Diverse population dynamics of three *Anopheles* species belonging to the Triannulatus Complex (Diptera: Culicidae)

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The Triannulatus Complex of Anopheles (Nyssorhynchus) *consists of at least three sibling species, namely* Anopheles triannulatus s.s., Anopheles halophylus *and a third undescribed member herein referred to as* An. triannulatus *"C". Sympatric anophelines belonging to species complexes, even though closely related, may exploit different environments such as larval habitats. In this paper we hypothesize that rainfall and seasonal flooding would distinctly influence the availability of larval habitats and consequently the seasonal population dynamics of sympatric members of the Triannulatus Complex. A reflection of this is distinct seasonal biting frequencies exhibited by three members of the Triannulatus Complex at a site in Central Brazil. Population dynamics seem to be influenced by the water level in the local rivers, although biting frequency of all three species was negatively influenced by rainfall.* An. triannulatus s.s. *was more abundant following the end of the rainy season, but notably 30 to 60 days after flooding. On the other hand,* An. halophylus *and* An. triannulatus *C peaked during the middle of the dry season, when water impoundments have no inflow, are somewhat reduced in size and the water becomes brackish. Differences in population dynamics were greater between* An. triannulatus s.s. *and* An. halophylus *and* An. triannulatus *C than between* An. halophylus *and* An. triannulatus *C. This might reflect differences in larval habitat exploitation and therefore spatial segregation among these members of the complex.*

Key words: *Anopheles triannulatus* - *Anopheles halophylus* - Triannulatus Complex - seasonal frequency

The Triannulatus Complex consists of at least three sibling mosquito species: *Anopheles triannulatus s.s.* (Neiva & Pinto), *Anopheles halophylus* Silva-do-Nascimento and Lourenço-de-Oliveira, and a third undescribed species, hereafter referred to as *An. triannulatus* "C", that can be differentiated by both isoenzyme and morphological characters (Silva-do-Nascimento et al. 2006). Molecular studies with specimens belonging to the Triannulatus Complex from a site in Central Brazil demonstrated that there is a barrier to gene flow among these three sympatric forms (Silva-do-Nascimento et al. 2006).

An. triannulatus s.l. is considered to be essentially zoophilic, exophilic and crepuscular, although it may bite humans indoors and play a secondary role in malaria transmission when in high densities (Deane et al. 1948, Charlwood & Wilkes 1981, Lourenço-de-Oliveira & Luz 1996, Tadei et al. 1998, Brochero et al. 2006, Zimmerman et al. 2006, Póvoa et al. 2006, Rosa-Freitas et al. 2007). Thus, low rates of *P. falciparum* and *P. vivax s.l.* circumsporozoite protein have been reported for *An. triannulatus s.l.* under natural conditions (Oliveira-Ferreira et al. 1990, Branquinho et al. 1993, Tadei & Dutary-Thatcher 2000, Póvoa et al. 2001, Galardo et al. 2007).

An. triannulatus s.s. is widely distributed throughout South and Central America, ranging from Argentina to Nicaragua, and was recently reported in Trinidad, West Indies (Faran & Linthicum 1981, Chadee & Wilkerson 2005). *An. triannulatus s.s.* breeds preferentially in partially shaded or sunlit habitats such as fresh water swamps, lakes, permanent ponds, ditches and river margins, generally associated with emergent graminaceous, submerged macrophytes and floating vegetation such as *Pistia* sp. (Cerqueira 1961, Faran 1980). This species may breed in residual freshwater left after gold mining, although exploitation of man-made environments is unusual (Lopes & Lozovei 1995, Ribeiro 2000, Silva 2002).

