

## Different responses of two floodwater mosquito species, *Aedes vexans* and *Ochlerotatus sticticus* (Diptera: Culicidae), to larval habitat drying

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Received 25 October 2005; Accepted 15 February 2006

**ABSTRACT:** Insect larvae that live in temporary ponds must cope with a rapidly diminishing resource. We tested the hypothesis that floodwater mosquitoes would react to diminishing water levels by accelerating larval development time and emerging as smaller adults. Since a reduction in habitat size leads to increased larval densities, we also included two larval densities. Newly-hatched floodwater mosquito larvae, *Aedes vexans* (87.9% of emerged adults) and *Ochlerotatus sticticus* (12.0% of emerged adults), were taken from the field and randomly assigned to one of three water level schedules. Survival to adult emergence was significantly affected by the water level schedule. *Ae. vexans* adults emerged later in the decreasing schedule than the constant water schedule, but time to emergence was not affected by larval density. In the drying water schedule, *Ae. vexans* adults emerged 6 to 14 days after complete water removal. Adult size was significantly affected by both water level schedule and larval density. Adults of *Oc. sticticus* emerged earlier in the decreasing than the constant water schedule which was in accordance with our hypothesis, but size was not affected. Our results indicate two different responses of two floodwater mosquito species to diminishing larval habitat. *Oc. sticticus* accelerated larval development while *Ae. vexans* larvae showed remarkable survival in humid soil. Both species are often numerous in inundation areas of large rivers, and climatic conditions after a flood might influence which species dominates the adult mosquito fauna. *Journal of Vector Ecology* 31 (1): 123-128. 2006.

**Keyword Index:** Floodwater mosquito, *Ochlerotatus sticticus*, *Aedes vexans*, larval survival, survival strategy.

### INTRODUCTION

Aquatic insects can have complex life cycles in which individuals undergo metamorphosis and often simultaneously shift habitats. Models of complex life cycles predict that an increased mortality at the immature stages should induce earlier metamorphosis at a smaller size (Rowe and Ludwig 1991). For mosquitoes and other insects with larvae in temporary habitats, potential mortality factors include biotic factors like predation and disease, and physical factors like drying of the ponds, extreme temperatures, and covering of the water surface by algae (Slater and Pritchard 1979). The two main causes for mortality of the immature stages are habitat drying and predation, although predation often has a minor impact on mosquito survival in temporary water bodies (Service 1977, Pritchard and Scholefield 1983).

Habitat drying could induce earlier metamorphosis and smaller size at pupation for mosquitoes. Juliano and Stoffregen (1994) tested for effects of decreasing water volume and increasing concentration of dissolved substances on developmental time of the tree hole mosquito *Ochlerotatus triseriatus* (Say). They found that decreasing water volume led to an earlier metamorphosis and a smaller size. In contrast, increasing concentration of dissolved substances showed the opposite effect, probably due to enhanced microbial growth that provided more food.

Density-dependent effects could also influence the timing of metamorphosis. Chodorowski (1969) observed that a

reduced time to metamorphosis in *Ochlerotatus communis* (deGeer) larvae was associated with habitat drying, but this effect disappeared at high mosquito densities. In a study of *Ochlerotatus cantans* (Meigen), Renshaw et al. (1993) found that larvae maintained at high densities showed increased mortality and reduced size. They suggested that the effect of habitat desiccation might be caused by increased larval densities during reduction of habitat size by desiccation. To disentangle the effect of desiccation from density-dependent effects, we included larval density as a factor in our experiments.

The aim of the present study was to examine the effect of decreasing water volume and habitat desiccation on floodwater mosquito development and to examine if these effects depended on larval density. Our hypothesis was that floodwater mosquitoes respond to decreasing water volume by accelerating their larval development time. Adults would emerge earlier but at a smaller size. High larval density could lead to delayed development and thus obscure the acceleration of development time, but enhance any reduction in adult mosquito size.

