Importance of Waste Stabilization Ponds and Wastewater Irrigation in the Generation of Vector Mosquitoes in Pakistan

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ABSTRACT The objective of the current study was to investigate the role of waste stabilization ponds (WSP) and wastewater-irrigated sites for the production of mosquitoes of medical importance. Mosquito larvae were collected fortnightly from July 2001 to June 2002 in Faisalabad, Pakistan. In total, 3,132 water samples from WSP and irrigated areas yielded 606,053 *Culex* larvae of five species. In addition, 107,113 anophelines, representing eight species were collected. *Anopheles subpictus* (Grassi) and *Culex* mosquitoes, especially *Culex quinquefasciatus* (Say) and *Culex tritaeniorhynchus* (Giles), showed an overwhelming preference for anaerobic ponds, which receive untreated wastewater. Facultative ponds generated lower numbers of both *Anopheles* and *Culex* mosquitoes, whereas the last ponds in the series, the maturation ponds, were the least productive for both mosquito genera. *An. subpictus* and *Anopheles stephensi* (Liston) were the dominant *Anopheles* species in wastewater-irrigated sites, with *Anopheles culicifacies* (Giles) recorded in low numbers. This was also the pattern in nearby sites, irrigated with river water. Among the *Culex* species, *Cx. tritaeniorhynchus* was by far the most frequently recorded in both wastewater- and river water-irrigated sites with *Cx. quinquefasciatus* as the second most abundant species but restricted to wastewater-irrigated areas. Univariate logistic regression analysis showed that presence of *An. subpictus* and *Culex* mosquitoes was significantly associated with emergent grass vegetation and low salinity. Regular removal of emergent grass along the margins of the anaerobic ponds and changes in the concrete design of the ponds are likely to reduce the mosquito production, especially of *Culex* species.

KEY WORDS irrigation, mosquito production, Pakistan, ponds, wastewater

With increasing water scarcity, wastewater from cities provides a valuable source of irrigation water for urban and periurban agriculture. However, urban wastewater contains contaminants that could pose risks to human health and agricultural sustainability, and it should therefore be treated before use in agriculture. In tropical developing countries, a system of waste stabilization ponds (WSP) is often promoted as a low-cost, effective, and robust treatment approach for the removal of organic material, helminth eggs, most bacteria and viruses (Mara 2000). WSP are shallow, artificial basins through which sewage flows. Treatment occurs through natural physical, chemical, and biological processes. One of the downsides of WSP is that they create large, stagnant open water bodies close to urban centers. The nutrient-rich wastewater may contribute to intense vegetation growth, creating opportunities for mosquitoes to oviposit. It also may provide food for larvae and protection to mosquito larvae against predators (Ikeshoji et al. 1967, Rutz and Axtell 1978, Kramer and Mulla 1979).

Limited attention has been paid to the potential role of WSP and wastewater-based irrigation systems in the generation of vector mosquitoes. The few studies that have been undertaken indicate that before such permanent water bodies are constructed close to large urban areas, consideration should be given to the possible health impact on the local communities. In Nigeria, for example, communities living around WSP were found to suffer more frequently from mosquito nuisance and malaria than those who lived >300 m away (Agunwamba 2001). Carlson et al. (1986) and Carlson and Knight (1987) recorded extremely high populations of *Culex quinquefasciatus* and *Culex nigripalpus* (Theobald) in WSP in Florida. A study in Southern Punjab, Pakistan, documented the important contribution of defunct wastewater disposal ponds and the surrounding wastewater irrigation system in the generation of vector mosquitoes (Mukhtar et al. 2003).

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A nationwide survey in Pakistan showed that in 80% of all cities with a population of >10,000 inhabitants used wastewater in agriculture, whereas only 2% of all cities had wastewater treatment facilities (Ensink et al. 2004). Faisalabad is one of the few cities in Pakistan with a functional WSP system. This offered a good opportunity to assess the importance of WSP and wastewater irrigation for the generation of vector mosquitoes. Such information would help in future design of WSP systems and in improving understanding of the potential health impacts of such facilities, especially in arid and semiarid regions.