Until now, *An. halophylus* and *An. triannulatus* C are known only from Salobra, Miranda municipality, Central-western region of Brazil, where they occur in sympatry with *An. triannulatus s.s.* (Silva-do-Nascimento & Lourenço-de-Oliveira 2002, Silva-do-Nascimento et al. 2006). Immature stages of *An. halophylus* were found in a sunlit swamp and in a temporary brackish water lake with abundant floating vegetation, primarily *Salvinia* sp. Both the swamp and temporary lake were formed during flooding of the Miranda River (MR) in the state of Mato Grosso do Sul (MS), Brazil (Silva-do-Nascimento & Lourenço-de-Oliveira 2002). Thus, the overall characteristics of *An. halophylus* larval habitats seem to be similar to those of *An. triannulatus s.s.*, except for water salinity. Chemical analyses of the water from an *An. halophylus* habitat showed a high concentration of NaCl (2 - 4 g/l). Laboratory rearing of *An. halophylus* was accomplished when larvae were kept in brackish water from such breeding sites, whereas *An. triannulatus s.s.* breeds in fresh water (Silva-do-Nascimento & Lourenço-de-Oliveira 2002). The larval habitats of *An. triannulatus* C are unknown.

Financial support: PAPES IV-Fiocruz
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Received 10 August 2007
Accepted 12 December 2007

In general, population densities of *An. triannulatus s.l.* in South America seem to increase during the rainy season or during the transitions between dry and rainy seasons (Deane et al. 1948, Lourenço-de-Oliveira et al. 1989, Rebêlo et al. 1997, Ianelli et al. 1998, Xavier & Rebêlo 1999, Souza-Santos 2002). High capture rates of *An. triannulatus s.l.* have been reported during periods of flooding in the rainy season or during reservoir replenishment in Brazil (Teodoro et al. 1995, Guimarães et al. 2004). Seasonal distribution and biting patterns of *An. halophylus* and *An. triannulatus* C remain unknown.

Closely related members of anopheline species complexes have been shown to exploit different environments and to exhibit distinct ecological attributes such as preference for fresh versus brackish water, especially when in sympatry (Coluzzi et al. 1979, Edillo et al. 2004). Therefore, although niche partitioning by members of species complexes is expected, it is thought that gravid females would preferentially select oviposition sites depending on species (Edillo et al. 2006). Very little is known about differences in larval and adult habitat usage by the three members of the Triannulatus Complex. In the present paper, we test the hypothesis that population dynamics of sympatric members of the Triannulatus Complex are distinctly influenced by rainfall and periods of peak of river flow that impact on availability and water characteristics of larval habitats.

MATERIALS AND METHODS

Study area - Mosquito collections were performed in Salobra (20°12'40" S, 56°29'30" W), Miranda municipality, MS, in Central-western Brazil. Salobra is located at the margin of the MR in the Brazilian Pantanal. Pantanal (literally 'huge swamp') is the world largest freshwater wetland, nearly 140,000 km^2, a seasonally flooded plain fed by tributaries of the Paraguay River, one of which is the MR (Galdino & Silva 2006). The MR basin occupies an area of approximately 47,000 km^2. It is 697 km long and runs in a southeast-northwest direction starting from Serra de Maracaju and empties into the Paraguay River. The MR and its tributaries usually carry a considerable concentration of alkaline earth cations. Salts are probably carried from abundant calcareous and dolomitic stone from the highlands of the Bodoquena and Maracaju-Campo Grande Mountains (Oliveira & Ferreira 2003, Galati et al. 2006). All along the margins of the MR and its tributaries, numerous lakes of various sizes, bordered by large swamps, are formed during the flood season. On the margins and flooded ground of these lakes and swamps, where deciduous seasonal alluvial forests grow, are found abundant Holocenic alluvial deposits (Mendes et al. 2004). The annual mean rainfall is 1,102 mm, with the highest indices reported from late October to March, and the driest period lasting from June to August-September (Galdino & Silva 2006). The nearly flat land surface and slight elevation gradient (3-15 cm/km north/south and 30-50 cm/km east-west) of the MR basin favor flooding. The climate is classified as sub-humid megathermic, with a monthly mean temperature ranging from 18 to 28°C, and an average annual relative humidity of 72.7%, with the lowest values registered from June to September (Artioli & Resende 2005, Galdino & Silva 2006). The soil is essentially composed of sediments that originated in the Bodoquena Mountains. Irrigated rice plantations and extensive cattle breeding are the most common economic activities.

