

Population control of the malaria vector *Anopheles pseudopunctipennis* by habitat manipulation

J. Guillermo Bond^{1,2}, Julio C. Rojas¹, Juan I. Arredondo-Jiménez², Humberto Quiroz-Martínez³, Javier Valle¹ and Trevor Williams^{1,4*}

¹ECOSUR, Tapachula 30700, Chiapas, Mexico

²Centro de Investigación de Paludismo-INSP, Tapachula 30700, Chiapas, Mexico

³Fac. de Ciencias Biológicas, U.A.N.L., San Nicolás de los Garza 66414, N.L., Mexico

⁴Depto. Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain

Insect vector-borne diseases continue to present a major challenge to human health. Understanding the factors that regulate the size of mosquito populations is considered fundamental to the ability to predict disease transmission rates and for vector population control. The mosquito, *Anopheles pseudopunctipennis*, a vector of *Plasmodium* spp., breeds in riverside pools containing filamentous algae in Mesoamerica. Breeding pools along 3 km sections of the River Coatán, Chiapas, Mexico were subjected to algal extraction or left as controls in a cross-over trial extending over 2 years. Initial densities of *An. pseudopunctipennis* larvae were directly proportional to the prevalence of filamentous algae in each breeding site. The extraction of algae brought about a striking decline in the density of *An. pseudopunctipennis* larvae sustained for about six weeks, and a concurrent reduction in the adult population in both years of the study. Mark-release experiments indicated that dispersal from adjacent untreated areas was unlikely to exert an important influence on the magnitude of mosquito control that we observed. Habitat manipulation by extraction of filamentous algae offers a unique opportunity for sustainable control of this malaria vector. This technique may represent a valuable intervention, complimenting insecticide spraying of households, to minimize *Plasmodium* transmission rates in Mesoamerica.

Keywords: habitat manipulation; *Anopheles*; filamentous algae; vector control

1. INTRODUCTION

Vector-borne diseases present a major challenge to human health and appear set to increase in importance in the future (Rogers & Randolph 2000; Hay *et al.* 2002). The success of insecticide-based control programmes in reducing the prevalence of vector-borne diseases at costs economically realistic for developing regions is irrefutable (Curtis & Lines 2000; Curtis & Davis 2001). However, chemical-based control has stimulated debate regarding the harmful effects of prolonged use of synthetic insecticides on human health and the environment (Attaran *et al.* 2000; Longnecker *et al.* 2001, 2002), and the priorities for funding in vector control research (Curtis 2000; Hoffman 2000). Mosquito resistance to insecticides is also a matter of concern (Sina & Aultman 2001).

Environmental management based on ecological principles represents an effective and expedient intervention for vector control (WHO 1982; Ault 1994). Programmes based on environmental modification such as drainage or filling in of pools and ponds, can be very effective for reducing mosquito populations (Kitron & Spielman 1989; Rose 2001). However, such programmes require considerable investment and may result in major changes to the ecology of these areas (Collins & Paskewitz 1995). By

contrast, habitat manipulation involving water management practices, irrigation controls or the elimination of aquatic vegetation offers the possibility to match the intervention to the specific ecological requirements of the local insect vector population (Kitron 1987; Ault 1994). The social and economic benefits of integrated control practices including physical barriers (bednets), pharmacological treatment and environment manipulation, have been clearly illustrated (Uttinger *et al.* 2001, 2002a,b; Shiff 2002).

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). For example, the presence of certain aquatic plants can profoundly affect the suitability of breeding sites (Marten 1986). Aquatic vegetation facilitates the reproduction and survival of anopheline mosquitoes through three mechanisms: an increased abundance of food resources (Smock & Stoneburner 1980; Rejmankova *et al.* 1991), providing physical shelter from disturbance and a refuge from predators (Gregg & Rose 1982; Orr & Resh 1989), and favourable conditions of microclimate and access to oviposition sites for adults (Hall 1972; Orr & Resh 1992).