The main objectives of the current study were to investigate the role of WSP in the generation of mosquitoes and the specific mosquito habitat and water quality characteristics of importance within the WSP system. Further, the study aimed to document the production of mosquitoes in irrigated areas surrounding the WSP, including irrigation systems using wastewater as well as river water. The potential health risks associated with mosquito breeding in WSP and wastewater-irrigated sites are discussed and the specific interventions for disease vector control of relevance for WSP and wastewater irrigation are highlighted.

Materials and Methods

Study Area. Faisalabad is the third largest city of Pakistan with >2,000,000 inhabitants. It is situated between two major perennial rivers, Ravi and Chenab, at 73.08′ E, 31.25′ N and at an altitude of 214 m above sea level. Meteorological information made available by the government station in Faisalabad from September 2001 to August 2002 showed a mean monthly maximum temperature ranging from 21°C in January 2002 to 43°C in May 2002 and a mean monthly minimum temperature ranging from 6°C in January 2002 to 30°C in July 2002. Recordings from September 2001 to August 2002 showed a total precipitation of 190 mm and a total pan evaporation of 1,992 mm. Land use in the area around Faisalabad is dominated by irrigated agriculture using water from the Ravi and Chenab rivers. In the city itself a survey identified nine sites, with a total area of >2,200 ha, where wastewater was used for irrigation.

Waste Stabilization Ponds. Wastewater from the western zone of the city is treated in a WSP system, which is fed by a large trunk sewer. This is mainly domestic wastewater, because most industries are located elsewhere in the city, and little industrial effluent ends up in the WSP. The WSP system is located in a densely populated periurban area of Faisalabad.

The WSP system had two series of six ponds in parallel, with each series consisting of three anaerobic ponds and three facultative ponds, with the last facultative pond in each series designed to act as a maturation pond. The anaerobic ponds were 150 m in length, 200 m in width, and 3.5 m in depth, and the facultative and maturation ponds were 500 m in length, 250 m in width and 1.5 m in depth. The ponds in each series were connected through open concrete channels. The daily inflow to the WSP was designed to be 90,000 m^3/d with a hydraulic retention time of 19 d, but the actual retention time at the time of study varied between 25 and 67 d. Because of poor maintenance and poor construction of the system, the cemented slanting walls of ponds, particularly around the anaerobic ponds had broken at different points. This led to the emergence of dense vegetation along the pond margins. The removal of the grids in the main sewage drain had allowed for large pieces of floating solid waste, including tree branches, plastic bags, and empty bottles to enter the anaerobic ponds. However, in the facultative and maturation ponds only grass, at a few points, and occasionally floating waste along the margins were visible.

Irrigated Sites. Just over one-half of the wastewater pumped to the WSP (45,000 m^3/d) was diverted and used in the adjacent fields before it could reach the anaerobic ponds. From November to April/May, wastewater use was high to allow for wheat and vegetable cultivation. The most commonly irrigated crops were fodder, sugarcane, wheat, and vegetables that included cauliflower, aubergine, spinach, and chilies.

Agricultural fields within a 2-km radius of the WSP were divided into two distinct zones according to the pattern of irrigation. In zone I, only untreated wastewater was used for irrigation and covered an area of ≈180–200 ha. In zone II, only water originating from the rivers was used for irrigation, covering an area of 125 ha. Within each irrigated zone, all water bodies were classified into the following three categories:

1. Irrigation channels: earthen channels that deliver water to fields, with flowing water, continuous standing water, or pools present.
2. Irrigated fields: fields that have been deliberately inundated with water for cultivation purposes, including field pools created after irrigation and standing water in the fields.
3. Small ponds: ditches that remained unfilled after, for example, roads or house construction activities and received rainwater, domestic water, or drainage water from agricultural fields.

Larval Collections. From July 2001 to June 2002, mosquito larvae were collected fortnightly from the WSP and surrounding irrigated area on separate consecutive days. The samples were collected by using a standard 350-ml aluminum dipper with a 1-m handle, and the dipping rate was six dips per m^2 of surface area for smaller habitats (Herrel et al. 2001). For larger sites, a "sample" representing 30 dips was taken within a 5-m^2 area (equivalent to six dips per m^2). For habitats with a surface area between 5 and 10 m^2, one sample was collected, whereas for habitats with a surface area between 11 and 20 m^2, two samples were taken, and for habitats with a surface area between 21 and 30 m^2, three samples, and so on. The upper limit of samples was six from a habitat with a size of 50 m^2 or more. For example, from a stabilization pond six well-dispersed samples were collected from different points along the margin. The percentage area of pond margin covered by vegetation also was calculated at the start of the study to decide on the number of samples to be taken from sites with and without vegetation, reflecting the habitat characteristics of the different ponds and establishing the basis for a sampling frame. On each collection date, three (three of six) samples were collected from emergent vegetation in the anaerobic ponds, but only one sample (one of six) was collected from emergent vegetation in the facultative and maturation ponds. For irrigated sites, the selection of samples from different sites was decided at the start of the study and was relative to the abundance of different site types. On each collection date, five irrigation canals, two irrigated fields, and two small ponds were sampled from both wastewater and river water-irrigated areas.