Biting frequency - Animal-bait mosquito captures were performed bimonthly, from May 2006 to Mach 2007, for five successive days from 17:00 to 20:00 h, at two sites ~200 m apart at the margin of the MR in Salobra. In May 2006, captures were also performed at a farm ~500 m from MR. Captures were done directly from horse bait at one site and simultaneously with a horse-baited Shannon trap at the other site. Captured *Anopheles* mosquitoes belonging to the Triannulatus Complex were divided into two groups based on time of capture: (a) 17:00 h until sunset (H1), and (b) sunset to 20:00 h (H2). Other anopheline species and non-identifiable specimens belonging to the Triannulatus Complex (females that died just after collection) (n = 687) were not considered.

Mosquito rearing and species identification - Most captured anophelines were already engorged. For those not engorged a blood source (sheep blood) was offered immediately after collection in order to obtain eggs. Adult females were identified to *An. triannulatus s.l.* (Consoli & Lourenço-de-Oliveira 1994) and individually separated into labeled vials to oviposite. Progenies were reared separately (Silva-do-Nascimento & Lourenço-de-Oliveira 2002). For each of the wild-caught females, morphological characters either from the P1 female or her progeny (eggs, fourth instar larva, male and female genitalia), as well as isoenzyme analyses (adult females), were performed for species identification (Silva-do-Nascimento & Lourenço-de-Oliveira 2002, Silva-do-Nascimento et al. 2006).

Meteorological and hydrological data - Temperature, humidity and daily rainfall were recorded at a meteorological station at Bodoquena Farm, in Miranda. Daily records on the MR water level were obtained by the Agência Nacional de Águas of MS at the "Miranda Station" (post 66910000). The Miranda station is the closest hydrological station to the mosquito collection sites in Salobra. Water from two larval habitats in Salobra was sampled bimonthly (May 2006 to March 2007). Analyses included determination of salinity (% of dissolved salt content), pH, conductivity (μS/cm), oxygen (DO) and total dissolved solids (TDS, mg/l).

Statistical analysis - For each of the seven weeks before collection, the rainfall was summed and the relationships between biting frequency and total rainfall were tested for significant Spearman correlations. In addition, correlation analyses (Pearson correlation coefficient) between species biting frequency and the maximum water level recorded for the MR in Salobra 15, 30 and 60 days before collection were performed.

RESULTS

A total of 2,207 mosquito specimens belonging to the Triannulatus Complex was sampled: 794 *An. triannulatus s.s.* (35.9%), 586 *An. halophylus* (26.6%)

and 827 *An. triannulatus* C (37.5%). All three species belonging to the Triannulatus Complex were collected throughout the year (Table I, Fig. 1). However, distinct population fluctuations were detected. The highest biting frequency for *An. triannulatus s.s.* was in May, when rainfall was decreasing and the highest levels of the MR were recorded in Salobra. On the other hand, both *An. halophylus* and *An. triannulatus* C showed the highest biting frequency in July, in the middle of the dry season, when both the MR water level and rainfall typically decreased. Significant positive Pearson correlations were found between biting frequency of *An. triannulatus s.s.* and water level of the MR (maximum level) recorded at 30 days (r = 0.824, r^2 = 68%), and 60 days (r = 0.908, r^2 = 83%). For *An. halophylus,* no Pearson correlation was observed between biting frequency and river level recorded at 15 (r = -0.23), 30 (r = 0.017) or 60 days (r = 0.189). Negative correlations were found between biting frequency of *An. triannulatus* C and water level of the MR recorded 15 (r = -0.256) and 30 days (r = -0.025) before mosquito capture; but no correlation was found for records 60 days before (r = 0.229, r^2 = 5%). The biting frequency of the three species was negatively influenced by rainfall. This can be seen more clearly in collection data from January, when the lowest anopheline biting frequencies and highest rainfall were recorded (Figs 1 and 2). Biting frequency of *An. triannulatus* was significantly inversely correlated with rainfall two and six weeks (between 8 and 14 days, and 36 and 42 days, respectively) before collection dates (Table II). Biting frequency of both *An. halophylus* and *An. triannulatus* C were significantly negatively correlated with rainfall except between 15 and 21 days.