The mosquito *Anopheles pseudopunctipennis* Theobald is widely distributed in Mexico, Central and South America and is an important vector of *Plasmodium* spp. in these

* Author for correspondence (trevor.williams@unavarra.es).

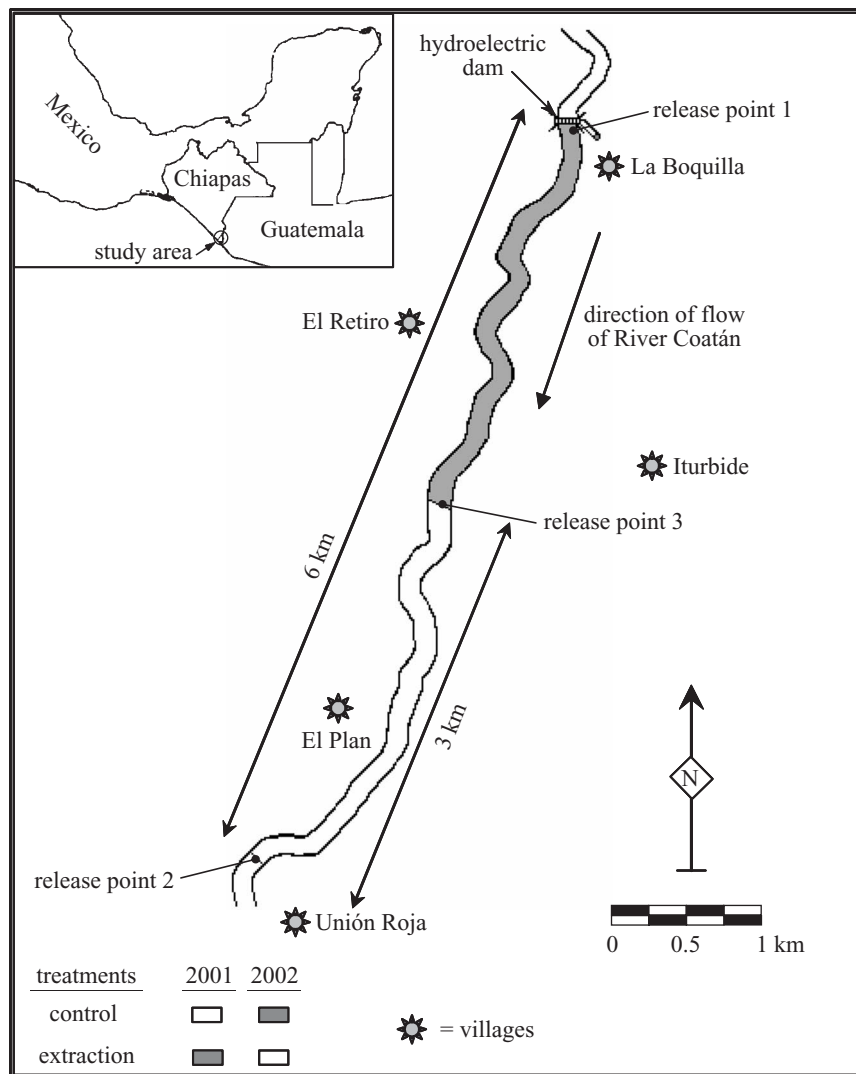


Figure 1. A section of River Coatán, Chiapas, Mexico between the hydroelectric dam near La Boquilla and the village of Unión Roja. This stretch of the river was divided into two sections, each 3 km long. The breeding pools within the upstream section (shaded grey) were subjected to algal extraction in 2001 and the downstream section was left as a control. In 2002, the situation was reversed with the downstream section treated and the upstream section left as a control. The release points (1–3) of the mark–recapture experiments on mosquito dispersal are also indicated.

regions (Fernández-Salas *et al.* 1994*a,b*). The immature stages of this species are commonly found in riverside pools colonized by filamentous algae of the genus *Spirogyra* (Rejmankova *et al.* 1991, 1993). In southern Mexico, the incidence of malaria is monitored continuously and outbreaks are controlled by spraying households with pyrethroid insecticide and dispensing prophylactic medicines to affected communities.

