LOCATION OF UNIVOLTINE *AEDES* EGGS IN WOODLAND POOL AREAS AND EXPERIMENTAL EXPOSURE TO PREDATORS

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Introduction. Mosquito eggs laid on water or upon soil would appear to be vulnerable to predacious arthropods, yet relatively few such predators are recorded. According to Christophers (1960), psocids fed on eggs exposed in storage, and Buxton and Hopkins (1927) reported that in Samoa eggs were removed from experimental pots by ants. Laird (1947), working in New Britain, found a hydrachnid mite that fed on mosquito eggs. In the United States, Stage and Yates (1939) obtained evidence of predation by carabid beetles.

Arthropod predators of mosquito eggs were investigated at Chatterton, Ontario, in studies of the natural control of pest mosquitoes, particularly *Aedes trichurus* (Dyar) and *Aedes stimulans* (Walk.). Both species lay their eggs during May and June in the moist soil surrounding shrinking woodland pools. These hatch in the following March when the pools are reflooded. Thus the eggs may remain exposed in or upon the soil from 3 to 5 months unless the water table is raised by early autumn rains. This paper reports on the site of egg populations and the presence of insect predators.

Materials and Methods. During September, 1958, preliminary soil samples 0.25 sq. meter in area and 2.0 cm. thick were taken along a transect in each of two pools, from a point at the highest water level of April 4 to the lowest of May 2. Each sample was placed in a plastic bag and stored for 4 months at 32–34° F. and later flooded in a pan with tap water.

More extensive sampling was done in October within three rectangular plots of 50, 12, and 12 sq. meters respectively, each of which enclosed a different pool area in the swamp. Samples were 0.25 sq. meter in area and 2.5 cm. thick, including debris, and were taken 0.5 m. apart along straight lines in order to sample one-quarter of the total area. Each sample was divided into four sub-samples to facilitate handling. The location of the samples in respect to pool contours and plant cover was indicated on a grid sketch as sampling proceeded. In 1959, an equal number of contiguous samples were removed from each plot as a further check on egg populations.

Radioactive phosphorus, P^{32}, obtained from Atomic Energy of Canada, Limited, Chalk River, Ontario, was used as a tagging agent to determine whether aedine eggs are subject to predation in the field. In an exploratory test made in June, 1959, approximately 10,000 conditioned (Christophers, 1960) eggs of *Aedes aegypti* (L.) were immersed in a solution of the isotope at a concentration of 250 μc./ml. for 4 days at 2.0° C. until the radioactivity of individual eggs reached 500 c.p.m. "Triton" (Rohm and Haas Company Inc., Philadelphia, Pa.) was employed as a wetting agent. A total of 1,300 activated eggs, in lots of 100, were then taken to the field and put out on small pieces of filter paper at random near pool margins. After 24 hours insects and other arthropods were collected within the pool area and tested for radioactivity, using a Nuclear-Chicago D47 gas flow counter and a M-5 sample changer connected to a Tracerlab Autoscaler.

In a later experiment, 5,800 of the tagged eggs were employed, although the average radioactivity per egg had decreased to 310 c.p.m. by the decay of the isotope. On June 16 the eggs were divided into 2 lots of 1,000, 2 of 500, and 14 of 200 and each lot placed on a piece of crumpled filter paper 3 cm. square. The papers were laid on the ground about 50 cm. apart along a well-defined pool margin where *Aedes* eggs are usually concentrated. Suspect predators were collected and tested as before.

In June, 1960, 4,800 eggs of *Aedes trichurus* were tagged with P^{32} to detect egg predators. Females of *A. trichurus* were captured in the Chatterton swamp after being allowed to engorge on hand-held guinea pigs, and were later transferred to oviposition cages in the laboratory. In this experiment the tagged eggs were distributed in lots of 200 on damp soil in petri dishes, and partly covered with fine sand. The petri dishes were taken to the swamp on June 26 and inserted in the soil near shaded pool margins where eggs of *A. trichurus* are usually laid. In later tests small flower pot saucers were substituted for the petri dishes.

