SURVIVAL AND EMERGENCE OF IMMATURE *ANOPHELES ARABIENSIS* MOSQUITOES IN MARKET-GARDENER WELLS IN DAKAR, SENEGAL

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Summary :

Anopheles arabiensis is the unique species of the *An. gambiae* complex observed in the wells dug by market-gardeners in the Dakar area. In order to relate the numbers of immature stages and emerging adults mosquitoes, population measurements were performed in eight wells in which *An. arabiensis* was the only mosquito species. Mean density of immature stages was measured using two sampling methods, the dipping with a tray by giving 50 dips in each well, and the quadrat with a frame on 2 or 3 m^2 in each well. The absolute number of emergent adults was obtained by collecting mosquitoes under net-trap covering entirely each wells.

The dipping method was quicker and more operational than quadrat method. Density estimations of larvae at stage I to IV did not significantly differed using dipping or quadrat methods. On the contrary, pupal density was underestimated when measured by dipping.

Mosquito nets placed over wells increased significantly emergence rate of adults, thus measurement of emerging mosquitoes was possible only the first day following the net putting up. The total number of immature stages in each well was significantly correlated with the number of emergent mosquitoes. The mean number of mosquitoes emerging daily from one well corresponded to 5 % of the total number of immature stages.

Stage distribution for larvae I to IV and pupae, estimated by quadrat, was respectively 29 %, 28 %, 22 %, 16 % et 5 % (total = 100 %). Taking account the mean duration of various immature stages and the number of emerging mosquitoes by day, the equation of the survivorship curve from larval hatch (excluded) to emergence included was : y = 427.2 - 136.8 Log x. Therefore the mean mortality at immature stages was 80% i.e. an emerging rate of 20 %.

The results of this study, associated with those of previous ones, permit to evaluate the average productivity of malaria vectors in market-gardener wells in the Dakar area.

KEY WORDS : *Anopheles arabiensis*, immature stages, survival, emerging rate, sampling methods, well, malaria, Dakar.

MOTS CLÉS : Anopheles arabiensis, *stades préimaginaux, survie, taux d'émergence, méthodes d'échantillonnage, puits, paludisme, Dakar.*

Résumé : SURVIE ET ÉMERGENCE DES STADES AQUATIQUES DU MOUSTIQUE *ANOPHELES ARABIENSIS* DANS LES PUITS MARAICHERS DE DAKAR, SÉNÉGAL

Anopheles arabiensis est la seule espèce du complexe An. gambiae rencontrée dans les puits maraîchers (localement dénommés céanes) creusés dans la région de Dakar. Dans le but de relier les nombres de stades préimaginaux et de moustiques émergents, des dénombrements de population ont été réalisés dans huit puits dans lesquels An. arabiensis a été le seul Culicidae rencontré. La densité moyenne des stades préimaginaux a été évaluée par deux méthodes d'échantillonnage : la méthode du dipping avec un plateau à raison de 50 coups de plateau par puits, et la méthode du quadrat avec un cadre de 1 m^2 à raison de deux ou trois quadrats par puits. Le nombre absolu d'adultes émergents a été obtenu en recouvrant chaque puits d'une moustiquaire et en collectant les moustiques néonates ainsi piégés. La technique du dipping s'est révélée plus rapide et plus opérationnelle que la technique du quadrat. Les estimations des densités de chacun des quatre stades larvaires n'ont pas différé significativement entre les méthodes du dipping et du quadrat. Mais la densité des nymphes a été sous-estimée avec le dipping.

La présence de moustiquaires-pièges sur les puits a significativement augmenté l'émergence, aussi, l'évaluation de l'émergence des adultes a seulement été possible lors de la première journée de mise en place des moustiquaires. Le nombre total de stades préimaginaux par puits a été significativement corrélé au nombre de moustiques émergeant. En moyenne, le nombre d'adultes émergents quotidiennement d'un puits a représenté 5 % du nombre total des larves.

Les stades larvaires I-IV et nymphal, estimés par quadrat, ont respectivement représenté 29 %, 28 %, 22 %, 16 % et 5 % (total = 100 %). Compte tenu de la durée moyenne de chaque stade préimaginal et du nombre de moustiques émergeant quotidiennement, l'équation de la courbe de survie depuis l'éclosion (exclue) jusqu'à l'émergence inclue a été : y = 427,2-136,8 Log x. En moyenne la mortalité préimaginale a donc été de 80 % i.e. un taux d'émergence de 20 %.