Mosquito Identification. Collected larvae were preserved in vials containing 70% isopropyl or alcohol for later identification. The third and fourth instars were identified, whereas early instars and damaged larvae were only counted, noted, and discarded. Pupae were neither counted nor recorded. The keys of Harbach (1988), Rao (1984), Reuben et al. (1994), and M. M. et al. (unpublished data) were used for *Culex* identification, and the key of Amerasinghe et al. (2002) was used for anopheline identification. To confirm specific identifications, representative samples also were reared in the laboratory to the adult stage.

Habitat Characteristics. On each WSP sampling visit, physical, chemical, and vegetation characteristics of individual habitats were noted in situ. The flora was characterized as algae, *Spirogyra* sp., grass, *Typha* sp., and debris.

Substrates were classified as either soil or cement, and water was recorded as flowing or standing. Additionally, in all sites the light conditions of habitats were recorded as exposed, partially shaded, or shaded. Predators included fish, water bugs (Hemiptera: Notonectidae; *Diplonychus* sp.), water beetles and water beetle larvae (Coleoptera: Dytiscidae; *Dytiscus* sp.), damselfly larvae (Odonata: Agrionidae; *Agrion* sp.), dragonfly larvae (Odonata: Libellulidae; *Pantala* sp.), water scorpions (Hemiptera: Nepidae; *Nepa* sp.), water boatman (Hemiptera: Corixidae; *Corixa* sp.), and backswimmers (Hemiptera: Notonectidae; *Notonecta* sp.).

Electrical conductivity (EC; model CO150, Hach Company, Loveland, CO) and pH (Hanna Instruments, Woonsocket, RI) were measured on freshly collected water samples in the dipper itself in situ. For each WSP, three readings were taken for each sample, and the average of these readings was recorded. To ensure the accuracy of water quality measurements, the instruments also were calibrated in the field once before actual readings took place. Additionally, to establish a nutrient-related water chemistry profile of the different ponds and their possible correlation with vector mosquito populations, the samples were analyzed in the laboratory monthly for biochemical oxygen demand (BOD), total phosphorus (P_2O_4), ammonium (NH_4), and turbidity by using standard methods (APHA et al. 1998). Samples for water quality analysis were stored on ice, transported in an icebox, and analyzed within 6 h of sampling.

Data Analysis. Abundance of mosquito larvae was highly skewed and therefore geometric means of the number of larvae per 1,000 dips were used with their respective 95% confidence intervals (CI). For the calculations a $\ln(x + 1)$ transformation was used to allow for samples with no larvae. Samples also were classified as positive or negative for each of the mosquito species. Presence or absence of mosquito larvae was used as binary outcome variable in logistic regression models to estimate the magnitude of the association of a particular species with site type and habitat characteristics. The analysis was carried out for each species individually, for all anophelines, and for all culicines. Logistic regression is a nonparametric method that generates regression coefficients that can be directly converted to odds ratios. Odds ratio in this context is defined as the odds of a certain factor being present in breeding sites positive for a particular species divided by the odds of the factor being present in breeding sites negative for that species. The results of the logistic regression analyses were reported as odds ratios with their 95% CI.

Although the hydraulic retention time in each pond was shorter than the sampling interval, one could argue that repeated samples from the same pond were not independent. The use of inferential statistics would then lead to an inappropriate increase in the precision of the estimates, such as means and differences between means. This is the issue of pseudoreplication, a potential problem in most ecological studies (Hurlbert 1984). To avoid this problem, we limited the use of inferential statistics on the data. For the logistic regressions, we adopted a robust (conservative) method in which each individual pond was included as a random effect. This approach resulted in wider CIs for the odds ratios than if fixed effects models would be used.

