

## AFFINITY AND DIVERSITY INDICES FOR ANOPHELINE IMMATURE FORMS

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### SUMMARY

As for the entire Amazon Region, malaria continues to be a major health public problem in Roraima that presented an Annual Parasitic Index of 85.4 in 2005, the highest in Brazil. Information on anopheline breeding sites is an essential component in malaria control strategies. Aiming to contribute to the limited knowledge on anopheline immature forms in Roraima, collections and breeding site observations were performed in 10 breeding sites around the capital city Boa Vista. Collections were carried out in the rainy and dry season periods between April 2004 and January 2005. Breeding sites comprised natural and artificial water reservoirs. A total of 623 immature forms were collected belonging to *Anopheles albitarsis* s.l., *An. triannulatus* s.l., *An. nuneztovari/dunhami*, *An. braziliensis*, *An. evansae*, *An. oswaldoi* s.l., *An. strodei* and *An. darlingi*. *An. albitarsis* and *An. braziliensis* were the most frequently found species. Eight larvae of *An. darlingi* were found in only one breeding site located in the forest. *An. triannulatus/An. nuneztovari* and *An. albitarsis/An. braziliensis* were the pairs of species that mostly occurred together. Both pair of species displayed the highest affinity index what might indicate a high compatibility for the same breeding conditions and/or a synergistic co-occurrence. Species diversity index was higher for the dry season.

**KEYWORDS:** Anophelines; Immatures; Roraima; Brazil.

### INTRODUCTION

As for the entire Amazon, malaria continues to be a major health public problem in Roraima where 31386 malaria cases were registered in 2005. These corresponded to an Annual Parasitic Index (API) of 85.4, the highest in Brazil, hitting the peak of a trend in one of the most malarigeneous states in the latest years (2<sup>nd</sup> API in 2004 with 71.2 and 3<sup>rd</sup> API in 2003 with 33.1)<sup>9</sup>. Information on anopheline immature forms and breeding sites is an essential component in malaria control strategies. In fact, vector control is one of the fundamental and most successful components of the malaria control strategy in the Amazon.

Immature forms and breeding site characterization literature for Roraima is limited<sup>3,5,17</sup> (National Health Foundation reports-FUNASA Entomological Reports, data not published). In this paper we perform breeding site observations and analyze affinity and diversity indices for the anopheline species collected around Boa Vista during the rainy and dry season of the years 2004-2005.

### MATERIAL AND METHODS

**Breeding site characterization:** Collections were performed in Boa Vista, Cantá, Bonfim and Alto Alegre municipalities in collections

performed in the rainy (April-June 2004) and dry seasons (November 2004-January 2005). Sampling was carried out by dipping three times in 30 points, located one m apart of each other until a total of 90 samples per site was reached. The total of 90 samples was irrespective of the total breeding site area. A plastic dipper with 350 mL capacity (standardized by the Pan American Health Organization) was used for this purpose. Breeding sites were photographed and some of their characteristics were observed based on FUNASA's guidelines for immature collection. These characteristics encompassed latitude, longitude, altitude (m), distance of the nearest domicile (m), width (m), length (m), area (km<sup>2</sup>), shading (%), depth at one m from the border (m), water flow (strong/medium/weak), type (lagoon, creek, water well, river side, water reservoir, bromeliad, water sewage, marsh, dam, excavation, ground depression, canal, river, flooded land, other), water clarity (clean, cloudy, polluted), vegetation (emergent, underwater, floating), date of the last rain, water temperature, water pH, number of collection points, number of positive collection points, average sampling per point, total number of samples, number and species of anopheline found, number of specimens per instar (1<sup>st</sup> - 4<sup>th</sup> larvae and pupae), anopheline species. Land cover type at the collection sites (savannah, alluvial forest, dense forest and transition areas) was also noted.

**Laboratory rearing and identification:** Larvae were transported to the laboratory (Núcleo Avançado de Vetores, Universidade Federal

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de Roraima-UFRR). First and 2<sup>nd</sup> instar larvae were reared for identification in the insectary room (26-28 °C mean room temperature and 70-80% humidity mean, NAVE-UFRR). Third and 4<sup>th</sup> instar larvae were identified by the use of taxonomical keys<sup>4,10</sup>. One pupa was reared to adult for identification using the same keys.

