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OVIPOSITIONAL RESPONSE OF *CULEX QUINQUEFASCIATUS* TO SOUTHEAST FLORIDA WASTEWATER

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ABSTRACT. The ovipositional response of *Culex quinquefasciatus* to water from 2 areas in a salt marsh impoundment receiving wastewater, holding pond water and distilled water was tested in a large screened outdoor enclosure. Gravid females did not significantly prefer water from the wastewater discharge point into an impoundment to water from a holding pond. They did prefer these 2 sources over both water taken 600 m into that impoundment and to distilled water. Nitrate or orthophosphate levels alone were not sufficient to determine relative ovipositional preference to these water sources.

INTRODUCTION

Since 1976, a 2 cell, 52 acre salt-marsh mosquito control impoundment system has provided tertiary treatment (i.e. nutrient removal) to secondarily treated nutrient-rich wastewater in Indian River County (IRC), Florida. This method of wastewater discharge is an economically attractive option to developers when large volumes of water are treated near impounded salt marshes. Impoundments used for wastewater retention can also benefit mosquito control districts because the need to pump water onto these areas can be reduced or eliminated (Carlson 1983).

Although some holding ponds (evaporation-percolation ponds) produce *Culex quinquefasciatus* Say and *Cx. nigripalpus* Theobald in large numbers in IRC (Carlson 1982), few mosquitoes were found in a 12-month survey in an impoundment system receiving secondarily treated wastewater from an activated sludge wastewater treatment plant (Carlson 1983). Smith and Enns (1967) demonstrated that mosquito production in wastewater ponds depends on numerous factors including types of wastes, dissolved oxygen content, available food and the presence of vegetation. In IRC, *Cx. quinquefasciatus* have been found in numerous habitats, including some devoid of vegetation but with heavy nutrient loads. Because various physical and chemical factors influence *Culex* oviposition, and since the use of impoundments for wastewater retention is increasing, an experiment was conducted to determine if impoundment water can be attractive to gravid *Cx. quinquefasciatus*. This paper describes a study which examined the ovipositional response of *Cx. quinquefasciatus* to water from 2 areas in a salt marsh impoundment system receiving wastewater, a holding pond and distilled water.

MATERIALS AND METHODS

Three replicates of an experiment designed to assess the ovipositional preference of *Cx. quinquefasciatus* to 4 water sources were conducted between February and May 1981 when *Cx. quinquefasciatus* is most common in south Florida wastewater ponds (Carlson 1982, O'Meara and Evans 1983). Egg rafts were field-collected in IRC, allowed to hatch and larvae were reared at 27°C, 85% RH and LD 12:12. Adults emerged in cages (37 × 64 × 50 cm) maintained at the same conditions and were supplied a 10% sugar solution.

Four days after emergence, 400 females were blood-fed on chickens. Another 400 were blood-fed on day 6. On the 6th and 7th day after blood-feeding, approximately 200 gravid females from each group of 400 (800 individuals) were released into a large, screened cage (2.3 × 3.7 × 2.6 m, volume = 22.1 m^3) located in a screened outdoor enclosure. This allowed mosquitoes to be constantly exposed to natural weather conditions. In each replicate, mosquitoes were added over 4 days to extend oviposition over several days. Oviposition occurred over as many as 12 days. Mosquitoes in the outdoor cage were supplied water but not sugar.

Water was collected daily from 4 sources and put into 16 black plastic containers each with a surface area of 100 cm^2 and placed in a 4 × 4 Latin square measuring 70 × 70 cm, similar to the method used by Suleman and Shirin (1981). Each container received 300 ml of water from one of the following 4 sources:

1) The point of entry of secondarily treated wastewater into a 2 cell, 52 acre salt-marsh mosquito control impoundment system in IRC (Imp 1),
2) Approximately 600 m from the point of wastewater discharge into that same 52 acre impoundment system (Imp 2),
3) A holding pond receiving discharge from the activated sludge wastewater treatment plant at Clemans Elementary School in IRC (HP),
4) Distilled water (DW).

Containers were examined daily for 12 days for egg rafts which were counted and removed.

RESULTS AND DISCUSSION

Table 1 reports ovipositional preference totals for each replication. The totals reveal that water from the school holding pond (HP) attracted the largest number of ovipositing mosquitoes (52.6%), followed by the wastewater entry point into the impoundment (Imp 1) (30.9%), 600 m into the impoundment sysem (Imp 2) (11.3%) and distilled water (DW) (5.2%).

