



Mosquito development in a macrophyte-based wastewater treatment plant in Cameroon (Central Africa)

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Received 10 December 2002; received in revised form 30 July 2003; accepted 15 August 2003

Abstract

Macrophyte-based wastewater treatment systems are recognized as an alternative for sewage purification in developing countries. Unfortunately, they also represent a favorable breeding ground for mosquitoes, thus a serious drawback that should be addressed despite the good promise of this technology.

A 1-year study of mosquito production in seven ponds of a *Pistia stratiotes*-based domestic wastewater treatment plant in Cameroon revealed that approximately 43 imagoes/m² per day rose up, among which 54% were female. *Mansonia* and *Culex* were the main breeding genera with about 55 and 42% of the total imagoes respectively. *Culex* bred mostly in the first three ponds (B1–3), characterized by a high organic pollution. *Mansonia* occurred in great number in the later ponds (B4–7), where the water quality was rather better and the roots of *P. stratiotes* well developed, thus permitting the fixation of a great number of larvae to the macrophyte roots. Though representing a favorable breeding ground for mosquitoes, only 0.02% of captured imagoes were *Anopheles gambiae*, suggesting that this wastewater treatment plant does not significantly contribute to the development of the malaria vector in this area.

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Keywords: Wastewater treatment; Cameroon; Macrophyte lagoons; *Pistia stratiotes*; Mosquito development

1. Introduction

The use of aquatic treatment systems, especially floating macrophytes, is recognized as an alternative method of wastewater treatment (Debusk and Reddy, 1987; Brix, 1997). The scientific basis governing this process is the symbiotic association between aquatic

macrophytes and microorganisms. The latter degrades the organic matter present in the water into simple nutrients that are absorbed by macrophytes. In return, macrophytes create favorable conditions for microbial activity (Wolverton, 1987; Vyzamal et al., 1998).

Among the advantages favoring this ecotechnology, especially in developing countries which lack capital and qualified manpower to run sophisticated methods, are its simplicity of construction and operation, low operation costs, and capability to withstand excess

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organic and hydraulic loads (Reddy and Smith, 1987). Furthermore, most developing countries have a warm tropical and subtropical climate that allows high biological activity and productivity year-round, which means high efficiency of the system (Denny, 1997; Kivaisi, 2001).

Despite these multiple advantages, many environmental questions are still to be addressed, among which is the development of mosquitoes, especially in warm regions. Indeed, the presence of large water bodies covered by macrophytes may represent a favorable breeding ground for such insects, thus providing nuisance pests and or disease vectors to the nearby communities (Martin and Eldridge, 1989; Dill, 1989).

In Cameroon, the use of aquatic macrophytes for sewage treatment is recent. A series of lagoons were built in 1986 to treat approximately 45 m³ per day of domestic sewage from a residential area in Yaounde. Several macrophytes were tested, among which *Pistia stratiotes* was selected and grown in ponds, since it has a good pollution removal capacity and can be easily harvested (Agendia et al., 1988). Indeed, the pollutant removal efficiency of the plant was quite satisfactory when using this macrophyte, with most COD, BOD and SS abatements being higher than 80% (Agendia, 1995). Furthermore, Agendia et al. (1997) estimated that approximately 27 t of compost could be obtained annually from the by-products (macrophytes and sludge), thus allowing the recovery of part of the operation cost while reducing the chemical fertilizer needs of the neighboring farmers.

However, in a preliminary survey aimed at assessing the environmental impacts of this plant, 65% of the population living in the vicinity estimated that its construction has led to the proliferation of mosquitoes, thus representing a serious threat that should be addressed when considering the diffusion of this technology. Efforts to control mosquitoes include vegetation management, the use of chemical and biological agents or construction design (Debusk and Reddy, 1987; Tchobanoglous, 1987; Kadlec and Knight, 1996). The success of these approaches depends mainly on a better knowledge of the mosquito species involved (Russell, 1999). But few studies have focused on mosquitoes breeding in aquatic treatment systems, especially in tropical regions where the warm climate favors the proliferation of a variety of fauna and flora.

