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Estimation and Comparison of *Anopheles maculipennis s.l.* (Diptera: Culicidae) Survival Rates with Light-trap and Indoor Resting Data

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Abstract

The main vector of malaria in Europe and Palearctic region is *Anopheles maculipennis* complex. In order to determine the survival rate of *An. maculipennis s.l.* this study was carried out in Garaboteh (Zanjan province, Iran) in July 2003. In this study anophelines were sampled for 31 consecutive days from five fixed animal shelters and five light traps. Out of 24481 collected *An. maculipennis s.l.*, 19703 (80.48%) were female. Relative density of female anopheline with 95% CI was 74.25-78.48 and 49.16-52.36 in light traps (LT) and pyrethrum space spray catch (PSC), respectively. A significant difference was in mean parous rate in LT (0.46) and PSC (0.50) samples ($P< 0.01$). Daily survival rate of anopheline mosquitoes in study area was 0.82-0.86. About 1.74% of female mosquitoes can transmit malaria after 10 d. In 4 d gonotrophic cycle, there was maximum correlation (>0.92) between parous and total females. Also there was significant correlation between nulliparous females and males in LT (r=0.96) and PSC (r=0.87) samples and with increasing anopheline abundance ratio of male/nulliparous significantly decreased (r=-0.35, P<0.05). Due to simplicity and feasibility of using graphical and parametric method, it can be placed in entomological surveys for monitoring parity, age structure and survival rate of vector anopheline in the world.

Keywords: Anopheles maculipennis s. l., *Survival rate, Malaria, Iran*

Introduction

Malaria is the most important vector borne disease which is transmitted by infected anophelinae mosquitoes, when they feed on human for their oviposition. Despite considerable progress in malaria control over the past decades, it is endemic in more than 100 countries of the world and kills 1.1-2.7 million people annually (1). Also more than 40% of the world populations live in areas with the risk of malaria transmission. The main vector of malaria in Europe and Palearctic region is *Anopheles maculipennis* complex (2). Out of nine known species of this complex, *An. maculipennis s.l.*, the historical vector of malaria in Europe and the Middle East, was the first discovered sibling species among mosquitoes (3, 4). This complex has been identified as the major vector of malaria in the Caspian Sea littoral and Central Plateau of Iran (5-8), higher altitudes in the north of Iraq, mainly from Zahko, Amadia, Rowanduz and Penjwin (9). Recently it has been identified as secondary vector in the Biga Plains of Turkey (10).

Control programs against anopheline vectors, such as large-scale use of insecticide impregnated bednets and indoor residual spraying reduce mosquito density and survival rate. The entomological impact of such programs can therefore be evaluated by age structure (11) and life expectancy of anopheline vectors (12).

McDonald (13) suggested that mosquito vectors mainly die of predation or environmental factors rather than old age. Clemens and Patterson

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(14) described two possible patterns of adult mosquito mortality. In the first pattern, mortality increases with age and there is a positive linear relationship between collection dates and mosquito age. Since anophelines are gonotrophically concordant after the first cycle, and the feeding cycle is equivalent to the oviposition cycle so parous rate can be used directly as an estimation of survival per feeding cycle (15-18).

In the second (Gillies' exponential model) the mortality rate does not vary with age. It can be estimated by mark-release-recapture experiments or laboratory multiple age-grading studies (19-23). The exponential model is one of the few methods applicable to gonotrophically discordant mosquito species. However, this model has several drawbacks, fundamental and potentially flawed assumption of the model is that the rate of female survivorship does not change with advancing age.

Pyrethrum space spraying and light-traps have been routinely used in studies of tropical and temperate mosquitoes (19, 24-28). Males of *An. maculipennis s.l.* like females are caught in light-trap. Indeed they rest commonly indoor and also are caught by pyrethrum space spray method. Ordinarily, male mosquitoes have low longevity (29, 30) and their activity is similar to nulliparous female specimens.

In this study the hypothesis that male anopheline frequency could estimate nulliparous female abundance, was tested in order to develop a more efficient use of the light trap and pyrethrum space spray for monitoring of age structure and parity of *An. maculipennis s. l.* and other malaria vectors in the world.

