

Ecology and habitat characterization of mosquitoes in Saudi Arabia

Khater, E.I.^{1,2*}, Sowilem, M.M.^{1,3}, Sallam, M.F.¹ and Alahmed, A.M.¹

¹Plant Protection Department, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, P.O. 2460, Saudi Arabia

²Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt

³Environmental Studies & Land Use Division, National Authority for Remote Sensing and Space Sciences (NARSS), P.O. Box: 1564 Cairo, Egypt

*Corresponding author email: eikhater@yahoo.com; ekhater@ksu.edu.sa

Received 11 January 2013; received in revised form 23 February 2013; accepted 24 March 2013

Abstract. The Kingdom of Saudi Arabia (KSA) contains many of the world's mosquito vectors of parasitic and arboviral diseases. However, few studies addressed their geographic distribution and larval habitat characteristics. We carried out a 14-months mosquito survey in three KSA regions: Makkah and Al-Baha (western) and Jazan (south-western). Larvae were collected by dipping from various water habitats and adults by CDC light and BG sentinel traps. Climatic conditions and physicochemical characteristics of collection sites were recorded. We collected a total of 3331 mosquitoes {larvae (n= 2766, 83%) and adults (n= 565, 17%)} of 21 species from six genera (8 *Anopheles*, 8 *Culex*, 1 *Aedes* and 3 others). Larval water habitats included streams, rocky pits, seepage, leakage and containers (plastic and concrete). Of the total larvae collected, 52% (n= 1439) were *Anopheles*, 44.3% (n= 1226) were *Culex*, 0.51% (n= 14) were *Aedes aegypti* and 3.1% (n= 87) were from four other species. The most abundant species were *Culex tritaeniorhynchus* (n=1008, ~36.3%) and *Anopheles dthali* (n= 976, ~35.3%). The medically-important species were *Anopheles arabiensis* (n= 128) and *Anopheles sergenti* (n= 58), vectors of malaria and *Culex tritaeniorhynchus*, *Culex quinquefasciatus* (n= 53) and *Ae. aegypti* (n= 14), vectors of arboviral diseases. Three species are new records in KSA and all from Jazan: *Anopheles superpictus* (n= 3), *Culex duttoni* (n= 1) and *Culex mimeticus* (n= 1), however the numbers were very low, which requires further investigations. Only two species were collected in the adult stage, *Cx. quinquefasciatus* (n= 561: 551 females and 10 males) from Makkah and *Culex theileri* (n= 4, all females) from Al-Baha. Only 3.8% (n= 21) of *Cx. quinquefasciatus* females were blood-fed. This study provides new information on the bionomics of 21 mosquito species in KSA including six dominant vector species and thus adds to the scarce data available on them. This information is essential to better understand mosquito population dynamics in relation to disease transmission and control.

INTRODUCTION

Countries in the Eastern Mediterranean Region of the World Health Organization (WHO), including the Kingdom of Saudi Arabia (KSA) bear ~11% of the world vector-borne diseases burden like malaria and arboviral diseases (WHO, 2004). In KSA malaria is endemic since 1900 (Mattingly & Knight, 1956), and health reports showed that there is active transmission albeit at small level, with <10% of the reported cases were

locally-transmitted and the rest were imported (MoH, 2007). The reported cases have been based on examination of blood films; however, there are no recent reports that linked malaria transmission to a specific mosquito vector. The only study (Al-Sheikh, 2011), reported malaria *Plasmodium falciparum* sporozoite rate of 0.61% in *Anopheles arabiensis* (21 positives of 1773 females tested by PCR). Generally, malaria transmission in KSA is considered low and unstable or absent, compared to areas in

Africa and South-East Asia, with high transmission rates (Hay *et al.*, 2009).

The major arboviral diseases in KSA are dengue fever (DF) and Rift Valley fever (RVF). Outbreaks of RVF were reported since 1995 in the Tehama Red Sea coastal plateau in the western and southern regions, due to floods following heavy rainfall and agricultural activities (Balkhy & Memish, 2003). These created ideal habitats for larvae of the RVF vectors, the floodwater mosquito *Aedes vexans arabiensis* and *Culex tritaeniorhynchus* (Jup *et al.*, 2002; Miller *et al.*, 2002). Outbreaks of DF were reported in Jeddah and transmitted by *Aedes aegypti* (WHO, 2004). Other arboviral diseases include Japanese encephalitis, West Nile fever, yellow fever and Chikungunya that are transmitted by many *Aedes* and *Culex* mosquitoes in the world; however, the transmission status in KSA is still unknown. Another virus called Alkhumra (Alkhurma) virus, was isolated from patients with dengue haemorrhagic-like fever from Alkhumra district in Jeddah, however the transmission mechanism of this virus is not known, and might be transmitted by mosquitoes or ticks (Madani *et al.*, 2005, 2012). Levels of transmission risk and incidence of these diseases fluctuate seasonally at the country and regional levels, due to the dynamics of mosquito vector, human populations and environmental changes (WHO, 2004; Murdock *et al.*, 2012).

Mosquito life cycle is affected by both biotic and abiotic factors. The biotic factors include adult blood and sugar meal types, species associations and interactions and natural enemies. The abiotic factors include physicochemical properties of larval habitats (water type and contents), surrounding vegetation and the prevailing ecological conditions (temperature and rainfall). There are complex interactions between these factors that significantly impact mosquito ecological fitness and vectorial capacity for disease transmission, with important implications for vector management and control at the local and regional levels (Blaustein & Chase, 2007; Juliano, 2009). Therefore, studying these factors for different mosquito fauna will help

monitor potential modifications of larval habitats due to rains, global climate change or man-made activities.

Previous studies on the ecology, distribution and abundance of mosquito species in KSA are generally few and sporadic, the most of which were in the western and southern regions. These studies included Asir province in 1993–1995 (Abdullah & Merdan, 1995) and 1999–2001 (Abdoon & Alshahrani, 2003), Jeddah in 2007–2008 (Al-Ghamdi *et al.*, 2008), Makkah, Jeddah & Al-Taif in 2004–2006 (Alahmed *et al.*, 2009), Madinah in 2004–2006 (Kheir *et al.*, 2010) and 2008–2009 (El-Badry & Al-Ali, 2010), Najran province in 2005–2006 (Alahmed *et al.*, 2011a), Tehama Red Sea coastal plain in 2007–2008 (Al-Sheikh, 2011) and Makkah in 2009–2010 (Aziz *et al.*, 2012). Fewer studies were in the Eastern region oases in 1979–1980 and 2009–2010 (Wills *et al.*, 1985; Ahmed *et al.*, 2011) and in the Central region (Riyadh) in 2002–2003 and 2003–2005 (Alahmed & Kheir, 2005; Alahmed *et al.*, 2007). These studies reported the presence of many species from many genera, the most important of which were *Anopheles*, *Aedes* and *Culex*. Of these studies, only few provided description of habitats of the collected larvae. Even fewer studies that provided evidence on the active role of some species in disease transmission mainly *Anopheles* vectors of malaria (Mattingly & Knight, 1956; Zahar, 1985, 1990; Al-Sheikh, 2011) and *Aedes* and *Culex* vectors of arboviruses like Sindbis (Wills *et al.*, 1985), RVF (Jup *et al.*, 2002; Miller *et al.*, 2002) and DF (WHO, 2004).

The KSA has peculiar zoogeographic position and economic importance and at potential risk of disease outbreaks due to the presence of many important mosquito species that are proven or suspected vectors of infectious diseases. Despite these facts, there is a serious lack of information on the bionomics of these species and the diseases they carry. Such information together with disease clinical and epidemiologic data is critical to understand the dynamics of disease transmission and identify the areas at the highest risk of disease outbreaks and put appropriate prevention and control plans.

That is what prompted us to conduct the present study to determine the distribution and ecology of mosquito fauna in KSA, especially in the southern and western regions; where malaria and arboviral disease epidemics have occurred in the last three decades.

MATERIAL AND METHODS

Mosquito collection, site selection and species identification

Mosquito collection sites were selected to represent three regions: Makkah (Jeddah, Arafat) and Al-Baha (western) and Jezan (south-western) (Fig. 1). These regions have been endemic for important diseases like malaria and arboviruses and where active transmission and outbreaks are taking place. In addition, Makkah region is a world destination for religious rituals and work, where millions of people visit every year, most of them are from disease endemic countries. Jeddah is a major harbour at the Red Sea and where intense activities of travelling and commercial activities take

place. Mosquito larval sites were selected based on initial directions from the field surveillance teams of the Department of Control of Vector-Borne Diseases of the Saudi Ministry of Health. Other sites were also visited through random selection. Mosquito samples were collected from the study regions in the period November, 2009-December, 2010, according to the standard methods (WHO, 2003). Mosquito larvae were collected by the dipping technique using 350 ml Clark's dippers and whirlpack bags. Adult mosquitoes were collected using carbon dioxide-baited CDC light and BG-sentinel (BGS) traps. Mosquitoes were identified by pictorial keys (Mattingly & Knight, 1956; Harbach, 1988).

