Winter survival of immature instars of *Mansonia indubitans* Dyar & Shannon and *Mansonia titillans* Walker (Diptera: Culicidae), in Buenos Aires, Argentina

Juan P Torretta/+, Pablo R Mulieri*/*, Luciano D Patitucci**, Valeria A Sander***, Patricia L Rodríguez**/***, Nicolás Schweigmann**/***

Cátedra de Botánica Agrícola, Facultad de Agronomía **Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina *ANLIS "Dr. C.G. Malbrán" Servicio de Vectores, Buenos Aires, Argentina ***Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

We conducted a whole year research on the ecology of Mansonia indubitans *and* Ma. titillans *in Macáes Pond, Costanera Sur Reserve, Buenos Aires, Argentina. The usage of different floating plants by immature instars and their overwintering was analized. The percentage of usage of the available floating macrophytes* (Pistia, Limnobium, *and* Salvinia) *by the larvae and pupae was studied. Also, we defined positivity (P+) as the percentage of plants with immature instars for each plant genus on a monthly basis.* Ma. *immature instars were captured throughout the year and* Pistia *was the resource most commonly exploited by the mosquitoes. The percentage of fourth-instar larvae and pupae on* Pistia *roots with respect to total immature instars captured was assessed on a monthly and seasonal basis. The proportion of fourth-instar larvae and pupae from both species of* Mansonia *on water lettuce roots, showed significant differences between months and seasons. Our results suggest that the populations of* Ma. indubitans *and* Ma. titillans *in Macáes Pond, survive during winter mainly as fourth-instar larvae.*

Key words: *Mansonia indubitans* - *Mansonia titillans* - *Pistia stratiotes* - winter survival - plant-animal interaction

The widespread genus *Mansonia* Blanchard is associated with aquatic macrophytes in the larval and pupal stage. They both possess modifications of their respiratory organs (siphons and trumpets, respectively) which enable them to attach to the roots of aquatic plants and obtain air from the aerenchyma (Dyar 1928, Forattini 1965). Adult females lay their characteristic egg masses on the leafs of aquatic macrophytes (Mattingly 1972a, b, Gass et al. 1983, Linley et al. 1986, Lounibos & Dewald 1989, Ferreira & Nunes de Mello 1999). Numerous floating and rooted aquatic plant species are associated with many *Mansonia* species (Slaff & Haefner 1985, Krishnamoorthy et al. 1994).

Water lettuce (*Pistia stratiotes* L., Araceae), considered as the third aquatic weed of world importance (Barreto et al. 2000), is used by the immature stages of a great diversity of insects (Poi de Neiff 1983, Escher & Lounibos 1993) and supports numerous *Mansonia* species in Africa (Laurence 1959, 1960), Asia (Krishnamoorthy et al. 1994), and America (Lounibos & Escher 1985, Lounibos & Dewald 1989, Ferreira 1999). The use of floating macrophytes for wastewater treatment is recognized (Brix 1997) as representing a favourable breeding ground for *Mansonia* mosquitoes (Kengne et al. 2003).

Ma. indubitans Dyar & Shannon and *Ma. titillans* Walker share a wide geographic range in America with Buenos Aires province, Argentina, being the southern limit (Mitchell & Darsie 1985). Females of both species are aggresively haematophagous (Almirón 2002) and potential vectors of filariosis (Forattini 1965).

García et al. (1995) reported that the immature instars of these species disappear during the winter in a relict of marginal jungle in Punta Lara Reserve (Buenos Aires province, Argentina) due to the death of *P. stratiotes*. Garcia et al. (1995) suggested the survival of *Mansonia* by overwintering adults.

The objectives of this work were: (a) study the winter behavior of immature instars of *Ma. indubitans* and *Ma. titillans* in Buenos Aires City and (b) determine the use of different plant species during overwintering of both species in a heterogeneous habitat.

MATERIALS AND METHODS

Study area - Field studies were conducted at the Macáes Pond, located in Costanera Sur Reserve (S 34º36'26,7" O 58º20'54,4"), Buenos Aires City, Argentina.

This man-made, 1-ha, freshwater habitat was overgrown with floating plants, mainly *Salvinia* spp. (*S. herzogii* de la Sota and *S. rotundifolia* Wild), *Pistia stratiotes* L. and *Limnobium laevigatum* (Humb. & Bonpl. ex Wild.) Heine. Due to the difficulty in identifying to the species level in the field, individuals of *Salvinia* were considered at generic level (hereafter *Salvinia*).