The characteristics substratum, exposure to light, and water flow were dropped from the analysis because all ponds had a concrete substratum, water was always standing, and all collected samples were exposed to sunlight. Also, only the presence or absence of emergent grass was analyzed, because the other vegetation types were found in very low numbers. The software package used for the data analysis was STATA 7.0 (Stata Corp., College Station, TX).

Results

Site Availability. WSP were permanent water bodies; therefore, they were sampled throughout the study period. From each pond, an equal number of samples ($n = 162$) was collected. More samples were collected from wastewater-irrigated areas ($n = 848$) than from river water-irrigated areas ($n = 340$), because river water-irrigated areas were affected by annual canal closure and inadequate water allocation.

Sample Positivity Rate and Mosquito Production in WSP. Of the total 1,944 water samples collected from the WSP, 20% contained mosquito larvae (Table 1). *Culex* and *Anopheles* immatures occurred in 19 and 12% of samples respectively. Overall, only two *Anoph-*

Table 1. Occurrence and abundance of *Culex* and *Anopheles* mosquitoes in waste stabilization ponds

Species with total no. collected	Anaerobic ponds (n = 972)		Facultative ponds (n = 648)		Maturation ponds (n = 324)		Total (n = 1,944)	
	Positive (%)	Mean (95% CI)	Positive (%)	Mean (95% CI)	Positive (%)	Mean (95% CI)	Positive (%)	Mean (95% CI)
An. stephensi (5,530)	48 (5)	0.25 (0.17–0.33)	42 (7)	0.43 (0.29–0.59)	9 (3)	0.17 (0.05–0.29)	99 (5)	0.29 (0.23–0.36)
An. subpictus (27,866)	153 (16)	1.41 (1.11–1.76)	21 (3)	0.17 (0.09–0.26)	2 (1)	0.02 (0.00–0.07)	176 (9)	0.64 (0.53–0.77)
An. culicifacies (1,443)			29 (5)	0.24 (0.15–0.34)	7 (2)	0.12 (0.03–0.22)	36 (2)	0.09 (0.06–0.13)
An. pulcherrimus (7)			1 (0)	0.01 (0.00–0.02)			1 (0)	0.00 (0.00–0.01)
An. peditaeniatus (39)			5 (1)	0.03 (0.00–0.05)	2 (1)	0.02 (0.00–0.05)	7 (0)	0.01 (0.00–0.02)
An. nigerrimus (99)			12 (2)	0.06 (0.03–0.10)	2 (1)	0.02 (0.00–0.06)	14 (1)	0.02 (0.01–0.04)
Total *Anopheles* (34,984)	163 (17)	1.55 (1.22–1.93)	52 (8)	0.62 (0.42–0.84)	10 (3)	0.20 (0.07–0.35)	225 (12)	0.93 (0.78–1.10)
Cx.quinquefasciatus (338,005)	304 (31)	9.25 (8.16–12.9)	9 (1)	0.06 (0.02–0.11)	2 (1)	0.03 (0.00–0.07)	315 (16)	2.28 (1.90–2.72)
Cx. tritaeniorhynchus (139,288)	218 (22)	3.55 (2.79–5.47)	42 (7)	0.49 (0.32–0.68)	4 (1)	0.06 (0.00–0.13)	264 (14)	1.46 (1.22–1.73)
Cx. pipiens (42,953)	203 (21)	2.65 (2.10–3.30)	2 (0)	0.02 (0.00–0.04)	1 (0)	0.01 (0.00–0.03)	206 (11)	0.92 (0.76–1.10)
Cx.pseudovishnui (247)			16 (3)	0.10 (0.05–0.16)	1 (0)	0.01 (0.00–0.04)	17 (1)	0.04 (0.02–0.05)
Total *Culex* (520,493)	309 (32)	13.4 (10.3–18.4)	46 (7)	0.54 (0.36–0.75)	12 (4)	0.10 (0.02–0.19)	367 (19)	3.46 (2.88–4.12)
Total mosquitoes	316 (33)	14.7 (11.3–19.1)	51 (8)	0.74 (0.50–1.02)	14 (4)	0.23 (0.09–0.40)	381 (20)	3.94 (3.28–4.70)