Ecological and physiochemical characteristics of study regions

Geographical, climatic and physiochemical characteristics of collection sites were recorded using global positioning system (GPS, Etrix Garmin). The geographical positions of the study areas Makkah, Al-Baha and Jezan are: NW, 23°45'14.55"N

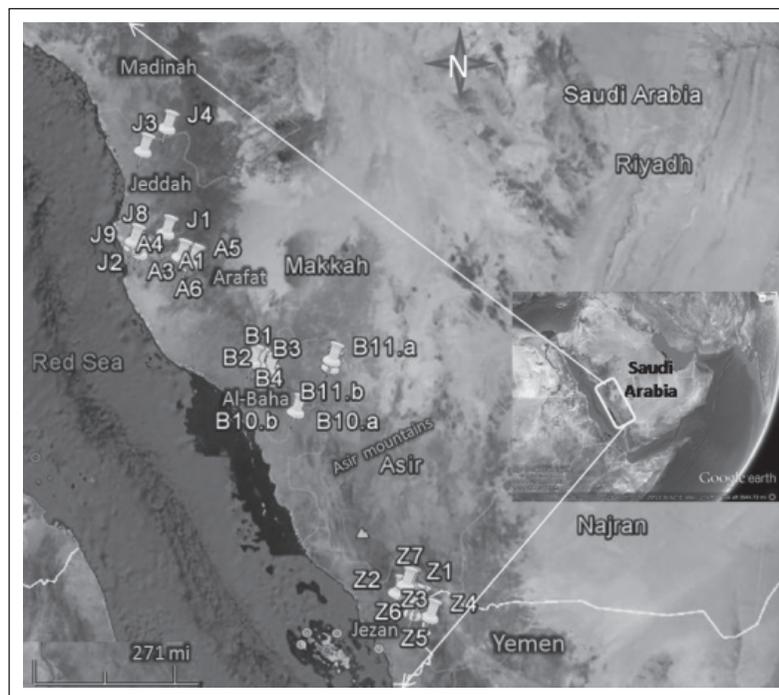


Figure 1. A map of Saudi Arabia showing the study regions and the collection sites of larvae and adults. Makkah (Jeddah: J1-4, 8, 9; Arafat: A1-6), Al-Baha: B1-4, 10, 11 and Jezan: Z1-7

& 38°26'47.18"E; NE, 24°8'5.98"N & 43°29'43.03"E; SE, 16°28'38.87"N & 43°56'6.42"E and SW, 16°24'27.39"N & 42°46'21.12"E. The characteristics of mosquito larval habitat and adult collection sites included positional coordinates (latitude and longitude), site area, depth, substrate type, distance from human dwellings, vegetation type, water turbidity, exposure to sunlight, and physicochemical properties like water temperature, total dissolved salts (TDS, salinity) and pH. Meteorological data especially temperature, rainfall (precipitation) and relative humidity (RH %) for the two years 2009 and 2010 were obtained from the Saudi National Meteorology & Environment Center. The study areas and collection sites were geo-referenced by Google Earth 7 (7.0.1.8244, beta), 2012. General information on the plants surrounding larval sites was obtained from Migahid (1978) and Alfarhan *et al.* (2005) as well as Wikipedia (<http://en.wikipedia.org/wiki>).

RESULTS

Ecological conditions and description of mosquito collection sites

Ecological conditions

The mean temperatures, rainfall and RH% in the study areas in a 14-months period (November 2009-December 2010) are illustrated in figure (2). The data showed slight difference in the mean temperature between the two years for each region (Fig. 2a). Arafat and Jazan were the hottest but had similar temperatures (30.9-31.9°C) over the two years, followed by Jeddah (28.8-29.4°C) and lastly Al-Baha (23.5-23.6°C). The hottest months were June in Arafat (~37°C), August in Jeddah (~34°C) and Al-Baha (~30°C) and September in Jazan (~36°C). The coolest months were January and December in Arafat (~25°C) and Al-Baha (~15°C), January and February in Jeddah (25°C) and January in Jazan (~26°C). For the mean of total annual rainfall, Jeddah received the highest rainfall (202.8 ± 100.2 mm) in 2010, followed by Al-Baha (196.0 ± 101.3 mm) in 2009, then Makkah (136.0 ± 86.6 mm) in 2009 and the

lowest was Jazan (8.9 ± 4.7 mm) in 2010 (Fig. 2b). On comparing 2009 to 2010, the data showed that there was a decrease in rainfall of 43% (1.7 fold), 62.6% (2.7 fold) and 88% (8 fold) in Arafat, Al-Baha and Jazan, respectively, while in Jeddah, there was an increase of 47.4% (1.5 fold) in rainfall. Regarding the means of annual RH%, there were slight variations between the two years in each region. The RH% in a descending order was Jazan (66.8%), Jeddah (58.9%), Arafat (46.8%) and Al-Baha (37.6%) (Fig. 2c).

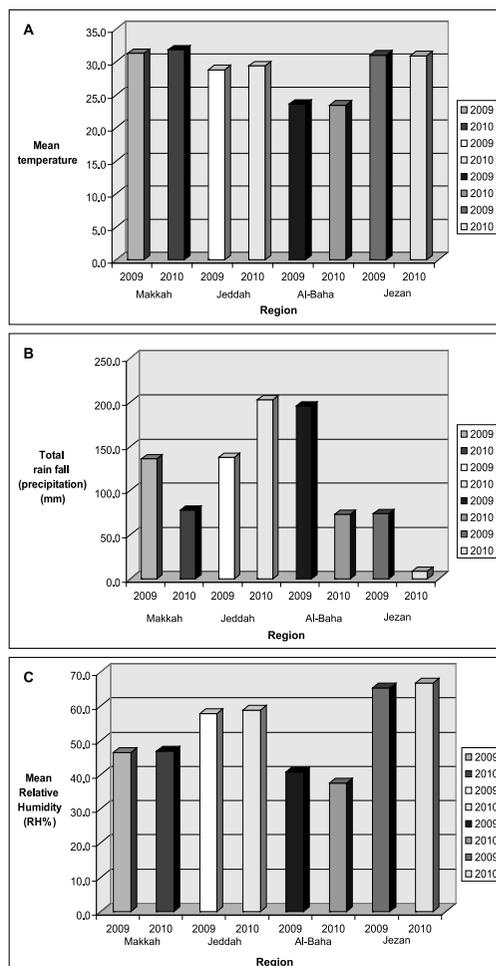


Figure 2. Summary of meteorological data a) temperature, b) rain fall and c) RH% in the study regions, Makkah, Al-Baha and Jazan from November, 2009 to December, 2010. Mean of twelve months, Mosquito collections were made for two months in 2009 (November-December) and for two months in 2010 (January to December). Me, Mean; Mn, Minimum; Mx, maximum. Data were obtained collated from the Surface Annual Climatological Report, the National Meteorology & Environment Centre, Saudi Arabia

Description of mosquito larval and adult collection sites

A total of 76 sites in 32 locations were visited for mosquito collections: 50 larval sites in 25 locations and 26 adult sites in 7 locations (Fig. 1). The larval positive sites were 38/50 (3/10 in Jeddah; 3/3 in Arafat; 21/21 in Al-Baha region; 11/16 in Jezan) with an overall positivity rate of 76%. The adult positive sites

were 7/26 sites (4/6 in Jeddah; 1/4 in Arafat; 2/5 in Al-Baha) with an overall positivity rate of 27%. In the other sites very few damaged or none adults were collected and could not be identified. The study areas and collection sites and those that were geo-referenced are shown (Fig. 1). Collection site names and positional coordinates are shown in Table (1).

Table 1. Coordinates of mosquito larval and adult collection sites in Makkah (Jeddah & Arafat), Al-Baha and Jezan regions, west and south-western Saudi Arabia (November, 2009-December, 2010)

| Region | Site code | Latitude | Longitude | Site name | Arabic/local name |
|---------------------------|-------------|---------------|---------------|---|-----------------------|
| Larval Collections | | | | | |
| 1. Jeddah | J1 | 21°42'51.00"N | 39°36'37.00"E | Madsos | وادي مدموس |
| | J2 | 21°27'28.57"N | 39°15'2.74"E | Sadd Ahdl (dam) | سد أهذل |
| | J3 | 22°44'0.61"N | 39°14'43.38"E | Hakkak | وادي حكاك |
| | J4 | 23°1'22.42"N | 39°33'56.50"E | Yanaeem | وادي بناعيم |
| | J5 | ND | ND | Meghnia | وادي مغنية |
| | J6 | ND | ND | Sadd Ain | سد عين |
| | J7 | ND | ND | Gaiza | وادي جيزا |
| 2. Arafat | A1 | 21°20'45.76"N | 39°57'48.19"E | Company Backyards | شرق عرفات |
| | A2 | 21°20'27.76"N | 39°58'50.16"E | Household (bird/animal sheds) | شرق عرفات |
| | A3 | 21°21'16.72"N | 39°59'2.83"E | Al-Rahma Mountain | جبل الرحمة-عرفات |
| 3. Al-Baha | B1 | 20°6'59.20"N | 40°53'57.71"E | Oleib valley | وادي عليب |
| | B2 | 20°2'4.64"N | 41°2'32.82"E | Samaa valley | وادي سمعة |
| | B3 | 20°8'30.00"N | 40°49'30.00"E | Helia valley | وادي حلية |
| | B4 | 19°55'0.00"N | 41°1'60.00"E | Mozairaa valley | وادي مزيرعة |
| | B5 | ND | ND | ND | ND |
| | B6 | ND | ND | ND | ND |
| | B7 | ND | ND | ND | ND |
| | B8 | ND | ND | ND | ND |
| | B9 | ND | ND | Nawan valley | وادي نوان |
| 4. Jezan | Z1 | 17°25'57.84"N | 42°51'49.62"E | Wesaa valley (S1) | وادي وساع |
| | Z2 | 17°19'47.70"N | 42°45'6.36"E | Wesaa valley (S2) | وادي وساع |
| | Z3 | 17°13'50.16"N | 42°57'4.86"E | Qusay valley (S3-6) | وادي قصي |
| | Z4 | 17°2'22.56"N | 43°2'39.48"E | Radd valley (S7-9) | وادي رد |
| | Z5 | 17°4'32.88"N | 42°57'2.76"E | Beesh valley (S10) | وادي بيث |
| | Z6 | 17°12'58.92"N | 43°1'44.46"E | Goura valley (S11) | وادي جورة |
| | Z7 | 17°26'21.00"N | 42°53'45.07"E | Harob valley (S12) | وادي هروب |
| Adult Collections | | | | | |
| 1. Jeddah | J8 | 21°35'59.09"N | 39°10'2.68"E | North Jeddah (Al-Bawadi district) | حي البوادي-شمال جدة |
| | J9 | 21°31'32.82"N | 39°9'41.74"E | | |
| 2. Arafat | A4 | 21°25'10.56"N | 39°49'22.44"E | Makkah Middle (Dam Area) | سد جرول-وسط مكة |
| | A5 | 21°21'46.98"N | 39°59'36.64"E | East Arafat/ Water station East Arafat/ Palm farm | شرق عرفات، وادي نعمان |
| | A6 | 21°19'22.15"N | 39°54'41.83"E | | شرق عرفات، مزرعة نخيل |
| 3. Al-Baha | B10.a | 19°32'9.24"N | 41°24'52.92"E | Lumah valley | وادي لومه |
| | B10.b | 19°32'18.60"N | 41°25'5.52"E | | |
| | B11.a | 20°10'24.24"N | 41°53'22.92"E | Ranya valley | وادي رائية |
| | B11.b | 20°5'16.80"N | 41°52'19.92"E | | |
| B11.c | 20°5'5.64"N | 41°52'6.96"E | | | |