Taking into account the results of all collections, we noted that activity of both *An. triannulatus* and *An. triannulatus* C peaked after sunset, while *An. halophylus* bit almost as frequently early as late in the evening (Fig. 3). In May, all three species showed a higher biting frequency at H1 rather than late in the evening (Figs 3 and

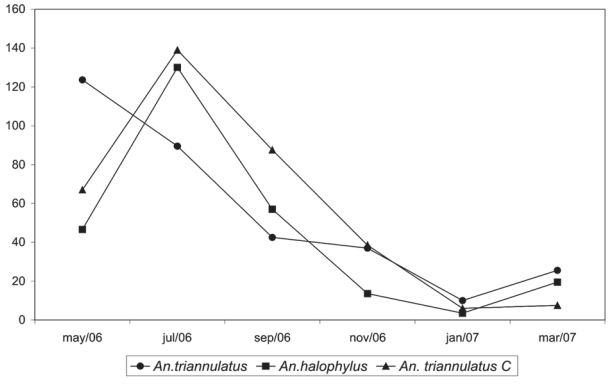

Fig. 1: bimonthly frequency of *Anopheles* species of the Triannulatus Complex in Salobra, Mato Grosso do Sul, Brazil, from May 2006 to March 2007. Frequency is given as mean of specimen per collector.

TABLE I

Number of specimens belonging to three *Anopheles* species of the Triannulatus Complex, collected from 17:00 h to sunset (H1) and from sunset to 20:00 h (H2) on horse bait, and in a horse-baited Shannon trap, in Salobra, state of Mato Grosso do Sul, Brazil, from May 2006 to March 2007

	May 2006				Jul 2006				Sep 2006				Nov 2006				Jan 2007				Mar 2007			
	H1	H2	ST	T	H1	H2	ST	T	H1	H2	ST	T	H1	H2	ST	T	H1	H2	ST	T	H1	H2	ST	T
An. triannulatus	218	115	38	371	58	87	34	179	2	82	3	87	0	63	10	73	0	33	0	33	10	24	17	51
An.halophylus	76	42	22	140	183	58	19	260	3	106	7	116	0	19	5	24	0	7	0	7	4	17	18	39
An. triannulatus C	126	51	24	201	169	77	32	278	5	159	11	175	0	62	16	78	0	12	0	12	3	11	2	16
Total	420	208	160	712	410	222	85	717	10	347	21	378	0	158	37	175	0	52	0	52	17	52	37	106

ST : Shannon Trap; T: total.

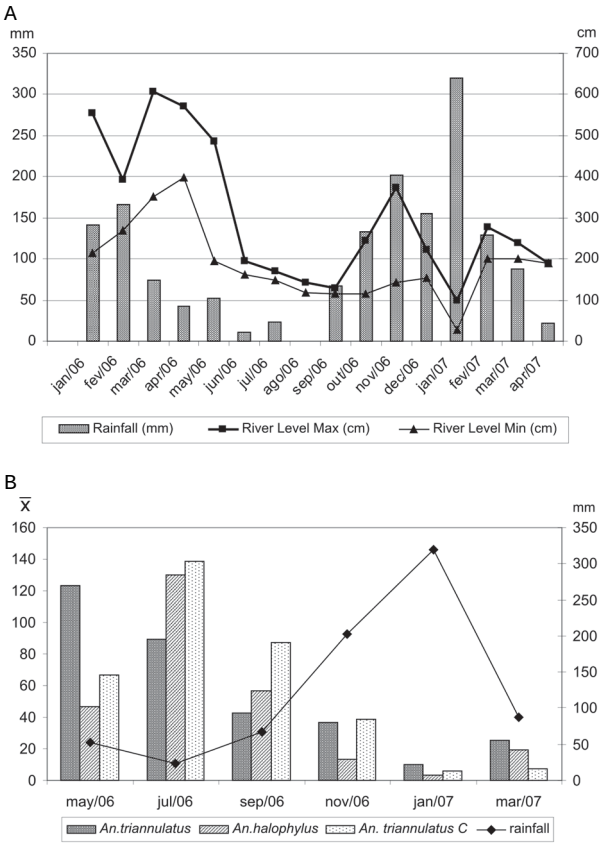

Fig. 2: Miranda River water level and rainfall (A) from January 2006 to April 2007, and bimonthly rainfall and the mean number of specimens of three *Anopheles* species of the Triannulatus Complex (B) from May 2006 to March 2007, in Salobra, Mato Grosso do Sul, Brazil.