As a first step to minimize the proliferation of such disease vectors or nuisance pests, a study aimed at assessing the biodiversity and the spatio-temporal dynamics of the mosquito breeding in a *P. stratiotes* based treatment plant was carried out from November 1997 to October 1998 in Yaounde (Cameroon).

2. Materials and methods

2.1. Site of study

This work was carried out in stabilization ponds devoted to treatment of approximately 45 m³ per day of raw sewage from the Biyem-Assi residential quarter of the capital city Yaounde (latitude 3°52'N, longitude 11°32'E, altitude 760 m). Yaounde enjoys a typical equatorial Guinean climate characterized by two rainy seasons (September to mid November and mid March to June) and two dry seasons (mid November to mid March and July to August). The annual rainfall is around 1600 mm and daily temperatures vary between 23 and 27 °C.

This plant has a total surface area of approximately 0.1 ha and is made up of eight ponds dug in lateritic soil and separated by dykes of compacted soil. Its design and operation is reported in Agendia (1995). Briefly, the first pond (B0), also called a decantation–digestion pond, is covered with a thick crust of organic matter that allows an anaerobic pre-treatment. The rest of the ponds (B1–7) are rectangular lagoons vegetated by the floating macrophyte *P. stratiotes* (Fig. 1). Water flows gently by gravity and the hydraulic retention time is comprised between 9 and 16 days. Dimensions of the ponds are presented in Table 1.

2.2. Wastewater quality monitoring

Water samples were collected every 5 days at the outflow of each pond from November 1997 to October 1998. pH was determined using a Schot Gerate 818 pH meter; temperature and conductivity by a Hach conductimeter; and dissolved oxygen content using a WTW Oxi 300 oxymeter. BOD₅ and SS were measured according to the general methods described in Hach handbook of water analysis (Hach, 1992).

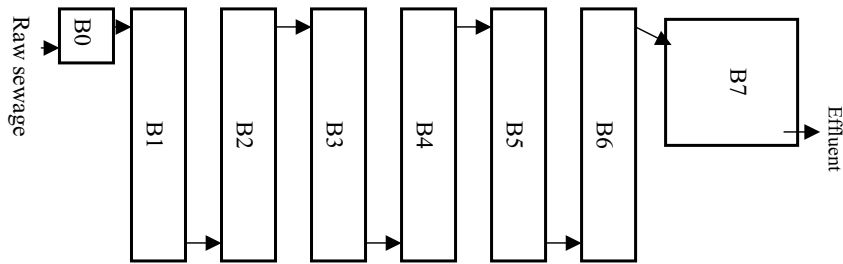


Fig. 1. Layout of the Biyem-Assi lagoon plant (B0: decantation–digestion pond; B1–7: lagoons).

2.3. Assessment of culicid biodiversity and dynamics

Biodiversity and dynamics of the culicids were assessed taking into account immature and adult mosquito stages breeding in B1–7 macrophyte ponds. Pond B0 was omitted, for it is anaerobic. Field investigations were carried out five times a month from November 1997 to October 1998.

2.3.1. Immature stages

A preliminary survey of the different mosquitoes breeding in the plant allowed us to distinguish free-living mosquitoes, i.e. mosquitoes whose immature stages breathe directly at air/water interface, and attached mosquitoes whose immatures live attached on the macrophyte roots. Immature free-living mosquitoes were sampled by randomly dipping at five stations in each lagoon with a 250 ml dipper. For attached mosquitoes, five water lettuces were haphazardly but rapidly picked up in each pond and conveyed into cut plastic bottles half-filled with water. Selected

plants were deprived of all secondary shoots by plucking off the stolons. Then the contents of each plastic bottle were poured into a small white basin. The roots of the plants were washed by gently swirling them in water to release the mosquito larva, nymphs, and other debris attached to the roots. Additionally, to assess whether there was a relationship between the immature stages and the macrophytes, we measured the fresh biomass of excised roots using a mini-scale balance.

The larvae and nymphs so collected were transferred into small-labeled plastic bottles with a small quantity of water, and carefully transported within 1 h to the laboratory for identification and counting. In general, this was done on living organisms using pipettes to aspirate the larva and nymphs. Due to their high number, only larvae from the 2nd to 4th stages and nymphs were considered. Identification was done according to the immature mosquito determination keys of the Ethiopian region (Hopkins, 1952). When determination was not possible at pre-imaginal stage, immature mosquitoes were allowed to develop to the adult stage where identification was easier.

