Oviposition habitat selection for a predator refuge and food source in a mosquito

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Abstract. 1. The influence of filamentous algae on oviposition habitat selection by the mosquito *Anopheles pseudopunctipennis* and the consequences of oviposition decisions on the diet, development, body size, and survival of offspring were examined.

2. A natural population of *An. pseudopunctipennis* in Chiapas, Mexico, oviposited almost exclusively in containers with filamentous algae. Algae represented 47% of the gut contents of mosquito larvae sampled from the natural population. Mosquito larvae fed on an exclusive diet of algae developed as quickly and achieved the same adult body size (wing length) as their conspecifics fed on a standard laboratory diet.

3. Multiple regression of survival of mosquito larvae on percentage surface area cover of algae (0–99%) and the density of predatory fish (zero to four fish per container) was best described by a second-order polynomial model. Increasing fish densities resulted in a reduction in mosquito survival in all algal treatments. The highest incidence of survival was observed at intermediate (66%) algal cover in all treatments.

4. The presence of fish significantly extended larval development times whereas algal cover had no significant effect. The presence of fish resulted in emergence of smaller adults due to reduced feeding opportunities and predator avoidance behaviour. Algal cover also affected mosquito wing length but differently at each fish density.

5. Oviposition habitat selection improves survival in the presence of predators and feeding opportunities for *An. pseudopunctipennis* larvae.

Key words. *Anopheles pseudopunctipennis*, *Cladophora glomerata*, feeding, fish, gut analysis, offspring survival, oviposition, predators, *Spirogyra majuscula*.

Introduction

Ovipositing insects should select sites that improve the survival, growth, and reproductive potential of the offspring, especially for species in which juveniles are incapable of migrating away from low quality habitats (Peckarsky *et al*., 2000). In the absence of inter- or intraspecific competition, progeny fitness will depend on two factors: an adequate food supply and the presence of natural enemies (predators, parasites, and pathogens). As a result, insects often avoid ovipositing in sites where there is a high risk of predation to their progeny (Blaustein, 1999; Angelon & Petranka, 2002; Spencer *et al*., 2002; Kiflawi

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et al., 2003). Oviposition habitat selection is particularly relevant in insect vectors of medical importance as it determines the localities to which larvicidal control measures are targeted (Service, 1993).

Understanding the factors that regulate the size of mosquito populations is considered fundamental to the ability to predict transmission rates and for vector population control (Service, 1989, 1995). Aquatic vegetation can exert an important influence on the reproduction of mosquitoes (Orr & Resh, 1989, 1992; Kant *et al.*, 1996). This may involve facilitating oviposition (Orr & Resh, 1992; Rejmankova *et al.*, 1996) or by providing a larval micro-habitat (Orr & Resh, 1992) from which to escape the attention of predators (Sih, 1986; Orr & Resh, 1989). Mosquitoes may also select oviposition sites based on the availability of larval food (Blaustein & Kotler, 1993). Studies of the factors that determine anopheline larval population densities have almost invariably assumed that aquatic vegetation represents an important food resource for larvae (Hall, 1972; Rejmankova *et al.*, 1992; Marten *et al.*, 1996), although anopheline feeding on filamentous algae is poorly studied.

Environmental management based on ecological principals represents an effective and expedient intervention for insect vector control (Ault, 1994). For example, a direct linear relationship between anopheline larval densities and percentage of breeding pool cover by filamentous algae in southern Mexico has recently been reported (Bond *et al.*, 2004b). Manual extraction of algae from anopheline breeding pools resulted in a marked reduction in larval and adult population densities that was sustained for approximately 2 months, by which time seasonal rains began to physically remove larvae from breeding sites.

The mosquito *Anopheles pseudopunctipennis* Theobald (Diptera: Culicidae) is an important vector of *Plasmodium* spp. in tropical regions of the Americas (Fernández-Salas *et al.*, 1994a,b). An intimate association between filamentous algae and immature stages of *An. pseudopunctipennis* has been reported throughout the geographic range of this mosquito (Rejmankova *et al.*, 1991, 1993; Manguin *et al.*, 1996). The present study was designed to examine the influence of filamentous algae on oviposition habitat selection by *An. pseudopunctipennis* and the consequences of oviposition decisions on the diet, development, body size, and survival of offspring in the presence of predators.

