Impact of Phlebotomine Sand Flies on U.S. Military Operations at Tallil Air Base, Iraq: 2. Temporal and Geographic Distribution of Sand Flies

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ABSTRACT CDC miniature light traps were used to evaluate the general biology of phlebotomine sand flies from April 2003 to November 2004 at Tallil Air Base, Iraq. Factors evaluated include species diversity and temporal (daily and seasonal) and geographic distribution of the sand flies. In addition, the abundance of sand flies inside and outside tents and buildings was observed. In total, 61,630 sand flies were collected during 1,174 trap nights (mean 52 per trap, range 0–1,161), with 90% of traps containing sand flies. Sand fly numbers were low in April, rose through May, were highest from mid-June to early September, and dropped rapidly in late September and October. More than 70% of the sand flies were female, and of these sand flies, 8% contained visible blood. *Phlebotomus alexandri* Sinton, *Phlebotomus papatasi* Scopoli, *Phlebotomus sergenti* Parrot, and *Sergentomyia* spp. accounted for 30, 24, 1, and 45% of the sand flies that were identified, respectively. *P. alexandri* was more abundant earlier in the season (April and May) than *P. papatasi*, whereas *P. papatasi* predominated later in the season (August and September). Studies on the nocturnal activity of sand flies indicated that they were most active early in the evening during the cooler months, whereas they were more active in the middle of the night during the hotter months. Light traps placed inside tents with and without air conditioners collected 83 and 70% fewer sand flies, respectively, than did light traps placed outside the tents. The implications of these findings to *Leishmania* transmission in the vicinity of Tallil Air Base are discussed.

KEY WORDS phlebotomine, Iraq, ecology, sandfly, distribution

Phlebotomine sand flies are of widespread importance in the transmission of *Leishmania* pathogens in Iraq. The sand flies of Iraq were described by Newstead (1920), Adler and Theodor (1929), and Pringle (1952). Most recently, Abul-Hab and Ahmed (1984) reported 14 species of phlebotomine sand flies in Iraq, to include six species of *Phlebotomus* and eight species of *Sergentomyia.* Of these 14 species, *Phlebotomus papatasi* Scopoli, *Phlebotomus sergenti* Parrot, and *Phlebotomus alexandri* Sinton are the only anthropophilic sand flies considered to be important vectors of *Leishmania* in Iraq. *P. sergenti* and *P. papatasi* are almost certainly the primary vectors of *Leishmania tropica* (Wright) and *Leishmania major* Yakimoff & Schokhor, respectively (Al-Azawi and Abul-Hab 1977, Killick-Kendrick et al. 1985, Al-Zahrani et al. 1988, Killick-Kendrick 1990). Sukkar (1978) suggested that *P. papatasi* was also the primary vector of visceral leishmaniasis [*Leishmania donovani* (Laveran & Mesnil) and/or *Leishmania infantum* Nicolle]; however, *P. papatasi* is a restricted vector and is incapable of transmitting any species of *Leishmania* other than *L. major* (Sacks and Kamhawi 2001). Killick-Kendrick (1990) reported that data from Li-Ren et al. (1986) indicated that *P. alexandri* should be considered a potential vector of visceral leishmaniasis in Iraq. Abul-Hab and Ahmed (1984) reported that *P. alexandri* was the second most abundant and widely distributed species of *Phlebotomus* in Iraq and that because it was frequently associated with humans it should be considered a potential vector of leishmaniasis. Azizi et al. (2006) found human blood in >30% of engorged *P.*

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alexandri collected in Iran and reported that five of 120 parous females were infected with *L. infantum*. *Phlebotomus tobbi* Parrot, *Phlebotomus wenyoni* Adler, Theodor & Lourie and *Phlebotomus perfiliewi* Perfiliev could potentially play a role in the transmission of leishmaniasis in Iraq; however, little is known about the distribution of these species nor have studies been conducted to assess their potential as vectors (Abul-Hab and Ahmed 1984).

Although both cutaneous ("Baghdad Boil") and visceral leishmaniasis are widespread in Iraq, relatively few studies have examined the temporal and geographic distribution of sand flies throughout the country. The earliest published studies on the sand flies of Iraq were conducted by Newstead (1920) and Adler and Theodor (1929). Sand flies were collected and described from a number of locations throughout Iraq; however, the majority of collections were made over only a few days at each site. In 1952, Pringle published results of the first comprehensive survey of the sand flies of Iraq. In total, 12 species were collected from 57 sites in 11 distinct areas; however, >70% of the specimens were collected in and around Baghdad. *P. papatasi* was the most commonly found species in 54 of the 57 sites and accounted for 68% of the total collection. *P. sergenti* and *P. alexandri* were relatively uncommon. Pringle (1956) subsequently conducted a limited survey for sand flies in the Zagros mountains and the central plains of Iraq. Only 155 phlebotomine sand flies were collected in the mountains, of which 28% each were *P. papatasi* and *P. sergenti*, whereas 2,536 sand flies were collected in the central plains, of which 67 and 0.6% were *P. papatasi* and *P. sergenti*, respectively.

Sukkar (1974, 1978, 1985), and Sukkar et al. (1983, 1985) published a series of studies on the role of sand flies as vectors of visceral leishmaniasis in Iraq. Sukkar (1974) collected six species of phlebotomine sand flies from six locations within 35 km of Baghdad. As with the earlier study of Pringle (1952), *P. papatasi* was the predominant species, accounting for 68% of the total collection. Although *P. alexandri* accounted for only 8% of the collection, Sukkar (1974) suggested that this species might be the vector of visceral leishmaniasis in Iraq. In this study, Sukkar also assessed both the seasonal abundance and the nocturnal periodicity of sand flies. In a subsequent study, Sukkar (1978) collected seven species of sand flies from a variety of *Leishmania*-endemic locations in northern and central Iraq. *P. papatasi* accounted for 62% of the sand flies collected. Only nine *P. alexandri* and one *P. sergenti* were collected. Sukkar et al. (1985) used a combination of indoor aspiration and human bait to collect seven species of sand flies from central Iraq. *P. papatasi* and *S. baghdadis* (Adler & Theodor) accounted for >99% of the collection, with *P. papatasi* comprising 68% of the aspiration collections and 99% of the biting collections. Sukkar (1985) and Sukkar et al. (1985) subsequently reported finding flagellates in the anterior midguts of 14 *S. baghdadis* and determined (based on morphological examination) that flagellates in three of the four sand flies examined were *Leishmania* spp. Sukkar (1985) and Sukkar et al. (1985) suggested that *S. baghdadis* might be a local vector of visceral leishmaniasis; however, isoenzyme electrophoresis characterization of two of the *Leishmania* isolates indicated that they were unlike *L. infantum, L. donovani, L. tropica, L. majo,* or *L. aethiopica.* In 1985, Sukkar found that *P. alexandri* accounted for 13% of the *Phlebotomus* spp. collected in central Iraq and reiterated that this species should be considered a potential vector of visceral leishmaniasis.

In the most comprehensive studies on the seasonal abundance and daily periodicity of sand flies in Iraq, Abul-Hab and colleagues (Abul-Hab and Mehdi 1970; Abul-Hab and Al-Baghdadi 1972a,b; Mohsen and Abul-Hab 1975; Al-Azzawi and Abul-Hab 1977; Mohsen 1983; Abul-Hab and Ahmad 1984; Abul-Hab and Al-Hashimi 1988) conducted a series of studies on the sand flies in and around Baghdad, with particular emphasis on *P. papatasi.* These studies included an evaluation of the geographical distribution (Abul-Hab and Ahmed 1984), seasonal abundance (Abul-Hab and Mehdi 1970; Abul-Hab and Al-Baghdadi 1972a,b), general biology (Mohsen and Abul-Hab 1975), and vector potential (Mohsen 1983, Al-Azzawi and Abul-Hab 1977) of *P. papatasi.* Abul-Hab and Al-Hashimi (1988) also evaluated the daily biting activity of sand flies, conducting studies from May to November at a site 50 km southeast of Baghdad. *P. papatasi* was the only sand fly collected on human bait during this study.