2.3.2. Adult stage

Adult mosquitoes breeding in the macrophyte lagoons (B1–7) were collected with the use of a mosquito trap derived from the model proposed by Aubin et al. (1973). Each trap has a pyramidal shape and covers 0.25 m². It holds, in its upper part, a 1.51 transparent box containing a funnel to channel the emerging mosquitoes (Fig. 2). In each pond, three traps were randomly located and inspected after 24 h. Mosquitoes that had emerged were then neutralized by using a commercial insecticide

Table 1
Characteristics of the Biyem-Assi sewage treatment plant

Ponds	Length (m)	Width (m)	Depth (m)	Surface (m ²)	Volume (m ³)
B0	7.5	3.5	1.8	26.3	47.3
B1	22	4.4	0.7	96.8	67.9
B2	22	4.3	0.8	94.6	75.2
B3	22	4.4	0.9	96.8	86.5
B4	22	4.3	0.8	94.6	76.0
B5	22	4.3	0.9	94.6	85.5
B6	22	4.4	0.9	96.8	86.4
B7	18	6.6	0.5	118.8	59.5

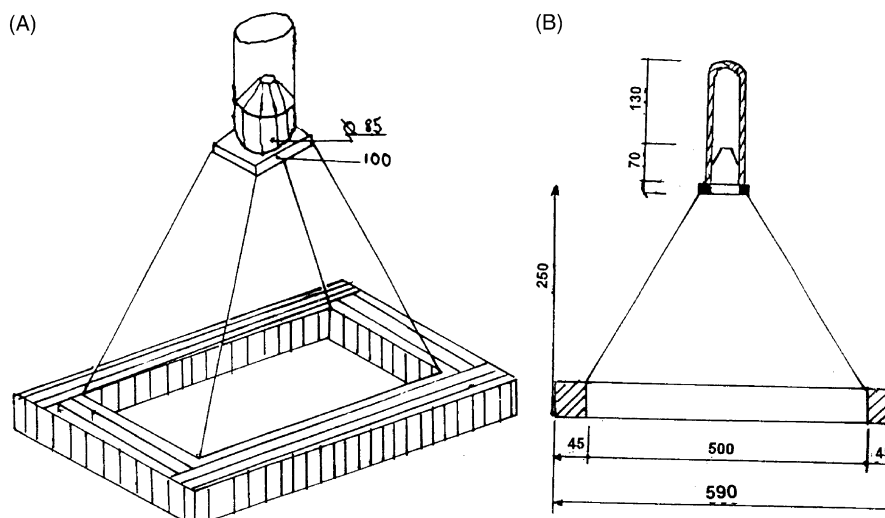


Fig. 2. Adult mosquito trap (adapted from Aubin et al., 1973) (dimensions in mm).

and identification done according to the adult mosquito key of the Ethiopian region (Edwards, 1941).

2.3.3. Expression and analysis of results

Population densities are expressed as follows: the average number of individuals per dipper for free-living immature mosquitoes, the average number of individuals per class of root biomass (RB) for attached mosquito larvae and nymphs, and the average number of imagoes/m² per day for adult stage. Non-parametric Kruskal–Wallis test was used for comparison between the main species, wetland ponds, and seasons.

3. Results

3.1. Physicochemical characteristics of wastewater

Wastewater physicochemical characteristics are presented in Table 2. Water in all the ponds was slightly warm and above neutrality, thus favorable for an intense biological activity. Indeed, the mean temperature was higher than 26 °C and pH ranged from 7.07 to 7.63. The first macrophyte ponds (B1 and B2) were extremely rich in organic and particulate matters as suggested by their BOD₅ and SS concentrations (respectively, 308 and 277 mg/l for B1, 212 and 149 mg/l

