HIGHLY POLLUTED LARVAL HABITATS OF THE *CULEX PIPIENS* COMPLEX IN CENTRAL SWEDEN

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ABSTRACT. Larvae of the *Culex pipiens* complex (*Cx. pipiens* and *Cx. torrentium*) were abundant in two highly polluted pools receiving sewage sludge in Uppsala, Sweden (early August through late September 1985). The water was characterized by high BOD, and high ion concentration of Cu, Fe, Al and much suspended matter. Maximum larval number at the pool surface area was 26.1/ml. The ratio between species was studied and *Cx. torrentium* comprised ca. 20% at the peak of abundance. Some egg rafts showed no embryogeny.

INTRODUCTION

Cosmosubtropical and cosmotropical species and subspecies of the *Culex pipiens* complex, as *Culex pipiens pallens* Coquillett and *Culex quinquefasciatus* Say, typically utilize man-made, highly polluted larval habitats (Ishii 1961, Kumada et al. 1972, Rutz and Axtell 1978).

Larvae of the nominate form, *Culex pipiens pipiens* L., however, in temperate areas of the Holarctic have been reported mainly from natural sites, such as more or less eutrophic lake shores, permanent ponds, pools or container-like sites (Natvig 1948, Carpenter and LaCasse 1955, Gutsevitch et al. 1971, Wood et al. 1979). In Sweden the species is distributed from Scania to Vasterbotten (Dahl 1977). Its ability to colonize highly polluted water has been reported from Denmark (Wesenberg-Lund 1920), the United Kingdom (Marshall 1938, Edwards et al. 1939) and Canada (Belton 1983).

Another species of the *Cx. pipiens* complex, *Culex torrentium* Martini, with primarily a western palearctic distribution, is frequently referred to as a clear water species. However, a record exists from Norway (Natvig 1948) of males and females reared from larvae collected in manure water. Hitherto from Sweden, larvae have only been reported from natural and man-made, unpolluted but more or less eutrophic, larval sites (Harbach et al. 1985).

In Scania and Uppland (C. Dahl, unpublished data) males of both species have been observed together in such sites. It was thus uncertain, whether such a highly polluted habitat as the sewage pools at Uppsala (Figs. 1 and 2) would yield both species. Therefore a general survey of the phenology, the ratio between the species and differences in egg raft sizes were made in two sewage pools at Uppsala, Central Sweden.

MATERIALS AND METHODS

In August–October 1985 many temporary pools were located on roads and in empty sewage sludge depositories of the Uppsala sewage plant near the river Fyrisan. This area was surrounded by fields, meadows and deciduous trees with an abundant bird fauna. Larvae of *Cx. pipiens* and *Cx. torrentium* were abundant in only two of the pools (URA and URB) (Fig. 3).

The two pools (depth, 15–20 cm) were about 4.5 m apart, nearly triangular and of slightly different size. The roadside margin of URA was vegetated and on the edges, abundant wasteland plants were present. Tomato seedlings were numerous on the edges around the pools, growing on the sludge.

Water quality for the sewage pools were compared to those formed by liquid oozing from a dumping ground in Uji, Japan. Both pools were characterized by high BOD, abundant suspended matter, high content of Cu, Fe and Al, nearly neutral pH, and high turbidity; URA was as black as Indian ink (transparency, <1 cm) while URB was yellowish (transparency, <5 cm). The water quality of URA was comparable with that of the oozed liquid sampled from the Uji dumping ground (Table 1).

Precipitation and air temperature data were obtained from the Institute of Meteorology, Uppsala University (Fig. 4). The maximum air temperature was 23.0°C, with daily mean temperatures fluctuating between 13.9–17.3°C. Air temperature (open star) and water temperature (solid star) measured at URA showed a close parallel to air temperature (Fig. 4). Daily thunderstorms with torrential rain from July 17–24 and July 30–August 5, caused formation of additional ground pools free of larval populations. Due to the low precipitation in late August and early September, water levels fell in the pools and their surfaces became yellowish green due to algal bloom.

A pipette was used for the quantitative collection of larvae and pupae from site URA. Samples (27–92 ml) of the surface water were taken from the zone of greatest apparent larval density from August 13 to 21. Five-hundred ml of water was dipped on and after August 24. Results were used for calculation of relative density.

