GONOTROPHIC CYCLE STUDY OF THE *Anopheles hyrcanus* SPECIES GROUP (DIPTERA: CULICIDAE) USING MARK-RELEASE-RECAPTURE EXPERIMENTS

Penelitian Siklus Gonotropik Anopheles Hyrcanus Group (Diptera : Culicidae) Dengan Percobaan Pewarnaan– Pelepasan – Penangkapan Kembali

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Abstrak. Telah dilakukan penelitian siklus gonotrofik kelompok *Anopheles hyrcanus* di desa Kebondowo, Kabupaten Semarang, Propinsi Jawa Tengah, Indonesia. Penelitian dilakukan dengan percobaan pewarnaan- pelepasan – penangkapan kembali *(Mark release recapture)* pada tahun 1996. Nyamuk yang ditangkap dibius dengan ether dan diidentifikasi. Nyamuk betina *An. peditaeniatus* dan *An. nigerrimus* kenyang darah dihitung dan disemprot dengan serbuk pewarna fluresen ("FIESTA" pigmen fluresen) menggunakan alat "insufflator" dan segera dilepas kembali. Spesies nyamuk *An. hyrcanus* yang tertangkap hari ke 2, 3 dan 4 diperiksa untuk melihat pewarnaan yang dilakukan pada malam sebelumnya, menggunakan lampu ultra violet (ULV) yang dioperasikan dengan batu baterai. Sampling nyamuk untuk penangkapan kembali dari setiap pelepasan dilakukan selama 8 malam berikutnya. Perkiraan ketahanan hidup dilakukan dengan perhitungan regresi jumlah nyamuk tertangkap setelah ditransformasi dengan ln (y + 1) sebagai fungsi waktu di dalam hari setelah pelepasan. Analisis data dilakukan dengan program komputer Systas untuk regresi jumlah nyamuk tertangkap dan hari setelah pelepasan. Rata-rata kehidupan harian kemudian dihitung sebagai antilog dari pada koefisien regresi. Percobaan pewarnaan – pelepasan – penangkapan kembali, ditemukan bahwa irama siklus gonotropik 3 hari yang merefleksikan pertumbuhan populasi kelompok *An. hyrcanus* lebih cepat.
Kata kunci : *An. hyrcanus* group; siklus gonotrofik; pewarnaan – pelepasan – penangkapan kembali

INTRODUCTION

Anopheles hyrcanus species group is readily distinguished from other mosquito species by the presence of a tuft of dark scales on each side of the clypeus, four pale bands on the palps and the ventral tuft of dark scales on the seventh abdominal segment in the adult. The stem of wing vein 5 always has a well defined dark mark towards the base (Harrison,1975, Reid,1953, Reid,1968). They are found almost everywhere in the country, from mountains to brackish water fringes. Initially this group was regarded as *An. hyrcanus* var. *sinensis* which is characterized by narrow hind-tarsal pale bands confined to the apex of each segment. *Anopheles hyrcanus* var. *nigerrimus* is distinguished by broad hind-tarsal pale bands, which cross the joints (Reid,1953). Past reference to the two variable varieties were in fact referred to eight species, namely: *An. sinensis, An. lesteri paraliae, An. peditaeniatus, An. Nigerri-mus, An. crawfordi, An. nitidus, An. Argyropus* and *An. Pursati* (Harrison, 1975, Reid,1968).

Gonotropic cycle of mosquito a repetition of the biting and egg – laying cycle. If mosquito vector survives long enough (say about 12 days) to transmit malaria, mosquito likely to have laid eggs 3 to 4 times (gonotrophic cycle for Anophelinae reported 3 days) (Reid,1968).