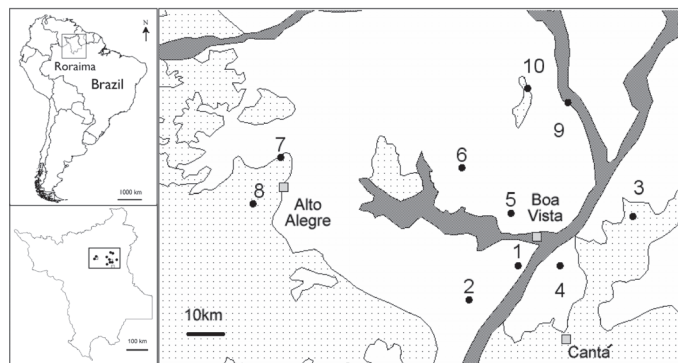
**Affinity index between species:** The index of affinity between pair of anopheline species occurring in the same breeding site was calculated using FAGER & MCGOWAN test<sup>8</sup> for all species pairs<sup>2,7,16,19</sup>. The test identifies objective groups of recurrent species defined by the strength of their association as  $[J (Na Nb)^{-1/2}] - [1/2 (Nb)^{1/2}]^{15}$ , where J is the number of co-occurrences, Na is the total number of occurrences of species A alone, Nb is the total number of occurrences of species B alone and species are chosen such that  $Na \leq Nb$ . The first term of the equation quantifies the degree of co-occurrence. The second term avoids higher association for rare findings and small sample sizes<sup>7,8,16</sup>. The resulting value provides a quantitative measure of species association. A positive inter-specific threshold value indicative of association was set to values  $\geq$  mean plus one standard deviation<sup>8,19</sup>.

**Diversity indices:** Species diversity for the eight anopheline species in the 10 breeding sites regarding type of land cover and between the rainy and dry seasons were evaluated by using the Shannon diversity index<sup>14</sup>. Shannon's index or species richness is  $(-\sum_{i=1}^S p_i \ln p_i)$  defined by that accounts for abundance and uniformity of the species present. The proportion of species *i* relative to the total number of species (*p<sub>i</sub>*) is calculated, and then multiplied by the natural logarithm of this proportion ( $\ln p_i$ ). The resulting product is summed across species and multiplied by -1. Chi-squared test for statistical significance of all associations was performed.

**Meteorological data:** Variables recorded were annual average rainfall (mm), temperature (°C, maximum and minimum) and water soil (%) (Boa Vista Air Force Base and Agritempo<sup>1</sup>). The climate presents two distinct seasons, a rainy season between April and November with high rainfall indices during the months of June and July and a dry season from December to March<sup>15</sup>.

## RESULTS

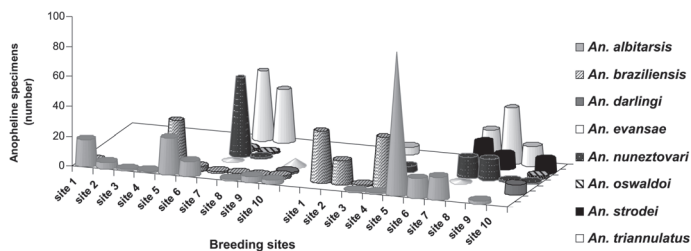
**Breeding site characterization:** Anopheline immature forms were collected in the municipalities of Boa Vista (six breeding sites), Cantá (one site), Bonfim (one site) and Alto Alegre (two sites) (Fig. 1 and Table 1). Immature form collections took place in the approximate 100 m peridomiciliary area of houses in permanent breeding sites in four Roraima municipalities (Table 2). Sampling was done in the morning period from 06:00 h to 11:00 h. Number of positive samples per point, number, species and instar of anopheline immature forms found were registered (Table 1). Latitude, longitude and altitude were determined (Table 1, GPS Garmin, Worldwide Geodesic System 1984). All sites had underwater or floating vegetation (Fig. 1). Most breeding sites were of the type water reservoir, had  $\geq 50\%$  of their area shaded and were in savannah areas (Table 2). Water temperature, pH and other breeding site characteristics were noted for rainy and dry seasons (Table 2). Breeding site variables during the rainy season were water temperature mean 28.9 °C (26.5-31.0  $\pm$  1.6) and pH mean 5.9 (5.5-6.5  $\pm$  0.3)



**Fig. 1** - South America, Brazil (upper left), Roraima (lower left) and the area (right) where anopheline immature forms were collected. Breeding sites numbered 1 to 10 (localities' names in Table 1). Squares represent the urban centers of the municipalities (Alto Alegre, Boa Vista and Cantá), dotted areas show forest, white areas show savannah and dark grey areas represent alluvial forest.

while during the dry season mean values registered were 29.7 °C (28-32.3  $\pm$  1.4) and 5.8 (4.9-6.5  $\pm$  0.6) (Table 2).