Fourth instar larvae were preserved in 75%

Fig. 1. Pool on the concrete sludge depository bed formed from oozed liquid near the Uppsala Sewage Plant (URA on August 16, 1985).

Fig. 2. A different sewage sludge depository near the Uppsala Sewage Plant (September 5, 1985).

ethanol and later mounted in Canada balsam with pieces of paper interposed between slide and coverslip to prevent distortion of the specimens by pressure. The identity of egg rafts was confirmed by rearing out larvae and males in the laboratory. Fourth instar larvae were identified by chaetotaxy (Harbach 1985).

Preliminary observations showed that an egg collected one day which did not hatch by 1600 h of the next day never produced a larva, even if an embryo was observed inside it. Thus all first instar larvae hatched out within ca. 1.5 days after oviposition. Therefore, eggs were mechanically separated at 2 days after deposition, counted and examined microscopically. They were then classified into the following three categories: i) hatched, ii) embryo, not hatched, and iii) no embryo. When an egg raft yielded more than one first instar larva or contained more than one embryo per egg, it was defined as a fertilized egg raft; the remainder were classed as unfertilized.

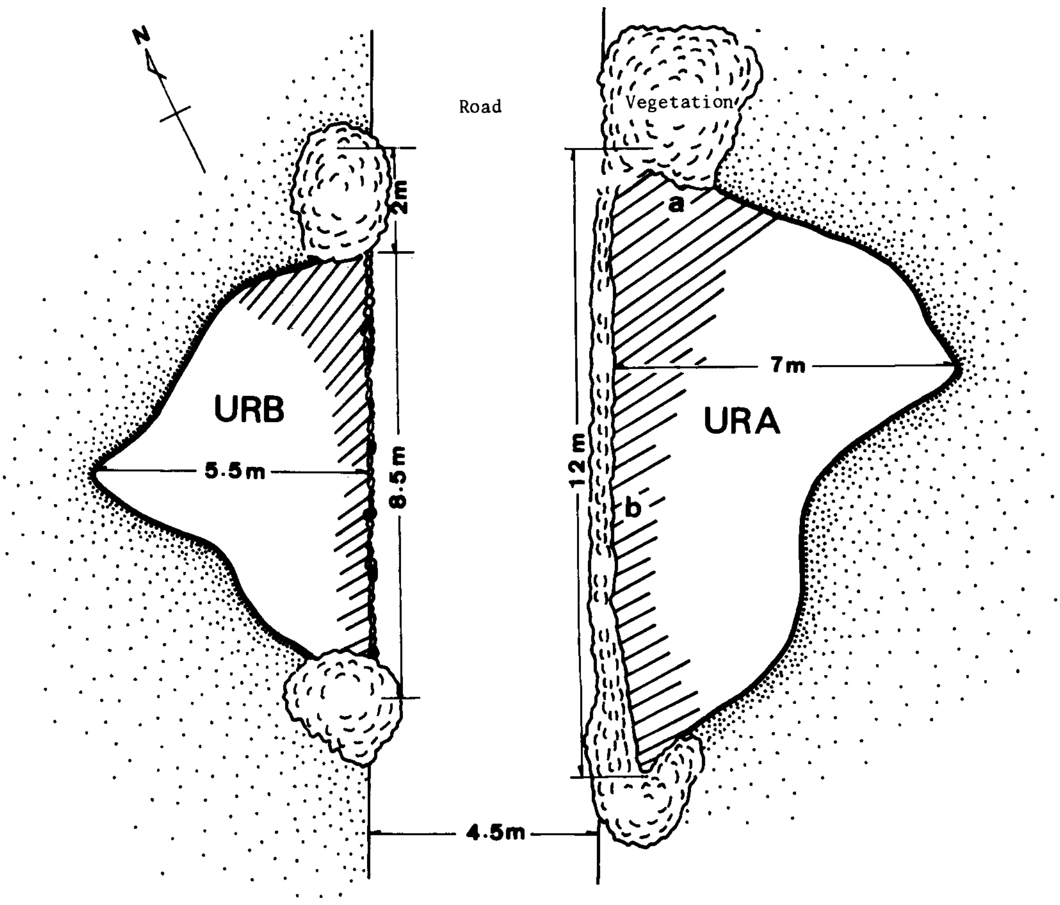

Fig. 3. Temporary pools (URA and URB; August 24, 1985) in sewage sludge depositories near the Uppsala Reningsverket (sewage plant), with abundant mosquito larvae (hatched area). a, b = main egg deposition area.

