Entomological studies on the impact of a small-scale irrigation scheme on malaria transmission around Zeway, Ethiopia.

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Abstract

To evaluate the impact of a small-scale irrigation scheme on the level of malaria transmission in a semi-arid area, entomological studies were conducted in Zeway area, Central Ethiopia. Larval and adult anophelines were sampled during the dry and short-rainy seasons from irrigated and non-irrigated villages. Overall, significantly higher density of *Anopheles* larvae were found during the dry season in the irrigated village (Mean = 38.3 larvae/100 dips) than the non-irrigated village (7.4 larvae/100 dips). Canal leakage pools, irrigated fields and irrigation canals were the major sources of *Anopheles* mosquitoes. Larval and adult *Anopheles pharoensis* and *An. arabiensis,* principal malaria vectors in Ethiopia, were more abundant in the irrigated village than the non-irrigated village throughout the study period. Hourly light trap catches revealed that peak indoor and outdoor biting activities of *An. arabiensis* and *An. pharoensis* occurred during the early period of the night before the local inhabitants retire to bed. The majority of blood-engorged *An. arabiensis* (0.78) and *An. pharoensis* (0.69) had fed on humans, suggesting that their highly anthropophilic nature in Zeway area. *Plasmodium falciparum* infection rates of 1.02% and 0.54% were determined for *An. arabiensis* and *An. pharoensis*, respectively, in the irrigated village. This study demonstrated that due to poorly maintained irrigation structures, the irrigation scheme created conducive breeding grounds for malaria vector species, particularly during the dry season. Consequently, the period of malaria transmission might possibly extend from seasonal to year-round, involving the dry season. Proper water management coupled with environmental management such as source reduction could reduce vector abundance and hence malaria transmission in the irrigation schemes.

6.

7. Key words: *Anopheles*, malaria transmission, small-scale irrigation scheme, *Plasmodium falciparum* sporozoite rate, Zeway, Ethiopia.

1. Introduction

Development of irrigation schemes is widely recognized as a key for promoting economic growth, ensuring food security and alleviating poverty in most developing countries (Lipton et al., 2003). However, past experience shows that inadequate consideration of both environmental and public health impacts can seriously undermine the sustainability of such schemes (Gratz, 1988; McCartney et al., 2007). Key among the potential negative impacts is the link between irrigation and malaria – a disease that affects between 300 and 500 million people each year globally and claims the lives of 1.5 to 2.5 million people annually (WHO, 2006).

By increasing the availability of surface water for breeding, irrigation favors the development of large populations of disease vectors such as anopheline mosquitoes responsible of transmission of malaria. Hence, there is great concern that irrigation can lead to increased malaria transmission especially in sub-Saharan Africa where 90%

of the global malaria burden exists and the prevailing climatic factors support proliferation of malaria vector mosquitoes and development of the parasite in the vector. However, the relationship between irrigation and malaria is not straightforward and varies according to endemicity and seasonality. In stable malaria endemic areas of sub-Saharan Africa, studies have shown that malaria transmission is equal or less in irrigated-rice growing areas compared with neighboring areas without irrigated rice cultivation (Josse et al., 1987; Lindsay et al., 1991; Boudin et al., 1992; Faye et al., 1993; Henry et al., 2003). The explanation for this finding is yet unresolved, but in some cases at least, could be attributed to displacement of the most anthropophilic (human blood seeking) malaria vector *Anopheles funestus* by *An. arabiensis* with lower vectorial capacity, as the later thrives more than the former in irrigated fields (Ijumba and Lindsay, 2001). It has also been suggested that many communities near irrigation schemes benefit from the greater wealth created by the schemes, often leading to better access to improved health care and hence receive fewer infective bites compared to those outside such schemes. On the other hand, in areas where malaria is absent or unstable, introduction of irrigation was found to place the non-immune population at a high risk of acquiring the disease, increasing malaria morbidity and mortality (El Gaddal et al., 1985; Ijumba et al., 1990). In such areas, irrigation, especially during the dry season, might alter the malaria transmission pattern from seasonal to annual, as observed in the Sahelian region of Mali (Sissoko et al., 2004) and in sub-arid irrigated areas of Madagascar (Marrama et al., 2004).

In Ethiopia, where three quarters of its land mass are potentially malarious, introduction or expansion of irrigation schemes can increase the burden of malaria in the country. A detailed epidemiological study in the highlands of Tigray, northern Ethiopia, has reported that malaria incidence in young children was sevenfold higher in communities near irrigation microdams than those further away (Ghebreyesus et al., 1999). A recent entomological study in the same area has reported 5.9-7.2 times more adult *An. arabiensis* (the main malaria vector in Ethiopia) in the dam villages than the controls, non-irrigated villages (Yohannes et al., 2005). The study also indicated that seepage water at the base of the dam, leaking irrigation canals and waterlogged fields were the main sources of *An. arabiensis* throughout most of the year. However, despite extensive development of irrigation schemes in semiarid fertile areas of the country with unstable disease transmission (MoWR, 2005), in-depth information on the link between irrigation and malaria in such environmental settings is lacking. The main objective of this study was to assess the possible impact of irrigation-based agricultural activities on malaria transmission in a semi-arid area with seasonal disease transmission.

2. Materials and Methods

The study area

The study was undertaken between February and May 2006, in two rural farming villages, Abene-Girmamo and Woshgulla, located in Zeway area ($8^{o}00`N$, $38^{o}40`E$), Central Ethiopia, 165 Km south of Addis Ababa, in the middle course of the Ethiopian Rift Valley (Figure 1). Both study villages are at a distance of 5-6 Km from Zeway town, which is situated alongside Lake Zeway. The area receives between 700-800 mm of annual rainfall, with the heavy rains during the months of June to September and short rains in April and May (National Meteorological Agency). The mean annual temperature is 20 ^{0}C, and February is the hottest month of the year.

Malaria transmission in Zeway area is generally unstable (seasonal), with peak transmission occurring between the months of September and November, immediately after the main rainy season, while the second less pronounced transmission period falls

between April and May in the short-rainy season. *Plasmodium falciparum* is the most prevalent malaria parasite in Zeway area, responsible for 60-70% of malaria cases. Vivax malaria is also common in the area, particularly in the dry season, but generally less prevalent. (Abose *et al*., 1998b; Zeway Malaria Control Unit, unpublished report). *Anopheles arabiensis* is the primary malaria vector in Zeway area, and elsewhere in Ethiopia, while *An*. *pharoensis* plays secondary role (Rishikesh, 1966; Abose *et al*., 1998a; Abose *et al*., 1998b; Ye-Ebiyo et al., 2000).

