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

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ABSTRACT

Background & objectives: Larval control is an integral part of malaria vector management in Ethiopia and elsewhere. For effective larval control, a sound understanding of the factors responsible for spatio-temporal variation in larval production is essential. A study was thus conducted to characterize larval habitats of anopheline mosquitoes in irrigation and major drainage areas between Adami Tulu and Meki towns, in the middle course of the Ethiopian Rift Valley.

Methods: Aquatic habitats were sampled for anopheline larvae and the associated environmental variables (water temperature, turbidity, water current, water pH and other variables) were measured, characterized and analyzed.

Results: Microscopic identification of the late instars (III and IV) of anopheline larvae collected throughout the study period yielded nearly 47.6% *Anopheles pharoensis,* 32.1% *An. arabiensis,* 17.1% *An. squamosus* and only 3.2% of other species (*An. coustani* and *An. cinereus*). Larvae of the local malaria vectors, *An. arabiensis* and *An. pharoensis* were most abundantly sampled from sand pools and natural swamps, respectively. Logistic regression analysis detected four best predictor variables associated with larval abundance of malaria vector species. Thus, relative abundance of *An. arabiensis* larvae was significantly and inversely associated with aquatic vegetation and water current, whereas the relative abundance of *An. pharoensis* larvae was significantly and positively associated with water temperature and the presence of algae in the water bodies.

Conclusion: Dry season anopheline larval habitats such as riverine sand pools that are created and maintained by perennial water bodies and their associated water development projects need to be considered in vector control operations.

Key words Anophelines; Ethiopia; irrigation and drainage; larval habitats

INTRODUCTION

Human malaria is transmitted by *Anopheles* mosquitoes originating from specific breeding habitats because transmission normally occurs within a certain radius (within flight range of adult vectors) from breeding habitats[1]. The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life cycle processes occur such as oviposition, larval development, and emergence take place[2]. Larval control will have to become an integral part of integrated vector management programmes if malaria elimination is the goal. Thus, the importance of larval interventions has recently regained attention in malaria control[3]. Factors that have stimulated renewed interest include opportunities to complement adulticiding with other components of integrated vector management, concerns about insecticide resistance and resurgence, the rising cost of insecticides and logistic constraints[3].

To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species[4]. Knowledge of the ecological characteristics of the breeding habitats and the environmental factors affecting mosquito abundance can help in designing optimal vector control strategies[2,5].

Although malaria is a major public health problem and anopheline larval control is an important component of malaria control programme in Ethiopia, usually by source reduction through management of larval habitats integrated with adult vector control[6–8], little is known about the larval ecology of *Anopheles* mosquitoes in the country. For example, the microhabitat factors that influence the occurrence and abundance of *Anopheles* larvae are not well-characterized even for the malaria vector species. The description of larval habitats have usually been given in more general terms such as marshes, rain pools, man-made pools and the like. Although somewhat informative, these habitat categories are not specific enough to define local environmental factors associated with specific anopheline species for planning and implementing appropriate larval control strategies. This study was undertaken to characterize the larval habitats of anopheline mosquitoes in irrigation and major drainage areas in the central Ethiopian Rift Valley and determine the association of some environmental factors of the larval habitats with the occurrence and abundance of mosquito larvae.

MATERIAL & METHODS

The study area

The study was conducted in irrigation and major drainage areas located in the middle course of the Rift Valley in Ethiopia between Meki and Adami Tulu towns. The area lies approximately between latitudes 07°51' and 8°90' N and longitudes 38°42' and 38°49' E at about 1700 m above the sea level. It covers about 1435 km^2 and is situated at about 150 to 170 km^2 south of Addis Ababa on the main road to southern Ethiopia. The total population of the study area was approximately 89,042 individuals in 2008. It was also overpopulated by local and migrant laborers from different parts of the country being attracted by job opportunities created by vegetable farms and other economic activities in the area. *Anopheles arabiensis* and *An. pharoensis* are the two malaria vectors in this epidemic prone area, the former being the major vector[6,9,10].