Les résultats de cette étude, associés à ceux d'études précédentes, permettent d'évaluer la productivité moyenne en vecteurs de paludisme dans les puits maraîchers de la région de Dakar.

Anopheles arabiensis is one of the two most potent malaria vectors of the *An. gambiae*. complex. It is prevalent in the most part of the sub-Saharan Africa and in much localities, like in Senegal, it is the main vector of human malaria (Fontenille *et al.*, 1997; Lemasson *et al.*, 1997). Its immature stages occur in a great variety of habitats, principally in temporary, sunlit pools such as rain puddles, but also in some (sub)permanent habitats like rice fields or wells (Gillies & Coetzee, 1987).

In the urban area of Dakar, the *An. gambiae* complex is only represented by *An. arabiensis* which is the major vector of malaria and the most abundant anthro-

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pophilic anopheline: about 95 % of anophelines biting man during the wait season from July to October belong to this species (Vercruysse & Jancloes, 1981; Trape *et al.*, 1992). In this area urban farming appears to be economically significant and any favourable zones are converted into market-gardens. Rudimentary wells (not reinforced with cement) are dug by the market-gardeners in the sand. Water is available in good supply and is used to irrigate allotments during the whole year. The term "céane" is used locally to denote these wells. More than 5,000 of them had been counted in the lowlands. These wells constitute effective breeding places for many mosquito species, and 33 % from them harbour immature *An. arabiensis*, especially from May to September with a maximum in June (Robert *et al.*, 1998).

In order to have a better understanding of population dynamics of that malaria vector in the market-garden wells this study aimed (i) to evaluate the survival of each immature stages from larval hatch to adult emergence, and (ii) to relate the numbers of immature stages and neonate adults.

MATERIALS AND METHODS

STUDY SITE

In the Dakar area climate is characterised by the alternation of (i) a rainy season during three months (July to September) with 200-500 mm of rainfalls, temperatures varying from 24 to 30°, and (ii) a dry season during the nine remaining months without any significant rainfall. Temperatures are relatively moderate (19-25°) between December and February.

This study was carried out in September-November 1995 and July-August 1997 in wells located in the town of Dakar within the quarters "Cerf-Volant" and "Bel-Air" in which mosquito species other than *An. arabiensis* were exceptional and were not considered further.

WELLS

Wells were selected with respect for their effective immature *An. arabiensis* and their large range of larval density. These wells measured between 6.3 and 11 m^2; their deeps were < 1 m. It must be noted that these selected wells are not representative for the Dakar area where 67 % of wells do not harbour any immature *An. arabiensis* and where mean larval density are weak (0.16/dip, Robert *et al.*, 1998).

SAMPLING IMMATURE STAGES

Amongst available sampling methods described by Service (1993), dipping method and/or quadrats method seemed the most appropriate for our purpose. These methods had been previously compared between them, but Service in his review (pp. 125-129) underlined the need for more studies comparing the efficiencies of different sampling methods. That is why we used two methods, dipping and quadrat, to sample immature stages of *An. arabiensis* in wells. The well known aggregation observed in the spatial distribution of larvae obliged to performed sampling for a relatively large surface.

Dipping was realised using one tray (L = 22 cm × l = 11 cm) which contained 500 ml and covered 0.024 m^2. It consisted in 50 dips among which 25 at the well periphery and 25 in the central area. The collected larvae were put back in the water after each dip. Total timing of this sampling varied between 20 and 60 minutes depending on the larval density within well.

Quadrat consisted to use a wooden and square frame with sides of one meter wide, to limit a space in the well surface in which all immature stages were collected and counted. In any case quadrat reached the bottom of the well. Quadrats were performed at least two times by well at the well periphery and in the centre ; if the well surface was large enough a 3rd quadrat was performed in an intermediate zone. Collected larva were put back in the well when observations on one quadrat were completed. Quadrat method took about one hour by quadrat i.e. 2-3 hours by wells.

PRODUCTION OF EMERGING ADULTS FROM WELLS

In the same wells than above and just after investigations made on immature stages, in the early afternoon, a large mosquito net was put up above the water surface, recovering totally each well (Service, 1977); it was maintained during three consecutive days and visited daily, early in the morning. Absolute number of neonate mosquitoes was obtained by catching male and female mosquitoes trapped under the net.

DURATION OF IMMATURE STAGES

The mean duration of pupal stage was measured in insectary using larvae IV collected from these wells; larvae which underwent pupal exdysis in the following hours after collection were individually placed and were observed every 30 minutes until emergence. The mean duration of the whole aquatic development was 11.2 days (Awono-Ambene & Robert, unpublished data). Following these observations and those of Service (1971) the mean durations of larvae I-IV used in this study for survival curve calculation were 1.5, 2.5, 2.5 and 3.5 days, respectively.