Buenos Aires is characterized by a subtropical climate with cold winters (mean temperature of 11°C) and hot, rainy summers (mean temperature is 25°C, Servicio Meteorológico Nacional 1993).

Fig. 1 shows maximum and minimum monthly average temperatures in Buenos Aires during 2003. By the fact

+Corresponding author: torretta@agro.uba.ar
Received 5 December 2005
Accepted 11 July 2006

that this study was undertaken in just one year, we compared maximum and minimum monthly mean temperatures with the mean values for 1991-2002.

Field samples - Surveys for immature instars were made from January to December 2003. Samples were taken weekly (January-April and November-December) or fortnightly (May-October), except july (one visit), using a 1404 cm^2 quadrangular sampling device. On each survey date, individuals of three genera were examined in 2 to 6 sampling units. The coverage (percentage of sample units) and number of individuals of *Pistia*, *Limnobium*, and *Salvinia* were recorded and the immature instars were captured by shaking plants vigorously in a plastic container with water (Lounibos & Escher 1985), and collecting them with plastic pipettes. They were fixed in situ with 80% ethanol. Fourth-instar larvae and pupae were identified to species according to Ronderos and Bachmann (1963). The other instars were treated together (called early instar larvae). The presence and number of the *Mansonia* immature instars were determined for each individual plant. Each plant with a single differentiated root was considered as an individual. Plants connected with a stolon were separated before immature *Mansonia* were collected. This disturbance caused by the harvesting of the plants could cause the loosening of some larvae from the roots of the floating plants

Taking into account the number and coverage of individuals from each plant genus, we estimated the mean monthly size of *Pistia*, *Limnobium*, and *Salvinia* using the formula:

$$TP_i = [\Sigma(cov_i * \#\,individuals/100) * 1404]/n$$

where cov_i: the coverage of the genus I; 1404: sampling unit area (in cm^2); and n: number of monthly sampling units.

Exploitation of plant resourses - The percentage of usage (PU_i) was defined as the number of *Mansonia* individuals collected on each plant genus (i) related to the total number of individuals caught each month. In addition, we calculated the mean total plants per sampling units (PT), mean plants with immature instars (PCI) and calculated the positivity (P+ = [PCI/PT]*100) for each plant genus monthly along the year.

Proportions of collected immature instars on *Pistia* roots were compared using the independent proportions test (Fleiss 1981). To assess differences among seasons we analyzed together the months corresponding to summer (January-March), fall (April-June), winter (July-September), and spring (October-December).

From September 2003 to December 2003, we measured the length of the longest root from each *Pistia* and *Limnobium* individuals and registered the number of *Mansonia* immature instars on each plant.

RESULTS

The coverage of floating plants at Macáes Pond changed along the year. The estimated mean size and the number of individual from each plant genera per sampling unit are shown in Table I. During winter the number of *Pistia* individuals per sampling unit was the lowest, also these individuals had the major mean size estimated. *Limnobium* and *Salvinia* individuals did not show any pronounced seasonal pattern (Table I). Other macrophytes, such as *Hydrocotile ranunculoides* L., *Lemna* spp., and *Wolffiella* sp. were also found in lower ratio.

Mansonia immature instars were collected throughout the year. Among the 2759 larvae and pupae captured, 78.5% were on *Pistia* roots, 19.5% on *Limnobium*, and only 2% on modified leafs of *Salvinia* (Table II). The percentage of usage (PU) varied during the year, with *Pistia* being the plant most used during all months (Table II). *Limnobium* roots usage was higher in spring and summer months while *Salvinia* was little used throughout the year.