Table 2
Physicochemical characteristics of the wastewater in the ponds

Ponds	pH	Physicochemical properties of wastewater (mean ± S.E.M.)					
		Temperature (°C)	Conductivity (μS/cm)	DO (mg/l)	BOD ₅ (mg/l)	SS (mg/l)	Turbidity (FTU)
B1	7.07 ± 0.04	26.4 ± 0.2	1292 ± 26	0.3 ± 0.0	308 ± 12	277 ± 13	270 ± 10
B2	7.12 ± 0.04	26.6 ± 0.2	1258 ± 27	0.8 ± 0.1	212 ± 13	149 ± 9	159 ± 6
B3	7.18 ± 0.03	26.7 ± 0.2	1215 ± 22	1.7 ± 0.2	173 ± 10	103 ± 7	122 ± 19
B4	7.22 ± 0.04	26.7 ± 0.2	1147 ± 23	2.2 ± 0.2	173 ± 9	70 ± 13	118 ± 4
B5	7.26 ± 0.04	26.8 ± 0.2	1094 ± 22	3.6 ± 0.3	135 ± 8	64 ± 4	93 ± 3
B6	7.30 ± 0.04	26.7 ± 0.2	1045 ± 23	4.4 ± 0.2	101 ± 7	45 ± 3	73 ± 3
B7	7.63 ± 0.06	26.7 ± 0.3	903 ± 28	9.6 ± 2.0	83 ± 2	30.1 ± 2	61 ± 2

Table 3
Biodiversity of adult mosquitoes in the plant

Immature stage characteristics	Genus	Number of imagoes captured during the whole period of study			Percentage with respect to the total number of imagoes
		Male	Female	Total	
Living attached to <i>Pistia stratiotes</i> roots	<i>Mansonia</i>	3969	5654	9623	54.78
	<i>Coquillettidia</i>	94	107	201	1.14
	<i>Ficalbia</i>	46	57	103	0.59
Free living	<i>Culex</i>	3839	3529	7368	41.94
	<i>Anopheles</i>	0	3	3	0.02
	<i>Aedes</i>	0	0	0	0
	Unidentified ^a	–	–	270	1.53
Total		7948	9350	17568	100

^a Parts essential for identification were lost during collection.

for B2). This high richness resulted in very low concentrations of dissolved oxygen (DO) (0.3 and 0.8 mg/l for B1 and B2, respectively) and high turbidity values (270 and 159 FTU for B1 and B2). Across the system, a significant amelioration of the water quality was noted. Dissolved oxygen, for example, increased to approximately 10 mg/l and SS was around 30 mg/l at the outlet.

3.2. Mosquito biodiversity

A total of 17,568 adult mosquitoes were captured during the investigation period, among which 54.8 and 41.9% belonged, respectively, to the genus *Mansonia* and *Culex* (Table 3). Identification of species both at the immature and mature stages indicated that most of the *Culex* belonged to *C. quinquefasciatus*, *C. decens* and *C. tigripes*, while for *Mansonia* it was both *M. africana* and *M. uniformis*. About 3% of captured imagoes were *Coquillettidia* (especially *C. metallica*), *Ficalbia*, and *Anopheles*. Although this plant represents a breeding ground for mosquito, only 0.02% of the total capture was the malaria vector *Anopheles gambiae*. They were found only in the last pond (B7) in the rainy season (late March to May). Though not found at adult stage, a few *Aedes* larvae were found in pond B1 and B2 only during the month of December 1997.

When considering the immature *Mansonia* attached to *P. stratiotes* roots, we generally noted in the various ponds a gradual increase of their number with respect

to root biomass (RB) (Fig. 3). For the whole plant, this number increased on average from 33 individuals for a macrophyte with RB class of (1–5 g) to 123 individuals for ones with RB class of (20–25 g). A sharp drop to about 60 individuals was observed for *Pistia* with RB > 25 g.

Fifty-four percent of the total number of imagoes captured during the whole period were females, therefore susceptible to creating a great nuisance when searching hosts for blood feeding. This proportion varied within the genera (59, 48, 53 and 55% of female, respectively, for *Mansonia*, *Culex*, *Coquillettidia* and *Ficalbia*). Captured *Anopheles* were all females.

3.3. Spatial distribution of mosquitoes

The spatial distribution of adult mosquitoes in the plant is represented in Fig. 4. The number of imagoes varied significantly between the ponds (Kruskal–Wallis test $H = 152.82$, d.f. = 6, $P < 0.001$). Very few mosquitoes underwent complete metamorphosis in ponds B1 and B7 with, respectively, an average of 11 and 19 imagoes/m² per day in comparison with the other ponds (B2–6) where the average numbers were higher than 47 imagoes/m² per day. *Anopheles*, *Ficalbia* and *Coquillettidia* were captured occasionally.