DATA ANALYSIS AND STATISTICAL TESTS

The efficiency of dipping versus quadrat for sampling immature stages was compared using the non parametric Wilcoxon test for two paired groups. Total number of immature stages in one well was evaluated from larval density estimation obtained with the quadrat method, multiplied by the measured well surface. The stage specific survivorship was estimated by dividing the total number of each of the five immature stages by their respective stage duration. The productivity of emerging adults was compared between wells by estimating the total number of immature stages using the correlation coefficient test. The equation of the survival curve which was the best fitted straight line was calculated with Microsoft Excel 5.0 software.

RESULTS

This study was realised in eight wells called A to H. Results concerned 6,866 larvae plus pupae, and 2,878 adult mosquitoes.

COMPARISON BETWEEN DIPPING AND QUADRAT

Total numbers of immature stages were got on one hand by 50 dips using a tray (corresponding to 1.20 m^2) related to 1 m^2, on the other hand by two or three quadrats related to 1 m^2 (Table 1). Comparing these two methods no significant difference were observed for each of the four larval stages (P always > 0.61 by Wilcoxon's test). On the contrary more pupae were collected using quadrat than using dipping ($z = -1.99$, $P = 0.046$ by the same test; Fig. 1). Larval stages I to IV and pupae, collected using quadrat, represented respectively 28.7 %, 28.4 %, 22.1 %, 15.5 % and 5.3 % of the whole immature population of *An. arabiensis*.

EMERGING ADULTS

The number of emerging adults was very variable between the different wells (Table II). It increased from the first to the third days after the net putting up; that was unexpected and brought evidence that the mosquito nets covering the wells increased the emerging rate of mosquitoes. This could be due to an increase (+ 1.2 °C in average) in the water temperature during the day as well as during the night. At any day after the net putting up no significant differences were observed between males and females (χ^2 always ≥ 1.06, $P \geq 0.30$).

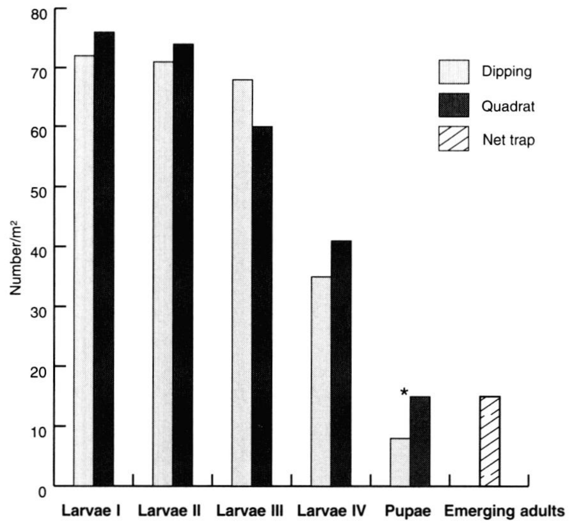

Fig. 1. – Mean densities per square meter of *An. arabiensis* collected from seven wells. The immature stages were sampled with dipping and quadrat; asterisc indicates significant difference between these two methods. The total emergent adult populations were traped under large nets positioned over wells.

DURATION OF PUPAL STAGE

Pupal exdysis were observed for 118 larval stage IV from 15 h to 23 h 30 with a mean (± SD) of 18.59 h ± 1.25. The emergence was observed from 21 h to 2 h 30 with a mean of 22.22 h ± 0.57 (Fig. 2). The mean pupal stage lasted 27.63 h ± 1.20 (i.e. 1.15 days). No differences were observed between males and females for the pupal exdysis time, for emergence time nor the total duration of pupal stage (data not shown).

Well	A		B		C		D		E		F		G		H		Total	
	d	q(3)	d	q(3)	d	q(2)	d	q(2)	d	q(2)	d	q(3)	d	q(2)	d	q(3)	d	q
Larvae I	42	91	57	99	138	305	32	31	18	39	220	650	94	136	0	0	601	1,351
Larvae II	39	74	53	67	159	296	43	40	15	29	213	685	85	142	0	0	607	1,333
Larvae III	28	39	27	67	240	212	37	27	16	56	153	543	58	95	1	1	560	1,040
Larvae IV	28	56	21	58	77	138	24	29	3	16	107	435	23	29	0	0	283	761
Pupae	4	21	5	18	10	33	12	20	0	0	32	158	4	13	0	0	67	263
Total	141	281	163	309	624	984	148	147	52	140	725	2,471	264	415	1	1	2,118	4,748

Table I. – Total number of immature stages of *An. arabiensis* collected by dipping (d) on 50 tray dippers or quadrat on two square meters (q2) or quadrat on three square meters (q3) in height market-garden wells in Dakar, Senegal.

