# DISTRIBUTION OF MOSQUITOES IN DIFFERENT WASTEWATER STAGES OF SECONDARILY TREATED DOMESTIC EFFLUENT AND UNTREATED CITRUS WASHWATER

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ABSTRACT. In Indian River County, Florida, mosquito populations were monitored weekly for 12 months in early and more advanced treatment stages of secondarily treated domestic wastewater and also in untreated primary and secondary citrus packing house washwater. Well defined differences in mosquito species abundances for early vs. late wastewater stages existed. In the early levels of secondarily treated effluent and in the primary citrus washwater, *Culex quinquefasciatus* populations were dominant. In the later wastewater stages of both systems, *Cx. nigripalpus*, *Anopheles* spp. and *Uranotaenia* spp. were more common. Seasonal *Culex* spp. abundance patterns were apparent at some but not all study sites. Water chemistry measurements demonstrated that both systems contained relatively low levels of nutrient-related parameters with large concentration variability.

## INTRODUCTION

In Indian River County (IRC), Florida, wastewater holding ponds receive: 1) secondarily treated effluent from activated sludge treatment plants and 2) untreated washwater from citrus packing houses.

Numerous mosquito species, especially *Culex* spp., inhabit some of these wastewater retention areas (Carlson 1982, Carlson 1983). O'Meara and Evans (1983) showed that a dairy barn lagoon system receiving nutrient-rich untreated wastewater was a prolific producer of *Cx. nigripalpus* Theobald and *Cx. quinquefasciatus* Say, and that seasonal switches in dominance between these species occurred. Carlson (1982) demonstrated that secondarily treated wastewater can also produce high *Culex* densities and also showed seasonal abundance patterns among *Culex* spp.

The 12-month study reported in this paper was conducted in IRC to quantify mosquito population differences between wastewater stages of both secondarily treated domestic effluent and citrus packing house washwater.

## MATERIALS AND METHODS

STUDY AREAS. LAMPLIGHTER MOBILE HOME PARK WASTEWATER SYSTEM (Fig. 1). The activated sludge wastewater treatment system servicing this park had a capacity of approximately 15,000 liters/day. Three sites within this study were examined.

a. The concrete aerobic digester tank (L1) measured 2.5 x 3 m.

b. One holding pond received secondarily treated effluent directly from the treatment plant (L2). It measured 15 x 34 m and filled to a depth of approximately 20 cm at peak capacity. Dense floating and emergent vegetation present included *Baccharis halimifolia* L. (saltbush), *Hydrocotyle umbellata* L. (water pennywort), *Ludwigia peruviana* (L.) Hara (peruvian primrose), *Panicum repens* L. (torpedo grass), and *P. maximum* Jacq. (guinea grass).

c. The second holding pond (L3) received overflow from L2 through a connecting pipe when water levels were high. Pond L3 measured 18 x 36 m and maintained a relatively constant depth of approx. 200 cm. No floating, but only emergent vegetation was present along its banks. The most common plants were *Cyperus odoratus* L. (flat sedge), *Pontederia lanceolatea* Nutt. (pickerelweed), *Panicum maximum*, *P. purpurascens* Raddi (para-grass) and *Salix floridana* Chapm. (a willow). *Gambusia affinis* Baird and Girard (mosquito-fish) were present but not observed in the densely vegetated, shallow areas where sampling occurred.

LEROY SMITH PACKING HOUSE WASHWATER SYSTEM (Fig. 1). Untreated washwater flowed from this citrus packing house into two holding ponds.

a. One pond (LS1), which measured 9.5 x 23 m with a 27 cm maximum depth, received discharge from the primary (soaking) stage of the fruit wash process. The most common emergent vegetation densely located near the banks included *Panicum purpurascens*, *P. maximum*, *Mikania cordifolia* (L.) Wild. (climbing hempweed) and *Bidens pilosa*.

b. Another holding pond (LS2) received water from the second wash stage. LS2 measured 10 x 13.5 m and filled to a depth of 30.5 cm. Dense emergent vegetation grew along the banks. The most common plants were *Panicum purpurascens*, *Ludwigia peruviana*, *Rumex verticillatus* L. (water dock) and *Colocasia esculentum* (L.) Schott. (elephant ears).

