Indian J Med Res 135, January 2012, pp 120-126

Mosquito biting activity on humans & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India

Nandini S. Korgaonkar, Ashwani Kumar[*], Rajpal S. Yadav[**], Dipak Kabadi & Aditya P. Dash[†,#]

National Vector Borne Disease Control Programme, Directorate of Health Services, Panaji, Goa, [*]National Institute of Malaria Research, Field Station, Panaji, Goa, [**]National Institute of Malaria Research, Field station, Nadiad, Gujarat & [†]National Institute of Malaria Research, New Delhi, India

Received February 22, 2010

***Background & objectives*: Knowledge of the bionomics of mosquitoes, especially of disease vectors, is essential to plan appropriate vector avoidance and control strategies. Information on biting activity of vectors during the night hours in different seasons is important for choosing personal protection measures. This study was carried out to find out the composition of mosquito fauna biting on humans and seasonal biting trends in Goa, India.**

***Methods*: Biting activities of all mosquitoes including vectors were studied from 1800 to 0600 h during 85 nights using human volunteers in 14 different localities of three distinct ecotypes in Goa. Seasonal biting trends of vector species were analysed and compared. Seasonal biting periodicity during different phases of night was also studied using William's mean.**

***Results*: A total of 4,191 mosquitoes of five genera and 23 species were collected. Ten species belonged to *Anopheles*, eight to *Culex*, three to *Aedes* and one each to *Mansonia* and *Armigeres*. Eleven vector species had human hosts, including malaria vectors *Anopheles stephensi* (1.3%), *An. fluviatilis* (1.8%), and *An. culicifacies* (0.76%); filariasis vectors *Culex quinquefasciatus* (40.8%) and *Mansonia uniformis* (1.8%); Japanese encephalitis vectors *Cx. tritaeniorhynchus* (17.4%), *Cx. vishnui* (7.7%), *Cx. pseudovishnui* (0.1%), and *Cx. gelidus* (2.4%); and dengue and chikungunya vectors *Aedes albopictus* (0.9%) and *Ae. aegypti* (0.6%). Two *An. stephensi* of the total 831 female anophelines, were found positive for *P. falciparum* sporozoites. The entomological inoculation rate (EIR) of *P. falciparum* was 18.1 and 2.35 for Panaji city and Goa, respectively.**

***Interpretation & conclusions*: Most of the mosquito vector species were collected in all seasons and throughout the scotophase. Biting rates of different vector species differed during different phases of night and seasons. Personal protection methods could be used to stop vector-host contact.**

Key words *Anopheles stephensi* - biting activity - circumsporozoite protein - entomological inoculation rate - landing rates - William's mean

Knowledge of bionomics of mosquitoes, especially of the disease vectors, is necessary to plan appropriate vector avoidance and control strategies. For example, information on biting activity of vectors during the night hours in different seasons could help in choosing personal protection measures that would prevent

[#]*Present address*: WHO/SEARO, New Delhi, India

human-mosquito contact. Earlier a study on the biting activity of disease vectors using human baits was conducted in the coastal urban areas of Goa, India[1]. Similar studies have generated valuable information on biting patterns of different vector species on bovine and human baits in India and neighbouring countries[2-5]. These studies point to the seasonal and temporal variations in mosquito biting rhythms. We undertook this study from October 2005 to September 2006 with two main objectives: (*i*) to determine the composition of mosquito fauna biting on humans and variation in their biting activity during different phases of night and seasons; and (*ii*) to measure sporozoite infection rates in *Anopheles stephensi*, which is considered primary malaria vector species, and other anophelines to find the existence of any secondary malaria vector species in Goa, India

Material & Methods

Study area: Goa is situated on the western coast of India with a population of 1.34 million[6] residing in two districts and 11 sub districts comprising of 15 towns and 398 villages. The study was conducted in these ecotypes covering both urban and rural areas of Goa within the jurisdiction of the Urban Health Centres of Panaji, Margao and Vasco and Primary Health Centres Candolim, Bicholim, Cansarvarnem, Ponda, Betki, Cortalim, Quepem, Canacona, Valpoi and Sanguem.