Occurrence and abundance expressed as the number (percentage in parentheses) of samples positive for larvae and the geometric mean (95% CI in parentheses) of larvae collected per 1,000 dips.

eles species, *Anopheles subpictus* (Grassi) and *Anopheles stephensi* (Liston), and three *Culex* species, *Culex quinquefasciatus* (Say), *Culex tritaeniorhynchus* (Giles), and *Culex pipiens* L. were found in high numbers. *Anopheles culicifacies* (Giles) was found less commonly, and *Anopheles nigerrimus* (Giles), *Anopheles peditaeniatus* (Leicester), *Anopheles pulcherrimus* (Theobald), *Culex bitaeniorhynchus* (Giles), and *Culex pseudovishnui* (Colless) were recorded in only trivial numbers.

The anaerobic ponds were clearly the most productive in terms of mosquito breeding. This applied to *Culex* as well as *Anopheles* species (Table 1). Among the *Culex* species collected from anaerobic ponds, *Cx. quinquefasciatus* was the most common followed by *Cx. tritaeniorhynchus* and *Cx. pipiens.* Among the *Anopheles*, *An. subpictus* was the most common species.

Cx. tritaeniorhynchus predominated among *Culex* species in the facultative ponds. Among the anophelines, *An. stephensi* was the dominant species associated with facultative ponds, but this habitat was also of importance for *An. subpictus* and *An. culicifacies.* For both *Culex* and anophelines, the maturation ponds were clearly the least productive.

Irrigated Sites. Of the total 1,188 collected samples, 38 and 44% were mosquito positive in wastewater- and river water-irrigated areas, respectively (Table 2), with *An. subpictus* and *An. stephensi* the dominant *Anopheles* species in both types of sites. In both sites, *An. subpictus* predominated in small ponds, and, to a lesser extent, irrigation channels, whereas *An. stephensi* was most common in irrigated fields. *An. culicifacies* was recorded from both wastewater- and river water-irrigated sites but in low numbers. Other *Anopheles* species, *Anopheles barbirostris* (van der Wulp), *An. peditaeniatus,* and *Anopheles maculatus* (Theobald), were collected in very small numbers and primarily in river water-irrigated areas. Among the *Culex*, *Cx. tritaeniorhynchus* was by far the species most frequently recorded in both wastewater- and river water-irrigated sites and primarily in irrigated fields and small ponds. *Cx. quinquefasciatus* was the second most abundant species of that genus, but almost exclusively restricted to wastewater-irrigated areas, occurring primarily in irrigation canals and small ponds.

Habitat Characteristics within the WSP and Occurrence of Mosquito Species. Anaerobic and facultative ponds are primarily designed for BOD removal, and as expected, BOD levels went down as water moved through the WSP system (Table 3). Compared with the maturation ponds, the anaerobic ponds were characterized by low levels of EC, pH, and high levels of ammonia, total phosphorus, and turbidity. For the parameters measured, the facultative ponds had intermediate values between those of the anaerobic and maturation ponds.

Univariate logistic regressions confirmed that the anaerobic and facultative ponds were the most important habitats within the WSP for anopheline and *Culex* mosquitoes (Table 4). *Culex* mosquitoes and *An. subpictus* were significantly associated with high levels of BOD, presence of emergent grass, and low salinity. *An. stephensi* was associated with the presence of grass and high values of total phosphorous. *An. culicifacies* showed no significant association with any of the parameters, possibly because of the low number of samples that were positive for this species.

Discussion

Wastewater, an Important Habitat for *Culex* Immatures and *An. subpictus*. The anaerobic ponds, with their high levels of BOD, ammonium, total phosphorous, and turbidity generated extremely high densities of three *Culex* species, namely, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *Cx. pipiens.* In these ponds, wastewater was at an early stage of treatment and extremely foul smelling. The preference of *Cx quin-*

Table 2. Mosquito species abundance by habitat type in irrigated areas of Faisalabad City

