (from 9.0 \pm 0.2 to 8.3 \pm 0.2 days) in the presence of the sibling species (Figure 5.1A; habitat sharing × species

interaction, to test whether the effect of the presence or absence of the sibling species on the development time varied between species: $F_{1,24}=28.2$, $P<0.001$). The same phenomenon was observed in the large water bodies (Figure 5.1B; habitat sharing \times species interaction: $F_{1,20}=10.1$, $P=0.005$), whereby the development time of *An. arabiensis* increased from 8.5 (± 0.1) to 9.0 (± 0.3) days and the development time of *An. gambiae* decreased from 7.8 (± 0.1) to 7.2 (± 0.1) days in the presence of the sibling species. *An. arabiensis* took significantly longer to develop from L1 larvae into pupae in both small (species: $F_{1,24}=43.6$, $P<0.001$) and large water bodies (species: $F_{1,20}=57.4$, $P<0.001$), compared to *An. gambiae*.

The size of the water body affected the development time significantly. In the small water bodies, the time to pupation was longer for both species in both single-species (habitat size: $F_{1,24}=35.0$, $P<0.001$) and mixed-species populations (habitat size: $F_{1,20}=43.8$, $P<0.001$), compared to the large water bodies. Larvae of *An. arabiensis* took significantly longer to develop into pupae in both single-species (species: $F_{1,24}=8.8$, $P=0.007$) and mixed-species populations (species: $F_{1,20}=94.9$, $P<0.001$), compared to larvae of *An. gambiae*.

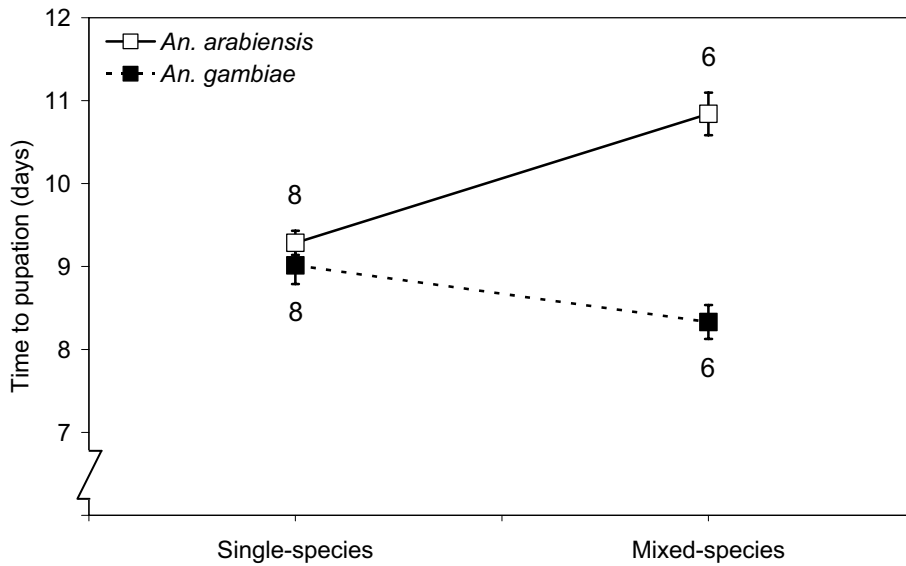
5.3.1.2 Effect on mortality

The effect of habitat sharing on the mortality of the immature stages was similar for both species in small (Figure 5.2A; habitat sharing \times species interaction: $F_{1,24}=1.4$, $P=0.247$) and large water bodies (Figure 5.2B; habitat sharing \times species interaction: $F_{1,20}=0.3$, $P=0.582$). In small water bodies, the proportion of larvae that did not reach adult stage were similar for *An. arabiensis* and *An. gambiae* in both single-species and mixed-species populations (species: $F_{1,24}=2.3$, $P=0.140$) and sharing of a habitat tended towards and increase of the mortality (habitat sharing: $F_{1,24}=4.1$, $P=0.055$) of the immature stages of *An. arabiensis* (from 22.1 ± 3.1 to $33.9\pm 3.3\%$) and *An. gambiae* (from 20.8 ± 4.4 to $23.9\pm 3.3\%$). In the large water bodies, habitat sharing had no effect on the mortality of the immatures of both species, but the mortality of the immatures of *An. arabiensis* ($37.5\pm 3.0\%$ in single-species and $34.4\pm 7.6\%$ in mixed-species populations) was significantly (species: $F_{1,20}=17.1$, $P=0.001$) higher than that of *An. gambiae* ($18.9\pm 2.0\%$ in single-species and $10.0\pm 6.1\%$ in mixed-species populations).

In the single-species populations, the effect of habitat size on the mortality differed between both species (habitat size \times species interaction, to test whether the effect of habitat size varied between species: $F_{1,24}=6.2$, $P=0.020$). The mortality of the immature stages of *An. arabiensis* increased in the large water bodies, compared to the small water bodies, whereas *An. gambiae* showed a similar mortality in both water bodies. The effects of mixed-species rearing was not affected by habitat size. *An. arabiensis* larvae showed a significantly higher

mortality in both single-species and mixed-species populations (species: $F_{1,24}=8.2$, $P=0.009$; $F_{1,20}=10.2$, $P=0.005$, respectively), compared to *An. gambiae*.

A



B

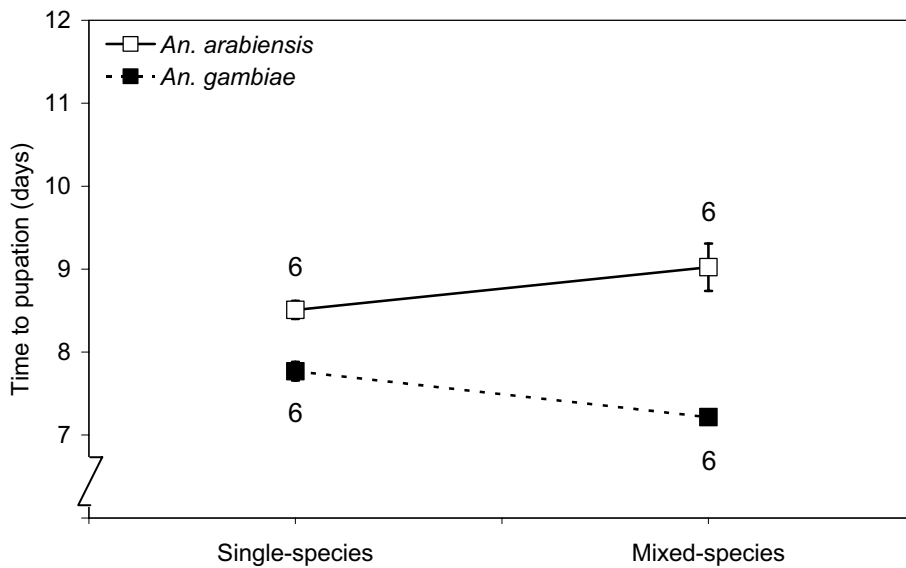


Figure 5.1 Interaction plots for the effect of habitat sharing by larvae of *An. arabiensis* (open squares) and *An. gambiae* (black squares) under ambient conditions on the mean time to pupation in **(A)** small water bodies and **(B)** large water bodies. The number near each data point indicates the number of replicates available for the analysis.

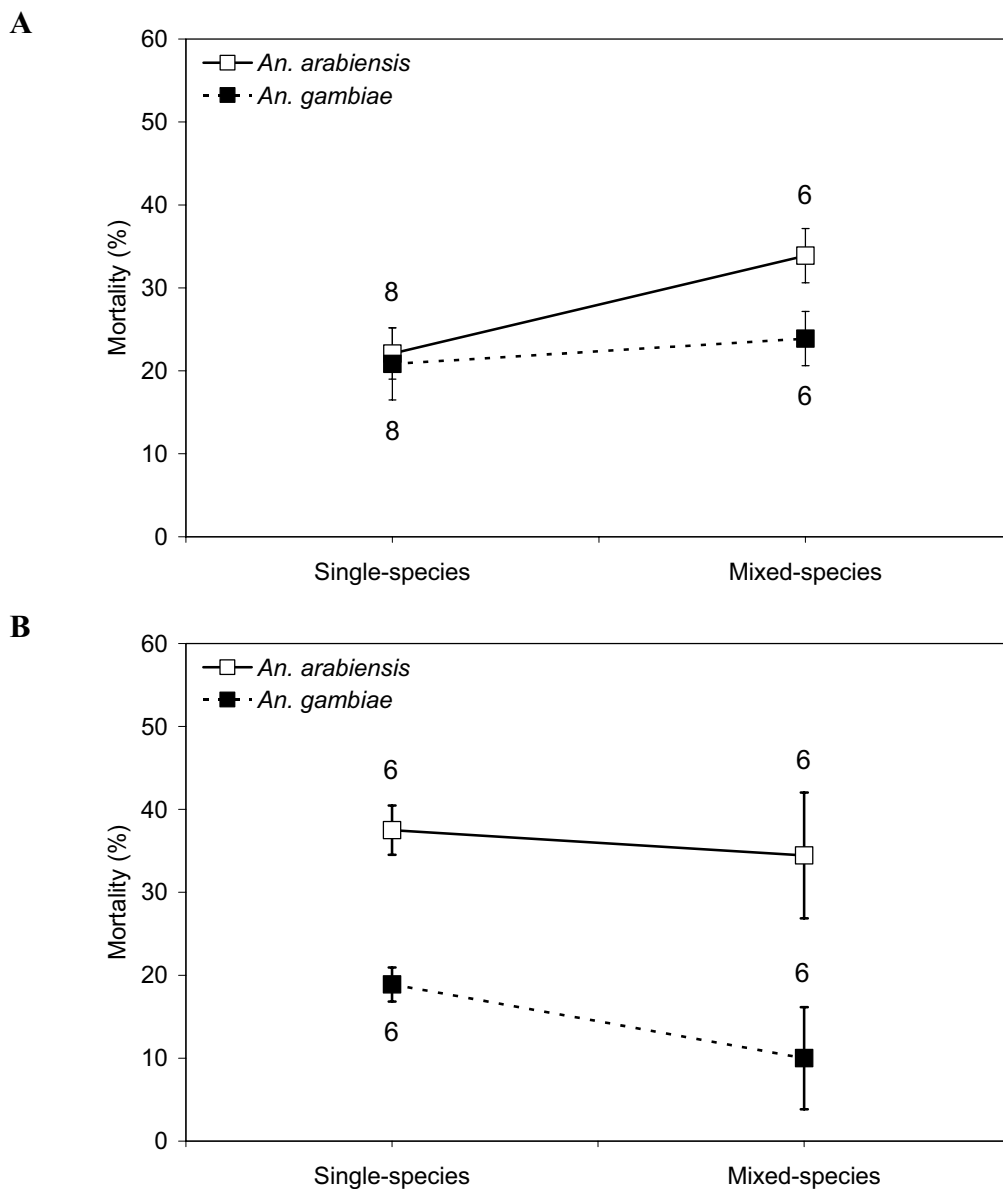


Figure 5.2 Interaction plots for the effect of habitat sharing by larvae of *An. arabiensis* (open squares) and *An. gambiae* (black squares) under ambient conditions on the mortality of the immature stages in **(A)** small water bodies and **(B)** large water bodies. Error bars represent the standard error of the mean. The number near each data point indicates the number of replicates available for the analysis.

5.3.1.3 Effect on sex ratio

There was no effect of habitat sharing and habitat size on the sex ratio for both species. The average male/female ratio was 0.8 (± 0.1) for *An. arabiensis* and 1.4 (± 0.2) for *An. gambiae*.

5.3.1.4 Effect on female size

Both habitat sharing and habitat size had no effect on the mean wing length of the female mosquitoes for both species. The average wing length of females of *An. arabiensis* (2.96 ± 0.02 mm) was significantly larger (Mann-Whitney U : $P < 0.001$) than that of females of *An. gambiae* (2.86 ± 0.02 mm).

5.3.2 Experiment 2: Effects of interactions at disproportionate species ratios

5.3.2.1 Effect on development time

When a higher proportion of the competing species was present in the population, the development time of *An. arabiensis* increased (from 10.6 ± 0.0 days when reared alone to 12.3 ± 0.2 days when 75% of the population consisted of its sibling species) whereas the development time of *An. gambiae* decreased (from 9.5 ± 0.2 days in single-species to 8.9 ± 0.2 days when 75% of the population consisted of its sibling species) (Figure 5.3; proportion competitive species \times species interaction, to test whether the effect of different proportions of the competing species present on the development time varied between species: $F_{3,16} = 17.4$, $P < 0.001$). Larvae of *An. arabiensis* took significantly (species: $F_{1,16} = 300.2$, $P < 0.001$) longer to develop into pupae than larvae of *An. gambiae*.