The area is semi-arid and relatively flat, with the natural vegetation mainly of scattered *Acacia* trees and thorn bushes. It has a fairly warm climate with mean annual temperature of 20.7°C and annual minimum and maximum temperatures of 14.2 and 27.2°C, respectively. The annual rainfall and the mean annual relative humidity is about 740 mm and 60%, respectively. The area is subjected to intensive grazing and agriculture where maize and other cereal crops are cultivated during the main rainy season (June–September) and the short rains (March–May). Vegetables (onion, tomato, potato, green pepper and cabbage) are mainly grown by irrigation during the dry season (October–February) including the short rains. The major drainages that are used for irrigation-based agriculture and other economic activities include Lake Ziway, Meki and Bulbula rivers. The present study covered irrigation and major drainage areas in three selected rural towns and three rural farming villages. The towns were Adami Tulu, Ziway and Meki whereas the farming villages included Gerbi, Abine-Germama and Edo-Gojola. These study localities were selected purposely, based on their proximity to the major drainage areas, presence of large and small irrigation farms, as well as past and recent reports of local malaria situation and its transmission, where *An. arabiensis* is the principal vector[6,9,10], and is the only member of the *An. gambiae* complex found in the area[6]. Thus, all anopheline positive habitats present within a 500 m radius of each irrigated village/town and 700 m along the major drainages (lake or river) which are located adjacent to the towns and villages were sampled to study larval habitats that are found closer to houses.

Larval sampling and processing

Mosquito larvae were sampled fortnightly from December 2007 up to early June 2008 which covered the dry season (December–February) and short rainy season (March–May). We assumed that mosquito breeding habitats associated with the drainages and irrigation systems are more accessible and suitable for larval survey and control during these periods of the year. During each survey, a habitat was first inspected for the presence of mosquito larvae visually, then by dipping using a standard dipper (11.5 cm diam and 350 ml capacity), pipettes, and white plastic pans[11,12]. When mosquito larvae were present, 10–30 dips were taken depending on the size of each larval habitat at intervals along the edge. Samplings were always done by the same individual in the morning (0900–1200 hrs) or afternoon (1400–1700 hrs) for about 30 min or less at each larval habitat. All III and IV instar anopheline larvae collected were preserved in 70% alcohol. In the laboratory, each larva was individually mounted in gum-chloral on a microscope slide and identified to species by morphological criteria[13, 14].

Larval habitat characterization and recording of environmental variables

Simultaneously with larval sampling, the environmental characteristics of each larval habitat were measured or estimated and recorded. The environmental variables recorded were water temperature, water pH, water depth, elevation, intensity of light, turbidity, vegetation type, water current, substrate type, distance to the nearest house, whether the habitat was natural or human made, the presence of algae and permanence of the habitat. Water temperature was measured using LCD portable Digital Multistem Thermometer (ST-9269 A/B/C-Model, USA), whereas, water pH was measured using pH indicator (Viac. Imbonati 2420159 Milano, Italy). Water depth was measured using a metal ruler at different points of each habitat and average depth was recorded. Water current was determined by visual inspection and categorized as slow flowing or still. Turbidity was estimated by taking water samples in glass test tubes and holding them against a white background to categorize them as either clear or turbid[15]. Intensity of light was visually categorized as light and shade. The type and presence of aquatic vegetation was observed and recorded as emergent, floating, emergent plus floating and none if no vegetation at all. Emergent plants included both aquatic and immersed terrestrial vegetation. The presence or absence of mats of algae (green algae) was visually determined. Distance to the nearest house was measured with a tape when it was shorter than 100 m and by foot steps when it exceeded 100 m. These were then categorized into 3 classes (e.g. 1 = 0–100 m, 2 = 100–300 m, and 3 for distances >300 m)[15].

Data analysis

Data analysis was done using SPSS software (version 13.0 for windows). Variations in larval counts (mean densities) among habitat types, among environmental factors (characteristics) of the larval habitats were analyzed using mean comparison and one way analysis of variance (ANOVA). Larval density was expressed as number of larvae per 100 dips since number of larvae sampled for some anopheline species was low and different numbers of dips (10–30) were taken based on the size of the habitats. When significant differences were observed in ANOVA, the Tukey test was used to separate the means. Pearson correlation analysis was used to determine the association among the environmental variables and also to assess the relationship between anopheline larval densities and environmental factors of the larval habitats, i.e. for each environmental variable, simple correlation between larval abundance and individual parameters were first checked and only significant associations were further examined by step-up multiple logistic regressions to determine the best predictor variables associated with relative abundance of the larval species of anophelines.