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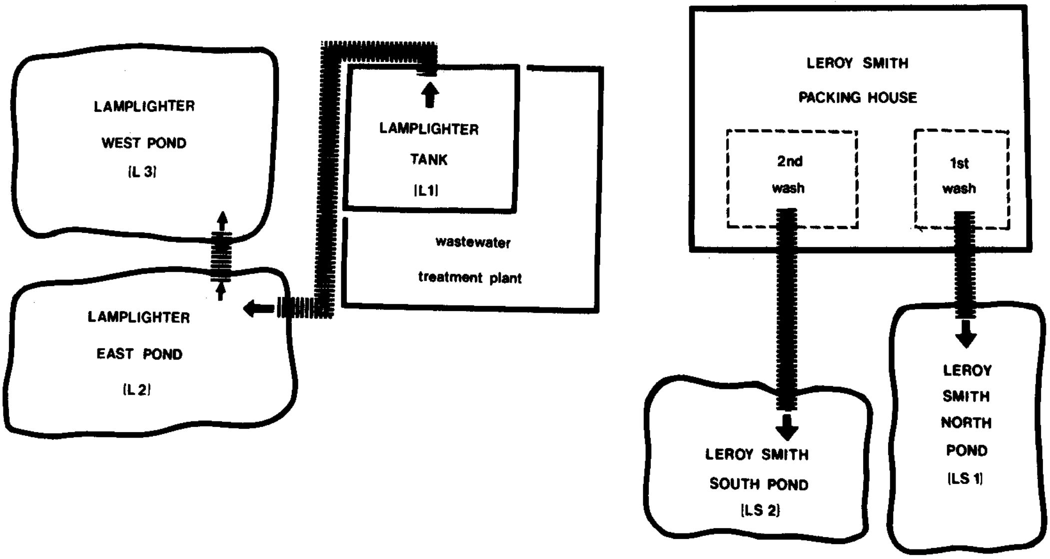


Fig. 1. Diagram of water flow in the two wastewater study areas.

SAMPLING. MOSQUITOES. In each study site (holding pond or tank), three permanent sampling stations were established widely dispersed over the water body. Sampling for immature mosquitoes (larva or pupa) was conducted once a week with a 350 ml dipper with a 1 m handle from the week of February 15, 1982 to the week of Feb. 7, 1983. In the holding ponds, 2 dips were taken at each station for a total of 6 samples per pond per week. In Lamplighter Tank (L1), 1 dip was taken per station per week, for a total of 3 dips. Larvae and pupae were returned to the laboratory where they were identified and counted. When agitating (part of its normal operation), L1 was not sampled for mosquitoes.

WATER CHEMISTRY. To establish a nutrient-related water chemistry profile of the study sites, surface water samples from each study site were collected once each week in the field at approximately the midpoint between the wastewater entry point and the distant-most point from the discharge pipe. Samples were returned to the laboratory and refrigerated. Analysis for nitrate ($NO_3$), nitrite ($NO_2$), orthophosphate ($PO_4$), ammonia nitrogen ($NH_3$-N), total Kjeldahl nitrogen (TKN) and acidity (pH) was completed within several days. Nitrates were measured using the Cadmium reduction method, nitrites and orthophosphate determined by the colorimetric method, total Kjeldahl nitrogen by the Kjeldahl method and ammonia nitrogen by the titration method; all described by A.P.H.A (1981). Acidity was determined using a Corning electronic pH meter (Model 610A).