ND, not determined

Characteristics of mosquito larval water habitats

In Arafat (A), three larval sites in the locations A1-3 were in East Arafat (Table 2, Fig. 1). One site was leakage water in A1 in a company backyard disposal area. It had turbid water with muddy substrate and was exposed to sunlight and close (~5 m) to the nearest worker rooms. The other two sites were shallow plastic dishes in a bird/animal shed of a household (A2) and a concrete water container in Al-Rahma Mountain area (A3). Both sites had clear water and covered from sunlight.

In Jeddah (J), the larval sites included one muddy site in Madsos valley (J1), one abandoned car tire filled with turbid water with muddy substrate and sunlight-exposed in Sadd Ahdl (J2) and two shallow streams in the valleys Hakkak (J3) and Yanae'm (J4) that were exposed to sunlight with clear water and rocky substrate (Table 2, Fig. 1). These sites were remote (>2 km) from inhabitant houses and surrounded by plants of the genus *Typha* (barda) (family Typhaceae), a perennial herbaceous plant associated with water or marshes and one of the genus *Ziziphus* (sedr) (family Rhamnaceae), spiny trees or shrubs with sugar-rich fruits.

Table 2. Characteristics of collection sites positive for larval mosquito species collected from Makkah, Al-Baha and Jazan regions, Saudi Arabia (November 2009 to December 2010)

| Location code | Location name | Type | Tur. | Sal. | Exp. | Veg. | Site area | Dist. | Depth | Sub. | T°C |
|--------------------|-------------------------------|-----------------|--------|-----------|---------|-------------------------------|-----------|-------|---------|----------|------|
| Region | | | | | | | | | | | |
| 1. Jeddah (Makkah) | | | | | | | | | | | |
| J1 | Madsos | Small spot | Turbid | ND | Exposed | ND | ND | ND | Shallow | Muddy | ND |
| J2 | Sadd Ahdl (dam) | Tire | Turbid | 7.3 /7.68 | Exposed | Null | <1 m | >2km | Shallow | Muddy | 36.2 |
| J3 | Hakkak | Stream | Clear | 7.5 /2.13 | Exposed | <i>Typha</i> | >1 m | >2km | Shallow | Rocky | 23.8 |
| J4 | Yanaeem | Stream | Clear | 7.5 /2.08 | Exposed | <i>Typha/ Ziziphus</i> | >1 m | >2km | Shallow | Rocky | 29.1 |
| J5 | Meghnia | Water Container | ND | ND | ND | ND | ND | ND | Shallow | Plastic | ND |
| J6 | Sadd Ain | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| J7 | Gaiza | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 2. Arafat (Makkah) | | | | | | | | | | | |
| A1 | Company Backyards | Leakage | Turbid | ND | Exposed | Null | 3 m | 5 m | <1m | Muddy | ND |
| A2 | Household (bird/animal sheds) | Water Container | Clear | ND | Covered | Null | 1.3 m | 0 m | <1m | Plastic | ND |
| A3 | Al-Rahma Mountain area | Water Container | Clear | ND | Covered | Null | 2.4 m | 0 m | <1m | Concrete | ND |
| 3. Al-Baha | | | | | | | | | | | |
| B5 | Site 1 | Seepage water | Clear | 0.63 | Exposed | <i>Commiphora/ Ziziphus</i> | 500m | 2km | <1m | Mud | ND |
| B6 | Site 2 | Seepage water | Turbid | 0.67 | Covered | <i>Commiphora/ Ziziphus</i> | 1000m | >2km | <1m | Mud | ND |
| B7 | Site 3 | Seepage water | Clear | 0.73 | Covered | <i>Commiphora/ Calotropis</i> | 500m | >2km | <1m | Mud | 18.8 |
| B8 | Site 4 | Seepage water | Turbid | 0.68 | Exposed | <i>Commiphora/ Calotropis</i> | 500m | >2km | <1m | Mud | 15.3 |
| 4. Jazan | | | | | | | | | | | |
| Z1 | Wesaa valley | Rocky pit | Clear | 1.06 | Exposed | Null | <1m | >1m | <1m | rocky | 26.8 |
| Z2 | Wesaa valley | Stream | Clear | 1.13 | Exposed | Null | >1m | >1m | <1m | rocky | 29.3 |
| Z3 | Qusay valley | Stream | Clear | 0.97 | Exposed | Null | >1m | <1m | <1m | rocky | 25.3 |
| Z4 | Radd valley | Stream | Clear | 0.66 | Exposed | Null | >1m | >1m | <1m | rocky | 23.7 |
| Z5 | Beesh valley | Stream | Clear | 0.66 | Exposed | <i>Typha/ Salix</i> | >1m | >1km | <1m | rocky | 24.6 |
| Z6 | Goura valley | Stream | Clear | 1.53 | Exposed | <i>Typha/ Salix</i> | >1m | <1m | <1m | rocky | 20.4 |

ND, not determined; Exp.= Sun Exposure, Dist.= Distance from nearest house, Sub.= Substrate type, T°C = Temperature (°C), Tur.= Turbidity, Sal.= Salinity, Veg.= Vegetation type, Typha= typha, Ziziphus= sedr, Commiphora= habak or habaq, Calotropis= milkweed, Salix= willow.

The sites visited in Al-Baha (B), included four wide (500-1000 m in diameter) sites in four locations (B1-4) in the valleys Oleib, Samaa, Helia and Mozeraa, respectively (Table 2, Fig. 1). These were seepage water with muddy substrates at ~2-km distance from inhabitant houses. These contained turbid (B2 and B4) or clear water (B1 and B3) and were exposed to sunlight (B1 and B4) or covered (B2 and B3). All sites were surrounded by plants from three genera, *Commiphora* (habaq or habak) (family Burseraceae), *Calotropis* (Usher, Ushar, Apple of Sodom or milkweed) (family Asclepiadaceae), a shrubby desert plant and *Ziziphus*. The other 17 sites in five locations in five valleys: including Nawan valley (B5-9) were undescribed as mosquitoes were delivered to us by collaborators.

In Jazan (Z), all 16 collection sites were in seven locations in the valleys Wesaa (Z1 & Z2); Quosay (Z3); Radd (Z4), Beesh (Z5), Goura (Z6) and Haroob (Z7) (Table 2, Fig. 1). All sites were water streams except that in Z1 was a rocky pit. All sites were shallow (<1m in depth) and contained clear water with rocky substrate and exposed to sunlight. Only two sites in Z5 and Z6 were surrounded by *Typha* and *Salix* (safsaf, willow) (family Salicaceae) shrubs or trees. All sites were close to houses, except Z5 that was at >1-km distance. This is in addition to one undescribed site in Z7.

Mosquito larval species collected

The larvae and species composition of each site are summarized in tables (3a-c). The mean numbers of larvae of dominant vector species (DVS) are shown in Table (4).

A total of 2766 larvae were collected from all study regions, of which 8.2% (n= 227) from Jeddah, 1.8% (n= 49) from Arafat, 33.5% (n= 927) from Al-Baha and 56.5% (n= 1563) from Jazan (Table 3a). These larvae belonged to 21 species (8 *Anopheles*, 8 *Culex*, 1 *Aedes*, 2 *Ochlerotatus*, 1 *Lutzia*, 1 *Uranotaenia*). A small number of larvae (n= 198, 7.2%) were only identified to the genus level including 123 *Anopheles* and 6 *Culex* from Al-Baha, 91 *Anopheles* and 69 *Culex* from Jazan. Jazan was the most productive for larvae followed by Al-Baha and Makkah (Jeddah and Arafat),

with 56.5% (n= 1563), 33.5% (n= 927) and 10% (n= 276) of all larval collections, respectively (Table 3a).

Anopheles larval species

Anopheles larvae from 8 species were the most abundant (n=1439) and represented 52% of all collected larval species, of which 870 (60.4%) from Al-Baha, 443 (30.8%) from Jazan and 126 (8.8%) from Jeddah (Table 3a). No anopheline larvae were collected from Arafat. The eight anopheline species collected were distributed as follows: 3 in Jeddah, 5 in Al-Baha and 6 in Jazan. *Anopheles dthali* was the most abundant species (n= 976, 67.8%), followed by *Anopheles arabiensis* (n= 128, 9%), *Anopheles sergenti* (n= 58, 4%) and *Anopheles azaniae* (n= 55, 3.8%). For the major malaria vectors, *An. arabiensis*, 63% (n= 81) were from Jazan and similar numbers were from Jeddah (n= 24, 19%) and Al-Baha (n= 23, 18%). For *An. sergenti*, 69% (n= 40) were from Al-Baha and 31% (n= 18) from Jeddah (Table 3a).

