EFFICACY OF *DUGESIA TIGRINA* (TRICLADIDA: TURBELLARIA) IN REDUCING *CULEX* NUMBERS IN BOTH FIELD AND LABORATORY

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ABSTRACT. The placement of 300 laboratory-reared planarians into each of 5 catch basins in Ontario, Canada during early July produced a significant reduction of 81% in *Culex* larvae in treated basins over that in the non-treated basins from August 23 until September 27, 1979 and an average reduction of 74% for the entire season (July–September). In laboratory tests, *Culex* adults reared from 100 first instar wild larvae in 2 liters of water were reduced by over 90% by the presence of 4 or more planarians, while even with 16 planarians an average of 1.3 adults still emerged. With 80 planarians and 500, 1000 or 2000 first instar larvae in 12 liters of water, emergence of adults was reduced by an average of 80, 89, and 88%, respectively. With 2000 feral larvae, emergence was reduced even in the absence of planarians due to overcrowding.

INTRODUCTION

Experimental field populations of *Culex* larvae were reduced by over 90% in 26 days in California by the planarian flatworm *Dugesia dorotocephala* (Woodsworth) (Legner et al. 1975). Another species, *Dugesia tigrina* (Girard), significantly reduced *Culex* spp. larvae in catch basins in Ontario (George 1978) but failed to control mosquitoes in temporary pools in North Dakota (Meyer and Learned 1981b). Ontario field tests were expanded and repeated in 1979 along with the development of a new means of monitoring the presence of planarians in catch basins. These results are reported here.

The consequence of many variables encountered in field experiments is reflected in large deviations in results. Some deviation is obviously due to variations in the deposition and hatch of feral mosquito eggs. Moreover, although size of planarians has little effect on numbers of mosquitoes taken, both the density and size of mosquito larvae does affect those numbers (Meyer and Learned 1981a). Neither density nor size of mosquito larvae can be maintained at constant levels during predation experiments. Finally, field counts of larvae and pupae were taken before their exposure to predation was ended. A more relevant measurement that circumvents some of these problems is the effect of planarians on the numbers of mosquito adults which develop from known numbers of first instar wild larvae. Such tests were conducted in the laboratory.

Reported here are the results of 3 sets of experiments conducted to obtain additional information on 1) the efficacy of *D. tigrina* in reducing feral *Culex* larvae in catch basins, 2) the efficacy of different numbers of *D. tigrina* in reducing adult emergence from 100 first instar *Culex* larvae, and 3) the efficacy of 80 planarians in reducing the adults that develop from 500, 1000 or 2000 first instar *Culex* larvae.

MATERIAL AND METHODS

A laboratory colony of *Dugesia tigrina*, established from specimens collected from the Thames River, London, Canada was the source of all planarians used in both field and laboratory experiments. In a field experiment, on July 12, 1979 five groups of 300 planarians were placed in each of 5 alternate catch basins not used in previous tests in Ailsa Craig, Ontario. Five catch basins in alternate succession without planarians served as controls. Populations of immature mosquitoes were monitored weekly in all 10 catch basins for 13 weeks (July 5–September 27) by counting the total numbers of larvae and pupae in each of five 300 ml dips from each catch basin. It was observed that planarians tended to collect on the undersides of floating objects such as leaves in catch basins. During late July, a piece of white Styrofoam® 2.5 cm thick and 25 cm square was allowed to float on the water surface in each of the 10 catch basins. Planarians gathered on the undersides of the floats where they were readily recognized and counted weekly from August 2 until September 27 inclusive.

For experiments conducted in the laboratory, first instar feral *Culex* larvae were hatched from egg rafts collected daily from outdoor artificial, sod-lined pools. Each egg raft was placed in a plastic cup containing 100 ml of water. Upon hatching first instar larvae were counted and placed in the appropriate experimental containers. During each of the experiments larvae were provided with appropriate amounts of unmedicated rabbit chow and powdered yeast, finely ground together in a coffee mill, and mixed in a ratio of 8:2 by weight.