RESULTS

Species composition and seasonal abundance of anopheline larvae

In total, 2134 late instars (III and IV) *Anopheles* larvae were collected and examined microscopically for species identification that yielded five *Anopheles* species among which *An. pharoensis* (47.6%), *An. arabiensis* (32.1%) and *An. squamosus* (17.1%) were the major species, whereas *An. coustani* (2.9%) and *An. cinereus* (0.3%) were generally scarce (Table 1). Larvae were observed during every month of the study period. Marked monthly variations were observed in densities of the anopheline larval populations with their minimum mean density in December and maximum mean density in March. In Meki River crossing Meiki town, larval densities of the major malaria vectors (*An. arabiensis),* gradually rose up during the short rainy season with its peak in March, which declined afterwards towards the beginning of the main rainy season in June (Figs. 1a & 2). In the other five localities which are almost adjacent to Lake Ziway, densities of *An. arabiensis* larvae increased towards the end of the short rainy season (May) and beginning of the main rainy season (June). On the other hand, peak mean larval densities of *An. pharoensis* and *An. squamosus* were during the initial larval survey of the dry season in all the localities (December–February) which gradually decreased during the short rainy season (Figs. 1b, c & 2).

Habitat diversity and larval abundance

Table 2 shows the spatial distribution of the anopheline larvae in different aquatic habitats in the study localities. *Anopheles arabiensis* and *An. pharoensis* larvae were the predominant species occurring in a wide range of habitats. *Anopheles arabiensis* larvae were collected most abundantly from sand mining pools (58.5%), whereas *An. pharoensis* and *An. squamosus* larvae were mostly coexisting in swamps (44.8 and 49.6%, respectively) followed by irrigation canals (34.1 and 35.6%, respectively). Larvae of *An. coustani* and *An. cinereus*, also occurred in swamps and irrigation canals co-existing with the former two species, but were scarce and generally absent from other habitat types. Expressed as number of larvae per number of sampling dips, the relative abundance of anopheline species in the different larval habitats was significantly variable. Thus, based on comparison of mean

Table 1. Number of late instar (III and IV) anopheline mosquito larvae collected from irrigation and major drainage areas in the six localities between Adami Tulu and Meki towns (December 2007–June 2008)

Larvae	Adami Tulu	Gerbi	Ziway	Abine Germama	Edo Gojola	Meki	Total	%
An. arabiensis	39	31	130	30	55	401	686	32.1
An. pharoensis	126	117	336	199	187	50	1015	47.6
An. squamosus	59	56	118	68	62	2	365	17.1
An. coustani	3	2	48	4	0	4	61	2.9
An. cinereus	0	0	0	7	0	0	7	0.3
Total	227	206	632	308	304	457	2134	100

