

# Oviposition habitat selection by the mosquito *Culiseta longiareolata* in response to risk of predation and conspecific larval density

MOSHE KIFLAWI<sup>1</sup>, LEON BLAUSTEIN<sup>1</sup> and MARC MANGEL<sup>2</sup> <sup>1</sup>Institute of Evolution, Faculty of Sciences, University of Haifa, Israel and <sup>2</sup>Department of Applied Mathematics and Statistics, Jack Baskin School of Engineering, University of California, Santa Cruz, U.S.A.

**Abstract.** 1. Most female *Culiseta longiareolata* (Diptera: Culicidae) avoid ovipositing in pools that contain the predatory backswimmer *Notonecta maculata*. Such oviposition habitat selection has been suggested to reflect a trade-off between the risk of predation on larvae and potential density-dependent fitness costs. This putative trade-off was examined. In particular, evidence was sought in support of direct female response to local heterogeneity in habitat quality.

2. Three habitat types were established using artificial outdoor pools: predator pools, and non-predator pools with either low or high densities of *Culiseta* larvae. During each experimental night, females were offered one of the three possible pair-wise treatment combinations.

3. The majority ( $\approx 88\%$ ) of females oviposited in low-density pools rather than in the predator- or high-density pools. Furthermore, a substantially higher proportion of females oviposited in predator pools when faced with the high-density alternative, however this was due largely to fewer females ovipositing in high- vs low-density pools.

4. Females of a second mosquito species (*Culex laticinctus*), the larvae of which are at a lower risk of predation, were predicted to exhibit weaker aversion to *N. maculata*; this prediction was supported only weakly.

5. Oviposition habitat selection by female *C. longiareolata* does not appear to involve a behavioural response that is based on individual assessment of local heterogeneity in *relative* pool quality, at least not at the spatial scale examined here; alternative explanations are discussed.

**Key words.** *Culex*, *Notonecta*, oviposition habitat selection, temporary pools, trade-off.

## Introduction

Parental capacity to enhance offspring survivorship and fecundity contributes, by definition, to parental fitness. In organisms with no parental care or juvenile dispersal, offspring survival and growth may depend strongly on the quality of the habitat in which they are deposited. Thus,

when potential habitats vary in their suitability for juveniles, females are expected to choose habitats that maximise their fitness. In mosquitoes, such oviposition habitat selection has been demonstrated in response to physical and chemical suitability for larval development (e.g. Trimble, 1979), habitat size and resource availability (e.g. Blaustein & Kotler, 1993; Heard, 1994), the presence and density of conspecific competitors (e.g. Edgerly *et al.*, 1998), and the presence of predators (e.g. Chesson, 1984; Blaustein *et al.*, 1995).

On the other hand, density dependence of fitness components such as survival and growth (e.g. Renshaw *et al.*, 1994; Lord, 1998; Mercer, 1999) implies that as more females

Correspondence: Leon Blaustein, Institute of Evolution, Faculty of Sciences, University of Haifa, Haifa 31905, Israel. E-mail: leon@research.haifa.ac.il

choose the same subset of available habitats, the value of preferred habitats will decline. Such density-dependent habitat quality is expected to lead to habitat utilisation that approaches the *ideal free* (*sensu* Fretwell & Lucas, 1970), guaranteeing fitness returns that are independent of oviposition site. Thus, at equilibrium, the ratio of females ovipositing in habitats with and without predators, for example, is expected to balance the risk of predation on offspring and density-dependent offspring growth and survival rates (Spencer *et al.*, 2002).

Females of the mosquito species *Culiseta longiareolata* avoid ovipositing in pools that contain the backswimmer *Notonecta maculata*, which preys on its larvae. Faced with a choice between an equal number of *predator* and *predator-free* pools,  $\approx 90\%$  of females oviposit in the pools that lack the predator (e.g. Blaustein *et al.*, 1995; Blaustein, 1998). This incomplete avoidance of predator pools has been hypothesised to reflect the trade-off between the risk of predation and the detrimental effects of larval density (Spencer *et al.*, 2002). The work reported here tested this hypothesis. In particular, evidence was sought in support of direct female response to local heterogeneity in habitat quality.