We have previously provided an overview of our efforts during Operation Iraqi Freedom to establish a "Leishmaniasis Control Program" at Tallil Air Base (TAB) (Coleman et al. 2006). Although the primary goal of this effort was to reduce sand fly populations at TAB, we also were able to conduct a variety of studies assessing the general biology and ecology of sand flies at the base. In this article, we report the temporal and geographic distribution of sand flies at TAB.

Materials and Methods

Collection of Sand Flies. Sand flies were collected in unbaited CDC miniature light traps (model 512, John W. Hock Company, Gainesville, FL) beginning in April 2003 and continuing through October 2004. The CDC miniature light trap is the standard trap used by deployed U.S. Army Preventive Medicine units to collect mosquitoes, sand flies, and other potential disease vectors. The CDC light traps used in 2003 were provided with collection cups with standard mesh (part no. 1.44) suitable for the collection of mosquitoes; therefore, a piece of paper was taped over the wire mesh at the bottom of the collection cup to prevent the escape of sand flies. In 2004, all light traps were equipped with collection cups with fine mesh (part no. 1.49) suitable for the collection of sand flies. Although the modification of the collection cups in 2003 may have affected the operation of the traps, a comparison of collection data (species numbers and diversity) from 2003 and 2004 suggests that there was

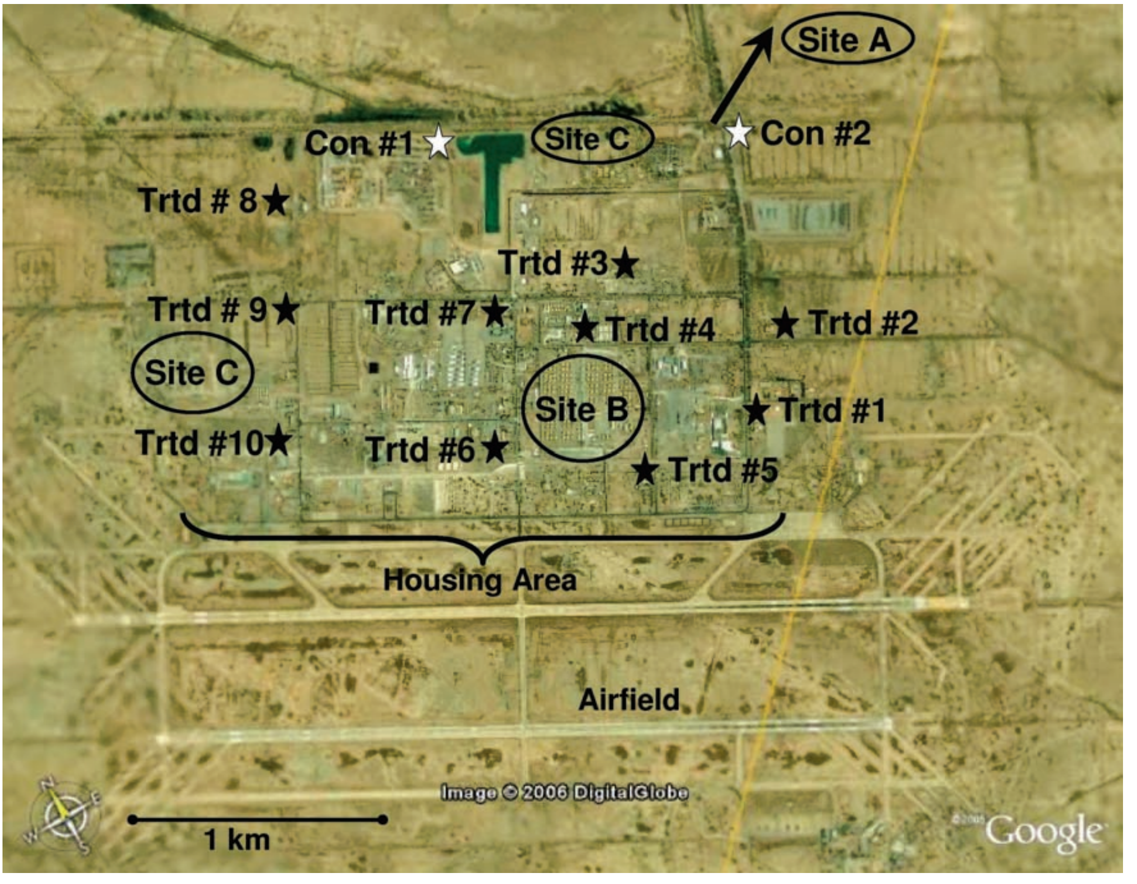

Fig. 1. Satellite image of Tallil Air Base, Iraq. The two untreated and 10 treated sites are identified by white and black stars, respectively. Other study sites include (A) the area 1.6 km northeast of TAB at which house studies were conducted, (B) the area in which studies were conducted using air-conditioned tents, and (C) the two areas in which studies were conducted using tents without air conditioning.

no effect. The CDC miniature light trap is most effective when CO_2 is used as a bait; however, neither dry ice nor compressed CO_2 was available at TAB during this study. Traps were placed at 1800 hours and collected by 0800 hours the next day (all times are local). The location of each trap was recorded using a hand-held global positioning system device. Upon return to the field laboratory, collection cups containing the sand flies were placed in a −70°C freezer. Sand flies were sorted and the numbers of male, unengorged female, and engorged female sand flies were recorded. Ten to 15% of the female sand flies and 95% of the male sand flies were shipped to the Walter Reed Army Institute of Research (Silver Spring, MD) where randomly selected specimens were identified to species. Eighty-five to 90% of the female sand flies and 5% of the male sand flies were placed in pools of one to 20 (separated by sex) for subsequent testing for *Leishmania* parasites by using a fluorogenic polymerase chain reaction (PCR) assay (male sand flies served as a negative control). These samples were stored at −70°C until tested. Results from PCR testing will be reported separately.

Temporal and Geographic Distribution of Sand Flies at TAB. Light traps were placed at 12 different sites at which military personnel were living at TAB. Ten of the sites were in areas where vector control activities (area spraying and application of residual insecticides) occurred regularly (treated sites). These sites were designated as Trtd #1 through Trtd #10. Two of the sites were in areas where vector control activities did not occur (control sites). These two sites were designated Con #1 and Con #2 (Fig. 1). Weather permitting, traps were placed one or more times per week beginning on 23 May 2003 and continuing through 25 October 2004. Traps were not set during the winter (November 2003–March 2004) when adult sand flies were absent. The primary goals of this surveillance program were to 1) monitor sand fly populations, 2) determine the species of sand flies that were present and identify changes in species composition over time, 3) evaluate the effectiveness of the sand fly control program that we established at TAB, and 4) test the sand flies for *Leishmania* parasites to aid in the identification of disease foci. In the present article, we report on the first two objectives.

Daily Activity of Sand Flies at TAB. We conducted a series of studies to determine the diel activity of phlebotomine sand flies. Unbaited light traps were set at 2000 hours on selected nights throughout the course of the sand fly season. Traps were set in an untreated site (Con #1). Every 2 hr, beginning at 2200 hours and ending at 0600 hours, the collection cups on each trap were removed and replaced with a new collection cup. Immediately after being removed from the trap, each collection cup was taken to the field laboratory and placed in a −70°C freezer until they were checked for sand flies as described above.