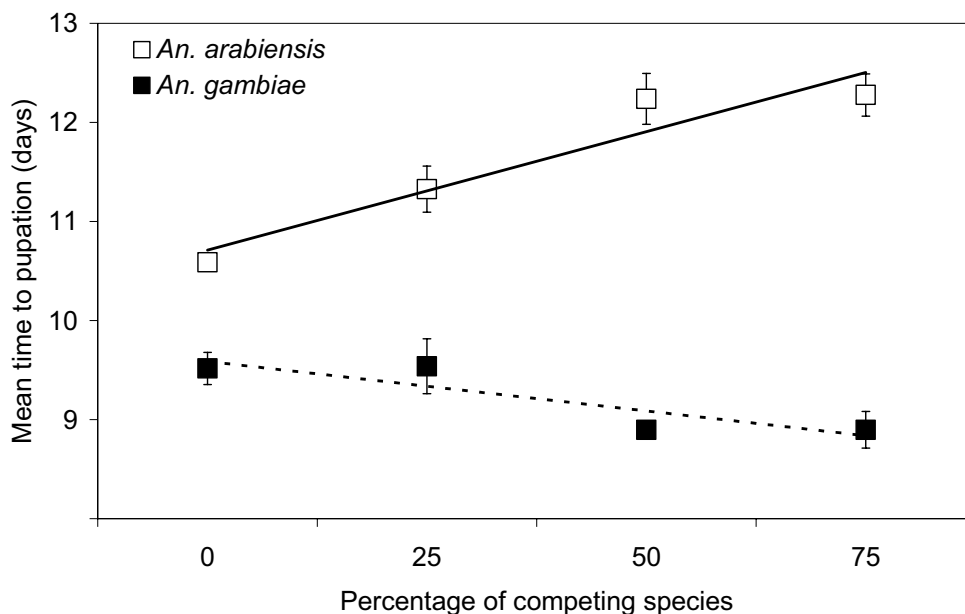


Figure 5.3 Interaction plot for the effect of an increased proportion of the competing species present on the development time for larvae of *An. arabiensis* (open squares) and *An. gambiae* (black squares). The error bars represent the standard error of the mean. The number of replicates per data point is three.

5.3.2.2 Effect on mortality

A higher proportion of the sibling species present in the population had no significant effect on the mortality of the immatures of both species. No difference was observed between the percentages of larvae of *An. arabiensis* (mortality: $15.6 \pm 2.1\%$) and *An. gambiae* (mortality: $16.4 \pm 1.6\%$) that did not reach adult stage.

5.3.2.3 Effect on sex ratio

There was no effect of the proportion of the sibling species present in the populations on the sex ratio for both species. The average male/female ratio was 1.0 (± 0.2) for *An. arabiensis* and 1.2 (± 0.1) for *An. gambiae*.

5.3.2.4 Effect on female size

Sharing a habitat with different percentages of the other species present in the populations had no effect on the wing length for both species. Overall, females of *An. arabiensis* (3.34 ± 0.02 mm) had significantly larger wings (Mann-Whitney *U*: $P < 0.001$) than females of *An. gambiae* (3.22 ± 0.02 mm).

5.4 Discussion

We demonstrated the existence of a competitive interaction between larvae of *An. arabiensis* and *An. gambiae* under natural conditions, which increased the time to pupation for *An. arabiensis* by 1.6 days in small water bodies. By contrast, the time to pupation for *An. gambiae* was decreased by 0.7 days. A similar trend (an increase of 0.5 days for *An. arabiensis* and a decrease of 0.6 days for *An. gambiae*) was observed in the large water bodies. Further investigations established that the effect became larger with an increasing proportion of the competing species present in the population.

The average time to pupation of larvae of *An. arabiensis* was consistently longer than that of larvae of *An. gambiae*. Schneider et al. [11] found a shorter development time for *An. arabiensis* larvae in both single-species and mixed-species populations compared to *An. gambiae*. Possible reasons for the differences between the two studies may include the mosquito strains that were used and the experimental circumstances. The present study used *An. arabiensis* populations that had recently been collected from the field and both sibling species were reared under ambient conditions. Schneider et al. [11] used laboratory colonies which were probably adapted to the laboratory conditions and might therefore have differed from natural field populations. Furthermore, the observed differences may have

originated from exposure of our mosquito larvae to ambient conditions (Table 5.1; daily variations in air temperature from 18.1 °C to 28.1 °C), whereas the experiments of Schneider et al. [11] were conducted at a constant air temperature of 27 °C.

Considering the time it took the larvae to pupate, the detrimental effect of habitat sharing for *An. arabiensis* was greater than the advantageous effect for *An. gambiae*. A possible reason might be that the development rates of the single-reared populations of *An. gambiae* were close to the highest rate possible under the given circumstances and therefore a further increase in development rate was not possible, as the species had reached its biological maximum. A shorter development time can be advantageous for the survival of the immature mosquitoes, as they are exposed for a shorter period to threats such as cannibalism, predation, pathogens and climatic variables such as rainfall resulting in flushing of breeding sites or periods of drought that result in desiccation of the larval habitat.

Similar to earlier findings by Schneider et al. [11], we observed that in both single-species and mixed-species populations the mortality of *An. arabiensis* was significantly higher than that of *An. gambiae*. In the small water bodies, sharing of a habitat seems to reduce the survivorship of both species. Schneider et al. [11] observed the same competitive disadvantage, but for *An. arabiensis* only.

There was no effect of habitat sharing on the sex ratio or female wing length for both species. The mean wing length of *An. arabiensis* females was significantly larger than of *An. gambiae* females in both our experiments. Size differences between *An. arabiensis* and *An. gambiae* have previously been reported in the field [1,32]. Interestingly, females of both *An. gambiae* and *An. arabiensis* that were reared in the greenhouse of experiment 2 were larger than the females of experiment 1, which were reared outdoors in our water bodies, even though the weather conditions during both experiments were similar. Also, the survival and the mean time to pupation of *An. arabiensis* larvae both increased when larvae were reared in the more protective environment of the greenhouse. Apparently, natural conditions such as direct sunlight and natural daily variations in water temperature affect the life-history traits of the immature mosquitoes, which are easily overlooked in laboratory studies and cannot be easily simulated.

The immediate cause of the different effects of habitat sharing between *An. arabiensis* and *An. gambiae* remains unknown. The experiments were designed such that competitive interactions could be studied with a constant density-dependent and food-resource factor, by placing the larvae in small cups of similar size that were floating in pools of different size. We propose two factors that may cause a competitive interaction between the two species.

First, competition could be driven by chemical or physical cues. Chemical cues have been observed in *Ae. aegypti* (L.), where larvae release growth retardants to the water [33-35] and pollute the water, probably with nitrogenous waste [36]. Besides chemical interference, physical interaction has also been proposed as a factor of importance [33]. We suggest that chemical cues may have resulted in the competitive interaction between *An. arabiensis* and *An. gambiae* in the present study. The observed differences between the species could be a result of intraspecific as well as interspecific competition. In theory, if *An. gambiae* produce growth retardants, fewer individuals of *An. gambiae* at the same larval density may result in the faster development of this species, while this growth retardant may inhibit the development of *An. arabiensis*.

A second explanation for the occurrence of competitive interactions may be the difference in foraging success between *An. arabiensis* and *An. gambiae*. Schneider et al. [11] proposed that the larger size of females of *An. arabiensis* could mean that this species requires a higher quantity of food. The species that is more efficient in food detection, ingestion and digestion may have a competitive advantage over the other species. Part of the food particles provided to the larvae sank to the bottom of the cups. *An. gambiae* might benefit from these particles, as this species crawls more often along the bottom substrate than *An. arabiensis* does [37]. Other explanations for differences in foraging success, that are shown to be of importance in culicine mosquitoes, are morphological differences of the mouth brushes [25], difference in stroke frequency of these mouth brushes and therefore different food quantity taken per unit time [13,25,26], a more active feeding behaviour with more time browsing and less time resting [27] or by a more efficient digestive enzyme system [26].

No water temperature data were recorded at the time of the experiments, due to the failure of the equipment. Over a period of 40 days during the same months in the previous year (Chapter 2), continuous water temperature measurements showed that the mean water temperatures of the small-sized and large-sized water bodies were similar (27.4 ± 0.2 and 28.1 ± 0.2 °C, respectively). However, considerably larger daily variations in temperatures occurred in the small-sized water body, where the mean difference between the average daily minimum (22.4 ± 0.1 °C) and maximum (36.8 ± 0.3 °C) water temperature was 14.4 °C. This difference was 7.1 (25.1 ± 0.1 - 32.1 ± 0.2) °C in the large-sized water body.

In laboratory experiments, no adults emerged when larvae were reared below 18 °C or above 32 °C [29,38]. Larval development rate peaked at a water temperature of 28 °C [29] and survival of the larvae was highest at 27 °C [30]. The minimum temperatures in the water bodies did not reach the lower lethal temperature of the larvae, but water temperatures daily

exceeded the optimum temperature for larval development and survival. From a review on previously observed development rates of larvae of *An. gambiae* s.l., it was estimated that the temperature at which mosquito larvae have the highest development rate is around 37 °C [39]. Above this temperature the development rate decreases sharply. Daily maximum water temperatures above 37 °C were frequently observed in the small-sized water body, at which some developmental processes might come to a partial or complete halt. However, under natural conditions these warmer temperatures are only reached for a short period of time (Chapter 2). Therefore, as our experimental data demonstrate, larvae of *An. gambiae* s.l. can readily survive short-term exposure to such high and unfavourable temperatures.

Considering the effect of habitat size, *An. arabiensis* in single-species populations took longer to develop into pupae but showed a higher survivorship in the small water bodies. It is therefore concluded that *An. arabiensis* can tolerate pools that are warmer during day-time and cooler during night-time, such as small puddles, better than larger water bodies, which show less variation in temperature during the day. *An. gambiae* experienced equal survival percentages but a longer development time in small water bodies. This may explain some of the differential variations that are observed in sibling species composition and temporal dynamics of sympatric adult populations.

Competitive suppression of *An. arabiensis* by *An. gambiae* may result in a behavioural change of *An. arabiensis*, causing females to avoid oviposition in breeding sites that are occupied by *An. gambiae*. However, this has not been observed in the field where both species are often found together in larval habitats [4-8]. Further studies should focus on the factors that govern competitive interactions and explore the existence of these interactions in the field. Oviposition experiments can provide information about female behaviour concerning habitat choice with presence or absence of the competing species at varying densities.

Future research should also aim at the effects of competition when early instars are competing with late instars of opposite species, as in a field situation all four larval instars can be found together in one habitat [10]. It has been demonstrated for *An. arabiensis* that the presence of older larvae may reduce the development rate of younger ones [12]. Furthermore, the effect of food quantity and quality and various lower larval densities on competition needs further investigation. Densities of the immature mosquitoes encountered in the field seem lower than those used in our experiment (e.g. 0.13 larvae/cm² [40], 0.016 larvae/cm² [12] and 0.009 larvae/cm² [10]). However, as *An. gambiae* s.l. larvae tend to aggregate [9,10], there will be frequent contact between individuals.

Understanding the exact mechanisms underlying the competitive interactions between *An. arabiensis* and *An. gambiae* will be required when proper mosquito control methods are applied. For example, in areas with a high ITN use and a proportionally higher number of *An. arabiensis* in the area [28], the *An. gambiae* larvae present may develop faster when they occur sympatrically with *An. arabiensis*, than they would on their own. Furthermore, understanding the competitive traits of both species would be an important step in the development of genetically modified mosquito populations [41]. The goal of such *Plasmodium*-resistant mosquito lines is to replace the current vector population and, therefore, the genetically modified mosquito needs to be a better competitor than current malaria vectors.

In conclusion, a competitive interaction between larvae of *An. arabiensis* and *An. gambiae* was observed, with *An. gambiae* being the better competitor. Larvae of *An. arabiensis* had a significant extension of their development time in both small and large water bodies, whereas *An. gambiae* developed faster into pupae. These effects were more pronounced when a higher proportion of the sibling species was present in the population. Under natural conditions, when they are vulnerably exposed to other biotic and abiotic factors, such competitive interactions are likely to affect the distribution and abundance of the resultant adult populations and can therefore affect the transmission of malaria, as *An. arabiensis* and *An. gambiae* have a differential vectorial competence [42].