Studies on ecology such as dispersal, survival rate and the length of the gonotrophic cycle are essential in understanding the epidemiology of transmission of vector-borne diseases such as

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malaria and filariasis. The survival rate determines the mosquito's longevity and correlate with the reproductive rate (Holmes,1987). With an estimate of the oviposition cycle in nature, it is possible to determine the average number of potentially infective bites that a female mosquito can inflict during her life-time (Charlwood, 1986, Charlwood,1985). The gonotrophic cycle of mosquitoes has been used to determine the survival rate based on the mean parous rate (Davidson,1954). The study was conducted to determine the gonotrophic cycle of the *An. hyrcanus* species group.

MATERIAL AND METHOD

Study Area

The study was conducted in the village of Kebondawa, in the subdistrict of Banyubiru, Semarang regency, Central Java province.This village is situated inland, about 40 km from the sea at an elevation of approximately 550 m, and is situated in the periphery of the Rawa Pening lake.The lake is more than 30 square and 5-10 meters deep. It has been used as the resource of electric power, for fish culture and as the water source for ricefields irrigation. Most of the water surface is covered with floating and emergent vegetations such as *Eichornia* sp., *Azolla* sp., *Pistia* sp., *Jussieua* sp., *Hydrilla* sp. and *Ipomoea* sp., and the surrounding area is overgrown with various species of grass. Other vegetations grown in the village are among others coconut palms, bamboo and cassava. Domestic animals such as chicken, ducks, geese, goats and water buffaloes are quite common. Most houses are built close to each other and made of wood or cement. The village area is about 15 square, and is surrounded by ricefields. Rice is only cultivated extensively during the dry season, whereas during the wet season the field is flooded with water. The lake and ricefields are the principal mosquito breeding habitats in the village.

METHOD OF THE STUDY

The gonotrophic cycle was studied by the mark-release-recapture method, with two replication in May and August 1996. Mosquitoes were collected with an aspirator while they were resting on the water buffalo shelters, placed in polyethylene cups, anesthetized with ether and immediately identified. Fully-blood-fed females of *An. peditaeniatus* and *An. nigerrimus* were counted and placed in the different polyethylene cups with 15 mos-quitoes in each cup. After the mosquitoes recovered, they were transferred into 300 ml polyethylene cylinders covered by mosquito netting at both open ends. They were then sprayed with fine fluorescent dust ("FIESTA" fluorescent pigments), blown through the polyethylene containers using an insufflator (Reisen,1979, WHO,1963), and then released immediately. This procedure was done according to that described by Reisen and Aslamkhan (Reisen,1979), in four consecutive nights using different colours for each night. All mosquitoes collected for the second, third and fourth releases were checked for markings from previous nights under a portable, battery-operated, ultra-violet light (UVL) lamp. Sampling for recaptures from each release was carried out over 8 consecutive nights, between 1800 to 2400 hours. Resting collections were conducted in the cattle sheds (buffalo shelters) and the surrounding vegetation. On nights five through nine, all samples collected were killed, screened under UV light and identified using a dissecting microscope. Experiments were conducted in the Kebondawa village, Central Java, Indonesia. Two species (*An. peditaeniatus* and *An. nigerrimus*) were chosen for the gonotrophic cycle study, due to the abundance of both species in the study area. The temperature and relative humidity of the surrounding areas were recorded daily throughout the period of the experiments. Temperature range was from 22.5 to

31.5°C and relative humidity fluctuated between 75 and 96%.

The survivorship was estimated by regressing the number of recaptured mosquitoes after transformed into ln(y+1) as a function of time in days after release (Reisen,1979). Data was analyzed using computer program Systat, for regression of the number of recaptured mosquitoes and day after released. The daily survival rate was then calculated as the antilog of the regression coefficient.

RESULTS AND DISCUSSION

Two species of *An hyrcanus* group were found in the study area. A total of 3,972 *An. peditaeniatus* and 2,946 *An. nigerrimus* blood fed individuals which were marked and released, 124 (3.12%) and 88 (2.99%) were respectively recaptured during 8 consecutive nights. The study was revealed that the peaks of recaptured occurred were on night three, followed by night four and five for both species *An. peditaeniatus* and *An. nigerrimus* (Tabel 1).