Distribution of Sand Flies Inside Buildings at TAB. We conducted a series of studies to determine the abundance of sand flies in and around a block of two-story townhouses located ≈1.6 km to the northeast of the main entrance to TAB (Fig. 1). A single unbaited light trap was placed in each of the following locations in each house: 1) in front of the house, 2) behind the house, 3) in a first floor room in the center of the house, and 4) on the flat roof (second floor) of the house. Traps were set in operation at ≈1800 hours and run until 0700 hours the next morning, when the collection cups were retrieved and taken to the field laboratory for cold storage and processing as described above. A generalized linear mixed model where trap location was treated as a "fixed" factor, and date and house were treated as "random" factors was used to evaluate differences in sand fly distribution in and around the buildings.

Distribution of Sand Flies Inside Tents at TAB. We conducted a series of studies to determine the abundance of sand flies in and around military tents with and without air conditioning. Two separate sites were used for studies with tents without air conditioning, whereas one site was used for tents with air conditioning (Fig. 1). A light trap was hung inside each tent and a second hung immediately outside each tent. All other procedures followed those outlined in the study evaluating sand fly distribution in buildings. A generalized linear mixed model where trap location was treated as a "fixed" factor and habitat (inside or outside of tent) and air conditioning (with or without) were treated as "random" factors was used to evaluate differences in sand fly distribution in and around the tents.

Statistical Analysis. The generalized linear mixed model that was used to assess the distribution of sand flies inside tents and buildings takes into account 1) the standard variability associated with counting processes (Poisson distribution), 2) what happens to a counting process in the presence of other unexplained sources of variation such as weather factors and time of day (Quasi-Poisson distribution), and 3) the relationship between repeated measurements at the same site (a mixture of fixed and random effects). Factors specifically selected for study (such as inside or outside of tents) are treated as fixed. Factors expected to influence sand fly counts but not of specific interest to the analysis (such as an exact location) are treated as random. The Generalized Linear Mixed Models used in this study automatically and consistently adjust for different numbers of measurements at different locations. Models were mechanistically built from simple to more complex. These models are fit under R (R Development Core Team 2005) by using the optional lme4 (Bates and Sarkar 2006) and Matrix (Bates and Maechler 2006) libraries. The Laplace optimization option was used for all models. An analysis of variance (ANOVA) procedure was used to assess effect of trap site on sand fly collections, with Student–Newman–Keuls test ($P < 0.05$) used to separate mean values.

Results

Temporal Distribution of Phlebotomine Sand Flies at Tallil Air Base. Traps were run a total of 68 nights—41 nights between 23 May and 31 October 2003 and on 27 nights between 25 April and 25 October 2004. Control site 1 was only used from 23 May to 5 August 2003. Use of this site was discontinued after 5 August because the site had been bull-dozed in preparation for construction of a "tent city." During this time, two traps were placed each night on a total of 20 nights, with four trap failures resulting in 36 successful trap nights. Control site 2 was selected as a replacement for Con #1; trapping at this site was initiated on 12 August 2003. From 12 August to 31 October 2003, two traps were set on each of 21 nights, resulting in 42 successful trap nights. From 25 April 2004 to 25 October 2004, three traps were set on each of 27 nights, with two trap failures resulting in 79 successful trap nights. For each of the 10 treated sites, a single trap was placed on each of the 68 nights that collections were made. Four of the sites (sites 1, 4, 7, and 10) had 68 successful collections, whereas at five sites (sites 2, 3, 5, 6, and 8) a single trap failed, resulting in 67 successful collections. Trapping at treated site 9 was discontinued after 45 nights of collecting due to construction in the area (Table 1).

During this study, 42,646 phlebotomine sand flies were collected in 809 trap nights (Table 2). Eighty-nine percent (723) of the traps contained sand flies, with 44% (352) of the traps containing <10 sand flies (Fig. 2). Totals of 18,883 and 23,763 sand flies were collected in the control areas (157 trap nights) and treated areas (652 trap nights), respectively. Significantly more sand flies were collected in each trap placed in control areas (mean 120 per trap night) than in treated areas (mean 36 per trap night). The temporal abundance of sand flies is presented in Fig. 3 (combined results from 2003 and 2004). The numbers of sand flies collected in both treated and control areas were low in late April, began increasing in early May, were highest from mid-June until early September, and decreased rapidly in late September and October. Seventy percent (29,634/41,807) of the sand flies whose sex was determined were female, whereas 8% (2,257/27,886) whose engorgement status was evaluated contained visible blood. The source of the blood was not determined.

Geographic Distribution of Phlebotomine Sand Flies at Tallil Air Base. The number of sand flies collected at each of the two control and 10 treated sites

Table 1. Numbers of sand flies collected in 12 locations at Tallil Air Base during 2003 and 2004

Site[a,b]	No. nights traps were set	No. sand flies collected	Mean no. of sand flies/trap (SEM)[c]
Control 1	36	5,073	140.9 (16.3) a
Control 2	121	13,810	114.1 (16.3) ab
Treated 7	68	5,255	77.3 (19.2) bc
Treated 2	67	3,754	56.0 (18.2) cd
Treated 8	67	3,700	55.2 (11.5) cd
Treated 6	67	2,714	40.5 (9.9) cd
Treated 3	67	2,071	30.9 (6.5) cd
Treated 9	45	1,113	24.7 (6.5) cd
Treated 1	68	1,608	23.7 (5.4) cd
Treated 5	67	1,576	23.5 (5.0) cd
Treated 10	68	1,209	17.8 (4.1) cd
Treated 4	68	763	11.2 (2.0) d
Total	809	42,646	52.7 (4.2)

[a] Collections were made on a total of 68 nights. Control site #1 was used from 23 May 2003 to 5 Aug. 2003, whereas control site 2 was used from 12 August 2003 to the end of the study. One or two traps were set at control site 1 and two traps at control site 2. Five and three traps failed on one night each at control site 1 and 2, respectively.

[b] One trap was placed in each of the treated sites. Traps at treated sites 2, 3, 5, and 8 each failed on one night. The site used for treated trap 9 was demolished and trapping discontinued after 45 collections had been made.

[c] Means followed by the same letter are not significantly different (Student–Newman–Keuls test; $P < 0.05$).

is presented in Table 1. The mean number of sand flies collected in each trap ranged from a low of 11 per trap night to a high of 141 per trap night. The number of sand flies collected in a single trap over one night ranged from 0 to 1,151. Significantly more sand flies were collected in traps at the two control sites than in any of the traps in the treated areas. The geographic distribution of phlebotomine sand flies during spring (April and May), summer (June–August), and fall (September and October) is presented in Fig. 4. Sand fly numbers were lowest in the light traps located in the southern part of the surveillance area (treated sites 1, 4, 5, 6, 9, and 10) and higher in the traps in the northern area (treated sites 2, 3, 7, and 8 and both control sites). The number of sand flies collected at each of the 12 trap sites in 2003 is presented in Fig. 5. For many of the traps, the majority of collections contained relatively few sand flies; however, for each trap a few collections contained substantially higher numbers than normally found in the trap.