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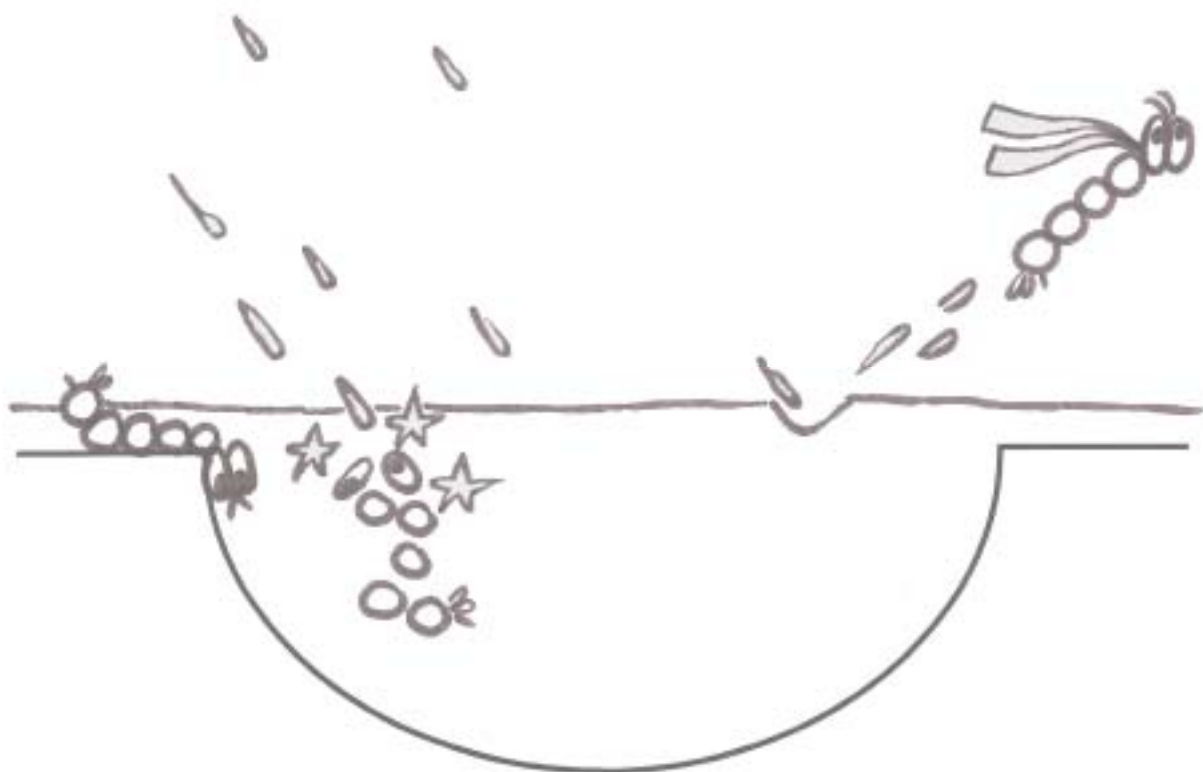
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Unexpected high losses of *Anopheles gambiae* larvae due to rainfall

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Abstract

The immature stages of malaria mosquitoes are vulnerable, as they are exposed to biotic and abiotic factors that may affect their survival. Rainfall, for instance, may have an impact on the population dynamics of immature malaria mosquitoes, but this effect is poorly understood. Here we show that rainfall significantly affected larval mosquitoes by flushing them out of their aquatic habitat and by killing them. Outdoor experiments under natural conditions in Kenya revealed that the additional nightly loss of larvae caused by rainfall was on average 17.5% for the youngest (L1) larvae and 4.8% for the eldest (L4) larvae; an additional 10.5% (increase from 0.9 to 11.4%) of the L1 larvae and an additional 3.3% (increase from 0.1 to 3.4%) of the L4 larvae were flushed away and larval mortality increased with 6.9% (from 4.6 to 11.5%) and 1.5% (from 4.1 to 5.6%) for L1 and L4 larvae, respectively, compared to nights without rain. This study demonstrates that immature populations of malaria mosquitoes suffer high losses during rainfall events. As these populations are likely to experience several rain showers during their lifespan, rainfall will have a profound effect on the productivity of mosquito breeding sites and, as a result, on the transmission of malaria. These findings are discussed in the light of malaria risk and changing rainfall patterns in response to climate change.

6.1 Introduction

With over a million deaths and between 350 and 500 million acute cases annually [1], malaria remains one of the most important and widespread tropical infectious diseases in the world. Over 75% of mortality cases occur among children living in sub-Saharan Africa [2]. Here, two sibling mosquito species *Anopheles arabiensis* Patton and *An. gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*) both belonging to the *An. gambiae* sensu lato complex and *An. funestus* Giles, are the principle vectors of malaria.

The immature stages of *An. gambiae* require an aquatic environment to develop and are often found in transient, sunlit and generally small pools [3-6]. The availability of these aquatic habitats depends on precipitation [6-8]. Precipitation creates new breeding sites and adds water to existing ones. The availability, persistence and dimensions of mosquito larval habitats depend to a large extent on the frequency, duration and intensity of precipitation.

Mortality during the development of the larval stages is very high. Various studies have reported that only a small fraction (2-8%) of the larvae that hatched, eventually survived to the adult stage and attributed this to the presence or absence of predators, parasites, pathogens [9-12] or cannibalism [13]. Other biotic factors that may affect survival are predation by sibling species [14] and other interactions between sibling species [15]. Abiotic factors such as temperature [16-18] may also affect larval mortality.

It has been suggested that precipitation could affect larval population dynamics by flooding habitats and consequently flushing larvae out [19-22]. Tuno et al. [23] observed a high larval mortality in open habitats in the western Kenya highlands and suggested a damaging effect of raindrops on larvae. The possible effect of mortality by the direct hit of a raindrop was studied by Mason [in: 19], who exposed larvae to rain showers and by Robert et al. [24], who exposed larvae to artificial rain. However, in both studies no damaging effect was observed. Russell et al. [19] proposed that the direct damage to anopheline larvae by precipitation may depend on raindrop size.

The Intergovernmental Panel on Climate Change (IPCC) expects significant variation in rainfall in tropical Africa in response to global warming whereby East Africa in particular is likely to experience an increase in annual mean rainfall [25]. Hulme et al. [26] predicted a similar pattern in equatorial East Africa but the expected increase in rainfall during December-February varies from 5-30% to 50-100% depending on the speed of global warming. A change in rainfall may result in a change in malaria transmission. Considering adult mosquitoes, changes in precipitation may lead to changes in species distribution, as the highly anthropophilic *An. gambiae* usually is the predominant mosquito species in

environments with high humidity, whereas *An. arabiensis*, which is zoophilic, is more common in zones with less rainfall [27-29]. An increase in rainfall will result in a higher near-surface humidity and this will favour malaria mosquitoes by extending their longevity [30]. Regarding the immature stages, an increase in rainfall will increase the availability, persistence and dimensions of larval habitats but also decrease mean temperatures and the amplitude of the diurnal temperature behaviour in these habitats [31]. Moreover, an increase in rainfall may have negative consequences for mosquito populations by impacting the immature life stages through excessive flooding or by direct hits.

The biotic and abiotic factors that affect life history traits such as growth, development and survival of the immature stages of *An. gambiae* s.l. require more attention, as they will affect the productivity in the breeding site and determine the abundance, distribution and fitness of the resultant adult mosquito populations, which will consequently affect the malaria transmission. Here we explore the effect of natural rainfall, a density-independent factor, on flushing, ejection and mortality of larvae of the malaria vector *An. gambiae* under ambient conditions in western Kenya.

6.2 Material and methods

6.2.1 *Anopheles gambiae* mosquitoes

Outdoor experiments were carried out on the grounds of the Kenya Medical Research Institute (KEMRI) in Kisumu, Kenya and started at 17:00 h (or one hour earlier at times when a rain shower was developing). Newly hatched L1 or L4 larvae of *An. gambiae* (maintained in the Vector Biology Control and Research Center at KEMRI) were used and they were fed 0.3 mg Tetramin®*Baby* fish food (TetraWerke, Melle, Germany) per larva at the start of each experiment. The tap water used in the experiments originated from a well at KEMRI and was stored in large containers for a few days prior to the experiments, to allow the sediments and other inorganic particles to settle. The next morning (09:00 h) all experimental basins were examined twice for larvae and their numbers (*dead*, *alive* and *not recovered*) recorded.

6.2.2 Experiment I - Flushing and mortality of *An. gambiae* larvae

To assess the extent of flushing of the larvae during rainfall and to see whether rainfall is a noticeable mortality factor, an experiment was carried out from April to July 2005, a period that covered part of the long rainy season that occurs annually in western Kenya. Small-sized (Ø 16 cm, 5 cm deep; hereafter referred to as *small basin*) or large-sized (Ø 30 cm, 9.5 cm deep; *large basin*) circular plastic basins were placed separately in the middle of a larger

basin (Ø 41 cm, 16 cm deep; *overflow basin*). By using a thin metal frame, the rims of the small/large basin and the overflow basin were placed at the same height. The experimental setup (Figure 6.1A) was levelled horizontally and the small and large basins were filled with water till overflowing. Each overflow basin was filled with 2 cm water, to prevent larvae from desiccating after flushing, and was provided with two screened (0.20 mm mesh size) outlets (Ø 1 cm in diameter) to allow excess rainwater to run off but prevent larvae from flushing out of the overflow basin during precipitation. The overflow basins were placed a few centimetres apart in a trench so that the topside of the experimental setup was at the same level as its surrounding soil and excess rainwater could run off freely. At the start of each experiment (17:00 h), twenty L1 or L4 larvae were placed in the small and large basins, each immature stage having four replicates in each size basin.

6.2.3 Experiment II - Ejection of *An. gambiae* larvae

Because ejection of larvae from the basins caused by the impact of raindrops was suspected, we next studied the possible occurrence of this phenomenon in a separate experiment from May to July 2005. A plastic basin (Ø 16 cm, 5 cm deep; hereafter referred to as *small basin*) was placed inside a larger plastic basin (Ø 30 cm, 9.5 cm deep; *medium sized basin*) and a thin metal frame kept the rims of the basins at the same level. The medium sized basin was placed inside a larger plastic basin (Ø 54.5 cm, 19 cm deep; *large basin*). For reasons mentioned in experiment 1, each basin was provided with two screened (0.20 mm mesh size) outlets (Ø 1 cm in diameter) and the large basins were placed a few centimetres from each other in a trench. The experimental setup (Figure 6.1B) was filled with water until the water level in all basins had reached the overflow outlets. At the start of each experiment (17:00 h), twenty L1 or L4 larvae were placed in the small basin, each immature stage having three replicates.

6.2.4 Meteorological data

The quantity of rainfall (mm) was measured with an automated rain-gauge (Eijkelkamp, The Netherlands; opening at 0.9 meters height; threshold 0.201 mm), wind speed (m/s) was recorded two meters above ground with a cup anemometer (Meteorology and Air Quality, Wageningen University, The Netherlands) and the air temperature (°C) was measured two meters above ground with a shielded and ventilated probe (Vaisala, Finland). Every 5 minutes, total quantity of precipitation and average wind speed and air temperature were stored on a data logger (21x MicroDataLogger, Campbell Scientific, U.K.).

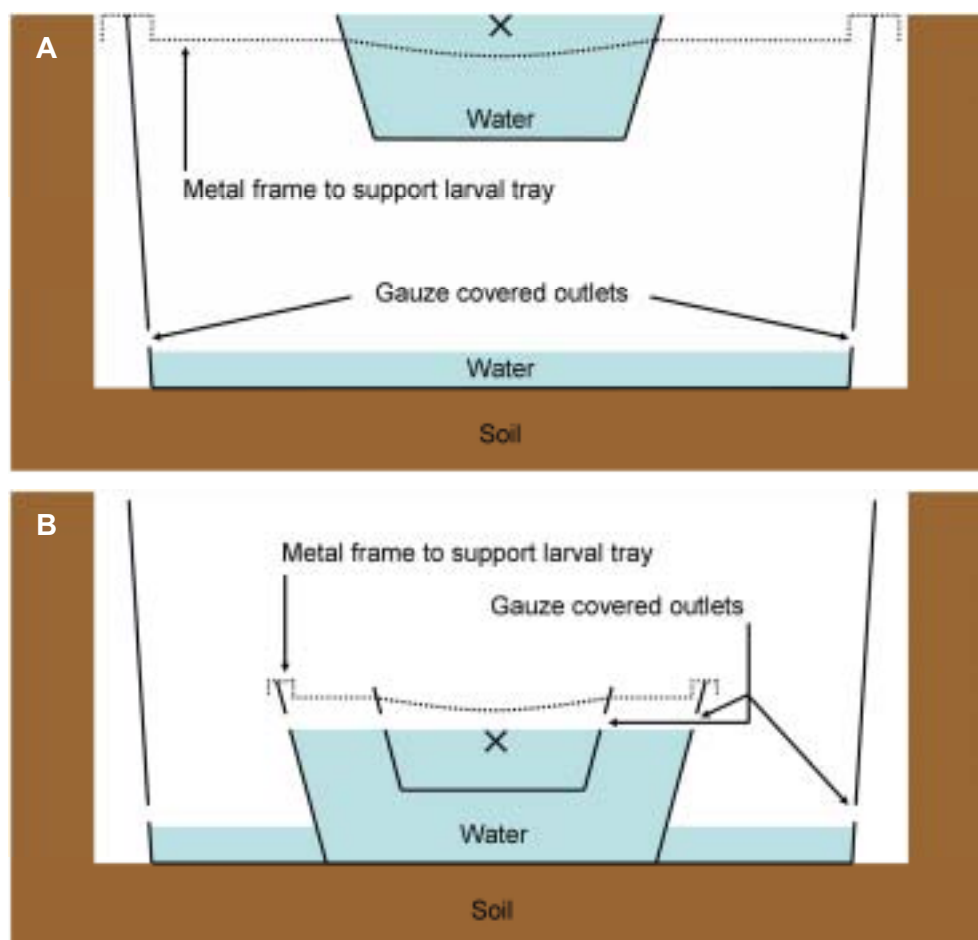


Figure 6.1 (A) Schematic side view of flush/survival experiment; (B) Schematic side view of ejecting-experiment. The solid lines represent the basins; the dotted lines the thin tripod metal frames and the coloured areas water. The openings in the basins are the screened with mesh-wire overflow holes; the black crosses indicate where the larvae were placed at the start of each experiment.