Anopheles arabiensis larvae were collected from a rocky stream in Hakkak (J3) in Jeddah (n= 19) and seepage muddy water in Lumah Valley (B1) in Al-Baha (n= 4) (Table 3b) and from rocky pit in Wesaa Valley (Z1) (n= 25) and from a stream in Beesh Valley (Z5) (n= 56) in Jazan (Table 3c). While *An. sergenti* larvae were collected from a small water site with muddy substrate in Madsos (J1) (n= 1), from a stream in Yana'eem (J4) (n= 2) and a plastic container in Meghnia (J5) in Jeddah (n= 15) and seepage muddy water in B1 (n= 34) and Ranya Valley (B7-8) (n= 3) in Al-Baha (Table 3b). Few larvae of both species were collected from unknown sites including five *An. arabiensis* from Sadd Ain (J6) in Jeddah and 19 from Oleib Valley (B1) in Al-Baha and three *An. sergenti* also from B1 in Al-Baha. Both *An. arabiensis* and *An. sergenti* were found together or singly or shared their water habitats with other anopheline species, mostly *An. dthali* and with *Culex* species, mostly *Cx. tritaeniorhynchus* in Jazan or *Culex theileri* in Al-Baha. Occasionally other genera like *Ochlerotatus* or *Lutzia* were also encountered in *Anopheles* larval habitats (Table 3b,c).

a. Culex larval species:

The eight *Culex* species collected were distributed as follows: three in Jeddah, one in Arafat, one in Al-Baha and five in Jeza. The collected *Culex* larvae comprised ~44.3% (n= 1226) of total larval species collected, the most of them (n= 1104, 90%) were from Jeza and comparable numbers from other regions (Table 3a). The most abundant species was *Cx. tritaeniorhynchus* (n= 1008, 82.2%), all from Jeza, followed by *Cx. theileri* (n= 59, 4.8%), from Al-Baha (n= 47) and Jeddah (n= 12) and *Culex quinquefasciatus* (n= 53, 4.3%), from Arafat (n= 35) and Jeza (n= 18). Few *Culex pipiens* (n= 9, 0.7%) were from Jeddah (Table 3a). The majority (94.6%, n= 954) of *Cx. tritaeniorhynchus* larvae were collected from stream water sites in

locations Z2-6 and the rest (5.4%, n= 54) were from rocky pits in Z1 (Fig. 1). All sites were with clear water and sunlit, with only two sites in Z5 and Z6 were surrounded by *Typha* and *Salix* plants (Table 2). All *Cx. quinquefasciatus* larvae (n= 53) were collected from a leakage water site in A1 in Arafat (n= 35) and a rocky pit in Z1 in Jeza (n= 18). In Z1 (Table 3c), *Cx. quinquefasciatus* was found together with *Cx. tritaeniorhynchus* and *An. arabiensis* at an approximate ratio of 1: 3:1.4. While *Cx. quinquefasciatus* from Arafat and *Cx. pipiens* from Jeddah (two members of *Cx. pipiens* complex) were found singly in their larval habitats. the nine *Cx. pipiens* were collected from unknown sites in Gaiza (J7) in Jeddah (Table 3b).

Table 3a. Summary of mosquito larval stages collected from Makkah, Al-Baha and Jeza regions of Saudi Arabia (November 2009–December 2010)

| Species | Region | Makkah | | Al-Baha | Jeza | Species Total |
|---------------------------------------|--------------------------|--------|--------|------------------|------|---------------|
| | | Jeddah | Arafat | | | |
| 1. <i>Anopheles dthali</i> | | 84 | 0 | 682 ² | 210 | 976 |
| 2. <i>An. arabiensis</i> ¹ | | 24 | 0 | 23 ³ | 81 | 128 |
| 3. <i>An. sergenti</i> | | 18 | 0 | 40 ² | 0 | 58 |
| 4. <i>An. azaniae</i> | | 0 | 0 | 0 | 55 | 55 |
| 5. <i>An. superpictus</i> | | 0 | 0 | 0 | 3 | 3 |
| 6. <i>An. multicolor</i> | | 0 | 0 | 0 | 2 | 2 |
| 7. <i>An. cinereus</i> | | 0 | 0 | 1 ² | 1 | 2 |
| 8. <i>An. turkhudi</i> | | 0 | 0 | 1 ² | 0 | 1 |
| 9. <i>Anopheles</i> spp. | | 0 | 0 | 123 | 91 | 214 |
| | Total genus ³ | 126 | 0 | 870 | 443 | 1439 |
| 1. <i>Culex tritaeniorhynchus</i> | | 0 | 0 | 0 | 1008 | 1008 |
| 2. <i>Cx. quinquefasciatus</i> | | 0 | 35 | 0 | 18 | 53 |
| 3. <i>Cx. theileri</i> | | 12 | 0 | 47 ² | 0 | 59 |
| 4. <i>Cx. laticinctus</i> | | 13 | 0 | 0 | 0 | 13 |
| 5. <i>Cx. pipiens</i> | | 9 | 0 | 0 | 0 | 9 |
| 6. <i>Cx. arbieeni</i> | | 0 | 0 | 0 | 7 | 7 |
| 7. <i>Cx. duttoni</i> | | 0 | 0 | 0 | 1 | 1 |
| 8. <i>Cx. mimeticus</i> | | 0 | 0 | 0 | 1 | 1 |
| 9. <i>Culex</i> spp. | | 0 | 0 | 6 ² | 69 | 75 |
| | Total genus ³ | 34 | 35 | 53 | 1104 | 1226 |
| 1. <i>Ochlerotatus caspius</i> | | 65 | 0 | 0 | 2 | 67 |
| 2. <i>Lutzia tigripes</i> | | 0 | 0 | 4 | 12 | 16 |
| 3. <i>Aedes aegypti</i> | | 0 | 14 | 0 | 0 | 14 |
| 4. <i>O. detritus</i> | | 2 | 0 | 0 | 0 | 2 |
| 5. <i>Uranotaenia unguiculata</i> | | 0 | 0 | 0 | 2 | 2 |
| | Total genus | 67 | 14 | 4 | 16 | 101 |
| | Grand total | 227 | 49 | 927 | 1563 | 2766 |

¹*Anopheles gambiae* s.l. (sensu lato or complex). ²Including larvae collected by others. ³This includes collections from all sites and excluding 2nd instar larvae and damaged insects.

Table 3b. Productivity of larval sites and species composition of mosquitoes collected from, Makkah region (Jeddah and Arafat) and Al-Baha regions, west of Saudi Arabia (November, 2009 and May, 2010)

| Region | Site code | Site name | Type | Mosquito Species | Larvae collected | Total/site | % of Total/site |
|---------------|--------------|-------------------|-----------------------|-----------------------------|------------------|------------|-----------------|
| 1. Jeddah | J1 | Madsos | Small spot | <i>Anopheles sergenti</i> | 1 | 39 | 2.6 |
| | | | | <i>An. dthali</i> | 32 | | 82.1 |
| | | | | <i>Culex laticinctus</i> | 6 | | 15.4 |
| | J2 | Sadd Ahdl | Tire | <i>Cx. theileri</i> | 12 | 30 | 40.0 |
| | | | | <i>Ochlerotatus caspius</i> | 16 | | 53.3 |
| | | | | <i>O. detritus</i> | 2 | | 6.7 |
| | J3 | Hakkak | Stream | <i>An. arabiensis</i> | 19 | 52 | 36.5 |
| | | | | <i>An. dthali</i> | 33 | | 63.5 |
| | J4 | Yanaeem | Stream | <i>An. sergenti</i> | 2 | 19 | 10.5 |
| | | | | <i>An. dthali</i> | 10 | | 52.6 |
| | | | | <i>O. caspius</i> | 7 | | 36.8 |
| | J5 | Meghnia | Container | <i>An. sergenti</i> | 15 | 22 | 68.2 |
| | | | | <i>Cx. laticinctus</i> | 7 | | 31.8 |
| J6 | Sadd Ain | ND | <i>An. arabiensis</i> | 5 | 5 | 100.0 | |
| J7 | Gaiza | ND | <i>Cx. pipiens</i> | 9 | 9 | 100.0 | |
| J10 | Mesfah | ND | <i>O. caspius</i> | 35 | 35 | 100.0 | |
| J11 | Regily | ND | <i>O. caspius</i> | 7 | 7 | 100.0 | |
| J12 | Gabalin | ND | <i>An. dthali</i> | 9 | 9 | 100.0 | |
| Total Jeddah | | | | | 227 | 227 | |
| 2. Arafat | A1 | Company Backyards | Leakage | <i>Cx. quinquefasciatus</i> | 35 | 35 | 100.0 |
| | A2 | House-hold | Container | <i>Ae. aegypti</i> | 10 | 10 | 100.0 |
| | A3 | Al-Rahma Mountain | Container | <i>Ae. aegypti</i> | 4 | 4 | 100.0 |
| | Total Arafat | | | | | 49 | 49 |
| Total Makkah | | | | | 276 | 276 | |
| 3. Al-Baha | B1 | Olieb valley | ND | <i>An. arabiensis</i> | 19 | 379 | 5.0 |
| | | | | <i>An. sergenti</i> | 3 | | 0.8 |
| | | | | <i>An. dthali</i> | 225 | | 59.4 |
| | | | | <i>An. turkhudi</i> | 1 | | 0.3 |
| | | | | <i>Anopheles*</i> | 123 | | 32.5 |
| | | | | <i>Cx. theileri</i> | 8 | | 2.1 |
| | B2 | Samaa valley | ND | <i>An. dthali</i> | 298 | 299 | 99.7 |
| | | | | <i>An. cinereus</i> | 1 | | 0.3 |
| | B3 | Helia valley | ND | <i>An. dthali</i> | 57 | 63 | 90.5 |
| | | | | <i>Culex spp.</i> | 6 | | 9.5 |
| | B4 | Mozeraa valley | ND | <i>An. dthali</i> | 102 | 102 | 100.0 |
| | B5-6 | Lumah valley | Seepage water | <i>An. arabiensis</i> | 4 | 58 | 6.9 |
| | | | | <i>An. sergenti</i> | 34 | | 58.6 |
| | | | | <i>Cx. theileri</i> | 16 | | 27.6 |
| | | | | <i>Lutzia tigripes</i> | 4 | | 6.9 |
| B7-8 | Ranya valley | Seepage water | <i>An. sergenti</i> | 3 | 26 | 11.5 | |
| | | | <i>Cx. theileri</i> | 23 | | 88.5 | |
| Total Al-Baha | | | | | 1128 | 1128 | |

ND, not determined; * damaged and 2nd larval instars.

