

THE DIFFERENCE IN THE INSTAR COMPOSITION OF IMMATURE MOSQUITOES SAMPLED BY THREE SAMPLING TECHNIQUES

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SUMMARY: Three techniques, namely a ladle, a metal quadrat and a D-shaped aquatic net, were used for comparison to sample immature stages of mosquitoes in woodland pools in northern England. The quadrat proved to be more reliable in giving a more accurate representation of the different instar proportions in the ponds than the ladle and aquatic net. The ladle showed bias of undersampling the earlier instars. Similar but not so severe bias also occurred for the D-net.

INTRODUCTION

Collections of mosquito immature stages are usually made to determine the presence or absence of various species in different habitats, to monitor population changes associated with seasonal abundance or control measures, and sometimes to estimate the size of the population in a habitat from number of larvae per dip or number enclosed in each quadrat. The objective of the present study was to compare the different proportions of larval instars and pupae of mosquitoes sampled with a ladle, a quadrat and a D-shaped aquatic net so as to select the best method to study the population dynamics.

The mosquitoes found breeding in the ground pools at Ness Woods in England were *Aedes cantans*, *Ae. punctor*, *Ae. rusticus* and *Culiseta annulata*. However, the data for *Ae. rusticus* and *Cs. annulata* were not analyzed due to insufficient numbers of immature stages sampled. The life history of *Ae. cantans* is that it is a one-generation species (univoltine) and eggs laid by the females during the summer among damp leaf litters of shaded woodland pools hatch in January or February with the result that 1st instar larvae appear in large numbers; pupae are found in late April or early May (Service, 1977).

According to Marshall (1938), when rainfall is average in the last quarter of the year, fourth-instar *Ae. punctor* larvae form in December, but pupation is deferred until about April; if rainfall is below average, hatching is postponed until the following year, but the date of pupation is little, if at all retarded and adults appeared in May.

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The study was undertaken in Ness Woods, a private woodland of some 6 ha at about 33 km southwest of Liverpool in the northwest of England.

MATERIALS AND METHODS

Larval instars and pupae were sampled by each of the following methods:

- i) a soup ladle (i.e. dipper) having a capacity of about 130 ml and a diameter of 9.5 cm.
- ii) a metal quadrat which in fact was a metal wastepaper basket with the bottom removed having an internal diameter of 28 cm at the top, 23 cm at the bottom and a length of 28 cm.
- iii) a D-shaped aquatic net, made of nylon mesh (Griffin, code YRF-700-G Net Beg, Scrim L05-049/075) fastened by popstuds over a D-shaped metal frame to which a 60 cm wooden handle was attached.

Comparative larval sampling at pond A: Pond A was a very large pond of an area of about 2,000 square meters. From March 7, 1979 to July 5, 1979, samples were taken at random by tossing a coin along the perimeter of the pond, each at a distance of 1 meter apart. Every two samples taken by a ladle was followed by one sample by a quadrat and one sample by the D-net. A total of 20 samples were taken by the ladle, 10 samples by the quadrat and 10 samples by the D-net each week.

Sampling using a ladle: The ladle was gently lowered until it reached the bottom of the pond and water allowed to flow into it, then it was drawn out of the pond. The contents were then tipped into a white plastic tray and larvae were transferred with a pipette to a 75×25 mm vial containing 70% alcohol as a preservative. Pupae in each sample were placed in a different vial with pond water, taken to the laboratory and adults allowed to emerge. Numbers of larvae were recorded according to species and larval instars, and the species of each pupa was determined by identification of the emerging adult.

Sampling using a quadrat: The metal quadrat was pushed into the ground and all enclosed larvae and pupae removed by means of a white plastic tray and then pipetted into vials, larvae being preserved and pupae kept alive. Numbers of larvae and emergent adults (from pupae) were recorded in the laboratory as in previous procedure. To ensure as far as possible that all larvae and pupae were removed from each quadrat, dipping with the white plastic tray in each quadrat was stopped only after the absence of larvae or pupae for five continuous dips.

Sampling using a D-net: The D-net was pushed into the pond until it reached the bottom, then drawn out and its contents quickly tipped into a white plastic tray containing pond water. The net was carefully flushed through with further pond water to remove any stranded larvae. Floating debris or leaf litter were vigorously shaken in the plastic tray to detach larvae or pupae before being discarded. Larvae and pupae were collected, preserved or reared and identified as before.