Artificial pools were used to look for a shift towards increased use of predator pools as *Culiseta* larval densities in the alternative, predator-free pools, were changed from practically nil to high. In addition, a comparison was made with a second mosquito species, *Culex laticinctus*, the larvae of which are less prone to predation by *N. maculata* (Blaustein, 1998). Reduced larval susceptibility, presumably due to lower movement rates (L. Blaustein, unpublished), was expected to result in a higher proportional use of predator pools by *Culex* females faced with a benign alternative. The potential for density-dependent fitness costs was assessed by comparing the size at metamorphosis of *Culiseta* reared under conditions of low and high larval densities.

## Methods

### *Experimental set-up and procedure*

A rectangular array of 18 plastic tubs ( $0.6 \times 0.4 \times 0.15$  m) was set up in the Mt Carmel Nature Reserve, Israel, on 14 May 2000. Each tub was separated by  $\approx 30$  cm from its two flanking neighbours. The water level in these artificial pools was established and maintained using aged (24 h) tap water. An initial inoculate of  $\approx 8$  l of re-suspended sediment and algae, obtained from artificial pools that had been standing for  $\approx 1$  month, was introduced into each of the 18 tubs. A large shading net (mesh density: 90%), placed  $\approx 2$  m above the entire array, kept water temperatures within the pools from rising above  $26^\circ\text{C}$ .

Proceeding around the array, pools were assigned sequentially to one of three treatments: (1) no predator, low *Culiseta* larval density (LO), (2) no predator, high *Culiseta* larval density (HI), (3) predator-present (NT) (see

details below). Prior to sundown on nights during which oviposition was monitored, the six pools belonging to one of the three treatments were covered, leaving females to choose between the two alternatives. On the following mornings, the egg rafts found in the exposed pools were recorded and removed, and all 18 pools were left uncovered. Egg rafts were brought back to the laboratory, and the emerging larvae were reared for species identification. Starting on 16 May, and over a period of 1 month, the low-density/predator, low-density/high-density, and high-density/predator combinations were examined on 6, 7, and 8 nights respectively. When interpreting the observed oviposition patterns, it was assumed that females of both mosquito species oviposit a single egg raft (van Pletzen & van der Linde, 1981; Clements, 1999).

High-density pools were stocked using larvae hatched from egg rafts oviposited in the field and reared in the laboratory. Initial densities of instar I–IV *Culiseta* larvae averaged  $89\text{ l}^{-1}$  ( $\text{SD} = 35$ ) (such high densities are often observed in nature; L. Blaustein, pers. obs.). Densities were sampled periodically and, in an attempt to account for natural mortality, stage I and II larvae were added regularly; however initial mortality rates greatly exceeded the rate at which the larvae were replenished, resulting in average densities of  $31 \pm 12$ ,  $34 \pm 10$ , and  $25 \pm 8\text{ l}^{-1}$ , 1, 2, and 3 weeks later respectively. Larval densities in the low-density pools were continuously well below  $11\text{ l}^{-1}$ . Density estimates were based on sampling with a  $5 \times 7$  cm net (mesh size:  $250\ \mu\text{m}$ ), having established, in controlled trials, that a single figure-of-eight sweep captures an average of 43% ( $\pm 5\%$ ) of the available larvae. The number of predators in predator pools, third- to fifth-instar *N. maculata*, was kept constant at three. Predator pools contained virtually no mosquito larvae, leaving *N. maculata* instars to feed on other aquatic invertebrates, as well as terrestrial invertebrates that found their way into the pools.

To assess density-dependent effects on larval performance, *Culiseta* pupae were collected from the low- and high-density pools, towards the end of the experimental period, transferred to emergence chambers, and left to metamorphose. The emerged adults were asphyxiated using chloroform, transferred to glass vials, and left to dry. The lengths of one wing of up to 10 males and 10 females, per pool, were measured to the nearest 0.1 mm using the WScanArray image analyser (Galai Production Ltd, Migdal Haemek Israel). Low pupation rates in high-density pools, and low larval densities in low-density pools, resulted in some pools having less than 10 wing measurements per sex, or even none at all (average number of *Culiseta* per pool, per sex:  $6.1 \pm 2.8$ ). A lack of sufficient subjects prevented a statistically rigorous analysis of *Culex*.