Overall, the positivity of *Pistia* (P+) was higher than for *Limnobium* (except for January and February) and *Salvinia* throughout the year (Fig. 2A), varying from 15 to 70%. The number of individuals of *Pistia* per sample

TABLE I

Means of size, coverage, and number of individuals of three plant genera in the Macáes Pond, Costanera Sur Reserve along the year 2003

	Pistia					*Limnobium*					*Salvinia*				
	Coverage		Individuals		cm^2	Coverage		Individuals		cm^2	Coverage		Individuals		cm^2
Month	Mean	SD	Mean	SD	Mean size	Mean	SD	Mean	SD	Mean size	Mean	SD	Mean	SD	Mean size
Jan	33,57	27,06	9,71	9,53	48,52	5,43	8,92	4,21	6,24	18,09	60,64	26,58	44,14	22,43	19,29
Feb	20,79	17,77	6,67	5,06	43,79	16,19	16,36	13,29	9,87	17,10	56,58	23,82	48,71	31,86	16,31
Mar	27,85	26,75	9,92	9,00	39,40	23,92	20,02	13,69	16,49	24,53	39,31	28,72	35,23	28,72	15,66
Apr	21,75	30,80	6,25	7,14	48,86	24,50	26,66	15,90	15,00	21,64	53,75	22,87	41,00	15,38	18,41
May	25,75	29,19	7,75	8,73	46,65	25,50	34,07	8,75	10,18	40,92	48,75	40,08	38,00	27,41	18,01
Jun	37,50	17,68	6,50	0,71	81,00	7,50	3,54	5,50	0,71	19,15	55,00	14,14	43,50	9,19	17,75
Jul	23,75	12,50	5,00	4,32	66,69	29,25	23,14	16,25	11,12	25,27	47,00	18,51	40,75	1,71	16,19
Aug	27,50	10,41	7,75	4,65	49,82	39,00	33,87	5,50	5,80	19,56	33,00	32,50	35,25	6,50	13,14
Sep	35,00	12,25	10,50	3,87	46,80	8,83	6,11	6,67	3,88	18,60	58,67	11,31	35,50	8,53	23,20
Oct	37,88	15,91	10,00	6,63	53,18	18,75	20,08	11,88	9,39	22,17	39,88	28,36	26,13	16,34	21,43
Nov	35,71	11,34	11,57	5,26	43,33	14,29	12,72	8,57	8,62	23,40	50,00	10,00	52,29	12,13	13,43
Dec	40,60	24,60	12,00	6,29	47,50	14,30	24,72	7,50	5,68	26,77	44,60	24,78	46,30	19,39	13,52

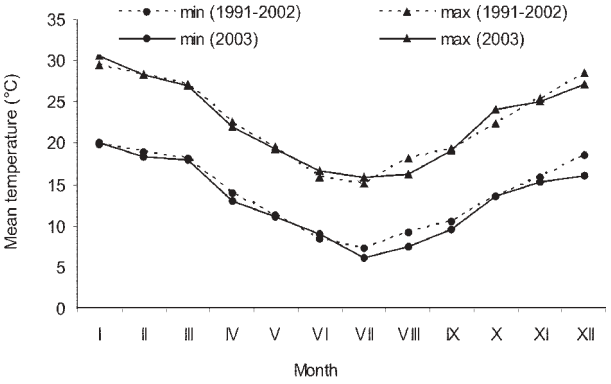

Fig. 1: mean temperatures in Buenos Aires City along the year 2003 (maximum and minimum) compared with the means of 1991-2002 period.