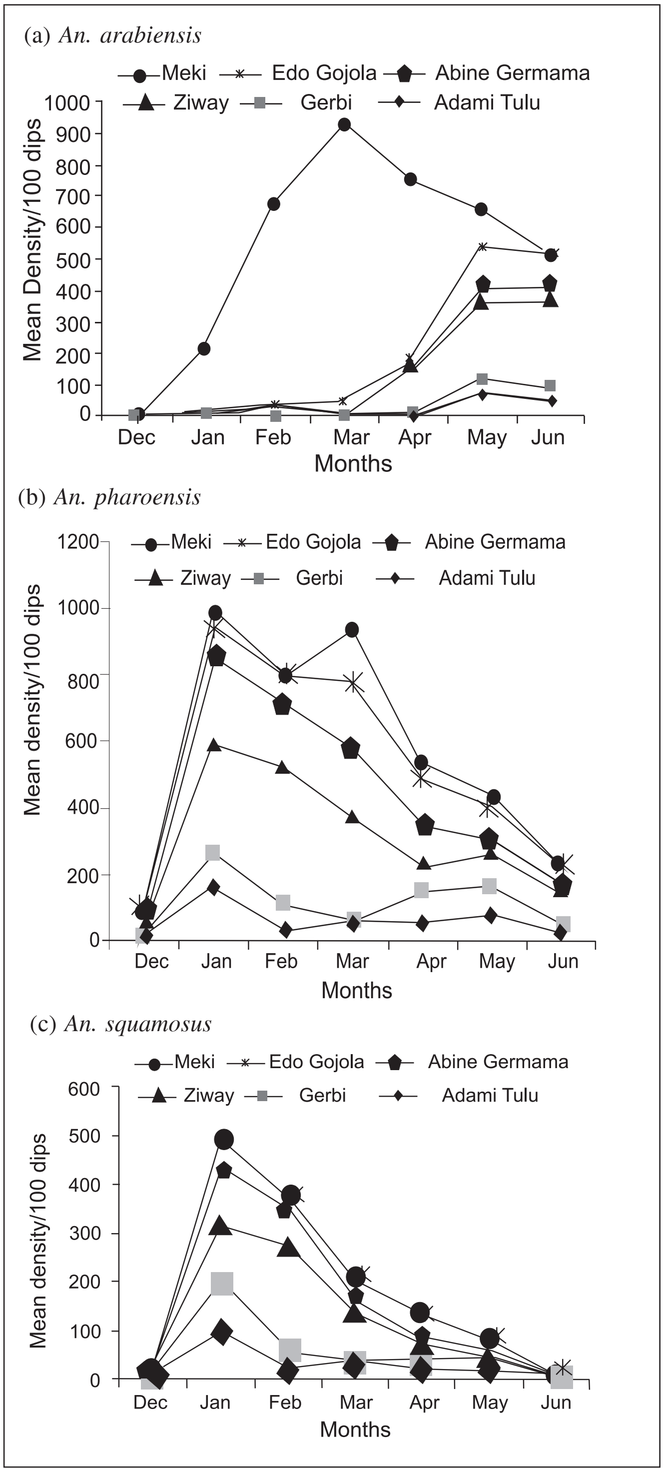

Fig. 1a,b & c: Monthly anopheline larval densities at six local sites during the study period.

densities of larvae, it was revealed that *An. pharoensis* (F = 3.212, df = 6257, p <0.05) and *An. arabiensis* (F = 13.370, df = 6257, p <0.05) were the most abundant larvae in swamps and sand pools, respectively. *Anopheles squamosus* (F=3.744, df = 6256, p <0.05) also colonized more swamps than the other habitat types.

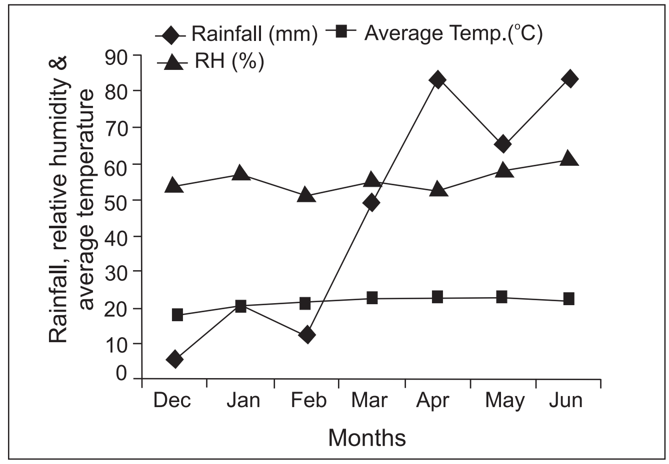

Fig. 2: Monthly rainfall, relative humidity and average temperature of the study area during the study period.

Environmental factors associated with larval occurrence/ abundance

Mean comparison and one-way analysis of variance (ANOVA) revealed the characteristics of larval habitats and mean densities of the anopheline larvae (Table 3). Significantly higher mean densities of *An. arabiensis* larvae were obtained from aquatic habitats that had clear and standing water, free of vegetation and temporary habitats near to human dwellings (<100 m). Higher mean densities of *An. pharoensis* larvae were also significantly collected from permanent and natural habitats that had clear and standing water with mats of algae. Likewise, significantly higher densities of *An. squamosus* larvae were collected from aquatic habitats which are clear, permanent, still, having emergent and floating vegetation as well as mats of algae which are further away from human dwellings (100–300 m).