## RESULTS

MOSQUITO SURVEY. GENERAL. Overall, 8 mosquito species, for a total of 67,891 individuals, were collected. They were: *Cx. quinquefasciatus* (71.9%), *Cx. nigripalpus* (25.0%), *Uranotaenia lowii* Theobald (2.0%), *Cx. salinarius* Coquillett (0.7%), *Anopheles crucians* Wiedemann (0.2%), *Ur. sapphirina* (Osten Sacken) (<0.1%), *Aedes taeniorhynchus* (Wiedemann) (<0.1%) *An. quadrimaculatus* Say (<0.1%) (Table 1).

LAMPLIGHTER MOBILE HOME PARK SECONDARILY TREATED WASTEWATER SYSTEM. LAMPLIGHTER TANK (L1). *Culex quinquefasciatus* was exclusively collected in this aerobic digester except for one visit (early August 1982) when two *Cx. nigripalpus* larvae were found. *Culex quinquefasciatus* were common except from mid-August to late November when the water was agitating (Fig. 2).

LAMPLIGHTER EAST (L2). Table 1 lists the seven mosquito species found in this first holding pond and their relative abundances. Figure 3 shows that *Cx. nigripalpus* were common and maintained steady densities throughout the study period. *Culex quinquefasciatus* individuals were collected from early in the study until late June, after which they were no longer encountered. Other mosquito species were found throughout the year.

LAMPLIGHTER WEST (L3). This pond, which received some overflow from L2, remained constantly flooded. Table 1 lists the six mosquito species present. Figure 4 shows that *Cx. nigripalpus* were significantly greater from

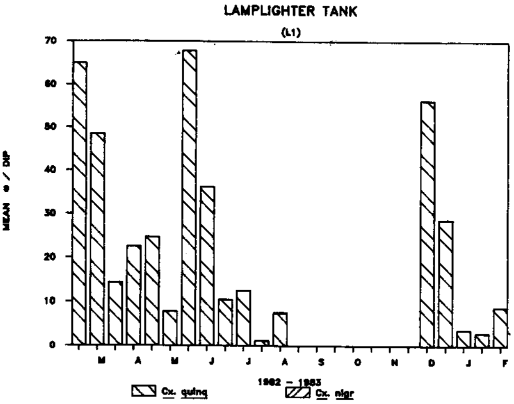


Fig. 2. Mosquito populations in the Lamplighter Tank study site (L1).

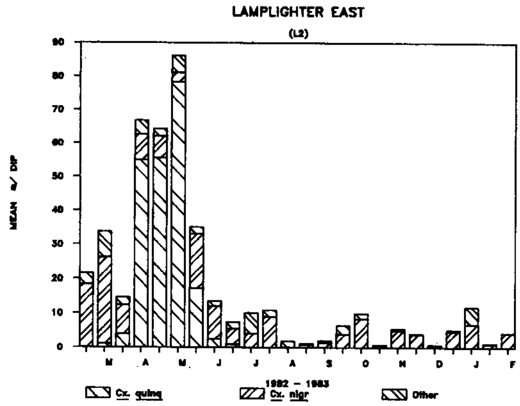


Fig. 3. Mosquito populations in the Lamplighter East study site (L2).

mid-April through the end of December ($t$ = 3.66, df = 50, p <0.001) as compared to the remainder of the study period. In contrast to L1, *Cx. quinquefasciatus* were rarely found here, and present in low densities in early 1982. Low numbers of *Anopheles crucians*, *An. quadrimaculatus*, *Cx. salinarius*, and *Ur. lowii* were collected throughout the year.

COMBINED LAMPLIGHTER RESULTS. (L1, L2, L3). These results show that at this study area, essentially only *Cx. quinquefasciatus* were collected in the early treatment level (L1) while in the later treatment stages (L2 and L3), *Cx. nigripalpus*, *Cx. salinarius*, *An. crucians* and *Ur. lowii* were more common (Table 1). From early March to the end of June, *Cx. quinquefasciatus* populations were significantly greater in L1 than L2 ($t$ = 2.85, df = 34, p <0.01) and L3 ($t$ = 5.83, df = 34, p <0.001). Also, during this same time period, *Cx. quinquefasciatus* populations were significantly greater at L2 than at L3 ($t$ = 3.01, df = 34, p <0.01). Similarly, *Cx. nigripalpus* was greater in L3 over L2 from early June to late December ($t$ = 4.07, df = 60, p <0.001).