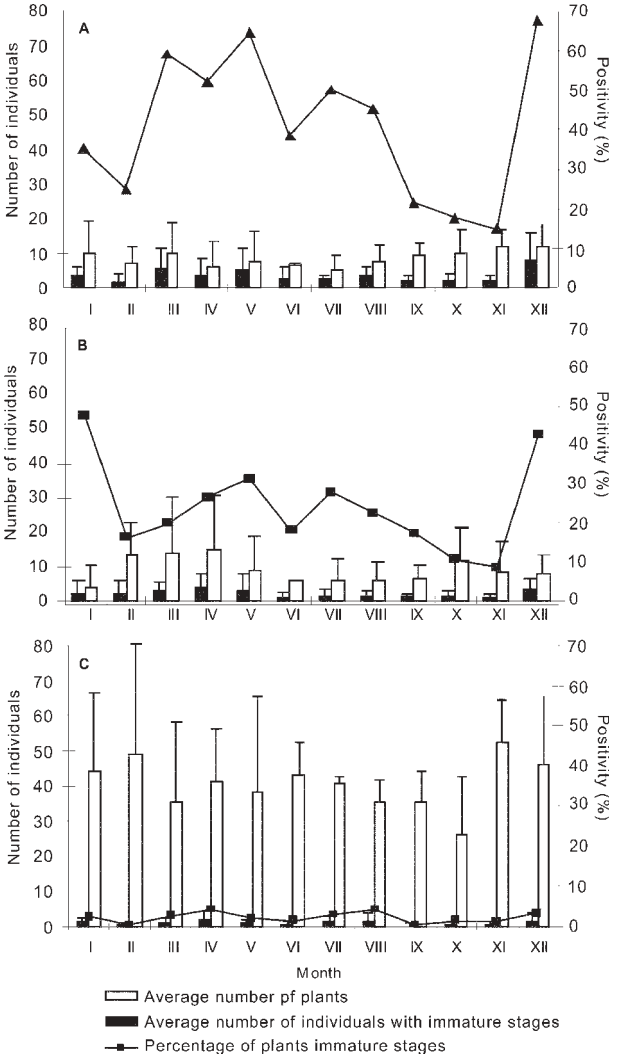

Fig. 2: positivity (P+), total number mean of individual for sampling unit (PT) and average of plant individual with *Mansonia* immature instars (PCI) for *Pistia* (A), *Limnobium* (B), and *Salvinia* (C) during the year in Macáes Pond, Costanera Sur Reserve, Buenos Aires City, Argentina. Seasons: I+II+III: summer; IV+V+VI: fall; VII+VIII+IX: winter; and X+XI+XII: spring.

TABLE II

Number of individuals of three plant genera examined and immature individuals of *Mansonia indubitans* and *M. titillans* collected in three plant genera in the Macáes Pond, Costanera Sur Reserve, Buenos Aires City, Argentina, during 2003

Month	n	*Pistia*			*Salvinia*			Limnobium			*Ma. indubitans*			*Ma. titillans*			Early instars		
		Total	P+	P–	Total	S+	S–	Total	L+	L–	Total	larvae	pupae	Total	larvae	pupae	Total	L I	L II	L III
Jan	14	136	48	88	618	15	603	59	28	31	113	71	42	73	57	16	148	92	30	26
Feb	24	160	40	120	1169	5	1164	319	52	267	47	39	8	41	40	1	129	78	35	13
Mar	13	129	76	53	458	12	446	178	35	143	60	55	5	112	110	2	304	146	114	42
Apr	4	25	13	12	164	7	157	60	16	44	23	23	0	32	32	0	60	7	37	16
May	4	31	20	11	152	3	149	35	11	24	183	183	0	19	19	0	31	3	13	15
Jun	2	13	5	8	87	1	86	11	2	9	11	11	0	26	26	0	22	0	14	8
Jul	4	20	10	10	163	5	158	65	7	58	92	92	0	83	83	0	28	0	8	20
Aug	4	31	14	17	141	6	135	22	5	17	50	50	0	35	35	0	26	1	16	9
Sep	6	56	12	44	213	1	212	40	7	33	69	69	0	14	14	0	13	0	5	8
Oct	8	80	14	66	209	3	206	95	10	85	43	39	4	15	15	0	16	0	9	7
Nov	7	81	12	69	366	3	363	60	5	55	9	7	2	7	7	0	11	3	2	6
Dec	10	120	81	39	463	15	448	75	32	43	105	98	7	72	65	7	637	446	140	51
Total	100	882	345	537	4203	76	4127	1019	210	809	805	737	68	456	503	26	1425	776	423	221

n: number of sample units; P+, S+ and L+ individuals of *Pistia*, *Salvinia*, and *Limnobium* with immature instars of *Mansonia* spp. P–, S– and L–, without immature instars. LI, LII and LIII, larvae first-, second-, and third-instar.

unit had the highest values in summer and spring and lower in fall and winter.

The monthly density of *Limnobium* showed an increase in February, March, and April and then diminished from May, rising again in October, with a slight decrease in November-December (Fig. 2B).

The mean monthly number of *Salvinia* per sampling unit was highest during the whole year. The maximum values of fern densities were found in January-February and November-December (Fig. 2C), but the percentage with immature instars was very low throughout the entire study.

Maximum and minimum monthly temperatures during 2003 were similar to those of the preceeding 13 years (1991-2002) (Fig.1).