Meteorological data: Weather data were obtained from the Regional Meteorological Observatory, Goa, and monthly means of various weather parameters *viz*., maximum and minimum temperatures, rainfall and relative humidity were worked out for the pre-monsoon (January-May), the monsoon (June-September), and the post-monsoon periods (October to December).

Mosquito collection: A preliminary survey was conducted to select suitable human dwellings for all-night bait collections in the study areas. The inhabitants of the dwellings were pre-informed about the purpose of mosquito collections and their informed consent was taken after study protocol was approved by Institutional Ehics Committee of National Institute of Malaria Research, New Delhi. Collections of female mosquitoes landing on human volunteers were carried out indoors from 1800 to 0600 h during 85 nights. Mosquitoes were etherized and identified in the laboratory to species level following the keys[7-10].

Entomological inoculation rate (EIR): All female anophelines were dried and stored individually in plastic vials containing dried silica gel under cold conditions (0-4^{o}C). These were tested by sporozoite enzyme-linked immunosorbent assay[11] using antibodies to circumsporozoite proteins (CSP) of *Plasmodium falciparum*, *P. vivax* 210 and *P. vivax* 247. End point results were read visually and confirmed at 450 nm using a Vmax kinetic microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The mean absorbance values of the five known negative controls was 0.207 (range: 0.119 - 0.305) for *P. falciparum;* 0.215 (range: 0.157 - 0.273) for *P. vivax* 210; and 0.095 (range: 0.092 - 0.103) for *P. vivax* 247. Twice the mean negative value was taken as cut-off value in this study, which has been earlier found to be most reliable[12]. Hence, samples with values above twice the mean of negative values were considered positives for the CSP of concerned *Plasmodium* species. EIR was calculated for Panaji and Goa using formula EIR = Number of landing mosquitoes/person/night x sporozoite rate.

Statistical analysis: Hourly data of landing mosquitoes of all the nights in each month were pooled by species and averages were worked out. The hourly data of number of mosquitoes collected from 1800 to 0600 h were pooled to study landing trends in different phases of night. Also to study seasonal biting trends, species-wise landing collections of various months were pooled for pre-monsoon, monsoons and post-monsoon period. Z - test of proportions was applied to study differences of vectors landing in different seasons. To study biting trends during the night in the entire study period covering all the collection hours and in different seasons species-wise, William's mean (M_w) was computed which is a modified geometric mean that compensates for the zero values, and is calculated using the formula Mw = anti log [sum (x+1)/N] – 1, where Mw = the modified geometric mean, x = number of mosquitoes caught during different hours and N = the number of observations[5].

The human volunteers and mosquito collectors were explained about the purpose, procedure and risk of infection during the collection and informed consent was obtained in writing from them. Volunteers and mosquito collectors were administered a weekly prophylactic dose of 600 mg chloroquine in a single dose. The study protocol was approved by the Ethics Committee of National Institute of Malaria Research, New Delhi.

Results

A total of 4,191 mosquitoes were collected during 85 nights from 14 different localities with an overall

mean mosquito landing rate of 49.3 mosquitoes per person/night. These mosquitoes belonged to 5 genera and 23 species, including the malaria vectors *An. stephensi* Liston (55, 1.31%), *An. fluviatilis* James (75, 1.79%), and *An. culicifacies* Giles (32, 0.76%); the filariasis vectors *Culex quinquefasciatus* Say (1710, 40.8%) and *Mansonia uniformis* Theobald (76, 1.81%); the Japanese encephalitis (JE) vectors *Cx. vishnui* Theobald, *Cx. pseudovishnui, Cx. tritaeniorhynchus* and *Cx. gelidus* (1262, 30.1%); and the dengue and chikungunya vectors *Aedes aegypti* L. and *Ae. albopictus* (Skuse) (63, 1.5%). Of the total 4,191 female mosquitoes, 3,273 (78.1%) were known vectors of different mosquito borne diseases and the remaining (918, 21.9%) were of non vector species (Table I). The mean number of female mosquitoes landing per person per night for malaria vectors was 1.89, filariasis vectors 20.9, JE vectors 14.83, and dengue/chikungunya vectors 0.73. The number of landing mosquitoes collected on human bait was maximum between 0300-0600 h (1622; 38.7%) followed by 1800 and 2100 h (1059; 25.3%), 2100-2400 h (830; 19.8%), and least (680; 16.2%) during 2400 to 0300 h (Table I).