Well	A		B		C		D		E		F		G		H		Total	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Day 1	23	14	22	18	58	44	40	42	4	0	186	253	5	5	0	0	338	376
Day 2	49	36	4	6	170	141	9	15	20	9	258	254	10	13	2	0	522	474
Day 3	16	13	23	34	69	54	20	13	70	73	390	357	19	16	0	1	607	561

Table II. – Number of emerging *An. arabiensis* males (m) and females (f) during three consecutive days from height market-garden wells covered by net traps in Dakar, Senegal.

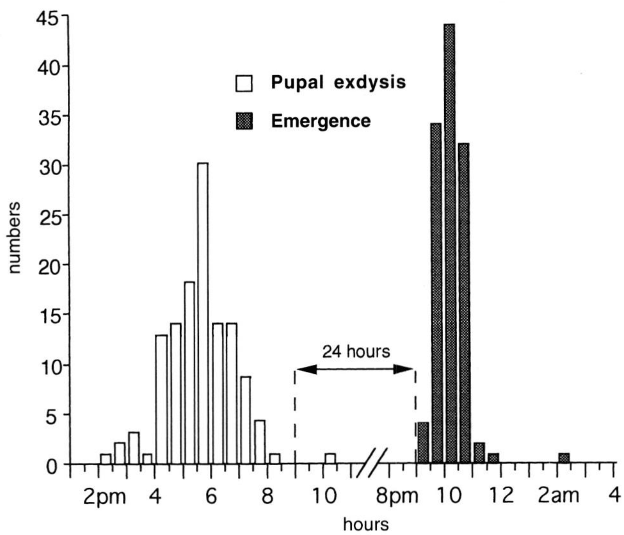

Fig. 2. – Timing of pupal exdysis and emergence in *An. arabiensis* at 27 °C (n = 118).

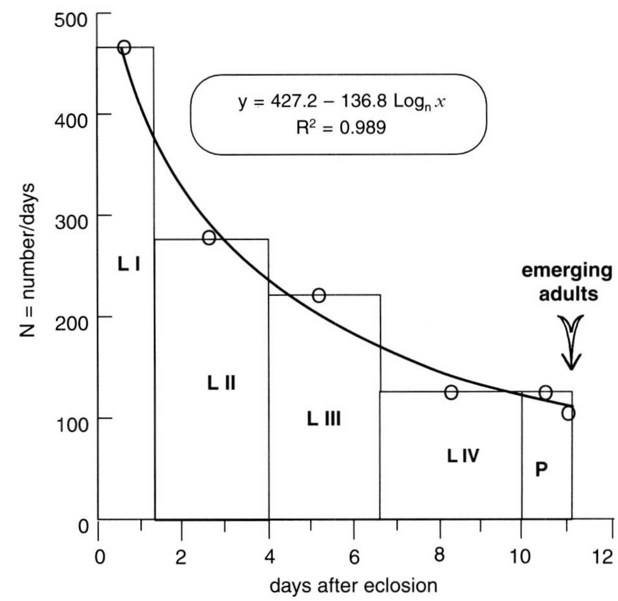

Fig. 3. – Age distribution and survivorship curve of the immature stages of *An. arabiensis* in market-gardener wells in Dakar (N = mean numbers of each stages by well divided by their instar duration, L I-IV = larval stages, P = pupae).

Survival curve

Due to its very low number of larvae the well H was excluded from that analysis which was made with the seven wells A to G. The number of larvae by stage divided by the mean duration of each stage (i.e. 1.5, 2.5, 2.5, 3.5 and 1.2 days respectively for larval stages I-IV and pupae) made the six basic points necessary to calculate the survival curve (Fig. 3). Various curves were searched using following models : simple regression, polynomial, power, logarithmic and exponential. Finally the best fitted curve (R^2 = 0.987) was obtained when using the following Log equation :

$$y = 375 - 117.5 \text{ Log}_n\ x$$

That is to say, in average, the number of neonates mosquitoes which emerged daily from one well represented about 20 % of the total number of stage I larvae, i.e. the survival rate from larval hatch (excluded) to emergence included was 0.20.