Table 3c. Productivity of larval sites and species composition of mosquitoes collected from Jezan region, west-southern of Saudi Arabia (December, 2009)

| Region | Site code | Site name | Type | Mosquito Species | Larvae collected | Total/site | % of Total/site |
|----------|---------------|--------------|-------------------|--------------------------------|------------------|------------|-----------------|
| 4. Jezan | Z1 | Wesaa valley | Rocky pit | <i>Anopheles arabiensis</i> | 25 | 244 | 10.2 |
| | | | | <i>An. azaniae</i> | 21 | | 8.6 |
| | | | | <i>An. dthali</i> | 39 | | 16.0 |
| | | | | <i>An. multicolor</i> | 2 | | 0.8 |
| | | | | <i>An. superpictus</i> | 3 | | 1.2 |
| | | | | <i>Anopheles</i> spp. | 40 | | 16.4 |
| | | | | <i>Culex duttoni</i> | 40 | | 16.4 |
| | | | | <i>Cx. mimeticus</i> | 1 | | 0.4 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 54 | | 22.1 |
| | | | | <i>Cx. quinquefasciatus</i> | 18 | | 7.4 |
| | | | | <i>Uranotaenia unguiculata</i> | 1 | | 0.4 |
| | Z2 | Wesaa valley | Stream | <i>An. azaniae</i> | 34 | 812 | 4.2 |
| | | | | <i>An. dthali</i> | 74 | | 9.1 |
| | | | | <i>Anopheles</i> spp. | 51 | | 6.3 |
| | | | | <i>Cx. arbieeni</i> | 7 | | 0.9 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 646 | | 79.6 |
| | Z3 | Qusay valley | Stream | <i>An. dthali</i> | 7 | 68 | 10.3 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 61 | | 89.7 |
| | Z4 | Radd valley | Stream | <i>An. dthali</i> | 7 | 90 | 7.8 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 14 | | 15.6 |
| | | | | <i>Culex</i> spp. | 69 | | 76.7 |
| | Z5 | Beesh valley | Stream | <i>An. arabiensis</i> | 56 | 82 | 68.3 |
| | | | | <i>An. cinereus</i> | 1 | | 1.2 |
| | | | | <i>An. dthali</i> | 17 | | 20.7 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 8 | | 9.8 |
| | Z6 | Goura valley | Stream | <i>An. dthali</i> | 7 | 244 | 2.9 |
| | | | | <i>Cx. tritaeniorhynchus</i> | 225 | | 92.2 |
| | | | | <i>Lutzia tigripes</i> | 12 | | 4.9 |
| Z7 | Haroob valley | ND | <i>An. dthali</i> | 59 | 59 | 100.0 | |
| | | | | Total | 1599 | 1599 | |

ND, not determined.

Table 4. Mean larval numbers collected of major mosquito dominant vector species in the western region of Saudi Arabia (November 2009 – December 2010)

| Mosquito Species | Mean number of larvae (\pm SEM)/Region | | | |
|----------------------------------|---|-----------------------|----------------------|-------------------------|
| | Jeddah | Arafat | Al-Baha | Jezan |
| 1. <i>Anopheles arabiensis</i> | 12 \pm 5.9 (24)* | 0 | 11.5 \pm 5.9 (23)* | 40.5 \pm 16.9 (81)* |
| 2. <i>An. sergenti</i> | 6 \pm 4.4 (18)* | 0 | 13.3 \pm 10 (40)* | 0 |
| 3. <i>Culex pipiens</i> * | 4.5 \pm 3 (9)* | 0 | 0 | 0 |
| 4. <i>Cx. quinquefasciatus</i> * | 0 | 17.5 \pm 11.8 (35)* | 0 | 9 \pm 6.1 (18)* |
| 5. <i>Cx. tritaeniorhynchus</i> | 0 | 0 | 0 | 168 \pm 148.2 (1008)* |
| 6. <i>Ae. aegypti</i> | 0 | 7 \pm 3.1 (14)* | 0 | 0 |

* Total mosquito number,

** Members of *Culex pipiens* complex.

b. Aedes and others larval species:

The other five reported species are from four genera and were distributed as follows: *Aedes* (1 species) from Arafat, *Ochlerotatus* (2 species) from Jeddah and Jezan, *Lutzia* (1 species) from Al-Baha and Jezan and *Uranotaenia* (1 species) were from Jezan only. Of these species, 101 larvae were collected and represented ~3.7% of the total larvae collected of all species from all regions (Table 3a). The most important of these species is *Ae. aegypti*; where only 14 larvae were collected, of which 10 larvae from small plastic dishes in a birds and animals shed in A2 and four larvae from a concrete water container in a mountainous area in A3 in Arafat (Table 3b, Fig. 1). Larvae of *Ae. aegypti* were found singly in their water habitats in Arafat. *Ochlerotatus caspius* larvae were collected from an abandoned vehicle tire with a muddy substrate site in Sadd Ahdl (J2) (n= 16) and from a stream rocky water in Yana'eem (J4) (n= 7) in Jeddah (Table 3b, Fig. 1). In addition *O. caspius* larvae were collected from unknown sites in Mesfah (J10) (n= 35) and Regily (J11) (n= 7) in Jeddah. Only two larvae of *Ochlerotatus detritus* were collected from (J2). Four larvae of *Lutzia tigrripes* were collected from seepage water sites in Lumah Valley (B5-B6) in Al-Baha, while in Jezan, 12 larvae were

collected from a stream water site in Goura Valley (Z6) (Table 3b,c, Fig. 1).

Mosquito adult collection sites

The adult collection sites were houses under construction in Northern Jeddah city (J8, J9). In Arafat, mosquitoes were collected from closed rooms in the vicinity of human houses near a dam (A4), one big hut for car maintenance in a water station (A5) at about 50 m from labour campus or in a dates palm farm (A6) of which only one was inhabited by the farm guard (Fig. 1, Table 5). Most of these rooms were free from human inhabitants at the time of collection. In Al-Baha, adult collections were made in five sites in locations B10 in Lumah Valley and B11 in Ranya Valley (Table 1). The positive sites (7/15) for adult collections were geo-referenced: two sites in J8 and J9 in Jeddah, three sites in A4-6 in Arafat and two sites B10 and B11 in Al-Baha (Fig. 1, Table 1).

Mosquito adult species collected

Thirty seven adult traps, 32 CDC and five BGS traps were placed in 32 sites. The CDC traps positive for mosquitoes were 1/6 in Jeddah, 4/6 in Arafat and 2/6 in Al-Baha with an overall positivity rate (38.8%). In Arafat, 1/5 BGS traps was positive for mosquitoes with an overall positivity rate (20%). The CDC light traps

Table 5. Mosquito adult collection sites and traps used in Makkah and Al-Baha regions

| Site code | Collection site/ type | Mosquito Species | Trap | Females (F) | | | Males (M) | F:M ratio | Total Species (%) |
|--------------|-------------------------------|-------------------------------|------------------|-------------|-------|-------|-----------|-----------|-------------------|
| | | | | Fed | Unfed | Total | | | |
| J8 | North Jeddah/ Building | | CDC | 0 | 1 | 1 | 0 | - | |
| A4 | Middle Makkah Dam area | <i>Culex quinquefasciatus</i> | CDC | 6 | 104 | 110 | 7 | 15.7:1 | 561 (99.3) |
| A5 | East Arafat/ Water station | | BGS ¹ | 0 | 152 | 152 | 0 | - | |
| A6 | East Arafat/ Palm farm | | CDC | 15 | 273 | 288 | 3 | 96:1 | |
| B10 | Al-Baha/ Lumah Valley | <i>Cx. theileri</i> | CDC | 0 | 4 | 4 | 0 | - | 4 (0.7) |
| Total adults | | | | 21 | 534 | 555 | 10 | - | 565 (100) |

¹Large number of mosquitoes was collected but were eaten by ants or damaged and could be identified.

collected 413 (73%) and the BGS traps 152 (27%) of total adults were collected, respectively (Table 5). The numbers of adults were 59 and 23 per trap of CDC positive and total traps used, respectively, and 152 and 30.4 per trap for BGS positive and total traps used, respectively. In the other traps very few unidentifiable damaged mosquitoes or none were collected including all 14 CDC traps distributed in Jazan.