When comparing the two main species breeding in the plant, most captured mosquitoes were *Culex* in the first ponds (B1–3), while it was *Mansonia* in the later ponds (B4–7). This gives *Culex/Mansonia* imago

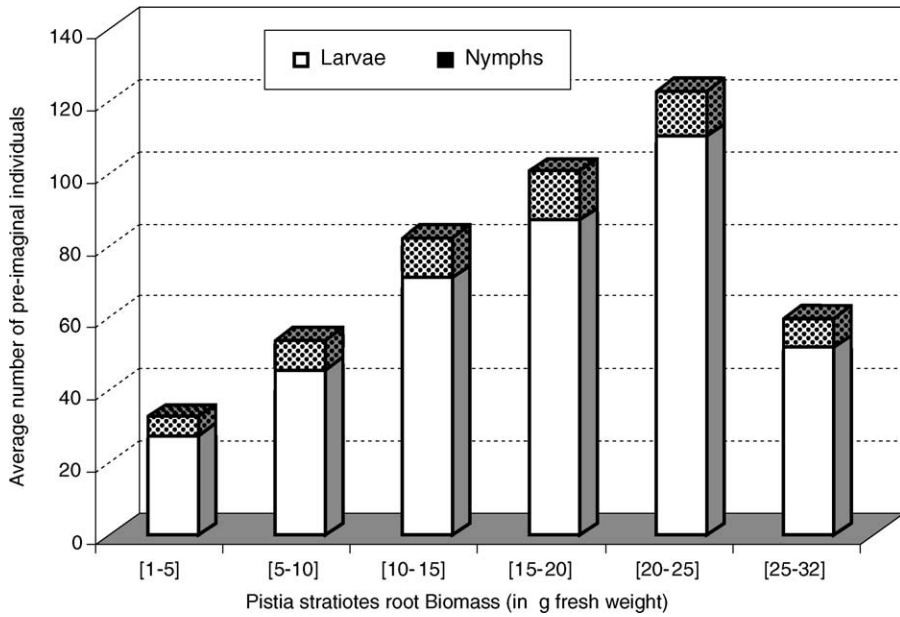


Fig. 3. Distribution of pre-imaginal stages of *Mansonia* with respect to root biomass.

ratios of 10/1, 39/7, 38/21, 22/37, 10/40, 5/41 and 1/16, respectively, in ponds B1–7. These variations tie in with those of their immature stages. Most *Culex* larvae were captured in the first three ponds (B1–3), whereas the number of *Mansonia* larvae found attached to the macrophyte roots was low in the first three ponds and high in the others (Fig. 4). Indeed, the number of immature *Culex* increased up to 30

individuals/dipper in B3, then dropped gradually to less than 10 and 3 larva/dipper in ponds B6 and B7, respectively. For *Mansonia*, the number of individuals found attached to macrophyte roots rather tended to increase from B2 with a mean of 13 individuals to attain a peak in B6 with a mean of 84 individuals/macrophyte even if a slight drop was noted in pond B7 (65 individuals/macrophyte).

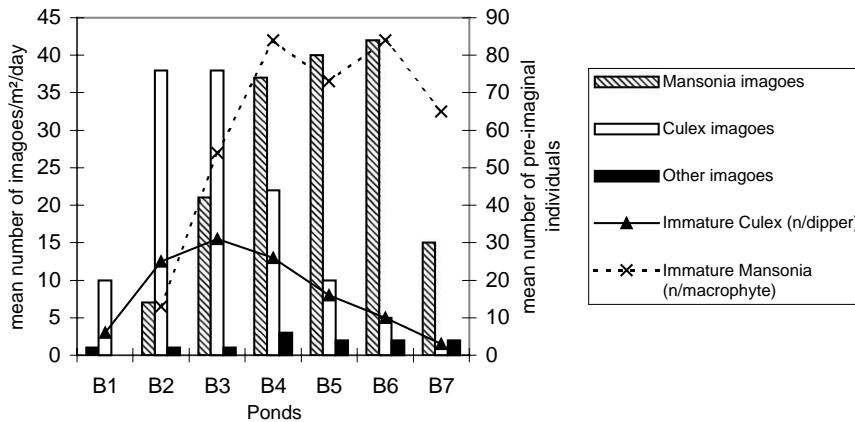


Fig. 4. Spatial distribution of the mosquitoes (others: *Ficalbia* + *Coquillettidia* + *Anopheles* + unidentified mosquitoes).