Methods

Study site

Field studies were performed in a large concrete water storage pool 20 m × 20 m × 1.5 m deep with water, in the village of El Plan – Las Palmas, Municipality of Tapachula Chiapas, Mexico (15 °03′81″N, 92 °12′40″W), at an altitude of 315 above sea level. The pool was continuously fed by a small stream, a tributary of the River Coatán, which flows past the village at a distance of ≈ 300 m

from the water storage pool. Temporary riverside pools along the River Coatán are primary breeding sites of *An. pseudopunctipennis* during the dry season. The climate in this region is tropical sub-humid, with two well-defined seasons: a rainy season from May to October and a dry season from November through April. The annual average rainfall is 3800 mm, the average annual temperature is 25 °C, and the relative humidity is 70–90% for most of the year. Field studies were performed from November 1996 to July 1997.

Influence of filamentous algae on oviposition habitat selection

Experimental units consisted of PVC tubes (25 cm diameter and 37.5 cm high) with two windows on the sides (5 cm high × 20 cm wide) and a polystyrene foam float glued to the sides of the tube. A fine nylon mesh was glued to the bottom and over the side windows to allow the flow of water and to prevent the escape of mosquito larvae. Ten tubes were tied to a string and fastened to the sides of the pool and submerged so that 10 cm of the tube remained above the water level. Five of the tubes were treated with algae and the remaining five tubes were left as untreated controls. Treatment with algae consisted of placing 170 g (wet weight) of *Spirogyra majuscula* Kützing in each tube to give approximately 66% cover of the water surface. Tubes were collected 3 days later and the number of *An. pseudopunctipennis* larvae was recorded by microscopic examination of the contents of each tube in a field laboratory. The algae from each tube were placed in trays covered with muslin and incubated in the field laboratory for a further 7 days to permit hatching of all viable eggs. An identical procedure was performed using *Cladophora glomerata* (L.). Overall, each species of algae was tested five times in an alternating sequence. The absence of oviposition in tubes lacking algae prevented statistical analysis; the total number of first-instar larvae observed in each replicate (data pooled for all five tubes) was compared for each species of alga by Mann–Whitney *U*-test using the SPSS v.10.0 package (SPSS, 1995).

Role of filamentous algae in the diet and development of larvae

(i) Analysis of larval gut contents. Fourth-instar *An. pseudopunctipennis* larvae were collected from 80 breeding pools (20 larvae per pool), randomly selected within a 6-km section of the River Coatán. Larvae were transported to the laboratory in a cool-box and killed by brief immersion in hot water. The food bolus enclosed in the peritrophic membrane was removed from each larval gut by dissection and placed in groups of 20 in plastic vials with 2 ml of sterile water. Vials were briefly sonicated in an ultrasonic bath to disrupt aggregates. Samples were then stained with DAPI (4′,6-diamidino-2-phenylindole) (Sigma, St Louis, Missouri) at a final concentration of 2 μg ml^{-1}, and placed in darkness at 4 °C for 25 min. The contents of each vial

were then passed through a 25-mm diameter black poly-carbonate filter (Millipore, Bedford, Massachusetts) of 0.45 μm pore size. Each black filter was placed on an oiled microscope slide, covered with immersion oil and a coverslip and examined using a Reichert–Jung Diastar epifluorescence microscope with a ×40 objective. Stained particles were counted in 50 randomly chosen fields of view for each slide. Particles could be differentiated into four categories by their staining: (i) bacteria (blue), (ii) protozoa (blue with distinctive nuclei), (iii) organic detritus (yellow), and (iv) algae (chlorophyll stains red). The number of debris particles, algal fragments and bacterial cells per gut were quantified by counting 50 fields of view, each of 40×40 μm area, from each polycarbonate filter following the method of Walker *et al.* (1988) but with no dilution factor correction. The process was repeated for each of the groups of larvae from each of the pools sampled.