**Laboratory rearing and identification:** A total of 623 immature forms were collected belonging to eight distinct species, all from the *Nyssorhynchus* subgenus. These species were *An. albitarsis* s.l., *An. triannulatus* s.l., *An. nuneztovari/dunhami*, *An. braziliensis*, *An. evansae*, *An. oswaldoi*, *An. strodei* and *An. darlingi* (Fig. 2 and Table 2). Anopheline specimens from the Albitarsis, Oswaldoi and Triannulatus species complexes cannot be separated morphologically. These species hereinafter will be cited as *An. albitarsis*, *An. oswaldoi* and *An. triannulatus*. Also by the absence of morphologic variations that allow to ready identification between *An. dunhami* and *An. nuneztovari*, the first one was identified as *An. nuneztovari* by its geographical distribution. *An. triannulatus*, *An. braziliensis* and *An. albitarsis* were the most frequent species found, respectively, while *An. evansae*, *An. oswaldoi* and *An. darlingi* were the least collected (Table 1). During the rainy season, *An. triannulatus* was the most abundant species (81 larvae) followed by *An. albitarsis* (65 larvae plus one pupa) and *An. nuneztovari* (53 larvae) (Fig. 2). In the dry season *An. albitarsis* was the most abundant species (127), followed by *An. braziliensis* (85) and *An. triannulatus* (76) (Fig. 2). *An. darlingi* was only found in site 10 located in the forest in the rainy season (one specimen), and in the dry season (seven specimens) (Tables 1 and 2) (Fig. 1 and 2). *An. albitarsis* was found in all 10 sites (Table 1 and Fig. 2).



**Fig. 2** - Anopheline species and number of specimens for 10 breeding sites during the rainy and dry seasons 2004-2005, Roraima, Brazil.

**Table 1**

Breeding sites (number, locality, season), total of positive sample points (out of 90 per site) and species found (discriminated by 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instars and pupa) in Roraima, Brazil, 2004-2005