Of the 2167 larvae and pupae collected on the *Pistia* roots, 703 were *Ma. indubitans*, 419 were *Ma. titillans*, and the remaining 1045 were early instar larvae. The percentage of total individuals captured on *Pistia* roots increased during the months that exhibited the lowest temperatures. The total percentage of usage on *Pistia* (PU_P) is shown in Fig. 3A. Fig. 3B shows the PU_P of fourth-instar larvae and pupae from both species.

The highest exploitation of *Pistia* roots by these immature instars occurs from May to October, when the parameter values were above 70%. The proportion of the fourth-instar larvae and pupae from both species of *Mansonia* on water lettuce roots showed significant differences among months ($\chi^2 = 953.29$, d.f. = 11, $p < 0.01$) and among seasons ($\chi^2 = 308.37$, d.f. = 3, $p < 0.01$).

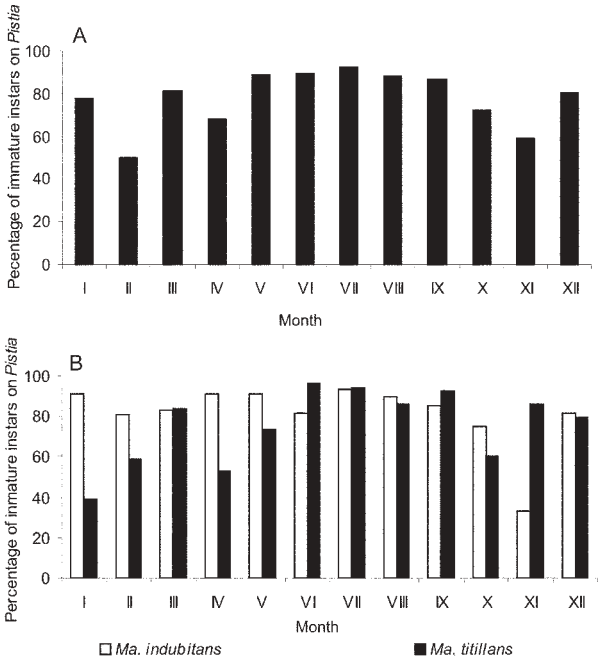

Fig. 3: percentage of immature instars (A) of *Mansonia* spp. (all instars within pupae) and (B) fourth-instar larvae and pupae of *Ma. indubitans* and *Ma. titillans* during the year in Macáes Pond, Costanera Sur Reserve, Buenos Aires City, Argentina; seasons: I+II+III: summer; IV+V+VI: fall; VII+VIII+IX: winter; and X+XI+XII: spring.

The number of *Ma. indubitans* and *Ma. titillans* individuals did not correlate with the length of the *Pistia* ($r = 0.17$, $p > 0.05$ and $r = 0.24$, $p > 0.05$, respectively) or *Limnobium* roots ($r = -0.17$, $p > 0.05$ and $r = 0.46$, $p > 0.05$, respectively).

DISCUSSION

The result that immature instars of *Ma. indubitans* and *Ma. titillans* in Macáes Pond, Costanera Sur Reserve were captured throughout the whole year differs from the results of others authors (Lounibos & Escher 1985, García et al. 1995). García et al. (1995) working in a nearby study area, found that *Mansonia* immatures disappeared in the absence of *P. stratiotes*. Lounibos and Escher (1985) reported a decline in *Ma. titillans* and *Ma. dyari* adult emergence in Florida (US) during January-February (boreal winter). They associated this fact with a decrease in water temperature and *P. stratiotes* dying off. Dewald and Lounibos (1990) have demonstrated that low temperature in winter was the most important variable affecting *P. stratiotes* growth. In Macáes Pond we observed a decrease in the number of water lettuce, although the population never disappeared completely.

Water temperature affects the longevity of the immature stages of Culicidae. Slaff and Haffner (1985) reported that *Ma. titillans* and *Ma. dyari* larvae diminish their metabolic activity when the temperature decreases. Moreover, under laboratory conditions, Tun-Lin et al. (2000) and Bayoh and Lindsay (2004) showed that the mortality of *Aedes aegypti* and *Anopheles gambiae* was 100% when bred at temperatures lower than 10 and 16ºC, respectively. Some species are clearly more cold tolerant than others (Bayoh & Lindsay 2004). Notwithstanding we did not measure water temperature, Young (1975) found that in a temperate pond the average air and water temperature followed the same pattern of seasonal fluctuations; therefore air temperature may reflect thermal conditions for the immatures instars.