4). In July, when the length of the day is short in the region, *An. halophylus* and *An. triannulatus* C presented even higher frequencies at H1, while *An. triannulatus* was collected in similar numbers at H1 and H2. From November to March, the period when days and nights are of almost equal length in Central Brazil, all species had basically a similar biting frequency regardless of capture times.

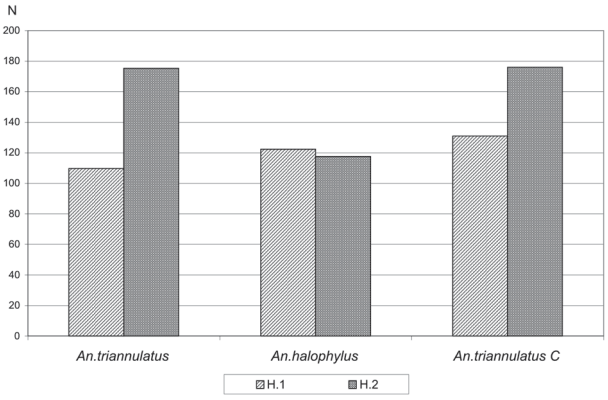

Fig. 3: specimens of *An. triannulatus s.s., An. halophylus* and *An. triannulatus* C collected from 17:00 h to sunset (H1) and from sunset to 20:00 h (H2) in Salobra, Mato Grosso do Sul, Brazil, from May 2006 to March/2007.

Meteorological and hydrological data – There were no differences in DO or pH from water samples from two larval habitats during the year. Conductivity and TDS were higher in the transition period between rainy and dry seasons. Salinity exhibited strong variation and was negatively influenced by rainfall (Fig. 5). The highest degree of salinity was recorded in May (1.3%). This corresponds to the beginning of the dry season and the highest levels of the MR. Salinity was slightly lower but still high in July (1.0%) but strongly decreased (0.1%) with increased rainfall from September to March.

DISCUSSION

Ecological and biological differences in types of larval habitats, seasonality and behavior may occur in species belonging to species complexes within their geographical distributions that can be related to local environmental characteristics. Thus, differences in population dynamics and behavior between sibling species are only suitably analyzed when these species occur in sympatry (Obsomer et al. 2007). Few sympatric *Anopheles* sibling species complexes have been studied. Herein, the population fluctuation of three sympatric members of the Triannulatus Complex was evaluated.

TABLE II

Results of Spearman's rank correlation analyses between frequency of three *Anopheles* species of the Triannulatus Complex and weekly rainfall during six intervals before the field collections

Week Intervals before collections	*An. triannulatus*		*An. halophylus*		*An. triannulatus* C	
	ρ	p	ρ	p	ρ	p
1 to 7	-0,314	0,103	-0,417[a]	0,027[b]	-0,425[a]	0,024[b]
8 to 14	-0,596[a]	0,000[b]	-0,589[a]	0,000[b]	-0,625[a]	0,000[b]
15 to 21	-0,173	0,377	-0,148	0,450	0,106	0,591
22 to 28	-0,267	0,168	-0,528[a]	0,003[b]	-0,541[a]	0,002[b]
29 to 35	-0,320	0,096	-0,529[a]	0,003[b]	-0,632[a]	0,000[b]
36 to 42	-0,542[a]	0,002[b]	-0,607[a]	0,000[b]	-0,707[a]	0,000[b]

a: Spearman's rank correlation coefficients significantly different from zero; *b*: $p \leq 0.05$.