### MATERIALS AND METHODS

First instar larvae of floodwater mosquitoes were collected in the field from two breeding sites close to the village of Österfärnebo (60° 17' N; 16° 50' E) in the lowlands of the River Dalälven, Central Sweden, on 28 June, 2003.

The larvae were only a few hours old at the time of sampling and the experiment was started that same day. The temperature in the laboratory varied between 18 and 20° C and the humidity between 65 and 80%. The photoperiod was 18L:6D, mirroring local natural light conditions. The larvae were kept in rectangular plastic containers (750 ml, 175 x 120 x 60 mm), each containing 100 g soil with a high clay content and 200 ml deionized water. Larvae were fed daily from the start of the experiment to day five with 0.5 mg fish food (TetraMin® Baby, Tetra, Melle, Germany) per larva, and from day six onwards with 1 mg fish food per larva. The fish food was suspended in water and the appropriate amount per container was added with an Eppendorff pipette. The amount of food for a container was adjusted if larvae died. From day 14 onwards, food was provided every second day. This feeding regime ensured that food was not a limiting factor for larval development.

The experiment used a two-way design with larval density and water level schedule as factors. Two larval densities were used: one larva and 20 larvae. Three water level schedules were used: constant, decreasing, and drying. In the constant water level schedule, water was added as needed to keep a constant water level. In the decreasing water level schedule, 60 ml of water was removed on day four, 60 ml on day five, 10 ml on day six, and 10 ml on day eight. In the drying water level schedule, water level was reduced as in the decreasing treatments until day five, but on day six all the remaining water was removed to strand any remaining larvae. These containers were then regularly sprayed with water to keep some humidity in the soil. This design resulted in six treatments with 20 replicates each (a total of 1,260 mosquito larvae). Mosquito larvae were randomly assigned to one of the six treatments.

The plastic containers were kept in a slight sloping position (angle of 23 degrees), thus a slight decrease of water level resulted in a rapid decrease of water surface area. They were stored on racks with 12 containers per rack. The racks were placed on shelves with one rack on the top, middle, and bottom shelves. Treatments were assigned randomly to each rack with shelf as a blocking factor to ensure that all replicates of a treatment would not end up on the same shelf.

Surviving larvae were counted every two days from day four onwards. Occurrence of pupae as well as day of emergence of adults was noted daily between day 9 and 14 and every second day afterwards. After appearance of the first pupae, containers were covered by nets and emerged adults were sampled with an aspirator, put into vials, and stored at -20°C. All adults were sexed and identified to species. Wing lengths were measured to represent adult mosquito size (Renshaw et al. 1994). Wings were removed, mounted in Euparal® (Fiebig Lehrmittel, Berlin, Germany), and measured in a stereomicroscope with a graduated eye-piece. Wing length was measured between the alula notch and the tip of the wing, excluding fringe scales.

Larval survival to day six of the experiment (before complete water removal in drying treatments) and survival to the adult stage was evaluated as relative survival per container, with no consideration of species or sex, and tested for effects

of water level schedule and larval density. For the response variables emergence day and wing length, mean value per container with 20 larvae was used to avoid pseudo-replication. Furthermore, separate analyses were done for females and males to assure that each replicate contributed with only one value to each analysis. The effects of larval density and water level schedule on adult emergence day and wing length of *Ae. vexans* (Meigen) were tested with factorial ANOVAs. The low number of emerged *Ochlerotatus sticticus* (Meigen) from containers with single larvae did not allow for testing of the effect of larval density. The effect of water level schedule on emergence day and wing length of *Oc. sticticus* was tested with a One-Way ANOVA for males and females separately. A One-Way ANOVA was used for testing the effect of the number of co-occurring *Oc. sticticus* larvae (0, 1-3, or 4-6) on emergence day and wing length of the main species *Ae. vexans*. Since only very few individuals survived to adulthood in the drying treatments, these treatments were excluded from these analyses. Statistical analysis was performed in Statistica 6.0 (StatSoft Inc. 2001).