Correlation analysis of each environmental variable with abundance of anopheline larvae revealed six variables to be significantly correlated with density of anopheline larvae (Table 4). *Anopheles arabiensis* larvae were significantly correlated with elevation, aquatic vegetation, habitat permanence, water current and distance to the nearest house. Eight of the 12 environmental variables (66.7%) analyzed were significantly correlated with the abundance of *An. pharoensis* larvae. *Anopheles squamosus* larval density was also significantly correlated with half of the environmental variables examined. From the continuous variables examined mean temperature was variable among different larval habitats (F = 3.215, df = 6258, p <0.05) and ranged from 20.4 to 30.5°C. Larval density was positively correlated with water temperature (r = 0.196, p <0.01).

Further multiple step-up regression analysis detected

Table 2. Distribution of *Anopheles* species in different types of larval habitats in irrigation and drainage areas between Adami Tulu and Meki towns (December 2007–June 2008)

Larval habitat	*An. arabiensis*	*An. pharoensis*	*An. squamosus*	*An. coustani*	*An. cinereus*	Total
Swamps	133 (19.4)	456 (44.8)	181 (49.6)	29 (47.5)	7 (100)	806 (37.7)
Irrigation canals	44 (6.4)	347 (34.1)	130 (35.6)	30 (49.2)	0	551 (25.8)
Sand pools	401 (58.5)	50 (4.9)	2 (0.5)	0	0	453 (21.2)
CLP	10 (1.5)	135 (13.3)	52 (14.2)	2 (3.3)	0	199 (9.3)
WHP	17 (2.5)	14 (1.4)	0	0	0	31 (1.5)
BMP	57 (8.3)	5 (0.5)	0	0	0	62 (2.9)
Rain pools	24 (3.5)	10 (1.0)	0	0	0	34 (1.6)
Total	686 (32.1)	1017 (47.6)	365 (17.1)	61 (2.9)	7 (0.3)	2136 (100)

CLP = Canal leakage pools; WHP = Water harvesting pools; BMP = Brick making pits; Figures in parentheses indicate percentages.

Table 3. Characteristics of larval habitats and mean densities of the three common species of anopheline larvae

Characteristics	Variables	*An. arabiensis*			*An. pharoensis*			*An. squamosus*		
		Mean ± SE	F	*p*	Mean ± SE	F	*p*	Mean ± SE	F	*p*
Intensity of light	Light	15.5 ± 2.9	0.001	0.971	16.7 ± 1.8	0.012	0.914	5.4 ± 0.8	2.455	0.119
	Shade	15.0 ± 11.5			15.8 ± 5.8			11.3 ± 4.3		
Turbidity	Clear	16.4 ± 3.4	0.516	0.473	18.4 ± 1.9	4.444	0.036	6.9 ± 0.9	9.745	0.002
	Turbid	10.9 ± 4.2			8.5 ± 4.5			0.2 ± 0.2		
Vegetation	Emergent	0.34 ± 0.34			7.5 ± 3.4			1.6 ± 1.0		
	Floating	20.9 ± 11.9	13.012	0	7.4 ± 3.0	8.648	0	0.3 ± 0.3	12.106	0
	Emergent+floating	5.3 ± 1.4			25.0 ± 2.9			10.1 ± 1.4		
	None	45.5 ± 9.7			7.0 ± 2.3			0.5 ± 0.3		
Permanence	Permanent	5.1 ± 1.3			50.0 ± 10			8.5 ± 1.2		
	Semi-permanent	27.6 ± 6.3	14.353	0	21.4 ± 2.6	7.023	0.001	1.8 ± 0.8	8.626	0
	Temporary	120 ± 40			9.4 ± 2.0			0 ± 0		
Water current	Still	19.5 ± 3.6	7.739	0.05	19.6 ± 2.1	10.342	0.001	6.5 ± 1.0	3.800	0.052
	Slow flowing	0 ± 0			5.5 ± 2.7			2.5 ± 1.0		
Distance to the nearest house	0–100 m	28.4 ± 7.7			11.5 ± 2.5			2.9 ± 0.8		
	100–300 m	12.4 ± 2.9	5.713	0.004	19.6 ± 3.4	1.963	0.143	6.1 ± 1.4	3.205	0.042
	>300 m	3.8 ± 1.7			18.4 ± 2.9			8.4 ± 1.9		
Origin of habitat	Natural	9.1 ± 2.7	1.653	0.2	26.2 ± 4.7	9.790	0.002	9.7 ± 2.0	8.465	0.004
	Human made	17.6 ± 3.7			13.39 ± 1.7			4.3 ± 0.8		
Presence of algae	Present	18.1 ± 4.8	0.892	0.346	28.7 ± 3.0	51.483	0	11.1 ± 1.5	49.9	0
	Absent	15.5 ± 2.8			5.0 ± 1.3			0.4 ± 0.3		

