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Breeding of *Anopheles* mosquitoes in irrigated areas of South Punjab, Pakistan

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Abstract. As part of investigations on potential linkages between irrigation and malaria transmission, all surface water bodies in and around three villages along an irrigation distributary in South Punjab, Pakistan, were surveyed for anopheline mosquito larvae (Diptera: Culicidae) from April 1999 to March 2000. Samples were characterized according to exposure to sunlight, substratum, presence of vegetation, fauna, inorganic matter and physical water condition (clear/turbid/foul). Also water temperature, dissolved oxygen (DO), electroconductivity (EC) and pH of sites were recorded. A total of 37 982 *Anopheles* larvae of six morphological types were collected from 2992 samples taken from irrigation/agricultural and village/domestic aquatic habitats. *Anopheles subpictus* Grassi *sensu lato* was by far the most abundant (74.3%), followed by *An. culicifacies* Giles *s.l.* (4.1%), *An. stephensi* Liston s.*l.* (2.6%), *An. pulcherrimus* Theobald (1.8%), *An. peditaeniatus* Leicester (0.3%) and *An. nigerrimus* Giles (0.1%). The four most abundant species were significantly associated with waterlogged fields and communal village drinking-water tanks. Habitat characteristics most correlated with occurrence of anophelines were the physical water condition and the absence/presence of fauna, particularly predators. Occurrence and abundance of *Anopheles* immatures were not significantly correlated with water temperature, DO, EC or pH. Malaria vectors of the *Anopheles culicifacies* complex occurred at relatively low densities, mainly in irrigated and waterlogged fields. In South Punjab, where rainfall is very low, it should be possible to reduce anopheline breeding through water management, as larvae develop mainly in water bodies that are directly or indirectly related to the extensive canal-irrigation system.

Key words. *Anopheles culicifacies*, *An. pulcherrimus*, *An. stephensi*, *An. subpictus*, canals, dissolved oxygen, environmental management, irrigation, larvae, malaria, mosquito breeding, pH, predators, salinity, temperature, water condition, Punjab, Pakistan.

Introduction

Irrigation development projects world-wide have been associated with negative impacts on human health, particularly with respect to vector-borne diseases. Evidence for a direct relationship between irrigation development and increased malaria transmission is inconsistent (Harrison & Scanlon, 1975; Oomen *et al*., 1994; Ijumba & Lindsay, 2001), with increased transmission in some situations (Coosemans, 1985; Goonasekere & Amerasinghe, 1988; Robert *et al*., 1992) but not others (Robert *et al*., 1988; Boudin *et al*., 1992). Pakistan is an intensively irrigated country where, during the colonial era, the Punjab province was considered one of the most malarious areas and experienced some of the most devastating epidemics in the Indian subcontinent (Christophers, 1911; Gill, 1927). Major epidemics of malaria in Pakistan have been attributed to long-term effects of the climatic cycle (Gill, 1928; Bouma & van der Kaay, 1996a, b). Despite expansion of the canal irrigation system and decline of the national Malaria Control Programme activities, due to economic constraints since the

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eradication efforts of the 1960s, malaria is not regarded as a major problem currently in the Punjab of Pakistan, perhaps reflecting sustained high levels of acquired immunity among the inhabitants as well as ecological factors governing vectorial capacity (Herrel *et al*., 2001). Just across the Indian border in Rajasthan, adjacent to our study area in South Punjab, recent irrigation of the Thar Desert has worsened the malaria situation. Malaria epidemics in this area are clearly associated with the extensive new irrigation network and above-average rainfall (Tyagi *et al*., 1995; Akhtar & McMichael, 1996) favouring efficient malaria vectors of the *Anopheles culicifacies* complex (Tyagi & Chaudhary, 1997).

There are claims that both endorse (Birley, 1990) and refute (Muneer, 1999) the view that irrigation development and malaria transmission are linked in Pakistan. As the anti-malaria house-spraying programme has been curtailed due to budgetary constraints, and because resistance is emerging in both *Anopheles* vectors and *Plasmodium* parasites in Pakistan, appropriate water management for the benefit of health as well as agriculture (i.e. saving water) should represent a feasible supplement to conventional vector control strategies**.**

The distribution and abundance of mosquito larvae reflect the oviposition preferences of adult females and the ability of immature stages to tolerate the conditions that prevail in aquatic habitats (Reisen *et al*., 1981). The only comprehensive attempt to characterize mosquito habitats systematically in the Punjab was made by Reisen *et al*. (1981). Prior to the 1980s, published research on mosquito ecology is scarce and remains largely qualitative (Ansari & Nasir, 1955; Aslamkhan & Salman, 1969). Studies from the Sindh province (Nalin *et al*., 1985) and North-west Frontier Province (Suleman *et al*., 1993) are also few and far between.

The objective of this study was to determine, in irrigated villages of South Punjab, which water bodies constitute breeding sites for anopheline larvae. The role of canal-irrigation was of particular interest. Our aim was to characterize *Anopheles* species preferences in terms of environmental characteristics and physical and chemical water quality, amplifying and updating the picture described by Reisen *et al*. (1981) The overall goal was to determine which sites and which site characteristics could best explain the occurrence and abundance of anophelines in order to define which environmental management strategies might be applicable for mosquito control in irrigated areas of Pakistan. Findings on adult anopheline population dynamics and malaria transmission are reported by Herrel *et al*. (2001).

Materials and methods

Study area

From 1 April 1999 to 31 March 2000 we surveyed irrigated villages within the Indus Basin Irrigation System (IBIS). About 130 billion m^3 of water are diverted annually into 62 000 km of irrigation channels and more than 70% of the Punjab is irrigated. The development of the IBIS, characterized by large water losses and the absence of a drainage system, has had profound ecological impacts such as rising groundwater tables, waterlogging, salinization and sodification. With the introduction of canal irrigation, groundwater tables have risen from 30 m to 0–1.6 m below surface level in many irrigated areas (PDWC, 1987). It is estimated that soil salinity deprives Pakistan of about 25% of its potential production of major crops (World Bank, 1994). As a result, despite an extensive irrigation network, crop yields in Pakistan are among the lowest in the world.

The study area lies within the command area of the Hakra 6R distributary, near Haroonabad (29°30′ N 73°08′ E) in Bahawalnagar District, ~300 km SW of Lahore (Fig. 1). This distributary, with a length of 45 km, is the sixth largest in Pakistan, serving an irrigated area of 42 000 ha. Via the Sulaimanke Headworks, water from the Sutlej River is diverted to the Hakra Branch Canal. Water is then channelled to the Hakra 6R distributary and further downstream through a network of lined tertiary canals. Finally, water is allocated through a fixed rotational system (*warabandi*) to farmers, who irrigate their fields via earthen field water courses.