Species of Phlebotomine Sand Flies Identified at Tallil Air Base. Sand flies in the genus *Phlebotomus* were identified to species, whereas *Sergentomyia* were only identified to genus. Fifteen percent (6,416/42,646) of the sand flies were identified—47% (3,021) and 53% (3,395) of the flies that were identified were collected in 2003 and 2004, respectively. *P. alexandri*, *P. papatasi*, and *P. sergenti* accounted for 30% (1,946), 24% (1,557), and 1% (56) of the sand flies that were identified, respectively, whereas 45% (2,857) were *Sergentomyia* spp. The relative abundance of each species of sand fly by month is presented in Fig. 6. *P. alexandri* was the predominant species identified in April and May, accounting for ≥50% of the collection each month, whereas from June until October it accounted for ≈25% of the total collection. In contrast, *P. papatasi* was less abundant early in the season (April–June), but it became more predominant later in the season (August and September) when it accounted for almost 50% of the specimens identified. *P. sergenti* was rare at all times, whereas *Sergentomyia* spp. accounted for 25–60% of the sand flies collected each month. The relative abundance of each species

Table 2. Phlebotomine sand flies collected at 12 trap sites at Tallil Air Base, Iraq

Yr	Mo	Untreated (control) area[a]			Treated area[a]		
		No. traps	No. sand flies	Mean no. of sand flies/trap (SEM)	No. traps	No. sand flies	Mean no. of sand flies/trap (SEM)
2003	May	3	171	57.0 (28.5)	29	958	33.0 (9.5)
	June	13	1,026	78.9 (18.9)	70	2,082	29.7 (5.6)
	July	16	3,071	191.9 (69.5)	78	4,857	62.3 (12.8)
	Aug.	12	1,836	153.0 (57.1)	55	3,499	63.6 (24.9)
	Sept.	18	2,003	111.3 (22.9)	81	2,105	26.0 (6.6)
	Oct.	16	205	12.8 (2.5)	72	326	4.5 (0.8)
	Total	78	8,312	106.6 (18.8)	385	13,827	35.9 (4.9)
2004	April	1	12	12.0 (N/A)	9	31	3.4 (1.9)
	May	15	1,140	76.0 (28.7)	50	752	15.0 (2.7)
	June	12	1,704	142.0 (76.8)	38	1,506	39.6 (8.9)
	July	12	4,363	363.6 (82.5)	40	4,853	121.3 (22.9)
	Aug.	15	1,712	114.1 (48.4)	50	1,703	34.1 (8.2)
	Sept.	12	1,036	86.3 (23.0)	40	430	10.8 (2.9)
	Oct.	12	604	50.3 (13.3)	40	661	16.5 (4.4)
	Total	79	10,571	133.8 (23.0)	267	9,936	37.2 (4.6)
2003 and 2004		157	18,883	120.3 (14.8)	652	23,763	36.5 (3.4)

N/A, not applicable.

[a] Normally, a single trap was set in each of the 10 treated sites and one or two in each of the two untreated sites. Trap failure due to batteries not holding a charge through the entire night is the primary reason for the unbalanced number of traps in some months. Treated areas received residual application of insecticides by using hand-held sprayers as well as ultralow volume spraying using truck-mounted sprayers on a routine basis.

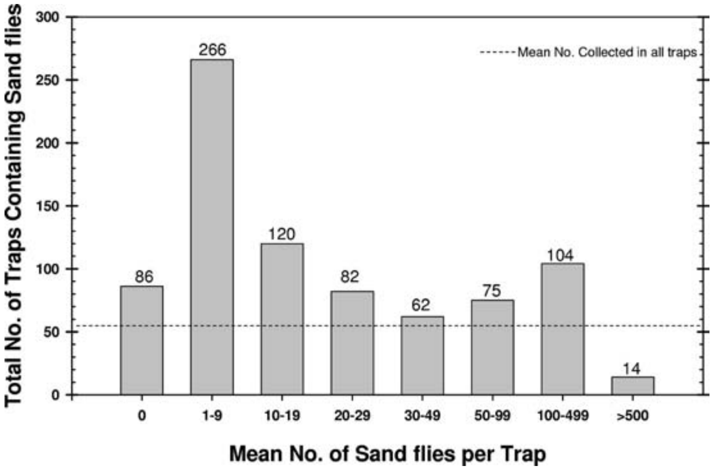

Fig. 2. Number of sand flies collected in each of 870 trap nights over the study (87 traps collected no sand flies, 276 traps collected one to nine sand flies, and so on).

of sand fly at the 12 different trap sites is presented in Fig. 7. *P. sergenti* accounted for <1% of specimens identified at all 12 trap sites. *Sergentomyia* spp. accounted for >50% of the collection at two sites (Trtd #2, 67%; Con #1, 53%), whereas *P. papatasi* (Trtd #3, 51%) and *P. alexandri* (Trtd #8, 52%) accounted for >50% of the collection at one trap site each.

Diel Activity of Sand Flies at Tallil Air Base. In total, 2,574 phlebotomine sand flies was collected during 25 trap nights between 6 May 2003 and 30 May 2004 (Table 3). Traps set in April and June collected relatively few sand flies (mean 4.8 per trap night and 14.8 per trap night, respectively), whereas traps set in May and October captured significantly more sand flies (mean 40.4 per trap night and 57.7 per trap night, respectively). The traps used in October were not standard CDC miniature light traps (as used in all other studies reported in this article) but were miniature downdraft blacklight (UV) traps (model 912, John W. Hock Company). The high number of sand flies collected in October presumably is a reflection of the effectiveness of this trap compared with the CDC miniature light trap (D.A.B., unpublished data). More than 80% (2,120/2,574) of the sand flies were collected from 2000 to 0200 hours, with 29% (738) collected before 2000 hours and 18% (454) collected after 0200 hours. Sand flies were more active early in the evening in April and October, whereas they were active throughout the night in May and June (Table 3). Thirteen percent (345/2,574) of the sand flies collected in this study were identified (Table 4). *Sergentomyia* spp., *P. alexandri, P. papatasi,* and *P. sergenti*

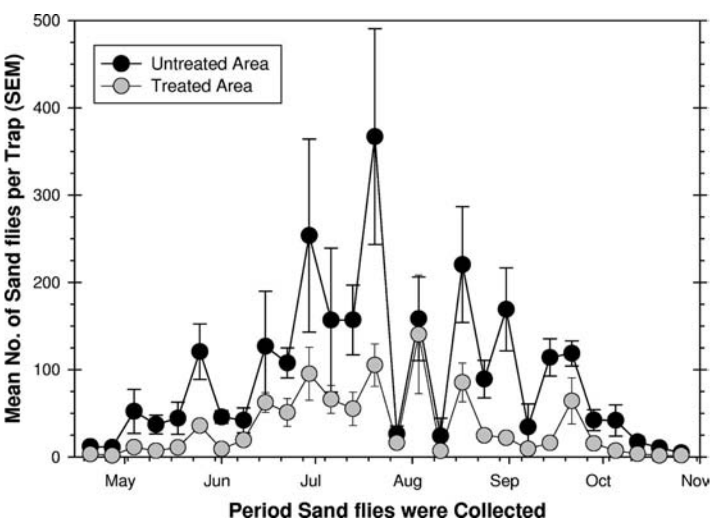

Fig. 3. Temporal distribution of phlebotomine sand flies at Tallil Air Base, Iraq. Data represent collections made in 2003 and 2004. The untreated area consisted of two sites that did not receive any insecticide treatment, whereas the treated area consisted of 10 sites that were routinely treated with insecticides.

