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Ideal time to evaluate infestation of breeding-sites of *Anopheles* spp. in Brazil

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Abstract Malaria is a serious problem for public health in Brazil, affecting more than 300,000 people annually. One of the options for controlling malaria is the treatment of breeding-sites infested by this mosquitoes larvae with biopesticides based on *Bacillus sphaericus*. For this proposal, it is necessary to have a good way for monitoring the presence of the larvae. This study aimed to verify the possibility of colleting *Anopheles* larvae at any time of the day, having flexibility in terms of collection times. The results from this study provide evidence to suggest that it is possible to collect the larvae at any time throughout the day, but the best time is between 7:00 and 9:00h in the morning. The larvae collected were identified at the level of species, and four species were detected: *Anopheles triannulatus*, *A. darlingi*, *A. peryassui* and *A. albitarsis*.

Keywords Malaria; Infestation; *Anopheles*; Brazil

Introduction

Malaria is a serious problem for public health, affecting more than 300,000 people annually in Brazil (Brasil - Ministério da Saúde, 2010). This disease is transmitted by several species of mosquito of the genus *Anopheles* and there are no vaccines to prevent it. Thus, one of the ways of avoiding the disease is to combat the transmitter mosquito.

The Health Vigilance Secretariat of the Ministry for Health (SVS/MS) recommends application of insecticide within the house, application of the same products in the air (fumigation) and drainage of natural breeding-sites for controlling *Anopheles* (Brasil - Ministério da Saúde, 2010). Another option is the treatment of breeding sites infested by the larvae of these mosquitoes with biopesticides based on *Bacillus sphaericus*. However, for this proposal, it is necessary to have a good way for monitoring the presence of the larvae, because the criteria for application of the products is the presence or absence of larvae in breeding sites.

According to the team from Acre State Health Department, monitoring the positivity of larval breeding sites should be done only during the first hours of the morning, between 6.00h and 9.00h, since at other times it would not be possible to detect the presence of larvae in the breeding sites. This short time limit restricts the execution of work. As this information is not scientifically documented, the present study aimed to verify if the observations of the local Acre team were pertinent and to support a monitoring program for *Anopheles* spp. larvae with greater flexibility in terms of collection times.

Results and Discussion

Evaluating the distribution of collections throughout the day in each sampling site (Figure 1) demonstrated three distinct collection patterns through the day. The time with the largest collection was between 7:00 and 10:00h in the morning, when 50% ± 15 of the total number of larvae present in the breeding-site were collected. This proportion fell to 22% ± 7 between 10:00 and 13:00h, 13% ± 8 between 13:00 and 16:00h and 12% ± 8 between 16:00 and 19:00h.

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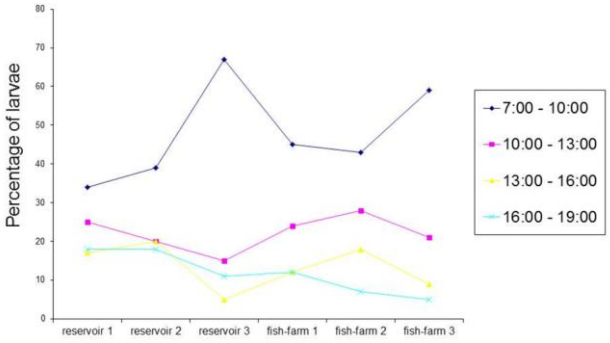

Figure 1 Percentage of larvae collected in 3 reservoirs and 3 fish-farm tanks in Cruzeiro do Sul, AC, at different times between 7:00h and 19:00h

These results demonstrate that the *Anopheles* spp. larvae are more active in the morning when the water temperature and sunlight on the breeding-site are lower. The number of larvae collected at each site and time throughout the day show that it is possible to monitor the larvae in the period from 7:00h to 19:00h for positive presence with precision higher than 90%, by means of sampling by the proposed method. Thus, sampling throughout the day is appropriate for monitoring larvae, when preparing guidelines for application or not of biolarvicide, due to the high precision rate in the method, especially in conditions of high larval infestation in the breeding-sites. Monitoring the water temperature allows observation of a consistent negative correlation between this variable and the number of larvae in each collection site (Table 1). This fact supports the inference that their activity, and therefore the chance to capture *Anopheles* larvae, decreases as temperature increases, although this study did not find a limiting temperature for larva collection. Apparently the absence of larvae in some collections, generating "false negative" results, is not related to temperature; other factors such as sampling effort, or collection spots within the breeding-site, should be investigated in the future.