Abene-Girmamo is an irrigated rural village, situated at an altitude of 1647 m. The village is inhabited by 934 people, mainly dependent on subsistence farming. The community is mainly comprised of the Oromo and Silte ethnic groups. Most families own livestock (mainly bovine, ovine and equine), with a human to cattle ratio of 1: 0.4. Woshgulla is a non-irrigated agricultural village, situated at an altitude of 1654 m, with a population size of 741. The village is located 8 Km away from the irrigation schemes in Abene-Girmamo. The inhabitants are dependent on subsistent rain-fed agriculture during the months of the wet seasons. They also keep livestock (mainly cattle, equine and ovine), with the mean human to cattle ratio of 1: 0.6. The domestic animals in both villages spend the night either indoors in the same homesteads with the owners or outdoors in open cattle enclosures. The main type of housing in these villages was circular huts, made of mud-brick walls and thatched roof. Mud-brick-making pits, partly covered with water, were commonly found at the backyards of households that commonly practice brick-making either for domestic use or for sale. These pits were mostly functional during the dry season but became non-functional in the wet seasons, because the rains could damage newly formed moist mud-bricks before they dry. Each village had a water-harvesting pool, i.e., collection of rainwater in a wide and deep well (volume ~ 2m width x 2m length x 6m depth) with corrugated iron-roofing.

The source of water for irrigation in Abene-Girmamo is Lake Zeway, located 5-6 Km away from the scheme. Water is pumped from the lake by three long plastic pipes (0.4 m diameter and 4-5 km long) that run underneath the ground to reach the unlined surface canals at uplifted soil mass. The surface irrigation canals feed smaller field canals to cover the entire agricultural field. However, due to poor construction and lack of maintenance, there were many leaking canals, causing leakage pools at unwanted places. These pools never dry because of continuous water leakage from the irrigation canals. Water logging also occurred in the agricultural field as a result of over-irrigation and water retaining characteristics of the soil. Sometimes, poor drainage led to water logging in the field. The uplifted soil walls of the surface canals were also frequently perforated and formed leakages, mainly due to the action of domestic animals while drinking water in the canals. Onion, cabbage and maize (*Zea m. mays*) were commonly grown under irrigation throughout most of the year.

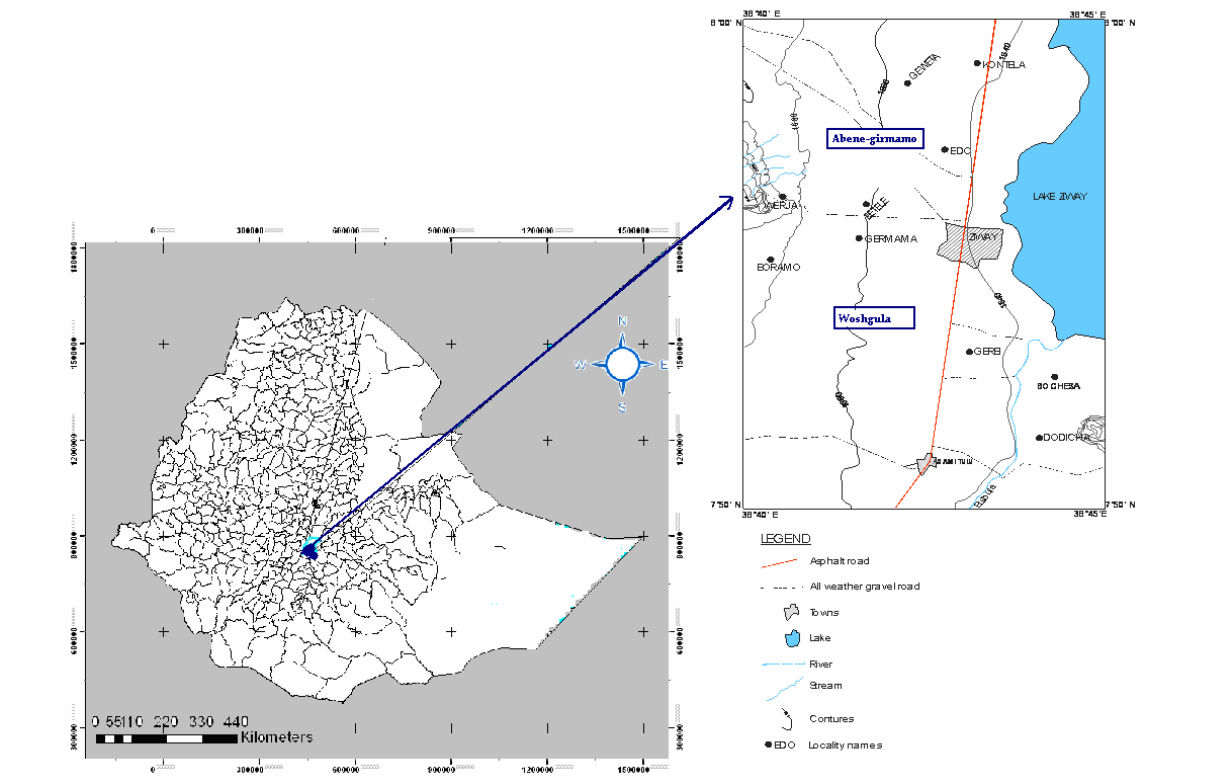

Figure 1. Location of the study area in Ethiopia and map of Zeway area (the two study villages are shown in bold rectangles).

Entomological surveys

Entomological surveys comprised larval and adult mosquito collections in the irrigated and non-irrigated study villages during the dry (February/March) and short-rainy (April/May) seasons of 2006.