## RESULTS

### Survival

Relative survival of larvae to day six (before drying out of treatments E and F) was neither affected by density (ANOVA:  $F_{1,114}=0.000$ ,  $p=1.000$ ), nor water schedule (ANOVA:  $F_{2,114}=0.321$ ,  $p=0.726$ ), and no interaction existed between density and water schedule (ANOVA,  $F_{2,114}=0.842$ ,  $p=0.434$ ). Survival to adult emergence was significantly influenced by water schedule (ANOVA:  $F_{2,114}=8.306$ ,  $p=0.000$ ). However, larval density did not affect survival to adults (ANOVA:  $F_{1,114}=0.000$ ,  $p=0.985$ ), and there was no interaction between water schedule and larval density (ANOVA,  $F_{2,114}=1.091$ ,  $p=0.339$ ). Survival to adult emergence was 91% in constant, 66% in decreasing and 3% in drying water schedule. In the drying water schedules, the number of larvae surviving decreased approximately 50% within two days of water removal, but then mortality rate slowed (Figure 1). In drying treatments with a single larva, the last living larva was observed 12 days after water removal. In drying treatments with 20 larvae, single larvae survived 14 days after water removal. The first adult from this treatment emerged six days after water removal, and the last adult 14 days after water removal.

### Adult emergence

Altogether, 676 adults (54%) emerged from the original 1,260 larvae. Of those, 588 individuals (87.9%) were *Ae. vexans*, and 80 individuals were *Oc. sticticus* (12.0%). One individual was identified as *Ae. cinereus* (0.1%, excluded from analyses). Seven individuals could not be identified to species (also excluded). For *Ae. vexans*, neither time to adult emergence (ANOVA:  $F_{2,75}=0.356$ ,  $p=0.701$ ) nor adult size (ANOVA:  $F_{2,71}=0.656$ ,  $p=0.525$ ) were significantly influenced by the number of co-occurring *Oc. sticticus* larvae.

Emergence of *Ae. vexans* males was neither significantly affected by water level schedule nor by larval density (Table

Table 1. Effects of larval density (1 and 20) and water level schedule (constant and decreasing) on emergence day of male and female *Aedes vexans*, results of factorial ANOVA.

	df	sum of squares	mean square	F	p
Males:					
Intercept	1	5650.159	5650.159	6201.073	0.000
density	1	0.383	0.383	0.420	0.520
water level	1	2.794	2.794	3.067	0.086
density*water level	1	0.275	0.275	0.302	0.585
Error	48	43.736	0.911		
Females:					
Intercept	1	5740.964	5740.964	2537.288	0.000
density	1	2.602	2.602	1.150	0.289
water level	1	29.047	29.047	12.838	0.001
density*water level	1	0.001	0.001	0.001	0.981
Error	47	106.344	2.263		

1). For *Ae. vexans* females, emergence day was significantly affected by water level schedule, but there was no significant effect of larval density and no interaction between larval density and water level schedule (Table 1). *Ae. vexans* adults emerged later in decreasing than in constant water level schedules (Figure 2).

In contrast, *Oc. sticticus* adults emerged earlier in decreasing than in constant water level schedules (Figure 2). No significant effect of water level schedule on emergence day of *Oc. sticticus* males (ANOVA:  $F_{1,23}=0.088$ ,  $p=0.770$ ) and females (ANOVA:  $F_{1,24}=3.672$ ,  $p=0.067$ ) was found.

#### Adult size

Adult size of both male and female *Ae. vexans* was significantly affected by both larval density and water level schedule, but there was no significant interaction of these two factors (Table 2). Adults of *Ae. vexans* emerging from containers with single larva were larger than adults that emerged from containers with 20 larvae (Figure 3). Also, larvae kept at a constant water level emerged as larger adults than larvae subjected to decreasing water. Size of *Oc. sticticus* males (ANOVA:  $F_{1,17}=1.16$ ,  $p=0.297$ ) and females (ANOVA:  $F_{1,18}=0.046$ ,  $p=0.833$ ) was not significantly affected by water level schedules.