Comparative larval sampling at pond B: Pond B was a small pond of about 30 m². From February 24, 1981 to June 3, 1981, samples were taken as described for pond A. Due to the small size of the pond there were fewer samples, but the total samples taken each week covered almost the whole perimeter of the pond. Every five samples by the ladle was followed by one sample with the quadrat and one sample by the D-net. A total of 10–20 samples by the ladle, 2–4 samples by the quadrat and 2–4 samples by the D-net were taken each week. The numbers of each larval instars and pupae were recorded as before in pond A.

RESULTS

Pond A

Two mosquito species namely *Ae. cantans* and *Cs. annulata* were found in pond A. However, the presence of *Cs. annulata* in pond A was observed only during the later part of the sampling period (from early June, 1979) and its density was low. Consequently the results of the comparative larval sampling are based entirely on sampling the predominant *Ae. cantans* population. The occurrence of various larval instars and pupae of *Ae. cantans* was seasonal. Results of comparative larval sampling at pond A in 1979 (Table I) shows that sampling with the ladle or a D-net when several larval instars were present often gave low proportions of earlier instars even when the population of the earlier instars was larger as determined by the other sampling method, the quadrat. This evidence is shown in the sampling with the ladle on April 11, 1979 (Table I), in which the proportion of 3rd-instar *Ae. cantans* was the highest (50.4%) but the proportion of 1st-instar was only 19.8%. Similarly, in the sampling with the D-net, the proportion of 3rd-instar larvae of *Ae. cantans* sampled was 47.3% and the proportion of 1st-instar larvae was only 22.3%. However, in the sampling with the quadrat, the proportion of 1st-instar *Ae. cantans* larvae was higher (41.3%) than the 3rd-instar (22.8%). The sampling on April 19, 1979 also showed that very low proportions of 1st-instar larvae were sampled by the ladle (3.8%) and D-net (2.3%) compared to that of the quadrat (19.1%). Both the ladle and the D-net sampled the 3rd-instar larvae as the highest proportion with 41.3% and 35.2%, respectively, but the quadrat sampled the 2nd-instar larvae instead of the 3rd-instar larvae as the highest proportion (34.8%).

Pond B

Results of comparative larval sampling at pond B in 1981 showed that there were mixed populations of three mosquito species, namely *Ae. cantans* (Table II), *Ae. punctor* (Table III), and a very small population of *Ae. rusticus*. Because of the very small population of *Ae. rusticus*, very few larvae were caught in a ladle (6.4%) and D-net (25.6%); by far the most were caught in the quadrat (67.9%). Results of sampling *Ae. cantans* and *Ae. punctor* with the ladle shows that as in pond A, usually the proportions of the earlier instars were less compared to the later instars. To a certain extent, the D-net also sometimes sampled

TABLE I

Results of comparative larval sampling of Ae. cantans at Pond A, Ness Woods in 1979

Date	Method	Number of samples	Total number and % of <i>Ae. cantans</i> immature stages				
			I	II	III	IV	Pupae
7.3.79	L	20	298 (100)	0	0	0	0
	Q	10	259 (100)	0	0	0	0
	D	10	519 (100)	0	0	0	0
11.4.79	L	20	26 (19.8)	33 (25.2)	66 (50.4)	6 (4.6)	0
	Q	10	560 (41.3)	468 (34.5)	309 (22.8)	20 (1.5)	0
	D	10	50 (22.3)	65 (29.0)	106 (47.3)	3 (1.3)	0
19.4.79	L	20	4 (3.8)	37 (35.6)	43 (41.3)	20 (19.2)	0
	Q	10	155 (19.1)	283 (34.8)	245 (30.1)	129 (15.9)	1 (0.1)
	D	10	11 (2.3)	135 (28.4)	167 (35.2)	152 (32.0)	10 (2.1)
28.4.79	L	20	0	0	27 (67.5)	13 (32.5)	0
	Q	10	0	0	75 (19.5)	299 (77.9)	10 (2.6)
	D	10	0	4 (1.2)	95 (29.5)	214 (66.5)	9 (2.8)
2.5.79	L	20	0	0	8 (30.8)	18 (69.2)	0
	Q	10	0	0	34 (10.6)	261 (81.6)	25 (7.8)
	D	10	0	0	15 (8.0)	155 (82.9)	17 (9.1)
8.5.79	L	20	0	0	0	14 (77.8)	4 (22.2)
	Q	10	0	0	3 (1.2)	190 (77.2)	53 (21.5)
	D	10	0	0	5 (4.1)	102 (83.6)	15 (12.3)
17.5.70	L	20	0	0	0	6 (50.0)	6 (50.0)
	Q	10	0	0	4 (1.6)	56 (22.9)	185 (75.5)
	D	10	0	0	3 (2.8)	19 (17.8)	85 (79.4)
23.5.79	L	20	0	0	0	2 (40)	3 (60)
	Q	10	0	0	0	25 (23.8)	80 (76.2)
	D	10	0	0	0	5 (10.4)	43 (89.6)
29.5.79	L	20	0	0	0	0	0
	Q	10	0	0	0	8 (24.2)	25 (75.8)
	D	10	0	0	0	1 (11.1)	8 (88.9)
7.6.79	L	20	0	0	0	0	0
	Q	10	0	0	0	1 (16.7)	5 (83.3)
	D	10	0	0	0	1 (33.3)	2 (66.7)
13.6.79	L	20	0	0	1 (100)	0	0
	Q	10	0	0	0	0	4 (100)
	D	10	0	0	0	0	5 (100)
23.6.79	L	20	0	0	0	0	0
	Q	10	0	0	0	1 (100)	0
	D	10	0	0	0	0	1 (100)