### *Oviposition analysis*

Normalised likelihood functions (SL) were calculated for the proportion ( $P$ ) of egg rafts oviposited in one treatment

of each of the paired alternatives. Corresponding to Bayesian posteriors with uniform priors (Hilborn & Mangel, 1997), these functions are given by:

$$SL(P_i|K, N) = \frac{P_i^K (1-P_i)^{(N-K)}}{\sum P_i^K (1-P_i)^{(N-K)}} \quad (1)$$

$$0 \leq P_i \leq 1$$

where  $N$  is total number of egg rafts oviposited during all of the days on which a particular treatment pair was offered, and  $K$  is the corresponding number of egg rafts oviposited in the pools belonging to one of the two treatments (note that in the denominator, the integral is approximated by summation in steps of  $dP=0.01$ ). These functions were used to determine confidence intervals that delimit the range of proportions that express the extent of female preference for the focal treatment. The maximum likelihood estimates of  $P$  ( $P_{MLE}$ ) equal the observed proportions.

Indication as to how likely the two species are to differ in habitat selection, when faced with any particular treatment pair, was obtained by evaluating the likelihood ratio:

$$R = \frac{L(P_{MLE,cs}|K_{cs}, N_{cs}) L(P_{MLE,cx}|K_{cx}, N_{cx})}{L(P_{MLE,cs+cx}|K_{cs+cx}, N_{cs+cx})} \quad (2)$$

where  $L$  is the likelihood of  $P_{MLE}$ . The subscripts  $cs$ ,  $cx$ , and  $cs+cx$  denote *Culiseta*, *Culex*, and the pooled data respectively. In other words, the likelihood that the two species differ in their preference was compared against the likelihood that they share the same preference. A similar analysis was used for within-species comparison of female tendency to oviposit in low-density pools, when paired with either predator or high-density pools. In all cases, higher values of  $R$  ( $R \geq 1$ ) imply stronger evidence in favour of a difference.

#### Diagnostic analysis

The above analyses assume that, throughout the experimental period, oviposition events are independent of each other. Two factors could violate this assumption. (1) Among experimental nights: some of the low-density pools are either more attractive (e.g. given possible heterogeneity in resource availability) and/or are more likely to be encountered (e.g. due to their position within the array). (2) Within experimental nights: as in other mosquito species (Clements, 1999), females may be attracted to freshly oviposited conspecific egg rafts. The assumption of independence was assessed by: (1) looking at the among-pool concordance in the daily number of egg rafts received, and (2) comparing the frequency distribution of the daily number of egg rafts oviposited, per pool, against the null expectation of a Poisson distribution; a tendency to aggregate egg rafts was expected to be manifested even when pooling data across experimental nights.

## Results

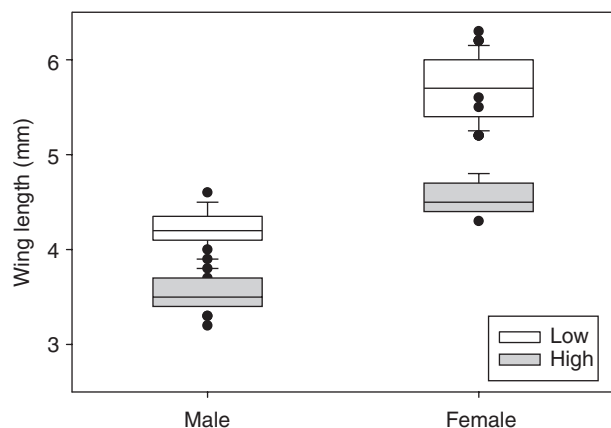
### The effect of larval density

High larval densities caused a significant decrease in the average (per pool) wing length of both males and females, with females suffering a larger decrease [split-plot ANOVA with sex and larval density (high and low) as the within- and between-pool factors respectively; density,  $F_{1,8}=142.3$ ,  $P<0.001$ ; density-sex interaction,  $F_{1,8}=15.9$ ,  $P<0.01$ ] (Fig. 1). In relative terms, the average wing length of males and females decreased by  $\approx 24$  and  $28\%$  respectively.