In this study, we demonstrate that habitat manipulation provides a unique opportunity for control of *An. pseudo-punctipennis*. Extraction of filamentous algae from breeding sites results in a dramatic and sustained decrease in the density of immature and adult stages. This represents a novel, safe and effective method of vector control that each community can adopt at minimal cost.

2. METHODS

(a) Ethical considerations

The procedures described in this study, specifically the use of human baits and the release of adult mosquitoes, were approved

by the bioethics committees of the National Institute for Public Health, part of the Mexican government's Department of Health, and the National Council for Science and Technology, Regional Research programme (CONACYT-SIBEJ), that funded the study. Human bait volunteers gave written consent to participate in the study and all had access to health care and prophylactic drugs (mefloquine hydrochloride, 250 mg week⁻¹). None of the volunteers became ill during the study. The density of adult mosquitoes was not significantly increased by mark–recapture trials because insects used in these studies were collected, as larvae, from the natural population already present in the study area.

(b) Study area

The study was performed during two dry seasons, November 2000 to July 2001 and January to July 2002. The study area was located in a 6 km section of the Coatán River, 25 km northeast of the town of Tapachula, Chiapas, between the villages of Unión Roja and La Boquilla (figure 1). This area is located between 15°03'81" N, 92°12'40" W and 15°03'52" N, 92°13'10" W, at an altitude between 297 and 675 m above sea level. The climate in this area is tropical subhumid with a wet season from May to

October and a dry season from November through to April. The annual average rainfall is 3800 mm, an average annual temperature of 25 °C, and relative humidity of 80–90% for most of the year.

The management of the Coatán River downstream from the hydroelectric plant 'Cecilio del Valle' (figure 1) over the past 20 years has created the environmental conditions that favour reproduction of *An. pseudopunctipennis* in pools of water along the river margins during the dry season. Similar conditions are observed in many other rivers in the region during this period when river discharge is diminished owing to lack of rainfall.

(c) Experimental design

The 6 km length of the river was divided into two zones, of 3 km each and marked at 100 m intervals by painting numbers on large boulders. The treatments were randomly assigned to each zone. In 2001, the upstream zone was selected for manual extraction of filamentous algae from all the riverside pools of water, whereas the downstream 3 km zone was left untreated as the control (figure 1). In 2002, the treatment and control zones were switched and the process of algal extraction repeated.

(d) Extraction of algae

Manual extraction of filamentous algae floating at the surface of pools was performed with the use of garden rakes covered with mosquito netting. This required 106 man hours of work per kilometre of river. Algae were extracted only once per year at the beginning of the dry season. The start of the study was taken to be the first day after extraction of algae from all breeding pools in the extraction zone had been achieved.

(e) Larval collections

Estimates of the density of *An. pseudopunctipennis* larvae were obtained using a standard 850 ml cup with a flat outer side attached to a wooden pole. Samples were taken by carefully immersing the cup at the edges of aquatic vegetation, or by dragging the dipper over the water surface. Larvae were collected, counted, and returned to their breeding site to avoid population disturbances from sampling. Prior to the extraction of algae, sampling was performed at 15 days and 7 days pre-treatment in both zones. Samples were taken at weekly intervals post-extraction.