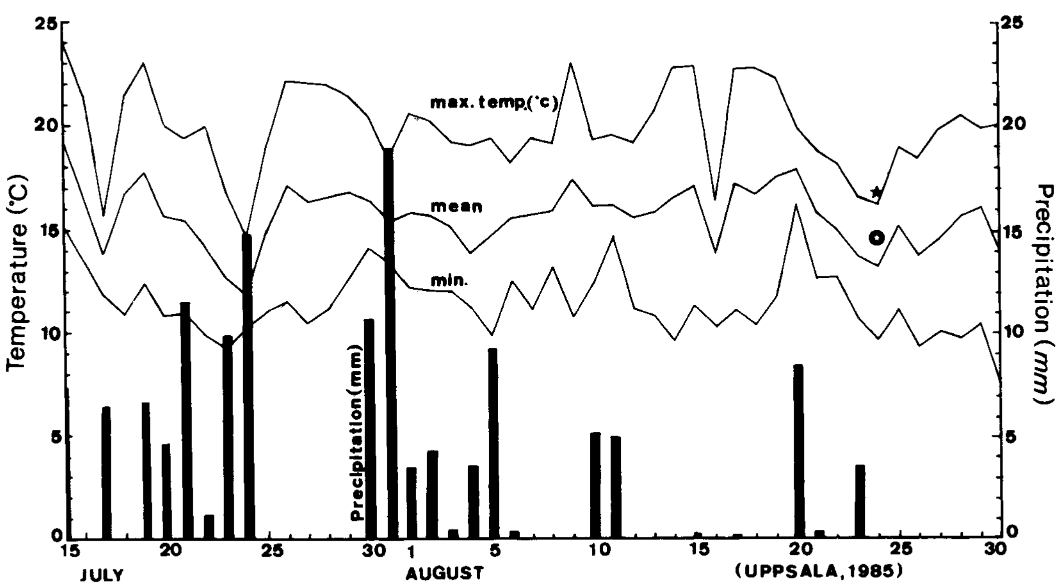

Fig. 4. Precipitation and air temperature during the main sampling period (data from the Institute of Meteorology, Uppsala University). Open star, air temperature and solid star, water temperature measured in URA at 1355 on August 24.

To express the shape of egg rafts numerically, the shape ratio (or c/b-ratio) (Ishii 1966) was calculated, with c being the number of eggs in the longest row longitudinally and b being that in the broadest transverse row.

RESULTS

Species-composition of Culex pipiens *and* Cx. torrentium. *Culex. p. pipiens* constituted 81.4% (n = 306) of dead males randomly selected from a caged population (n = 895). This population was established from URA from two egg rafts collected on August 3, and from larvae and pupae collected from August 13 through September 8.

The mean percentage of *Cx. pipiens* from the samples collected from August 13 to 24 was 77.7%, and ranged between 65.0 and 84.5% (Fig. 5b). The mosquito population of the pools consisted of approximately 80% *Cx. pipiens* at peak abundance. Such a value is close to the ratio 80:20 (n = 10), determined on *Cx. pipiens* and *Cx. torrentium* egg rafts collected from August 3 to 24.

In the Uppsala area *Cx. pipiens* does not outnumber *Cx. torrentium* during October because in a natural larval locality (two ground pools in a grassy field at Starbo, suburb of Uppsala) in early October 1985, a large *Cx. torrentium* population (44% of the larvae) (n = 115) was observed.

General abundance of larvae and pupae. An initial search on August 3 revealed no larvae and pupae in URA or URB, whereas two *Cx. pipiens* egg rafts were found among vegetation along the roadside margin of URA. Intensive searching failed to disclose any adults.

When visited for the second time on August 13, URA harbored abundant larvae, so densely packing the areas (hatched in Fig. 3) that the color of the surface water was darkened. Also, many egg rafts were found among vegetation (Fig. 3 a and b). The maximum number was 10 per 40 cm^2 area. All egg rafts found were of the boat- (or raft-) shape, typical of the *Cx. pipiens* complex.