Anopheline larvae were sampled for eight days between February and March in the dry season and also between April and May in the short-rainy season of 2006. At each survey, all available potential mosquito breeding habitats such as irrigation canals (unlined surface canals with still water due to back-flow), canal leakage pools (pools formed from leaking main canals), irrigated field paddies (water logging in the field due to over-irrigation and poor drainage canals), water-harvesting pools, mud-brick-making pits and rain pools within one kilometer radius from each study village were surveyed using standard dippers (350ml). The surface area of each potential mosquito breeding site was estimated in square meter (m^2) and sampling was made at a rate of 6 dips/m^2. One 'sample' was defined as 30 dips (or less, in smaller sites) taken over a surface area of 5 m^2. For sites in the range of 5-10 m^2, one sample was taken, whereas two samples were taken from sites in the range of 11-20 m^2 and so forth. An upper limit of six samples was set for all sites with water surface area exceeding 50 m^2 (Amerasinghe and Munasingha, 1988). Larval anophelines sampled from each type of breeding habitat were transferred to separate vials and killed by gently heating and preserved in 70% alcohol for later species identification.

Adult anophelines were sampled from indoors and outdoors for ten consecutive nights between February and March in the dry season and between April and May in the short-rainy season of 2006. Three

techniques: i.e., CDC light traps, mouthpiece aspirators and space-spray, were used for adult sampling. In order to obtain a representative sample of the mosquito population for the irrigated village, a total of 6 houses were randomly selected (2 from the edge of the village at close proximity to the irrigated field [~300 m], 2 from the middle [~600 m] and 2 from the far side of the village [~800m]). Similarly, the non-irrigated village was roughly divided into three sampling zones based on proximity to the non-irrigated agricultural field and two houses were randomly selected from each zone. Untreated bed nets were distributed for the households and the same houses were used throughout the study period. A total of six outdoor sites (~100 m away from occupied houses) was also selected in each village for outdoor light trap collection.

A total of twelve (six indoors and six outdoors) CDC light traps (Model 512; J. W. Hock Co., Atlanta, USA) was operated in each village from 1800 to 0700 hours throughout each sampling night. Each indoor light trap was hung on a wall; with the bulb about 45 cm above the head of a person sleeping under an untreated bed net (Lines *et al*., 1991). Outdoor light traps were hung on trees at close proximity (~50 to 100 m) to open cattle enclosures where some individuals spend the evening keeping their livestock from theft. To determine peak activity of anophelines during the period of the night, hourly mosquito collections were also conducted indoors and outdoors by light traps. Using mouthpiece aspirators and space-spray, female *Anopheles* mosquitoes were collected from their daytime resting sites both indoors and outdoors (Service, 1993). While collecting light traps the following morning, a team of three collectors holding aspirators and torchlight searched for resting mosquitoes from the six light trap houses and from possible outdoor-resting sites (burrow pits, ground holes, tree holes, open cattle sheds and among vegetations) in each village. For the purpose of comparison, the team of collectors spent the same period of time (20 minutes) indoors and outdoors (Abose *et al*., 1998b). Using white sheets of cloth and an aerosol of pyrethroids (Mobil flit; Mobil Africa Sales Inc., Belgium; Composition [% weight]: Tetramethrin 0.12; Phenothrin 0.12; Allethrin 0.25; Solvents, Propellants and essential oils 99.43), indoor-resting anophelines were also collected from six selected houses in each village. Before spraying the houses, all openings that could allow mosquito escaping (such as doors, windows, and holes on the walls) were closed, and the entire floor was covered with the white cloth. The houses were then sprayed with Mobil flit for about 5 minutes and left closed for 10 minutes. Thereafter, the sheets were brought outside the rooms to inspect and collect the knock-down mosquitoes. Mosquitoes collected by the different techniques were counted and kept in separate paper cups for latter identification and mosquito processing.

Species identification and dissection

At Zeway Malaria Control Laboratory, preserved anopheline larval samples were counted and individually mounted on microscope slides for species identification based on morphological characteristics (Verrone, 1962b). Only third and fourth larval instars were used for species identification of anopheline larvae. Adult anophelines collected by the different sampling methods were also sorted out into species based on morphological characteristics (Verron, 1962a). One-third of unfed female *Anopheles* mosquito collections obtained from light trap catches and all unfed female anophelines caught resting indoors and outdoors were dissected to determine parity rates for each species during the dry and short-rainy seasons. Ovaries with coiled tracheal skeins were considered as nulliparous (did not lay eggs), while those with stretched out tracheoles were taken as parous (laid eggs) as described by Lewis (1958). All the remaining female anopheline samples and the head-thorax region of dissected mosquitoes were stored in the silica-gel

dessicator and transported to Addis Ababa University, Biomedical Science Laboratory, and kept at room temperature (19-22 ^{o}C) for later mosquito processing.

Mosquito processing

The head-thorax region of each dried female anopheline was tested for the presence of *P. falciparum* and *P. vivax* sporozoite antigens using Enzyme-Linked Immunosorbent Assay (ELISA) (Wirtz *et al.*, 1987). The direct ELISA procedure described by Beier and colleagues (Beier *et al*, 1988) was used to determine the sources of blood meals (human *vs.* bovine) of the blood-engorged female anophelines.

Data analysis

Daily larval and adult mosquito collections were entered into Microsoft Excel Database and log-transformed ($\log_{10}$ [n+1]), and tested for normality before analysis. The abundance of larval and adult anophelines was compared between villages and seasons using nonparametric Mann-Whitney *U*-test. The same test was applied to compare the indoor and outdoor density of adult anophelines. The relative abundance of *Anopheles* species in the larval and adult collections was compared using Kruskal-Wallis Test, a non-parametric test for ascertaining significance among more than two variables. Larval density was expressed as the mean number of anopheline larvae per 100 dips. Sporozoite infection rate of each *Anopheles* species was expressed as the proportion of mosquitoes containing malaria sporozoite antigen from the total samples of a species tested by ELISA. The Human Blood Index (HBI) for each *Anopheles* species was calculated as the proportion of samples positive for human blood from the total blood meals of a particular species tested. The level of significance was determined at 0.05. All analyses were done using Microsoft Excel 2003 and statistical software, SPSS version 13 (SPSS Inc, Chicago, IL, USA).

3. Results

Larval habitats and abundance

Total number of positive larval habitats, number of *Anopheles* larvae collected and larval density in the irrigated and non-irrigated study villages during the two sampling seasons are presented in Table 1. Four-times more positive *Anopheles* larval sites were encountered in the irrigated village (n = 51) compared to the non-irrigated village (n = 12) during the study period. Consequently, higher *Anopheles* larval densities were found in the irrigated village (mean no. larvae per 100 dips = 36.0; 95% CI = 25.4–48.5; z = -3.196, $P < 0.001$) than the non-irrigated village (mean no. larvae per 100 dips = 14.9; 95%CI = 9.1–20.8) throughout the study period. The difference in *Anopheles* larval abundance and positive larval sites between the dry and short-rainy seasons was significant in the non-irrigated village whereas there was insignificant seasonal difference in the irrigated village. Overall, *Anopheles* larval production in the non-irrigated village was associated with the wet seasons while high larval production in the irrigated village was evident both in the dry and wet seasons.