A total of 565 adults belonging to two *Culex* species were collected: *Cx. quinquefasciatus* from Makkah (n= 561, 99.3%) including 560 from Arafat (A4-6) and only one from Jeddah (J8) and *Cx. theileri* from Al-Baha (B10 & B11) (n= 4, 0.7%) (Table 5, Fig. 1). Of the 561 *Cx. quinquefasciatus* collected, 551 (98.2%) were females and 10 (1.8%) were males. Of the collected females, 21 (3.8%) were blood-fed and 534 (96.2%) were unfed (Table 5). In Al-Baha, all four *Cx. theileri* collected were blood unfed females (Table 5). All females were collected from rooms outside the main household or from buildings under construction. The overall *Cx. quinquefasciatus* female:male ratio was 55:1 with the ratio 15.7:1 in the dam area (A4) and 96:1 in the palm farm (A6) (Table 5).

DISCUSSION

The KSA is the largest country in the Arabian Peninsula (~1.9 m Km²) with diverse ecology and geography and peculiar position in the world zoogeographic regions, the Palaearctic region from the north, the Ethiopian (Afrotropical) region from the south-west and the Oriental region from the east (Lane & Crosskey, 1995). This rendered KSA as the border of many mosquito species gradient zones and ecological adaptations. In spite of these eco-biological characteristics, the ecology of mosquitoes present in KSA is largely unknown. Here we attempted to study mosquitoes of three western and southern regions of KSA, where active malaria and arboviruses transmission with periodic outbreaks have been reported (Mattingly & Knight, 1956; Zahar, 1985; Jup *et al.*, 2002; Miller *et al.*, 2002; Balkhy & Memish, 2003; WHO, 2004; MoH, 2007).

The presence of plants and climatic changes such as ambient temperature and rainfall significantly affect the type and abundance of larval habitats. This in turn affects the number of mosquito species and larval stages, the life span, the behaviour and development of adults with direct implications for disease transmission (Juliano, 2009; Murdock *et al.*, 2012). The major change observed in this study between 2009 and 2010 was the decrease in rainfall by 43%, 62.6% and 88% in Arafat, Al-Baha and Jazan, respectively, while in Jeddah, there was an increase of 47.4% in the same period. This could lead to the creation/disappearance of larval habitats for specific mosquito species and therefore, influence their distribution and abundance.

We were able to record five plants from five genera that were associated with larval sites: *Typha* (Jeddah, Jazan), *Ziziphus* (Jeddah, Al-Baha), *Commiphora* (Al-Baha), *Calotropis* (Al-Baha) and *Salix* (Jazan). In all previous studies on mosquitoes from KSA, there was no mention of specific plant genus with specific mosquito species, except for the general terms vegetation, aquatic plants or algae. Plants represent important source of sugar nutrients and influence both larval and adult development and survival as reported for *Anopheles gambiae s.l.* and *An. sergenti* in Africa (Ye-Ebiyo *et al.*, 2003; Müller *et al.*, 2010). Increased survival rate will allow infected female mosquitoes to live longer than the extrinsic incubation period of malaria parasite, which in turn increases malaria incidence (Kebede *et al.*, 2005; Gu *et al.*, 2011).

In this study, most of the visited larval sites were in areas where previous but sporadic mosquito surveys were conducted either by researchers or by the governmental surveillance teams for mosquito control operations. The low numbers of many species collected in this study might be due to the routine governmental surveillance and insecticide spraying schemes. For adult collections, only two species, *Cx. quinquefasciatus* and *Cx. theileri* were collected by two types of traps, CDC and BGS traps. The difference in efficiency of the CDC and BGS traps in mosquito collection, might be due

to the number of traps assembled (32 CDC vs. 5 BGS), trap positioning and species attractiveness to each trap. The reasons for the absence of anopheline species from adult trap collections are unknown. These low numbers of individuals and species collected might be due to the inability to access houses or house spraying. This is an important obstacle to study adult mosquitoes resting, feeding and host-seeking behaviour and further analysis of their vectorial status for disease transmission.

The 21 species reported in this study raise the total species reported from KSA to about 35 species, of which 28 species records in the WRBU (last accessed December, 2012) and 33 species records by Alahmed *et al.* (2011b). This number is subject to increasing or decreasing due to the confusions and lack of accurate identification (mis-identification or missed-identification) of samples, as observed in the published reports from KSA. For example, the case of *An. arabiensis*, and despite it is the only member of *An. gambiae* complex recorded in KSA outside Africa, some authors reported the presence of other members of the complex and named it *An. gambiae* (Al-Ghamdi, 2008; Alahmed *et al.*, 2011a). One study reported the presence of two species *An. gambiae* (larval collections) from Makkah and *An. arabiensis* (adult collections) from Al-Taif (Alahmed *et al.*, 2009). In this study we also reported species that are members of complexes such as *Cx. pipiens/Cx. quinquefasciatus*, members of *Cx. pipiens* complex and *Ae. aegypti* complex. These species were identified differently by different authors based on morphological keys. To avoid such confusions, molecular approaches must be developed for accurate identification and establish their true species status and identify new species. This is particularly important as not all members of species complexes are vectors of diseases and such information is lacking in the Arabian Peninsula. In this respect, and to our knowledge, in this study three species were recorded in KSA for the first time, and all from Jazan: *Anopheles superpictus*, *Culex duttoni* and *Culex mimeticus*. Due to the few (n= 1-3) mosquito numbers collected of these species, more

studies are needed to provide robust evidences on their presence, distribution and medical importance in KSA.

Out of eight anopheline species reported in this study (Table 3a), only two species are proven malaria vectors, *An. arabiensis* and *An. sergenti* and from the major world DVS (Sinka *et al.*, 2010). *Anopheles arabiensis* is a major malaria vector and a member of *An. gambiae s.l.* in East Africa of the Ethiopian zoogeographic region, which extends to the western-southern regions of Arabia. *Anopheles sergenti* has wide geographical distribution as an important malaria vector, especially in oases habitats in Europe, the Middle East, the Jordan Valley and southern and western regions of Asia. In KSA, it acts as a secondary malaria vector in the absence of *An. arabiensis* (Mattingly & Knight, 1956; Zahar, 1985, 1990). Our results and previous reports showed that the two species *An. arabiensis* and *An. sergenti* have adapted to a broad range of natural and artificial water habitats with various physicochemical characteristics (Abdullah & Merdan, 1995; Alahmed *et al.*, 2007, 2009, 2011a; Al-Sheikh, 2011).

The physicochemical characteristics of larval water habitats regulate the abundance of a mosquito species, for example *An. gambiae* was collected from natural water habitats, of which 79% were in villages compared to man-made habitats in cities (Alahmed *et al.*, 2011a). Recent studies showed that mosquitoes (*Aedes*, *Culex*, *Anopheles* and the predator *Toxorhynchites*) inhabiting sites like discarded vehicle tyres have “proclivity” to occupy small containers (Yee *et al.*, 2010). The abundance and distribution of these mosquitoes were correlated with tyre detritus contents, size and proximity and density of human populations (Yee *et al.*, 2010). *Anopheles gambiae s.s.* larvae well-tolerated highly polluted waters with heavy metals, but with biological costs of reduced survivorship, birth rate and population doubling time (Mireji *et al.*, 2010). Sibling species of *An. subpictus* complex in S.E. India and Sri Lanka have variable salinity preferences (Surendran *et al.*, 2011).

The other six species reported in this study (Table 3a) are widely distributed but not malaria vectors in KSA, and might be important vectors in other countries, and therefore, their abundance and vectorial roles must be monitored regularly. *Anopheles dthali* was the most abundant anopheline species collected in this study and comprised 67.8% of total anophelines collected and 60% of the species collected from the valleys of Asir (Abdoon & Alshahrani, 2003). Its larvae were collected from different habitats and in most cases were associated with other *Anopheles* and *Culex* species. Al-Sheikh (2011) reported that *An. dthali*, *Anopheles pretoriensis*, *Anopheles multicolor* and *Anopheles turkhudi* from Tehama occupied habitats with similar characteristics to those of *An. gambiae s.l.*

Similarly, *Culex* mosquitoes in KSA live in a broad range of water habitats including canals, water collections due to rain, agricultural and irrigation practices and from fresh water reservoirs, brackish and stagnant water. These water collections contained aquatic plants, algae or highly organic matters and were of moderate to high salinity at higher levels than reported in this study. *Culex* larval species were found singly or in association with more than one species from culicine or other genera, the most important of which were *An. arabiensis* and *An. sergenti* (Abdullah & Merdan, 1995; Alahmed & Kheir, 2005; Alahmed *et al.*, 2007, 2009; Kheir *et al.*, 2010; Ahmed *et al.*, 2011). Of the species reported, 3 species are the most important as arboviral vectors: *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*.

In spite of its high medical importance as the main vector of DF in the world and KSA, few studies were carried out on *Ae. aegypti*, mainly from the western region: Makkah, Jeddah and Al-Taif (Alahmed *et al.*, 2009) and Madinah (Kheir *et al.* 2010; El-Badry & Al-Ali, 2010). In these studies the *Ae. aegypti* larval breeding sites were similar to those reported for *Anopheles* and *Culex* above. The only comprehensive study on the bionomics of *Ae. aegypti* was conducted in Makkah for two years, 2009-2010 in response to the increased dengue cases in the city in

2008-2009 (Aziz *et al.*, 2012). The number of larvae in the wet season (WS) (May to October) was eight fold that of the dry season (DS) (November – April), with a peak in March. The collected *Ae. aegypti* larvae represented 35.5% of total mosquito genera collected (*Culex*: 50%; *Anopheles*: 14.5%). This was due to the adverse effects of DS conditions of high temperature and decreased rainfall on availability of oviposition sites, larval abundance and lifespan. Larvae were collected from various natural and artificial water sites in-house and peridomestic. They reported an average larval index Breteau Index (BI) of 3.24 (DS: 1.66; WS: 4.83) that lies in the BI range (2-5%) that alarms the risk for dengue outbreaks (Tun-Lin *et al.*, 1996), which requires continuous monitoring of species seasonal abundance in the city and other cities where dengue is endemic in KSA.