With the arrival of the warm temperatures, fourth-instar larvae pupate and emerge as adults. This is consistent with our finding of pupae only during spring and summer. We suggest, that fourth-instar larvae better tolerate adverse temperature conditions than early instars, although the accumulation of fourth-instar may be solely a product of earlier instars molting to the final instar, where metamorphosis is suspended until temperatures rise up.

At Macáes Pond the reproduction of *Mansonia* species starts by the end of the spring (unpublished data). There are two factors that cause the proportion of fourth-instar larvae to diminish with rising temperature: (1) during the reproductive season there is an increase in early instar larvae and (2) fourth-instar larvae develop into pupae.

The association between *Mansonia* and *Pistia* in Macáes Pond is strong. Mulieri et al. (2005) found a selective *P. stratiotes* pattern of root utilization by *Ma. indubitans* and *Ma. titillans* in Macáes Pond during the warm season.

Nevertheless, during low temperature months, when the average number of *Pistia* individuals per sampling unit diminishes, the proportion of larvae on water lettuce

roots increases until reaching maximum values during winter. The largest individuals of *P. stratiotes* are whom survive during winter. Based on our results, we suggest a special importance of *P. stratiotes* for the winter survival of *Ma. indubitans* and *Ma. titillans* in its southernmost distribution edge, corroborating with García et al. (1995).

We showed that populations of *Ma. indubitans* and *Ma. titillans* in Macáes Pond, Buenos Aires City, survive during the cold period principally as fourth-instar larvae. The existence of an overwintering adult population, as suggested by García et al. (1995), was not examined during our study.

The winter association between *Mansonia* larvae and water lettuce would allow control of these mosquitoes by careful management of this aquatic weed.

ACKNOWLEDGMENTS

To Marc Slaff and Phillip Lounibos for reviewing earlier drafts of this manuscript, the personnel of Reserva Ecológica Costanera Sur for logistic support, Victoria Botazzi for her valuable cooperation in the field work, and Ramiro Saurral for revising the language and anonymous reviewers, for their critical suggestions of this manuscript.

REFERENCES

Almirón WR 2002. Culicidae (Diptera) de la provincia de Córdoba. In O Salomón *Actualizaciones en Artropodología Sanitaria Argentina*, Mundo Sano Ediciones, Buenos Aires, p. 97-113.

Barreto R, Charudattan R, Pomella A, Hamada R 2000. Biological control of neotropical aquatic weeds with fungi. *Crop Prot 19*: 697-703.

Bayoh MN, W Lindsay 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Med Vet Entomol 18*: 174-179.

Brix H 1997. Do macrophytes play a role in constructed wetlands. *Wat Sci Technol 35*: 11-17.

Dewald LB, Lounibos LP 1990. Seasonal growth of *Pistia stratiotes* L. in south Florida. *Aquat Bot 36*: 263-275.

Dyar HG 1928. *The Mosquitoes of the Americas*, Carnegie Inst. Wash. Publ. Nº 387, Washington, DC, 616 pp.

Escher RL, Lounibos LP 1993. Insect associates of *Pistia stratiotes* (Arales: Araceae) in southeastern Florida. *Fla Entomol 76*: 473-500.

Ferreira RLM 1999. Densidade de oviposição e quantificação de larva e pupas de *Mansonia* Blanchard, 1901 (Diptera: Culicidae), em *Eichhornia crassipes* Solms. e *Pistia stratiotes* Linn. na ilha da Marchantaria, Amazônia Central. *Acta Amazonica 29*: 123-134.

Ferreira RLM, Nunes de Mello JAS 1999. Aspectos biológicos de três espécies de *Mansonia* (*Mansonia*) Blanchard, 1901 (Diptera, Culicidae) em laboratório. *Rev Bras Ent 43*: 29-34.

Fleiss JL 1981. *Statistical Methods for Rates and Proportions*, 2nd ed, J Wiley & Sons, New York, 320 pp.

Forattini OP 1965. *Entomologia Médica. 3º Volume. Culicini: Haemagogus, Mansonia, Culiseta. Sabethini. Toxorhynchitini. Arboviroses. Filariose bancroftiana, Genética*, Universidade de São Paulo, São Paulo, 416 pp.