Anopheles larval collections were composed of five species, among which *Anopheles arabiensis*, *An. pharoensis* and *An. coustani* were the major species. The distribution of *Anopheles* species in different larval habitats in the irrigated and non-irrigated villages is shown in Table 2. Among the five types of larval habitats in the irrigated village, canal leakage pools and irrigated field puddles were the most important sources of *An. arabiensis*, accounting for nearly 60% of the larval collection during the study period. For *An. pharoensis*, canal leakage pools and irrigation canals were the major larval habitats as more than 90% of larval collection of this species were obtained from these habitats. In the non-irrigated village, brick-making pits and rain pools were the most important *Anopheles* larval habitats. Overall, around 80% of the total *Anopheles*

larval production in the irrigated village was from three types larval habitats (irrigated field puddles, canal leakage pools and irrigation canals) associated with the irrigation scheme.

Adult anopheline collections

A total of 1271 adult anophelines was collected from the two study villages during the study period, of which 94% (n = 1213) and 6% (n = 58) were from the irrigated and non-irrigated villages, respectively (Table 3). *Anopheles pharoensis* was the major species predominantly sampled in the irrigated village during the dry season (56.9%; n = 340; X^2 = 52.294; df = 2; P < 0.001) while *An. arabiensis* predominated in short-rainy season (50.2%; n = 309; X^2 =17.751, df = 2, P < 0.001). Of the few adult anophelines collected in the non-irrigated village during the short-rainy season, the majority (65.5%, n = 38) were *An. arabiensis*. No mosquito was collected in the non-irrigated village during the dry season.

The density of *An. pharoensis* per light trap-night was higher during the dry season (mean no. mosquito/trap/night = 2.24; 95% CI = 1.21–3.17; Mann-Whitney U = 2422.0, z = -5.244, P < 0.001) than the short-rainy season (mean no. mosquito/trap/night = 1.48). The difference between indoor and outdoor densities was significant, being higher outdoors (Mann-Whitney U = 4646.0, z = -4.257, P < 0.001) than indoors. In contrast, the mean density of *An. arabiensis* was higher during the short-rainy season (mean no. mosquito/trap/night = 1.70; Mann-Whitney U = 1840.0, z = -2.569, P = 0.01) than the dry season (mean no. mosquito/trap/night = 1.23; 95%). There was also significant difference between the indoor and outdoor densities, being higher indoors (Mann-Whitney U = 3849.5, z = -5.849, P < 0.001) than outdoors. The density *An. coustani* was higher outdoors (Mann-Whitney U = 5772.5, z = -2.342, P = 0.001) than indoors during the two sampling seasons. Although the densities of *Anopheles* mosquitoes were generally very low in the non-irrigated village during the study period, similar indoor-outdoor trends were noted for the three species. Overall, *An. arabiensis* was more endophagic while *An. pharoensis* and *An. coustani* were more exophagic in the study area.

Peak hourly activity of Anopheles *species*

Peak indoor and outdoor activities of *An. arabiensis* were observed during the early period of the night, between 18:00-19:00 and 19:00-20:00 hours, respectively (Figure 2). Thereafter, its activity steadily decreased both indoors and outdoors throughout the rest of the night. Peak indoor and outdoor activities of *An. pharoensis* occurred between 20:00-21:00 and 19:00-20:00 hours, respectively, which gently declined thereafter, but with a remarkable increase between 22:00-23:00 hours at outdoors (figure 3). For *An. coustani*, its peak indoor and outdoor activities were recorded between 18:00-19:00 hours, which sharply dropped thereafter but with a remarkable peak between 22:00-23:00 and 05:00-06:00 hours, indoors and outdoors, respectively (Figure 4). Overall, about 75%, 66%, and 69% of the biting by *An. arabiensis*, *An. pharoensis* and *An. coustani* occurred during the early period of the night (before 22:00 hours), before the local people retire to bed.

Parous rate

Parous rate of *Anopheles* species in the irrigated and non-irrigated study villages during the dry and short-rainy seasons is presented in Table 5. In the irrigated village, the parous rate of *An. arabiensis* was higher during the dry season (58.7%, n = 46) than the short-rainy season (22.2%, n = 81). In contrast, the parous rate of *An. pharoensis* did not vary significantly between the two sampling seasons; 43.3% (n = 39) and 41.2% (21/51) in the dry and short-rainy season, respectively. For *An. coustani*, higher

parous rate was recorded during the dry season (18.2%, 4/22) than the short-rainy season (3.4%, 1/29). In the control village, among few anophelines caught during the short-rainy season, only two parous *An. arabiensis* females (5.3%; 2/38) were found. Overall, *An. arabiensis* and *An. pharoensis* had higher parous rate during the dry season, suggesting higher longevity of these species in this season.

Host feeding preference

Table 6 shows the sources of mosquito blood meals in the irrigated village. Among 120 blood-fed *An. arabiensis* specimens tested, 70.8% (n = 85) and 14.2% (n = 17) were positive for only human and bovine bloods, respectively. Some (7.5%, n = 9) were mixed blood meals originated from human and bovine, and the remaining were unidentified (7.5%, n = 9) - possibly originated from other domestic hosts (e.g. equines and ovines). Overall, the Human Blood Index (HBI) for *An. arabiensis* was found to be 0.78. Out of 142 blood-engorged female *An. pharoensis* specimens tested, 61.3% (n = 87) and 20.4% (n = 29) had human and bovine blood meals, respectively. Some blood meals (7.7%, n = 11) were composed of both human and bovine bloods, and 10.6% (n = 15) of *An. pharoensis* blood meals were not identified. Overall, the HBI for *An. pharoensis* was found to be 0.69. From a total of 16 blood-engorged *An. coustani* specimens, only one specimen (6.2%) was positive for human blood while the majority (75%, n = 12) gave positive result for bovine blood. The overall HBI for *An. coustani* was 0.06. The ELISA results showed that *An. arabiensis* and *An. pharoensis* are the most important anthropophagic species in Zeway area.