Similar to *Ae. aegypti*, most of *O. caspius* (= *Aedes caspius*) larvae were collected from the western and southern regions: Asir, Jazan and Abha (Abdullah & Merdan, 1995), Makkah, Jeddah and Al-Taif (Alahmed *et al.*, 2009), Madinah (Kheir *et al.*, 2010). It was also collected from the Central region (Riyadh) (Alahmed *et al.*, 2007) and Al-Ahsaa (Ahmed *et al.*, 2011). The water habitats were variable and included seepage and sewage water, stagnant and slow-running clear water, brackish water pools and ditches. It was found in association with *Cx. quinquefasciatus*, *Cx. pipiens*, *Cx. theileri* and *An. multicolor*. Fewer number (n < 50) of *O. caspius* adults were collected from Makkah, Jeddah and Al-Taif (Alahmed *et al.*, 2009) and Madinah (Kheir *et al.*, 2010). There are no reports on the medical importance of this species in KSA; however, it is an efficient vector of arboviral diseases like RVF (Turell *et al.*, 1996) and Tahyna and WN viruses (Milankov *et al.*, 2009) in other countries.

An important phenomenon in mosquito ecology is the presence of controphic species, those species that live in the same larval habitats. Those species are either conspecific (i.e. from one genus) or heterospecific (i.e. from different genera) (Juliano, 2009). These species associations affect mosquito development and life span due to competition for food, exposure to

predators and susceptibility to pesticides, which favour certain vector species on the others (reviewed in Blaustein & Chase, 2007 and Juliano, 2009). Generally, single species develop better than controphic species (Kweka *et al.*, 2012). *Anopheles* development was negatively affected when co-inhabited *Culex* water habitats (Impoinvil *et al.*, 2008; Surendran *et al.*, 2011; Kweka *et al.*, 2012). Small-size females could increase the biting rate and frequency of blood-feeding, while increased wing-length or body size will increase mosquito flight for host-seeking and egg laying and increase fecundity (or parity rate as a measure of blood-feeding behaviour) (Kweka *et al.*, 2012). In contrast, small male numbers and body size could implicate their flight ability and mating potential, which in turn will reduce the female reproductive potential (Kweka *et al.*, 2012). The presence of larval fourth instars with first instars led to cannibalism (same species), or predation (different species), as reported for *An. arabiensis*, *An. gambiae* s.s. and *Anopheles quadriannulatus*, the three members of *An. gambiae* complex (Koenraadt & Takken, 2003). Similarly the predation of *Toxorhynchites* or *Lutzia* larvae on *Anopheles*, *Aedes* and *Culex* larvae was also reported (Kweka *et al.*, 2012). In addition, the introduction of *Aedes notoscriptus* in Australia led to the replacement/ extinction of *Ae. aegypti* (Russell, 1986), which might be due to behavioural or chemical factors, yet to be identified.

Few studies were carried out on the transmission of arboviruses and incrimination of the principal mosquito vectors in the Arabian Peninsula. Wills *et al.* (1985) made 16 isolations of Sindbis virus from *Culex* mosquitoes in the Eastern region in KSA: 13 from *Culex univittatus* and one each from *Cx. tritaeniorhynchus*, *Cx. pipiens* complex and *Culex* spp. The studied species also included *Ae. vexans arabiensis* and *Cx. tritaeniorhynchus*, vectors of RVF (Jup *et al.*, 2002; Miller *et al.*, 2002). Studies showed that *Ae. aegypti* (L.), *Cx. pipiens* (L.), and *Cx. quinquefasciatus* Say, were experimentally-susceptible to the infection of RVF virus (Turell *et al.*, 2008). In KSA,

three of four dengue virus serotypes, DENV1-3, were responsible for dengue outbreaks occurred in Jeddah (Zaki *et al.*, 2008), however, the vector(s) still unincriminated. This is in addition to *Cx. quinquefasciatus* that is a vector of filarial and arboviral diseases in many parts of the world (reviewed in Harbach, 2011), and a link to transmission in KSA is yet to be established. This is particularly important as a *Cx. pipiens* laboratory colony from KSA was highly susceptible to the filarial worm *Wuchereria bancrofti* under laboratory conditions (Omar, 1996). In that study, mosquitoes were allowed to feed on a microfilaraemic expatriate Indian worker living in Abha, Asir region. A recent study also showed the successful propagation of Alkhumra virus (ALKV) in an *Ae. albopictus* mosquito cell line (C6/36), suggesting that mosquitoes not ticks could be the natural vector of this virus in KSA (Madani *et al.*, 2012).

In summary, six of the mosquito species recorded in KSA (Table 4) are considered important DVS according to the description of Sinka *et al.* (2010). These include *An. arabiensis*, *An. sergenti*, *Cx. quinquefasciatus*, *Cx. pipiens*, *Cx. tritaeniorhynchus* and *Ae. aegypti*. The mosquito DVS are characterized by their well-adaptation to a broad range of habitats and climatic conditions, highly anthropophilic, variable adult behaviour of resting and vectorial capacity (Sinka *et al.*, 2010). Full characterization of the bionomics of these DVS under local eco-demographic conditions for example in the Arabian Peninsula, will add important information to the global disease maps established like those for malaria (Hay *et al.*, 2010). This will help to minimize the risk of disease transmission and outbreaks in KSA due to changing human activities or climate change. This is also especially important for control strategies that aim at reducing vector-human contacts like indoor residual spraying and insecticide-treated nets; monitor the spread of insecticide resistance and development of innovative integrated vector management strategies (Hemingway *et al.*, 2006).

Acknowledgement. We deeply thank the Research Center and the Centre for Excellence for Biotechnology Research (CEBR), College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia for partial funding this research project.

REFERENCES

- Abdoon, A.M. & Alshahrani, A.M. (2003). Prevalence and distribution of anopheline mosquitoes in malaria endemic areas of Asir region, Saudi Arabia. *The Eastern Mediterranean Health Journal* **9**(3): 240-247.
- Abdullah, M.A. & Merdan, A.I. (1995). Distribution and ecology of the mosquito fauna in the southwestern Saudi Arabia. *Journal of the Egyptian Society of Parasitology* **25**(3): 815-837.
- Ahmed, A.M., Shaalan, E.A., Aboul-Soud, M.A., Tripet, F. & Al-Khedhairi, A.A. (2011). Mosquito vectors survey in the AL-Ahsaa district of eastern Saudi Arabia. *Journal of Insect Science* **11**: 176.
- Alahmed, A. & Kheir, S. (2005). Seasonal activity of some haematophagous insects in the Riyadh region, Saudi Arabia. *Journal of the Saudi Society of Agricultural Sciences* **4**(2): 95-105.
- Alahmed, A., Al Kheriji, M. & Kheir, S. (2007). Distribution of habitats of mosquito larvae (Diptera: Culicidae) in Riyadh Region, Saudi Arabia. *Journal of King Saud University-Agricultural Sciences* **19**: 35-55.
- Alahmed, A.M., Al Kuriji, M.A., Kheir, S.M., Alahmedi, S.A., Al Hatabbi, M.J. & Al Gashmari, M.A. (2009). Mosquito fauna (Diptera: Culicidae) and seasonal activity in Makka Al Mukarramah Region, Saudi Arabia. *Journal of the Egyptian Society of Parasitology* **39**(3): 991-1013.
- Alahmed, A.M., Kheir, S.M., Al Kuriji, M.A. & Sallam, M.F. (2011a). Breeding habitats characterization of *Anopheles* mosquito (Diptera: Culicidae) in Najran Province, Saudi Arabia. *Journal of the Egyptian Society of Parasitology* **41**(2): 275-288.
- Alahmed, A.M., Sallam, M.F., Khuriji, M.A., Kheir, S.M. & Azari-Hamidian, S. (2011b). Checklist and pictorial key to fourth-instar larvae of mosquitoes (Diptera: Culicidae) of Saudi Arabia. *Journal of Medical Entomology* **48**(4): 717-737.
- Alfarhan, A.H., Al-Turki, T.A. & Basahy, A.Y. (2005). Flora of Jizan Region, Final Report of project AR-17-7, *King Abdulaziz City for Science and Technology (KACST) vol.1*: pp 545.
- Al-Ghamdi, K., Alikhan, M., Mayhoub, J. & Afifi, Z.I. (2008). Studies of identification and population dynamics of anopheline mosquitoes from Jeddah Province of Saudi Arabia. *Bioscience Biotechnology Research Communications* **1**(1): 19-24.
- Al-Sheik, A.A. (2011). Larval habitat, ecology, seasonal abundance and vectorial role in malaria transmission of *Anopheles arabiensis* in Jazan Region of Saudi Arabia. *Journal of Egyptian Society of Parasitology* **41**(3): 615-634.
- Aziz, A., Dieng, H., Ahmad, A., Mahyoub, J.A., Turkistani, A.M., Mesed, H., Koshike, S., Satho, T., Salmah, MR C., Ahmad, H., Zuharah, W.F., Ramli, A.S. & Miake, F. (2012). Household survey of container-breeding mosquitoes and climatic factors influencing the prevalence of *Aedes aegypti* (Diptera: Culicidae) in Makkah City, Saudi Arabia. *Asian Pacific Journal of Tropical Biomedicine* **2**(11): 849-857.
- Balkhy, H.H. & Memish, Z.A. (2003). Rift Valley fever: an uninvited zoonosis in the Arabian Peninsula. *International Journal of Antimicrobial Agents* **21**(2): 153-157.
- Blaustein, L. & Chase, J.M. (2007). Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology* **52**: 489-507.
- El-Badry, A.A. & Al-Ali, H.H. (2010). Prevalence and seasonal distribution of dengue mosquito *Aedes aegypti* (Diptera: Culicidae) in Al-Madinah Al-Munawara, Saudi Arabia. *Journal of Entomology* **7**(2): 80-88.