Habitat	Wastewater irrigated				River water irrigated			
		IC	IF	SP		IC	IF	SP
Total samples	848	268	329	251	340	118	142	80
Mosquito positive (%)	38	20	48	44	44	20	54	59
Species	Total collected	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Total collected	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
An. stephensi	9,447	0.30 (0.13–0.50)	7.36 (4.85–10.9)	4.11 (2.53–6.39)	10,041	0.83 (0.32–1.53)	49.70 (26.0–94.3)	13.20 (5.7–29.2)
An. subpictus	38,446	1.87 (1.05–3.02)	1.88 (1.24–2.71)	3.89 (2.24–6.37)	6,225	2.01 (0.78–4.07)	0.23 (0.02–0.48)	7.03 (2.73–16.3)
An. culicifacies	2,417	0.14 (0.03–0.26)	2.49 (1.63–3.62)	0.68 (0.36–1.08)	3,732	15.00 (7.98–27.4)	0.11 (0.00–0.26)	2.68 (1.03–5.67)
An. pulcherrimus	176		0.36 (0.19–0.56)	0.14 (0.03–0.25)	532		1.61 (0.79–2.83)	
An. peditaeniatus	177		0.28 (0.13–0.46)	0.07 (0.00–0.15)	108		0.57 (0.22–1.01)	0.07 (0.00–0.22)
An. nigerrimus	154		0.34 (0.17–0.52)	0.16 (0.04–0.29)	475	0.05 (0.00–0.14)	3.15 (1.74–5.29)	0.41 (0.05–0.91)
An. barbirostris	0				111		0.70 (0.30–1.20)	
An. maculatus	0				88		0.38 (0.11–0.73)	
Total *Anopheles*	50,817	2.50 (1.46–3.99)	23.9 (15.9–35.8)	18.3 (11.3–29.6)	21,312	4.12 (1.89–8.10)	77.4 (39.1–153)	69.0 (28.8–163)
Cx. quinquefasciatus	17,489	0.61 (0.28–1.03)	0.88 (0.51–1.33)	2.37 (1.35–3.82)	99		0.10 (0.00–0.22)	0.32 (0.00–0.74)
Cx. tritaeniorhynchus	52,534	1.32 (0.73–2.11)	16.90 (10.8–25.9)	13.50 (7.96–22.4)	12,927	0.70 (0.20–1.41)	27.1 (13.8–52.1)	13.70 (5.51–32.4)
Cx. pseudovishnui	635	0.04 (0.00–0.10)	1.05 (0.66–1.52)	0.30 (0.12–0.51)	880	3.16 (1.70–5.42)	0.10 (0.00–0.26)	1.42 (0.52–2.86)
Cx. pipiens	633	0.12 (0.00–0.24)		0.33 (0.12–0.58)	23		0.05 (0.00–0.15)	
Cx. bitaeniorhyanchus	233	0.04 (0.00–0.10)	0.14 (0.04–0.26)	0.11 (0.01–0.23)	107		0.43 (0.13–0.83)	0.08 (0.00–0.24)
Total *Culex*	71,524	2.19 (1.26–3.49)	21.1 (13.5–32.6)	19.4 (11.3–33.8)	14,036	0.88 (0.29–1.72)	29.1 (14.7–56.7)	17.0 (6.84–40.5)

IC, irrigation canal; IF, irrigated field; SP, small pond.
Data are number of larvae collected and geometric mean number of larvae collected per 1,000 dips (95% CI in parentheses).

Table 3. Water quality characteristics of different ponds within the waste stabilization pond system of Faisalabad City

	n	Anaerobic ponds		Facultative ponds		Maturation ponds	
		Mean	Range	Mean	Range	Mean	Range
BOD (mg/liter)	72	193 (32)	129–247	149 (32)	88–203	110 (41)	39–188
EC (dS/m)	324	2.0 (0.6)	0.1–3.5	3.6 (0.6)	0.7–5.6	3.7 (0.4)	2.4–5.0
NH_4 (mg/liter)	72	40 (12)	16–62	31 (11)	10–52	21 (10)	4–38
Total phosphorus (mg/liter)	72	5.2 (1.6)	3.3–8.4	4.6 (1.2)	3.3–7.2	4.1 (1.0)	2.9–6.0
pH	324	7.2 (0.3)	5.7–8.0	7.6 (0.3)	6.4–8.8	7.7 (0.3)	6.2–8.6
Turbidity (FAU)	72	272 (93)	83–398	128 (48)	30–216	67 (24)	19–128

FAU, formazine attenuation unit.
Data are arithmetic means with standard deviations in parentheses.

quefasciatus and *Cx. pipiens* for such wastewater sites agrees with findings of Carlson et al. (1986) and Carlson and Knight (1987). Similarly, Mukhtar et al. (2003) and O'Meara and Evans (1983) found anaerobic components of wastewater treatment systems to be major habitats for *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* in South Punjab, Pakistan, and for *Cx. quinquefasciatus* in south Florida, respectively. Of four *Culex* species found in irrigated sites, *Cx. tritaeniorhynchus* was by far the most abundant, especially in irrigated fields, in agreement with previous observations elsewhere in Asia (Reuben 1971, Floore et al. 1971, Gould et al. 1974, Reisen et al. 1976).