(ii) Suitability of Spirogyra majusoula for larval development. Groups of 50 first-instar *An. pseudopunctipennis* larvae were obtained from a continuously reared laboratory colony maintained in the Centro de Investigación de Paludismo, Tapachula, Chiapas, Mexico (Villarreal *et al.*, 1998). These larvae hatched within the previous 24 h and were transferred to plastic trays $(25 \times 15 \times 5$ cm) with 250 ml of distilled water. Larvae were offered either 1250 mg day^{-1} *S. majuscula* or a standard laboratory diet offered at the quantities used for rearing the laboratory colony (30 mg day^{-1} during the first and second instars and 90–120 mg day^{-1} during the third and fourth instars). To eliminate bacteria, algae were subjected to an antibiotic treatment involving 50 U ml^{-1} penicillin G and 50 μg ml^{-1} streptomycin sulphate for 48 h followed by extensive washes prior to use in experiments. The standard laboratory colony diet consisted of ground rodent food, mainly comprising ground cereals and soya (23% protein, 6.0% oil, 60% fibre, 4.9% sucrose, 6.1% mineral salts, by weight). Larvae were reared in the laboratory at 28 ± 2 °C and L:D 14:10 h photoperiod. Trays were checked daily and pupae were transferred to water filled plastic cups in emergence cages. The immature development time, from being placed in rearing trays to adult eclosion was recorded. The number and sex of emerging adults was noted daily. Following emergence, the wing length of each mosquito was measured from the axial incision to the apical margin. The fimbrial scales were not included in this measurement and the wing was selected from the left or right side of the body at random. Wing length was taken as an indicator of body size; the cubic function (a dimensionless number) was calculated for statistical analysis as recommended by Briegel (1990a,b). The experiment was performed five times. Results were subjected to *t*-tests with equal and unequal variances, as required.

Filamentous algae as a predator refuge

(i) Survival in the presence of predators. The effects of black molly, *Poecilia sphenops* Valenciennes, a fish predator of mosquito larvae, and filamentous algae cover on the survival of *An. pseudopunctipennis* larvae were examined in a crossed-design experiment involving four levels of filamentous algae cover (0, 33, 66 and 99%) and four densities of *P. sphenops* (zero, one, two, and four fish per container). Each treatment was replicated four times.

Samples of *Spirogyra* spp. were transported from the River Coatán and were carefully washed and examined to eliminate the presence of predators and other invertebrates. Algae were placed in perforated metallic trays sealed with nylon mesh within the pool, and allowed to establish and recover from the manipulation process for 1 week. Experimental containers consisted of PVC tubes (described above) and were floated with 20 cm of the tube above the water line to prevent fish jumping into or out of the tubes. Sixteen tubes were placed in the water storage pool in groups of four and were held in place by a string fastened to the edge of the pool. Once established, 0, 80, 170, and 250 g algae (wet weight) were added to tubes resulting in 0, 33, 66, or 99% surface cover within the tubes respectively. These quantities of algae were assigned randomly to the experimental tubes and were left to establish for 3 days.

Medium-sized examples of *P. sphenops*, 4–6 cm in total length, were assigned randomly to PVC tubes at densities of zero, one, two, and four fish per tube. Fish were allowed to acclimatise for 24 h. Once acclimatised, 100 first-instar *An. pseudopunctipennis* from the laboratory colony, 24–48 h post-hatching, were added to each container. The number of insects that survived to pupation in each container was recorded daily. The absence of mosquito survival in the treatments involving fish with no algae present resulted in an unbalanced design and precluded a standard factorial analysis of variance. Consequently, the percentage of insects that survived to pupation was $\sqrt{(x + 1)}$ transformed to normalise the distribution and subjected to quadratic linear regression against the percentage of algal surface cover and fish density. Both algal cover and fish densities were subjected to $(x - \text{mean } x, y - \text{mean } y,$ respectively) transformation to reduce colinearity of standard and squared terms in the regression (Sokal & Rohlf, 1981).