The introduction of irrigation and settlement schemes in the late 1920s promoted migration to the area, which was previously a sparsely populated desert. With the (1947) partition of India and Pakistan, which subdivided the Punjab internationally, a second influx of settlers arrived, mostly from the Indian state of Punjab. The current population of the command area is estimated at 160 000 inhabitants, for which agriculture is the dominant activity. In the *rabi* season (May–September), sugarcane, cotton and rice are grown. Rice cultivation is possible only in areas where irrigation water is abundant, for example, in villages located in the head of the system. In the *kharif* season (October–April), the main crops are wheat and fodder.

Out of the 94 villages located within the Hakra 6R command area, villages 149/6R, 111/6R and 438/6R were selected for this study. These particular villages were selected from the head, middle and tail of the distributary to reflect a continuum of environments, from severely waterlogged to desert conditions. Groundwater tables are 0.5–2 m below soil surface in the head and middle villages and at a depth of more than 10 m in the tail-end of the distributary. These levels remained stable for the study period. Villages are not levelled and do not have roads nor any drainage systems for the disposal of excess rain water and household wastewater. Agricultural drains for the collection of runoff from irrigated fields are also non-existent. No vector control activities were carried out for the duration of the study and both villagers and the District Health Office confirmed that this had been the case for at least the past 5 years. Our census in November 1999 established population size in these villages as 900, 1530 and 970 inhabitants, respectively.

The study area is situated in the Cholistan portion of the Thar Desert, which stretches into the neighbouring state of Rajasthan in India. The area lies below the 200 mm rainfall isohyet and the climate is arid. Rainfall, humidity, potential evaporation and temperature for the study area are given in Fig. 2. Total rainfall per village for the 12 months under study was particularly low (132 mm) as the region experienced a

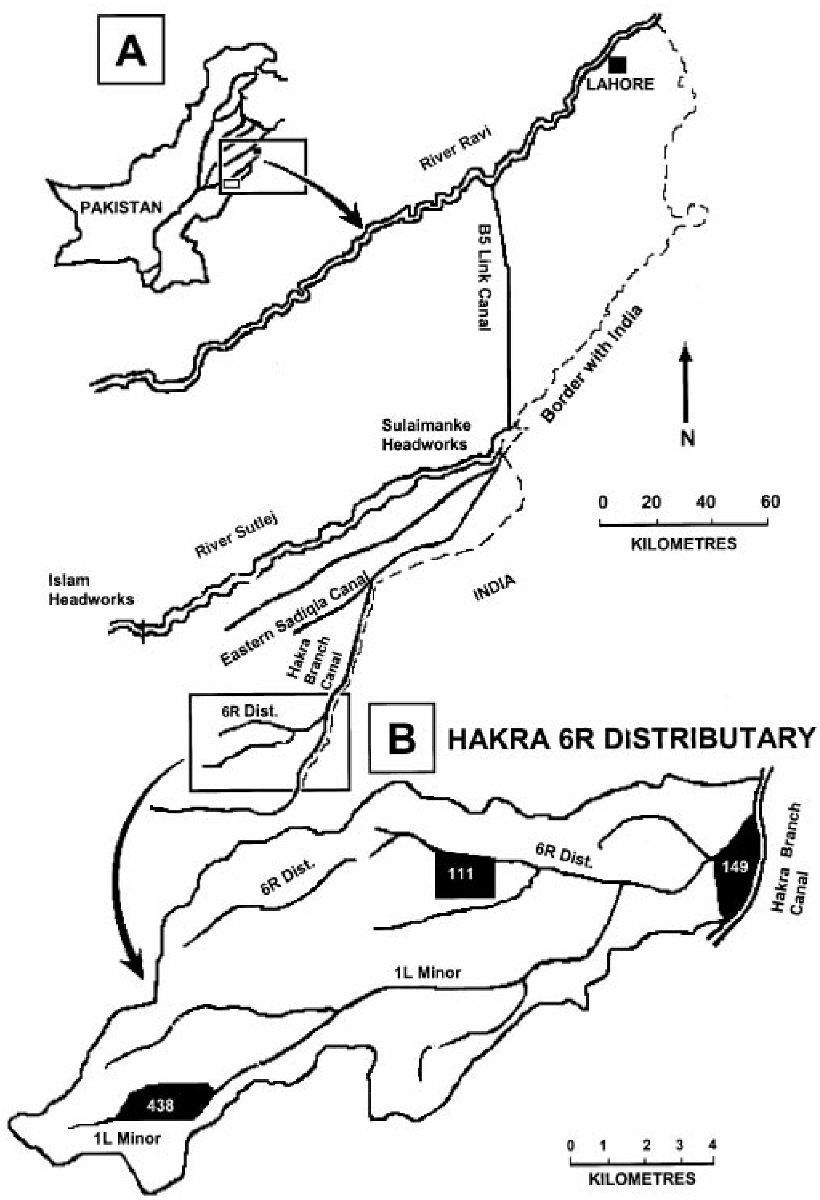

Fig. 1. (A) Outline map of Pakistan, showing inset: location of the Hakra-6R distributary and (B) the three study villages (111, 149, 438) along the distributary.

severe drought. Measurements from the Haroonabad meteorological station confirmed that 1999 was indeed an exceptionally dry year (53 mm) as compared with 1998 (258 mm). Using data from this station, total potential evaporation during our study was estimated at 2470 mm.

Mosquito sampling

Four field assistants were trained in larval collection and recording methods and were supervised on most field visits by an additional researcher. On each field visit, sites were sampled as they were encountered by the entomology team as they walked through the village. To avoid clustering of samples, particularly for site types that were common, collections were spaced throughout the village.

The surface area of every sampled site was estimated in m^2. Samples were taken in proportion to area with a standard 350 mL aluminium dipper (Amerasinghe *et al*., 1997). Six dips were collected per m^2. One 'sample' was defined as 30 dips (or less, for smaller sites) taken over a surface area of 5 m^2. For sites in the 5–10 m^2 range, one sample was collected, whereas two samples were taken from sites in the 11–20 m^2 range and so forth. An upper limit of six samples was set for all sites with