García JJ, Campos RE, Macia A 1995. Observaciones ecológicas sobre *Mansonia indubitans* y *Mansonia titillans* (Diptera: Culicidae) y sus enemigos naturales en Punta Lara, Argentina. *Revta Soc Ent Arg 54*: 43-50.

Gass RF, Deesin T, Surathin K, Vutikes S, Sucharit S, Harinasuta C 1983. Studies on oviposition characteristics of *Mansonia* (*Mansonioides*) mosquitoes in southern Thailand. *Ann Trop Med and Parasit 77*: 605-614.

Kengne IM, Brissaud F, Akoa A, Eteme RA, Nya J, Ndikefor A, Fonkou T 2003. Mosquito development in a macrophyte-based wastewater treatment plant in Cameroon (Central Africa). *Ecol Eng 21*: 53-61.

Krishnamoorthy K, Rajendran G, Panicker KN 1994. Aquatic vegetation and their natural hospitability to the immatures of *Mansonia* mosquitos, the vectors of *Brugia malayi* in Shertallai, Kerala, India. *Southeast Asian J Trop Med Public Health 25*: 760-765.

Laurence BR 1959. Oviposition by *Mansonioides* mosquitoes in the Gambia, West Africa. *Proc Roy Ent Soc Lond (A) 34*: 161-170.

Laurence BR 1960. The biology of two species of mosquito, *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald), belonging to the subgenus *Mansonioides* (Diptera: Culicidae). *Bull Entomol Res 51*: 491-507.

Linley JR, Linley PA, Lounibos LP 1986. Light and scanning electron microscpy of the egg of *Mansonia titillans* (Diptera: Culicidae). *J Med Ent 23*: 99-104.

Lounibos LP, Escher RL 1985. Mosquitoes associated with water lettuce (*Pistia stratiotes*) in southern Florida. *Fla Entomol 68*: 169-178.

Lounibos LP, Dewald LB 1989. Oviposition site selection by *Mansonia* mosquitoes on water lettuce. *Ecol Ent 14*: 413-422.

Mattingly PF 1972a. Mosquitos eggs. XIX. Genus *Mansonia* (Subgenera *Rhynchotaenia* Bréthes and *Mansonia* Blanchard) with a further note on genus *Ficalbia* Theobald. *Mosq Syst 4*: 45-49.

Mattingly PF 1972b. Mosquitos eggs. XVIII. Genus *Mansonia* (Subgenera *Mansonioides* Theobald). *Mosq Syst 4*: 50-59.

Mitchell CJ, Darsie RF 1985. The mosquitoes of Argentina. Part II. Geographic distribution and bibliography (Diptera, Culicidae). *Mosq Syst 17*: 279-334.

Mulieri PR, Torretta JP, Schweigmann N 2005. Host plant selection of two *Mansonia* Blanchard species (Diptera: Culicidae) in a heterogeneous habitat of Buenos Aires City, Argentina. *J Vector Ecol 30*: 201-205.

Poi de Neiff A 1983. Observaciones comparativas de la mesofauna asociada a *Pistia stratiotes* L. (Araceae) en algunos ambientes acuáticos permanentes y temporarios (Chaco, Argentina). *Physis 41*: 95-102.

Ronderos RA, Bachmann AO 1963. Mansoniini neotropicales. I (Diptera: Culicidae). *Revta Soc Ent Arg 26*: 57-65.

Servicio Meteorológico Nacional. 1993. *Argentina: Estadísticas Climatológicas 1981-1990*, Vol. 37, Buenos Aires, Argentina.

Slaff M, Haffner JD 1985. Seasonal and spatial distribution of

Mansonia dyari, *Mansonia titillans*, and *Coquilletidia perturbans* (Diptera: Culicidae) in the Central Florida, USA, phosphate region. *J Med Ent 22*: 624-629.

Tun-Lin W, TR Burkot, BH Kay 2000. Effects of temperature and larval diet on development rates and survival on the dengue vector *Aedes aegypti* in north Queensland, Australia. *Med Vet Entomol 14*: 31-37.

Young JO 1975 .Seasonal and diurnal changes in the water temperature of a temperate pond (England) and a tropical pond (Kenya). *Hydrobiologia 47*: 513-526.