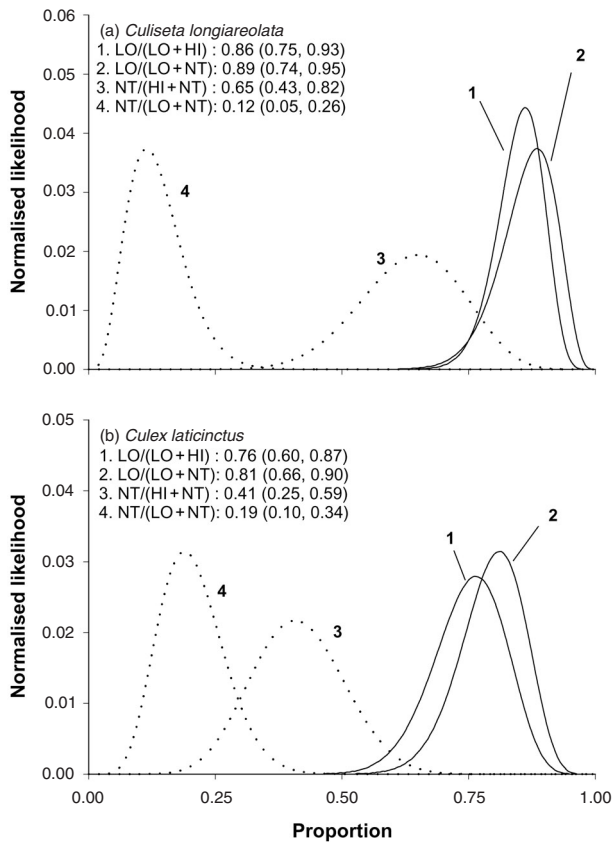
### Oviposition analysis

Both *Culiseta* and *Culex* oviposited preferentially in low-density pools, discriminating against both predator and high-density pools (Fig. 2), with only weak evidence in support of *Culiseta* exhibiting stronger preference in either case (predator:  $R=1.5$ ; high density:  $R=2.0$ ). For neither species did the proportion of females ovipositing in low-density pools seem to depend on whether the alternative comprised predator or high-density pools (*Culiseta*:  $R=1.05$ ; *Culex*:  $R=1.12$ ).

The proportion of females ovipositing in predator pools was markedly higher when these were paired with high-density, rather than low-density, pools, with *Culiseta* showing the more pronounced difference (*Culiseta*:  $R=2 \times 10^3$ ; *Culex*:  $R=6.2$ ; Fig. 2), however this change did not result from the redistribution of a fixed number of egg rafts among paired treatments. Rather, the average daily number of egg rafts oviposited in predator pools was independent of the alternative treatment (two-way ANOVA of square-root transformed numbers, density effect:  $F_{1,24}=0.02$ , NS; species-density interaction:  $F_{1,24}=0.01$ , NS), whereas significantly fewer egg rafts were oviposited in high-density pools



**Fig. 1.** Box plots (median, 10th, 25th, 75th, and 90th percentiles) depicting per-pool average wing lengths of male and female *Culiseta longiareolata* that, as larvae, developed under conditions of high and low conspecific densities.



**Fig. 2.** Normalised likelihood functions of the proportion of *Culiseta longiareolata* and *Culex laticinctus* females that oviposit in pools belonging to one of two treatments. Solid curves compare oviposition in low *Culiseta* larval density pools (LO), when paired with either predator pools (NT) or high *Culiseta* larval density pools (HI). Dotted curves depict the shift in the proportion of females that oviposit in predator (NT) pools, as *Culiseta* densities in the alternative set of pools are changed from low to high. The maximum likelihood estimate ( $P_{MLE}$ ) is provided for each treatment pair, together with the lower and upper confidence intervals (in parentheses).

than in low-density pools, lowering the total (density effect:  $F_{1,24} = 10.24$ ,  $P < 0.01$ ; species–density interaction:  $F_{1,24} = 0.83$ , NS) (Fig. 3). Note that even if a true difference in the mean number of egg rafts oviposited in predator pools went undetected due to low power ( $\beta = 75\%$ ; Fig. 3a), it could not account for the difference observed between the low-density and high-density pools (Fig. 3b).