Table 4. Correlation coefficients between environmental variables and densities of anopheline larvae

Environmental variables	Total anophelines	*An. arabiensis*	*An. pharoensis*	*An. squamosus*
Water temperature	0.196†	0.029	0.349†	0.145*
Water depth	–0.129*	–0.067	0.150*	–0.088
Elevation	–0.082	0.131*	–0.106	–0.136
Intensity of light	0.003	–0.002	–0.007	0.102
Turbidity	–0.214†	–0.047	–0.137*	–0.201
Aquatic vegetation	0.069	–0.304†	0.284†	0.152
Habitat permanence	–0.035	0.302†	–0.172†	0.262
Water current	–0.264†	–0.180†	–0.207†	–0.261†
Distance to house	–0.043	–0.213†	0.101	0.164*
Natural habitats	–0.147*	–0.078	0.201†	–0.188†
Presence of algae	0.330*	0.084	0.426†	0.421†
Water pH	0.018	–0.062	0.048	0.198†

*Correlation significant at 0.05 level; †Correlation significant at 0.01 level.

Table 5. Multiple step-up regression for three common species of anopheline larvae in relation to habitat characteristics

Species		R^2	Coefficient	SE	Standard coefficient	t	P
An. arabiensis	(constant)		74.82	10.39		7.19	0
	Aquatic vegetation	9.3	–12.92	2.22	–0.36	–5.82	0
	Water current	15.6	–28.06	6.74	–0.26	–4.17	0
An. pharoensis	(constant)		–58.28	18.27		–3.14	0
	Presence of algae	18.2	19.09	3.49	0.34	5.47	0
	Water temperature	22.3	2.64	0.76	0.22	3.49	0
An. squamosus	(constant)		–3.45	2.13		–1.62	0.11
	Presence of algae	17.7	10.37	1.51	0.41	6.89	0
	Distance to house	19.3	2.09	0.99	0.13	2.1	0.04

key environmental variables associated with the occurrence and abundance of anopheline larval species (Table 5). Accordingly, the relative abundance of *An. arabiensis* larvae was negatively associated with aquatic vegetation and water current while that of *An. pharoensis* was positively associated with the presence of mats of algae and water temperature. *Anopheles squamosus* larval abundance was also positively associated with the presence of algae in the water bodies and distance to the nearest house.

DISCUSSION

This study has documented the occurrence of five species of *Anopheles* larvae (*An. pharoensis, An. arabiensis, An. squamosus, An. coustani* and *An. cinereus*) the former two were the predominant species in the area. Cytogenetic studies have previously confirmed that *An. arabiensis* is the sole member of the *An. gambiae* Complex present in the Ziway area[6]. All the species identified here have previously been documented[6,16]. Seven larval habitat types were identified in the area (swamps, irrigation canals, canal leakage pools, sand pools, water harvesting pool, brick making pits and rain pools), of which the former two habitats were the most common breeding sites. The availability, persistence and dimensions of all the larval habitats except rain pools are dependent on water from the Lake Ziway, Meki and Bulbula rivers. All of these habitat types were previously reported from the area and elsewhere in the country[6,7,10] except sand mining pools. Riverine sand mining pits block water flow and create pools which offer ideal habitats for the proliferation of anopheline mosquitoes. To our knowledge, this habitat type had not been reported so far from this country although it is a common anopheline larval habitat elsewhere. In this regard, Surendran and Ramasamy[5] and Robert *et al*[17] reported that open pit mining has altered the natural ecosystem in many countries and paved the way for the emergence of different malaria vectors, with increased vector breeding sites. In Sri Lanka, for example, *An. culicifacies* larvae were observed to breed abundantly in rock pools and sand pools along river margins[5].