Malaria vectors

An. stephensi - The maximum numbers of *An. stephensi* females were caught from 0300-0600 h (23; 41.8%) and the least during 2400-0300 h (6; 10.9%) (Table I). The trends of landing mosquitoes based on William's mean values also suggested that distinct peak activity of *An. stephensi* was in the early hours of the morning. However, the temporal difference in biting behaviour was apparent during different seasons. A distinct peak in biting activity in the early hours of the morning was seen in the pre-monsoon months while in the monsoons there was accelerated biting between 2000 to 2400 h and in the early morning hours from 0300 to 0600 h. In the post-monsoons, a small peak in the evening was followed by another at midnight. Of the total 55 *An. stephensi* collected, 15 (27.3%), 17 (38.9%) and 23 (41.8%) were collected during pre-monsoons, monsoons and post-monsoon seasons, respectively. However, the seasonal differences were found to be non significant (Table II).

Of the 831 female anophelines, evaluated for CSP by ELISA, only 2 *An. stephensi* females were found to contain *P. falciparum* sporozoites. Among anophelines tested, the plasmodial infection rate was 0.2 per cent, while amongst *An. stephensi* alone, it was 3.6 per cent. The two infected females were among 18 *An. stephensi* collected from Panaji city. The EIR of *P. falciparum* was calculated as 18.11 for Panaji and 2.35 for entire Goa.

An. fluviatilis - Similar to *An. stephensi*, the peak number of females of *An. fluviatilis* were caught during early hours of the morning from 0300 to 0600 (30; 40%) and least during 2400 to 0300 h (10; 13.3%). *An. fluviatilis* showed bimodal peaks one from 2100-2300 h and a higher peak from 0300 to 0600 h during the monsoons while maximum biting was noticed from 2100 to 2200 h during post-monsoon period and biting during monsoons was very less. Of the total 75 *An. fluviatilis* collected, most (54, 72%) were collected

Table I. Total number (%) of landing mosquitoes collected on human bait during various phases of 85 all night (1800 - 0600 h) in 14 different localities of Goa

Species	1800:2100 h	2100:400 h	2400:0300 h	0300:0600 h	Mean no.	Total no.
*Anopheles stephensi**	15 (27.3)	11 (20)	6 (10.9)	23 (41.8)	0.65	55
*An. fluviatilis***	18 (24)	17 (22.7)	10 (13.3)	30 (40)	0.88	75
*An. culicifacies***	11 (34.2)	5 (15.6)	9 (28.1)	7 (21.9)	0.38	32
*Culex quinquefasciatus****	397 (23.2)	335 (19.6)	280 (16.4)	698 (40.8)	20.11	1710
Cx. tritaeniorhynchus#	170 (23.3)	155 (21.2)	104 (14.3)	300 (41.2)	8.58	729
Cx. vishnui#	82 (25.3)	71 (21.9)	53 (16.4)	118 (36.4)	3.81	324
Cx. pseudovishnui#	3 (75)	1 (25)	0	0	0.05	4
Cx. gelidus#	53 (25.9)	35 (17.1)	36 (17.5)	81 (39.5)	2.41	205
Aedes aegypti Φ	9 (34.6)	3 (11.5)	3 (11.5)	11 (42.4)	0.31	26
Ae. albopictus Φ	19 (51.4)	8 (21.6)	0	10 (27)	0.44	37
*Mansonia uniformis****	21 (27.6)	11 (14.5)	23 (30.3)	21 (27.6)	0.89	76
Other anophelines	173 (25.9)	132 (19.7)	120 (17.9)	244 (36.5)	7.87	669
Other culicines	88 (35.3)	46 (18.4)	36 (14.5)	79 (31.7)	2.93	249
Total mosquitoes	1,059(25.3)	830 (19.8)	680 (16.2)	1,622 (38.7)	49.31	4191

Urban &**rural malaria vectors, ***Filariasis vector, #JE vectors and Φ Dengue/ Chikungunya vectors

during pre-monsoon which were significantly more than 5 (6.7%) collected during monsoons and 16 (21.3%) collected during post-monsoons (Table II). None of the 75 *An. fluviatilis* were positive for *Plasmodium* infection during sporozoite ELISA studies.