Materials and Methods

Study area The investigation was carried out in over period of one month (July, 2003) in district of Gharaboteh (47° 40′ 10″ E, 37° 7′ 15″ N), Province of Zanjan. This district lies about 85 km Southwest of Zanjan, center of province, and has about 5100 inhibitants. The district is about 1160 m above sea level and lies near the Ghezel-Ozan River. Rice fields, swampy places arising from irrigation waters, stagnant water, pools, stream fed pools, streams and fish rearing pools are the major sources of mosquito breeding in this area. The averages of maximum and minimum temperatures in summer are 32- 15° C and 5.3- -2.5° C in winter, respectively. The average yearly rainfall is about 300 mm. The district had not been under vector control program during the study period.

Light trap collections Five miniature CDC light-traps (Hausherr's Machine Works, Toms River, New Jersey and John W. Hock Company, Gainesville, Florida) were hung adjacent to mosquito nets. Light-traps operated by rechargeable batteries, were fitted with 12 V halogenated bulbs and 0.7 cm mesh grids to exclude larger insects. Traps were switched on at 21: 00 h local time, and were instructed to be switched them off at 06:00AM.

Pyrethrum space spray catches Five fixed animal shelters were used in this program. Every morning indoor rested mosquitoes in five animal shelters were collected by the standard method (25), using 0.2% pyrethrum spray.

Mosquito processing All collected mosquitoes were transferred into the plastic jars. The house and trap number and date of collection were recorded on the jar label and then jars were transferred into the cool box with ice packs. In the laboratory collections were identified under dissecting microscope (at x40) against standard taxonomic keys frequency of female and male of *An. maculipennis s.l.* in each sample were recorded and females were classified according to the blood digestion stages (abdominal conditions). Empty and freshly feed *An. maculipennis s.l.* were dissected for parity and classified as nulliparous and parous on the basis of the tracheolar skeins of the ovaries (31, 32).

Statistical analysis Survival rate and blood-meal frequency were estimated by using linear regression, to relate the numbers of mosquitoes biting on one day, to parous individuals collected one oviposition cycle later (17, 18, 33).

To test the efficiency of mosquito male frequency in estimating nulliparous females, graphical and parametric method were utilized (34) to examine bias and error in methods.

Results

A total of 24481 *An. maculipennis s.l.* were collected during July 2003. There was not significant difference in sex ratio of collected mosquitoes (X^2= 0.65, P= 0.42) in both methods. Out of collected specimens, 19703 (80.48 %) were female. There were not significant differences in abundance of mosquitoes in five light traps (LT) and five indoor pyrethrum space spray catch (PSC) (Table 1). Relative density of *An. maculipennis s.l.* female and male within indoor resting with 95% confidence limits was 74.25-78.45 and 17.68-18.96, respectively. In light trap samples, the density was 49.16- 52.36 and 11.91-13.11, respectively. In both studying methods, density of male anopheline was correlated with females (Fig. 1) and Pearson correlation coefficient between them was 0.7149.

Comparison of blood feeding stages of *An. maculipennis s.l.* caught by LT and PSC (Table 2) indicated that the proportion of blood fed captured mosquitoes by LT was lower (10%) than PSC (72.5%), (X^2 =14468.77, df = 2, P=0). Out of PSC captured *An. maculipennis s.l.*, 8476 females were dissected for parity. In dissected females, 4295 (51%) were parous and in LT samples out of 7869 dissected females, parous rate was 0.46. A significant difference (z= 6.67, P<0.01) in the mean parous rates was detected between LT and PSC captured mosquitoes (Table 3).

In 4 days gonotrophic cycle, there was maximum correlation (r= 0.92, for LT and r=0.99, for PSC) between parous and total female mosquitoes. Mean parous and survival rate during gonotrophic period in LT and PSC sampling methods were 0.46 and 0.50, respectively. This rate was unstable in first 10 days but afterwards was fixed (Fig. 2).

Pearson correlation coefficients for the relationship between abundance of log transformed nulliparous females and males in LT and PSC collections were calculated. There was significant correlation (Fig. 3) between nulliparous females and males in LT (r= 0.96) and PSC (r= 0.87) samples of *An. maculipennis s.l.* The potential bias in estimating nulliparous anopheline mosquito abundance by LT and PSC was examined graphically. There was significant decrease tendency (Fig. 4) for the ratio of male/nulliparous with increasing mosquito abundance in PSC (r= -0.35, P< 0.05) and LT (r = -0.58, P< 0.001) samples.