Table 1 Pearson correlation coefficient between water temperature and the number of larvae collected by sampling in different breeding-sites (fish-farm tanks and reservoirs) in Cruzeiro do Sul, AC, from 6:00h to 19:00h

Breeding-sites	Number of larvae collected	Correlation coefficient (R)	Probability
Fish-farm 1	157	−0,547	0,053
Fish-farm 2	86	−0,691	0,009
Fish-farm 3	71	−0,703	0,007
Reservoir 1	24	−0,679	0,011
Reservoir 2	28	−0,652	0,016
Reservoir 3	53	−0,903	0,000

The larvae collected were identified to the species level, and four species were detected: *A. triannulatus*, *A. darlingi*, *A. peryassui* and *A. albitarsis* (Figure 2). The predominant species was *A. triannulatus*, followed by *A. peryassui*, *A. darlingi* and *A. albitarsis*. It is interesting to note that in reservoir 3 only *A. peryassui* was present, and in large numbers. In reservoirs number 1 and 2 and in fish-farm tank 2, this species was also detected, but in much lower numbers. Previous studies reported the presence of this species but in lower numbers (Furlaneto et al., 2007; Silva et al., 2006). According to Deane et al. (1988), Oliveira-Ferreira et al. (1990), Tadei et al. (1998) and Tadei & Thatcher (2000), among others, *A. darlingi* is the main species transmitting malaria in the region. However, these authors comment that *A. triannulatus*, *A. braziliensis* and *A. nuneztovari* have been found infected with *Plasmodium falciparum* and/or *P. vivax*, and so are also involved, on a lesser scale, in transmitting

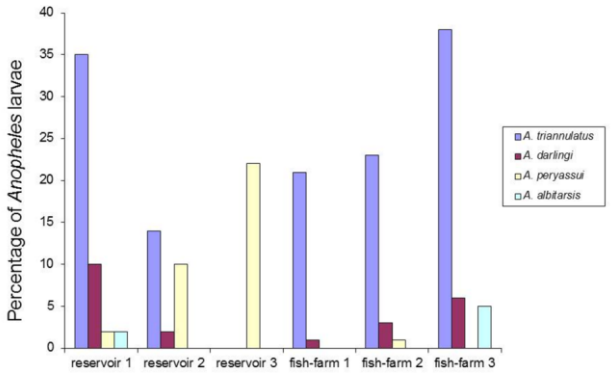

Figure 2 Percentage of species of *Anopheles* present in three breeding-sites of the type "reservoir" and "fish-farm tanks"

malaria. It is important to carry out studies on the ecology of each species found, to check their vectorial capacity. According to Silva et al., (2006) the presence of *Plasmodium falciparum* was detected in *A. peryassui*, so this may be a species involved in transmitting malaria.

Throughout the day, during collections, the relationship was confirmed as random between the times and the species found, both in the reservoir (Figure 3), and in the fish-farm tank (Figure 4).

The results obtained suggest that the evaluation of positive presence in the breeding-sites can be carried out at any time of the day, but studies that need a greater number of larvae should be done in the morning, preferably.