Sporozoite rate

The *P. falciparum* sporozoite rates of *Anopheles* species in the irrigated village is presented in Table 7. None of the samples tested were positive for *P. vivax* sporozoites. Among 424 female *An. arabiensis* specimens collected from the irrigated village and tested for *P. falciparum* sporozoites, 5 (1.18%) were found to be positive. None of the thirty-one *An. arabiensis* specimens caught in the non-irrigated village were positive for *P. falciparum*. Among the total of 509 *An. pharoensis* collected from the irrigated village, three (0.59%) were tested positive for *P. falciparum* sporozoites. None of the four *An. pharoensis* and sixteen *An. coustani* specimens collected in the non-irrigated village was positive for malaria sporozoites. Seasonally, higher *P. falciparum* sporozoite rate of *An. arabiensis* was recorded in the short-rainy season (1.47%; 4/272) than the dry season (0.66%; 1/152). The *P. falciparum* sporozoite rate of *An. pharoensis* was 0.92% (3/325) in dry season, while none (0/184) were positive in the short-rainy season. Overall, the *P. falciparum* sporozoite rate of *An. arabiensis* and *An. pharoensis* suggests the potential of these species in malaria transmission in the irrigated study village during the dry and the short-rainy seasons.

Table 1. Total number of positive larval habitats, number of *Anopheles* larvae collected and larval density (mean no. larvae/100 dips) in irrigated (Abene-Girmamo) and non-irrigated (Woshgulla) villages in Zeway area, Central Ethiopia, during the dry (February/March) and short-rainy (April/May) seasons of 2006.

	Irrigated village			Non-irrigated village	
Season	Total no. positive larval habitats	No. of *Anopheles* larvae collected (%)	Larval density[δ] (95%CI)	Total no. positive larval habitats	No. of *Anopheles* larvae collected (%)	Larval density (95%CI)
Dry	38	797 (46.0)	38.3 (26.2–50.5)*	5	69 (22.8)	7.4 (4.4 –10.5)
Short-rainy	33	936 (54.0)	34.9 (24..9–45.9)*	11	233 (77.2)	15.2 (9.3–21.1)
Overall	51[a]	1733 (100)	36.0 (25.4–48.5)*	12[a]	302 (100)	14.9 (9.1– 20.8)

[δ] Larval density refers to mean number of *Anopheles* larvae per 100 dips. 95% confidence interval is shown in brackets. * The difference in seasonal larval density between the two villages was significant ($P < 0.001$).

[a] The overall total number of larval habitats in each village is not equal to the sum of positive larval habitats in the two sampling seasons since the same larval habitat can occur in both seasons.

Table 2. Distribution of *Anopheles* species* in different types of larval habitats in irrigated (Abene-Girmamo) and non-irrigated (Woshgulla) villages in Zeway area, Central Ethiopia, between February and May 2006.

* Only third and fourth larval instars were sorted out into species

Village	Larval habitats	*An. arabiensis* (%)	*An. pharoensis* (%)	*An. coustani* (%)	*An. cinere*
An. squamosus	Total (%) (Total no. of positive sites)				
Irrigated	Brick-making pits (5)	70 (18.5)	2 (0.4)	72 (21.5)	0
0	144 (12.3)				
	Canal leakage pools (12)	108 (28.5)	242 (52.8)	57 (17.1)	0
0	407 (34.6)				
	Irrigated field puddles (23)	118 (31.1)	41 (9.0)	66 (19.8)	0
0	225 (19.1)				
	Irrigation canals (4)	45 (11.9)	173 (37.8)	85 (25.4)	0
1	304 (25.9)				
	Rain pools (7)	38 (10.0)	0 (0.0)	54 (16.2)	3
0	95 (8.1)				
	Total (51) (%)	379 (32.2)	458 (39.0)	334 (28.4)	3
(0.3)	1 (0.1) 1175 (100)				
Non-irrigated	Brick-making pits (7)	57 (52.8)	8 (88.9)	14 (45.2)	-
-	79 (53.4)				
	Rain pools (4)	51 (47.2)	0 (0.0)	17 (54.8)	-
-	68 (45.9)				
	Water harvesting pools (1)	0 (0.0)	1 (1.1)	0 (0.0)	-
-	1 (0.7)				
	Total (12) (%)	108 (73.0)	9 (6.1)	31 (20.9)	-
-	148 (100)				

Table 3. Number of adult *Anopheles* mosquitoes collected from irrigated (Abene-Girmamo) and non-irrigated (Woshgulla) villages in Zeway area, using different sampling methods, during the dry (February/March) and short-rainy (May/April) seasons of 2006.

Village	Season	*An. arabiensis*	*An. pharoensis*	*An. coustani*	Total (%)
Irrigated	Dry (%)	182 (30.4)	340 (56.9)	76 (12.7)	598 (49.3)
	Short-rainy (%)	309 (50.2)	212 (34.5)	94 (15.3)	615 (50.7)
	Total (%)	491 (40.5)	552 (45.5)	170 (14.0)	1213 (100)
Non-irrigated	Dry (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Short-rainy (%)	38 (65.5)	4 (6.9)	16 (27.6)	58 (100)
	Total (%)	38 (65.5)	4 (6.9)	16 (27.6)	58 (100)
Grand Total (%)		529 (41.6)	556 (43.8)	186 (14.6	1271 (100)

Table 4. Indoor and outdoor density of *Anopheles* species (mean no. mosquitoes /light trap/ night) in irrigated (Abene-Girmamo) and non-irrigated (Woshgulla) villages in Zeway area, during the dry (February/March) and short-rainy (April/May) seasons of 2006.