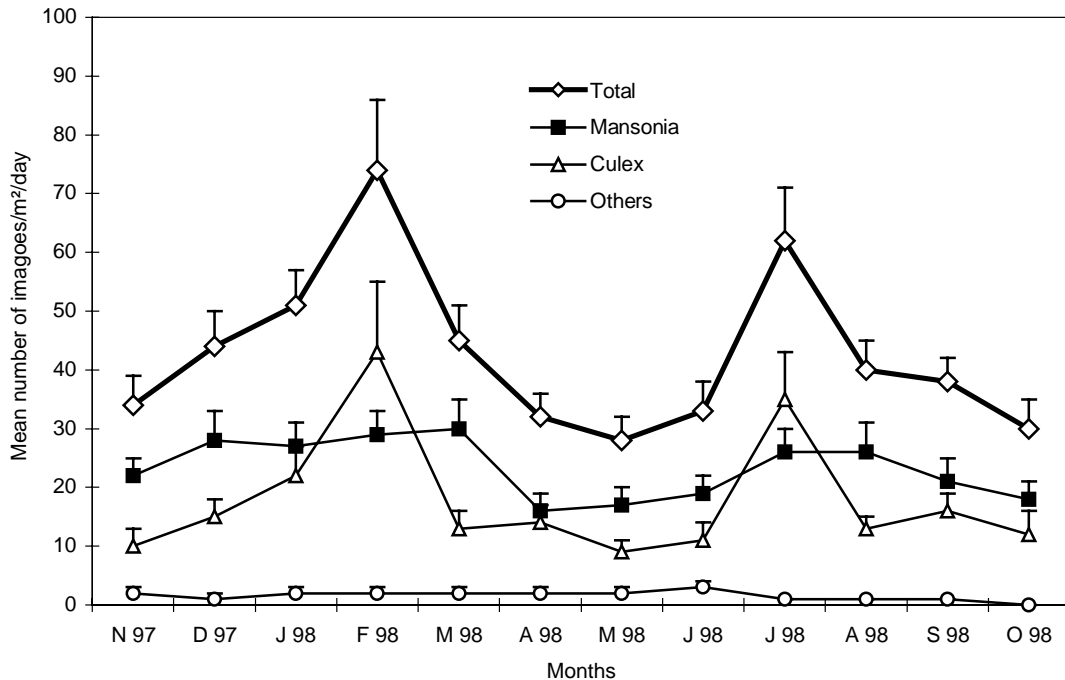


Fig. 5. Seasonal dynamics of mosquito imagoes in the whole plant (others: *Ficalbia* + *Coquillettia* + *Anopheles* + unidentified mosquitoes).

3.4. Seasonal dynamics

The study of the seasonal dynamics of mosquitoes breeding in the plant (Fig. 5) permitted a distinction of two periods of high breeding all during the dry seasons, one during the month of February and the other during the month of July (74 and 62 imagoes/m² per day, respectively). This evolution was attributed mainly to *Culex* ($H = 35.39$, d.f. = 11, $P < 0.001$), with a production higher than 40 and 30 imagoes/m² per day, respectively, for the 2 months. For *Mansonia*, the number of imagoes captured during the different months did not show any statistical difference ($H = 12.11$, d.f. = 11, $P = 0.356$), even if a slight decrease of their number was observed from May to July 1998 with less than 20 imagoes/m² per day.