An. culicifacies - *An. culicifacies* was found biting throughout the night at variable frequencies (Table I). There was no definite biting trend shown by the species although during the post-monsoons, maximum biting occurred before 0200 h. Maximum number of landing *An. culicifacies* mosquitoes were collected during post-monsoon *i.e.* 25 of 32 (78.1%) which were significantly more than the other two seasons (Table II). Of 32 *An. culicifacies* collected, none were found positive for *plasmodium* infection during sporozite ELISA studies.

Filariasis vectors: *Cx. quinquefasciatus* - This species was captured throughout the year though maximum landing females were collected from 0300-0600 h (698; 40.8%) and the least (280; 16.4%) during 2400-0300 h (Table I). Biting activity of *Cx. quinquefasciatus* increased gradually from 0200 h and peaked between 0500 and 0600 h both during pre- and post-monsoon seasons although peak in the post-monsoon season was comparatively much higher. Of the 1710 *Cx. quinquefasciatus* mosquitoes, 636 (37.2%) were collected during the pre-monsoon, 200 (11.7%) during the monsoon and 874 (51.1%) during the post-monsoon and the difference between the different seasons was significant (Table II).

Mansonia uniformis: William's mean suggested that there was no time preference for biting shown by *M. uniformis* in general although during the post-monsoon biting pattern showed variability temporally and biting increased past mid night during this season. *M. uniformis* was found in much lesser numbers as compared to *Cx. quinquefasciatus.* This vector of Brugian filariasis was more prevalent during post monsoon season when maximum number of these mosquitoes *i.e.* 54 (71.1%), followed by 20 (26.3%) during pre- monsoon and only 2 (2.6%) were encountered during the monsoon period and the difference between the seasons was significant (Table II).

Japanese encephalitis vectors: *Cx. tritaeniorhynchus* along with other 2 JE vectors *viz*., *Cx. vishnui* and *Cx. gelidus* were found feeding throughout the night with maximum biting occurring from 0300-0600 h and the least during 2400-0300 h (Table I). Another suspected vector of JE in this area, *Cx. pseudovishnui* was found feeding in negligible numbers with biting activity between 1800 and 2100 h (3; 75%) and from 2100-2400 h (1; 25%). Of the four JE vectors, *Cx. tritaeniorhynchus,* showed distinct peak in biting activity after 0300 h during the post-monsoon while during pre- and post-monsoons there was no such distinct peak. In the case of *Cx. vishnui* there was much more biting activity up to midnight compared with later hours during the pre-monsoon months while during the monsoon there was very less landing populations and no definite choice

Table II. Seasonal landing populations of mosquitoes on human baits and inter- seasonal comparison

Species	Pre-monsoon (Jan.-May) No. (%)	Monsoon (June-Sept.) No. (%)	Post-monsoon (Oct.-Dec.) No. (%)	Total no.
Anopheles stephensi	15 (27.3)	17 (30.9)	23 (41.8)	55
An. fluviatilis	54 (72)*	5 (6.7)†	16 (21.3) δ	75
An. culicifacies	5 (15.6)	2 (6.3)†	25 (78.1)δ	32
Culex quinquefasciatus	636 (37.2)*	200 (11.7)†	874 (51.1)δ	1710
Cx. tritaeniorhynchus	217 (29.8)*	104 (14.3)†	408 (55.9)δ	729
Cx. vishnui	103 (31.8)*	15 (4.6)†	206 (63.6)δ	324
Cx. pseudovishnui	2 (50)	0	2 (50)δ	4
Cx. gelidus	71 (34.6)*	10 (4.8)†	124 (60.5)δ	205
Aedes aegypti	11 (42.3)	5 (19.2)	10 (38.5)δ	26
Ae. albopictus	0	0	37 (100)	37
Mansonia uniformis	20 (26.3)*	2 (2.6)†	54 (71.1)δ	76
Other anophelines	356 (53.2)*	70 (10.5)†	243 (36.3) δ	669
Other culicines	96 (38.6)*	19 (7.6)†	134 (53.8)δ	249
Total mosquitoes	1,586 (37.8)*	449 (10.7)†	2,156 (51.4)δ	4,191