- Gu, W., Müller, G., Schlein, Y., Novak, R.J. & Beier, J.C. (2011). Natural plant sugar sources of *Anopheles* mosquitoes strongly impact malaria transmission potential. *PLoS One* **6**(1): e15996.
- Harbach, R.E. (1988). The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). *Contributions of American Entomology Institute* **24**(1): 1-240.
- Harbach, R.E. (2011). Classification within the cosmopolitan genus *Culex* (Diptera: Culicidae): the foundation for molecular systematics and phylogenetic research. *Acta Tropica* **120**(1-2): 1-14.
- Hay, S.I., Guerra, C.A., Gething, P.W., Patil, A.P., Tatem, A.J., Noor, A.M., Kabaria, C.W., Manh, B.H., Elyazar, I.F., Brooker, S., Smith, D.L., Moyeed, R.A. & Snow, R.W. (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Medicine* **6**(3): e1000048. Erratum in: *PLoS Medicine* **6**(10).
- Hay, S.I., Sinka, M.E., Okara, R.M., Kabaria, C.W., Mbithi, P.M., Tago, C.C., Benz, D., Gething, P.W., Howes, R.E., Patil, A.P., Temperley, W.H., Bangs, M.J., Chareonviriyaphap, T., Elyazar, I.R.F., Harbach, R.E., Hemingway, J., Manguin, S., Mbogo, C.M., Rubio-Palis, Y. & Godfray, H.C.J. (2010). Developing global maps of the dominant *anopheles* vectors of human malaria. *PLoS Medicine* **7**(2): e1000209.
- Hemingway, J., Beaty, B.J., Rowland, M., Scott, T.W. & Sharp, B.L. (2006). The innovative vector control consortium: improved control of mosquito-borne diseases. *Trends in Parasitology* **22**(7): 308-312.
- Impoinvil, D.E., Keating, J., Mbogo, C.M., Potts, M.D., Chowdhury, R.R. & Beier, J.C. (2008). Abundance of immature *Anopheles* and culicines (Diptera: Culicidae) in different water body types in the urban environment of Malindi, Kenya. *Journal of Vector Ecology* **33**(1): 107-116.
- Juliano, S.A. (2009). Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology* **54**: 37-56.
- Jup, P.G., Kemp, A. & Grobbelaar, A. (2002). The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology* **16**(3): 245-252. Erratum in: *Medical and Veterinary Entomology* **16**(4): 464.
- Kebede, A., McCann, J.C., Kiszewski, A.E. & Ye-Ebiyo, Y. (2005). New evidence of the effects of agro-ecologic change on malaria transmission. *American Journal of Tropical Medicine and Hygiene* **73**(4): 676-680.
- Kheir, S.M., Alahmed, A.M., Al Kuriji, M.A. & Al Zubyani, S.F. (2010). Distribution and seasonal activity of mosquitoes in Al Madinah Al Munwwrah, Saudi Arabia. *Journal of the Egyptian Society of Parasitology* **40**(1): 215-227.
- Koenraadt, C.J. & Takken, W. (2003). Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Medical and Veterinary Entomology* **17**(1): 61-6.
- Kweka, E.J., Zhou, G. & Beilhe, L.B. (2012). Effects of co-habitation between *Anopheles gambiae* s.s. and *Culex quinquefasciatus* aquatic stages on life history traits. *Parasites and Vectors* **5**: 33.
- Lane, R.P. & Crosskey, R.W. (1995). General introduction. In: *Medical Insects and Arachnids*. R.P. Lane and R.W. Crosskey, eds., Chapman and Hall, **Ch1**: p 1-29.
- Madani, T.A. (2005). Alkhumra virus infection, a new viral hemorrhagic fever in Saudi Arabia. *Journal of Infection* **51**(2): 91-97.
- Madani, T.A., Kao, M., Azhar, E.I., Abuelzein el-T.M., Al-Bar, H.M., Abu-Araki, H. & Ksiazek, T.G. (2012). Successful propagation of Alkhumra (misnamed as Alkhurma) virus in C6/36 mosquito cells. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **106**(3): 180-5.
- Mattingly, P.F. & Knight, K.L. (1956). The mosquitoes of Arabia. *International Bulletin of British Museum and Natural History (Entomology)* **4**(3): 89-141.

- Migahid, A.M. (1978). Flora of Saudi Arabia, Second Edition, Vol. 1 Dicotyledons, pp 607, Riyadh University Publication, Published by Sunmoon Sunj.
- Milankov, V., Petric, D., Vujic, A. & Vapa, L. (2009). Taxonomy, biology, genetic variability and medical importance of *Ochlerotatus caspius* (Pallas, 1771) and *O. dorsalis* (Meigen, 1830) (Diptera: Culicidae). *Acta Entomologica Serbica* **14**(2): 195-207.
- Miller, B.R., Godsey, M.S. & Crabtree, M.B. (2002). Isolation and genetic characterization of Rift Valley fever virus from *Aedes vexans arabiensis*, Kingdom of Saudi Arabia. *Emerging and Infectious Diseases Journal* **8**(12): 1492-1494.
- Mireji, P.O., Keating, J. & Hassanali, A. (2010). Biological cost of tolerance to heavy metals in the mosquito *Anopheles gambiae*. *Medical and Veterinary Entomology* **24**(2): 101-107.
- MoH (2007). Report of the Saudi Ministry of Health, 2007.
- Müller, G.C., Beier, J.C. & Traore, S.F. (2010). Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. *Malaria Journal* **20**(9): 262.
- Murdock, C.C., Paaijmans, K.P., Cox-Foster, D., Read, Andrew F. & Thomas, M.B. (2012). Rethinking vector immunology: the role of environmental temperature in shaping resistance. *Nature Reviews Microbiology* **10**: 869-876.
- Omar, M.S. (1996). A survey of bancroftian filariasis among South-East Asian expatriate workers in Saudi Arabia. *Tropical Medicine and International Health* **1**(2): 155-60.
- Russell, R.C. (1986). Larval competition between the introduced vector of dengue fever in Australia, *Aedes aegypti* (L.), and a native container-breeding mosquito, *Aedes notoscriptus* (Skuse) (Diptera: Culicidae). *Australian Journal of Zoology* **34**: 527-534.
- Sinka, M.E., Bangs, M.J. & Manguin, S. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**: 117.
- Surendran, S.N., Jude, P.J. & Ramasamy, R. (2011). Variations in salinity tolerance of malaria vectors of the *Anopheles subpictus* complex in Sri Lanka and the implications for malaria transmission. *Parasites and Vectors* **24**(4): 117.
- Tun-Lin, W., Kay, B.H., Barnes, A. & Forsyth, S. (1996). Critical examination of *Aedes aegypti* indices: correlations with abundance. *American Journal Tropical Medicine and Hygiene* **54**: 543-547.
- Turell, M.J., Linthicum, K.J., Patrican, L.A., Davies, F.G., Kairo, A. & Bailey, C.L. (2008). Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *Medical and Veterinary Entomology* **45**(1): 102-8.
- Turell, M.J., Presley, S.M., Gad, A.M., Cope, S., Dohm, D.J., Morrill, J.C. & Arthur, R.R. (1996). Vector competence of Egyptian mosquitoes for Rift Valley fever virus. *The American Journal of Tropical Medicine and Hygiene* **54**: 136-139.
- Wills, W.M., Jakob, W.L. & Francly, D.B. (1985). Sindbis virus isolations from Saudi Arabian Mosquitoes. *Transactions of the Royal Society and Tropical Medicine and Hygiene* **79**: 63-66.
- WHO (World Health Organization) (2003). Malaria entomology and vector control, Learner's Guide. *WHO/CDS/CPE/SMT/2002.18*, Rev.1, Part I. 2003.
- WHO (World Health Organization) (2004). Integrated Vector Management: Strategic Framework for the Eastern. The Mediterranean Region 2004-2010. The WHO Regional Office for the Eastern Mediterranean, Cairo. 2004.
- Yee, D.A., Kneitel, J.M. & Juliano, S.A. (2010). Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Entomology* **47**(1): 53-62.

- Ye-Ebiyo, Y., Pollack, R.J., Kiszewski, A. & Spielman, A. (2003). Enhancement of development of larval *Anopheles arabiensis* by proximity to flowering maize (*Zea mays*) in turbid water and when crowded. *American Journal of Tropical Medicine and Hygiene* **68**(6): 748-752.
- Zahar, A.R. (1985). Vector bionomics in the epidemiology and control of malaria, Part I, the WHO African Region and the southern WHO Eastern Mediterranean Region, Section III: South-western Arabia, *WHO/VBC/85.3-MAP/85.3*, p.211-244, WHO, Geneva.
- Zahar, A.R. (1990). Vector bionomics in the epidemiology and control of malaria, Part II, the WHO European and Eastern Mediterranean Region, Section III, A: the Mediterranean Basin, *WHO/VBC/90.2-MAP/90.2*, pp.225, WHO, Geneva.
- Zaki, A., Perera, D., Jahan, S.S. & Cardoso, M.J. (2008). Phylogeny of dengue viruses circulating in Jeddah, Saudi Arabia: 1994 to 2006. *Tropical Medicine and International Health* **13**(4): 584-592.

Web sites accessed:

1. WRBU, <http://www.wrbu.org/>, (last accessed December, 2012)
2. <http://en.wikipedia.org/wiki>.
3. (http://www.bg-sentinel.com/downloads/BG-Sentinel_Manual.pdf).