LEROY SMITH CITRUS PACKING HOUSE WASHWATER SYSTEM. LEROY SMITH NORTH (LS1). *Culex quinquefasciatus* occurred in this primary washwater in a normal seasonal pattern: abundant in the spring, disappearing in late July and reappearing in October. *Culex nigripalpus* were found in the spring and infrequently collected until early winter (Fig. 5). Four species were collected at this site with *Cx. salinarius* and *Ur. lowii* found in low numbers.

*Culex quinquefasciatus* populations were significantly reduced during the period from mid-July to mid-November as compared to the remainder of the study period ($t$ = 3.71, df = 50, p <0.001).

LEROY SMITH SOUTH (LS2). This holding pond, which received water from the second wash stage, exhibited greater species diversity

Table 1. Mosquito production (x̄/dip) in two wastewater study areas in Indian River County, Florida, February 1982–February 1983.

| | Lamplighter secondarily treated wastewater system | | | Leroy Smith citrus packing house washwater system | |
|---|---|---|---|---|---|
| Species | Tank (L1) | East (L2) | West (L3) | North (LS1) | South (LS2) |
| *Cx. quinquefasciatus* | 18.3 | 8.4 | 0.6 | 117.4 | 11.8 |
| *Cx. nigripalpus* | <0.1 | 6.8 | 11.3 | 10.2 | 26.2 |
| *Cx. salinarius* | 0.0 | 0.8 | 0.1 | <0.1 | 0.6 |
| *Ur. lowii* | 0.0 | 0.9 | 1.5 | 0.3 | 1.8 |
| *Ur. sapphirina* | 0.0 | <0.1 | 0.0 | 0.0 | <0.1 |
| *An. crucians* | 0.0 | 0.4 | 0.2 | 0.0 | <0.1 |
| *An. quadrimaculatus* | 0.0 | <0.1 | <0.1 | 0.0 | <0.1 |
| *Ae. taeniorhynchus* | 0.0 | 0.0 | 0.0 | 0.0 | <0.1 |
| Total | 18.3 | 17.3 | 13.7 | 127.9 | 40.4 |

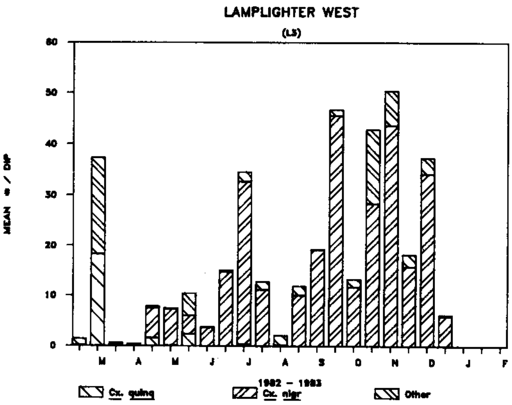


Fig. 4. Mosquito populations in the Lamplighter West study site (L3).

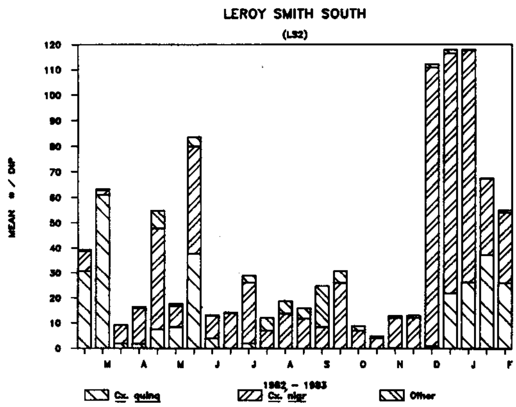


Fig. 6. Mosquito populations in the LeRoy Smith South study site (LS2).

than LS1 (Table 1). *Culex quinquefasciatus* followed the seasonal pattern of being common in the winter and spring and not present in the summer. However, *Cx. nigripalpus* was common throughout the study reaching peak numbers in December (Fig. 6).