(ii) Effects of algae and predators on development time and adult wing length. Pupae obtained from each treatment were transported daily to the laboratory in plastic containers in an insulated box. Pupae were placed in plastic cups in mesh cages to permit adult emergence. The number and sex of emerging mosquitoes was noted daily. Adult wing length of 10–30 adults of each sex (depending on survival in each treatment) were measured from each replicate, from the axial incision to the outer margin excluding the fimbrial scales (Armbruster & Hutchinson, 2002). Mean larval development time (the interval from first instar to pupation) and mean adult wing length data were calculated for each replicate and subjected to two-way ANOVA. Mean separation was performed by Tukey test (SPSS, 1995).

Results

Influence of filamentous algae on oviposition habitat selection

When tested with *S. majuscula*, mosquitoes oviposited exclusively in containers with algae; the mean ($\pm$ SE) number of first-instar *An. pseudopunctipennis* was 21.0 ± 11.4 larvae per container. No larvae were observed in containers containing water alone. Similarly, when offered *C. glomerata*, oviposition was observed in the treatment involving algae in four out of five replicates (mean of all replicates was 16.2 ± 7.2 larvae per container), whereas larvae were observed in only one replicate (a single oviposition container) in which no algae were present. As a result, the influence of filamentous algae on larval feeding and development and survival in the presence of predators was then studied. A non-parametric comparison between the number of larvae observed in containers with *S. majuscula* and *C. glomerata* indicated no significant difference in the attractiveness of these two species of algae for oviposition of *An. pseudopunctipennis* (Mann–Whitney $U = 18$, $P > 0.05$).

Role of filamentous algae in the diet and development of larvae

(i) Analysis of larval gut contents. Of the 80 groups of larvae (20 larvae per group) subjected to gut content analysis, all were positive for the presence of filamentous algae. The mean ($\pm$ SE) number of particles observed for each group of 20 larval guts was $70\,180 \pm 2348$ fragments of organic debris, $64\,197 \pm 2446$ fragments of algae, and 1227 ± 276 bacterial cells. No protozoa were observed. In total, algae represented 47% of the gut contents of mosquito larvae, suggesting that algae comprise an important part of the diet in all the riverside pools from which larvae were sampled.

(ii) Suitability of Spirogyra majuscula for larval development. Larval diet had no significant effect on mean wing length (length$^3 \pm$ SD) of adult females (control $= 58.74 \pm 0.47$ mm; algae $= 58.24 \pm 0.09$ mm; $t_8 = 0.941$, $P = 0.077$, unequal variances), or adult males (control $= 45.06 \pm 0.29$ mm; algae $= 46.70 \pm 2.54$ mm; $t_8 = 0.830$, $P = 0.222$, unequal variances) in the laboratory. Immature development times were also similar for insects reared on each type of diet for both females (control $= 8.23 \pm 0.10$ days; algae $= 8.19 \pm 0.08$ days; $t_8 = 0.623$, $P = 0.511$, equal variances) and males (control $= 8.55 \pm 0.11$ days; algae $= 8.52 \pm 0.24$ days; $t_8 = 0.443$, $P = 0.807$, equal variances), indicating that *S. majuscula* and the standard diet used to continuously maintain the laboratory colony were equally suitable for mosquito development.

Filamentous algae as a predator refuge

(i) Survival in the presence of predators. Multiple regression of survival of mosquito larvae [expressed as the square root of the percentage ($x + 1$) of larvae that survived to pupate] on percentage algal cover and fish density revealed a highly significant relationship ($F_{4,59} = 49.66$, $P < 0.0001$; Table 1). The minimal significant model was a second-order polynomial with no interactions between variables.