P *<0.001 compared to monsoon, †<0.001 compared to post-monsoon, δ<0.001 compared to pre-monsoon

of biting hours. During the post-monsoon however, there were two clear peaks one from 2100 to 2200 h and other from 0500 to 0600 h. The third JE vector *Cx. gelidus* showed trimodal biting pattern during the pre-monsoon, the first peak from 2000 to 2100 h, the second from 2300 to 2400 h and the third much higher at 0500-0600 h. This species showed higher biting rate before 2100 h and decline thereafter. During the post-monsoon months there were three peaks like the pre-monsoon months, the first up to 2100 h, the second from 0100 to 0200 h and much higher peak from 0400 to 0500 h. Overall, significantly more number of JE vectors *viz*., *Cx. tritaeniorhynchus, Cx. vishnui* and *Cx. gelidus* were collected during the post-monsoon season being 408 (55.9%), 206 (63.6%) and 124 (60.5%), respectively than the pre-monsoon *i.e*. 217 (29.8%), 103 (31.8%) and 71 (34.6%), respectively.

Dengue and chikungunya vectors:

Although *Aedes aegypti* and *Ae. albopictus* are primarily diurnal species, they were also found active biting human host especially during the early phase of night and early morning hours as compared to the second and third phases of the night. Although *Ae. aegypti* did not show any preferred time, it was encountered in all the three seasons in small numbers *i.e.* 11 (42.3%), 5 (19.2%) and 10 (38.5%), respectively and the difference was found to be non significant (Table II). *Ae. albopictus* was only found during post-monsoon season and of the 37 landing mosquitoes collected, the species showed crepuscular feeding up to 2100 h and then during early hours from 0300 to 0600 h.

The non vector anophelines showed temporal biting variations during different seasons although overall maximum biting occurred from 0300-0600 h and least during 2400-0300 h (Table I). These species of anophelines were significantly more prevalent during the pre-monsoon compared with other two seasons (Table II). During the pre-monsoon months, the biting started during the first phase of night but declined after 2300 h and picked up after 0200 and peaked from 0500 to 0600 h. During the monsoon, the populations of these anophelines declined drastically, whereas during the post-monsoon, the pattern of biting appeared quite similar to pre-monsoon months.

The non vector culicines were most prevalent during post-monsoon *i.e.* 134 (53.8%) followed by significantly less numbers *i.e.* 96 (38.6%) in the pre-monsoon and 19 (7.6%) in the monsoon. The seasonal biting pattern revealed that during the pre-monsoon months there were two peaks, one in the early phase of night and second lower peak during early morning. During the monsoon, the populations declined significantly although maximum biting was noticed during early morning hours. During the post-monsoon months, there were two prominent peaks one crepuscular and the second in the early morning.

Overall, about half of the mosquitoes were collected during post-monsoon season *i.e*. 2156 (51.4%) followed by pre-monsoon 1586 (37.8%) and the least *i.e*. 449 (10.7%) during the monsoon and this difference was statistically significant (Table II).

Discussion

The study revealed that the vectors of malaria, filariasis and Japanese encephalitis were actively feeding on humans throughout the year between 1800 h to 0600 h. Differences were observed in the biting rhythms of different vector species during different phases of the night and seasons. The data also revealed that vector biting was the least in the monsoon months compared with other seasons which could be due to the flushing effect of heavy rainfall on immature populations although the temperature and humidity conditions were ambient during this season.

An. stephensi, a primary malaria vector, was found in the urban coastal localities of Goa *viz*., Panaji, Candolim, Margao and Cortalim. It was found actively pursuing the host throughout the night with distinct peak activity in the early hours of the morning and in all the three seasons. However, a similar study[1] carried out in Goa had observed peak biting of *An. stephensi* from 2100 to 2400 h. The biting habits of *An. stephensi* are found to be multi-modal or arrhythmic[2,16]. The previous studies[3-5] also suggest that the biting activity of the same species in a particular location can be influenced by sleeping behaviour of the host, microclimatic conditions and the lunar cycle.