Table 1: Analysis of deviance for the mean number of *An. maculipennis s. l.* collected in light traps and pyrethrum space spray method from Garaboteh (Zanjan, Iran), July 2003

	Pyrethrum space spray		**Light trap**	
	Female	**Male**	**Female**	**Male**
Total number	11835	2839	7868	1939
Mean (95%CL)	74.25-78.45	17.68-18.96	49.16-52.36	11.91-13.11
F (4,151) ns*	0.14	0.05	0.16	0.06

ns* = not significant, P<0.001

Table 2: Blood feeding condition of *An. maculipennis s. l.* caught by light trap and pyrethrum space spray from Garaboteh (Zanjan, Iran), July 2003

Abdominal condition	**Light trap (%)**	**Pyrethrum space spray (%)**	**Total**
Empty	6685 (85)	189 (1.5)	874
Freshly-fed	790 (10)	8586 (72.5)	9376
Gravid	393 (5)	3060 (26)	3453

Table 3: Parameter estimates for *An. maculipennis s. l.* collected from Garaboteh (Zanjan, Iran) in July 2003

Time delay	Survival rate	Correlation index
Pyrethrum space spray captured mosquitoes		
0	0.506	0.38
1	0.508	0.11
2	0.509	0.19
3	0.509	0.55
4	0.508	0.92
5	0.509	0.69
6	0.5011	0.44
7	0.5014	0.12
8	0.5015	0.33
9	0.5012	0.56
10	0.508	0.65
light trap captured mosquitoes		
0	0.461	0.26
1	0.463	0.05
2	0.463	0.35
3	0.462	0.68
4	0.460	0.99
5	0.459	0.68
6	0.460	0.36
7	0.461	0.32
8	0.464	0.35
9	0.462	0.41
10	0.457	0.39

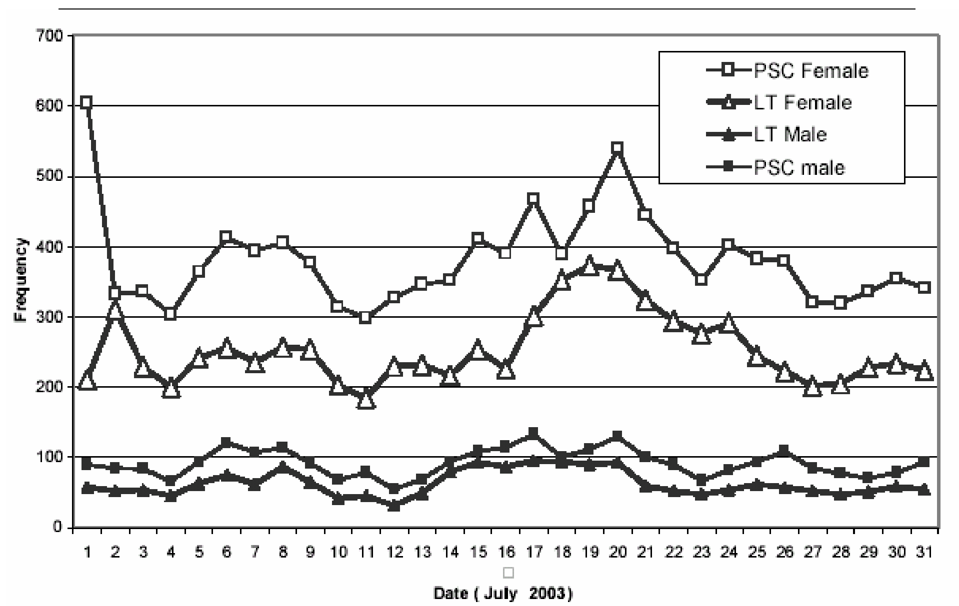

Fig. 1: Abundance of *An. maculipennis s.l.* collected by light traps (LT) and pyrethrum space spray (PSC) in Garaboteh (Zanjan Iran) in July 2003.