Breeding site	Locality (Latitude, longitude, altitude-m)	Season	Positive points	Species found	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	Pupa	Total
1	Centenário, Boa Vista (02°47'34", 60°43'04", 80)	Rainy	15	<i>An. albitarsis</i>	1	13	4	0	0	18
		dry	19	<i>An. braziliensis</i>	0	1	0	0	0	1
2	BR-174 Km 21, Boa Vista (02°42'26", 60°50'26", 87)	rainy	4	<i>An. albitarsis</i>	4	0	0	0	0	4
		dry	11	<i>An. braziliensis</i>	6	3	5	2	0	16
3	BR-401, São Francisco Village, Bonfim (02°54'50", 60°25'41", 100)	rainy	1	<i>An. albitarsis</i>	0	0	1	0	0	1
		dry	13	<i>An. albitarsis</i>	0	1	0	0	0	1
				<i>An. braziliensis</i>	0	1	0	0	0	1
				<i>An. triannulatus</i>	3	1	1	0	0	5
		<i>An. nuneztovari</i>	1	3	0	0	0	4		
4	BR-401, Santa Cecília, Cantá (02°47'34", 60°36'43", 84)	rainy	1	<i>An. albitarsis</i>	0	1	0	0	0	1
		dry	32	<i>An. albitarsis</i>	0	1	0	0	0	1
				<i>An. braziliensis</i>	13	17	2	2	0	34
				<i>An. nuneztovari</i>	4	0	0	0	0	4
5	Monte Cristo III, Refazenda Farm, Boa Vista (02°55'18", 60°44'08", 80)	rainy	33	<i>An. albitarsis</i>	15	7	1	0	1	24
		dry	37	<i>An. braziliensis</i>	17	13	2	0	0	32
				<i>An. albitarsis</i>	16	43	18	18	0	95
6	Bamerindus Farm, N.Sra. Aparecida Ranch, Boa Vista (03°02'04", 60°51'31", 99)	rainy	12	<i>An. albitarsis</i>	4	1	1	4	0	10
		dry	10	<i>An. braziliensis</i>	2	1	0	0	0	3
				<i>An. albitarsis</i>	2	5	3	3	0	13
7	RR-205, São Sebastião Ranch, Alto Alegre (03°03'37", 61°18'45", 115)	rainy	42	<i>An. braziliensis</i>	1	0	0	0	0	1
				<i>An. triannulatus</i>	37	3	5	1	0	46
				<i>An. nuneztovari</i>	33	5	12	2	0	52
				<i>An. oswaldoi</i>	2	0	0	0	0	2
				<i>An. evansae</i>	0	0	1	0	0	1
		dry	40	<i>An. albitarsis</i>	10	4	1	0	0	15
				<i>An. triannulatus</i>	14	4	2	1	0	21
				<i>An. nuneztovari</i>	13	1	0	0	0	14
		<i>An. evansae</i>	0	1	0	0	0	1		
		<i>An. strodei</i>	14	2	1	0	0	17		
8	RR-343, São José Farm, Alto Alegre (02°56'42", 61°22'52", 100)	rainy	25	<i>An. albitarsis</i>	0	0	0	1	0	1
				<i>An. braziliensis</i>	0	0	1	0	0	1
				<i>An. triannulatus</i>	13	15	5	2	0	35
				<i>An. nuneztovari</i>	0	1	0	0	0	1
				<i>An. oswaldoi</i>	0	1	0	0	0	1
		dry	48	<i>An. triannulatus</i>	8	22	5	2	0	37
		<i>An. nuneztovari</i>	2	10	1	1	0	14		
		<i>An. strodei</i>	6	0	2	2	0	10		
9	Passarão, Boa Vista (03°11'57", 60°35'30", 91)	rainy	4	<i>An. albitarsis</i>	1	1	0	2	0	4
				<i>An. braziliensis</i>	0	0	1	0	0	1
		dry	13	<i>An. albitarsis</i>	0	1	0	1	0	2
				<i>An. triannulatus</i>	3	6	2	2	0	13
		<i>An. nuneztovari</i>	0	0	1	0	0	1		
10	Serra da Moça Creek, Boa Vista (03°14'01", 60°41'38", 117)	rainy	10	<i>An. darlingi</i>	1	0	0	0	0	1
				<i>An. albitarsis</i>	3	0	0	0	0	3
				<i>An. braziliensis</i>	1	1	0	0	0	2
				<i>An. evansae</i>	0	2	2	1	0	5
		dry	10	<i>An. darlingi</i>	0	2	2	3	0	7
				<i>An. nuneztovari</i>	0	0	0	1	0	1
				<i>An. oswaldoi</i>	1	0	1	0	0	2
		<i>An. strodei</i>	0	6	3	0	0	9		
TOTAL	10		380	8	261	214	94	53	1	623

**Table 2**  
Characteristics of 10 permanent breeding sites in four municipalities of Roraima, 2004-2005

Breeding site	Type	Surrounding vegetation	Water speed	Water clarity	Water vegetation	Nearest domicile (m)	Approximate dimensions			Shading (%)	Depth <sup>1</sup> at 1m (m)	Water temperature (°C)		pH	
							Width (m)	Length (m)	Area (km <sup>2</sup> )			Rainy season	Dry season	Rainy season	Dry season
1	lake <sup>2</sup>	savannah	none	cloudy	underwater	100	200	100	2	70	0.3	28	28.2	6.14	4.92
2	lake	savannah	none	clean	underwater	100	80	80	6.4	60	0.15	29.2	32.3	5.80	6.33
3	lake	savannah/forest <sup>3</sup>	none	cloudy	floating	150	100	100	10	100	0.15	31	31	5.90	5.38
4	lake <sup>2</sup>	savannah	none	cloudy	underwater	200	30	35	1.05	50	0.20	26.5	28.5	5.70	5.23
5	lake <sup>2</sup>	savannah	weak	clean	underwater	50	40	80	3.2	25	0.40	27.9	28	6.12	6.50
6	lake	savannah	none	cloudy	floating/ underwater	50	250	500	125	90	0.30	28	29	5.60	5.20
7	cistern	cultivated savannah	none	cloudy	underwater	100	18	35	0.063	15	0.20	29.2	29.3	5.80	6.46
8	lake <sup>2</sup>	cultivated savannah <sup>4</sup>	none	cloudy	underwater	150	10	60	0.6	25	0.40	31	30.4	6.30	6.24
9	lake	savannah/ forest <sup>3</sup>	weak	clean	floating/ underwater	400	1000	1000	1000	80	0.50	27.4	30.3	6.50	6.17
10	lake	Seasonal forest/ dense forest <sup>3</sup>	none	clean	underwater	400	30	70	2.1	50	0.50	31	30.2	5.50	5.54