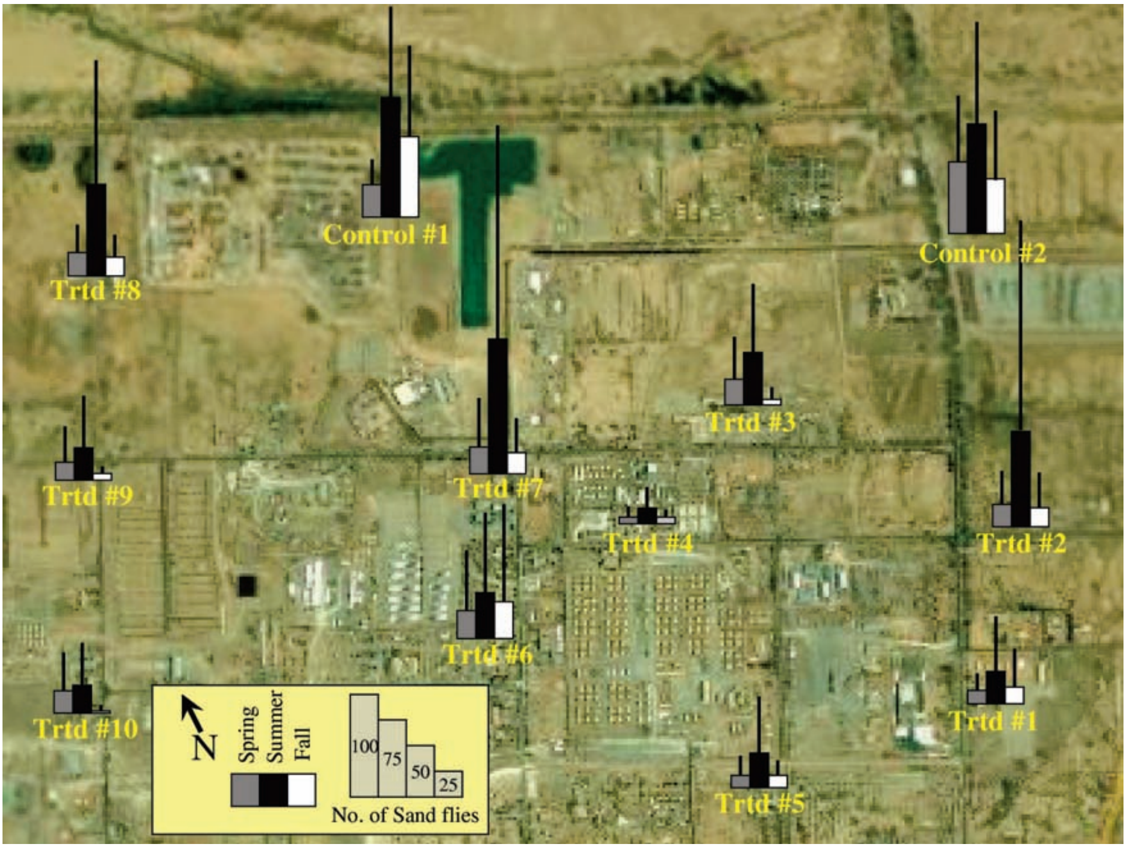

Fig. 4. Abundance of phlebotomine sand flies during the spring, summer, and fall at two untreated and 10 treated sites at Tallil Air Base, Iraq.

accounted for 55% (191), 24% (82), 15% (52), and 6% (20) of the specimens identified, respectively. *Sergentomyia* spp., *P. alexandri,* and *P. papatasi* were most active from 2000 to 0400 hours, with relatively few collected from 0400 to 0600 hours, whereas all 20 *P. sergenti* were collected between 2200 and 0400 hours (Table 4).

Distribution of Sand Flies Inside Buildings at TAB. This study was conducted in two houses on 14 and 16 June 2003 and in three houses on 15 June 2003, for a total of seven replicates. Each replicate consisted of four light traps set in and around each house. In total, 225 sand flies (mean 8.0 per trap night) were collected. There was no significant difference ($P = 0.21$) in the number of sand flies collected on the roof, inside, in front of, or behind each house (Table 5). Almost 50% (33/69) of the sand flies that were identified in this study were *P. papatasi,* with *P. alexandri* and *Sergentomyia* spp. accounting for 12% (8/69) and 40% (28/69) of the collection, respectively.

Distribution of Sand Flies Inside Tents at TAB. This study was conducted between 25 May and 30 October 2003. Ninety-five replicates were conducted in tents that had no air conditioning and 61 replicates in tents with air conditioning (Table 5). A total of 16,185 sand flies (mean 51.9 per trap night) was collected in this study. Significantly fewer sand flies were collected inside both air conditioned (mean = 4.7 per trap night) and nonair-conditioned (mean 34.8 per trap night) tents then outside the same tents (mean 28.5 per trap night and 114.2 per trap night, respectively). A lower proportion of sand flies were collected inside tents with air conditioners (14.2%) than were collected inside tents without air conditioners (23.3%), relative to the number collected outside the tents at each site. Both habitat (inside or outside of tents) and air conditioning (with or without) had a significant impact ($P < 2.2 \times 10^{16}$ and $P = 0.0093$, respectively) on sand fly numbers.

More than 70% (314/437) of the sand flies that were identified in the tent study were *P. papatasi,* with *P. alexandri* and *Sergentomyia* spp. accounting for 14% (59/437) and 15% (64/437) of the collection, respectively. The sex of 87.7% (14,190/16,185) of the sand flies collected in this study was determined, with females accounting for 65% (9,260/14,190) of the collection. There was no significant difference in the proportion of female versus male sand flies inside (68%; 2,074/3,032) or outside (64%; 7,186/11,158) of tents, nor was there a difference in female versus male sand flies inside or outside of tents with and without air conditioning (data not presented).