Increasing fish densities resulted in a reduction in mosquito survival in all algal treatments (Fig. 1). The highest incidence of survival was observed at 66% algal cover in all treatments, such that the relationship between mosquito survival and algal cover was curvilinear at all fish densities (zero to four fish). In the control treatment without fish, mean survival increased from 29% in the absence of algae to 53% at 66% algal cover and then fell to 35% at maximal algal cover (99%). In the presence of fish, mean survival increased from 0 in the absence of algae to maximum values of between 9.0 and 19.5%, in the presence of 66% algal cover, at densities of one and four fish per tube respectively, and then fell slightly (to 5.5 and 16% survival respectively) at the highest (99%) algal cover. The density of two fish per tube was intermediate in all treatments. None of the fish died or escaped during the experimental period.

(ii) Effects of algae and predators on development time and adult wing length. The absence of surviving insects in fish treatments without algal cover precluded the determination of development time. These treatments were therefore excluded from the analysis. Development time of larvae, from being released to pupation, was not significantly affected by the percentage of algal cover in any treatment ($F_{3,39} = 0.017$; $P = 0.974$) (Fig. 2). The presence of fish, however, resulted in a significantly extended larval development time ($F_{3,39} = 42.5$; $P < 0.001$), that increased steadily from an average of 8.4 days in treatments without fish, to a maximum of 12.1 days in treatments involving a density of four fish (mean values pooled for 33, 66, and 99% algal treatments within each fish density).

The cube of adult female wing length in millimetres (mean $\pm$ SD) was 58.15 ± 0.16 in mosquitoes that emerged from the treatment involving no algal cover and no fish. The absence of surviving insects in fish treatments without algal cover precluded measurement of wing length. These treatments were therefore excluded from the analysis. The presence of fish resulted in emergence of adult females with consistently shorter wings compared with those that developed in the no-fish treatments ($F_{3,46} = 11048.5$; $P < 0.0001$) (Fig. 3). Algal cover also affected female mosquito wing length ($F_{2,46} = 773.6$; $P < 0.0001$) but differently at each fish density reflected in a significant interaction effect ($F_{6,46} = 538.4$; $P < 0.0001$). Algal cover at 66 and 99% resulted in a steady decrease in wing length with increasing fish density. At 33% cover, however, wing length was lowest at the densities of one and two fish but increased slightly but significantly at a density of four fish. A similar pattern was observed between treatments in the wing lengths of adult males (results not shown).

Table 1. Multiple linear regression (quadratic model) of square-root transformed percentage survival of mosquito larvae on percentage of surface cover of filamentous algae ($x - $ mean) and number of predatory fish per container ($y - $ mean).

Function $= 3.64906 - 1.21921x + 0.02283y + 0.44572x^2 - 0.000843355y^2$
No. of cases $= 64$, adjusted $r^2 = 0.7622$

Source	Sums of squares	d.f.	Mean	F	P
Regression	261.12	4	65.28	49.66	<0.0001
Residual	77.56	59	1.31		
Total	338.69				

Term	Coefficient	SE	$t(59)$	P
Intercept	3.64906	0.289675	12.5971	<0.0001
x (algae)	−1.21921	0.109960	−11.0878	<0.0001
y (fish)	0.02283	0.003885	5.8763	<0.0001
x^2	0.44572	0.080844	5.5134	<0.0001
y^2	−0.00084	0.000132	−6.4081	<0.0001

Both x and y were transformed to ($x - $ mean) and ($y - $ mean) to reduce colinearity between normal and squared terms in regression.

Discussion

Oviposition habitat selection is influenced by a diversity of chemical, physical, and physiological factors. Once attracted to the oviposition site, gravid females use visual (colour, texture, brightness), and olfactory cues (semio-chemicals) to decide the suitability of a potential habitat for egg laying (Bentley & Day, 1989). However, it is

Fig. 1. Graphical representation of multiple linear regression of the square root of percentage survival of *Anopheles pseudopuncti-pennis* larvae, of percentage surface cover of *Spirogyra majuscula* ($x - $ mean), and density of predatory fish ($y - $ mean).