<sup>1</sup>Depth at 1m from the border (m); <sup>2</sup>Artificial lake, excavated for water storage; <sup>3</sup>Savannah/forest and seasonal forest/dense forest are contact areas; <sup>4</sup>Secondary vegetation and pasture.

**Affinity index between species:** Affinity indices between pair of species had significant result ( $\geq 0.544$ ) for the pair *An. triannulatus*/*An. nuneztovari* only (Tables 3 to 5). The pair *An. triannulatus*/*An. nuneztovari* and *An. albitarsis*/*An. braziliensis* were the pair of species that displayed the highest affinity indices (Table 5).

located in contact areas/cultivated savanna (sites 3, 7, 8 and 10) and the savannah (sites 1, 2, 4, 5 and 6) were not statistically significant (Table 6). However, species diversity index was statistically significant higher for the dry season when compared to rainy season ( $df = 7$ ,  $\chi^2 = 60.32$ ,  $p \geq 0.001$ ).

**Diversity indices:** Species diversity indices between breeding sites

**Meteorological data:** During the rainy season maximum

**Table 3**  
Anopheline species and number of specimens occurring in 10 breeding sites during the rainy and dry seasons, 2004-2005, Roraima, Brazil

Site Nb.	Season	Species of <i>Anopheles</i>								Total
		<i>albitarsis</i>	<i>triannulatus</i>	<i>braziliensis</i>	<i>nuneztovari</i>	<i>strodei</i>	<i>darlingi</i>	<i>evansae</i>	<i>oswaldoi</i>	
1	Rainy	18	0	1	0	0	0	0	0	0
2		4	0	0	0	0	0	0	0	0
3		1	0	0	0	0	0	0	0	0
4		1	0	0	0	0	0	0	0	0
5		24	0	32	0	0	0	0	0	0
6		10	0	3	0	0	0	0	0	0
7		0	46	1	52	0	0	1	2	
8		1	35	1	1	0	0	0	1	
9		4	0	1	0	0	0	0	0	
10		3	0	2	0	0	1	5	0	
1	Dry	0	0	34	0	0	0	0	0	0
2		0	0	16	0	0	0	0	0	0
3		1	5	1	4	0	0	0	0	0
4		1	0	34	4	0	0	0	0	0
5		95	0	0	0	0	0	0	0	0
6		13	0	0	0	0	0	0	0	0
7		15	21	0	14	17	0	1	0	
8		0	37	0	14	10	0	0	0	
9		2	13	0	1	0	0	0	0	
10		0	0	0	1	9	7	0	2	
TOTAL		193	157	126	91	36	8	7	5	623

**Table 4**  
Single occurrence matrix for pairs of anophelines species in 10 breeding sites in both rainy and dry seasons (20 total possible occurrences)

Species of Anopheles	<i>albitarsis</i>	<i>triannulatus</i>	<i>braziliensis</i>	<i>Nuneztovari</i>	<i>strodei</i>	<i>Darlingi</i>	<i>evansae</i>	<i>oswaldoi</i>
<i>albitarsis</i>		2	3	3	2	1	1	2
<i>triannulatus</i>	11		8	2	1	2	1	1
<i>braziliensis</i>	7	3		4	3	1	1	1
<i>nuneztovari</i>	10	0	7		0	1	1	0
<i>strodei</i>	14	4	11	5		1	2	2
<i>darlingi</i>	14	6	10	7	2		2	2
<i>evansae</i>	13	4	9	6	2	1		2
<i>oswaldoi</i>	14	4	9	5	2	1	2	

Numbers showing above the diagonal bars are total number of occurrences for the species given in the first row alone, when paired to the species given in the first column. Numbers showing below the diagonal bars are total number of occurrences for the species given in the first column alone, when compared to the species given in the first row.