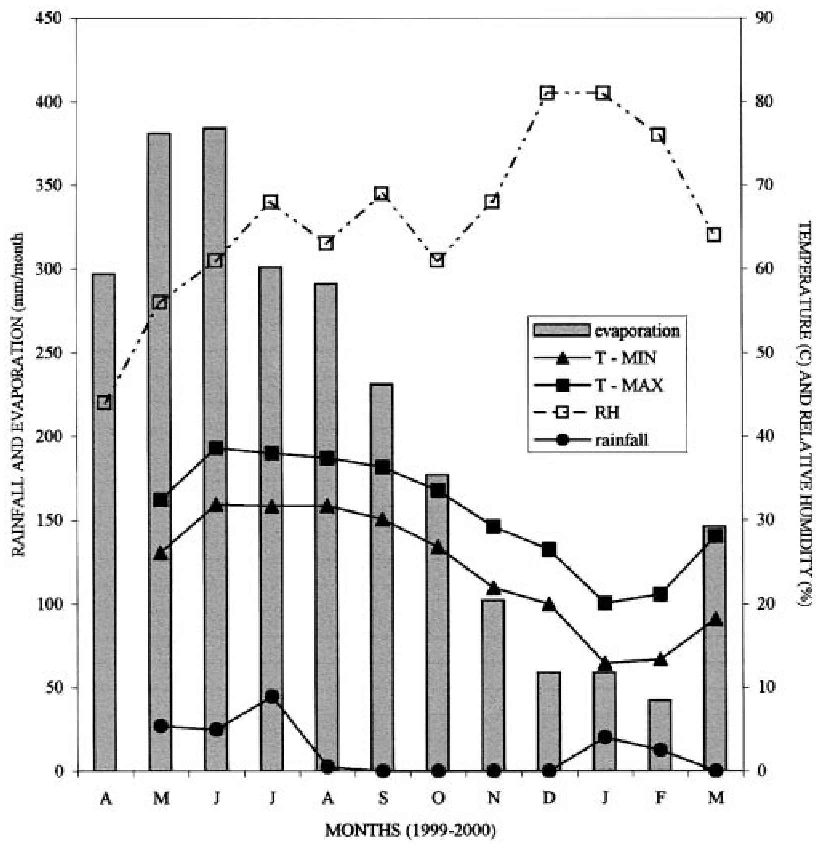

Fig. 2. Climatological data for the study area, April 1999–March 2000. Potential evaporation and percentage relative humidity (r.h.) were calculated from data collected at the nearest meteorological station in Haroonabad (45 km away from the farthest village). Rainfall and temperature data (T-MIN, minimum; T-MAX, maximum) represent monthly averages measured in the study villages.

a surface area exceeding 50 m^2. All anopheline larvae were preserved in 70% isopropyl alcohol and counted.

Since partition from India there have been no new keys published for the larval stages of anopheline mosquitoes of Pakistan. Therefore, based on the most recent checklist of Pakistan *Anopheles* contained in Glick (1992), existing larval keys (Puri, 1931; Christophers, 1933; Mattingly & Knight, 1956; DuBose & Curtin, 1965; Reid, 1968; Harrison & Scanlon, 1975; Rao, 1984; Amerasinghe, 1992) were adapted for the Pakistan fauna. These keys (Amerasinghe *et al*., in preparation) were further refined by rearing larvae to adulthood and confirming adult identities using Glick (1992). Taxonomic identifications were limited to third and fourth instars. Younger instars and damaged larvae were noted in a separate category and were left unidentified.

Habitat characterization

Sampled sites were described in terms of vegetation, including algae, reeds, grasses, rice and other crop plants. Fauna was subdivided into non-predators and predators, with the latter category comprising larvivorous fish, giant water bugs (*Diplonychus* sp., Hemiptera: Belostomatidae), water beetles and water beetle larvae (*Dytiscus* sp., Coleoptera: Dytiscidae), damselfly (*Agrion* sp., Odonata: Agrionidae) and dragonfly larvae (*Pantala* sp., Odonata: Libellulidae), water scorpions (*Nepa* sp. and *Ranatra* sp., Hemiptera: Nepidae), water boatmen (*Corixa* sp. and *Sigara* sp., Hemiptera: Corixidae) and backswimmers (*Notonecta* sp., Hemiptera: Notonectidae.). Substratum was classified as either soil or cement. Physical water condition was assessed by eye as clear, turbid or foul, and water was recorded as flowing or standing. In addition, the exposure of the site to sunlight was scored as exposed, partially shaded or shaded. The temperature of all samples was measured by taking readings from a thermometer held submerged at the edge of the site. When it was not possible to measure temperature directly from the site (e.g. from wells and shallow water tanks), measurements were made from a freshly collected water sample in the dipper itself. The chemical water quality parameters: dissolved oxygen (DO) (mg/L) (Hach® Company, Loveland, CO U.S.A.; DO175 Meter), electro-conductivity (EC) (mS) (Hach® company; EC20 Meter) and pH (Hanna® Instruments; Woonsocket, RI, U.S.A.) were measured in the field for most samples.