Village	Species	Mean no. mosquito/ light trap/ night				
		Dry season			Short-rainy season	
		In	Out	Mean	In	Out
Mean						
Irrigated	*An. arabiensis*	1.53	0.93	1.23	2.14	1.22
1.70						
	An. pharoensis	1.74	2.72	2.24	1.31	1.65
1.48						
	An. coustani	0.50	0.73	0.62	0.57	1.00
0.79						
	Any anopheline	3.77	4.38	4.09	4.02	3.87
3.97						
Non-irrigated	*An. arabiensis*	0.00	0.00	0.00	0.63	0.21
0.32						
	An. pharoensis	0.00	0.00	0.00	0.07	0.00
0.03						
	An. coustani	0.00	0.00	0.00	0.05	0.23
0.13						
	Any anopheline	0.00	0.00	0.00	0.75	0.44
0.59						

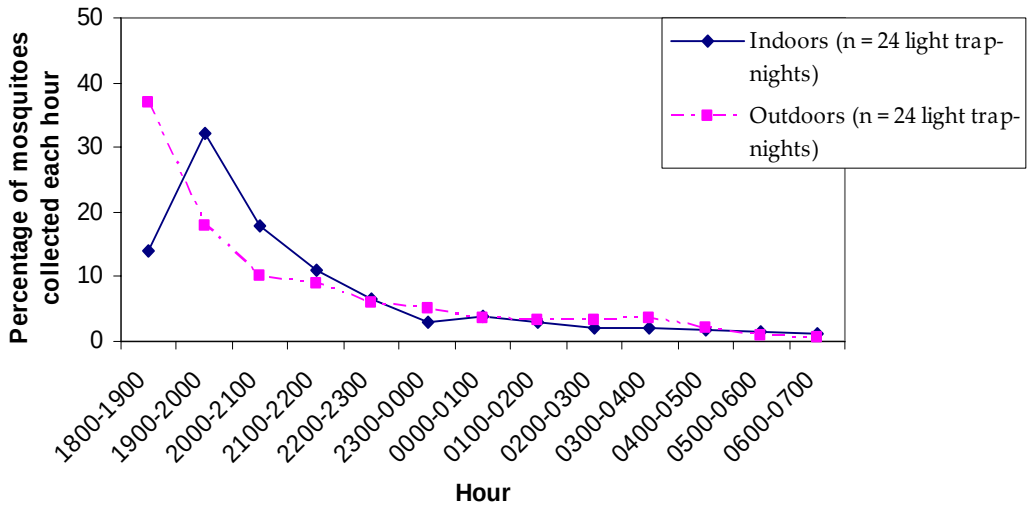

Figure 2. Hourly activity of *Anopheles arabiensis* indoors and outdoors from light trap catches (as percentage of mosquitoes collected each hour) in an irrigated village in Zeway area, during the study period (February to May 2006).

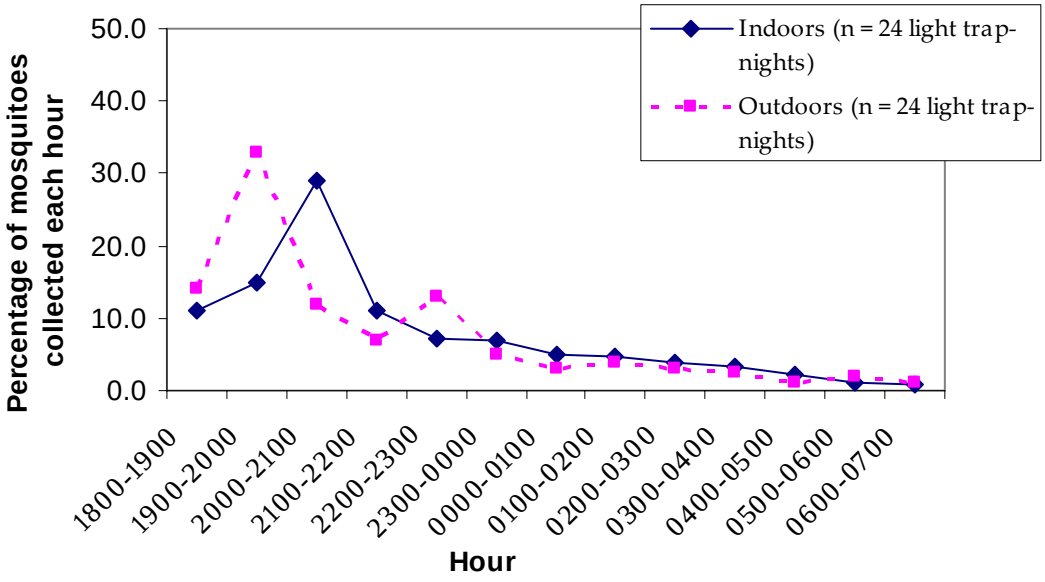

Figure 3. Hourly activity of *Anopheles pharoensis* indoors and outdoors from light trap catches (as percentage of mosquitoes collected each hour) in an irrigated village in Zeway area, during the study period (February to May 2006).

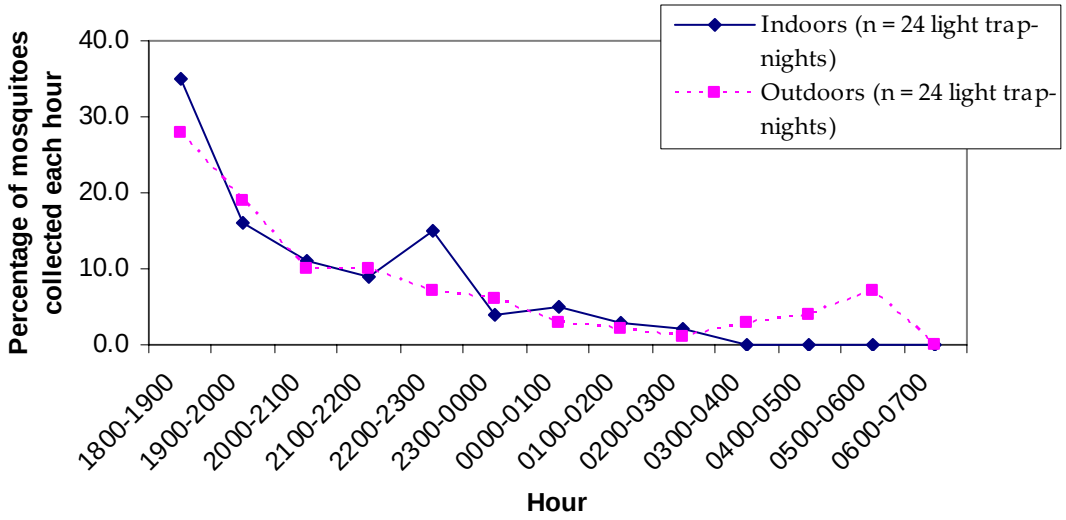

Figure 4. Hourly activity of *Anopheles coustani* indoors and outdoors from light trap catches (as percentage of mosquitoes collected each hour) in an irrigated village in Zeway area, during the study period (February to May 2006).