**Table 5**  
Matrix of co-occurrence (above diagonal) and affinity index (below diagonal) between pair of anophelines species in 10 breeding sites pair of anopheline species

Species of Anopheles	<i>albitarsis</i>	<i>triannulatus</i>	<i>braziliensis</i>	<i>nuneztovari</i>	<i>strodei</i>	<i>darlingi</i>	<i>evansae</i>	<i>oswaldoi</i>
<i>albitarsis</i>		4	8	5	1	1	2	1
<i>triannulatus</i>	0.218		3	6	2	-	2	2
<i>braziliensis</i>	0.494	0.165		4	-	1	2	2
<i>nuneztovari</i>	0.327	<b>0.662</b>	0.276		3	1	2	3
<i>strodei</i>	0.020	0.267	-	0.436		1	1	1
<i>darlingi</i>	0.053	-	0.062	0.073	0.120		1	1
<i>evansae</i>	0.169	0.183	0.197	0.231	0.045	0.055		1
<i>oswaldoi</i>	0.020	0.267	0.062	0.324	0.045	0.055	0.045	

<sup>1</sup> Significant value in bold, i.e.  $\geq 0.544$  corresponding to average + 1 standard deviation ( $0.350 + 0.194$ ); - pair of species did not occur together.

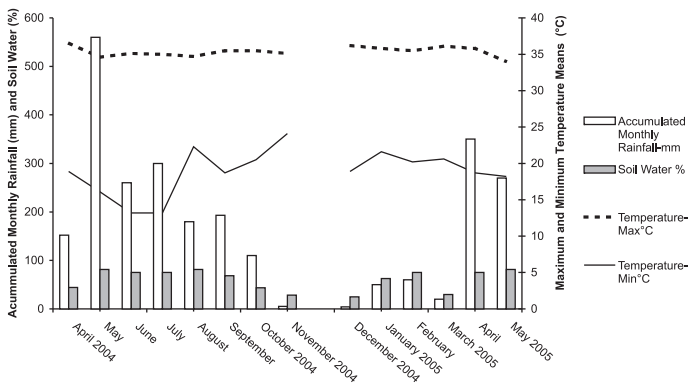
temperature mean was 35.3 °C (varied from 34.6 to 36.5 ± 0.6), minimum temperature mean was 17.6 °C (13.2-22.3 ± 3.5), rainfall mean 45.7 mm (17-83 ± 23.9), available soil water mean 67% (43.4-81.3 ± 16.5). For the dry season values for maximum temperature mean was 35.7 °C (35.1-36.2 ± 0.5), minimum temperature mean was 21.1 °C (18.9-24.1 ± 1.9), rainfall mean 13.5 mm (0-31.7 ± 15.4), available

soil water mean 44.2% (24.9-75 ± 23) (Fig. 3). Date of the last rain varied from zero to three days during the rainy season and from three to 24 days in the dry season.

## DISCUSSION

Anopheline immature form collections were performed in permanent water reservoirs in the Boa Vista surroundings for its importance as most populated city and administrative center and, for logistic reasons. Cantá, in the Southeast border of Boa Vista, was the most malarigeneous municipality out of the 15 Roraima municipalities with API of 398.1 (2005) and 317.8 (2004), followed by Bonfim to the East (2<sup>nd</sup> API in 2005 with 189.8 while held the 10<sup>th</sup> API position in 2004 of 81.2). Alto Alegre, in the West border, presented an API of 67.7 in 2005 (65.5 in 2004). Boa Vista had an API of 32.6 in 2005 (25.1 in 2004). High malaria risk areas are those which API is  $\geq 50$  according to Ministry of Health of Brazil. All localities where breeding sites were located reported malaria cases (Table 4).

Anophelines can exploit a broad range of water bodies as breeding places. Some species present a high ecological valence and not show preferences for a certain kind of breeding place while others are stricter on their choices. While *An. albitarsis* and *An. braziliensis* where



**Fig. 3** - Meteorological variables prevailing in the study area depicted in Fig. 1. Rainy season is between April and November and dry season is from December to March.