Of the three *Anopheles* species identified in substantial numbers in this study, only *An. subpictus*, which predominated in wastewater-irrigated sites, was significantly associated with highly polluted anaerobic ponds. This species is generally considered to tolerate a wide range of physicochemical conditions, including a high content of organic matter (Ansari and Shah 1950, Reisen et al. 1981) and has previously been found in the Punjab to breed in foul and polluted habitats, including street drains, street pools, and septic tanks (Reisen et al. 1981, Herrel et al. 2001). The association of *An. stephensi* with cleaner water is in line with previous studies from the Punjab (Reisen et al.

Table 4. Association of major mosquito species with habitat characteristics of the waste stabilization ponds

	n	*An. subpictus*		*An. stephensi*		*An. culicifacies*		Total *Culex*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Pond									
Maturation	324	1.0		1.0		1.0		1.0	
Facultative	648	5.4[a]	1.3–23	2.4[a]	1.2–5.0	2.1	0.9–4.9	2.0[a]	1.0–3.8
Anaerobic	972	30[a]	7.4–122	1.8	0.9–3.7			12[a]	6.7–22
Grasses									
No emergent grass	1,367	1.0		1.0		1.0		1.0	
Emergent grass	577	2.2[a]	1.2–4.3	1.3[a]	1.0–1.5	0.5	0.2–1.4	2.9[a]	1.7–5.0
EC (dS/m)									
0–1.5	203	1.0		1.0		1.0		1.0	
>1.5–2.5	674	0.7[a]	0.7–0.8	0.8[a]	0.8–0.9			0.7[a]	0.6–0.8
>2.5–3.5	421	0.5	0.1–2.1	1.6[a]	1.3–1.9			0.3[a]	0.1–0.7
>3.5	646	0.1[a]	0.0–0.2	0.9	0.4–2.0			0.1[a]	0.1–0.2
BOD (mg/liter)									
0–120	252	1.0		1.0		1.0		1.0	
>120–175	648	17	0.6–451	2.4	0.9–6.9	2.1	0.5–8.6	6.7[a]	2.1–21
>175	756	38[a]	2.3–646	1.9	0.9–3.8	0.1	0.0–2.2	13[a]	6.2–25
TP (mg/liter)									
< 4.0	396	1.0		1.0		1.0		1.0	
4.0–4.3	432	2.2	0.7–6.6	1.7[a]	1.1–2.6	1.2	0.4–3.6	1.7	0.6–4.7
4.4–6.0	444	1.4	0.6–3.5	1.0	0.6–1.9	0.8	0.1–4.5	2.6[a]	1.5–4.6
> 6.0	384	1.5	0.5–4.2	3.1[a]	1.1–8.6	1.6	0.7–3.9	1.2	0.5–3.1
NH_4 (mg/liter)									
< 26	396	1.0		1.0		1.0		1.0	
27–37	360	1.1	0.3–4.6	1.1	0.6–2.1	1.1	0.5–2.2	1.4	0.3–6.2
38–45	588	2.3	0.3–16	1.5	0.7–3.1	0.5	0.1–2.9	2.3	0.5–10
> 45	312	2.6	0.5–14	1.7	0.9–3.2	0.8	0.2–3.6	2.2	0.5–9.7
Turbidity (FAU)									
0–80	294	1.0		1.0		1.0		1.0	
81–160	558	1.6	0.1–42	0.6[a]	0.3–0.9	0.7	0.5–1.1	1.4	0.2–13
161–300	444	3.0	0.3–27	0.6	0.2–2.4			3.4	0.7–17
301–400	360	4.4	0.6–34	0.9	0.3–2.6			4.1[a]	1.1–16

FAU, formazine attenuation unit; TP, total phosphorus.
Numbers are odds ratios (OR) with their 95% CI from univariate logistic regression analysis. The first category for each habitat characteristic is the reference category.
[a] Significant association ($P < 0.05$) by using chi-square test.