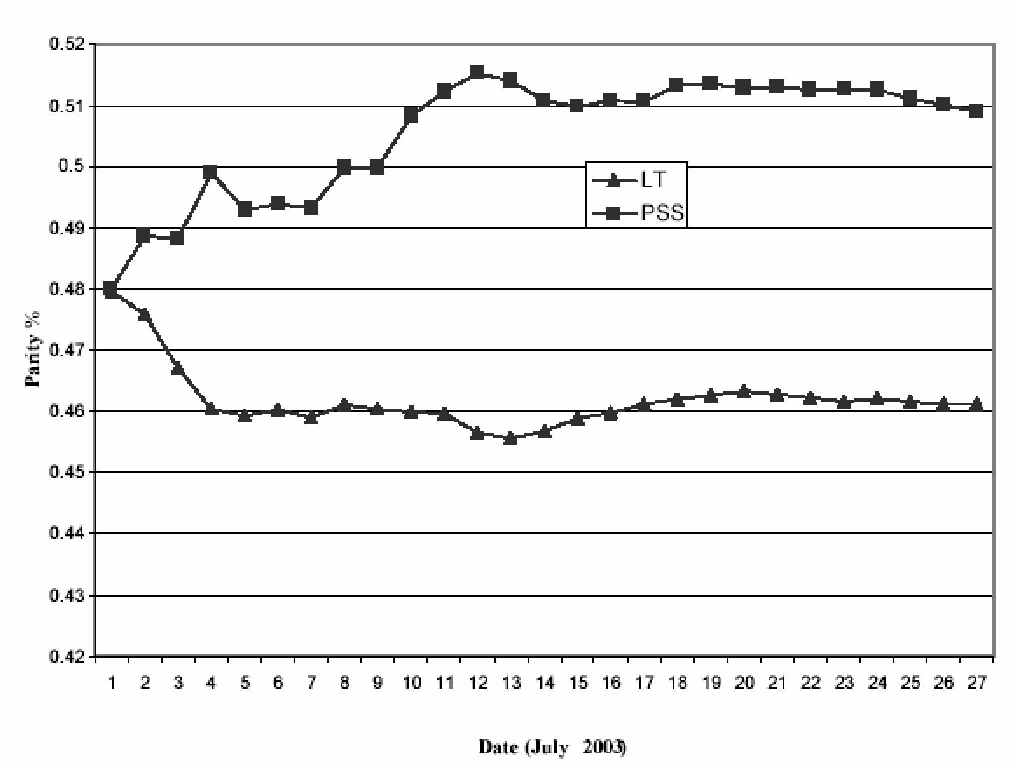

Fig. 2: Parity levels in *An. maculipennis s. l.* collected by light traps (LT) and pyrethrum space spray (PSS) in Garaboteh (Zanjan, Iran) in July 2003.