#### Diagnostic analysis

Because low-density pools received the vast majority of all egg rafts, the analysis was limited to these six pools. During the 13 nights in which low-density pools were offered, each pool received between 5 and 27% of the 81 egg rafts oviposited by *Culiseta*; however, there was no statistical indication for consistent differential use of pools

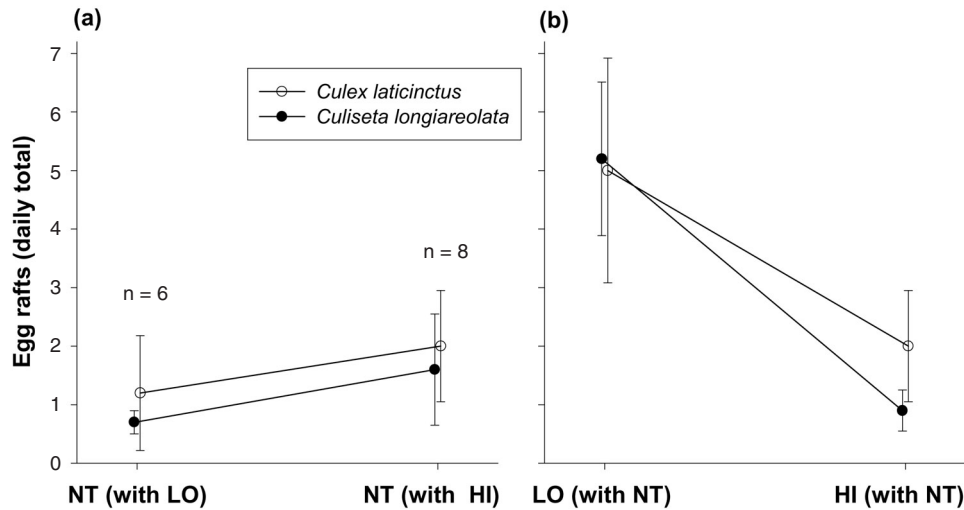
across nights (Kendall's coefficient of concordance,  $W = 0.25$ ,  $P = 0.116$ ). Forty-eight per cent of the 56 *Culex* egg rafts oviposited during the same period were found in a single pool. The remaining pools received between 6 and 25% of the egg rafts, with no statistical indication for consistent differentiation among pools other than the one idiosyncratic pool ( $W = 0.18$ , NS).

The frequency distribution of the number of *Culiseta* egg rafts per pool did not differ significantly from the Poisson [Chi square goodness of fit:  $\chi^2 = 7.5$ , d.f. = 5,  $P > 0.15$ ; with 9% probability of type II error at the  $\alpha = 0.20$  level (power analysis followed Cohen, 1988)]. The distribution for *Culex* did differ significantly from the Poisson, regardless of whether or not the idiosyncratic pool was included in the analysis ( $\chi^2 = 18.4$ , d.f. = 5,  $P < 0.01$  and  $\chi^2 = 70.1$ , d.f. = 4,  $P < 0.001$  respectively). In both cases, however, the main contribution to the deviation was a preponderance of single egg rafts and the absence of double egg rafts. Thus, it would seem that such a tendency to aggregate egg rafts, to the extent that it exists in *Culiseta* and *Culex*, is of little consequence to inferences regarding oviposition habitat selection.

#### Discussion

Both *Culiseta* and *Culex* females oviposited preferentially in low-density, predator-free pools, avoiding pools that contained either predators or high larval densities (Fig. 2). This result is not unexpected. Mosquito larvae experience high mortality rates in the presence of notonectids (Chesson, 1984; Blaustein, 1998; Eitam *et al.*, 2002), whereas high densities decrease larval survivorship, development rate, and size at metamorphosis (e.g. Bradshaw & Holzapfel, 1992; Renshaw *et al.*, 1994; Lord, 1998; Mercer, 1999; this study: Fig. 1). Adult body size, in turn, often correlates positively with longevity and female fecundity (e.g. Bradshaw & Holzapfel, 1992; Lyimo & Takken, 1993; Ameneshewa & Service, 1996). The main goal in this study, however, was to examine whether females trade off between the two potential risk factors based on their assessment of local oviposition-habitat availability.