Relationship between larval stages and emerging adults

The number of adults emerging from one well was directly linked to its immature population size. This link between (i) the total number of immature stages by well, estimated by quadrat and related to the well surface, and (ii) the daily number of emerging adults was significant (r = 0.95, n = 8, P < 10-4). The equation of the best fitted straight line was :

$$y = 0.047\ x - 13.4 \text{ with } R^2 = 0.92.$$

That is to say, the number of adults mosquitoes which emerge daily from a well represented an average of 5 % of the total number of immature stages present in that well.

DISCUSSION

Our study in the market-garden wells of Dakar permitted : to compare the dipping and the quadrat as sampling method of immature stages of *An. arabiensis*, to document the duration and the survival of these immature stages, to define the limits of utilisation for net-traps in order to evaluate the emergence rate of adult mosquitoes, and to establish a relationship between immature stage density and emerging rate.

Larval population measurements in the wells did not differ when performed with dipping or quadrat. The difference observed only for pupae might be due to a greater speed of movement in the water thus permitting to escape with dipping. Finally the dipping remain a reliable and operational method to sample larval population of *An. arabiensis* in the wells. But, for pupal sampling the quadrat appear to be more powerful, and must be preferred.

Continuous presence of the mosquito net placed over the wells was associated, at least for three days, with a regular increase of the *An. arabiensis* emergence rate. That can be due to an increase in the water temperature, the net acting as a lid by a greenhouse effect. Nevertheless it is also possible that the absence of renewal for youngest larval stages, due to the inaccessibility of the oviposition site for gravid female mosquitoes, would facilitate the development of the older larval stages possibly by reducing the intraspecific competition for alimentary resources. For these various reasons emerging rate was calculated in our study considering only the results obtained after the first night following the net trap putting up. Our observations on the exact time of emergence of *An. arabiensis* during the late afternoon are in total agreement with those of Jones & Reiter (1975).

Calculation of the survival curve was marred by two causes. First the estimation of larval density had been confronted to larval aggregation and movements (Russel *et al.*, 1945; Wada & Mogi, 1974; Okazawa & Mogi, 1984). Second the mean duration of each larval stage was difficult to measure in the fields and the observations realised in insectaries must be use with caution because numerous parameters vary between natural and artificial environments (nutriment, larval density, mean temperature, temperature variation, etc.). It is of interest to note in our study that a curve in Log presented the best fit. That makes sense with the generally admitted notion of a constant mortality rate during aquatic development, unrelated to age. The survival during immature stages was estimated to 20 %. This percentage is high regarding those reported by various authors: survival from larval hatch to adult emergence was 4 % for *An. gambiae* s.l. in borrow-pits in Kenya (Service, 1971), 1-5 % for anophelines in Philippine rice fields (Mogi *et al.*, 1984), 0-6 % in Thailand rice fields (Mogi *et al.*, 1986). In our study the high survival rate is possibly related to the absence of main predators such as larvivorous fishes, ordinary abundant in the market-garden wells of the Dakar area and very efficient in the control of immature mosquito populations (Robert *et al.*, 1998).

Noticeable variability in the adult mosquito productivity had been observed between various wells ; that agree with Bailey *et al.* (1980) for whom the variations of population size for adult mosquitoes are not only due to larval density. Physicochemical characteristics of each well are also factors which contribute to explain this variability (Robert *et al.*, 1998). Nevertheless the number of adults *An. arabiensis* emerging from the wells was correlated with the number of immature stages ; this finding permit, in the context of market-garden well of the town of Dakar, to hypothesise on the productivity of these wells directly from immature population measurement. Previous study (Robert *et al.*, 1998) observed a maximal density (per m^2) of 28.4 larvae of *An. arabiensis* in June (mean obtained from 48 wells distributed within 5 different quarters in the town of Dakar) ; with a mean surface of 10 m^2 by well, and 5 % of the total number of immature stages which emerge daily, the daily productivity in adult at the maximal larval density should be $28.4 \times 0.05 \times 10 = 14.2$. With a sex-ratio of 1:1 the number of *An. arabiensis* females emerging daily from each well during the density peak is thus estimated to be 7.1. This estimation must be regarded as tentative but from there it underlines that the 5,000 market-garden wells of the Dakar area constitute effective breeding places for anopheline vectors. This information is crucial in the perspective of malaria vector control in the urban Dakar.

ACKNOWLEDGEMENTS

This study was funded by Orstom (Institut français de recherche scientifique pour le développement en coopération) and by the French Ministry for Cooperation and Development (programme paludisme).

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Reçu le 31 décembre 1998
Accepté le 23 février 1999