L: Ladle, Q: quadrat, D: D-net, (): percentage.

TABLE II

Results of comparative larval sampling of Ae. cantans at Pond B, Ness Woods in 1981

Date	Method	Number of samples	Total number and % of <i>Ae. cantans</i> immature stages				
			I	II	III	IV	Pupae
24.2.81	L	10	35 (100)	0	0	0	0
	Q	2	217 (100)	0	0	0	0
	D	2	153 (100)	0	0	0	0
3.3.81	L	10	36 (100)	0	0	0	0
	Q	2	519 (100)	0	0	0	0
	D	2	128 (100)	0	0	0	0
10.3.81	L	10	18 (29.0)	44 (71.0)	0	0	0
	Q	2	237 (72.7)	89 (27.3)	0	0	0
	D	2	202 (43.3)	265 (56.7)	0	0	0
17.3.81	L	20	54 (58.1)	38 (40.9)	1 (1.0)	0	0
	Q	4	167 (44.8)	193 (51.7)	13 (3.5)	0	0
	D	4	198 (56.9)	140 (40.2)	10 (2.9)	0	0
25.3.81	L	15	16 (18.2)	57 (64.8)	15 (17.0)	0	0
	Q	3	90 (15.8)	395 (69.5)	79 (13.9)	4 (0.7)	0
	D	3	54 (18.5)	183 (62.7)	53 (18.2)	2 (0.7)	0
2.4.81	L	15	0	4 (5.8)	59 (85.5)	6 (8.7)	0
	Q	3	1 (0.2)	324 (50.2)	286 (44.3)	34 (5.3)	0
	D	3	1 (0.3)	139 (45.6)	152 (49.8)	13 (4.3)	0
7.4.81	L	15	0	0	10 (62.5)	6 (37.5)	0
	Q	3	0	104 (27.0)	212 (55.1)	69 (17.9)	0
	D	3	0	5 (5.9)	53 (62.4)	27 (31.8)	0
15.4.81	L	15	0	0	0	13 (100)	0
	Q	3	0	3 (1.2)	30 (12.2)	212 (86.5)	0
	D	3	0	0	20 (14.6)	117 (85.4)	0
22.4.81	L	15	0	0	0	14 (100)	0
	Q	3	0	0	32 (11.3)	249 (88.0)	2 (0.7)
	D	3	0	0	16 (10.5)	133 (87.5)	3 (2.0)
29.4.81	L	15	0	0	0	11 (68.8)	5 (31.2)
	Q	3	0	0	0	100 (84.7)	18 (15.3)
	D	3	0	0	0	25 (67.6)	12 (32.4)
6.5.81	L	15	0	0	0	12 (66.7)	6 (33.3)
	Q	3	0	0	0	50 (54.3)	42 (45.7)
	D	3	0	0	0	43 (67.2)	21 (32.8)
13.5.81	L	15	0	0	0	1 (3.8)	25 (96.2)
	Q	3	0	0	0	31 (16.6)	156 (83.4)
	D	3	0	0	0	23 (13.5)	148 (86.5)
20.5.81	L	15	0	0	0	0	15 (100)
	Q	3	0	0	0	0	112 (100)
	D	3	0	0	0	0	30 (100)
27.5.81	L	15	0	0	0	0	3 (100)
	Q	3	0	0	0	0	9 (100)
	D	3	0	0	0	0	6 (100)

L: ladle, Q: quadrat, D: D-net, (): percentage.