Table 5. Parous rate of *Anopheles* species in the irrigated (Abene-Girmamo) and non-irrigated (Woshgulla) villages in Zeway area, Central Ethiopia, in the dry (February/March) and short-rainy (April/May) seasons of 2006.

Village	Season	*An. arabiensis*		*An. pharoensis*		*An. coustani*	
		N[1]	P (%)*	N	P (%)	N	P (%)
Irrigated	Dry	46	27 (58.7)	90	39 (43.3)	22	4 (18.2)
	Short-rainy	81	18 (22.2)	51	21 (41.2)	29	1 (3.4)
Non-irrigated	Dry	-[#]	-	-	-	-	-
	Short-rainy	38	2 (5.3)	4	0 (0.0)	16	0 (0.0)

[1] N = number of specimens dissected

* P is the number of parous females, and the percentage of P is calculated from N.

[#] - (minus sign) indicates that no mosquito was collected during that season and thus no dissection.

Table 6. Number (and percentage) of blood-fed *Anopheles* mosquitoes tested positive for human and/or bovine bloods by direct ELISA, from collections obtained from an irrigated village in Zeway area, Central Ethiopia, during the study period (February to May 2006).

Species	No. of mosquito tested	Human only (%)	Bovine only (%)	Mixed blood* (%	Unidentified (%)
An. arabiensis	120	85 (70.8)	17 (14.2)	9 (7.5)	9 (7.5)
An. pharoensis	142	87 (61.3)	29 (20.4)	11 (7.7)	15 (10.6)
An. coustani	16	1 (6.2)	12 (75.0)	0 (0.0)	3 (18.8)

* Mixed blood meal refers to a blood meal containing both human and bovine bloods.

Table 7. *Plasmodium falciparum* sporozoite rate of *Anopheles* species collected from an irrigated village (Abene-Girmamo) in Zeway area, Central Ethiopia, during the dry (February/March) and short-rainy (April/May) seasons of 2006.

Season	*An. arabiensis*		*An. pharoensis*		*An. coustani*	
	N	SR (%)	N	SR (%)	N	SR (%)
Dry	152	1 (0.66)	325	3 (0.92)	61	0 (0.00)
Short-rainy	272	4 (1.47)	184	0 (0.00)	70	0 (0.00)
Overall	424	5 (1.18)	509	3 (0.59)	131	

N – number of mosquitoes tested by ELISA.
SR – number of mosquitoes tested positive for *P. falciparum* sporozoites.

Note: none of the mosquitoes collected from the non-irrigated village were positive for malaria sporozoites.

4. Discussion

The present study revealed that the small-scale irrigation scheme in Zeway area has created breeding sites for the two malaria vector species, namely, *An. arabiensis* and *An. pharoensis*. The most important prolific *Anopheles* larval habitats were found to be poorly constructed irrigation canals (that allow water to stand for a period of time), canal leakage pools (formed due to perforated soil walls of the irrigation canals) and waterlogged fields (field puddles formed due to over-irrigation). The same breeding habitats have been shown to create conducive breeding grounds for *An. arabiensis* in the dam villages of Tigray, where microdam-based irrigation is practiced during the dry season (Yohannes *et al*., 2005). In agreement to our findings, in Mwea irrigation scheme, Kenya, it has been reported that *An. arabiensis*, *An. pharoensis* and *An. coustani* thrive well in irrigated fields where rice was commonly grown (Ijumba *et al*., 1990; Muturi *et al*., 2006). In irrigation schemes of Faiyum Governorate, Egypt, irrigation ditches, seepage water collections and irrigated fields with moderate crop growth were shown to be the major sources of *An. pharoensis* during the dry season (Soliman *et al*., 1967), in line with our finding for the same species in the present study.

We also observed that larvae of *An. arabiensis* were predominantly abundant in newly formed canal leakage pools and field puddles, while larval *An. pharoensis* preferred canal leakage pools and irrigation canals covered with vegetations. Even in the same larval habitats where the two species coexisted (such as canal leakage pools), *An*opheles *arabiensis* mostly preferred the shallow, sunlit and disturbed (muddy) margins of the habitat while *An. pharoensis* was frequently sampled around the shaded and deeper parts of the habitat with encroaching vegetation. This indicated that *An. arabiensis* and *An. pharoensis* have different larval habitat requirements. Previous studies have shown that *An. arabiensis* prefers open, shallow and temporary breeding habitats while *An. pharoensis* thrives in shaded, permanent water bodies with emergent vegetation (Snow, 1983; Gillies and Coetzee, 1987).

Rains are known to have dual effect on the development of mosquito larvae. When it rains, new mosquito-breeding sites are created; at the same time at other previously existing sites, some individuals will be washed away. We observed that newly formed breeding sites were sooner colonized by *An. arabiensis* and *An. coustani* (as these species prefer such habitats) while older permanent larval habitats of *An. pharoensis* diminished. Similar observations were reported in Mwea irrigation scheme in Kenya, where larval *An. arabiensis* were found abundantly in newly flooded rice fields in the wet season but a few weeks later, when the rice moderately grew, *An. pharoensis* was the one predominated (Mukiama and Mwangi, 1989).

This study generally confirmed that the irrigation scheme in Zeway area has created good *Anopheles* mosquito breeding conditions by restoring the lost surface water during the dry season. Thus, *Anopheles* larval production in the irrigated villages of Zeway area is no longer restricted to the wet seasons; rather continuous breeding of *Anopheles* mosquitoes throughout most of the year is possible as the crucial linkage between the rainy seasons is provided by the irrigation activities. Therefore, there is a potential for dry season malaria transmission in the irrigated villages of Zeway area, as malaria vector mosquitoes (*An. arabiensis* and *An. pharoensis*) thrive well in breeding sites created by the irrigation scheme coupled with the prevailing climatic factors that could facilitate development of the aquatic stages of the vector as well as the malaria parasites inside the female anopheline.

Consistent with the observed seasonal trend in larval abundance, variations in seasonal adult densities were also evident during the study period. The density of adult *An. arabiensis* was higher in the short-rainy season than the dry season while the densities of *An. pharoensis* and *An. coustani* peaked in the dry season. Similar seasonal trend was observed in villages at close proximity to Lake Zeway, where *An. arabiensis* outnumbered *An. pharoensis* during the wet season while the latter dominated the former in the dry season. These species are common in irrigated villages elsewhere in Africa where they occur sympatrically (Snow, 1983; Mukiama and Mwangi, 1989; Ijumba *et al*., 1990; Muturi *et al*., 2006).