Significant differences in ovipositional attractiveness among water sources was demonstrated by comparing egg raft subtotals using the Kruskal-Wallis test ($H' = 30.8$, df = 3, $p < 0.001$). The Kruskal-Wallis multiple comparison technique showed a significant ovipositional preference for HP compared with Imp 2 and DW ($p<0.05$). Likewise, Imp 1 was significantly preferred ($p<0.05$) over Imp 2 and DW. There was no significant ovipositional preference between sites HP and Imp 1 or Imp 2 and DW (Hollander and Wolfe 1973). To summarize, HP = Imp 1 > Imp 2 = DW. These observations of oviposition in water from HP and Imp 1 differed from concurrent field observations on the occurrence of preimaginal mosquitoes. Field sampling of immature *Cx. quinquefasciatus* individuals at Clemans School holding pond during the period that this oviposition study was in progress showed a $\bar{x} = 36.7$ per 350 ml dip (Carlson 1982). Weekly sampling for immature mosquitoes during that same period averaged <0.3 per dip at Imp. 1. No larvae or pupae were collected at Imp 2 (Carlson 1983).

Nutrient-rich wastewater provides ovipositing females with an immature habitat containing an adequate larval food supply. Oviposition by *Culex* mosquitoes can be enhanced by high concentrations of chemical stimulii such as organic nitrogen, ammonia nitrogen, nitrates and organic carbon or a slightly alkaline pH (Hagstrum and Gunstream 1971, Sinha 1976, Kaul et al. 1977). Studies have also shown proteins, the metabolites of certain bacteria and the presence of fatty acids can influence ovipositional behavior (Ikeshoji et al. 1967, Hwang et al. 1982). These influencing factors can be common in wastewater such as the secondarily treated wastewater sources used in this study.

There can be significant daily wastewater chemistry concentration fluctuations. However, a water quality profile of the sites compiled over several years is useful in comparing these different wastewater locations. Water quality measurements taken between June 1980 and February 1981 at Clemans School holding pond showed a mean nitrate concentration of 6.8 parts per million (ppm) (range = 0–34.7 ppm) and a mean pH of 7.1 (range = 6.8–7.4) (Carlson 1982). The mean orthophosphate level in early 1982 measured 1.7 ppm (range = 0.5–0.8 ppm).

Nitrate ($\bar{x} = 2.0$, range = 0.8–3.5 ppm) and orthophosphate levels ($\bar{x} = 2.5$, range = 1.1–3.8 ppm) taken between June 1981 and April 1982 at Imp 1 were significantly higher than at 7 other salt-marsh mosquito control im-

Table 1. *Culex quinquefasciatus* egg raft distribution totals from 4 water sources in Indian River County, Florida.

Replication	Wastewater entry point into salt marsh impoundment (Imp 1)	600 m from wastewater entry point into impoundment (Imp 2)	Holding pond (HP)	Distilled water (DW)	Total
1	63	13	53	6	135
2	61	10	200	3	274
3	105	61	137	30	333
Total	229[a](30.9%)	84[b](11.3%)	390[a](52.6%)	39[b](5.2%)	742

[a,b]—different letters indicate a significant difference at the 0.05 level.

poundments not receiving wastewater studied in IRC. Mean pH measured 7.8 (range = 7.5–8.3). At Imp 2, orthophosphate levels were still significantly higher than the 7 other impoundments examined ($\bar{x}$ = 2.5, range = 1.1–7.0 ppm) in that study but nitrate levels did not differ significantly. Mean pH measured 7.7 (range = 7.5–8.3) (Carlson 1983).

Analysis of variance conducted on nitrate and orthophosphate levels between HP, Imp 1 and Imp 2 revealed a significant difference only in nitrates between HP and Imp 2 ($F_{[1,8]\ 0.05}$ = 9.51). The present study showed that HP is significantly higher than Imp 2 in egg raft totals and nitrate levels. Egg raft totals at Imp 1 were significantly greater than at Imp 2 but nitrate levels did not differ significantly. Nitrate or orthophosphate levels alone were not sufficient to explain the ovipositional attractancy of these water sources.