6.2.5 Statistical analysis

Flushing was calculated as the percentage of living larvae found in the overflow basin from the total number of larvae that survived and *mortality* as the percentage of dead larvae in all basins from the total number of larvae at the start of the experiment. *Ejection* was calculated as the percentage of living larvae recovered outside the small basin from the total number of larvae that survived. Larvae that were not recovered were assumed dead. Larvae that had died were excluded from the *flushing* and *ejection* analysis as they were unable to respond actively to the rainfall. Occasionally an L4 larva moulted to a pupa. These pupae were excluded from the analysis. Data were analyzed with the SPSS software (v. 14.0, SPSS Inc., Chicago, IL), using the Mann Whitney *U* test. Correlations between *flushing*, *mortality* and

ejection on the one hand and various rainfall and other weather variables during an experimental night on the other hand were obtained with the Spearman's rank correlation test.

6.3 Results

6.3.1 Flushing and mortality of *An. gambiae* larvae

6.3.1.1 Meteorological data

Experiments were carried out on 45 nights without and on 26 nights with rainfall. The total quantity of rainfall varied from 0.2 to 39.8 mm per night and the maximum rainfall intensity recorded was 9.5 mm in 5 minutes. During nights with rainfall, the total rainfall quantity was significantly correlated with the highest rainfall intensity ($\rho=0.95$, $P<0.001$) and with the duration of precipitation ($\rho=0.93$, $P<0.001$). Highest precipitation intensity and precipitation duration were also significantly correlated ($\rho=0.79$, $P<0.001$). Figure 6.2 shows the total quantity of rainfall and the maximum rainfall intensity (per 5 minutes) per night during the study period.

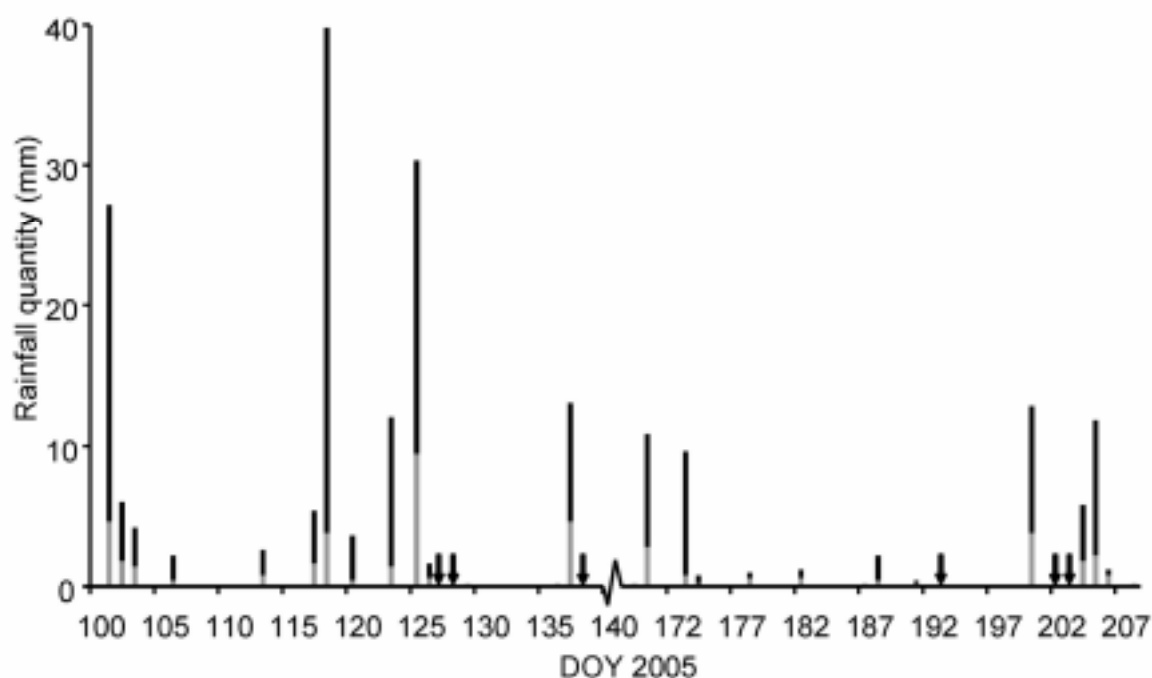


Figure 6.2 Total rainfall quantity, including maximum rainfall intensity (in grey), per night from April 10 (Day Of Year 100) up to July 27 (DOY 208). The arrows indicate missing data. Note that DOY 141 up to 167 are omitted from the figure, as no experiments were carried out.

Comparing nights with and without rainfall, there was no significant difference in the average air temperature and the average wind speed. The average air temperature was 21.6 (± 0.1 SEM, range 18.8-24.2) °C and 20.8 (± 0.1 , range 18.5-24.1) °C and the average wind speed 0.7 (± 0.0 , range 0.4-1.0) m/s and 0.7 (± 0.0 , range 0.5-1.0) m/s on nights without and with rainfall, respectively.

The average maximum recorded wind speed was significantly ($P < 0.05$) higher during nights with rainfall (3.4 ± 0.3 , range 1.6-6.3 m/s) than during nights without rainfall (2.7 ± 0.3 , range 1.0-10.9 m/s).

6.3.1.2 Flushing

Although this was unexpected, some larvae (on average 0.9 ± 0.3 % and 0.1 ± 0.1 % of the first instar (L1) and fourth instar (L4) larvae, respectively) were swept out of the basins by flushing during nights without rainfall (Figure 6.3). There was no significant difference between the percentages of larvae (for both L1 and L4 larvae) that flushed out of the small and out of the large basins. L1 larvae had a higher chance ($P < 0.001$) of being flushed than L4 during a night without rainfall (Figure 6.3). Flushing of L1 larvae during nights without rainfall was significantly correlated with average wind speed ($\rho = 0.13$, $P < 0.05$), but not significantly correlated with highest wind speed recorded that night. Flushing of L4 larvae was not significantly correlated with either of those variables.

During nights that experienced rainfall, there was no significant difference between the percentages of larvae (for both L1 and L4 larvae) that flushed out of the small and out of the large basins. During nights with rainfall, significantly ($P < 0.001$) more L1 and L4 larvae were swept away compared to nights without rainfall (Figure 6.3). The increase in flushing was 10.5% for L1 larvae and 3.3% for L4 larvae. During nights with rainfall, L1 larvae had a significantly ($P < 0.001$) higher chance of being flushed out than L4 larvae.

6.3.1.3 Mortality

Mortality of larvae after a night with rainfall was considerably higher than after a night without rainfall for both first and fourth larval stages (Figure 6.3). The mean increase in mortality during rainy nights was 6.9% (from 4.6 ± 0.3 to 11.5 ± 0.9 %) and 1.5% (from 4.1 ± 0.6 to 5.6 ± 0.8 %) for L1 and L4 larvae, respectively. On all nights, with or without rain, the survival of L1 larvae was significantly ($P < 0.001$) lower than that of L4 larvae (Figure 6.3).

6.3.1.4 Overall loss

Adding the average percentage of mosquito larvae that flushed out of their habitat to the average percentage of larvae that did not survive an experimental night gives the average total loss of the immature mosquito population per night. This amounted to a loss of 23% for L1 larvae and 9% for L4 larvae during a night with rainfall. During nights without rainfall these losses were 5.5% and 4.2% for L1 and L4 larvae, respectively, caused mostly by natural mortality. Corrected for the latter, the average increase in overall loss was 17.5% for L1 and 4.8% for L4 larvae during rainfall events.

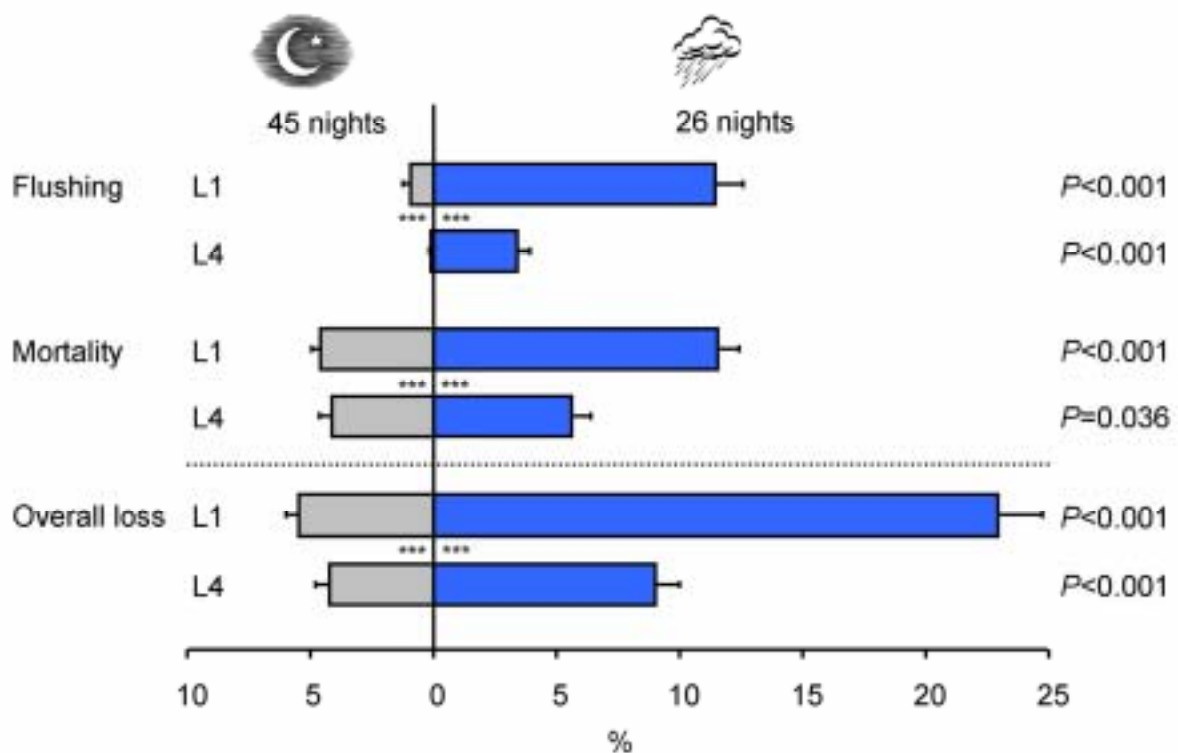


Figure 6.3 Percentage of L1 and L4 larvae of *An. gambiae* that flushed away or died and the overall loss during nights without rainfall (on the left) and nights with rainfall (on the right). The asterisks (***) indicate the level of significance between L1 and L4 larvae ($P < 0.001$). The error bars indicate the standard error of the mean.

6.3.2 Ejection of *An. gambiae* larvae

6.3.2.1 Meteorological data

Experiments were carried out during 29 nights without and 16 nights with rainfall. The total quantity of precipitation varied from 0.2 to 30.4 mm per night. The maximum rainfall intensity

recorded was 9.5 mm in 5 minutes. Comparing nights with and without rainfall, there was again no significant difference in the average air temperature and the average wind speed.

6.3.2.2 Ejection

No mosquito larvae were ejected from their original basin during nights without rainfall. During nights with rainfall 1.3 (± 0.6) % of the L1 larvae and 0.7 (± 0.4) % of the L4 larvae were ejected from their original basin. The difference between nights without and with rainfall was significant for L1 ($P=0.001$) and L4 ($P<0.05$) larvae but the chance of being ejected was similar for L1 and L4 larvae. When ejection occurred, more larvae were recovered from the medium tray (short distance) than from the large tray (long distance). There was no significant difference between the total percentage of larvae being ejected and the percentage of larvae being ejected into the medium basin.

6.4 Discussion

Precipitation flushed, ejected and killed a significant proportion of larvae of *An. gambiae* in different stages of development. Young larvae (L1 stage) experienced the highest flushing, ejection and mortality, meaning that the oldest larvae (L4 stage) were better able to withstand the effects of precipitation. We did not investigate the impact of rainfall on the second and third instar larval stages, but assume that their respective loss values lie within those found for the L1 and L4 stages.