A shift in proportional use of predator pools, which is due to a difference in larval densities in the non-predator alternative (Fig. 2), would have implied that females adjust their choice to suit *relative* habitat quality only if the total number of oviposition events remained constant. This was not the case here (Fig. 3). Rather, considerably fewer egg rafts were oviposited, on average, when females had to choose between predator and high-density pools, than when choosing between predator and low-density pools. This pattern does not negate the proposition that oviposition habitat selection reflects the trade-off between the risk of predation and density-dependent effects. It is possible, for example, that at least some of the females that visited the experimental site considered it as only part of their available options. Conversely, females may not compare their options, *per se*, but rather utilise the different habitat



**Fig. 3.** (a) The daily average ( $\pm 1$  SE) number of egg rafts collected from the six *Notonecta maculata* pools (NT), for the  $n$  nights on which they were offered alongside six other pools containing either high (HI) or low (LO) densities of larval *Culiseta longiareolata*. (b) The corresponding daily average number of egg rafts collected from either the high-density or the low-density pools.

types with specific probabilities that, potentially, balance the risk of predation and the effect of high larval densities (Spencer *et al.*, 2002).

Consider the second scenario, assuming that females arrive at the array at a rate that is independent of the treatment combination on offer. Assume further that individual females limit the number of pools they visit before foregoing oviposition and leaving the array. Given, for example, a 0.1 probability of ovipositing in predator and high-density pools (compared with 1.0 in low-density pools), and an average of two oviposition attempts per female, it can be shown that: (1) the expected oviposition rates given low-density/high-density and low-density/predator combinations should be four times higher than with the high-density/predator combination, (2) the expected daily average number of egg rafts oviposited in predator pools should be twice as high with the high-density/predator combination than with the low-density/predator combination, and (3) the proportion of egg rafts oviposited in low-density pools, paired with either predator or high-density pools, should be  $\cong 0.91$ . All three predictions are at least qualitatively consistent with the data (Figs 2 and 3).

The avoidance of predator pools by *Culex*, when faced with the low larval-density alternative, was weaker than that of *Culiseta* (81 vs 89% respectively). While the latter is expected to show the greater avoidance, based on differential larval susceptibility to predation, the two species are only 1.5 times more likely to differ than to coincide in the extent of their aversion to *N. maculata*. Concomitantly, female *Culex* exhibited lower aversion to the high, vs low, larval density treatment (76 vs 86% respectively), possibly due to differential dietary requirements (van Pletzen, 1981; Clements, 1992). Thus, the difference between the two species in their proportional use of predator pools, when paired

with the high-density alternative (Fig. 2), may reflect a difference in their response to each risk factor separately, rather than a difference in their perception of relative pool quality.

In summary, while oviposition habitat selection in *C. longiareolata* may reflect an adaptive balance between two risk factors, predation and density-dependent larval growth/survival, individual females are not adjusting their decisions to suit local heterogeneity in habitat quality. Rather, individuals appear to respond, in a probabilistic manner, to the absolute value of potential oviposition sites, not to their relative value. Oviposition habitat selection can have significant demographic consequences (Spencer *et al.*, 2002), which may carry over to the community level (Blaustein, 1999). Understanding the mechanism of oviposition habitat selection would not only enhance understanding of the structure of ephemeral communities, such as those found in temporary pools, but may also inform pest-control policies.

#### Acknowledgements

We thank A. Eitam, L. Green, and Y. Maclade for assistance during fieldwork; and M. Spencer, J. E. Cohen, and J. S. Brown for valuable discussions. M. Spencer and two anonymous referees offered helpful comments on an earlier version of the manuscript. The study was supported by U.S.–Israel Binational Science Foundation grant 98-390, awarded to L. Blaustein and M. Mangel, and benefitted greatly from the assistance offered by A. Luria and the Nature Reserve Authority of Israel.