**Table 6**  
Shannon diversity index for 10 breeding sites for the rainy and dry seasons, 2004-2005, in Roraima, Brazil

Species of <i>Anopheles</i>	Breeding site									
	1 savannah	2 savannah	3 savannah/ forest	4 savannah	5 savannah	6 savannah	7 cultivated savannah	8 cultivated savannah	9 savannah/ forest	10 Seasonal forest/ dense forest
<b>Rainy season</b>										
<i>albitarsis</i>	-0.367	-0.322	-0.207	-0.092	-0.292	-0.368	0	-0.046	-0.316	-0.230
<i>triannulatus</i>	0	0	0	0	0	0	-0.354	-0.367	0	0
<i>braziliensis</i>	-0.075	0	0	0	-0.329	-0.249	-0.030	-0.046	-0.145	-0.181
<i>nuneztovari</i>	0	0	0	0	0	0	-0.362	-0.046	0	0
<i>strodei</i>	0	0	0	0	0	0	0	0	0	0
<i>darlingi</i>	0	0	0	0	0	0	0	0	0	-0.113
<i>evansae</i>	0	0	0	0	0	0	-0.030	0	0	-0.299
<i>oswaldoi</i>	0	0	0	0	0	0	-0.052	-0.046	0	0
<b>Dry season</b>										
<i>albitarsis</i>	0	0	-0.207	-0.092	-0.292	-0.347	-0.214	0	-0.224	0
<i>triannulatus</i>	0	0	-0.365	0	0	0	-0.258	-0.368	-0.297	0
<i>braziliensis</i>	-0.285	-0.179	-0.207	-0.138	0	0	0	0	0	0
<i>nuneztovari</i>	0	0	-0.366	-0.230	0	0	-0.206	-0.275	-0.145	-0.113
<i>strodei</i>	0	0	0	0	0	0	-0.230	-0.230	0	-0.361
<i>darlingi</i>	0	0	0	0	0	0	0	0	0	-0.340
<i>evansae</i>	0	0	0	0	0	0	-0.030	0	0	0
<i>oswaldoi</i>	0	0	0	0	0	0	0	0	0	-0.181
Shannon <b>Rainy</b>	0.442	0.322	0.207	0.092	0.621	0.617	<b>0.829</b>	0.552	0.461	<b>0.823</b>
Shannon <b>Dry</b>	0.285	0.179	<b>1.145</b>	0.461	0.292	0.347	<b>0.939</b>	<b>0.873</b>	0.666	<b>0.995</b>

<sup>1</sup> Highest diversity indices in bold

collected in all 10 breeding sites with high specimens numbers in breeding sites located in the savannah, *An. darlingi* was found in breeding site 10 only, a shaded permanent water body in a savannah/ forest contact area, conditions known as ideal for its breeding<sup>4,6</sup>. FUNASA reports *An. darlingi* to be widely distributed all over the state. Nonetheless, *An. darlingi* was found in breeding site 10 only. This fact suggests that *An. darlingi* does not occur uniformly all over the region but presents limitation to specific landscapes.

The presence of some anopheline species could synergistically help other species. *An. triannulatus/An. nuneztovari* and *An. albitarsis/An. braziliensis* had the highest affinity indices (0.662 and 0.494, respectively) (Table 5). Even though the pair *An. albitarsis/An. braziliensis* was observed together in eight occasions rather than six occasions seen for the pair *An. triannulatus/An. nuneztovari*, the number of occasions each one of the species in both pairs were observed alone (Table 4) was decisive in the index outcome. High affinity index might indicate that species share preferences for the same breeding site conditions and/or that the presence of one species somehow facilitates the other. The same might happen at lower intensity for the pair of species *An. nuneztovari/An. strodei* and *An. nuneztovari/An. oswaldoi*.

Usually, contact areas are zones of ecological tension that tend to display higher plant diversity (CURTIS 1959 *apud* VAN DER MAAREL<sup>18</sup>). Whether plant diversity is related to anopheline diversity seen in those breeding sites is a subject that deserves further

investigation. Although not statistically significant, disturbed areas represented by the cultivated savannah (breeding site 7 and 8) presented higher species diversity both during rainy and dry seasons (Table 6). Breeding site 7 in the cultivated savannah is a man-made excavation for rain water storage.