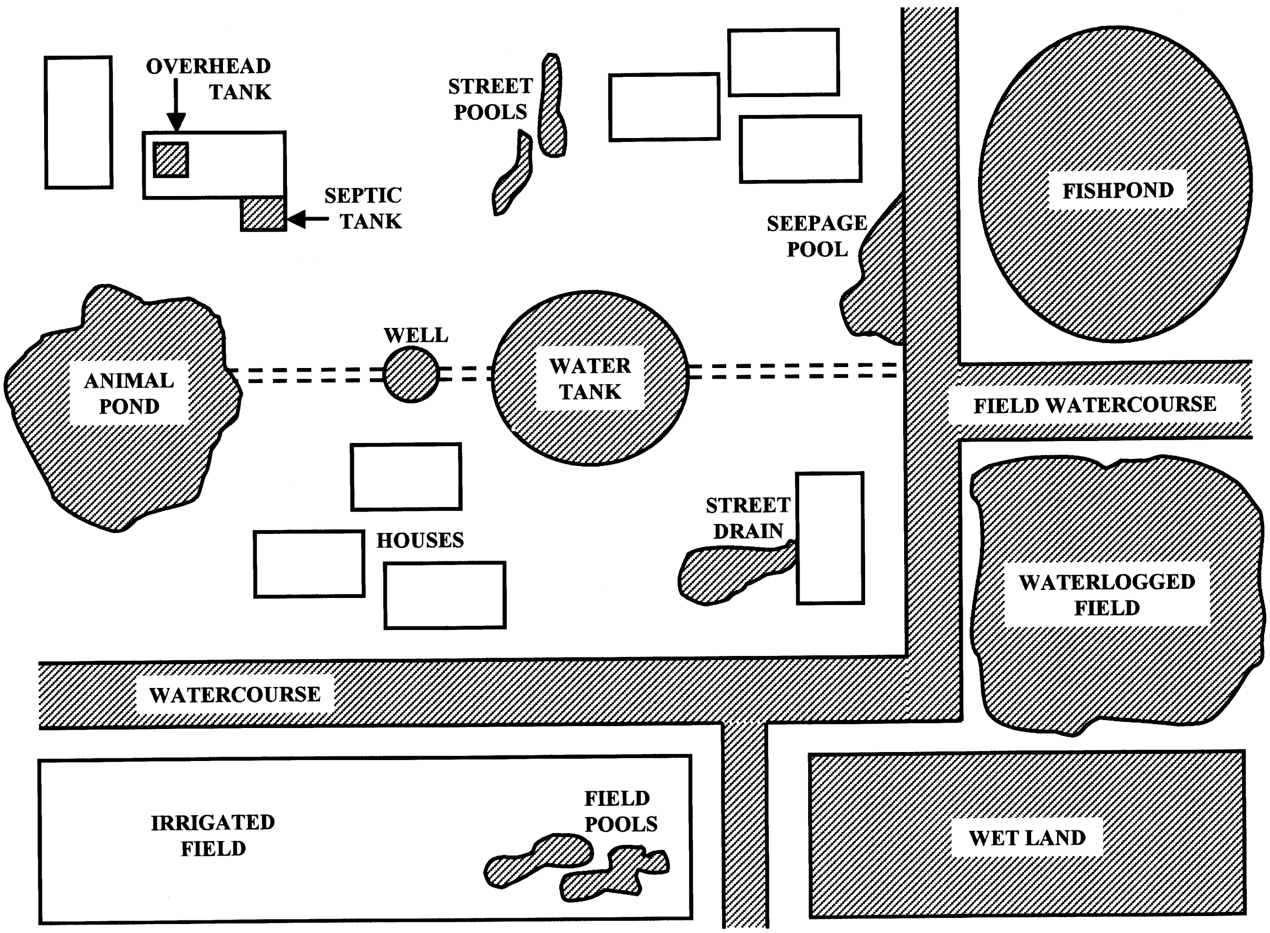

Fig. 3. Simplified diagram of villages along the Hakra-6R distributary, showing the various water bodies sampled for anopheline larvae, illustrating that houses are in close proximity to canals and other perennial surface water bodies associated with irrigation.

Habitat selection

Villages were sampled consecutively on separate days every fortnight, between 07.00 and 12.00 hours. Larvae were collected from a minimum of 23 sites within the village and in agricultural areas within a 1 km radius (Fig. 3). Sites were selected to capture, as far as possible, the diversity of potential breeding habitats in villages. The apportionment of samples between site types was based on the abundance of the different site types at the start of the study. Most habitat categories were present in the different villages but wetlands, waterlogged fields and fishponds were found exclusively in the head of the system, in village 149/6R. This is because head villages in general receive more water than those located further along distributaries.

At the beginning of the study, sites were classified into the following categories:

1 Water tank – near the middle of every village is a main water tank (diggi), at least 50 m^2 wide, with bricked or plastered sides. Water is received on a fixed rotational basis from the Hakra 6R distributary and then pumped from the diggi to individual households for drinking and domestic use.

2 Well – this category includes seepage wells, which are groundwater wells, and connected wells, which are linked to water tanks via a conduit and contain irrigation water.

3 Animal pond – villages have one main animal pond located at the periphery of the village, which receives water from Hakra 6R. Animal ponds are at least 30×40 m^2 and are always earthen. They are used for the watering and washing of animals.

4 Watercourse – a lined irrigation watercourse delivering water to field water courses. This category also includes pools in watercourses.

5 Seepage pool – a pool that forms from seepage losses due to leakage from a watercourse.

6 Field watercourse – an unlined, earthen irrigation channel that takes off from a lined watercourse to deliver water to fields. This category also includes pools located in the field watercourse.

7 Irrigated field – a field inundated due to deliberate action by a farmer. If a crop was present, e.g. rice, it was recorded. This category included field pools.
8 Waterlogged field – an inundated field with a layer of excess water that stands for a period of time due to the presence of an impermeable clay layer or over-irrigation[1].
9 Street pool – a pool in a street that forms due to rainfall, high groundwater table, seepage from the water tank or a nearby hand pump.
10 Street drain – a pool in a street that is clearly connected to and caused by effluents from a household drain.
11 Septic tank – a brick-lined construction that collects human wastes. These are under 3 m^2 and are often uncovered. This definition includes true septic tanks as well overflow tanks.
12 Overhead tank – a concrete or brick tank placed on a rooftop, typically covered and with a grid along the upper edges. These usually contain about 1 m^3 of water, which is used for domestic purposes within a single household.
13 Fishpond – a pond to which irrigation water has been diverted for the purpose of commercial fish farming. It always has an earthen substratum and is typically larger than 50 m^2.
14 Wetland – an area that has become extensively waterlogged due to the proximity of the groundwater table and/or an impermeable clay layer. Because of the high salinity, the area cannot be cultivated for agricultural purposes.

Data analyses

Species occurring in at least 3% of the total number of samples collected were included in the analysis. The association of particular species with site types was investigated using logistic regression analysis (SPSS 8.0 for Windows). For each species, site categories that were always negative were excluded. The logistic regression model included the remaining site types only and was carried out for each species separately. The occurrence of a species was defined as the presence of that species, regardless of the density at which it was collected. Odds ratios (ORs) were derived for each species, calculated as the odds of finding a particular species in a site type divided by the odds of finding that species in animal ponds (the reference habitat).

The associations of particular species with site characteristics was also investigated using logistic regression analysis (SPSS 8.0 for Windows). The characteristics 'substratum' and 'water flow' were dropped from the analysis because most sites had a soil substratum and standing water. Samples that were 'partially shaded' and 'shaded' were merged as one category and were included in the model so that sites were classified as being either 'shaded' or 'exposed'. The remaining parameters were included in the logistic regression model to correct for possible confounding effects and the analysis was done for each species individually. Results are reported as ORs, calculated as the odds of a particular factor being present in sites positive for a species divided by the odds of it being present in the absence of this species.

Anopheline larval densities are reported as the total collected and the number of immatures per 1000 dips per sample for each of the site types. The abundance data were not used in the analysis because they remained highly skewed and could not be normalized through any transformation. No relationships could be discerned between larval abundance data and changes in temperature, DO, EC and pH. No further analysis was carried out and it was therefore only possible to report on the ranges at which anophelines occurred.

Results

Site availability

Most sites were present throughout the year, despite the fact that the area experienced a severe drought. For all three study villages, irrigation structures (e.g. watercourses) were obvious permanent water bodies, as were street drains, street pools and animal ponds. This fact would tend to support the view, expressed by previous workers (de Zulueta *et al*., 1980; Birley, 1990), that because rainfall is minimal in South Punjab and evaporation rates very high, breeding is likely to be confined to water bodies that are directly or indirectly related to canal irrigation.