RESULTS AND DISCUSSION. *Egg Populations.* Counts of larvae from flooded soil samples taken along a transect of a semi-permanent pool site indicated that 84.8 percent of the eggs were deposited on a gently sloping margin 2 to 3 meters wide. In contrast, 92.6 percent of the eggs in a more transient pool originated on a steep bank about one meter in width. From the former site the number of larvae per sample varied from 0 to 141 (average 48.8) and from the latter from 0 to 192 (average 67.1). Samples taken near the April 11–16 water levels in sandy loam among mosses and sedges produced more larvae than those from the pool bottom or from high margins with perennial ground cover.

Egg populations in the rectangular plots varied considerably (Table 1). Site 1 produced more than three times as many *Aedes stimulans* as *A. trichurus* whereas the other two more shaded sites contained larger proportions of *A. trichurus*. Larvae of other univoltine *Aedes* were few.

The egg population in sites 1 and 3 was actually greater than that shown as many larvae were destroyed by larvae of the dytiscid *Agabus erichsoni* G. & H. before their presence was discovered in the rearing pans. The predators occurred in 14 samples from site 1 and in 3 from site 3 all from pool margins. Eggs of this species overwinter apparently in moss with the *Aedes* eggs and hatch in the following March or April (James, 1961).

In general, mosquito eggs were deposited at or near the margins rather than on pool bottoms that had little or no plant cover. Likewise, few eggs were present in soil above the high water contours though these were often well covered with vegetation later in the summer.

Aedes eggs were similarly located in 1959 as shown from larval counts obtained

TABLE 1.—Number of larvae from soil samples containing eggs of univoltine *Aedes*, collected in three woodland pool sites at Chatterton, Ont., 1958.

Location	Total samples	Total larvae	Number of larvae per sample: Average	Number of larvae per sample: Range	*A. trichurus*	%	*A. stimulans*	%	Other spp.	%
Site 1—Southern edge of swamp	50	4396	87.9	1–439	927	21.0	3150	71.7	319	7.3
Site 2—100 meters within swamp	12	1662	138.5	0–757	983	59.1	594	35.7	87	5.2
Site 3—100 meters within swamp	12	725	60.4	5–342	310	42.8	361	49.8	54	7.4

by flooding contiguous soil samples. For instance, in site 1, 96.1 percent of the eggs were from marginal and sub-marginal areas and the remainder from pool bottoms and high knolls. In site 2, 98.9 percent came from the pool margins and the remainder from bottom samples, but in site 3 the margins contained 89.5 and bottoms 10.5 percent. This suggests that the eggs are concentrated each year in the same marginal and sub-marginal areas.

The location of some of the eggs in the soil was evident from the examination of a marginal sample. This contained 133 intact eggs of which 59 occurred on the surface and on moss while the remainder were concealed among debris. In the laboratory also, series of wild *Aedes trichurus* deposited a total of 2,622 eggs in lantern globes set over moist sand. Of these, 1,812 (69.1 percent) were laid on the surface and the remainder were partially inserted beneath it. On the other hand, females of *A. stimulans* laid only 47.1 percent on the surface and the remainder in the sand. In detailed studies of the distribution of eggs of *A. stimulans* in soil samples, McDaniel and Horsfall (1963) showed that the majority of the eggs occurred at depths of from 5 to 10 mm. and that large proportions of these were found in the first 5 mm. All these data suggest that many eggs of univoltine *Aedes* are exposed to terrestrial predators for several months after they are deposited unless the pools are flooded in early autumn.

PREDATORS. An investigation of possible predators was begun at Chatterton in June, 1959, when P^{32}, was used to identify predators of the eggs. In the exploratory test, 13 species of arthropods were collected and tested but only 2 species were found sufficiently radioactive to be classed as predators: an ant, *Myrmica lobicornis fracticornis* Emery, and a carabid beetle, *Pterostichus (Poecilus) lucublandus* (Say). Four other small beetles were mildly radioactive. A count of the remaining eggs showed that from 11 to 100 (mean 58.2) eggs were missing from the various lots, probably due to predators. That removal of eggs to nearby soil by predators was negligible was shown by flooding a thin layer of soil taken within a 15 cm. radius of the egg papers and collecting hatched larvae.