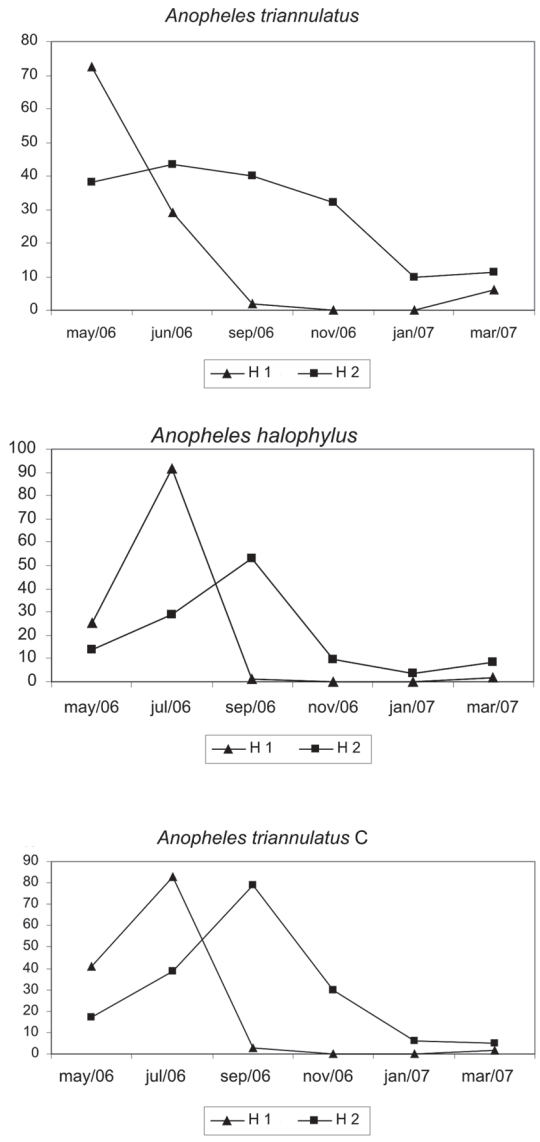

Fig. 4: bimonthly frequency of three *Anopheles* species of the Triannulatus Complex. Frequency is given as mean of specimens per collector captured from 17:00h to sunset (H1) and from sunset to 20:00 h (H2), in Salobra, Mato Grosso do Sul, Brazil, from May 2006 to March 2007.

Our year-round collections in Salobra showed that all three species bit more frequently late in the evening, although *An. halophylus* was collected in similar numbers early and late in the evening (Fig. 3). Distinct hourly biting patterns were recorded for the species throughout the year (Fig. 4). The biting time of a mosquito species at a given location is dependent on the relationship between inherited endogenous oscillators and local environmental and physiological factors such as age of the population, moon phase, rainfall, and distance from the oviposition or mating site (Charlwood 1996, Obsomer et al. 2007). During seasonal population peaks of a mosquito species, a large proportion of the biting individuals consists of young nuliparous females. Early evening biting activity has been observed during population peaks for other Neotropical anophelines (Elliot 1972, Klein & Lima 1990). Charlwood and Wilkes (1979) found that the individuals of *Anopheles darlingi* that were biting in the crepuscular peaks observed in Mato Grosso consisted largely of nulliparous females. In considering the population peak of each species of the Triannulatus Complex, we observed that a higher number of specimens were collected at H1. Contrasting, in off peak periods, these three species tended to be more frequent after sunset (H2). Although hourly captures were not conducted in the present study, *An triannulatus s.l.* biting activity peaked shortly after sunset, however, it continued into the night (data not shown).