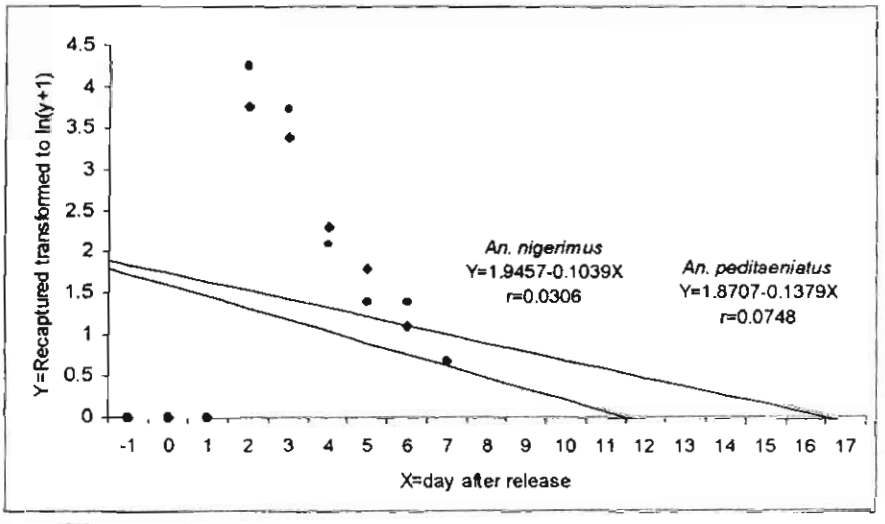
The number of recaptured mosquitoes, after transformed into ln (y+1) decreased significantly as a linear function of time in days after release for eight days recapture sequence. Unfed and fed recaptured mosquitoes were analyzed (half gravid and gravid recaptured were resting mosquitoes not for blood feeding). Low numbers of recaptured were recorded 124 (3.12%) and 88 (2.99%) were respectively for *An. peditaeniatus* and *An. nigerrimus* (two experiments), which are mostly caught on day three (Table 1).

Survivorship was not estimated for each experiment separately, due to very low numbers of recaptures were recorded for both experiments. Therefore the data of both species from the two experiments were pooled and used for regression of recaptures on time (day) for the estimation of survivorship of the *An. hyrcanus* species group. The regression coefficient was calculated to be r = 0.0748 (*An. peditaeniatus*) and r = 0.0306 (*An.* nigerrimus). The daily survival rate was then calculated as the antilog of the regression coefficient, as 0.7883 for *An. Peditaeniatus* and 0.1098 for *An. nigerrimus*.

Tabel 1. Number of recaptured *An. peditaeniatus* and *An. nigerrimus* #

DAY AFTER RELEASED	*An. peditaeniatus* (3,972)		*An. nigerrimus* (2,946)	
	UF / F (%)	HG / G (%)	UF / F (%)	HG / G (%)
1	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
2	0 (0.00)	1 (0.03)	0 (0.00)	1 (0.03)
3	69 (1.74)	27 (0.68)	42 (1.43)	18 (0.61)
4	41 (1.03)	16 (0.40)	29 (0.98)	11 (0.37)
5	7 (0.18)	1 (0.03)	9 (0.31)	3 (0.10)
6	3 (0.08)	0 (0.00)	5 (0.17)	0 (0.00)
7	3 (0.08)	0 (0.00)	2 (0.07)	0 (0.00)
8	1 (0.03)	0 (0.00)	1 (0.03)	0 (0.00)
TOTAL	124 (3.12)	45 (1.12)	88 (2.99)	33 (1.12)

\# : Total number of two experiments
UF : Unfed
F : Freshly blood fed



HG : Half Gravid (resting mosquitoes, no analyzed)
G : Gravid (resting mosquitoes, no analyzed)