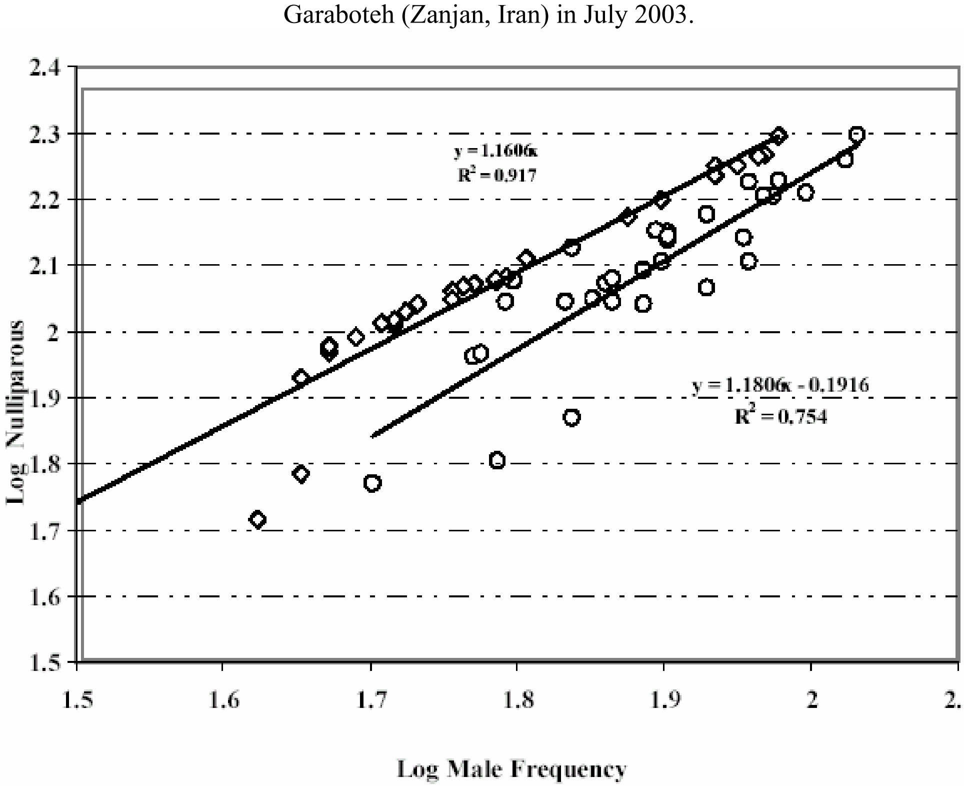

Fig. 3: Scatter distribution for the relationship between log transformed males and nulliparous females of *An. maculipennis s. l.,* collected with light traps (◊) and pyrethrum space spray (O) in Garaboteh (Zanjan, Iran) in July 2003.

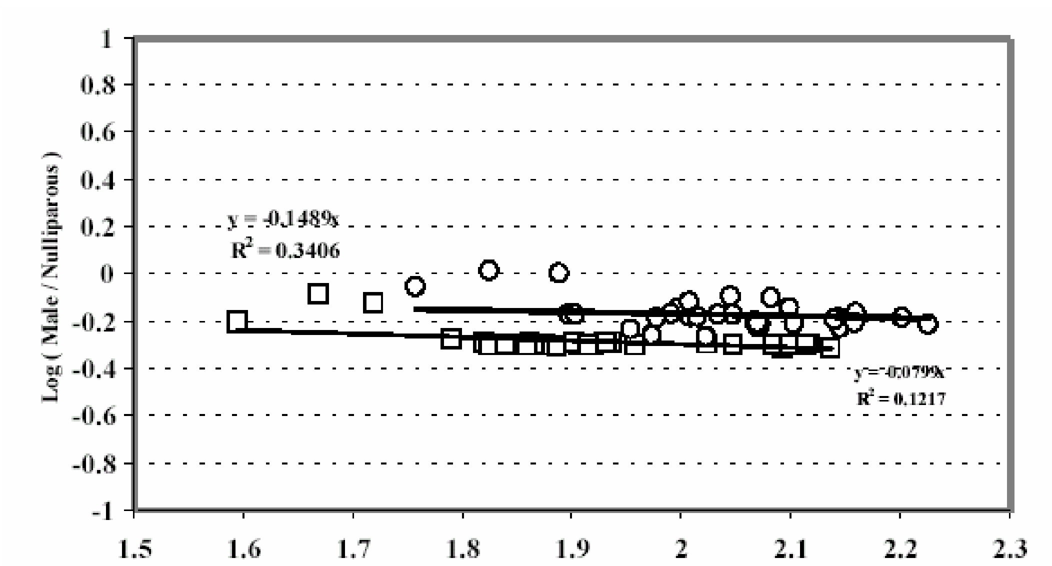

Fig. 4: Scatter distribution for the relationship as log ratio between male and nulliparous abundance and the William's mean (on logarithmic scale) of male and nulliparous females of *An. maculipennis s. l.,* catch by light traps (□) and pyrethrum space spray (O) in Garaboteh (Zanjan, Iran) in July 2003

Discussion

Although malaria was officially declared eradicated from central plateau of Iran in 1975, its former vectors, mainly members of the *An. maculipennis* complex, are still distributed throughout the plateau and Caspian Sea littoral. The present situation of anophelism without malaria indicates that current socioeconomic and environmental conditions maintain the basic case reproduction number (Ro) below 1. Recently, it has been speculated that predicted climate changes may increase anopheline abundance and biting rates (as well as reduce the *Plasmodium* parasite extrinsic incubation period), allowing the reemergence of malaria transmission.

The findings showed that *An. maculipennis s.l.* had high density in the study area. These observations are consistent with the results of Manoucehri et al. (5) in central Plateau and Caspian Sea littoral. High reletive density has been reported for *An. gambiae* as 44-65 (33) and for *An. punctipennis* as 16-72 (35).