The observed flushing of larvae on nights without rainfall was significantly correlated with wind speed. Occasionally it was observed that the water rippled due to gusts of wind and drops of water went over the rim of the basins. Because larvae tended to be situated at the air-water interface at the rim of the basins (personal observations) and it is known that they aggregate [7,9], larvae might have flushed out of the basin by the turbulence caused by wind.

Under natural circumstances, breeding sites must fill up before runoff of water takes place. The basins in the present study were filled to the brim, which may have resulted in a larger number of larvae that were flushed out during rainfall compared to sites that need to fill up first. On the other hand the basins were levelled, which may have led to an underestimation of the proportion of larvae that were flushed out. Under natural circumstances, water runoff will be stronger, as this will be concentrated at the lowest point of the edge of a breeding site instead of all around the edge as was the case in the experimental setup.

It appeared difficult to recover dead larvae because of the soil particles that had accumulated in the basins due to splashing of soil around the basins. Therefore, larvae that were unaccounted for could either have died in the setup or have been ejected from the experimental basins. Theoretically, larvae that were categorized as flushed could also have been ejected into the overflow basins. Ejection may have occurred when larvae were present at the air-water interface, just outside the centre of a raindrop-impact. When a raindrop hits the water surface, water is splashed away. It is conceivable that those spatters contain a mosquito larva and that this larva is flung away. However, our second experiment showed that this ejection did not contribute greatly to larval flushing and mortality. This is similar to an earlier finding of Robert et al. [24] who observed a weak dispersion by ejection of larvae of *An. arabiensis* when they were exposed to artificial rain.

Being flushed out of or ejected from their habitat onto the muddy surroundings does not necessarily imply the death of mosquito larvae. Larvae of *An. gambiae* s.l. are able to move actively over moist soil [32,33] and may therefore reach a new water body or return to the same one. Moreover, larvae may flow passively with runoff over the soil [33]. The percentage of immatures that reaches a new habitat remains unknown and will depend on a variety of factors, such as the duration and quantity of rainfall, geographical parameters and distance to nearest water body. However, larvae that are swept out of their natural habitat are likely to suffer higher mortalities than those that are left in the breeding site.

The observed higher mortality of mosquito larvae after rainfall may have several causes. Serious damage may be inflicted by the force of the impact of the drop when a larva is present near the water surface in the centre of a raindrop-impact. A small experiment whereby *An. arabiensis* was exposed to artificial rain showed no mortality as a result of the shock due to raindrops [24]. Russell [19] mentioned that the direct damage done to anophelines by rain will conceivably depend on size of the raindrops.

Another explanation for the observed mortality is the occurrence of water currents during rainfall. A longer period of turbulent water may exhaust mosquito larvae if they actively try to move away from the water surface to avoid being hit by a raindrop or to avoid being flushed out of their habitat or if they actively try to reach the air-water interface for oxygen. It was observed that the larvae were less present at the air-water interface during rainfall when they were not shielded from rain (KPP, unpublished data) and it is known that the diving behaviour of larvae of *An. gambiae* can kill them [34], as such behaviour has energetic costs. When larvae are not able to obtain oxygen at the air-water interface, they can survive from

8.5 up to 10.6 hours, depending on the larval stage [35], but this has not been tested in turbulent waters.

The significant loss of larvae due to rainfall will as a result decrease the larval density in a breeding site, which will lead to a lower competitive pressure for food and space. Whether such lower densities are advantageous for the development time and survival of the immatures of *An. gambiae* is not clear since various studies, where the effect of density on mosquito life-history traits was examined, are ambiguous. In two laboratory studies, survivorship decreased at higher densities [15,16], although the latter study showed that density strongly interacted with the temperature of rearing. By contrast, another laboratory study [36] showed that increasing densities (up to 2.6 larvae/cm²) had no effect on survivorship and similar results were obtained when larvae were reared outdoors in artificial habitats in Kenya [37]. In the same outdoor experiment, the development time reduced when larval densities were lower [37]. One laboratory study showed a similar result [36], whereas in another study the age at pupation, under laboratory conditions, was shorter when densities increased [16]. The observed differences are probably due to different setups of the studies and therefore more studies are required to examine the relationship between the density and the development and survival of the immatures.

Rainfall may also affect the mosquito larvae indirectly, by flushing out the predators and pathogens, which had previously colonized the same habitats. This will increase the chances of mosquito larvae to survive. Furthermore, rainfall decreases the water temperature [31] and this effect, which is larger in smaller water bodies (KPP, unpublished data), may affect larval development and survival as well.

The effects of rainfall on other malaria mosquito species *An. arabiensis* and *An. funestus* may be different and need to be studied in more detail. A recent study [35] showed that both *An. arabiensis* and *An. gambiae* did not differ mutually in their diving behaviour, but both species differed in this behaviour from *An. funestus*, which dived less frequently. Therefore *An. funestus* may be more affected by rainfall than *An. gambiae*. Charlwood & Edoh [38] suggested a difference in rainfall tolerance between larvae of *An. gambiae* and *An. arabiensis* based on their numbers prior to and after heavy rainfall.

Besides investigating other malaria mosquito species, the effects of rainfall on the pupal stage of *An. gambiae* needs to be examined. This life stage is very important, as the emergence of adult mosquitoes determines the productivity of a breeding site. Romoser and Lucas [39] suggested that pupal diving behaviour during heavy pelting rains helps them to avoid drowning and being flushed away. Pupae have a ventral air space containing gas,

which may be disrupted by a direct hit from a raindrop, causing the loss in hydrostatic balance. Once this balance and buoyancy are affected, they cannot be restored and the pupae eventually drown [40].

Furthermore, the long-term effects of precipitation on *An. gambiae* immatures require more attention. Rainfall may result in larval stress and larvae may have to consume more energy during rainfall, which may affect life history traits such as development time, survival and adult size.

This study showed that rainfall kills *An. gambiae* larvae and flushed them out of their habitat resulting in additional losses of 17.5% of L1 larvae and 4.8% of L4 larvae. Mortalities of the second and third instar larvae are likely to lie in between these values. Our data showed the loss experienced by rainfall during one night only. It is likely that larvae will be exposed to more frequent rain showers during their lifespan, resulting in a large population decrease due to flushing and mortality. During our study it rained on average once every three nights, although there were periods with daily rainfall and periods of several days without rainfall. Combined with the knowledge that larvae of *An. gambiae* s.l. may take between one and three weeks to develop into adult mosquitoes under ambient condition in the field [22,37], larvae may experience three or more nights with rainfall during their lifespan. The proportion of larvae of one generation that may be flushed away and killed during their lifespan will be substantial and therefore rainfall per se will affect larval population dynamics dramatically.

These effects of precipitation on mosquito populations should be considered in the light of climate change and malaria risk. Small changes in temperature and precipitation would directly affect the development of parasites and the behaviour and geographical distribution of the vectors [41,42]. An increase in malaria transmission due to the creation of a more suitable environment will be most pronounced at the borders of endemic malaria areas and at higher altitudes within malaria areas [41]. Increased frequency of malaria outbreaks may be seen in countries where it is not endemic at present [43]. Some areas that will experience a decrease in precipitation may see a shift from currently year-round transmission to only seasonal transmission in the future [44]. When the predicted increases in rainfall in East Africa [25,26] do occur, the spacing of the rains will be an important determinant affecting the rates and efficiency with which mosquito populations will grow and transmit diseases [45]. Increases in rainfall will directly result in an increase in the number of and a longer persistence of aquatic habitats. However, such increases will result in higher numbers of larvae that are flushed away and a higher larval mortality, as we observed moderate but

significant correlations between larval flushing, ejection and mortality (especially of L1 larvae; Table 6.1) and rainfall quantity, maximum intensity and duration. Therefore more rainfall not only results in more mosquito breeding sites which may in turn lead to an increase in malaria vectors, but it will also result in a large decrease of the existing immature populations, which leads to a reduction in emerging adults of that generation. Therefore, spacing of rainfall events should be viewed as an important determinant in the productivity of *An. gambiae* breeding sites, and hence in the mosquito population dynamics and transmission of malaria. Climate change, causing increased precipitation and frequency of rain showers, may thus indirectly affect malaria and other mosquito-borne diseases by impacting the larval populations.

Table 6.1 Correlation between flushing, mortality and ejection of L1 and L4 larvae of *An. gambiae* and various rainfall variables, during nights with rainfall.

	Flushing	Mortality	Ejection
L1 larvae			
Rainfall quantity (mm night ⁻¹)	0.49***	0.25***	0.49***
Rainfall max. intensity (mm 5min ⁻¹)	0.36***	0.18*	0.47**
Rainfall duration (minutes night ⁻¹)	0.57***	0.28***	0.36*
L4 larvae			
Rainfall quantity (mm night ⁻¹)	0.30***	ns	0.39**
Rainfall max. intensity (mm 5min ⁻¹)	0.23***	ns	0.35*
Rainfall duration (minutes night ⁻¹)	0.38***	ns	0.33*

Spearman's rank correlation coefficients and their level of significance are given. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$; (ns) not significant.

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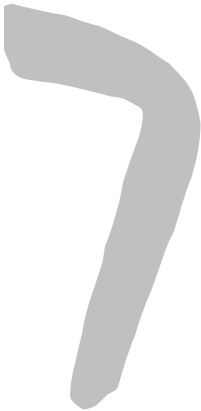
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Discussion

Krijn Paaijmans



7.1 Introduction

The work presented in this thesis expands our knowledge on the biology and ecology of immature stages of the Afro-tropical malaria vector *An. gambiae* s.l. by assessing the impact of physical (water temperature - Chapters 4, 5; water turbidity - Chapter 4; rainfall - Chapter 6) and biological factors (competition between sibling species - Chapter 5) on the growth, development and survival of *An. arabiensis* and *An. gambiae*. By combining studies on these life-history characteristics with the results obtained from detailed observations of the weather and the associated water temperature (Chapters 2, 3), an overview of the interactions between weather, water and malaria mosquito larvae is presented in the present chapter. The most important results of the field studies are summarized and perspectives for future work are presented, arranged by objective of this thesis (see Chapter 1). Finally, I discuss how the results can lead to improvement of (i) current models that look at malaria mosquito dynamics, (ii) larval control strategies and (iii) the understanding of the effects of climate change on malaria mosquito dynamics, and hence on the transmission of malaria.

7.2 Summarizing the most important results and perspectives for future work

7.2.1.1 Objective 1: To assess the diurnal fluctuations of water temperature in aquatic habitats of malaria mosquitoes and relate them to meteorological data.

The growth and development of larvae of *An. gambiae* s.l. is temperature-dependent [1,2]. For accurate calculations of the growth and development of immature stages, the temperature of the water that surrounds them should be known, by direct measurements or estimates. In tropical, small, shallow and open pools, which are typical breeding sites of *An. gambiae* s.l. [3-7], this temperature can fluctuate over a large range throughout the day [Chapter 2; 8,9-14]. Therefore, larvae will be exposed daily to a wide range of water temperatures. From a review on previously-observed development rates of larvae of *An. gambiae* s.l., it was estimated that the temperature at which mosquito larvae have the highest development rate is approximately 37 °C [15]. The development rate decreases slightly when the temperature gets lower but above this temperature it decreases sharply. In laboratory experiments at constant temperatures, the larval development rate peaked at a water temperature of 28 °C [2]. This suggests that the development rate of larvae that experience temperatures that exceed 28 °C is higher but could be disadvantageous: Larvae may develop too fast, which prevents them from reaching a certain critical body mass needed before pupation [16].

As temperature is an important determinant in the development of malaria mosquito

larvae, an understanding of the temperature fluctuations to which these larvae are exposed daily is necessary. To my knowledge, the daily variations in water temperature in natural breeding sites have neither been studied in detail nor over a long period. Therefore, the daily temperature fluctuations in three semi-natural water bodies of different size were first studied and linked to local meteorological data (Chapter 2).

Stratification was not observed in clear water bodies, but the top water layer (a thickness of 2 mm) of each water body differed slightly in temperature from the layers underneath. At night, the temperature of the top layer was up to 2 °C lower than the layers below, and during the day the temperature of this layer was up to 1 °C higher, depending on the weather conditions. The top water layer of a water body is the most important layer in the larval population dynamics of *An. gambiae* s.l., as larvae generally live horizontally near the air-water interface of aquatic habitats [17]. They may dive when mechanical disturbance takes place [17,18], but in small, shallow and clear water bodies they will not experience extreme differences in water temperature during this behaviour, as these water pools are homothermic. The mean temperature of the differently-sized water bodies was on average 27.4-28.1 °C throughout the study period. This suggests that *An. gambiae* s.l. mosquitoes have evolutionarily adapted to the temperatures in such breeding sites, as it corresponds with the temperature at which larval development rate peaks [2] and larval survival is highest [1] in the laboratory.