1981, Herrel et al. 2001), but the occurrence of *An. stephensi* in WSP also indicates that in our studied conditions it exploits polluted habitats as well.

Although *An. culicifacies* was found in low numbers, its presence in water of relatively high organic content in both WSP and wastewater-irrigated sites is different from the findings of most other studies in South Asia and may indicate some habitat flexibility of this species (Amerasinghe et al. 1995, Tyagi and Chaudhary 1997, Herrel et al. 2001). Of the five *Culex* species, *Cx. tritaeniorhynchus* was most prevalent in the intermediate-state wastewater, confirming previous findings (Reisen et al. 1981).

When comparing the findings from this study with previous studies, it is important to point out that the BOD levels recorded were below values often found in ponds with untreated wastewater in developing countries where values between 400 and 800 mg/liter are common (Cairncross and Feachem 1993).

Role of Facultative and Maturation Ponds. The prolonged stay of the wastewater in facultative and maturation ponds changed water quality significantly with reductions in levels of BOD, ammonium, total phosphorus, and turbidity and increase in EC. In the cleaner water, the larval density was lower, but the diversity of species collected higher than in the anaerobic environment. The overall lower density of mosquitoes collected from the facultative and maturation ponds is likely to be influenced by the very small area of these pond margins that were covered by vegetation (1–2%). By contrast, the anaerobic ponds, with an average of ≥50% of their margins covered by emergent vegetation, were presumably more attractive for oviposition, likely to be rich in food, and provided protection against predators.

Public Health Importance. The WSP and irrigation sites surveyed accommodated a substantial number of mosquito species with the potential for increasing the occurrence of vector-borne diseases among the urban communities, especially those close to WSP or wastewater-irrigated sites. However, it may be important to point out that the WSP do not seem to result in the introduction of species not already present in the area.

Cx. quinquefasciatus has been found naturally infected with *Wuchereria bancrofti* in Pakistan (Aslamkhan and Pervez 1981). *Cx. pipiens* and *Cx. quinquefasciatus* have been implicated as vectors of West Nile virus in the Indo-Pakistan subcontinent (Burney and Munir 1966, Peiris and Amerasinghe 1994). *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* are vectors of Japanese encephalitis in Asia and also vectors of West Nile virus on the Indo-Pakistan subcontinent (Barnett 1967, Amerasinghe and Ariyasena 1990, Peiris and Amerasinghe 1994). The location of Pakistan next to endemic areas for West Nile virus in India and China increases the risk of outbreaks (WHO 1995). Also, the attractive environment created by the WSP and irrigation sites for a number of bird species that may act as zoonotic hosts may have to be considered when evaluating the risk of arbovirus transmission in the area.

Both of the major vectors of malaria in Pakistan, *An. stephensi* and *An. culicifacies*, were identified, and the diversity of water quality habitat types created by the irrigation system and the different treatments ponds is likely to favor colonization by these two species. However, the findings suggest that if wastewater is the source of irrigation in an existing system, then the overall abundance of malaria vectors may actually be reduced, because this irrigation system will become a less productive site for the key malaria vectors.

Species Complex and Habitat Preferences. In the current study, we have not identified which of the sibling species within the *An. culicifacies* and *An. subpictus* complexes or ecological variants of *An. stephensi* occurred in the surveyed sites. Because the different siblings have different vector potential, it will be important in future studies to establish whether the siblings in wastewater habitats are any different from those in irrigated areas or in other habitats (Subbarao et al. 1988, Herrel et al. 2004).

Vector Control. A few design changes of the system of WSP at Faisalabad are worth considering when designing future system. The WSP are very big and rectangular with slanting walls. Owing to their large size, water moves at negligible speed, and their rectangular shape maintains standing water in the corners. Additionally, slanted walls enhance the free growth of vegetation along the pond margin. If the WSP could be designed and managed to reduce the amount of emergent vegetation, this is likely to reduce mosquito production in these systems.

It is clear that to reduce the potential impact of WSP on *Culex* production, the focus should be on the anaerobic ponds. During the peak periods of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* production, larvicides and vegetation management may be practiced to mitigate both disease risks and nuisances linked with the very high number of mosquitoes generated.

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