According to findings, outdoor light traps underestimated indoor resting anopheline. This observation is consistent with works of Charlwood et al. (36) who found that the relative density of *An. farauti* collected in light traps was 25-80 from Papua New Guinea. High density of *An. maculipennis s.l.* in Garaboteh (Zanjan) is expected, because in this area vector control program has not been performed over the past 30 yr and study area has potential breeding sites. Because the use of light traps in connection with mosquito nets decreased the number of blood fed mosquito caught, so 90% of females caught from light traps were unfed. Wekesa et al. (37) showed that only 28% of *Culex tarsalis* collected from different habitats were blood fed. In their studies this percentage for *An. freeborni* was 23%. Pyrethrum space spray unlike light traps caught >72% of freshly fed females. Sadandene et al. (28) in study of Korapat, Orisa, India: found that 82% of anopheline mosquitoes caught from indoor light traps were freshly fed.

In this study an oviposition cycle of 4 d had high correlation coeficiency in parous and total *An. maculipennis s.l.* A significant oviposition cycle of 4 d was obtained for *An. arabiensis* at Mwea with potential breeding sites, however that was on inland site with relatively low mean temperature (33). Overestimate on gonotrophic cycle of *An. freeborni* in Sacramento Valley, *An. punctipennis* in Maryland and *An. quadrimaculatus* in Florida were reported to be 4-6, 4-5, and five d (38- 40). Unlike this study, lower estimation of gonotrophic cycle was reported from tropical areas. The length of oviposition cycle for *An. gambiae* and *An. merus* in rainy seasons was 2 d. In both cases the breeding places were close to sampling sites. An oviposition cycle of 3 d was obtained for *An. gambiae* at Misha, Kenya during dry seasons while there were not potential breeding sites (33). The additional time required for oviposition in Garaboteh (Zanjan) may reflect the influence of environmental conditions especially mean temperature.

A significant difference was seen in mean survival rate per oviposition cycle in the population of *An. maculipennis s.l.* caught from light traps (0.46) and pyrethrum spray catch (0.50). Lower parity was reported by Githeko et al. (41) in light trap samples. A significant difference in the mean parous rate later confirmed by McHug (39, 42) between unfed (0.36.4) and blood-fed (42.5) of *Culex tarsalis.* This discrepancy may be explained by difference in the genetic composition of two vector population with respect to the member of *An. maculipennis* complex, special behavior of nulliparous or parous mosquitoes and lowered parity of unfed females. However, low survival rate was reported for *An. labranchiae,* an important malaria vector of Italy (43, 44), some population of *An. punctipennis* from Pupua New Guinea (45). Mean daily survival rate obtained in this study (0.82-0.84) was higher than some populations of *An. maculipennis* from Iran (5) and *An. labranchiae* from Italy (43, 44). In case of other *Anopheles* species, the probability of daily survival was 0.89 for *An. pharensis* and 0.80 for *An. multicolor* in Egypt (46), 0.80-0.83 for *An. pulcherrimus* in Iran (47), 0.88 for *An. psudopunctipennis* in southern Mexico (48), 0.80- 0.88 for *An. gambiae s.l.* in Sudan (21) and 0.45- 0.68 for *An. vestitipennis* in southern Mexico(49). The importance of daily survival of vectors for efficient transmission of infection is obvious. Mean temperature at Garaboteh (Zanjan) in July was 25°C and duration of sporogonic cycle of *Plasmodium vivax* is 10 d at 25° C. Therefor the probability of *An. maculipennis s.l.* survival through 10 d was 0.174, which means that 1.74% of female mosquitoes respectively may live long enough to transmit malaria.

The findings showed significant correlation between males and nulliparous data. There was significant tendency for the ratio of male/ nulliparous, so decrease arises with increasing mosquito abundance. Also there was difference in the log ratio on male/nulliparous between light trap and pyrethrum space spray catch data. The mean log ratio for LT and PSC data were -0.3 and -0.2, whose antilogies were 2 and 1.5, respectively. This means that on average the catch 1 male from LT or PSC method were 2 and 1.5 times that of nulliparous in that sample. Graphical and parametric method has been successfully used for detecting relationship between anopheline abundance in light traps and indoor resting or outdoor human landing collections (50-54). Due to its simplicity and feasibility on anopheline parity detection, this method can be placed in public health evaluation plans and declare the age structure and survival rate of vector anopheline in different geographical areas.

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