An. fluviatilis was reported earlier as a main vector of malaria in the rural eastern hilly regions of Goa[13]. In the present study, this species was collected in the entire scotophase and in all seasons from both hills and foothills of Sanguem and Valpoi in the east, Quepem in the south central and Canacona in the south western parts of Goa. *An. culicifacies* was recorded earlier from the middle sub coastal belt of Goa[13]. In the present study also, this species was captured biting from the three sub coastal areas of Bicholim, Cansarvarnem and

Quepem and did not appear to play any significant role in malaria transmission. The species also did not show much seasonal and temporal variability. Earlier studies had however, shown that the feeding patterns of *An. culicifacies* were highly variable and dependent on local ecology, particularly the climatic factors[14-16].

The landing catches of *Cx. quinquefasciatus* was the most predominant of all the species. The brugian filariasis vector *M. uniformis* was found comparatively in much lesser numbers than *Cx. quinquefasciatus*. In the absence of *Brugia malayi* infections in Goa, this species has only nuisance potential.

The JE vectors pose a serious challenge to the public health in Goa as sporadic outbreaks of JE have been frequently reported especially in the sub coastal belt of Goa. Of all the JE vectors, *Cx. tritaeniorhynchus* was found to be the predominant vector species in Goa. This and other two species, *viz*., *Cx. vishnui* and *Cx. gelidus* were also similarly active throughout the night irrespective of seasons. However, the fourth Japanese encephalitis vector, *Cx. pseudovishnui* was collected in negligible numbers.

Ae. aegypti and *Ae. albopictus* are well-known vectors of dengue and chikungunya respectively in urban and rural areas in India. Small numbers of both these vectors were active after dusk and around dawn. Although these two vectors are known to be primarily diurnal species and Goa being endemic to both dengue and chikungunya, the present study suggests that preventive measures undertaken against other vector species would also be partly effective against these vectors especially during the early hours of the evening and morning when their biting was noticed.

The incrimination studies have confirmed that only *An. stephensi* is the primary malaria vector in Goa as two of the 831 female anophelines, evaluated for CSP by ELISA, were females of this species found positive for *P. falciparum* sporozoites. Panaji continues to be high malaria risk area in Goa as both the positive females were captured from the city where EIR was also found to be quite high in previous two studies, *An. stephensi* was incriminated as malaria vector from Panaji and Candolim in Goa[17,18].

A study on the biting rhythm of mosquitoes[2] postulated the possibility of genetic factors influencing the biting behaviour of mosquitoes and variations in the degree of anthropophily and endophagy among different populations of anophelines and aedine species of mosquitoes. Other studies have indicated that feeding pattern of disease vectors varies widely and is dependent on climatic factors[19,20]. Vector mosquito population densities and the extent of their contact with human hosts are important factors in determining the transmission rates of vector borne diseases[4]. Hence, the circadian rhythm of biting cycles is of great epidemiological significance.

The vector-host contact can be prevented by using available personal protection methods such as repellents, proper clothing and long lasting insecticide nets (LLINs). The latter act as mechanical and insecticidal barrier which can repel or knockdown mosquitoes upon contact thus providing effective protection against vector borne diseases during most part of the night[21]. In the present study, about a quarter of all mosquitoes were found biting in the evening and early hours of the night. During this period, application of suitable repellents and proper clothing can provide protection against vectors.

Acknowledgment

The authors acknowledge the assistance rendered by the field and laboratory staff of National Vector-borne Disease Control Programme, and National Institute of Malaria Research (ICMR), Field Station (FS), Goa, India. Authors thank Dr Hemanth Kumar, Sr. Research Scientist of NIMR, FS Goa and also acknowledge the help of Goa Meteorological Observatory for providing weather data and also the Indian Council of Medical Research for the facilities.