The pH differed significantly between HP and Imp 1 ($F_{[1,24]\ 0.01}$ = 75.91) and between HP and Imp 2 ($F_{[1,24]\ 0.01}$ = 113.95). Even though they differ, the mean pH values for all 3 locations can be considered slightly alkaline, a condition favorable for the presence of high numbers of *Cx. quinquefasciatus* individuals (Sinha 1976).

Laboratory studies by Suleman and Shirin (1981) demonstrated an ovipositional preference by *Cx. quinquefasciatus* for water in which larvae and pupae of the same species developed. This behavior corresponds with laboratory observations of Andreadis (1977) that gravid *Cx. salinarius* Coquillett prefer oviposition water in which pupae have developed. The preference for rearing water, which Andreadis attributed to an oviposition attractant of pupal origin, could have been a contributing factor for Clemans School holding pond water being the preferred ovipositional medium in this experiment.

CONCLUSIONS

In this large, outdoor cage, water from the point of wastewater discharge into a salt-marsh mosquito control impoundment was the preferred medium for over 30% of the *Cx. quinquefasciatus* egg rafts deposited even though this site contained few mosquitoes in the field. Nitrate and orthophosphate levels and oviposition in this water were not significantly different as compared to water from a holding pond which often contained large numbers of *Culex* mosquitoes.

In the field, the lack of mosquitoes at Imp 1 and Imp 2 was probably due to several factors. Larvivorous fish are present in this impoundment system which may limit preadult mosquito populations. An apparent lack of nearby oviposition sites for *Cx. quinquefasciatus* may also limit their presence. There may possibly be some oviposition sites in the vicinity which are more attractive than the impoundment to ovipositing females. Nevertheless, this experiment indicated that nutrient loads near the discharge point of the salt marsh impoundment studied are adequate to attract gravid *Cx. quinquefasciatus*. Monitoring and quite possibly special management of these areas may be necessary to assure that impoundments used for wastewater retention do not become significant producers of fresh-water mosquitoes.

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A COMPARISON OF THREE MOSQUITO SAMPLING TECHNIQUES IN NORTHWESTERN NEW JERSEY[1,2]

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ABSTRACT. The similarity of 3 mosquito sampling methods was compared in northwestern New Jersey for 2 summers. The collecting techniques were the standard New Jersey light trap, dry ice baited CDC miniature light trap and human bite count. The data indicated that the dry ice baited CDC trap closely agreed with the mosquito collections made with the bite count. Collections from the standard light trap, however, did not accurately reflect the nuisance mosquito populations at the study sites.

INTRODUCTION

The need to accurately assess mosquito population levels has long been a major component of control efforts. Early attempts at measuring mosquito populations consisted of counting, capturing and identifying the species attracted to humans (Smith 1904). Although the technique provided a useful overview of the mosquitoes causing nuisance to the residents of a given area, the results obtained were inherently variable due to differences among the collectors. The development of the New Jersey light trap marked the beginning of efforts to overcome human induced variability by using consistent, mechanical mosquito collecting devices (Headlee 1932).

Although the light trap is currently employed as a standard mosquito sampling device, critics of the trap indicate that the collections do not reflect the true mosquito nuisance levels (Huffaker and Black 1943, Service 1976). Reeves (1951) demonstrated that carbon dioxide might be a major factor in attracting mosquitoes to mechanical traps in proportion to those sampled by landing rates and animal-baited traps. Extensive research since that time has shown that adding dry ice as a source of carbon dioxide will greatly increase the number and variety of mosquito species captured (Newhouse et al. 1966, Morris and DeFoliart 1969, Magnarelli 1975). Frequently, the dry ice supplement is used in conjunction with CDC portable light traps (Sudia and Chamberlain 1962), so that collections may be made without the need for a nearby electrical outlet. The current study was undertaken to quantitatively compare mosquito collections made with 3 techniques that are commonly used to measure mosquito population levels; landing/biting rates, the standard New Jersey light trap and dry ice baited CDC light traps.

MATERIALS AND METHODS

The study was conducted in 4 state parks and forests in northwestern New Jersey. The area is characterized by gently rolling hills, mixed deciduous and pine forests, and limestone, sandstone and shale rock formations. Much of the region has an underlying clay pan, and during the spring, melting snow forms numerous woodland pools that produce sizeable populations of univoltine mosquito species. As the season progresses, the pools dry and reflood, leading to the emergence of several floodwater mosquito species.

A standard New Jersey light trap was operated nightly at each site from early June through August, 1979 and 1980. Collections

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