The magnitude of the daily fluctuations in water temperature was negatively correlated with habitat size. The recorded difference between the daily minimum and maximum water temperature was on average 14.4 (\pm 0.3) °C in the smallest (\varnothing 0.16 m, water depth 0.04 m) and 7.1 (\pm 0.2) °C in the largest water body (\varnothing 0.96 m, water depth 0.32 m) during a study period of 40 days. Therefore, mosquito larvae will be exposed to a larger range of temperatures in smaller water bodies each day. Moreover, the speed at which the water temperature increased before and decreased after reaching its maximum was negatively correlated with the size of the water body. Such aspects are likely to affect some life-history traits of the larvae of *An. gambiae* s.l., which will be discussed below (section 7.2.3.1).

The significant correlations between various weather variables and the daily minimum, average and maximum water temperature enables us to estimate the average and extreme water temperatures in anopheline habitats. Such correlations should be useful where accurate equipment for detailed meteorological observations is not widely distributed, such as in Africa.

Larvae of *An. gambiae* are encountered in turbid water collections, but the effects of

turbidity on life-history traits of the larvae and on the water temperature are poorly understood. The latter was demonstrated in Chapter 3: Suspended soil particles increased the near-surface water temperature and affected the diurnal temperature behaviour of small water bodies during daytime. In addition to affecting the water temperature, turbidity may interfere with larval feeding [Chapter 4; 19] and hence affect the life-history traits of larvae of *An. gambiae* s.l. This will be elaborated on below.

Turbidity may be a highly variable and changeable parameter throughout the day, decreasing due to the sinking of fine particles over time or increasing because of the disturbance of the pools by e.g. human activities, drinking cattle or rainfall. When water turbidity is taken into account in the assessment of larval population dynamics, I argue that turbidity should be monitored frequently and accurately in natural habitats to arrive at firm conclusions about the impact of this parameter. Clean and transparent water obviously appears clear, but a few particles do not automatically make it turbid. The simple division into clear and turbid water as observed by the human eye [13,20,21] may be too subjective for a water property that affects the life-history characteristics of *An. gambiae* s.l. larvae to a large extent (Chapter 4) and therefore it is necessary to measure the degree of turbidity accurately.

7.2.1.2 Perspectives

Breeding sites of anopheline mosquitoes come in different shapes and sizes, they differ in turbidity, and are encountered at different latitudes and altitudes during different seasons. Therefore, precise temperature measurements in various breeding sites are still required to gain a better insight into the diurnal temperature fluctuations to which larvae are exposed during their lifespan. Turbid water bodies require extra attention to improve our understanding of the effect of water turbidity on the temperature of all water layers, during both day- and nighttime. Moreover, as malaria vectors are still able to breed in the dry season in puddles along lakes and rivers and in irrigation systems [22-25], when meteorological conditions differ from those in the wet seasons, long-term measurements to assess the diurnal behaviour of the water temperature during other seasons are required. Regarding altitude, various studies have shown a reduced development rate and survival of larvae of *An. gambiae* s.l. in highland areas in western Kenya, compared to lowland areas [26,27]. In the highlands, larvae are subjected to a different thermal regime. However, apart from the altitude, the effect of the type of land cover (vegetation) in the area, which can influence habitat temperature and the occurrence of *An. gambiae* s.l. [28-30] needs to be considered as well.

With this extra information, the model estimates of water temperatures in various types of habitats can be verified and improved. Moreover, it may enable us to give estimates of the average water temperatures and temperature extremes under global warming. More on the modelling of water temperatures will be discussed below, and more on the possible effects of climate change on the water temperature can be found in section 7.5.

7.2.2.1 Objective 2: To develop a physical model that simulates the diurnal fluctuations of the water temperature in malaria mosquito breeding sites using meteorological data and to compare the model with continuous measurements of water temperatures in the field.

To my knowledge, no attempts have been made to model the temperature of typical, small and shallow habitats inhabited by tropical malaria mosquito larvae. Only large bodies of water, such as lakes, aquaculture ponds and reservoirs have been modelled [31-36]. Recently, a first step was made towards the modelling of temperature fluctuations in a small, shallow pool (\varnothing 1.1 m, water depth 0.25 m). This pool was, however, situated in The Netherlands [37] and stratified during daytime, which required a different modelling approach as the water body was divided into various horizontal water layers of different temperature. Because there was no stratification observed in the clear water bodies investigated in this thesis project, each one was considered as a box (Chapter 2).

The model, which is described in Chapter 2, estimated the daily course of the water temperature accurately. The air-water interface appeared to be the most important boundary for energy exchange processes and on average 82-89% of the total energy was gained and lost at this boundary. Although rainfall did affect the water temperature directly, the energy exchange caused by rainfall was negligible. The horizontal soil heat flux needs to be included in the model. Besides the loss of energy due to longwave radiation, energy loss due to evaporation, which continued throughout the day, was large in all pools (average energy loss of 15-18%). When the estimated latent heat flux was converted to evaporation in millimetres per day, the estimated average daily evaporation ranged from 4.2 mm in the small-sized to 3.7 mm in the large-sized water body. Such evaporation rates can have large consequences for the longevity of larval breeding sites that are only a few centimetres deep. Larvae of *An. gambiae* s.l. may take between one and three weeks to develop into adult mosquitoes under ambient conditions in the field [8,38]. Although larvae can survive for a few days on moist soil [39], they need water to complete their development and various studies have shown that habitat stability is positively associated with pupal productivity [40,41].

7.2.2.2 Perspectives

In order to better understand the physics behind small and shallow water bodies some aspects need to be studied in greater detail. First, soil appeared to be an important factor in the model, as the breeding sites were small and shallow. Quantifying the soil heat flux can easily be achieved by placing soil heat flux plates in the soil, just beneath the water-soil interface. In addition to investigating the soil heat flux, measuring soil moisture helps to understand soil heat conductivity. Moreover, soil moisture affects the infiltration of water from the pool into the surrounding soil and thus may also contribute to habitat longevity. This brings me to a very important limitation of the experimental setup: The water bodies were lined with a thin layer of plastic. At natural breeding sites, the soil at the water-soil interface will be completely saturated. In our model it was assumed that the soil was saturated, which is most likely, as the measurements were conducted during the rainy season. During dry periods, the soil that surrounds breeding sites can dry out rapidly and temperatures of the soil adjacent to the breeding sites can exceed the temperature of the water [42]. This may result in cracks in the soil and, as a consequence, water could run off from the pools. If soil properties are better understood, we may furthermore be able to clarify the soil heat flux and thus simplify the model, which will be discussed below.

Second, the reflection and refraction of incoming solar radiation by water and soil need to be studied in more detail. The albedo of water and, due to the shallow nature of the habitats, the albedo of the soil at the water-soil interface determine the net incoming solar radiation. The albedo of a water body is complex and depends on solar elevation, the so-called Fresnel albedo [43], on the diffusive component of irradiation and on the conditions of the water surface.

Third, the predictive powers of the model can be improved by adding some aspects of the hydrological balance of a pool. This can be achieved by incorporating the water level, which was kept constant in the experimental setup, into the model. In reality, the water level will vary between days due to the loss of water by evaporation and the increase of water by rainfall and water influx. The inclusion of these parameters can improve the predictions of the diurnal variation in water temperature and longevity of natural breeding sites.

Some other possible improvements of the model concern the local setting of natural breeding sites. Examples are parameters that include (i) corrections for the location of a water surface, as pools that lie deeper will have different wind and radiation conditions (reduced view factor), (ii) similar corrections for the land cover of an area, which also affects the incoming radiation and the fetch length of the wind: This is important as breeding sites of *An. gambiae* s.l. are not situated in large open areas, but are often surrounded by vegetation, houses etc., (iii)

the height of aquatic vegetation, e.g. the developmental stage of a rice crop, which can cause a decrease of incoming solar radiation depending on the height of the crop and could affect the wind fetch as well, (iv) the effects of the turbidity of a pool on the incoming radiation and (v) the presence of algae at the air-water interface, which may also affect the incoming radiation load.

When stratification occurs, however, a different model must be used, such as that described by Losordo and Piedrahita [35] and adapted for smaller pools by Jacobs et al. [37].

Our model can be verified and improved with actual data of the water temperature in various natural aquatic habitats (see perspectives of the previous objective). As soon as the physics behind the water temperature is better understood, the model can be simplified. As mentioned before, good and accurate equipment for meteorological measurements are not easily available in Africa. Therefore, a simplification of the model by making estimates of the incoming solar radiation and the complicated soil heat flux may prove useful in areas where these meteorological data are not readily available.

Moreover, one can go to a larger scale by using meteorological satellite sensors, which provide mechanisms for routine measurements of weather where meteorological stations are scarce [44,45]. Combining these data with an overlay of a geographic information system (GIS) grid on remotely sensed high resolution data, gives the opportunity to organize and characterize mosquito larval habitats. [46-49]. When this system is combined with biological data on the growth, development and survival of the immature stages of mosquitoes in relation to water temperature and other factors that affect their life-history characteristics, the mosquito abundance over time in a given area can be estimated. However, verification of the GIS outcome and the actual monitoring of larval densities in natural habitats will still be required to obtain proper input data.

7.2.3.1 Objective 3: To study the effect of diurnal fluctuations of water temperature on the life-history traits of the immature stages of the Afro-tropical malaria vectors *An. arabiensis* and *An. gambiae*, reared in single-species and in mixed-species populations, under ambient conditions.

There are only a few studies that have investigated the growth, development and survival of larvae of *An. gambiae* s.l. under different magnitudes of temperature fluctuations [8,50] or that have investigated the effects of water turbidity on these life-history characteristics [19]. However, in these studies other factors such as predators, parasites, pathogens, physiochemical water properties or nutrition may have affected the outcome.

By excluding or standardizing these factors in our experiments and by creating pools that had approximately similar daily average water temperatures, we assessed the effects of various diurnal temperature fluctuations on the life-history traits of *An. gambiae* s.l. Moreover, by conducting the experiments under ambient conditions and by creating semi-natural breeding sites, we were able to imitate the natural situation, with respect to daily temperature fluctuations, quite realistically.

In Chapter 4 the effects of habitat size, which is related to different diurnal fluctuations of the water temperature (Chapter 2), and habitat turbidity, which affects habitat temperature (Chapter 3) and possibly larval feeding [19], were investigated. Larval development rate of *An. arabiensis* and *An. gambiae* was positively correlated with habitat size, regardless of water turbidity. There was no effect of habitat size on larval survivorship or on the size of the emerging females. It is very likely that the observed differences in development rate between the differently-sized habitats were due to spatial and temporal differences in water temperature, which are described in Chapter 2.

In turbid water, larvae of *An. arabiensis* and *An. gambiae* took longer to develop into pupae and emerged as smaller female mosquitoes, compared to larvae that were reared in clear water. Moreover, water turbidity affected the survival of *An. gambiae*: Larvae in clear water showed a higher survivorship. Larvae in turbid waters would be exposed to higher temperatures during the daytime on both a spatial and temporal scale (Chapter 3). Apart from these effects of water temperature, a second factor may have contributed to the observed differences in life-history characteristics between clear and turbid water collections: inedible particles in turbid water may interfere with an efficient uptake of nutrients (see perspectives below).

In addition to the different diurnal fluctuations of the water temperature and habitat turbidity, competitive interactions between *An. arabiensis* and *An. gambiae* affected the development rate of the two species. In Chapter 5, it was observed that *An. arabiensis* and *An. gambiae* competed when they coexisted in a breeding site, which often occurs in the field [4,13,50-54]. Habitat sharing significantly affected the larval development time of both species, but in an opposite way: The development time of larvae of *An. arabiensis* increased, whereas the development time of larvae of *An. gambiae* decreased. *Anopheles gambiae* appears to be the better competitor. In the small-sized water bodies the mortality of both species seemed to increase when they shared one habitat.

7.2.3.2 Perspectives

To further elaborate on the suggestion that differences in the fluctuations of the water

temperature will lead to different effects on the life-history characteristics of *An. gambiae* s.l., extra tests under laboratory conditions are required. This can be achieved by imitating the various magnitudes of water temperature fluctuations presented in this thesis (Chapter 2) around the exact same mean temperature.

Furthermore, larval feeding of *An. arabiensis* and *An. gambiae* needs to be studied in further detail. The observed differences in larval development between the two sibling species may be explained by differences in feeding rate, feeding activity or other feeding-related mechanisms. Although both species are morphologically indistinguishable [55], their feeding efficiency may have evolved differently.

To address whether chemical cues could have induced the different effects of competition between the sibling species, larvae of both species should be reared together in a single tray whereby the species are separated by mesh wire and food is abundant. If the same results are obtained, the possible effect of different feeding mechanisms or a possible physical interaction between larvae can be excluded.

Finally, if more field experiments are conducted, it would be important to measure the microclimatic conditions, such as the diurnal water temperature dynamics, simultaneously. Unfortunately, due to failure of the micrometeorological equipment in the year 2006, we were unable to give solid evidence for the effects of variations in water temperature on the life-history traits of *An. gambiae* s.l.