The numbers of larvae at the pool surface water of what seemed to be the most densely populated area was 26.1/ml on August 13, 8.0/ml on August 18, and 2.4/ml on August 21 (Fig. 5b). The population diminished greatly by Au-

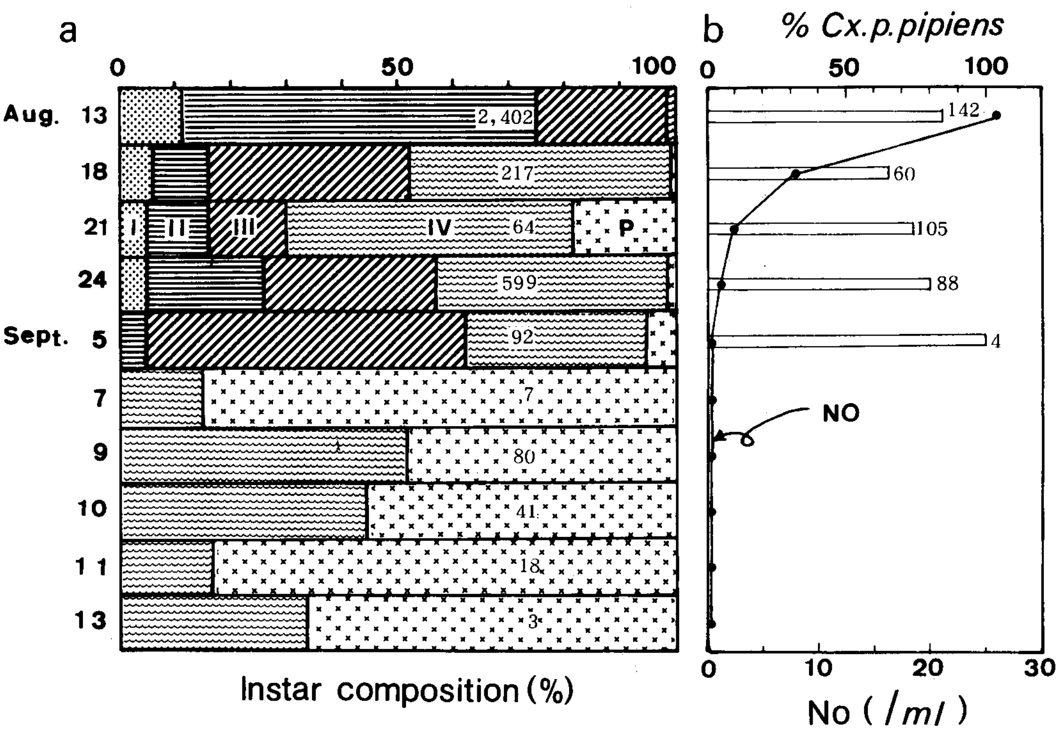

Fig. 5. a: Instar distribution, species composition, and no. larvae/ml of the immature stages of the *Culex pipiens* complex in URA. I–IV = larval instar; P = pupa; numerals = number of specimens observed. b: no. larvae/ml (line); 1 on the bottom scale indicates a number between 0 and 1/ml. For calculating no. larvae/ml, 27–92 ml of surface water of the zone of greatest apparent larval density were sucked by a pipette from August 13–21 and 500 ml water was dipped on and after August 24.

gust 24, making it difficult to collect larvae by the pipetting technique. Thus 500 ml of surface water was dipped and the amount of larvae (1.2/ml) calculated from this. It declined further toward the end of August. Therefore, after September 5 all larvae and pupae that could be located within 30 min were collected with values of 0–1 at the most populated area. No egg rafts were found on and after September 5.

Larvae were first observed in URB on August 18. They were present thereafter at low density only, thus sampling was concentrated to the URA pool.

Pupae first appeared in large numbers during late August, reaching a peak of abundance in mid-September. Only fourth instars and pupae were found after mid-September, when the moulting and emergence rates declined due to low temperature (mean air temperature, 6.5°C; maximum, 13.9°C, minimum, −1.4°C, September 21–30).

A few resting adults of *Cx. pipiens* and/or *Cx. torrentium* were found among vegetation of the margin of the pools on August 18. Three days later emergence was at its peak and resting adults of *Cx. pipiens* and/or *Cx. torrentium* had become numerous not only on the margin of the pools but also on more distant vegetation.

Characteristics of egg rafts. By separately culturing 10 egg rafts among 68 collected in URA from August 3 to 24, eight were *Cx. pipiens* while the other two were *Cx. torrentium*. From the remaining 58 egg rafts only *Cx. pipiens* or *Cx. torrentium* adults were obtained by laboratory rearing.