Higher species diversity was found for the dry season (Table 3). Dry season has been assumed to offer most stable environmental conditions. During rainy season rainfall values can reach 83 mm per day and it rains almost every day<sup>12</sup>. Accumulated rainfall of 560 mm per month was registered in May 2004 for Boa Vista<sup>12</sup>. In fact, during the time frame of our study, rainfall and soil water had the highest variance between the rainy and dry seasons. During the rainy season rainfall mean was 571.2 mm while this value decreased to 238.6 mm mean in the dry season. Interestingly, recent data shows that, contrary to the most of the vegetation around the world that follows a general pattern of growth and lush during the rainy season, the Amazon rainforest productivity is enhanced during the dry season<sup>11</sup>. In our view this indicates that environmental conditions are more stable during the dry season suitable for species to increase activity and population sizes.

Further taxonomical studies will help to understand species distributions for the three species complexes found (*Albitarsis*, *Triannulatus* and *Oswaldoi*). Recent studies show that *An. albitarsis* species E is the prevailing species in Boa Vista<sup>13</sup>.

*An. darlingi* breeding sites exist all over the Roraima territory most likely in patches of suitable ecological conditions for the species. Even though *darlingi* was found only in a forest breeding site (number 10) and in low numbers (one larva in the rainy season and seven larvae in the dry season) malaria case numbers remained the same throughout the period (Fig. 2). This finding is in accordance with low density numbers seen for *An. darlingi*<sup>15</sup>. Natural infection investigations on adult anophelines in Boa Vista showed that *An. darlingi* even in low density is able to maintain malaria endemicity<sup>15</sup>.

Higher species diversity of anopheline species in contact areas of ecological tension, and anopheline species diversity boost in the dry season are fascinating issues that deserve attention as they are linked to malaria epidemiology. Further work in these areas might substantiate indications seen in this study and are under way.

### CONCLUSIONS

*An. braziliensis* and *An. albitarsis* were the most frequently found species during the rain and dry seasons in savannah, savannah-forest and forest areas. Breeding sites in savannah-forest contact areas presented higher species diversity. *An. darlingi* was found in only one breeding site located in one of those savanna-forest contact areas. Even though *An. darlingi* is regarded as a mosquito of broad distribution in Roraima, it is most likely that the species is restricted to patches of suitable ecological conditions. *An. triannulatus*/*An. nuneztovari* and *An. albitarsis*/*An. braziliensis* were the pairs of species that mostly occurred together what might indicate a high compatibility for the same breeding conditions and/or a synergistic co-occurrence. Dry season displayed a higher number of specimens collected. This is in accordance with recent plant ecology studies<sup>11</sup> that show an overall vegetation spur in the Amazon Forest during the dry season. *An. darlingi* larvae were found rarely. Nonetheless, malaria cases were reported throughout the study period. These larval observations corroborate previous adult data on anopheline *Plasmodium* infection<sup>15</sup> that concluded that *An. darlingi* specimens even in low numbers since accompanied by *An. albitarsis* are able to maintain malaria endemicity.

### RESUMO

#### Afinidade e diversidade de formas imaturas de anofelinos

Como para toda a Amazônia, a malária representa um importante problema de saúde pública em Roraima. Roraima apresentou um índice parasitário anual de 85,4 em 2005, o maior do Brasil. O conhecimento dos criadouros de anofelinos constitui-se num componente primordial nas estratégias de controle da malária. No intuito de contribuir para o limitado conhecimento dos criadouros de anofelinos em Roraima, coleções de imaturos e observações dos criadouros foram realizadas no entorno da capital Boa Vista. As coletas foram feitas nas estações seca e chuvosa entre abril de 2004 e janeiro de 2005. Os criadouros observados foram reservatórios de água naturais e artificiais. Um total de 623 formas imaturas foram coletadas pertencentes às espécies *An. albitarsis s.l.*, *An. triannulatus s.l.*, *An. nuneztovari/dunhami*, *An. braziliensis*, *An. evansae*, *An. oswaldoi s.l.*, *An. strodei* e *An. darlingi*. *An. braziliensis* e *An. albitarsis* foram as espécies mais frequentemente encontradas. Oito larvas de *An. darlingi* foram encontradas em apenas um criadouro na floresta. *An. triannulatus*/*An. nuneztovari* e *An.*

*albitarsis*/*An. braziliensis* foram os pares de espécies de maior co-ocorrência. Ambos pares de espécies apresentaram o maior índice de afinidade o que pode indicar compatibilidade para as mesmas condições prevalentes nos criadouros e/ou sinergismo. O índice de diversidade de espécies foi maior para a estação seca.

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