Of all the five anopheline species, *An. pharoensis* larvae were abundant in herbaceous swamps in the major drainage systems and irrigation areas preferring the permanent lakeshore vegetated water body for breeding. Studies elsewhere also revealed that *An. pharoensis* breeds in large vegetated swamps and along lakeshores among floating plants[18]. In such habitats, *An. squamosus* larvae, were usually sympatric with *An. pharoensis*. Co-existence of these two species in the same breeding site has previously been reported[6]. In contrast, *An. arabiensis* larvae preferred seasonal habitats such as sand pools, brick making pits and rain pools, consistent with the previous observation in the same general area[6] (E. Aklilu, unpublished data). *Anopheles arabiensis* is a typical r-strategist, colonizing temporary habitats in which selection favours rapid population surge since larval predation is less prevalent in temporary habitats than in large permanent habitats[19]. It was observed that larvae of the principal malaria vector in the country (*An. arabiensis)* were most abundant in sand pools along the edge of the Meki River during the dry and small rainy season before the onset of the main rainy season (June–September). This period coincided with the drying out of Meki river water, due to intensive sand mining activities, which in turn resulted in formation of several river water residual pools as sand pools. These were generally clear water and sunlit which are favourable for *An. arabiensis* larvae.

Step-up multiple regression results demonstrated key environmental variables significantly associated with the relative abundance of *An. arabiensis*, *An. pharoensis* and *An. squamosus* larvae. Thus, vegetation and water current were negatively associated with abundance of *An. arabiensis* larvae. In the relatively dry Ethiopian environment, larval *An. arabiensis* are present mainly in small, temporary rain pools that are free of vegetation[16]. Deforestation and cultivation of natural swamps created conditions favourable for *An. gambiae s.l.* breeding in Kenya[19]. The negative association between *An. arabiensis* larval abundance and water current is also consistent with previous reports[20,21] which pointed out that *An. gambiae s.l.* usually prefer still water in which they can stay close to the surface with their orifice open to the air for breathing. Miller *et al*[22] also demonstrated that *An. gambiae s.l.* larvae are capable of terrestrial displacement whereby they can reach in standing water. The presence of mats of algae was a key environmental factor positively associated with the abundance of *An. pharoensis* and *An. squamosus* larvae, as they were most commonly sampled (above 85%) from swamps and irrigation canals where algae were well-established during most of the study period. These larval species usually share the same habitat[6]. Previous studies have shown that algal growth is a key factor for the growth of some anopheline species[23]. *Anopheles pharoensis* larval abundance was also positively associated with water temperature, as previously observed elsewhere [24].

Anopheles squamosus larval abundance was also significantly and positively associated with the distance to the nearest house. This would be expected since the species is not known to be involved in malaria transmission, although Abose *et al*[6] reported its occurrence both in indoor and outdoor resting collections. The medical importance of this species remains to be explored.

In conclusion, the study demonstrates the diversity of larval habitats in this relatively small but economically important area of rural community which occasionally experiences malaria epidemics[6]. Larval monitoring and control measures are the part and parcel of the overall anti-malarial campaign. Thus, the importance of dry season larval habitats such as irrigation canals and sand mining pits which are responsible for continuous production of the adult vectors throughout most of the year need to be considered in larval vector control operations. In a nearby locality of the Rift Valley (Koka), brick-making pits that were created during the dry season were the most productive larval habitat for the main malaria vector, *An. arabiensis*. These habitats are usually discrete and limited in number, so that anti-larval measures against them are very well suited during the dry season, thus, reducing the overall number of mosquitoes before the increased larval habitats during the rainy season. To our knowledge, this study is the first attempt to analyze the complex environmental variables that determine anopheline larval occurrence/abundance especially in drainage and irrigation areas in Ethiopia. Further, detailed year-round investigation in different ecosystems of the country, emphasizing on the biotic, physico-chemical and other non-biotic factors in both productive and non-productive larval habitats is needed towards a sound understanding of anopheline larval ecology and application of appropriate larval control measures.

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