7.2.4.1 Objective 4: To assess the direct impact of rainfall on the survival of larvae of *An. gambiae*.

Mosquitoes are *r*-strategists, which are characterized by a rapid development but a low probability of surviving to adulthood [56]. The observed mortality among larvae of *An. gambiae* s.l. in the field is extremely high: Various studies report that only a small fraction (2-8%) of the larvae that hatched, eventually survived to the adult stage. The high mortality is most often attributed to predators, parasites and pathogens [57-60], although since cannibalism within malaria mosquito species has been demonstrated [61,62], this factor has also been pointed to as a contributor to larval mortality in the field [63]. It has furthermore been suggested that rainfall may also affect larval population dynamics by flushing the larvae out their habitats when these habitats get flooded [8,41,64,65], but this effect has never been shown quantitatively in a large field trial.

In Chapter 6, we reported the results of outdoor experiments under ambient conditions in Kenya and showed that rainfall is a very important contributor to larval mortality: The

additional nightly loss of larvae caused by rainfall was on average 17.5% for the youngest (L1) larvae and 4.8% for the eldest (L4) larvae, compared to nights without rainfall. Most of the larvae were flushed out of the habitat, some were ejected from the habitat and the remainder could have died due to various mechanisms, such as (i) direct mortality by the hit of a raindrop or (ii) indirect mortality caused by stress or by exhaustion due to the rain itself or associated water movements or currents that are created by rainfall.

Larval flushing, ejection and otherwise-caused mortality were positively correlated with rainfall quantity, maximum intensity and duration. As mosquito larvae may take one to three weeks to develop into adult mosquitoes (see above), it is highly likely that they will experience several rainfall events during their lifetime. Although the effects were more severe for L1 larvae than for the L4 larvae, the high mortality in the younger stages will automatically result in a lower abundance of the older larvae a few days later. Moreover, various field experiments have shown that L4 larvae of *An. gambiae* s.l. take the longest time to develop into the next stage and have a reduced survivorship in general, compared to the younger larval stages [26,57,59,66]. Therefore, the seemingly small proportion of L4 larvae that is lost during rainfall will still have a profound effect on the productivity of mosquitoes from breeding sites and, as a result, on the transmission of malaria.

There are several other aspects concerning rainfall that should be discussed, which will affect the life-history characteristics of *An. gambiae* s.l. First, rainfall will not solely affect the larvae of *An. gambiae*. When *An. arabiensis* is present in the same pool, rainfall may change the anopheline species composition, hence affecting the development rate of both species (Chapter 5). Second, flooding of water pools is likely to flush out other organisms as well, including predators, parasites and pathogens of *An. gambiae* s.l., but also food particles. Third, rainfall may affect the ease with which larvae can consume edible particles (Chapter 4), as rainfall may increase habitat turbidity. Finally, rainfall will affect the temperature of a pool either immediately (Chapter 2) or over a longer period [67]. A sudden decrease of temperature may inflict larval stress, but the exact effect of the sudden transition to cooler conditions is not known.

7.2.4.2 Perspectives

The long-term effects of rainfall on the surviving mosquito larvae remain to be investigated. It is conceivable that frequent and long rains will affect the larvae, as they dive and stay submerged during rainfall (unpublished data). This may be stressful and energy consuming, especially when currents caused by rainfall bring about continuous diving or 'swimming'

behaviour to stay away from the surface. Furthermore, larvae may get damaged by raindrops, which may inhibit their development into adults.

Apart from the long-term effects of rainfall, other immature stages of *An. gambiae* and the effects of rain on other anopheline species need to be studied. Pupae could also be greatly affected by rainfall, as they have a ventral air space containing gas, which may be disrupted by a direct hit from a raindrop. This loss of their hydrostatic balance results in drowning [68].

From a physical point of view it would be helpful to study (i) the mechanism of raindrop impact and larval ejection and (ii) the course and intensity of currents in the water bodies caused by rainfall.

7.3 Mosquito population dynamics models

The growth, development and survival of the immature stages of *An. gambiae* s.l. affect the productivity of a breeding site. The time of development from an egg into an adult, combined with larval survivorship, determines the numbers of emerging mosquitoes over time. The size of the emerging adults is of importance, as size affects adult survivorship and fecundity [69-71]. Moreover, recent experiments suggest that habitat quality may influence malaria parasite transmission potential by females of *An. gambiae* [72].

Biological models for malaria transmission focus mainly on the temperature-dependent blood feeding intervals and longevity of the adult mosquitoes and development of malaria parasites within the mosquito [73]. Accurate estimates of changes in mosquito abundance in large areas as a result of changes in temperature, rainfall and humidity are very difficult to obtain [74,75]. Immature stages are rarely considered in predictive epidemiological models. Breeding habitats and/or the larval development and survival have only been incorporated in a few models [15,76]. The latter model used the air temperature at two meters height as input parameter for larval development and survival. We showed in Chapter 2, however, that the use of air temperature is not a sound method of approach for the water temperature, as water temperature is higher than air temperature throughout the day.

The inclusion of variation in the local eco-epidemiology of malaria is expected to aid the development of more effective control strategies and improve the predictions of malaria transmission in response to climate change. As the inclusion of the larval stages into malaria transmission models may be difficult due to the wide range of aquatic habitats they exploit, which will have varying effects on the life-history characteristics of the larvae, they are, however, more easy to sample in the field, as they are confined to their aquatic habitats. By monitoring representative breeding sites of the area and by estimating the types and numbers of those

aquatic habitats (see section 7.2.2), baseline data of larval populations can be obtained, which can be used as input in mosquito population models.

These baseline data can be coupled to models that estimate the temperatures in anopheline breeding sites (Chapter 2) where the larvae are found. By combining the known relationships between larval growth and development on the one hand and the daily thermal regimes of different habitats on the other hand (Chapter 4), adult mosquito abundance in an area over time can be predicted, although various other factors that affect the life-history traits of larvae of *An. gambiae* s.l. (Table 1.1) must be included. All these data together will lead to better malaria risk assessments and, therefore, to improved planning of health management control strategies. Furthermore it will enable us to better predict the effects of climate change on the transmission of malaria (see section 7.5).

7.4 Larval control strategies using biolarvicides

Larvae are, in contrast to adult mosquitoes, confined to their habitat, which makes them easier targets for control strategies [77]. In recent years, two different species of bacterial larvicides, *Bacillus thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs), have been applied in field trials and have been shown to be effective in larval control [78]. Furthermore, several fungal species appear to be promising biolarvicides [see review: 79]. Reducing the burden of malaria by implementing larval control could be useful in combination with adult control strategies, such as the use of insecticide-treated bednets [78]. However, in order to design efficient larval control methods, mechanisms that regulate the adult productivity in natural habitats must be understood [40].

The observations that development rate was lower (*An. arabiensis*) or higher (*An. gambiae*) when larvae were exposed to competition (Chapter 5), and that the development rate was faster in larger compared to smaller habitats and faster in clear compared to turbid water (both for *An. arabiensis* and *An. gambiae*, Chapter 4) need to be considered in the design and implementation of larval control, notably the frequency at which breeding sites are visited for control. This frequency will furthermore depend on the dose and the residual effect of the larvicide [78]. Moreover, the persistence of the breeding sites may be important. The transient nature of breeding sites of *An. gambiae* s.l. will affect the efficiency of an applied larvicide [80].

Reports on whether all or only certain breeding sites need to be targeted are ambiguous. Some groups suggest that only pupae-producing habitats closest to homes should be treated [41], others suggest all potential breeding sites should be targeted [6,81]. The data in this

thesis support the latter groups, as all the semi-natural water bodies produced pupae during the studies. However, other factors that affect the life-history characteristics of larvae of *An. gambiae* s.l. (Table 1.1), which were controlled for in these experiments but do occur in nature, may change this outcome. As field studies showed that even small breeding sites still produce pupae [41,50], my philosophy would be that one mosquito may still transmit malaria and that therefore all breeding sites should be targeted.

The physical data obtained with our studies may also contribute to a successful design and implementation of larval control. The fact that bacterial and fungal larvicides have their own temperature range in which they grow, develop and survive, with their own species-specific optimum temperatures, needs to be considered. Therefore, it is important to know the diurnal temperature fluctuations in the pools or puddles that are treated, with respect to the life-history traits of the control agents.

Not only temperature, but also rainfall will have an impact on larvicides. When breeding sites overflow, part of the larvicides flush out of the habitat, changing the concentration of the larvicides and hence decreasing their lethal effect. However, rain may also wash the biolarvicides into other non-treated breeding sites, killing the larvae there [80]. Therefore, the frequency of visits to the field will depend on the frequency and quantity of rainfall as well. When there is a long period of drought, shallow sites will dry out soon so that larvicides may not need to be applied there, depending on the age of the present immatures: Using estimates of the evaporation rates from aquatic habitats, it is possible to establish a threshold water depth per aquatic habitat, below which the application of larvicides is useless due to future desiccation of the habitat.

7.5 Climate change

Climate is one of the main factors determining distribution and prevalence of vector-borne diseases such as malaria, dengue and West Nile virus [82]. Climate change will have a serious effect on the transmission of malaria, as the disease is sensitive to climate: Small changes in temperature and precipitation can directly affect the development of parasites and the behaviour and geographical distribution of the vectors [83,84]. Climate change will result in a more suitable environment and an increase in malaria transmission may be most pronounced at the borders of endemic malaria areas and at higher altitudes within malaria areas [83]. The influence of meteorological variables on vector populations, e.g. absolute mosquito abundance, is poorly understood [73,85]. A discussion on the impact of climate change on the smallest scale within the malaria vector ecology, the breeding site, is urgently

needed.

Increases in rainfall will directly result in an increase in number and persistence of aquatic habitats, although the impact of rainfall will depend on parameters such as local evaporation rates, the soil percolation and the slope of terrain [86]. However, the assumption that an increase in rainfall due to global warming, as is predicted in East Africa [87,88] will lead to an increase in malaria may not be as straightforward as always assumed. Considering the impact of rainfall on the existing larval populations, we demonstrated that rainfall accounts for high losses during the immature lifespan of *An. gambiae* s.l. These extra losses during rainfall events may result in a reduction in emerging adults of that generation. Spacing of rainfall events should be viewed as an important determinant in the productivity of *An. gambiae* breeding sites (Chapter 6), in mosquito population dynamics and the transmission of malaria [89].

As has been discussed frequently in this thesis, temperature and larval development are positively correlated. The immature stages of malaria mosquitoes rarely receive attention in models that predict mosquito population dynamics (see section 7.3). Various model predictions indicate a median increase of 3-4 °C of the air temperature by the year 2100, as a result of global warming [88]. The effects of such changes in air temperature on the water temperature and consequently on the growth, development and survival of the immatures of malaria mosquitoes require attention.

With our model it will be possible to predict the diurnal fluctuation of water temperature of pools in the future, by using climate change scenarios of various Global Circulation Models (GCMs) as input. An overview and evaluation of these models can be found in the report "Climate Change 2007" [90]. Although both the model and the various climate change scenarios have their uncertainties, they provide an indication of the effects of climate change on water temperature.

As the impact of climate change on the water temperature has not yet been assessed, I will simplify the situation for the purpose of this discussion. Considering the linear relationship between the average air and average water temperature (Chapter 2), I expect an increase in the average water temperature due to global warming. This results in an average water temperature above 27-28 °C, thus higher than the optimum temperature for larval development and survival.

The maximum temperature that larvae are able to endure for some time is 41 °C [8,14]. However, when larvae were exposed to constant temperatures above 32 °C in the laboratory, larvae did not complete development [2,91]. At the upper temperature range at which larvae

developed into adults (30–32 °C), mortality was still high (approximately 70%). As there seems to be a 'delicate' relationship between temperatures above the optimum temperature for larval development and survival on the one hand and the duration at which larvae are exposed to them on the other hand, I argue that a change of the upper temperature limit in water bodies will become important in larval population dynamics under a changing climate. The temperature in small and shallow water collections, such as rain puddles and footprints filled with rain water, may become unfavourably high. Temperatures near or beyond the upper lethal temperature may affect larval survival. Moreover, the larval development rate may accelerate, causing larvae to develop too fast, preventing them from building up the necessary reserves prior to pupation [16]. When this elongates the time to pupation, larvae exposed to predators, pathogens, parasites and rainfall or to larval biopesticides for longer periods.

The effects of climate change on the temperature of natural breeding sites of *An. gambiae* s.l. should be investigated in the future. Special care must be taken with the potential change in solar radiation, which is hardly discussed in the report "Climate Change 2007" [88]. However, a change in atmospheric greenhouse gasses may result in a change in incoming solar radiation. This can result in an increase of solar radiation, which is referred to as global brightening [92], or in a decrease, the so-called global dimming [see review 93]. Changes in incoming solar radiation load may have large consequences for the temperature in water pools, as solar radiation is the driving force behind energy exchange processes.

Finally, a big question will remain: are malaria mosquito larvae capable of adapting to a changing environment?