Mosquito species

Among 2992 samples collected, 1005 were mosquito-positive (Tables 1 and 2). *Culex* and *Anopheles* mosquito larvae occurred in 19.2% and 24.7% of all samples, respectively. Anopheline larvae were collected from all surveyed sites with the exception of overhead tanks and wetlands. The most frequently occurring and by far the most abundant species was *An. subpictus*. *Anopheles culicifacies* and *An. stephensi* were encountered much less often. Overall abundance of these species was also much lower than *An. subpictus*. Species that were present but much more rarely collected were *An. pulcherrimus*, *An. peditaeniatus* and *An. nigerrimus*.

[1]Covell (1941) distinguishes two types of water-logging, as follows: ''Irrigation and water-logging – The term 'water-logging', as defined by Gill (1917), is confined to areas where owing to the height of the subsoil water the superimposed soil is kept permanently damp by moisture derived from the subsoil, as contrasted with 'false water-logging', which refers to dampness of the soil caused by the presence of an impenetrable stratum preventing for a considerable time the downward percolation of rain water.

The question of the influence of irrigation and water-logging on the incidence of malaria is a complex one, and its aspects differ widely in different localities. In India, in the Punjab, it has been repeatedly shown that where canal irrigation gives rise to water-logging a grave degree of endemic malaria associated with a constantly high spleen rate results (Gill, 1927). Here irrigation, if unaccompanied by adequate drainage schemes, will tend to raise the level of the subsoil water and favour the formation of swamps; whilst by raising the relative humidity it will create conditions favourable for mosquito life.''

Table 1. Percentage occurrence of mosquito and *Anopheles* larvae per site type for the period April 1999–March 2000. Numbers represent the percentage of positive samples out of the total number of samples taken from each site type.

	Group or species (%) (No. of positive samples)							
Site type (No. of samples)	Mosquito (1005)	*Anopheles* (740)	*An. subpictus* (521)	*An. culicifacies* (218)	*An.stephensi* (203)	*An.pulcherrimus* (109)	*An.peditaeniatus* (34)	*An.nigerrimus* (12)
DOMESTIC (2196)	34.5	26.4	19.7	7.6	6.8	2.9	0.7	0.2
Animal pond (786)	38.2	27.1	26.1	2.9	4.1	0.3	0.8	0.1
Street drain (404)	19.1	11.6	10.4	1.0	2.5	0	0	0
Street pool (399)	27.8	22.6	20.8	2.0	6.3	0.5	0	0
Septic tank (73)	26.0	4.1	2.7	0	1.4	0	0	0
Well (84)	27.4	14.3	4.8	7.1	9.5	1.2	0	0
Water tank (401)	56.9	53.4	24.2	31.4	18.2	14.5	2.5	0.7
Overhead tank (49)	0	0	0	0	0	0	0	0
AGRICULTURAL (796)	31.0	20.2	11.1	6.4	6.8	5.8	2.3	1.0
Waterlogged field (57)	38.6	28.1	15.8	15.8	10.5	24.6	0	0
Irrigated field (313)	32.3	22.4	11.5	6.7	7.7	8.9	5.1	2.6
Fishpond (61)	26.2	24.6	8.2	24.6	4.9	0	0	0
Wetland (24)	50.0	0	0	0	0	0	0	0
Field watercourse (114)	30.7	23.7	17.5	2.6	7.9	0.9	0.9	0
Watercourse (204)	27.0	15.2	8.3	1.5	5.4	1.5	0.5	0
Seepage pool (23)	26.1	8.7	4.3	0	4.3	0	0	0

Site types and occurrence of anophelines

The logistic regression model with site types explained quite well the occurrence of the four main species we encountered (Table 3). All species showed significant associations with both agricultural and domestic sites located within villages. *Anopheles subpictus* was collected from all *Anopheles*-positive site types. Habitats that were most significantly associated with this species were: animal ponds, water tanks and waterlogged fields. It also frequently occurred in street pools and field water courses but significantly less often in all other sites. *Anopheles culicifacies* was very strongly associated with water tanks, fishponds, waterlogged and irrigated fields. Wells were also frequently positive for this species. A significant negative relationship was found with street drains. The species was never collected from septic tanks and seepage pools. Like *An. culicifacies*, *An. stephensi* showed significant associations with water tanks, waterlogged and irrigated fields and wells. *Anopheles pulcherrimus* was significantly found breeding in water tanks, waterlogged and irrigated fields but was never collected from street drains, septic tanks, fishponds and seepage pools. No anophelines, of any species, were ever collected from wetlands and overhead tanks.

Site characteristics and occurrence of anophelines

Characteristics of different site types are summarized in Table 4. The major characteristics that differentiate sites include salinity levels, physical water condition and the presence of fauna, predators and vegetation. Wetlands and waterlogged fields were highly saline, in contrast with drinking water sources such as overhead tanks and water tanks, which, not surprisingly, had the lowest EC values. Physical water condition was also an important parameter, as the main breeding sites were overwhelmingly clear. Vegetation occurred least often in overhead tanks, as did fauna and predators. Predators were also only rarely found in septic tanks.

The logistic regression model using environmental characteristics explained quite well the occurrence of the four species in aquatic habitats (Table 5). The occurrence of *An. subpictus* was highly correlated with presence of vegetation, inorganic matter, predators and other fauna. Exposed sites with turbid water were also preferred by this species. *Anopheles culicifacies* and *An. stephensi* were similar, occurring significantly more often in habitats with fauna, predators and inorganic matter. Both species preferred sites with clear, rather than turbid or foul water. *Anopheles pulcherrimus* was found significantly more frequently in sunlit habitats with clear water that contained predators.

Most larvae occurred in sites with a DO below 10 mg/L, within a pH range of 7–11 and with low salinity, below 5 mS. The variation in temperature between sites is more likely a reflection of the time, between 07.00 and 12.00 hours, at which they were sampled on one sampling day, rather than real differences between sites. In general, however, anophelines occurred in the range 15–35°C.

Discussion

Understanding where mosquitoes breed and why they prefer certain water bodies over others is vital for the elaboration of sound mosquito control strategies and is particularly important

Table 2. Abundance of anopheline larvae by site type for the period April 1999–March 2000. Total number of immatures collected and the mean number of larvae per 1000 dips per sample (± SE).Unidentified larvae totalled 6355 first and second instars and damaged larvae.