ovipositional decision making, modulated by the detection of semiochemicals indicating the presence of predators (Stav *et al.*, 1999; Kiflawi *et al.*, 2003; Eitam & Blaustein, 2004; Blaustein *et al.*, 2004), or immature mosquitoes (Bentley *et al.*, 1976; Laurence & Pickett, 1985; Zahiri & Rau, 1998; Reiskind & Wilson, 2004), including oviposition pheromones (Laurence & Pickett, 1985; Mboera *et al.*, 1999), that has attracted most attention from insect ecologists. In this study, an absolute preference by a natural population of *An. pseudopunctipennis* to oviposit in containers with *S. majuscula* over containers with water alone was observed. This behaviour was almost identical when offered a choice of containers with open water or *C. glomerata*. The consequences of the ovipositional decision to the diet, development rate, and body size of offspring, and the role of filamentous algae as a refuge from predatory fish was then examined.

Anophelines usually feed on microorganisms and detritus (Merritt *et al.*, 1992). Algae may also be common components of the gut contents (Howland, 1930), although their nutritional value, and specifically their digestibility, has been questioned (Laird, 1988). The degree of selectivity of mosquito larval feeding is also a subject of debate with various authors suggesting that gut contents largely reflect the size of the larvae, method of feeding and the availability of different food items in the larval habitat (Laird, 1988; Clements, 1992). A number of studies have indicated that bacterial cells are a major constituent of the larval diet (Walker *et al.*, 1988; Merritt *et al.*, 1990; Rejmankova *et al.*, 2000), although bacteria only represented a minor component of the diet of *An. pseudopunctipennis* in this study. Particle analysis of gut contents does not take into account colloidal or dissolved foodstuffs, although such materials may be important to larval nutrition (Wotton, 1990). Indeed, algal cells may produce nutritionally important exudates that are not apparent by gut particle analysis (Wotton, 1990). In addition, particle counts did not allow
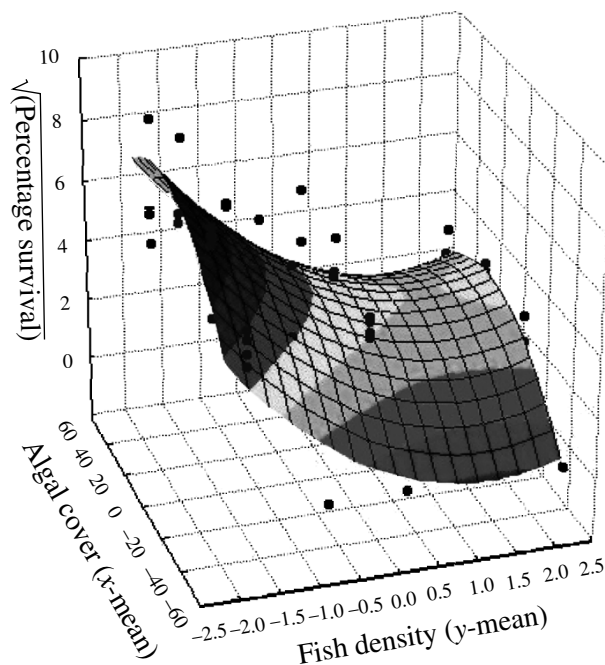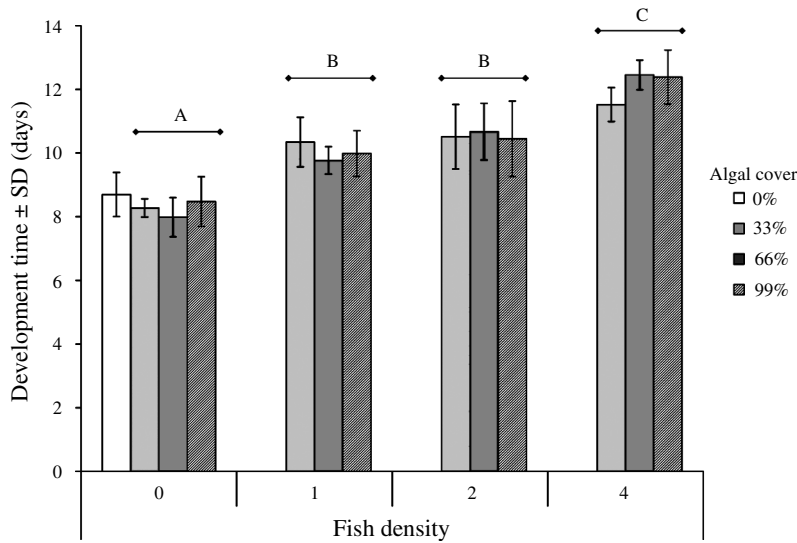
Fig. 2. Mean development time (first instar to adult eclosion) of *Anopheles pseudopunctipennis* larvae exposed to 0–99% surface cover of filamentous algae and densities of zero to four predatory fish per tube. Columns headed by identical letters are not significantly different (ANOVA, Tukey, $P > 0.05$). As no mosquitoes survived in the 0% algal cover treatment in the presence of fish, this was excluded from the analysis.