*Culex quinquefasciatus* populations were significantly reduced from mid-June to late November as compared to the remainder of the study period ($t$ = 3.02, df = 50, p <0.01).

COMBINED LEROY SMITH RESULTS (LS1, LS2). These results show that overall in this system, *Cx. quinquefasciatus* were much more common in LS1, the pond receiving the primary washwater (Table 1). Conversely, *Cx. nigripalpus* were more common in LS2 which received effluent from the second wash stage.

From early February to mid-July *Cx. quinquefasciatus* were very common at both sites but significantly greater at LS1 ($t$ = 5.13, df = 42, p <0.001). From early November to the end of the study, *Cx. nigripalpus* peaked and were significantly greater at LS2 ($t$ = 3.30, df = 28, p <0.01).

WATER CHEMISTRY SURVEY. Results of water chemistry analysis for all study sites are summarized in Table 2. It is important to note that the Lamplighter and LeRoy Smith wastewater systems did not contain the extremely high organic nutrient levels measured in mosquito abundance studies in swine and poultry waste lagoons in North Carolina (Rutz et al. 1980) or dairy barn wastewater in central Florida (O'Meara and Evans 1983).

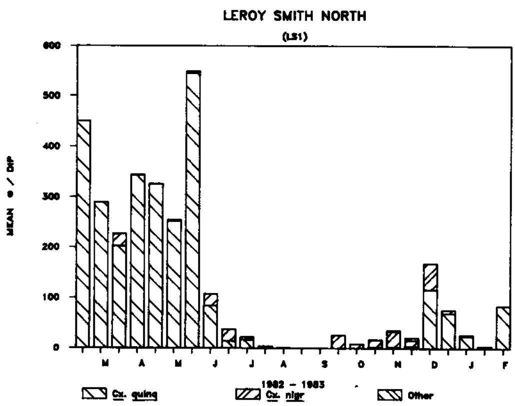


Fig. 5. Mosquito populations in the LeRoy Smith North study site (LS1).

## DISCUSSION

MOSQUITO DISTRIBUTION. Gross larval mosquito abundance preferences for different wastewater sources have been reported in the literature. Provost (1969) stated that *Cx. quinquefasciatus* favors more polluted water than does *Cx. nigripalpus*. Although often found in wastewater habitats, *Anopheles* and *Uranotaenia* spp. generally prefer ponds and lakes (Carpenter et al. 1946). However, factors other than water conditions, in particular nearby mosquito sources, or localized meteorological conditions, can greatly influence the mosquito complex of a wastewater retention area with respect to both species composition and preadult densities thus accounting for mosquito differences between study areas.

Sampling sequential wastewater stages within both the Lamplighter and LeRoy Smith study areas has better defined patterns of mosquito abundance by wastewater-inhabiting mosquitoes between water from different but adjacent treatment levels. *Culex quinquefasciatus* were most common in the early wastewater stages of secondarily treated wastewater (L1) and citrus washwater (LS1) while in later wastewater stages (L2, L3, LS2), *Cx. nigripalpus*, *Cx.*