	Species											
	An. subpictus		*An. culicifacies*		*An. stephensi*		*An. pulcherrimus*		*An. peditaeniatus*		*An. nigerrimus*	
Site type	Total	Average	Total	Average	Total	Average	Total	Average	Total	Average	Total	Average
DOMESTIC												
Animal pond	11355	482.2 ± 102.7	43	1.8 ± 0.4	88	4.3 ± 1.3	4	0.2 ± 0.1	19	0.8 ± 0.4	2	0.1 ± 0.1
Street drain	2368	698.1 ± 413.5	14	1.7 ± 1.1	62	9.8 ± 3.7	0	0	0	0	0	0
Street pool	8222	789.5 ± 191.3	13	2.3 ± 1.1	177	17.5 ± 8.8	4	0.5 ± 0.4	0	0	0	0
Septic tank	8	29.7 ± 23.7	0	0	1	0.8 ± 0.8	0	0	0	0	0	0
Well	13	15.2 ± 9.4	10	10.6 ± 4.9	48	50.3 ± 23.0	1	1.0 ± 1.0	0	0	0	0
Water tank	1324	110.1 ± 21.9	1237	102.8 ± 14.8	246	20.4 ± 3.5	180	15.0 ± 3.1	32	2.7 ± 1.1	3	0.2 ± 0.1
Overhead tank	0	0	0	0	0	0	0	0	0	0	0	0
AGRICULTURAL												
Waterlogged field	57	33.3 ± 16.0	25	14.6 ± 6.1	64	37.4 ± 21.7	243	142.1 ± 58.3	0	0	0	0
Irrigated field	305	35.3 ± 19.2	106	12.1 ± 3.8	252	95.2 ± 47.5	274	30.5 ± 10.1	47	5.0 ± 1.4	19	2.0 ± 0.9
Fishpond	16	8.7 ± 5.3	111	60.7 ± 21.3	5	2.7 ± 1.6	0	0	0	0	0	0
Wetland	0	0	0	0	0	0	0	0	0	0	0	0
Field watercourse	4325	3367 ± 1676.0	6	2.6 ± 1.6	30	12.8 ± 6.6	1	2.9 ± 2.9	2	0.6 ± 0.6	0	0
Watercourse	207	63.4 ± 21.4	4	0.7 ± 0.4	38	8.8 ± 3.5	6	1.0 ± 0.7	1	0.2 ± 0.2	0	0
Seepage pool	8	38.6 ± 38.6	0	0	1	4.8 ± 4.8	0	0	0	0	0	0
Totals	28208	479.5 ± 93.2	1569	18.0 ± 2.2	1012	20.8 ± 5.2	713	8.2 ± 1.6	101	1.1 ± 0.2	24	0.3 ± 0.1

Table 3. Associations of anopheline larvae with particular site types as derived by logistic regression analysis. Odds ratios with 95% confidence intervals in parentheses, using animal ponds as the reference category (OR = 1).

	Species			
Site type	*An. subpictus*	*An. culicifacies*	*An. stephensi*	*An. pulcherrimus*
DOMESTIC				
Animal pond	1.0	1.0	1.0	1.0
Street drain	0.33 (0.23–0.47)	0.33 (0.11–0.97)	0.60 (0.29–1.23)	0*
Street pool	0.74 (0.56–0.99)	0.68 (0.30–1.53)	1.57 (0.92–2.70)	1.97 (0.27–14.03)
Septic tank	0.08 (0.02–0.33)	00*	0.33 (0.04–2.43)	0*
Well	0.14 (0.05–0.39)	2.55 (1.00–6.46)	2.48 (1.10–5.57)	4.71 (0.42–52.49)
Water tank	0.90 (0.68–1.19)	15.19 (9.54–24.20)	5.24 (3.39–8.10)	66.15 (16.09–272.04)
Overhead tank	0*	0*	0*	0*
AGRICULTURAL				
Waterlogged field	0.53 (0.25–1.10)	6.22 (2.73–14.18)	2.77 (1.11–6.93)	127.37 (28.08–577.63)
Irrigated field	0.37 (0.25–0.54)	2.38 (1.30–4.38)	1.96 (1.13–3.38)	38.43 (9.11–162.16)
Fishpond	0.25 (0.10–0.64)	10.82 (5.29–22.12)	1.22 (0.36–4.10)	0*
Wetland	0*	0*	0*	0*
Field watercourse	0.60 (0.36–1.00)	0.89 (0.26–3.03)	2.02 (0.94–4.35)	3.46 (0.31–38.46)
Watercourse	0.26 (0.15–0.43)	0.49 (0.15–1.66)	1.34 (0.66–2.71)	5.84 (0.97–35.14)
Seepage pool	0.13 (0.02–0.96)	0*	1.07 (0.14–8.19)	0*

*Denotes sites excluded from the logistic regression analysis because samples collected were all species-negative.

in areas where large-scale irrigation is being practiced (Birley, 1991; Ijumba & Lindsay, 2001). Our study showed that, in three irrigated villages of South Punjab, four *Anopheles* complexes dominated among larval collections. In order of abundance these were: *An. subpictus*, *An. culicifacies*, *An. stephensi* and *An. pulcherrimus*, but our investigations were compromised by inability to distinguish between sibling species of these complexes (Subbarao & Sharma, 1997). Apart from the absence of *An. annularis* from our collections, the anopheline fauna on which we report is consistent with

Table 4 Characteristics of sampled sites. Numbers represent the percentage of samples with each characteristic. For DO, EC, pH and temperature, the mean values (± SE) for each site type are indicated.

	Characteristic											
					Inorganic matter		Physical water condition			Chemical water quality		
Site type	Light Exposed	Vegetation Present	Fauna Present	Predators Present	Present	Clear	Turbid	Foul	pH	DO (mg/L)	EC (mS)	Temperature (°C)
DOMESTIC												
Animal pond	78.6	97.5	97.2	84.5	26.7	16.8	81.3	1.9	8.1 ± 0.1	5.7 ± 0.2	1.9 ± 0.1	25.7 ± 0.2
Street drain	92.3	96.8	65.6	23.8	56.4	5.7	38.4	55.9	8.3 ± 0.1	3.0 ± 0.2	5.2 ± 0.3	26.0 ± 0.3
Street pool	93.7	92.7	74.9	58.6	42.4	21.8	71.9	6.3	8.5 ± 0.1	5.9 ± 0.4	13.1 ± 1.7	27.6 ± 0.3
Septic tank	54.8	84.9	60.3	5.5	37.0	6.8	16.4	76.7	8.0 ± 0.1	2.6 ± 0.3	2.7 ± 0.3	25.3 ± 0.7
Well	47.6	71.4	51.2	19.0	38.1	85.7	6.0	8.3	8.0 ± 0.1	3.8 ± 0.3	1.2 ± 0.5	24.3 ± 0.6
Water tank	97.3	98.0	94.5	80.8	55.6	97.3	2.7	0	8.8 ± 0.1	7.7 ± 0.2	0.4 ± 0	25.3 ± 0.3
Overhead tank	55.1	57.1	8.2	4.1	0	98.0	2.0	0	8.2 ± 0.2	7.0 ± 0.5	0.8 ± 0.2	25.7 ± 0.9
AGRICULTURAL												
Waterlogged field	96.5	93.0	73.7	66.7	1.8	86.0	14.0	0	8.7 ± 0.3	4.3 ± 0.7	32.1 ± 7.8	33.0 ± 0.5
Irrigated field	73.5	98.4	71.9	65.5	4.8	88.5	11.2	0.3	8.5 ± 0.1	5.3 ± 0.3	1.5 ± 0.2	30.0 ± 0.3
Fishpond	100	77.0	83.6	62.3	4.9	90.2	9.8	0	7.9 ± 0	7.7 ± 0.4	2.3 ± 0.4	26.3 ± 1.0
Wetland	100	66.7	58.3	41.7	0	95.8	4.2	0	10.4 ± 0.1	15 ± 0	50.3 ± 3.8	34.5 ± 1.1
Field watercourse	80.7	96.5	70.2	56.1	17.5	58.8	41.2	0	8.4 ± 0.2	7.3 ± 0.7	1.4 ± 0.5	27.2 ± 0.5
Watercourse	75.5	89.7	54.4	37.3	18.1	54.9	42.6	2.5	8.5 ± 0.1	6.2 ± 0.4	1.0 ± 0.1	25.9 ± 0.4
Seepage pool	95.7	95.7	82.6	73.9	8.7	47.8	43.5	8.7	9.0 ± 0.4	3.8 ± 2.6	2.4 ± 1.1	32.0 ± 0.6