Sampling effort was defined in previous studies (Savage *et al.* 1990; Southwood & Henderson 2000) based on Taylor's power law (Taylor 1979). This consisted of 5–20 dips from each breeding site, depending of the numbers of larvae in the initial five dips which was used to determine the sample size (total number of dips). If the number of larvae captured in the first five dips averaged 0–5 larvae dip⁻¹, a maximum of 20 dips were taken. If the average was 5–20 larvae, then 15 dips were taken, if 20–50 larvae, then 10 dips were taken, and if more than 50 larvae dip⁻¹, then only five dips were taken. A total of 50 pools were sampled in each zone. The larval density index was calculated by dividing the total number of larvae by the sampling effort (total number of dips). In each treatment the number of *An. pseudopunctipennis* larvae was registered by stage and breeding site. Larvae were transported to the laboratory in sealed plastic bags for identification.

(f) Physical factors and vegetation

For all habitats, the surface area of the breeding site was estimated using a tape measure and an appropriate geometrical shape as a model (rectangle, rhombus, etc). The depth of water (average of five measurements per pool) and percentage surface area cover of filamentous algae were noted. Meteorological data were

obtained from a weather station located at Finca San Andrés Nexapa, next to the village of El Retiro (figure 1).

(g) Adult collections

Adult *An. pseudopunctipennis* populations were estimated prior to extraction of algae and at weekly intervals for 15 weeks post-treatment at four sites in each zone using light traps and human baits.

(i) Light traps

CDC Updraft Model 1312 (J. W. Hock Co., Gainesville, FL, USA) light traps fitted with 4W ultraviolet (UV) lamps were placed at four sites in each zone. Ten traps were placed at four sites around the villages on each side of the river during two nights a week at each site from 1800 to 0600. Trapping was performed weekly. Each morning, the number of adult female *An. pseudopunctipennis* caught in each trap during the previous night was registered. Captures of *An. pseudopunctipennis* males and other mosquito species were low and were ignored.

(ii) Human baits

Weekly trapping using human baits was performed in each zone (WHO 1975). Two volunteers were seated by different houses, one person inside and the other outside. The trousers were rolled up to the knee and adult female *An. pseudopunctipennis* that landed to feed on the exposed skin were collected using a flashlight and a mouth aspirator. Collections were made for periods of 50 min followed by a 10 min break from 1800 to 2400. The mosquitoes collected were sealed in plastic tubes and were later counted and checked for species in the laboratory. Sampling was performed weekly at two sites in the control zone and two sites in the treated zone.

(h) Dispersal

The probability of immigration of *An. pseudopunctipennis* adults from the untreated zone into the treated zone was estimated using mark–release–recapture methods. Immature stages of *An. pseudopunctipennis* were collected from breeding sites along the Coatán river and reared to adulthood in the laboratory. When adults of 1–3 days post-emergence were available, these were placed in plastic containers sealed with muslin. The containers were lined with paper previously treated with fluorescent powder (Lumogen, BASF, Holland, MI) as described by Ulloa *et al.* (2002). A different colour powder was used for each liberation. The first liberation involving 1800 adults was performed at the northernmost part of the study zone (release point 1 in figure 1). At the moment of release the wind was northerly at 2.1 m s⁻¹ and continued to be northerly during the trapping period. The second liberation, performed 20 days after the first, involved 1450 marked adults and occurred at the southernmost point of the study area (release point 2 in figure 1). At the moment of the second release the wind was northerly at 0.6 m s⁻¹ and was northerly or easterly during the trapping period. The third liberation, 20 days later, involved 500 mosquitoes released in the centre of the study area (release point 3 in figure 1). At the third release the wind was easterly at 0.3 m s⁻¹ but was northerly for all the remaining trapping period.

Trapping of marked mosquitoes was performed immediately following liberation at intervals of 50, 100 or 500 m up to a distance of 3–6 km from the point of release. Marked mosquitoes were identified using a UV lamp. Trapping continued for 10 days after each release.