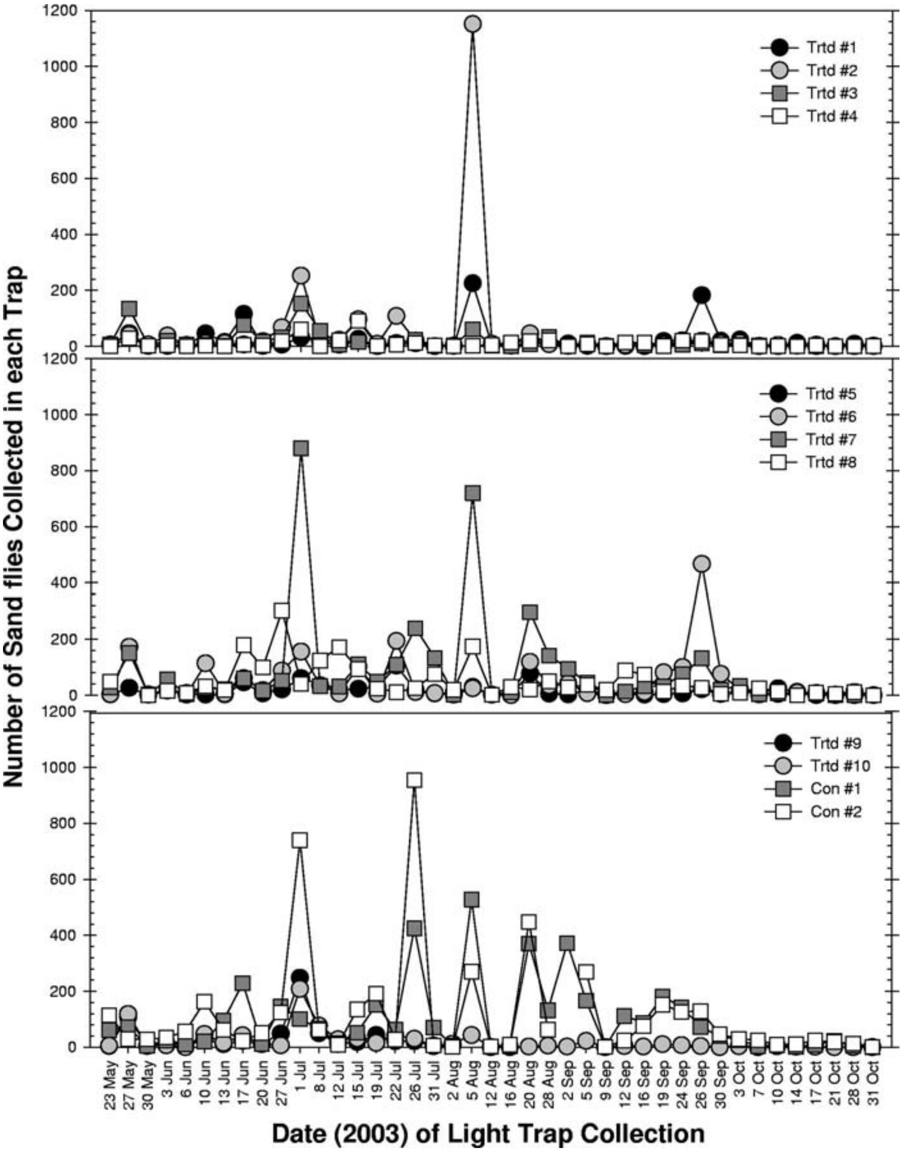

Fig. 5. Number of sand flies collected each day at the two untreated and ten treated trap sites in 2003

Discussion

We provide the first in-depth examination of the phlebotomine sand fly fauna of southern Iraq. The sheer numbers of phlebotomine sand flies collected at TAB was our most notable result. We collected >65,000 sand flies in 1,235 trap nights (mean 53 per trap night), whereas most other published studies in Iraq reported collecting fewer than 5,000 sand flies (Adler and Theodor 1929; Pringle 1952, 1956; Sukkar 1974, 1978), albeit using different collecting methods. United States Army and Navy preventive medicine units stationed throughout Iraq in 2004 and 2005 collected far fewer sand flies in six other locations than we did at TAB (Table 6). The mean numbers of sand flies collected per trap at TAB were 3–100 times higher

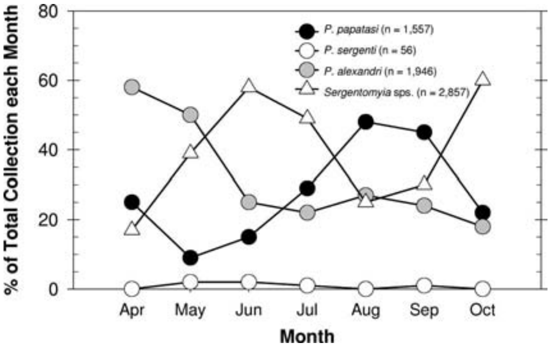

Fig. 6. Relative proportion of *P. papatasi*, *P. sergenti*, *P. alexandri*, and *Sergentomyia* spp. by month at Tallil Air Base, Iraq. Data represent sand flies collected in 2003 and 2004.

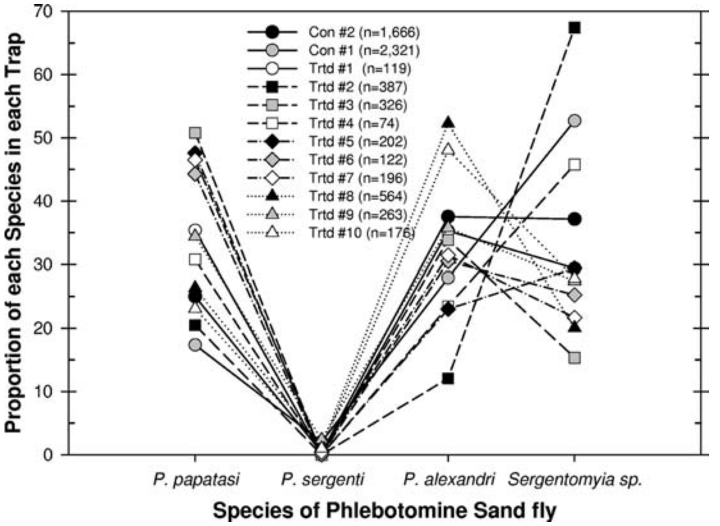

Fig. 7. Relative proportion of *P. papatasi*, *P. sergenti*, *P. alexandri*, and *Sergentomyia* spp. at each of the two untreated and 10 treated trap site at Tallil Air Base, Iraq. Data represent sand flies collected in 2003 and 2004.

than at these other locations. Light traps placed in our two control sites collected >100 sand flies per trap per night during 217 trap nights in 2003 and 2004, with a peak of >360 flies per trap per night collected during 12 trap nights in July 2004 (Tables 1 and 2). The abundance of the sand flies was reflected in the high number of bites received by U.S. military personnel stationed at TAB. In 2003, up to 75% of soldiers in certain units reported receiving insect bites, with a number of soldiers receiving an estimated 1,000 or more bites in a single night (Coleman et al. 2006). Examination of the bites, interviews with the soldiers, and collection of biting arthropods in areas where bites occurred all suggested that sand flies were responsible for almost all of these bites. A review of sick call records from the Battalion Aid Station of the first Battalion of the 293rd Infantry Regiment revealed that 47% (172/377) of all sick call visits from 1 April to 15 May 2003 were for sand fly bites. Although we did not determine the species of sand fly that was responsible for these bites, both *P. papatasi* and *P. alexandri* are abundant at TAB. Although *P. papatasi* commonly feeds on humans (Sukkar et al. 1985, Killick-Kendrick 1990, Sawalha et al. 2003), the blood-feeding habits of *P. alexandri* are less well known. However, this species has been implicated as a vector of visceral leishmaniasis (Sukkar 1974, 1985; Li-ren et al. 1986; Killick-Kendrick 1990) and Azizi et al. (2006) reported that >30% of parous *P. alexandri* had fed on human blood. Visceral leishmaniasis due to *L. infantum* is common in the vicinity of TAB (H. Ali, personal communication), whereas initial sequencing of the glucose-6-phosphate isomerase gene indicated that 20% (3/15) of *Leishmania* isolates from sand flies were *L. infantum* (Coleman et al. 2006). If *P. alexandri* is indeed the vector of this parasite, the abundance of this species of sand fly at TAB is not surprising.

A major difference between our study and other published studies from Iraq is that we used CDC miniature light traps, whereas manual aspiration, sticky traps or biting collections were previously used. Although light traps have been commonly used for the collection of sand flies (Hilmy et al. 1989, Kaul et al. 1994, Robert et al. 1994, Alexander 2000, Orndorff et al. 2002), few studies have compared them to landing and/or biting collections. Alexander (2000) reported that a potential disadvantage of light traps was that they might preferentially sample females of certain

Table 3. Diel activity of phlebotomine sand flies collected at Tallil Air Base during 2003 and 2004

Hour collected	April (6 traps[a])		May (3 traps[a])		June (13 traps[a])		Oct. (3 traps[b])		Total no. collected (%)
	Total no. collected (%)	Mean/trap (SEM)	Total no. collected (%)	Mean/trap (SEM)	Total no. collected (%)	Mean/trap (SEM)	Total no. collected (%)	Mean/trap (SEM)	
2000–2200	59 (41)	9.8 (4.1)	74 (12)	24.7 (11.3)	180 (19)	13.8 (3.8)	425 (49)	141.7 (64.5)	738 (29)
2200–2400	57 (40)	9.5 (5.7)	168 (28)	56.0 (6.2)	314 (33)	24.2 (13.5)	310 (36)	103.3 (34.3)	849 (33)
2400–0200	14 (10)	2.3 (0.8)	238 (39)	79.3 (18.6)	193 (20)	14.8 (3.9)	88 (10)	29.3 (18.1)	533 (21)
0200–0400	3 (2)	0.5 (0.3)	114 (19)	38.0 (13.1)	206 (21)	15.8 (3.5)	18 (2)	6.0 (5.0)	341 (13)
0400–0600	10 (7)	1.7 (1.1)	12 (2)	4.0 (4.0)	67 (7)	5.2 (1.4)	24 (3)	8.0 (5.7)	113 (4)
Total	143 (100)	4.8 (1.5)	606 (100)	40.4 (8.2)	960 (100)	14.8 (3.0)	865 (100)	57.7 (19.5)	2,574 (100)

[a] Unbaited CDC-style light trap.
[b] Unbaited ultraviolet light trap.