Table 2. Water quality at two wastewater study areas in Indian River County, Florida ($\bar{x}$±S D).[1]

| | Lamplighter secondarily treated wastewater system | | | Leroy Smith citrus packing house washwater system | |
|---|---|---|---|---|---|
| Parameter | Tank (L1) | East (L2) | West (L3) | North (LS1) | South (LS2) |
| $NO_2$ | 0.25±0.18 | 0.32±0.41 | 0.45±0.52 | 0.15±0.16 | 0.15±0.15 |
| $NO_3$ | 2.51±6.34 | 2.50±3.38 | 2.17±2.84 | 0.28±0.19 | 0.32±0.51 |
| $NH_3$ | 8.86±17.45 | 4.19±9.28 | 2.14±4.46 | 1.01±2.49 | 0.42±0.71 |
| TKN | 12.84±19.92 | 4.49±9.28 | 2.77±5.00 | 1.57±2.62 | 0.92±1.39 |
| $PO_4$ | 1.12±1.00 | 1.07±1.45 | 1.26±1.69 | 1.01±1.38 | 0.91±1.08 |
| pH | 7.63±0.49 | 7.48±0.33 | 7.59±0.55 | 7.23±1.09 | 7.48±0.37 |

[1] All values expressed in parts per million (ppm) except pH. N = 52 for all parameters at all study sites except L1, where N = 51.

*salinarius*, *An. crucians* and *Ur. lowii* were most common (Table 1).

SEASONALITY. The typical south Florida *Culex* abundance pattern is that *Cx. quinquefasciatus* are most common in the winter and spring with *Cx. nigripalpus* dominant in the summer and fall (Provost 1969). This seasonal pattern was vividly observed at LS1 and LS2, to a less distinct degree at L2 and L3, but not occurring at all at L1, where *Cx. quinquefasciatus* were exclusively present year-round. The seasonal pattern observed at most of the study sites follows O'Meara and Evans (1983) findings in a south Florida dairy barn lagoon system. They also showed that seasonal *Culex* abundance patterns were apparent.

OTHER OBSERVATIONS. Rutz et al. (1980) reported positive associations between *Cx. quinquefasciatus* and "intermediate pollution levels" of the swine and poultry waste lagoons they investigated. However, "intermediate pollution levels" as defined by their study (TKN = 175 ppm) are far in excess of the values measured in the two wastewater systems we investigated (Table 2).

In contrast, the water sources we investigated were characterized by low nutrient-related concentrations along with high variability. These conditions make attempts to associate water chemistry and mosquito abundances problematic. However, even though concentration variability was large, it is noteworthy that mean values for $NH_3$ and TKN were higher in earlier treatment stages where *Cx. quinquefasciatus* was the dominant mosquito species. This finding is similar to that of Hagstrum and Gunstream (1971) who associated high concentrations of $NH_3$-N and organic nitrogen with *Cx. quinquefasciatus*, and also Sinha (1976) who associated oviposition by this species with ammonia-rich water. Laboratory studies have indicated that the presence of certain amino acids, proteins or bacterial metabolites can positively influence *Culex* ovipositional behavior (Ikeshoji et al. 1967, Kramer and Mulla 1979). Further studies to enable field identification of suitable preadult mosquito habitats should focus on the relationship in the field of microorganisms with mosquito occurrence.

All of the study sites except L1 were water bodies with large areas of dense vegetation in close proximity to open water areas. The presence of vegetation can enhance mosquito production not only by making the pond more attractive ovipositionally (Rutz and Axtell 1978), but also by denying larvivorous fish access to immature mosquitoes.

Bay (1967) reported that *Gambusia affinis* were ineffective predators in larval habitats that were heavily vegetated. This inability of fish to control mosquito larvae was observed at L3 where *G. affinis* and immature mosquitoes occurred simultaneously. In order to provide mosquito control benefits through fish predation adequate to eliminate larviciding, larvivorous fish must be maintained in sufficiently high numbers and have free access to larvae, a condition frequently not occurring in nature (Todd and Giglioli 1983, Carlson and Vigliano 1985).

## SUMMARY

Well defined mosquito species abundance patterns in early versus late wastewater stages existed in both secondarily treated wastewater and untreated citrus washwater. *Culex quinquefasciatus* populations were most common in early treatment levels with *Cx. nigripalpus*, followed by *Uranotaenia* and *Anopheles* spp. more commonly occurring in later treatment levels. Seasonal abundance patterns occurred in some but not all study sites.

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