## References

- Amenesheva, B. & Service, M.W. (1996) The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia. *Medical and Veterinary Entomology*, **10**, 170–172.
- Blaustein, L. (1998) Influence of the predatory backswimmer, *Notonecta maculata*, on invertebrate community structure. *Ecological Entomology*, **23**, 246–252.
- Blaustein, L. (1999) Oviposition site selection in response to risk of predation: evidence from aquatic habitats and consequences for population dynamics and community structure. *Evolutionary Theory and Processes: Modern Perspectives* (ed. by S. P. Wasser), pp. 441–456. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Blaustein, L. & Kotler, B.P. (1993) Oviposition habitat selection by the mosquito, *Culiseta longiareolata*: effects of conspecifics, food and green toad tadpoles. *Ecological Entomology*, **18**, 104–108.
- Blaustein, L., Kotler, B.P. & Ward, D. (1995) Direct and indirect effects of a predatory backswimmer (*Notonecta maculata*) on community structure of desert temporary pools. *Ecological Entomology*, **20**, 311–318.
- Bradshaw, W.E. & Holzapfel, C.M. (1992) Reproductive consequences of density-dependent size variation in the pitcher plant mosquito, *Wyeomyia-smithii* (Diptera: Culicidae). *Annals of the Entomological Society of America*, **85**, 274–281.
- Chesson, J. (1984) Effect of *Notonecta* (Hemiptera: Notonectidae) on mosquitoes (Diptera: Culicidae): predation or selective oviposition? *Environmental Entomology*, **13**, 531–538.
- Clements, A.N. (1992) *The Biology of Mosquitoes, Vol. 1: Development, Nutrition and Reproduction*. Chapman & Hall, London.
- Clements, A.N. (1999) *The Biology of Mosquitoes, Vol. 2: Sensory Reception and Behaviour*. CABI Publishing, Wallingford, U.K.
- Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates, Hillsdale, New Jersey.
- Edgerly, J.S., McFarland, M., Morgan, P. & Livdahl, T. (1998) A seasonal shift in egg-laying behaviour in response to cues from future competition in a tree hole mosquito. *Journal of Animal Ecology*, **67**, 805–818.
- Eitam, A., Blaustein, L. & Mangel, M. (2002) Effects of *Anisops sardea* (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia*, **485**, 183–189.
- Fretwell, S.D. & Lucas, H.J. (1970) On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica*, **19**, 16–36.
- Heard, S.B. (1994) Imperfect oviposition decisions by the pitcher plant mosquito (*Wyeomyia-smithii*). *Evolutionary Ecology*, **8**, 493–502.
- Hilborn, R. & Mangel, M. (1997) *The Ecological Detective: Confronting Models with Data*. Princeton University Press, Princeton, New Jersey.
- Lord, C.C. (1998) Density dependence in larvae of *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, **35**, 825–829.
- Lyimo, E.O. & Takken, W. (1993) Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles-gambiae* females in Tanzania. *Medical and Veterinary Entomology*, **7**, 328–332.
- Mercer, D.R. (1999) Effects of larval density on the size of *Aedes polynesiensis* adults (Diptera: Culicidae). *Journal of Medical Entomology*, **36**, 702–708.
- van Pletzen, R. (1981) Larval and pupil behaviour in *Culiseta longiareolata* (Macquart) (Diptera: Culicidae). *Journal of the Limnological Society of Southern Africa*, **7**, 24–28.
- van Pletzen, R. & van der Linde, T.C.K. (1981) Studies on the biology of *Culiseta longiareolata* (Macquart) (Diptera: Culicidae). *Bulletin of Entomological Research*, **71**, 71–79.
- Renshaw, M., Service, M.W. & Birley, M.H. (1994) Size variation and reproductive success in the mosquito *Aedes cantans*. *Medical and Veterinary Entomology*, **8**, 179–186.
- Spencer, M., Blaustein, L. & Cohen, J.E. (2002) Oviposition habitat selection by mosquitoes *Culiseta longiareolata* and consequences for population size. *Ecology*, **83**, 669–679.
- Trimble, R.M. (1979) Laboratory observations on oviposition by the predaceous tree-hole mosquito, *Toxorhynchites rutilus septentrionalis*. *Canadian Journal of Zoology*, **57**, 1104–1108.

Accepted 14 December 2002