TABLE III
Results of comparative larval sampling of Ae. punctor at Pond B, Ness Woods in 1981

Data	Method	Number of samples	Total number and % of <i>Ae. punctor</i> immature stages				
			I	II	III	IV	Pupae
24.2.81	L	10	10 (66.7)	5 (33.3)	0	0	0
	Q	2	48 (84.2)	9 (15.8)	0	0	0
	D	2	28 (87.5)	4 (12.5)	0	0	0
3.3.81	L	10	0	4 (100)	0	0	0
	Q	2	43 (87.8)	6 (12.2)	0	0	0
	D	2	33 (68.8)	15 (31.2)	0	0	0
10.3.81	L	10	2 (20.0)	7 (70.0)	1 (10.0)	0	0
	Q	2	25 (25.0)	75 (75.0)	0	0	0
	D	2	21 (27.6)	55 (72.4)	0	0	0
17.3.18	L	20	6 (8.8)	18 (26.5)	44 (64.7)	0	0
	Q	4	66 (19.2)	164 (47.7)	114 (33.1)	0	0
	D	4	66 (24.0)	115 (41.8)	94 (34.2)	0	0
25.3.81	L	15	0	22 (47.8)	20 (43.5)	4 (8.7)	0
	Q	3	6 (2.6)	141 (60.5)	62 (26.6)	24 (10.3)	0
	D	3	6 (3.4)	85 (47.8)	45 (25.3)	42 (23.6)	0
2.4.81	L	15	0	0	11 (52.4)	10 (47.6)	0
	Q	3	0	13 (5.7)	97 (42.2)	120 (52.2)	0
	D	3	1 (0.7)	7 (5.2)	59 (43.7)	68 (50.4)	0
7.4.81	L	15	0	0	5 (21.7)	18 (78.3)	0
	Q	3	0	0	69 (28.0)	174 (70.7)	3 (1.2)
	D	3	0	0	10 (18.9)	43 (81.1)	0
15.4.81	L	15	0	0	0	9 (39.1)	14 (60.7)
	Q	3	0	0	4 (1.2)	223 (66.6)	108 (32.2)
	D	3	0	0	0	65 (63.1)	38 (36.9)
22.4.81	L	15	0	0	0	1 (14.3)	6 (85.7)
	Q	3	0	0	0	15 (18.5)	66 (81.5)
	D	3	0	0	0	6 (21.4)	22 (78.6)
29.4.81	L	15	0	0	0	5 (83.3)	1 (16.7)
	Q	3	0	0	0	25 (43.9)	32 (56.1)
	D	3	0	0	0	10 (71.4)	4 (28.6)
6.5.81	L	15	0	0	0	0	1 (100)
	Q	3	0	0	0	3 (8.8)	31 (91.2)
	D	3	0	0	0	3 (30.0)	7 (70.0)
13.5.81	L	15	0	0	0	0	0
	Q	3	0	0	0	0	3 (100)
	D	3	0	0	0	0	0

L: ladle, Q: quadrat, D: D-net, (): percentage.

the later instars higher than the earlier instars but not as severe as the ladle. However, the quadrat usually sampled the earlier instars higher than the later instars during the time when earlier instars are in abundance.

DISCUSSION

The main difference between the results was that the ladle and, to a lesser extent the D-net, sampled mostly the later instars compared to the earlier instars. In contrast the quadrat always sampled the earlier instars more than the later instars during the time where the population of the earlier instars was high. According to Service (1976), dipping usually catches larvae and pupae at the water surface. Since both in the presence or absence of alarm reactions, different species and also different instars of the same species may remain at the water surface for varying periods, it follows that dipping may frequently be biased for a particular species or instar. Nielsen and Nielsen (1953) observed the 1st-instar, and to a lesser extent 2nd-instar, larvae of *Aedes taeniorhynchus* came up to the water surface much less frequently than older larvae. Differences between submersion times of the different immature stages will probably result in sampling bias when the age-structure of the population is derived from dipping. Hagstrum (1971) in sampling larval instars of *Culex tarsalis* Coquillett by comparing a quadrat made of an aluminium frame (20×25 cm and 25 cm high) open at both ends and a pint dipper of surface area 95 cm², he concluded that the quadrat was more efficient in collecting 1st-instar larvae than dipping. On the contrary Chubachi (1976) used a dipper (8.4 cm in diameter and 1.8 cm in depth) for sampling larvae and pupae of *Culex tritaeniorhynchus summorosus* Dyar and *Anopheles hyrcanus sinensis* Wiedemann in rice fields and an artificial container in Japan. He found that the efficiencies for sampling larvae and pupae of *Cx. t. summorosus* in the artificial container and the rice fields were identical. In the case of *An. h. sinensis*, the same finding was reported from the artificial container but not in the rice fields because the younger instars of *An. h. sinensis* were concentrated around the rice plants.