Materials and methods

The experiment was carried out in the town of Cruzeiro do Sul in Acre, at three sampling points for two types of breeding-site: "fish-farm tank" and "reservoir". The difference between them is the presence of the fishes. At each site samples were collected at five random points. Every hour for 12 hours (between 7:00h and 19:00h) the number of larvae present in the breeding-sites was evaluated by means of counting the larvae were collected using a dipper (Consoli & Lourenço de Oliveira, 1994; Vilarinhos, et al., 1997). The assay was repeated three times, on three different days. The collected larvae were counted and identified using the taxonomic key

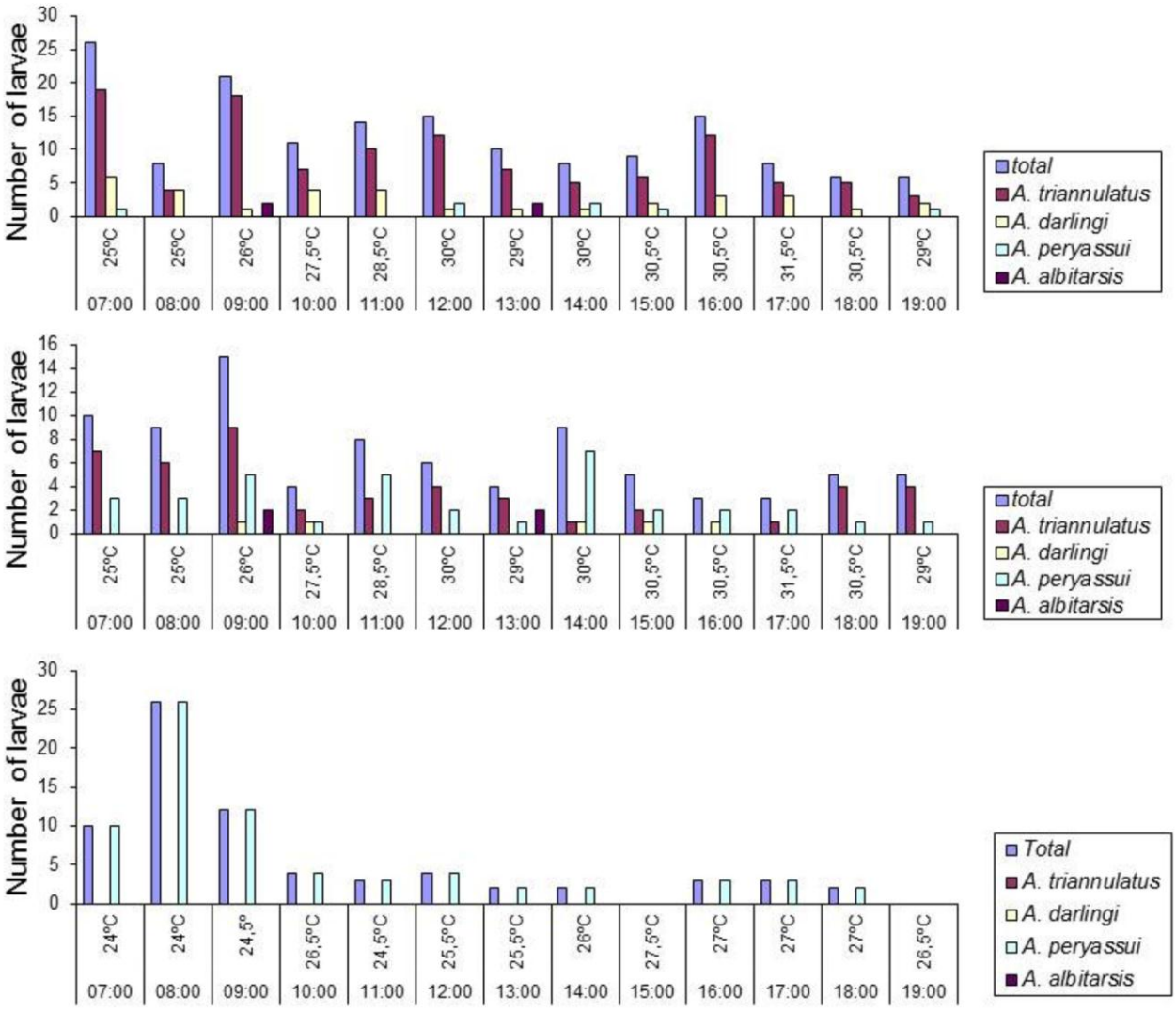

Figure 3 Distribution of *Anopheles* species found during collections per hour in three reservoirs (1, 2, 3)