The seasonal frequency of *An. triannulatus s.l.* is known to vary throughout South America. Brazilian populations of *An. triannulatus s.l.* generally peak during the rainy season or during the transitions between rainy and dry seasons (Ianelli et al. 1998, Xavier & Rebêlo 1999, Souza-Santos 2002). In Venezuela and Argentina, larval density increased in the beginning and in the middle of the dry season, respectively (Rubio-Palis et al. 2005, Stein et al. 2004). In Bolivia however, larval density peaked in the rainy season (Rejmánková et al. 1999). These distinct seasonal variations may be due to characteristics of different members of the Triannulatus Complex. Additionally, the influence of geographical variation in seasonal rainfall, river levels, temperatures, abundance of larval habitats and of floating vegetation, may affect population dynamics for any given species of the Triannulatus Complex. *An. triannulatus s.s.* seems to be more common and widespread in South and Central America than the other members of the complex (Deane et al. 1948, Faran 1980, Faran & Linthicum 1981, Silva-do-Nascimento & Lourenço-de-Oliveira 2002). Nevertheless, specimens morphologically identifiable to *An. halophylus* and/or to *An. triannulatus* C have been collected in three states in the Brazilian Amazon (Rondônia, Acre, Amazonas and Pará) and in Paraguay and Bolivia (Deane et al. 1947, 1948, Root 1926).

In the present study, distinct seasonal biting frequencies were exhibited by three sympatric members of the Triannulatus Complex in Salobra. Biting frequency of all three species was shown to be negatively influenced by rainfall. A noteworthy reduction in biting frequency for the three species was detected during the rainy season (November to March). The decrease in biting frequency of *An. triannulatus s.l.* was striking for January 2007, when an unusual increase in rainfall was recorded (Fig. 2). Increasing precipitation in the rainy season probably impacts negatively on their population density in Salobra due to flushing and strong physicochemical changes in water characteristics at breeding sites. High numbers of temporary larval habitat mosquito breeders such as *Psorophora* and *Aedes* were noticed in January (data not shown). Strong decrease in biting frequency for *An. triannulatus s.l.* during the rainy season in Salobra was also described approximately 60 years ago: 2,666 specimens were collected at the beginning of the dry (and malarious) season, whereas only three were obtained in the summit of the rainy season (Travassos & Teixeira de Freitas

1940, Travassos 1940, Galvão & Lane 1941). We observed that frequency of all three species tended to decline as water impoundments subsided late in the dry season.

In Salobra, the population of *An. triannulatus s.s.* seems to peak immediately after the long rainy season. Notably, this peak occurs just after flooding of the lowlands by the MR and tributaries that induce the formation of large and permanent marshes with abundant vegetation. On the other hand, *An. halophylus* and *An. triannulatus* C populations peak later, when natural water impoundments no longer receive inflow from the MR and are therefore somewhat reduced in size. Water impoundment size reduction is probably related to the combined effect of evaporation and low rainfall (Figs 1 and 2). *Anopheles halophylus* and *An. triannulatus* C seem to be more closely related to each other than either is to *An. triannulatus s.s.* (Silva-do-Nascimento et al 2006). Coincidently, differences in population dynamics were greater between *An. triannulatus s.s.* and *An. halophylus* and *An. triannulatus* C than between *An. halophylus* and *An. triannulatus* C.

The highest biting frequency for *An. triannulatus s.s.* occurred in May 2006 (Figs 1 and 2). Peaks of high water levels in MR have been recorded in Salobra since March 2006. These peaks of river flow were found to be high enough to flood large low-lying expanses around Salobra. Therefore, an increase in *An. triannulatus s.l.* population in Salobra seems to be positively correlated with the formation of abundant large stable and permanent marshes subsequent to flooding of the MR and tributaries. Coincidently, higher biting frequencies of *An. triannulatus s.l.* following flooding during reservoir replenishment periods have been reported in Southwest and Central Brazil (Teodoro et al. 1995, Guimarães et al. 2004). During flow peak and river flooding periods in Salobra, great variation in river level is observed within short periods (2 - 3 days). Perceptible differences were detected by monthly low and high MR levels records (Fig. 2).