In the later experiment a total of 115 suspect predators were collected from the ground in the test area. Ants were the most conspicuous insects. Workers of two species were found later to be radioactive well above background: *Lasius sitkaensis* Pergande (82, 43, 38 and 35 (pupa) c.p.m.) and *Camponotus herculeanus* (Linné) (40, 39, 37 and 36 c.p.m.). One small staphylinid beetle also was radioactive (35 c.p.m.). Such low radiation counts, however, suggest that the ants became radioactive by contact in transporting the eggs rather than by ingesting them since layers of the chorion, particularly of some aedine eggs, are tough and impervious (Christophers, 1960) and the contained embryos would transmit little radioactivity to the predator unless ingested.

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stated that, of the three species of ants, *Lasius sitkaensis* was more likely to be a predator than were the others, particularly of eggs laid on the surface of the soil. Radioactive prey might also be transferred from one ant to another, which would account for the presence of radioactive pupae discovered in a nest of *L. sitkaensis*.

Counts of the eggs remaining from this experiment showed that more were missing from the 1,000-egg lots than from lots with fewer eggs, shown below:

Total no. eggs	No. damaged	Percent	No. missing	Percent	No. remaining	Percent
2 x 1,000	114	5.7	1,637	81.9	249	12.4
14 x 200	118	4.2	862	30.8	1,820	65.0
2 x 500	73	7.3	216	21.6	711	71.1

Additional predators were recorded in 1960 by exposing tagged eggs of *Aedes trichurus* in the Chatterton swamp. Forty-eight hours after the eggs were put out a total of 163 arthropods were collected in the 25 sq. meter test area. These included 91 ants, 63 Coleoptera and 9 insects of other orders. A total of fourteen specimens of the following species showed radioactivity appreciably above background: *Lasius sitkaensis, Myrmica lobicornis fracticornis,* and four small carabids, *Agonum* sp., *Bembidion frontale* (Lec.), *Bembidion muscicola* Hayw., and *Pterostichus* sp. These data confirm in part records by Stage and Yates (1939) who reported *Agonum pusillum* (Lec.) and *Bembidion* sp. as predators of *Aedes* eggs in the Pacific Northwest.

Non-activated eggs of *Aedes aegypti* were also exposed to predators. Five saucers, each with 200 eggs, were inserted along the abrupt margin of a pool and five others at sub-margins and pool bottoms. The control consisted of 200 eggs in a screened vial placed in the test plot. A canopy of plastic cloth was erected to shield the saucers from rain. After 5 days the saucers were returned to the laboratory and flooded and the numbers of remaining eggs determined from counts of the hatched larvae. Large numbers of eggs were missing from saucers in each pool area as compared to a mortality of 2.5 percent in the control. Mean numbers lost from 200 eggs per saucer were 110 (range 66–128) for the high margin adjacent to dense ground cover, and 76 (range 29–162) for the sub-margin and bottom. Foraging ants and adults of *Bembidion* spp. were observed in both test areas during the experitment.

Adults of *Bembidion* spp. and workers of *Myrmica l. fracticornis* were tested in the laboratory to determine their potential for feeding on *Aedes* eggs. Specimens of the former were collected from the swamp floor during July largely among two mosses, *Climacium dendroides* (Hedw.) Web. and Mohr and *Mnium affinis* Bland. In the first test, six adults of *Bembidion frontale* were isolated in 2-inch shell vials with cotton plugs and containing a moistened split raisin. For the first two days each bettle was supplied daily with 10 eggs of *Aedes trichurus* and after this up to 20 of the smaller eggs of *A. aegypti* as required. All vials were checked daily and missing or damaged eggs replaced to restore the total.

In the absence of other prey, the beetles consumed or damaged an average of 5.5 eggs (range 0–10) of *A. trichurus* and 13.4 (range 5–20) of *A. aegypti* daily. As observed under a binocular microscope, a beetle would seize an egg with its mandibles and cut it in two near the middle before feeding on the contents. In one instance, only very small fragments of chorion remained after it had finished feeding on an egg.

In a similar test the feeding potential of two species of *Bembidion* was compared with that of the ant, *Myrmica l. fracticornis*. It was found that the adults of *Bembidion frontale* disposed of 15.7 eggs of *A. aegypti* per day as compared to 15.3 by *Bembidion muscicola* and 5.1 by the ant. These results suggest that species of *Bembidion* may be more important than ants as predators of aedine eggs, and warrant further research on their field ecology.

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