Table 5. Associations of anopheline larvae with particular habitat characteristics. Odds ratios (with 95% confidence intervals in parentheses) derived by logistic regression, showing reference category value 1.0 for each type of association.

		Species			
Characteristic		*An. subpictus*	*An. culicifacies*	*An. stephensi*	*An. pulcherrimus*
Water quality	Clear	1.0	1.0	1.0	1.0
	Turbid	1.51 (1.23–1.85)	0.14 (0.09–0.20)	0.35 (0.25–0.48)	0.06 (0.03–0.13)
	Foul	0.41 (0.24–0.68)	0.02 (0.00–0.16)	0.16 (0.06–0.41)	0*
Light	Shaded	1.0	1.0	1.0	1.0
	Exposed	1.40 (1.03–1.91)	1.59 (0.96–2.63)	1.06 (0.68–1.64)	2.18 (1.03–4.61)
Vegetation	Absent	1.0	1.0	1.0	1.0
	Present	2.54 (1.26–5.10)	1.90 (0.67–5.36)	1.53 (0.61–3.87)	1.10 (0.38–3.18)
Fauna†	Absent	1.0	1.0	1.0	1.0
	Present	2.77 (1.69–4.54)	6.13 (2.30–16.33)	6.84 (2.60–18.09)	0.74 (0.18–3.05)
Predators	Absent	1.0	1.0	1.0	1.0
	Present	2.46 (1.82–3.32)	2.14 (1.37–3.34)	1.85 (1.20–2.86)	8.54 (2.67–27.28)
Inorganic matter	Absent	1.0	1.0	1.0	1.0
	Present	1.69 (1.37–2.08)	2.14 (1.58–2.89)	1.47 (1.08–2.00)	0.98 (0.63–1.53)

†General aquatic fauna of potential predators (see line below) as well as non-predators (e.g. Baetidae, Chironomidae, Hydrometridae).*Excluded from logistic regression analysis because samples with this characteristic were all negative.

findings of other authors (Aslamkhan, 1971), as recorded previously from parts of the Punjab nearer Lahore (Ansari & Nasir, 1955; Aslamkhan & Salman, 1969; Reisen *et al*., 1981) and across the Indian border in neighbouring Rajasthan (Tyagi, 1998).

Each *Anopheles* complex was found to be associated with particular site types and environmental characteristics, which suggests that the categories we developed were a valid framework for investigating larval ecology. In other parts of Punjab, these species were found to breed mainly in ponds and pools (Reisen *et al*., 1981). In our study area, water tanks and waterlogged fields supported highest densities of the four major species complexes collected. Irrigated fields were also important for all species, except for *An. subpictus*. We found that irrigated rice fields supported *Anopheles* breeding, whereas anophelines were completely absent from wetlands and from overhead tanks. This is probably attributable to the very high salinity levels detected in wetlands. In overhead tanks anopheline larvae were probably absent due to the frequent cycling of water and the relative absence of vegetation, fauna and sunlight.

For the major malaria vector of Pakistan, *An. culicifacies*, we report that water tanks followed by fishponds, waterlogged and irrigated fields, were the key habitats in terms of occurrence and abundance. More polluted habitats such as street drains were negatively associated with this species. Previous researchers have noted higher densities of *An. culicifacies* at pond margins and lotic habitats (Reisen *et al*., 1981). Under similar climatic conditions in the Thar Desert of Rajasthan, India, irrigation development has created suitable sites, including waterlogged fields, which resulted in the widespread introduction of *An. culicifacies* to the area (Tyagi & Chaudhary, 1997). There, *An. culicifacies s.l.* has become the predominant anopheline (Tyagi & Verma, 1991). In our area, we found that *An. culicifacies* outnumbered *An. stephensi* but our collections remained overwhelmingly dominated by *An. subpictus* larvae.

In Rajasthan, there is a clear distinction in the breeding preferences of *An. culicifacies* and *An. stephensi*, with the latter species preferring deep, earthen, well-like freshwater storage reservoirs (Tyagi & Verma, 1991). In agreement with these observations and those of others (Ansari & Nasir, 1955), we also found significant *An. stephensi* breeding in wells. However, we noted considerable overlap in the site preferences of these two species. This was confirmed by both species' preference for sites with clear, over turbid, water as reported previously (Reisen *et al*., 1981).

Although the overall number of *An. culicifacies* collected was low, it was significantly associated with both irrigated and waterlogged fields in agreement with other researchers (Tyagi & Chaudhary, 1997), as were *An. stephensi* and *An. subpictus*. The presence of all four major species in waterlogged fields with an average electroconductivity of around 32 mS does seem to indicate a certain degree of salt tolerance but anophelines were absent from wetlands where salinity was higher (50.3 on average). In many areas of the Punjab, canal irrigation has led to a rise in water tables and extensive waterlogging and salinization. If the data from our 'wetlands' are indicative of the wider situation in severely waterlogged and salinized areas of the Punjab then it would seem that mosquito productivity is indeed offset by high salinity levels. Since the 1970s various Salinity Control and Reclamation Projects (SCARP) are underway in Pakistan to reclaim land by lowering groundwater tables. It is possible that such reclaimed lands, as compared with saline lands, might in fact favour mosquito breeding, as surface water habitats would tend to be fresh because they would be generated by rainfall and irrigation. Preliminary investigations have shown no relationship between depth to groundwater and malaria prevalence nor between the percentage of land under rice cultivation and malaria prevalence (Donnelly *et al*., 1997).