It is thought that because earlier instars of *Ae. cantans* prefer to remain submerged for a longer time than later instars, they were sampled less by the ladle and D-net. This can be explained as follows, when the ladle was slowly drawn upwards from the bottom of the pond towards the surface, the disturbance created might have caused more of the earlier than later instars to escape from the ladle, thus causing sampling bias against the earlier instars. With the D-net it is more difficult to see why the younger instars appeared to be under-sampled. In contrast sampling with the quadrat enable almost all larvae of all instars trapped within the quadrat to be removed. Therefore, there was a better chance of recovering the earlier instars from each sample than with the ladle or D-net. Thus, although the results of sampling with the ladle and D-net show more similarities in the proportion in the various instars than obtained with the quadrat, I believe the quadrat method reflected better the actual instar popula-

tions present in the ponds. However, the disadvantage of using the quadrat is the time spend on dipping from each quadrat. Because of the seasonal nature in the life history of *Ae. cantans*, the immature stages decline in numbers towards late May due to adult emergence, sampling with the ladle in late May onwards became even more unreliable. But sampling with the quadrat was more appropriate with low density populations encountered at this time of the year. Service (1976) compared the efficiency of the ladle and open-ended cylinders for collecting *Ae. cantans* and *Ae. rusticus* immature stages in a ditch in England and concluded that both 4th instar larvae and pupae were seriously underestimated by dipping. Downing (1977) in the U.S.A. found that in New Jersey woodland pools, the dipper was efficient only at collecting *Ae. canadensis* larvae from the shallow parts of the pools and Knight (1964) considered that the static quadrat has the advantage over dipping in that more opportunity existed to collect larvae that were frightened from the surface by the approach of the collector.

To conclude, among the three methods of sampling mosquito immature stages in ponds at Ness Woods, the quadrat proved more reliable in giving a more accurate representation of the different instar proportions in the ponds than the two other methods. The ladle showed considerable sampling bias especially in undersampling its earlier instar. Similar, but not so severe, bias occurred when sampling was with the D-net.

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REFERENCES

- CHUBACHI, R. (1976): The efficiency of the dipper in sampling of mosquito larvae and pupae under different conditions. *Sci. Rep. Tohoku Univ. ser. IV (Biol.)*, 37, 145-149.
- DOWNING, J. D. (1977): A comparison of the distribution of *Aedes canadensis* larvae within woodland pools using the cylindrical sampler and the standard pint dipper. *Mosquito News*, 37, 362-366.
- HAGSTRUM, D. W. (1971): Evaluation of the standard pint dipper as a quantitative sampling device for mosquito larvae. *Ann. Entomol. Soc. Amer.*, 64, 537-540.
- KNIGHT, K. L. (1964): Quantitative methods for mosquito larval surveys. *J. Med. Entomol.*, 1, 109-115.
- MARSHALL, J. F. (1938): *The British Mosquitoes*. British Museum (Natural History), London. 341 p.
- NIELSEN, E. T. AND NIELSEN, A. T. (1953): Field observations of the habits of *Aedes taeniorhynchus*. *Ecology*, 34, 141-156.
- SERVICE, M. W. (1969): Observations on the ecology of some British mosquitoes. *Bull. Entomol. Res.*, 59, 161-194.
- SERVICE, M. W. (1976): *Mosquito Ecology: Field Sampling Methods*. Applied Science Publishers, London. 583 pp.
- SERVICE, M. W. (1977): Ecological and biological studies on *Aedes cantans* (Meig.) (Diptera: Culicidae) in southern England. *J. Appl. Ecol.*, 14, 159-196.