The findings of this thesis demonstrate that biotic and abiotic factors significantly affected the growth, development and survival of the aquatic stages of malaria mosquitoes in an important but complex way. Future studies on malaria epidemiology and malaria control should consider these factors, which are summarized in Table 7.1, in order to improve malaria risk predictions and to successfully incorporate the immature stages of the mosquitoes in malaria intervention strategies. As species-specific interactions occur, which may give rise to differential development, detailed knowledge at species level is required before interventions can be implemented.

Table 7.1 Impact of abiotic and biotic factors on the life-history characteristics of larvae of *An. gambiae* s.l. as studied in this thesis under constant density and nutrition and under ambient conditions.

Abiotic factors	Growth	Development	Survival
Fluctuating water temperature			
<i>An. arabiensis</i>	-	+	-
<i>An. gambiae</i>	-	+	-
Water turbidity			
<i>An. arabiensis</i>	+	+	-
<i>An. gambiae</i>	+	+	+
Precipitation			
<i>An. gambiae</i>	ns	ns	+
Biotic factor	Growth	Development	Survival
Interspecific competition			
<i>An. arabiensis</i>	-	+	+/-
<i>An. gambiae</i>	-	+	+/-

(+) indicates a significant effect; (-) indicates no effect was observed; (+/-) indicates a tendency to affect a life-history characteristic; (ns) not studied

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SAMENVATTING

Het succes van malariabestrijding met medicijnen, geïmpregneerde klamboes of door het behandelen van rustplaatsen van malariamuggen met lang-werkende insecticiden, neemt steeds meer af. Omdat malaria een van de meest belangrijke infectieziekten ter wereld is (jaarlijks komen meer dan 350 miljoen mensen in aanraking met de ziekte en sterven er ruim één miljoen mensen), worden alternatieve bestrijdingsmethoden toegepast of ontwikkeld. Methoden waarbij muggenpopulaties worden gereduceerd door de bestrijding van de larvale stadia, krijgen meer aandacht. Deze larvale stadia bepalen voor een belangrijk deel de verspreiding, dynamiek en fitness van volwassen muggen, en daarmee indirect de dynamiek en verspreiding van malaria. Een beter fundamenteel inzicht in de biologie en ecologie van de larvale stadia van malariamuggen zou kunnen bijdragen aan een verbetering van huidige en aan de ontwikkeling van nieuwe bestrijdingsmethoden. Daarnaast kan meer kennis over larvale stadia resulteren in een verbetering van huidige modellen die malariarisico in kaart brengen.

Het doel van dit onderzoek is om de effecten van fysische en biologische factoren op de biologie en ecologie van larven van de malariamuggen *Anopheles arabiensis* en *An. gambiae*, de meest wijdverspreide en efficiënte vectoren van malaria in Afrika ten zuiden van de Sahara, te bestuderen. In dit proefschrift laat ik zien hoe meteorologische variabelen (i) de biologische eigenschappen van larven van *An. arabiensis* en *An. gambiae* en (ii) de temperatuur van hun directe leefomgeving, zoals kleine waterplasjes, beïnvloeden. Meteorologische data en de watertemperatuur in seminatuurlijke plassen van verschillende afmetingen, blootgesteld aan de natuurlijke omgevingsfactoren, zijn gemeten in West Kenia, samen met de groei, ontwikkeling en overleving van larven van malariamuggen in identieke waterplasjes.

Omdat de watertemperatuur een belangrijke factor is in de groei, ontwikkeling en overleving van de larven, zijn betere metingen van de temperatuurschommelingen waaraan larven in de natuur dagelijks worden blootgesteld, nodig. Dit soort metingen in heldere seminatuurlijke waterplasjes zijn beschreven in Hoofdstuk 2. Het dagelijkse temperatuurverloop in de kleinste waterplas verschilde op zowel een ruimtelijke als een tijdelijke schaal van die in de grotere plassen. Verder is, met gebruikmaking van lokale weer- en bodemdata, een model ontwikkeld dat het dagelijkse verloop van de watertemperatuur nauwkeurig voorspelt (Hoofdstuk 2). Watertemperatuur wordt ook beïnvloed door troebelheid van het water: Troebele waterplasjes hebben in de middag (tussen 13 en 17 uur) een hogere

watertemperatuur nabij het wateroppervlak dan heldere plasjes met dezelfde afmetingen (Hoofdstuk 3).

Naast deze fysische experimenten, zijn biologische experimenten uitgevoerd in identieke waterplasjes. Larven van *An. arabiensis* en *An. gambiae* zijn hierbij opgekweekt in identieke doorzichtige plastic bekertjes, die dreeven in waterplasjes van verschillende dimensie. Op deze manier zijn de effecten van verschillende dagelijkse fluctuaties in de watertemperatuur op de biologische eigenschappen van de muggenlarven bepaald (Hoofdstuk 4, 5). De ontwikkelingssnelheid van beide muggensoorten was positief gecorreleerd aan de grootte van de broedplaats: Larven ontwikkelden zich sneller tot volwassen insecten wanneer de dagelijkse amplitude in watertemperatuur kleiner was (Hoofdstuk 4). Wanneer de muggenlarven in troebel water werden opgekweekt, daalden de groei- en ontwikkelingssnelheid, vergeleken met muggenlarven in helder water. De sterfte van *An. gambiae* larven was bovendien groter in troebel water. Aangezien beide soorten vaak samen in dezelfde plas voorkomen, zijn ook de effecten van mogelijke competitie op groei en ontwikkeling bestudeerd. Wanneer beide soorten een habitat deelden, veranderde de ontwikkelingssnelheid significant, maar in tegenovergestelde richting; de ontwikkelingssnelheid van *An. arabiensis* nam af, terwijl die van *An. gambiae* toenam. Bovendien leek in de kleinste waterplas de sterfte van beide soorten toe te nemen wanneer ze samen voorkwamen (Hoofdstuk 5). Tenslotte is het directe effect van regenval op de overleving van *An. gambiae* larven bepaald. Een hoger verlies van muggenlarven tijdens regenachtige nachten is waargenomen, door het wegspoelen van larven uit hun habitat en door een hogere sterfte, vergeleken met droge nachten (Hoofdstuk 6).

De resultaten uit dit proefschrift demonstreren dat biotische en abiotische factoren de groei, ontwikkeling en overleving van de aquatische stadia van malariamuggen significant kunnen beïnvloeden. De interacties tussen de mug en deze factoren zijn complex en niet altijd eenduidig. Toekomstige studies aan malaria-epidemiologie en malariabestrijding zouden rekening moeten houden met deze factoren om modelvoorspellingen en strategieën van bestrijding te verbeteren. Omdat soortspecifieke interacties plaatsvinden, die bijdragen aan verschillen in larvale ontwikkeling, is gedetailleerde kennis op soortsniveau noodzakelijk voordat malaria-interventies worden uitgevoerd.

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CURRICULUM VITAE

On 11 June 1978, I, Krijn Petrus Paaijmans, was born in 's-Hertogenbosch (also known as Den Bosch), The Netherlands. After having finished the Dutch equivalent of high school at the Saint Odulphus Lyceum in Tilburg in 1997, I studied biology at the Wageningen Agricultural University (now Wageningen University), The Netherlands. Through two courses at the Laboratory of Entomology during this study, I came in contact with Willem Takken and Sander Koenraadt. Fascinated by the biology and ecology of malaria mosquito larvae, I carried out three MSc-projects on this topic. First, I studied the ecology of anopheline mosquito larvae in a Kenyan highland area together with Andrew Githeko at the Kenya Medical Research Institute (Kisumu, Kenya) and worked for a month at the International Centre for Insect Physiology and Ecology (Mbita, Kenya) with Bart Knols, carrying out a small experiment to study the effect of habitat desiccation on the survivorship of *Anopheles gambiae* larvae. Second, I worked with Pete Billingsley and John Dallas at the Department of Zoology (University of Aberdeen, Scotland) and investigated the kinship among larvae of *Anopheles arabiensis* and *Anopheles gambiae* collected from different breeding habitats in Miwani, western Kenya, using microsatellite markers. After a third study in The Netherlands, assessing the occurrence and ecology of the former European malaria vector *Anopheles maculipennis* s.l. with Willem Takken, I obtained my MSc-degree in Biology, with a specialization in ecosystem biology, in November 2002. I continued to work on this last subject for one year as a junior scientist at the department of Animal Ecology and Ecophysiology with Jan-Peter Verhave and Gerard van der Velde (Catholic University of Nijmegen, now Radboud University Nijmegen, The Netherlands) in close collaboration with Willem Takken. In September 2003, I was given the opportunity to start my own doctoral research on my favourite creature: the malaria mosquito larva. It was an interdisciplinary PhD-project, the aim of which was to assess the impact of meteorological variables on water temperatures and malaria mosquito larvae, under the supervision of Adrie Jacobs (Meteorology and Air Quality, Wageningen University) and Willem Takken. Experiments were carried out during three visits to the Kenya Medical Research Institute in the past four years (duration of 6, 7 and 1½ months, respectively) under the supervision of Andrew Githeko. The results of my PhD-project are presented in this dissertation.





PUBLICATIONS

Peer-reviewed papers

1. Paaijmans KP, Wandago MO, Githeko AK & Takken W (2007) Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. *PLoS ONE* 2(11): e1146. DOI:10.1371/journal.pone.0001146.
2. Jacobs AFG, Heusinkveld BG, Kraai A & Paaijmans KP (2007) Diurnal temperature fluctuations in an artificial small shallow water body. *International Journal of Biometeorology* DOI:10.1007/s00484-007-0121-8.
3. Huijben S, Schaftenaar W, Wijsman A, Paaijmans K & Takken W (2007) Avian malaria in Europe: an emerging infectious disease? In: Emerging pests and vector-borne diseases in Europe. Takken W & Knols BGJ, editors. Wageningen: Wageningen Academic Publishers. pp 59-74.
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6. Koenraadt CJM, Paaijmans KP, Githeko AK, Knols BGJ & Takken W (2003) Egg hatching, larval movement and larval survival of the malaria vector *Anopheles gambiae* in desiccating habitats. *Malaria Journal* 2:20.

Non peer-reviewed papers

1. Jacobs AFG, Heusinkveld BG & Paaijmans KP (2005) Shallow water temperatures and malarial mosquitoes *Bulletin of the American Meteorological Society* 86: 25-26.
2. Takken W, Huijben S, Wijsman A, Paaijmans KP, Schmidt H & Schaftenaar W (2003) Avian malaria in Rotterdam. *Dutch Wildlife Health Centre Newsletter* 2: 3-4.
3. Paaijmans KP, Koenraadt CJM, Dallas JF, Billingsley PF & Takken W (2003) Use of microsatellites for kinship analysis of sympatric populations of the malaria mosquito larvae of *Anopheles gambiae* Giles s.s. and *An. arabiensis* Patton. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV)* 14: 17-23.

(To be) Submitted

1. Paaijmans KP, Jacobs AFG, Takken W, Heusinkveld BG, Githeko AK, Dicke M & Holtslag AAM. Observations and model estimates of diurnal water temperature dynamics in mosquito breeding sites in western Kenya.
2. Paaijmans KP, Takken W, Githeko AK & Jacobs AFG. The effect of water turbidity on the near-surface water temperature of malaria mosquito breeding sites.
3. Paaijmans KP*, Huijben S*, Githeko AK & Takken W. Competitive interactions between larvae of *Anopheles arabiensis* and *Anopheles gambiae* under ambient conditions in western Kenya.
4. Paaijmans KP, Githeko AK & Takken W. Water temperature fluctuations and water turbidity affect life-history traits of *Anopheles arabiensis* and *Anopheles gambiae* under ambient conditions in western Kenya.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of Literature (5.6 ECTS)

- Micrometeorological parameters influencing the growth, development and survival of African malaria mosquito larvae.

Laboratory Training and Working Visits (4.3 ECTS)

- Meteorology and vector ecology; Kenya Medical Research Institute, Kenya (2004, 2005, 2006)
- Plant-insect interactions, chemical ecology and vector diseases; London, UK (2007)

Courses (6.4 ECTS)

- Meteorology and climate (2003)
- Atmosphere-land interactions (2004)
- Field experiment (2004)

Discussion Groups / Local Seminars and Other Scientific Meetings (5.4 ECTS)

- Autumn Symposium of the Buys Ballot Research School (2003)
- Two-weekly PhD Malaria-meeting; Kenya Medical Research Institute, Kenya (2005-2006)
- Two-weekly PhD-discussion group; PE&RC, ENT (2005-2007)
- Meeting Netherlands Entomological Society (2006)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.5 ECTS)

- PE&RC annual meeting (2003, 2005)
- PE&RC introduction weekend (2004)

Symposia, Workshops and Conferences (6 ECTS)

- 4th International Congress of Vector Ecology; Reno, Nevada, USA (2005)
- 5th European Congress on Tropical Medicine and International Health; Amsterdam, The Netherlands (2007)

Courses in which the candidate has worked as a teacher

- Biology and Management of Plant Pathogens, Insects and Weeds II (2005, 2006); 40 days

Supervision of MSc students (s)

- Vector ecology; 40 days; 2 students

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