4. Discussions and conclusion

As any other standing pool, this *Pistia*-based treatment plant represents a favorable breeding ground for

mosquitoes. Taking into consideration the whole plant, approximately 43 mosquitoes emerged on an average basis per square meter per day, mainly *Mansonia* and *Culex*. Luckily, bearing in mind the endemic malaria situation of the region (Manga et al., 1992), this plant can be considered as not favoring the development of the malaria vector *A. gambiae*, since it was found only accidentally and in small numbers. This rareness of *Anopheles* may be due to the thick mat formed by *P. stratiotes* over the ponds and the poor water quality. This species has been reported to breed generally in clean and sunny water (Goma, 1982).

The dynamics of the two main species are certainly influenced by the nature of the macrophytes used and the water quality in the ponds. Indeed, *Mansonia* larvae, though found in water with other aquatic macrophytes such as water hyacinth (Gopal, 1987), are generally associated with the presence of water lettuce (Holstein, 1949). They live generally attached to the macrophyte roots by means of their siphon (for larva) or trumpet (for nymph), which are modified respiratory apparatuses through which they are able

to draw O₂ directly from the lacunal spaces in the root tissues (Goma, 1982). The increase of *Pistia* root biomass with the stream (data not reported), therefore, provides a greater space for the attachment of even a greater number of pre-imaginal mosquitoes. Up to 300 larvae have been counted in single macrophyte with a RB equal to 20 g in pond B6. The sharp drop of the average number of larvae when RB > 25 g could be explained by the fact that these macrophytes were usually too old, and having stayed long in the pond, the roots were overloaded with organic debris and other particles which probably disturbed the normal fixation of larvae or nymphs to the roots. Furthermore, the movement of oxygen from the leaves to the roots is greatly reduced in such old aquatic plants (Moorhead and Reddy, 1988).

When considering the water quality, wastewater from the first ponds (B1–3) contained high concentrations of organic and particulate matters, which rather favor the complete metamorphosis of *Culex* from larvae to adults by hindering the colonization of predators. This also probably reduced the survival of *Mansonia* larvae, as they do not generally withstand highly polluted environments. In the downstream ponds (B4–7), the water quality gradually improved, allowing a better development of the *Pistia* root system on which immature *Mansonia* attached. A large number of predators also colonize these ponds, but contrarily to free-living mosquito larvae such as *Culex*, immature *Mansonia* easily hide themselves in the abundant root system. Brix (1997) and Russell (1999) reported this dissimulation of mosquito larvae as one of the drawbacks generally linked with the presence of macrophytes in constructed wetlands.

The small population of mosquitoes in pond B1 was probably due to the excess of sludge in this pond, which even hindered the development of *Culex* though reputed as the ‘most dirty mosquito’ by Holstein (1949) while in pond B7, it was the experimental cultivation of the mosquito-fish *Gambusia* sp.

The seasonal variations observed could be due to the fact that most mosquitoes oviposited during the dry seasons. Indeed, most temporary pools dried up, leaving the treatment ponds as one of the major shelters in the area. Rickenbach (1981) also mentioned that high temperatures generally encountered during these periods increase the rate of hatching of mosquito eggs and reduce their aquatic life cycle.

These results highlight the dilemma of sanitary engineers and local public health authorities that have to treat water at low-cost while reducing the nuisance pest. Research needs to be directed mainly at determining the following:

- The appropriateness of using floating macrophytes for wastewater treatment, especially *P. stratiotes* since it favors the proliferation of *Mansonia*.
- The use of biological agents such as the mosquito fish *Gambusia* sp.; successful trials of their acclimatization in most of the ponds of this wastewater treatment plant and the low level of mosquito in pond B7 where they were present during the whole period of study could be viewed as promising. Indeed, except in ponds B1 and B2 where the high level of pollution does not permit their survival, the acclimatization rates of this fish varies from 70 to 100% in the other macrophytic ponds (Kengne Noumsi, 2000). Today this fish is present in most African rivers and could be efficiently domesticated for the purpose.
- The association of different treatment techniques: a hybrid system combining lagoons with subsurface flow wetlands, which are recognized as minimizing mosquito development, should be examined.

Acknowledgements

This work was supported by the International Foundation for Science, IFS (grant no. 1580/3F), and the French Ministry of Foreign Affairs (Programme CAMPUS, grant no. 94 016 400). Special thanks to Clement GOUAGNA and Jean Yves Meunier, researchers at OCEAC, for their contribution in the determination of mosquito species.

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