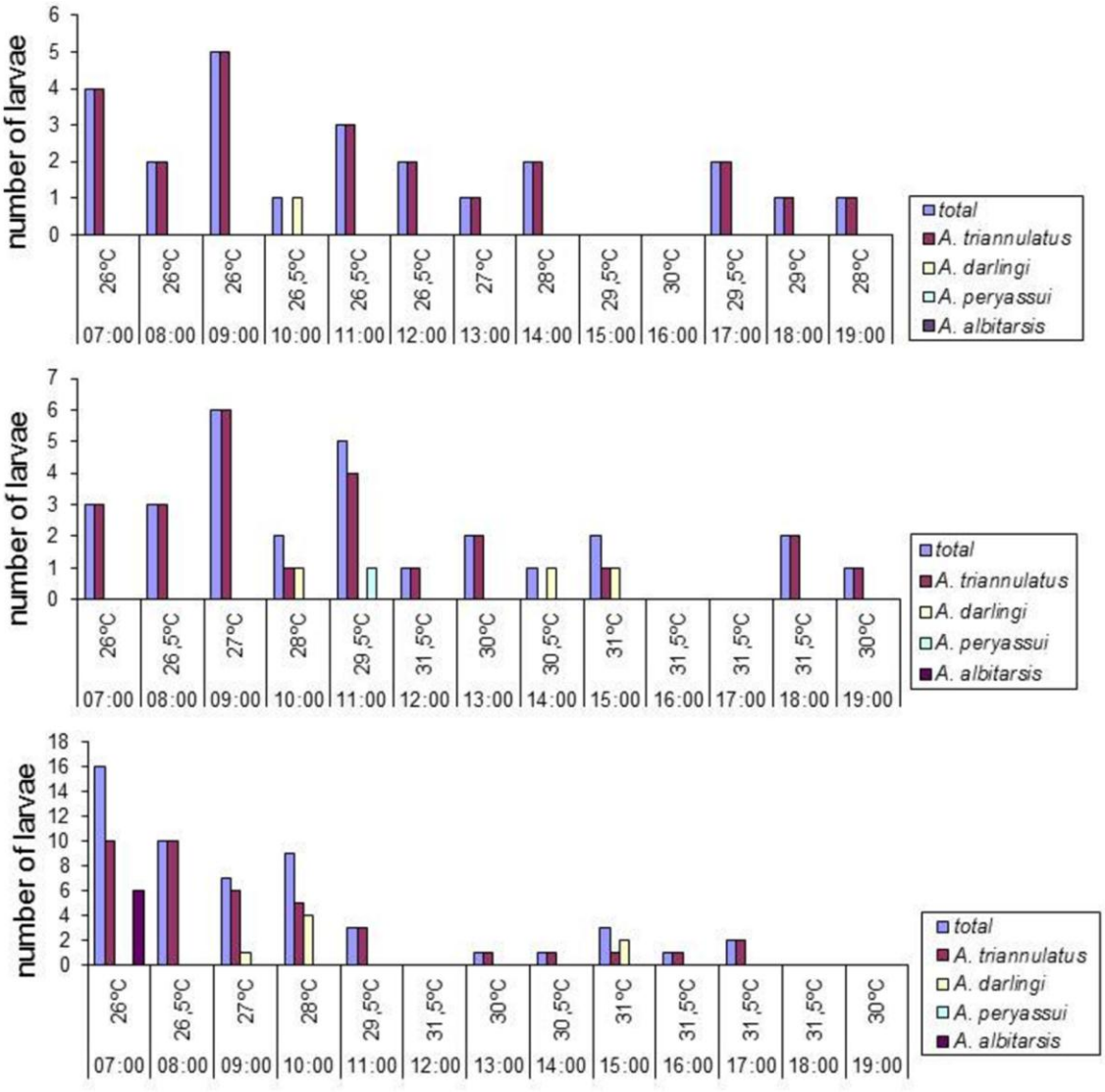

Figure 4 Distribution of *Anopheles* species found during collections per hour in three fish-farm tanks (1, 2, 3)

described in Consoli & Lourenço de Oliveira (1994).

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