Table 4. Diel activity of several species of phlebotomine sand flies collected at Tallil Air Base during June 2003

Hour collected	No. of sand flies collected (% of total in each column)				
	P. papatasi	*P. sergenti*	*P. alexandri*	*Sergentomyia* sp.	Total
2000–2200	11 (21)	0 (0)	15 (18)	35 (18)	61 (18)
2200–2400	8 (15)	8 (40)	20 (24)	31 (16)	67 (19)
2400–0200	16 (31)	4 (20)	21 (26)	52 (27)	93 (27)
0200–0400	14 (27)	8 (40)	22 (27)	58 (30)	102 (30)
0400–0600	3 (6)	0 (0)	4 (5)	15 (8)	22 (6)
Total	52 (100)	20 (100)	82 (100)	191 (99)	345 (100)

Sand flies were collected from 4 to 11 June 2003.

species that are highly phototropic and suggested that light traps had limited value in ecological studies of sand flies. However, Fryauff and Modi (1991) and Davies et al. (1995) determined that light trap collections were comparable to biting collections for *P. papatasi* in Egypt and sand flies in the Peruvian Andes, respectively, whereas Rioux et al. (1982) reported that adhesive traps provided results similar to human bait. In a preliminary study, we compared the sand fly trapping efficacy of the standard CDC light traps against sticky traps, an updraft light trap and a miniature downdraft blacklight trap at TAB but did not determine the species composition of the flies collected on sticky traps, nor have we compared light traps to manual aspiration or bite-landing collections. It will be necessary to conduct appropriate comparative studies in southern Iraq to determine whether light trap collections in general accurately represent the phlebotomine fauna in the region and more specifically approximate human landing/biting rates.

The appearance of adult sand flies at TAB in early April and their disappearance in early November are consistent with other published studies on sand flies in Iraq (Abul-Hab and Al-Baghdadi 1972a,b; Abul-Hab and Mehdi 1970; Pringle 1952; Sukkar 1974, 1978). Although this seasonality seems to be related to temperature, we did not attempt to determine the precise relationship between environmental factors and sand fly abundance. Abul-Hab and Baghdadi (1972a,b) reported that populations of *P. papatasi* and *S. squamipleuris* (mistakenly identified as *P. squamipleuris*) in the vicinity of Baghdad had a minor peak in the spring (April and May) and a major peak in the fall (September and October), with low numbers collected in the summer (June–early September). Abul-Hab and Baghdadi (1972b) suggested that high temperatures in the summer caused the sand flies to aestivate and reduced breeding activity, with falling temperatures in September and October resulting in a resumption of activity. In contrast, data from our study (Table 1; Figs. 3 and 4) support those of Pringle (1952) who found that sand fly populations were low in the spring and fall and peaked during the summer. Pringle (1952) thought that a hot season in which rain was rare resulted in deep cracks in the soil that supported *P. papatasi* populations during the heat of the summer. During 2003 and 2004, daily temperatures at TAB exceeded 40°C on 137 and 145 d, respectively, of the 184 d between 1 May and 31 October. During this time, measurable precipitation occurred on only 1 d in 2003. These exceedingly hot, dry climatic conditions are similar to those that Pringle (1952) reported, and, at TAB, did not seem to adversely affect sand fly abundance or activity.

Although sand flies overall were extraordinarily abundant at TAB during the summer, significant day-to-day variation occurred (Fig. 5). For example, on 2 August 2003, we collected a mean of five sand flies per trap night, whereas 3 d later (5 August) we collected a mean of 296 sand flies per trap night. The number of sand flies collected was unusually high (number collected on a given trap night exceeded the mean plus one standard deviation for all collections made at that particular trap site) for ≈9% (72/809) of the light trap collections, whereas no sand flies were collected in 11% (86/809) of the light trap collections. Although the observed fluctuations presumably reflect changes in environmental conditions (e.g., no sand flies collected on windy nights, whereas high numbers were collected on very calm nights), the fluctuations were not always consistent from site to site. For example, unusually high numbers of sand flies (see above definition) were collected from at least one trap site on

Table 5. Distribution of phlebotomine sand flies in and around building and tents at Tallil Air Base, Iraq

Trap location	No. traps	No. sand flies (% of total)	Mean no. of sand flies/trap (SEM)	No. of each species identified (% of total in row)			
				P. papatasi	*P. alexandri*	*Sergentomyia* spp.	Total
House study							
Inside	7	45 (20)	6.4 (1.9)	8 (47)	2 (12)	7 (41)	17 (100)
Roof	7	54 (24)	7.7 (2.5)	5 (63)	0 (0)	3 (37)	8 (100)
Outside, front	7	59 (26)	8.4 (4.0)	10 (45)	2 (10)	10 (45)	22 (100)
Outside, back	7	67 (30)	9.6 (3.1)	10 (45)	4 (20)	8 (35)	22 (100)
Total	28	225 (100)	8.0 (1.4)	33 (48)	8 (12)	28 (40)	69 (100)
Tents without air conditioners							
Inside	95	3,303 (23)	34.8 (4.0)	165 (73)	19 (8)	43 (19)	227 (100)
Outside	95	10,853 (77)	114.2 (17.7)	149 (71)	40 (19)	21 (10)	210 (100)
Total	190	14,156 (100)	74.5 (9.5)	314 (72)	59 (14)	64 (15)	437 (100)
Tents with air conditioners							
Inside	61	288 (14)	4.7 (1.2)				
Outside	61	1,741 (86)	28.5 (7.7)	Not determined			
Total	122	2,029 (100)	16.6 (4.0)				

Table 6. Comparison of sand fly collections made at Tallil Air Base with those at various other locations in Iraq

Collection site	General area	Yr	No. trap lights[a]	Collection period	No. sand flies	Mean no. of sand flies/trap (SEM)
Tallil Air Base	An Nasiriyah	2003	524	9 May–31 Oct.	25,926	49.5 (4.8)
		2004	345	25 April–25 Oct.	20,633	59.8 (6.7)
Al-Asad Air Base	Al-Haqlaniyah	2004	356	5 April–26 Oct.	1,536	4.3 (0.6)
Camp Anaconda	Balad	2004	772	12 April–21 Oct.	4,791	6.2 (1.0)
		2005	410	29 April–30 Sept.	957	2.3 (0.2)
Camp Victory	Baghdad	2004	638	5 May–6 Oct.	6,030	9.5 (1.0)
		2005	204	3 May–26 Oct.	2,563	12.6 (1.6)
Camp Babylon	Babylon	2004	2,060	4 May–9 Nov.	5,503	2.7 (0.1)
Camp Taji	Taji	2004	293	11 May–21 Oct.	4,399	15.0 (1.4)
Camp Speicher	Tikrit	2004	584	23 Mar.–4 Nov.	347	0.6 (0.1)
		2005	800	21 Mar.–7 July	6,667	8.3 (1.3)

All collections were made by U.S. military preventive medicine units deployed to Iraq during Operation Iraqi Freedom.
[a] Standardized traps (CDC miniature light traps) and collection procedures were used for the collection of sand flies at all sites.