Table 2. Effects of larval density (1 and 20) and water level schedule (constant and decreasing) on adult body size measured by wing length of male and female *Aedes vexans*, results of factorial ANOVA.

	df	sum of squares	mean square	F	p
Males:					
Intercept	1	408.1844	408.1844	10986.82	0.000
density	1	0.3097	0.3097	8.34	0.006
water level	1	1.4207	1.4207	38.24	0.000
density*water level	1	0.0177	0.0177	0.48	0.493
Error	43	1.5975	0.0372		
Females:					
Intercept	1	406.2473	406.2473	10177.74	0.000
density	1	0.4648	0.4648	11.65	0.001
water level	1	1.4671	1.4671	36.76	0.000
density*water level	1	0.0190	0.0190	0.48	0.493
Error	42	1.6764	1.6764		

## DISCUSSION

Floodwater mosquito species show several adaptations for a life in temporary wetlands (Becker 1989, Becker et al. 2003). They produce drought-resistant eggs that can survive for several years. Subsequent larval hatching is triggered by several factors like microbial-induced decline in dissolved oxygen and appropriate water temperature. However, not all larvae hatch uniformly and this so-called “hatching in installments” ensures survival of populations in case initial larval populations are killed by drying of breeding sites. The larval development is temperature-dependent and can be completed within very short time frames. In addition, floodwater mosquito species are known for massive egg production and long-range adult dispersal. We investigated an additional potential adaptation of floodwater mosquito species to temporary wetlands, the responses of larvae to a diminishing habitat.

Our results for *Ae. vexans* were not in accordance with our hypothesis. Time to adult emergence increased under a decreasing water schedule and adult size was reduced. However, some adults emerged long after water removal (6 to 14 days) and showed remarkable survival ability in humid soil. Chodorowski (1969) tested the ability of *Oc. communis* larvae to survive short waterless periods and found 25% of larvae and 100% of pupae alive after 18 h. He suggested that this ability might be of greater importance for survival than an acceleration of development time by desiccation. Therefore, the survival ability of *Ae. vexans* larvae in humid soil might not require accelerated developmental time. A survival probability of 3% would still result in large numbers of mosquitoes, considering the high larval densities that can be found in temporary water. Sharkey et al (1988) reported mean numbers of 327 *Ae. vexans* larvae in dip samples containing 350 ml water. However, larval densities can be much higher, and densities of approximately 10,000 *Ae. vexans* larvae per 1,000 ml has been observed in inundation areas in the Upper Rhine River Valley (M. L. Schäfer, personal observation).

Our results for *Oc. sticticus* indicated that this species responded to decreasing water levels by decreasing the time to metamorphosis. However, this did not result in smaller adults. Juliano and Stoffregen (1994) observed smaller sizes induced by decreasing water volume only for females of *Oc. triseriatus*. Thus, this response might not always be measurable.

The large number of larvae that can be found in temporary wetlands also indicates that the density of 20 larvae in 200 ml of water used in this study might not be sufficient to create density-dependent effects on floodwater mosquito species. Reported density-dependent effects include increased mortality of immature stages, delay of development, and smaller adults (Agnew et al. 2000, Gleiser et al. 2000, Agnew et al. 2002). Agnew et al. (2002) tested a minimalist approach using trials with one, two, or three larvae of *Ae. aegypti* (L.) in 5 ml water and found density-dependent effects despite the low number of larvae. In our experiment, delayed development was not due to density-dependent effects, but mean size of