Figure 1. Regression line of mosquito recaptured and day after release of *An. peditaeniatus* and *An. nigerrimus* over two experiments[1]

[1]Number of mosquitoes recaptured was transformed to ln (y + 1) pooled as a function of time in days after release 9999

The regression functions of the present study showed indication that 100% mortality of *An. hyrcanus* group were 11-12 days (*An. peditaeniatus*) and 16-18 days (*An. nigerrimus*) (Figure1). This results longer with the curve presented by Quraishi *et al.*(Quraishi et al.,1966) who estimated for *An. stephensi* about 11.5 days after release. The indication of 12 to 13 days for *Cx. tritaeniorhynchus* was presented by Reisen and Aslamkhan (Reisen,1978).

Out of 6,918 *An. hyrcanus* species group (represent 2 species *An. peditaeniatus* and *An. nigerrimus*), blood fed individuals which were marked and released, only 212 (3.06%) unfed and freshly blood fed and 78 (1.13%) half gravid and gravid, were recaptured during the study. In many tropical areas, temperature fluctuations are relatively small, and the gonotrophic cycle of the *Anopheles* species was usually two to three days, however, in cooler seasons or at higher altitudes, the gonotrophic cycle may be extended [12]. In East Africa, *Anopheles funestus* normally had a two-day cycle when the temperature is about 25.6°C and a three-day cycle when the temperature fell below 24.7°C (Gilles,1953). *Anopheles stephensi* (Pakistan), *An. punctulatus* and *An. koliensis* (Papua New Guinea), *An. darlingi* (Brazil), *An. maculatus* and *An. campestris* (Malaysia) was reported to have a 2-3 days gonotrophic cycle (Charlwood et al.,1986, Reisen,1979, Roberts et al.,1983), whereas it was 4 days for *An. culicifacies* and *An. Albimanus* (Rodriguez et al.,1992). Three to four days gonotrophic cycles were reported for *An. stephensi* (Iran), *An. minimus* (Philippines) (Catangui,1971, Quraishi et al.,1966). In general, the gonotrophic cycle of *Anopheles* in the tropical countries was reported at 2-4 days. Present studies revealed similar results, where the peaks of recapture occurred on night three, followed by night four and five. The obvious 72 hours rhythm is clearly an indication of the oviposition and gonotrophic cycles of *An. hyrcanus* group.

both species *An. peditaeniatus* and *An. nigerrimus* in the study area..

A mark-release-recapture experiments using *An. nigerrimus* and *An. Peditaeniatus* in Kebondawa village, Banyubiru subdistrict, Semarang regency, Central Java, exhibited a three days rhythm. This also indicated the gonotropic cycle of the *An. hyrcanus* species group. This result was less than those for *An. albimanus* in Southern Mexico with a 4 days gonotrophic cycle (Rodriguez et al.,1992), *An. stephensi* in Pakistan with (2-3 days) (Reisen, 1979). *An. minimus flavirostris* in Philippines (4 days) (Catangui,1971), *An. Culicifacies* in Pakistan (4 days) (Reisen, 1979, Reisen and Mahmood,1979), *An. punctulatus* and *An. koliensis* in Papua New Guinea (2.7-3.7 days) and (2.4-3.2 days), respectively (Charlwood et al.,1986). However, the present result appears to be longer to that of *An. lesteri paraliae* in Peninsular Malaysia which has a 48 hours gonotrophic cycle (Loong et al.,1990). This result reflects faster population development for both the species *An. nigerrimus* and *An. peditaeniatus*.

CONCLUSION

A mark-release-recapture experiments of *An. hyrcanus* group, using *An. nigerrimus* and *An. peditaeniatus* in Kebondawa village, Banyubiru, Central Java, exhibited a three days rhythm. This result indicated the gonotropic cycle of the *An. hyrcanus* species group, and reflects faster population development for both species of *An. nigerrimus* and *An. peditaeniatus*.

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