20 of 68 d during the course of this study; however, on 11 of these nights high numbers of sand flies were only collected from three or fewer of the 12 trap sites, whereas on only four nights (1 July 2003, 5 August 2003, 16 June 2004, and 24 July 2004) were high numbers collected from six or more trap sites. We will conduct a thorough evaluation of the impact of environmental conditions on sand fly activity in a subsequent article.

Another notable difference between this and previous studies in Iraq was that *P. alexandri* was the most abundant species of *Phlebotomus* encountered at TAB, whereas in all other published studies *P. papatasi* predominated. *P. alexandri* made up >50% of the phlebotomine population at certain times of the year at TAB and 30% over the entire study period (Fig. 6). This species never made up >13% of the overall sand fly population in other areas (Pringle 1952, 1956; Abul-Hab and Mehdi 1970; Abul-Hab and Al-Baghdadi 1972a,b; Sukkar 1974, 1978; Sukkar et al. 1985; Abul-Hab and Al-Hashimi 1988). In contrast, *P. papatasi* accounted for only 24% of the total phlebotomine sand fly collection at TAB (Fig. 6), whereas in other studies in Iraq this species accounted for 60–95% of the total collection when using sticky traps or manual aspirators (Pringle 1952, 1956; Sukkar 1974, 1978; Sukkar et al. 1985) and close to 100% of biting collections (Abul-Hab and Al-Hashimi 1988). As with the majority of other studies in Iraq, *P. sergenti* was relatively uncommon at TAB, accounting for <1% of the sand flies that were identified.

Due to the sheer number of sand flies collected during this study (61,630), we were only able to identify ≈10% (6,416) of the specimens that were collected. Although our method for selecting sand flies to be identified does not allow us to compare absolute numbers of each species over time, we can evaluate the relative abundance of each species during any given period (Fig. 6). Although rare at all times, *P. sergenti* was most abundant in May and June, when 95% (53/56) of the specimens were collected (Fig. 6). Surprisingly, all 56 *P. sergenti* that were identified in this study were collected in 2003, with none detected in 2004. We are uncertain why no *P. sergenti* were identified in 2004; however, possibilities include 1) changes in habitat due to the U.S. military occupation at TAB resulted in a reduction in *P. sergenti* populations; 2) sampling bias resulted in the failure to capture *P. sergenti* in 2004, whereas they were collected in 2003; or 3) natural seasonal fluctuations in 2004 resulted in reduced *P. sergenti* populations that were below our detection level. *P. papatasi, P. alexandri,* and *Sergentomyia* spp. were present in high numbers throughout the year at all collections sites at TAB; however, the proportion of each species relative to the others varied over time and space (Figs. 6 and 7). *P. alexandri* was most abundant early in the year (April and May), whereas *P. papatasi* predominated later in the year (August and September). These data were consistent for both 2003 and 2004. Further studies will be needed to determine why *P. alexandri* predominated early in the year, whereas *P. papatasi* was more abundant later in the summer.

The site at TAB where the majority of personnel lived and where all of our traps were placed encompassed an area of ≈3.5 km^2. The minimum and maximum distances between any two of our 12 trap sites were 0.31 and 2.6 km, respectively. Although this is a relatively small area, some general differences in sand fly abundance were noted. Traps placed in sites to the north-east (both control sites and treated sites 2, 3, 7, and 8) generally collected more sand flies than did those traps to the southwest (treated sites 1, 4, 5, 6, 9, and 10) (Table 1; Fig. 4). Although traps placed in the two control sites collected significantly more sand flies than did traps in seven of the 10 treated sites, it is difficult to determine whether the lower numbers in the treated sites resulted from sand fly control activities or whether this is a reflection of a natural gradation in sand fly abundance at TAB. The effect of our sand fly control efforts, to include several controlled experiments, will be presented in a subsequent article in this series.

Diel/circadian activity of sand flies has historically been evaluated using landing collections on humans (Abul-Hab and Al Hashimi 1988), sticky traps (El Said et al. 1986), and truck traps (Roberts 1994). This is the first published Old World evaluation to use hourly light trap collections as a means of assessing sand fly activity. Our data revealed that peak activity periods in April and October differed significantly from those in May and June, with peak activity periods shifting from early in the evening in April and October to later in the night in May and June. In April and October, the highest proportion of sand flies were collected between 2000–2200 hours, whereas in May and June the peak activity periods were 2400–0200 hours and 2200–2400 hours, respectively (Table 3). There did not seem to be any significant differences in the diel activity of the different species of sand flies from the June collection (Table 4). Our findings agree with those of El Said et al. (1986) who used sticky traps and found that *P. papatasi* was most active between 0000 and 0400 in August in Alexandria, Egypt. Mohsen (1983) reported similar results in central Iraq, whereas Roberts (1994) found that sand flies (predominantly *P. alexandri* and *S. clydei*) in Oman were active throughout the night with no significant periods of peak activity. These data suggest that personnel in southern Iraq will be exposed to sand flies throughout the night and accordingly must use appropriate personal protective measures (deet-based repellents, permethrin-treated clothing and bed-nets) that will last all night.

Accommodations for U.S. military personnel deployed to Iraq vary tremendously. In 2003, the majority of soldiers at TAB frequently slept outdoors (on cots or on top of vehicles), in tents without air conditioning, or in buildings (normally without air conditioning or window screens) that had been abandoned by Iraqi military forces. In 2004 accommodations had improved tremendously and the majority of personnel slept in air-conditioned tents or trailers. The goal of our tent and building study was to determine whether the type of accommodation affected sand fly activity and potential risk of sand fly bites. Somewhat surprisingly, our data clearly demonstrated that there were no differences in sand fly abundance in four locations in and around two-story townhouses (Table 5). Although we anticipated that there would be no differences in abundance in the three ground level sites in each building, given the sand fly's reputation as a weak flyer (Alexander 2000), we had expected that numbers would be lower on the roof of the building. Guidance provided by Preventive Medicine personnel in 2003 (when air conditioning was generally not available) suggested that sleeping as high as possible (i.e., on the roof of a house or on top of a truck) could potentially provide some degree of protection from sand flies. Our data clearly demonstrated that this was not the case. Conversely, our data show that significantly fewer sand flies were found inside of tents (with or without air conditioning) than in areas immediately outside of the tents, with 84 and 70% fewer sand flies found inside of tents. It is not clear whether this reduction in total numbers was accompanied by a reduction in biting activity. It is possible that biting activity inside of the air-conditioned tents may have been totally eliminated if the temperatures were cool enough.

Our data clearly demonstrate that medically important sand flies are a significant threat to military personnel stationed at TAB. Important vector species (e.g., *P. papatasi* and *P. alexandri*) are abundant from April to October in all sites that were examined. Although we have not presented results from testing of sand flies for leishmaniasis in this paper, our data suggest that *Leishmania* parasites are present in a significant (>1%) proportion of sand flies at TAB (Coleman et al. 2006). In the absence of a vaccine or chemoprophylactic drugs, the best method of protection against leishmaniasis remains a combination of a sand fly control and effective use of personal protective measures.

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