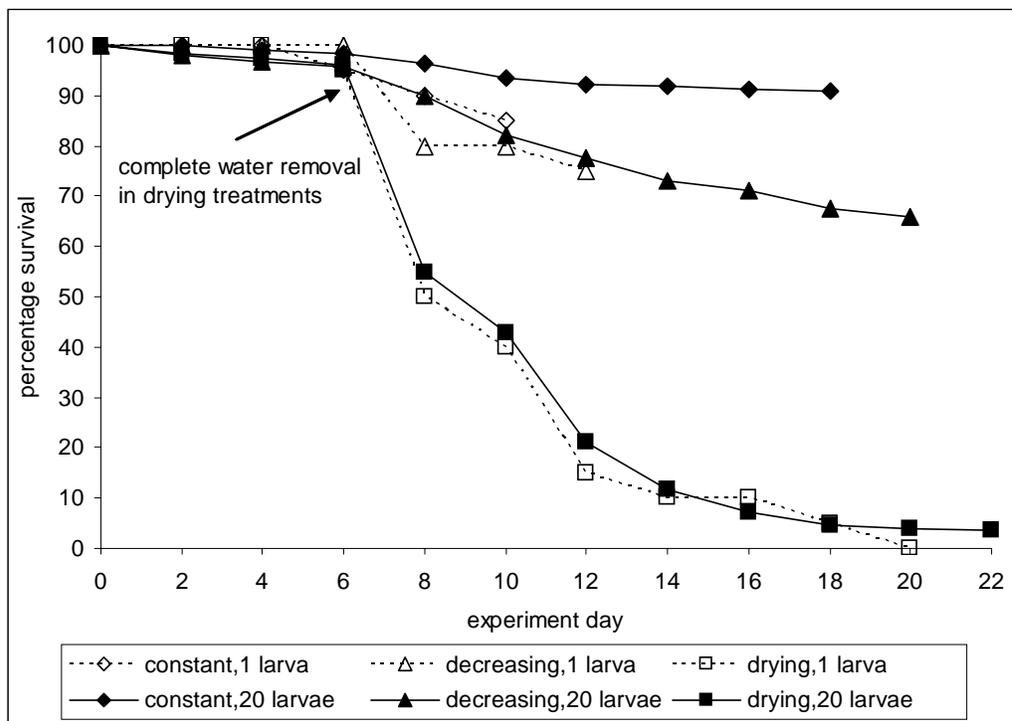


Figure 1. Percentage survival over time of floodwater mosquitoes subject to three water schedules (constant, decreasing and decreasing followed by drying out) and two larval densities (1 and 20 larvae).

*Ae. vexans* adults was smaller in the treatments with 20 larvae.

The observed differences in the responses of *Ae. vexans* and *Oc. sticticus* to decreasing water levels might indicate alternative survival strategies by co-existing species. In inundation areas of large rivers, one of these two species is often predominant. The water level of the River Dalälven was continuously decreasing after larval collections. In the experiment, *Ae. vexans* was the dominant species. However, adult sampling by CDC miniature light traps at a location approximately 1,500 m away from our larval collection sites at the end of July resulted in higher numbers of *Oc. sticticus* (42% of total catch) than *Ae. vexans* (5% of total catch) (unpublished data). One might speculate that the natural conditions during June and July 2003 favored the species with fast development (*Oc. sticticus*) over the one with the "sit and wait" strategy (*Ae. vexans*). It is unclear to what extent the reactions of the two floodwater species represent strategic responses or plasticity. From this respect, it would also be desirable to test the responses of other mosquito species, such as *Culex pipiens* that utilize more stable water bodies, to diminishing larval habitat and to test larval survival ability in humid soil.

In the field, the effect of diminishing water levels could be mediated through increased water temperature as the water volume decreases. Larval development time decreases with increasing water temperature, and this temperature effect was not included in our experiment. At temperatures around 20–25°C, it takes *Ae. vexans* about 9–11 days to complete adult emergence (Read and Moon 1996). Trpis and Shemanchuk (1970) reported that the fastest larval development of *Ae.*

*vexans* occurred at 25°C with pupation after 7 days. Larval development time becomes crucially important when larval mosquito control measurements should be conducted. During the summer months, control measures could be restricted to less than 7 days after observations of first stage larvae (Trpis and Shemanchuk 1970). The results of this study indicate that decreasing water levels followed by larval habitat drying might not necessarily result in low adult mosquito population densities. Since larval survival ability in humid soil is high, rainfall within 14 days after desiccation could still enable adult mosquito emergence. Further experiments are needed to clarify the ability of different immature stages to survive waterless periods and to complete metamorphosis with adult emergence.