In the first laboratory experiment, 31 planarians of about the same size (8 ± 1 mm) were held for 1 week without food and then divided into 5 lots of 1, 2, 4, 8 and 16. Each lot was placed in 2 liters of water in one of five, 25 cm diam enameled basins, together with 100 first instar mosquito larvae per basin. A sixth basin without planarians served as a control. The numbers of adult mosquitoes to emerge from each basin were recorded daily by counting and removing the pupal exuviae. This test was replicated 6 times.

The second laboratory experiment consisted of 80 planarians (5–8 mm) in 12 liters of water in 18 liter plastic garbage containers with 500, 1000 and 2000 first instar larvae, respectively. Duplicate containers without planarians served as controls. Adult emergence was determined daily by exuviae counts. Each treatment with a control was replicated 4 times with the exception of that with 500 larvae which had 2 replicates. Treatments and controls were analyzed and compared with a *t*-test.

RESULTS AND DISCUSSION

CATCH BASINS. From August 23 until September 27, 1979 inclusive, the numbers of mosquito larvae and pupae were significantly lower ($p<0.01$) in dips from catch basins with planarians than in those without (Fig. 1). During this period the overall average number of larvae and pupae per dip was 190.4 ±51 SE in the control basins and only 36.4± 17 SE in catch basins with planarians, a reduction of 81%. From July 12 until September 27 there was an average of 74.6% fewer larvae in the basins with planarians than in those without. This was the second consecutive year that a significant reduction in larvae occurred with planarians in catch basins, the first having occurred on August 28, 1978 (George 1978) and the second on August 23, 1979 (Fig. 1). Large standard deviations resulted from uncontrollable large variations in larvae numbers between both catch basins and dips from the same catch basin. Even with these variations significant differences did occur once in 1978 (George 1978) and for 6 consecutive weeks, August 23–September 27 inclusive, in 1979 (Fig. 1).

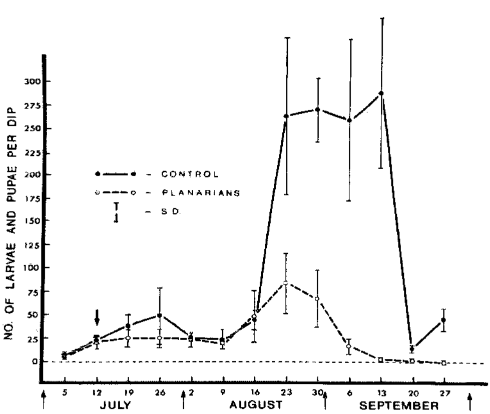

Fig. 1. Mosquito larvae and pupae sampled in catch basins with and without planarians. Downward directed arrow indicates date that planarians were introduced while upward directed arrows indicate months. S.D.-standard deviation.

Samples of 10 larvae taken for identification each week revealed over 95% to be either *Culex pipiens* Linn. or *Cx. restuans* Theobald. On August 2 only 10% were *Cx. pipiens* and by September 6 all were *Cx. pipiens.*

From August 9 until September 27 in 1979, *D. tigrina* was present on the Styrofoam® floats only in catch basins to which planarians had been added and absent from the floats in control basins. The rounded averages of the numbers of planarians of all sizes per float for the 9-week period of August 2 to September 27 inclusive was 8, 10, 17, 22, 15, 5, 9 and 28. It is not known how closely these numbers represented the actual population or reflected the behavior of planarians in the catch basins. The numbers did increase and decrease with the numbers of mosquito larvae and pupae in the treated basins except on the last date when the numbers of planarians were the highest for the season. It is possible that the high densities of mosquitoes were required for effective predation and predator increase. The constant occurrence of many small planarian buds, together with the posterior shape of larger planarians that had obviously undergone fission revealed the planarians to be both feeding and reproducing. Ostracods were present in some catch basins and planarians were observed feeding on insects, such as Collembola, stranded on the water surface. The effects of such potential alternate hosts, especially at low mosquito densities, on mosquito reduction by planarians is suggested as a project for future investigation.