In terms of occurrence and abundance, animal ponds and street pools were important habitats for *An. subpictus*, which attained quite high densities in relatively polluted sites such as street pools and drains. These site preferences are in agreement with previous research in the Punjab, which recorded that this species was able to tolerate a wide range of physico-chemical conditions (Reisen *et al*., 1981) including higher organic matter content (Ansari & Shah, 1950). We confirm that *An. subpictus* is significantly associated with turbid water (Reisen *et al*., 1981). Although it was occasionally present in irrigated fields, we do not find evidence for a clear association with irrigated rice fields, as has been previously reported for this species in Pakistan (Ansari & Nasir, 1955). According to Suguna *et al*. (1994), the *An. subpictus* complex comprises at least four sibling species, provisionally termed A, B, C and D. Of these, Species B breeds in brackish water and is an important vector of malaria and filariasis in coastal zones, probably distributed from India to Indonesia (Barodji *et al*., 2000), whereas the freshwater sibling species apparently have much lower vectorial capacity due to their zoophily. We have not determined which sibling species of *An. subpictus s.l.* occur(s) in our study area.

Both *An. culicifacies* and *An. stephensi* have been implicated as malaria vectors in irrigated areas of the nearby Thar Desert (Tyagi & Chaudhary, 1997). Although both *An. culicifacies* species A and B occur in rural areas of the Pakistani Punjab (Mahmood *et al*., 1984), field and laboratory observations indicate that species A is the likely key malaria vector, whereas *An. stephensi* is considered of lesser or no importance in transmission (Mahmood *et al*., 1984; Mahmood & Macdonald, 1985; Subbarao *et al*., 1988). Recent evidence from Sheikhupura District in Northern Punjab, suggests that *An. stephensi* may be a more important vector than previously believed (Rowland *et al*., 2000). It was five times more prevalent than *An. culicifacies*, and falciparum malaria cases peaked in October, after *An. culicifacies* had disappeared but when *An. stephensi* was still present (Rowland *et al*., 2000). There are three ecological variants of *An. stephensi*: 'type', 'intermediate' and 'mysorensis' (Sweet & Rao, 1937; Subbarao *et al*., 1987), with only the latter two occurring in rural areas. We do not yet know which of the five sibling species of the *An. culicifacies* complex (Kar *et al*., 1999), nor which ecological variants of *An. stephensi*, occur in our study area (Tyagi & Verma, 1991).

Results from monthly active case detection surveys in our study villages revealed that malaria prevalence remained very low. We are not in a position to discern whether this is attributable to the low densities of adult *An. culicifacies* (Herrel *et al*., in preparation) or other factors such as the harsh climatic conditions, which may have limited *Plasmodium* development and/or shortened the lifespan of female anophelines.

We observed a temporal pattern (data not shown) in the occurrence of anopheline larvae with the greatest proportions of positive samples collected from August to December, which broadly corresponds to the period with progressively cooler temperatures. Proportions of positive samples declined in winter (January–March) and summer (April–June). There was no clear temporal trend for *An. stephensi*, unlike previous findings (Reisen *et al*., 1981). *Anopheles culicifacies* was more frequently collected in the months September to December. Our observations support previous findings, which show that temperature changes result in a temporal shift of *An. subpictus*, which dominates in the warmer season (Ansari & Nasir, 1955; Reisen *et al*., 1981) and attains a peak occurrence of 45% in October. This has been attributed to its inability to tolerate low temperatures (Ansari & Nasir, 1955), as well as its ability to withstand higher conductivity, turbidity, pH and organic pollution with ammonia of sites that dessicate during the warmer season (Reisen *et al*., 1981). Previous workers have found that *An. subpictus* larvae and adults disappear completely from January to June and conclude that the species is reintroduced annually with the onset of the monsoon (Ansari & Nasir, 1955; Reisen, 1978; Reisen *et al*., 1982; Reisen & Milby, 1986). Although numbers were reduced, we still found *An. subpictus* larvae in this period, which suggests that immatures of this species are able to overwinter.

In terms of chemical water quality, we did not find conclusive evidence for associations with anopheline abundance nor occurrence. Other studies indicate that *An. culicifacies* abundance is positively associated with dissolved oxygen in Sri Lanka (Amerasinghe *et al*., 1995), India (Russel & Rao, 1942) and Pakistan (Reisen *et al*., 1981). The range 28–32°C has been shown to be an important determinant for the development of immature stages of *An. culicifacies* in India (Pal, 1945). *Anopheles stephensi* is inversely correlated with temperature and positively correlated with pH (Reisen *et al*., 1981). The preference of *An. stephensi* for slightly acidic sites has been noted in Rajasthan (Tyagi *et al*., 1992) and in Pakistan it has been collected in highly saline sites (unpublished, reported in Reisen *et al*., 1981).

Our study suffered from the usual limitations associated with larval sampling as described by Service (1993). It is well established that there is unequal dispersion of larvae and clustering occurs within habitats but, for practical reasons, we assumed homogenous distribution of larvae within habitats. Although samples were taken in proportion to surface area, the maximum number of dips from any single site was 180, even when sites far exceeded 50 m^2. Especially for larger habitats, which are ecologically more diverse than small water bodies, it is possible that we missed a substantial proportion of the larval population. Although we found significant relationships between species occurrence and site types as well as environmental variables, we experienced difficulties in interpreting our abundance data. Factors such as predator densities, inter- and intraspecific competition and other variables undoubtedly influenced the abundance of *Anopheles* immature stages in each site. However, apart from the general associations summarized in Table 5 we were unable, for logistical reasons, to determine correlations (negative or positive) with each type of potential predator and other aquatic fauna, as interpreted by Victor & Reuben (1999) and Sunish & Reuben (2001) for mosquito immature populations in rice fields of South India. Moreover, we ignored the possible influences of fertilizers (Victor & Reuben, 2000; Sunish & Reuben, 2001), pesticides and insecticide resistance.

We found some interesting contrasts in the anopheline fauna between villages. *Anopheles culicifacies* larvae and most of the six anopheline species were collected from village 149/6R at the head of the distributary. In contrast to the other villages, it was characterized by high groundwater tables and had waterlogged fields and fishponds. In the tail-end village, species diversity was much lower and *An. subpictus* overwhelmingly dominated larval collections. The differences in the species encountered were dependent on the site types present in villages, which are directly related to the irrigation water management practices and groundwater levels. These contrasting patterns merit further research in order to establish how they might related to the large-scale situation in the Punjab.

To facilitate the planning of environmental control measures, there is a need for further investigations on bionomics and ecology of anopheline mosquito developmental stages, especially in irrigated agricultural systems. Detailed small-area studies as presented here should be combined with assessments of land use over large areas through remote sensing and satellite imagery within a Geographical Information System framework (Thomson & Connor, 2000).

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