Larvae of *An. halophylus* were collected in sunlit marshes with abundant floating vegetation and high concentrations of sodium chloride (Silva-do-Nascimento & Lourenço-de-Oliveira 2002). One of the important tributaries of the MR is the Salobra River (SR), which has water with a notable chemical characteristic. It has high concentration of electrolytes (Ca^{++} and Mg^{++}), and bicarbonate ions are the main anion (Oliveira & Ferreira 2003). The high concentration of alkaline earth cations carried by the SR waters probably is a result of an abundance of calcareous and dolomitic rock in the Botoquena Mountains (Oliveira & Ferreira 2003). The SR joins the MR just at Salobra, where large marshes and lakes of fresh to brackish water with sparse to abundant floating vegetation are formed during the flooding period due to overflow of a mixture of waters from the SR and MR. Coincidently, both *An. halophylus* and *An. triannulatus* C peaked in the dry season (July), when these marshes and lakes are smaller, since there is no water coming from the surrounding rivers and precipitation is scarce. As a result there is an increase in ion and salt concentrations and that become stable in most larval habitats, which may be favorable to *An. halophylus* and *An. triannulatus* C. Additionally, the soil at Salobra is essentially composed of salty and calcareous sediments originated in the Bodoquena Mountains. Thus salts from the soil may also be dissolved in the water of flooded plains, and consequently several local marshes have moderate to high salt concentrations. These water bodies in Salobra are often classified as brackish water. Waters are classified as brackish when salinity is from 0.05 to 3% (= 500 - 30,000 ppm) (CONAMA 2000). In fact, continuous evaluation in one of the water impoundments at Salobra showed salinity ranging from 0.1 to 1.3% (Fig. 5).

Published biological and ecological data in the literature regarding to *An. triannulatus* from South and Central America may potentially correspond to one, two, or more species of the Triannulatus Complex. The distinctive population dynamics reported herein suggest either the exploitation of different larval habitats or ecological niches by the Triannulatus Complex species during the speciation process. Ayala and Coluzzi (2005) suggested that complete reproductive isolation in the *Anopheles gambiae* Complex was subsequent to specialization of the different species to separate niches. Immature niche partitioning for the three species of the Triannulatus Complex has not been evaluated yet. Diabaté et al. (2005) suggested that spatial segregation between the M and S forms of *An. gambiae s.s.* may be not determined by differences in their exploitation of the physical and chemical characteristics of larval habitats. Baimai et al. (1988) showed that sympatric populations of two species in the *Anopheles dirus* complex may share identical larval habitat and even the same pools in Thailand, while a third member is more often found in pools that dried out in dry spells during the rainy season. Our data showed similar seasonal population density of the most closely related forms in the Triannulatus Complex - *An. halophylus* and *An. triannulatus* C. This may suggest that these two closely related forms may exploit larval habitats with similar characteristics, i.e. with the same physicochemical conditions, or even share the same larval habitats. Associations between immatures of *Anopheles* belonging to the Triannulatus Complex within larval habi-

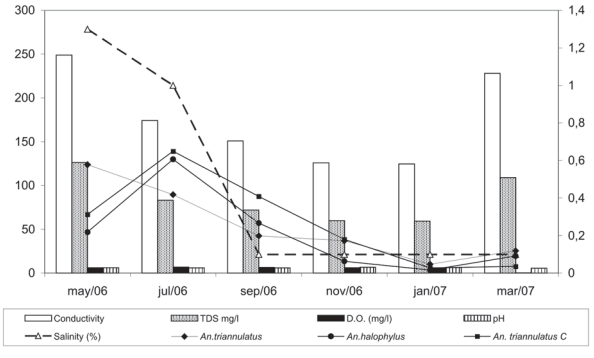

Figure 5: chemical parameters of the water from two larval habitats and number of specimens of three *Anopheles* species of the Triannulatus Complex collected in Salobra, Mato Grosso do Sul, Brazil, from May 2006 to March 2007.

tats need to be investigated. Larval habitats need to be characterized, taking in account varying conditions of water physicochemical characteristics, as well as rainfall and inundation dynamics in flood-prone areas.

ACKNOWLEDGMENTS

To Maria Goreti Rosa-Freitas for critical reading of the manuscript, Rafael Maciel-de-Freitas for the help in the statistical analysis, Andiaria Ramos da Silva and Maycon S A S Neves for laboratory assistance, Marcelo Quintela Gomes and Glauber Rocha for field and laboratory assistance, Fazenda Bodoquena for providing meteorological and environmental data, Darcílio F Baptista and Thaissa Alves for chemical analysis of water, and Agência Nacional de Águas for the daily records of MR water levels.

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