#### Acknowledgments

We thank Karin Lindström and Darold Batzer for valuable comments on the manuscript. This study was financially supported by grants from the Zoological Foundation of Uppsala University to M.L.S. and by a grant from the Swedish Environmental Protection Agency to J.O.L.

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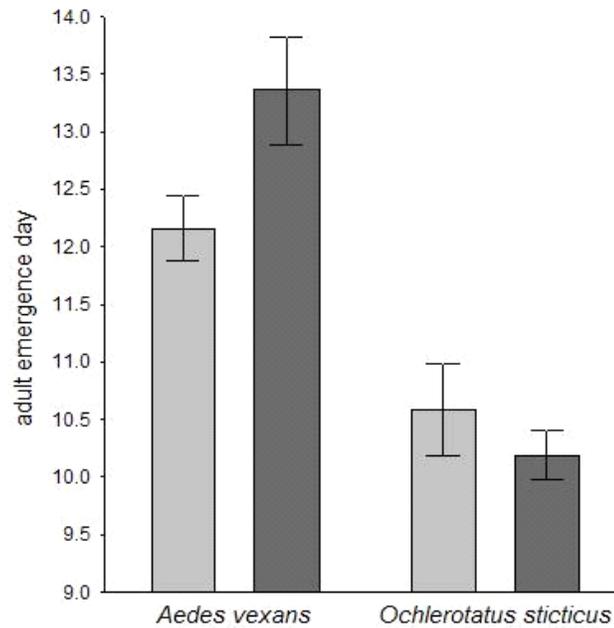


Figure 2. The effect of water schedule on mean time to adult emergence of *Aedes vexans* and *Ochlerotatus sticticus*. Light grey bars represent constant water level schedule, dark grey bars represent decreasing water level schedule. Error bars show 95% confidence intervals.

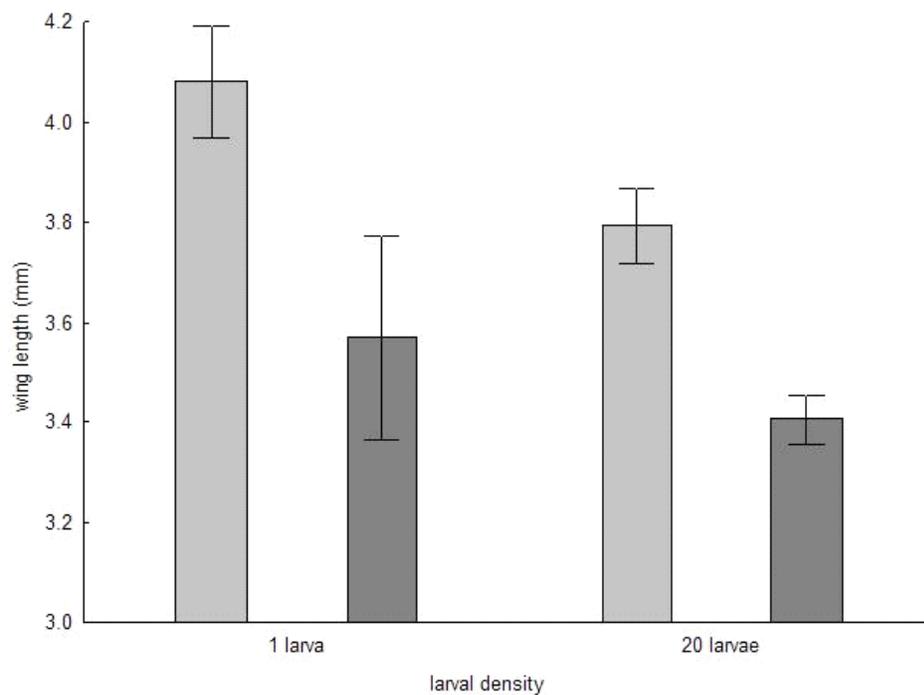


Figure 3. The influence of water schedule and larval density on mean adult size measured as wing length of *Aedes vexans*. Light grey bars represent constant water level schedule, dark grey bars represent decreasing water level schedule. Error bars show 95% confidence intervals.

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