us to estimate the biovolume of ingested matter, which may be a more accurate measure of the importance of each constituent of gut contents (Wetzel & Likens, 1991).

These findings demonstrated that filamentous algae were abundant in the guts of larval *An. pseudopunctipennis* in natural breeding pools. Moreover, laboratory-reared larvae developed as quickly and attained the same adult size (as judged by wing length) given a diet of *S. majuscula* or the standard diet of the laboratory colony, indicating that filamentous algae alone are nutritionally suitable to support the entire development of *An. pseudopunctipennis* larvae. Several other aquatic insects have been shown to be capable of development on a diet of filamentous algae monocultures including ephydrid flies (Zack & Foote, 1978) and Trichoptera (Keiper, 2002).

In the absence of predators, the highest survival of larvae was observed at 66% algal cover. Greater quantities of algae reduced survival possibly by presenting a physical barrier to larval respiration or feeding activity close to the air – water interface. Lesser quantities of algae may have reduced mosquito survival by exposing larvae to harmful levels of ultraviolet light (Donahue & Schindler, 1998; Buma *et al.*, 2003).

The black molly (*P. sphenops*) proved to be a voracious predator of *Anopheles* larvae, completely eliminating them from treatments in which no algal refuge was available. However, filamentous algae functioned as an effective refuge from fish predation at densities of one to four fish per container. Survival was highest in containers with 66% algal cover in all treatments involving fish. Submerged
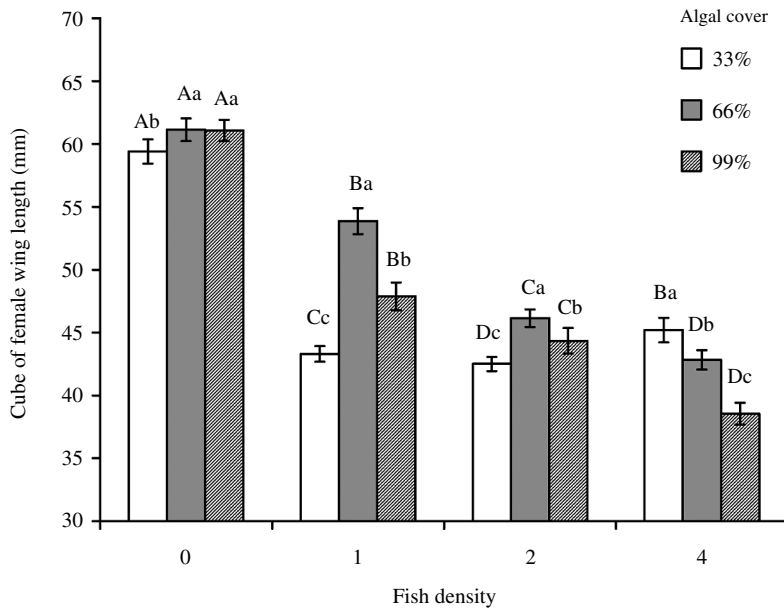