References

1. Kumar A, Thavaselvam D, Sharma VP. Biting behaviour of disease vectors in Goa. *J Parasitic Dis* 1995; *19* : 73-6.
2. Reisen WK, Khan A. Biting rhythm of some Pakistan mosquitoes (Diptera Culicidae). *Bull Entomol Res* 1978; *68* : 313-30.
3. Ghosh KK, Chakraborty S, Bhattacharya, Palit A, Tandon N, Hati AK. *Anopheles annularis* as a vector of malaria in rural West Bengal. *Indian J Malariol* 1985; *26* : 65-9.
4. Gillies HM, Warrell DA. *Bruce Chwatt's essential malariology*, 3rd ed. London: Edward Arnold; 1993.
5. Shriram AN, Ramaiah KD, Krishnamoorthy K, Sehgal SC. Diurnal pattern of human biting activity and transmission of sub periodic *Wuchereria bancroftian* (Filariidia: *Dipetalonematidae*) by *Ochlerotatus niveus* (Diptera: *Culicidae*) on the Andaman and Nicobar islands of India. *Am J Trop Med Hyg* 2005; *72* : 273-7.
6. Singh RP, Sikri DK. *Administrative atlas*. Delhi: Controller of Publications; 2005.
7. Barraud PJ. *The fauna of British India - including Ceylon and Burma, Diptera,* vol. 5- Family *Culicidae*, Tribe *Megarhini* and *Culicini*, London. Taylor and Francis; 1934.
8. Christophers SR. *The fauna of British India including Ceylon and Burma. Diptera, vol. IV Family Culicidae, Tribe Anophelini.* London. Taylor and Francis; 1933 vi. p. 371.

9. Nagpal BN, Sharma VP. *Indian anophelines*. New Delhi: Baba Barkha Nath Printers and Lebanon, NH: Science Publishers, Inc.,1995.

10. Das BP, Rajgopal R, Akiyama J. Pictorial key to the species of Indian *Anophline* mosquitoes. *Zoology* 1990; *2* : 131-62.

11. Burkot TR, Williams JL, Schneider I. Identification of *Plasmodium falciparum* infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 1984; *33* : 783-8.

12. Beier JC, Asiago CM, Onyango FK, Koros JK. ELISA absorbance cut-off method affects malaria sporozoite rate determination in wild Afro tropical *Anopheles*. *Med Vet Entomol* 1988; *2* : 259-64.

13. Borcar PA, Srivastva HML, Roy RG, Mukherji NL. Malaria eradication programme in Goa. *Bull Indian Soc Mal Com Dis* 1967; *4* : 45-54.

14. Brooke Worth C. Notes on the Anopheline fauna of a hill tract in Mysore State India. *Indian J Malariol* 1953; *7* : 125-82.

15. Bhombore SR, Sitaraman NL, Achuthan C. Studies on the bionomics of *An. fluviatilis* in Mysore State, Part II, India. *Indian J Malariol* 1956; *10* : 23-32.

16. Rao TR. *The Anophelines of India*, revised ed. Delhi: Malaria Research Centre, (ICMR); 1984. p. 37-40.

17. Korgaonkar NS, Kumar A, Yadav RS, Kabadi D, Dash AP. Sampling of adult mosquito vectors with Mosquito Magnet® PRO in Panaji Goa, India. *J Am Mosq Control Assoc* 2008; 24 : 604-7.

18. Sumodan PK, Kumar A, Yadav RS. Resting behaviour and malaria incrimination of *Anopheles stephensi* in Goa, India. *J Am Mosq Control Assoc* 2004; *20* : 317-8.

19. Bhatt RM, Srivastava HC, Pujara PK. Biology of malaria vectors in Central Gujarat. *Indian J Malariol* 1994; *31* : 65-76.

20. Devi NP, Jauhari RK. Relationship between *Anopheles fluviatilis* & *Anopheles stephensi* (Diptera: Culicidae) catches & the prevalence of malaria cases at Kalsi area in Dehradun district (Uttaranchal). *Indian J Med Res* 2006; *123* : 151-8.

21. Curtis CF, Jana-Kara B, Maxwell CA. Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance. *J Vector Borne Dis* 2003; *40* : 1-8.

Reprint requests: Dr Ashwani Kumar, Scientist 'E' & Officer-in-Charge, National Institute of Malaria Research Field Station, DHS Building, Campal, Panaji 403 001, Goa, India
e-mail: ashwani07@gmail.com