LABORATORY PREDATION TESTS. 1–16 Planarians and 100 *culex* larvae. When 100 first instar *Culex* larvae were placed in 2 liters of water in each of 6 containers with 0, 1, 2, 4, 8 or 16 planarians respectively, 4 or more planarians reduced emergence by over 90% (Fig. 2). However, not even 16 planarians prevented all larvae from attaining the adult stage and an average of 1.3 adults per container emerged. As larvae were consumed, their density decreased along with planarian predation. This is similar to the relationship of consumption to prey density reported by Meyer and Learned (1981a) and the functional response to prey density for

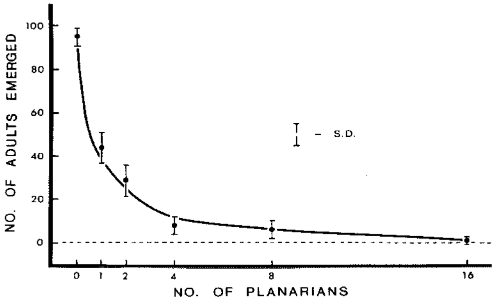

Fig. 2. Emergence of *Culex* adults from 100 first instar larvae reared with different numbers of planarians in 2 liters of water in the laboratory.

invertebrates reported by Holling (1965). Although the apparent encounter dependent trend displayed in Fig. 2 indicates it might not be difficult to reduce moderate densities of mosquito larvae in catch basins for prolonged periods with a few *D. tigrina*, it could be difficult to provide enough planarians to achieve and maintain eradication of immature mosquitoes in all basins.

80 PLANARIANS AND 500, 1000 or 2000 *CULEX* LARVAE. When 80 planarians were placed with each of 500, 1000 or 2000 first instar *Culex* larvae in 12 liters of water, only 15, 9 and 6% respectively of the larvae reached adulthood (Table 1). A comparison of the numbers of adults from containers with planarians to those without, revealed that 80 planarians reduced adult emergence of 500 larvae by 80%, of 1000 by 89% and of 2000 by 80%. The overall reduction for all tests with 80 planarians was 88%. This reduction is only slightly below the 92% reduction obtained with only 8 planarians and 100 larvae in the previous experiment.

The density of first instar larvae per liter was 50 in the previous experiment and one of 42, 83 or 166, respectively in this experiment. Such differences are not believed to greatly affect predation rates as most encounters occur on the substrate at the bottom of the containers (Meyer and Learned 1981a, George 1978). Even in the absence of planarians, in containers with 2000 larvae, only 51% of the larvae emerged as adults compared to 80% with 1000 larvae and 76% with 500. Hence, crowding of larvae even without predators reduced emergence. Moreover, it was determined in preliminary tests that densities of over 100 larvae per liter reduced emergence. Even though 90% or more pupation occurs routinely at much higher densities with colonies laboratory-reared for prolonged periods, this reduction occurred consistently with all larvae hatched from eggs collected outdoor. The overall reduction by planarians at high densities of feral larvae, therefore, is even more remarkable when it is realized that the initial effect of predation is to reduce larval density and competition. It follows that with mosquito infestation levels above 100 larvae per liter, predation in the wild may actually promote mosquito survival by reducing larval density. Hence the emergence of mosquito adults from a given volume of water seems to be buffered in such a manner that the effects of planarian predators are minimized at high larval densities. This provides a possible explanation for failure to always obtain reductions of feral mosquito larvae by planarians (Meyer and Learned 1981b) while failure at low levels may be due either to reduced encounters, as indicated, or to alternate hosts, which have yet to be investigated. Like *D. dorotocephala* in California, *D. tigrina* in Ontario is certainly capable of regulating *Culex* mosquitoes. The ability of *D. tigrina* to survive from one year to the next in catch basins in Ontario is under investigation.

Table 1. *Culex* adults from indicated numbers of first instar larvae reduced by 80 planarians in 12 liters of water.

	Numbers of adults emerged**	
First instars	Controls	Planarians
500*	378 ± 10.6	75 ± 32.0
1,000	795 ± 108.5	87 ± 57.7
2,000	1,028 ± 164.7	121 ± 96.5

* Two replicates. All others were replicated four times.
** Means ± SD.

ACKNOWLEDGMENT

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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