Fig. 3. Effects of algal cover (*Spirogyra* spp.) and fish density (*Poecilia sphenops*) on cube of adult female wing length in millimetres (mean ± SD) of *Anopheles pseudopunctipennis*. Columns headed with different capital letters are significantly different (ANOVA, Tukey, $P < 0.001$) for comparisons between fish densities within each algal treatment, columns headed with different lower case letters are significantly different (ANOVA, Tukey, $P < 0.001$) for comparisons between algal cover treatments within each fish density. Larval survival was zero in the treatments involving no algal cover in the presence of fish (excluded from analysis).

vegetation impedes the movement and obstructs the vision of predatory fish (Manatunge *et al.*, 2000) and the density and architectural complexity of the macrophytes are important in that respect (Savino & Stein, 1989; Persson & Eklov, 1995). It was observed that mats of filamentous algae were almost impenetrable to *P. sphenops* limiting them to foraging at the edges of the algal mass.

The results of this study resemble those of Orr and Resh (1989, 1992) who reported a positive relationship between the density of emergent stems in monospecific beds of *Myriophyllum aquaticum* (Vell.) and the density of eggs and larvae of *Anopheles hermsi* Barr. In choice experiments, larvae also selected high density patches of *M. aquaticum* stems as microhabitat. Similarly, emergence of *Culex erythrothorax* Dyar was positively correlated with the density of emergent bulrush stems and larvae were virtually absent from open areas of water in an experimental wetland (Workman & Walton, 2000). Survival of *Anopheles* larvae in the presence of mosquito fish, *Gambusia affinis* (Baird and Girard), was dependent on the density of predators and the density of macrophyte stems available as refuge (Orr & Resh, 1989). In contrast, Blaustein and Byard (1993) observed no evidence that a submergent plant (*Ceratophyllum demersum* L.) provided a refuge from a fish predator for *Culex pipiens* L. larvae.

Aquatic arthropods can respond to the presence of predators by reducing time spent in foraging and feeding behaviours (Macchiusi & Baker, 1992; Peckarsky *et al.*, 1993), increasing their propensity to use water currents to drift away (horizontally or vertically) from potential predators (Peckarsky & Penton, 1989; Brancelj & Blejec, 1994; McIntosh *et al.*, 2002), and by modifying life-history traits to reduce their period of occupancy of a dangerous habitat (Stibor, 1992). When such experiments involve exposure only to water previously inhabited by predators, the causes of changes in aquatic arthropod behaviour and life-history traits can be assigned to specific anti-predation responses (Sih, 1986; Burks *et al.*, 2000; Peckarsky *et al.*, 2002), whereas evaluation of direct exposure to predation involves a combination of antipredator responses and the result of selection of survivors with possibly specific phenotypic characteristics; survivors represent those less vulnerable prey that managed to avoid predators (Hechtel & Juliano, 1997).

The presence of fish resulted in emergence of adult females with consistently shorter wings compared with those that developed in the no-fish treatments. There was an interaction with algal cover in that, in the 33% algal cover treatment, wing lengths at the density of four fish were slightly greater than those observed in the one and two fish densities. A similar pattern was observed between treatments in the wing lengths of adult males, indicating that these effects are not sex-specific, in contrast to the sexual differences in responses to predators reported by Ball and Baker (1996) in a chironomid.

The identification of food sources and their relative importance in the larval diet are particularly relevant to certain novel approaches to vector control. The use of biologically derived insecticides (Federici *et al.*, 2003; Bond *et al.*, 2004a), encapsulation of bacterial toxins in mosquito food items (Manasherob *et al.*, 1997), genetic modification of potential food plants (Wu *et al.*, 1997), and habitat manipulation (Knight *et al.*, 2003; Bond *et al.*, 2004b; Jiannino & Walton, 2004), all depend on knowledge of mosquito oviposition habitat selection and the ensuing consequences for larval diet selection. In conclusion, *An. pseudopunctipennis* selects oviposition habitats to ensure a suitable larval food source, filamentous algae. The intricate architecture of these algae represent a physical barrier to predation and thereby act as an effective refuge from key predators, such as *P. sphenops*.

Acknowledgements

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