Indoor and outdoor light trap catches revealed that *An. arabiensis* was more endophagic while *An. pharoensis* and *An. coustani* showed a more exophagic behavior. We observed that the local people in the study area spend the early part of the night (on average up to 10 pm) outdoors either working on their field or taking care of their cattle. Such night time behavior of the local people might increase the chance of receiving more bites by the inherently exophagic populations of *An. arabiensis* and *An. pharoensis* in the study area. Similar suggestion for *An. arabiensis* was previously made by Ameneshewa (1995) who worked in Gergedi (Awash valley, about 80 Km from Zeway) reported that the biting behavior of this species depends strongly on the availability of host either indoors or outdoors during the period of its biting activity in the evening. *An. pharoensis* and *An. coustani* are well known exophagic species in Ethiopia (Nigatu *et al*., 1994; Adugna and Petros, 1997; Abose *et al*., 1998b; Taye *et al*., 2006) and elsewhere in Africa, such as Kenya (Ijumba *et al*., 1990; Mukiama and Mwangi, 1989), Sudan (El Gaddal et al., 1985) and Cameroon (Antonio-Nkondjio *et al*., 2006).

We found that peak indoor and outdoor activities of *An. arabiensis*, *An. pharoensis* and *An. coustani* occurred during the early period of the night (before 22:00 hours), coinciding with the night time behavior of the local people in the study area. Similar early biting behavior was previously reported for *An. arabiensis* and *An. pharoensis* in Zeway (Abose *et al*., 1998b), and *An. arabiensis* in Tigray (Yohannes *et al*., 2005). In Sille, an irrigated village in southern Ethiopia, Taye *et al*., (2006) reported that peak biting activities of *An. pharoensis* and *An. coustani* occurred between 18:00 and 20:00 hours, which is in agreement with the present findings for the two species. In contrast to the observed early biting periodicity of *An. arabiensis* in the present study area, these authors reported a peak biting activity between 23:00 and 3:00 hours for the same species in Sille. Interestingly, 40 years ago, in Zeway area most *An. gambiae* s.l. (presumably *An. arabiensis*) fed readily after 23:00 hours and little early evening biting activity was recorded (Rishikesh, 1966), suggesting that the early biting behavior of this species has evolved since then. The early biting activity of *An. arabiensis* is likely to be a consequence of long-term application of residual insecticides, particularly DDT, selecting for early biting behavior as it has also been suggested recently in Tigray (Yohannes *et al*., 2005). Moreover, such early biting activity of the malaria vector populations in the current study area is likely to compromise the efficacy of insecticide-treated bed nets as large proportion of bites occurred before the local people, including children, go to sleep under their bed nets.

In the present study, *An. arabiensis* had a higher parous rate during the dry season than the short-rainy season while *An. pharoensis* showed insignificant variation in its parous rate during the two seasons. This report is inconsistent with a previous finding in Upper Awash that recorded a

higher parous rate for *An. arabiensis* during the wet season than the dry season (Ameneshewa, 1995). The explanation for this discrepancy is that following the unusual heavy rains in April, high recruitment of young ones into the existing older population might have resulted in a higher proportion of nulliparous females and hence lower parous rate. The parity rate of *An. arabiensis* and *An. pharoensis* thus suggested higher longevity during the dry season, hence likely to maintain malaria transmission during this season of the year.

The reported Human Blood Index (HBI) for *An. arabiensis* (0.78) and *An. pharoensis* (0.69) in the present study reaffirmed the importance of these species in malaria transmission in Zeway area. Yohannes *et al* (2005) reported an HBI of 0.72 for indoor-resting *An. arabiensis* in Tigray, northern Ethiopia, which is a comparable finding for the same species in the present study. An HBI of 0.66 was reported for *An. arabiensis* in Konso, southern Ethiopia, (Tirados *et al*., 2006), which is lower than the present finding, as the species population in Konso was reported to be exclusively exophagic. Adugna and Petros (1996) reported higher HBI for *An. pharoensis* (0.84) and *An. coustani* (0.26) from samples collected in mixed dwellings. In our study, *An. coustani* had shown an exceptionally high preference of *An. coustani* (75%) for bovine blood – hence less likely to play significant role in malaria transmission. Hence, the present study confirmed that *An. arabiensis* and *An. pharoensis* are the two most important anthropophagic species in Zeway area, which is in agreement with previous reports from the same area (Rishikesh, 1966; Abose *et al*., 1998b).

The *P. falciparum* sporozoite rate of 1.18% for *An. arabiensis* in the present study is comparable to the 1.1% sporozoite rate reported from Arbaminch (Habtewold *et al*., 2001) and Sille (Taye *et al*., 2006; Tirados *et al*., 2006), but lower than a 1.52% sporozoite rate in the adjacent, Wonji area (Ameneshewa, 1995). A 0.88% *P. falciparum* sporozoite rate of *An. pharoensis* in the dry season confirms the vectorial role of this species in malaria transmission in the irrigated villages of Zeway area particularly during the dry season. On the other hand, *Anopheles arabiensis* was found infected with *P. falciparum* sporozoites both in the dry and short-rainy seasons, suggesting that this species play significant role in malaria transmission both in the dry and wet seasons of the year. These findings could be the first report for the dry season and also for *An. pharoensis*. Hence, the role of *An. pharoensis* in transmitting *P. falciparum* should not be underestimated in areas where this species is abundant.

The major short-coming of the current study was that larval and adult collections were made merely on seasonal basis, only focusing on the dry and short-rainy seasons, due to which monthly variations at different periods of the year were not shown. Hence, further longitudinal studies in the same area are required to ascertain the present findings.

In conclusion, although development of irrigation schemes is of paramount importance to increase crop yield and hence to ensure food security and economic growth in Ethiopia, its adverse health problems may pose significant public health concerns. The findings of the present study underscore the importance of irrigation schemes in semi-arid areas like Zeway in maintaining malaria transmission particularly during the dry season, when mosquito abundance is normally presumed to be limited. Proper water management and control measures such as source reduction through environmental management could help to reduce mosquito-breeding sites and thus malaria transmission.

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