Because of the small number of egg rafts separately identified, no distinct differences between the measurements of the egg rafts of the two species except for the egg number could be confirmed.

The following values were determined for 29 *Cx. pipiens*-complex egg rafts (Mean ± SE, variation range): c = 29.2 ± 1.3, 16–44; b = 10.2 ± 0.4, 7–15; and c/b = 2.9 ± 0.1, 1.6–4.0. The mean number of eggs per raft in *Cx. torrentium* (190 ± 16, 179–201; n = 2) was significantly ($P < 0.05$) smaller than that of *Cx. pipiens* (291 ± 15, 240–336; n = 8). For 68 rafts the mean % eggs hatched per raft was 93.2 ± 2.5, 0.0–100.0. Mean % eggs with embryos and not hatched per raft was 1.0 ± 0.3, (0.0–14.3); and mean % eggs with no embryos per raft was 5.8 ± 2.5, 0.0–100.0.

DISCUSSION

Female *Cx. pipiens* and *Cx. torrentium* depositing eggs on these pools were probably the first generation of the year, because overwintered females deposit eggs by early July. Their larvae were mostly found in clean water pools. However, they seem also to be attracted by foul water, as are their counterparts distributed in temperate and tropical regions. In developed countries this is a very restricted source. This means that, the prevailing concept that *Cx. pipiens* in Europe might select clean water for oviposition is doubtful. If there are foul water pools in dumps, sewage ditches, lagoons, etc. as often are found in Japan (Table 1), *Cx. pipiens* in Europe might select such foul water as *Cx. p. pallens* commonly does in Japan.

The two sibling species coinhabited these pools at an unbelievably high larval density. While most larvae emerged into adults before mid-September, some of them failed probably due to the cold climate. The emerged adults will become the overwintering population.

The mean number of eggs per raft was comparable with that reported by Ishii (1966) of 53 field-collected *Cx. p. pallens* rafts (271, 75–425) and Dobrotworsky (1953) of *Cx. p. australicus* Dobrotworsky and Drummond rafts (256, 136–503). The large raft size of the Swedish *Cx. pipiens* complex suggests they attacked avian hosts. *Culex p. pallens* lays more eggs when they take chicken blood (Ishii 1966). There were no

Table 1. Water quality of URA and URB (analyzed by Uppsala Reningsverket (sewage plant) compared to that of an oozed liquid sample from a dumping ground in Uji, Japan.

	Color*	pH*	Al* (mg/liter)	Cu* (mg/liter)	Fe* (mg/liter)	Conductivity* (mS/cm 25°C)	BOD** (mg/liter)	TSS** (mgPt/liter)	Transparency (cm)
URA, Sweden	black	7.5	0.34	4.71	18.3	471	181	650	<1##
URB, Sweden	yellowish brown	7.7	0.85	3.19	29.1	319	121	600	<5##
U2#, Japan	black	7.5	—	0.10	77.4	—	(COD) 1,610	844	<1

* in water sampled August 21, 1985.
** in water sampled August 28, 1985.
\# Yashima et al. (1977).
\## estimated.
— not measured.

complaints about mosquito attacks from the sewage plant workers and people nearby. No females obtained from the pools studied, deposited autogenous eggs in the laboratory (T. Ishii, unpublished data).

The egg raft size was significantly ($P < 0.05$) larger in *Cx. pipiens* than in *Cx. torrentium.* However, this needs further study, because this conclusion is based on very small numbers.

The b, c, and c/b values of both species were comparable with those of *Cx. p. pallens* (b = 11.1, 5–16; c = 33.1, 17–47; c/b ≐ 3.0, 1.8–5.3; n = 53) (Ishii 1966), a close similarity being observed in the egg raft between European *Cx. pipiens* and *Cx. p. pallens* of northeastern Japan.

The fact that three out of 68 rafts failed to produce embryos shows that in Uppsala some females of the complex deposit unfertilized egg rafts or genetically incompatible ones. Among the hatched rafts, only one had a high percentage of eggs without embryos, while others had <4%. This particular raft probably resulted from an incompatible combination of gametes similar to a case reported by Yen and Barr (1974) in which about 1% of eggs had embryos which did not hatch and no embryos were observed for 6% of the eggs.

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