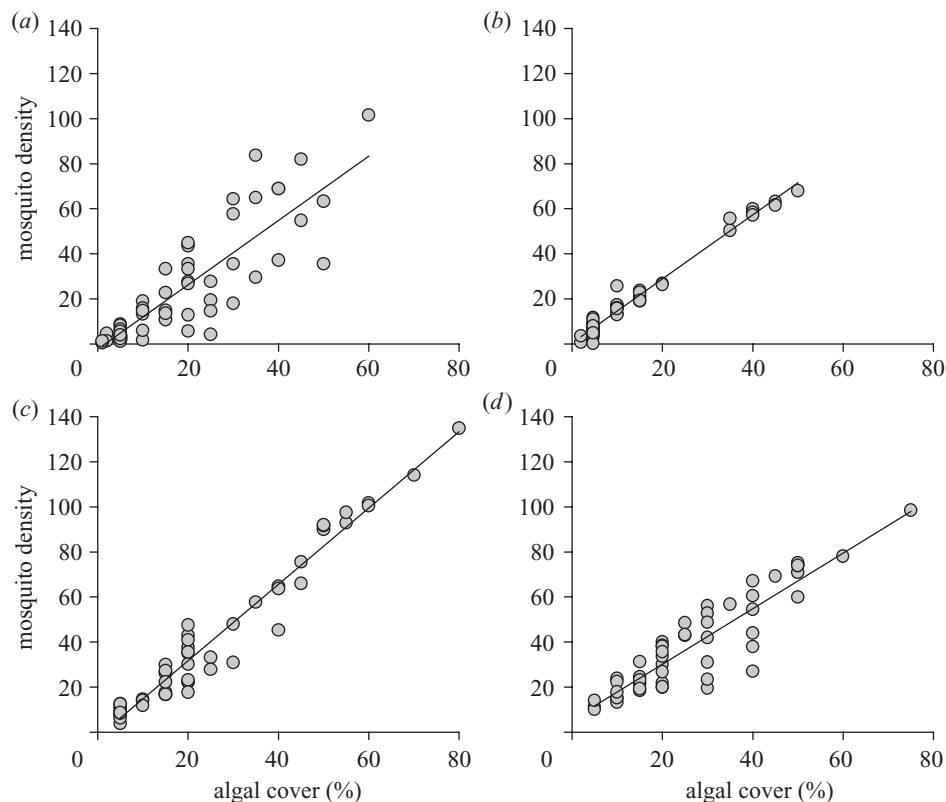


Figure 2. Linear regression of larval mosquito density (average number of larvae per dip) against the percentage cover of filamentous algae in breeding sites within the (a) control ($y = 1.43x - 2.35$, $r^2 = 0.70$) and (b) treated section ($y = 1.43x + 0.33$, $r^2 = 0.98$) of the River Coatán prior to algal extraction treatment in 2001, and (c) control ($y = 1.69x - 2.21$, $r^2 = 0.95$) and (d) treated section ($y = 1.23x + 5.45$, $r^2 = 0.82$) of the river in 2002.

(i) Data analysis

The mean numbers of larvae per dip were transformed to $\log(x + 1)$ to normalize the distribution and eliminate heteroscedasticity. Differences in larval abundance between zones were analysed by univariate repeated-measures analysis of variance (ANOVA). Captures of adult *An. pseudopunctipennis* per light trap per night and numbers of mosquitoes per person per night for human baits were similarly $\log(x + 1)$ transformed and subjected to repeated-measures ANOVA. Tests of sphericity and normality were performed to verify the univariate ANOVA model. Physical characteristics and algal cover were compared by *t*-test. Transformations were not required to normalize data distributions.

The analysis of mosquito dispersal did not focus on the rate of dispersal but rather the absolute distances from the point of release at which marked individuals were recovered. Mark-recapture data were pooled over the 10 days on which trapping was performed following each release. Linear regression of the number of marked mosquitoes captured against $\log_e(\text{distance})$ from point of release was performed in a generalized linear interactive modelling (GLIM) program with a Poisson error structure specified (Numerical Algorithms Group 1993). The accuracy of regression models was determined by examining the distribution of fitted values and residuals.

3. RESULTS

In 2001, *An. pseudopunctipennis* breeding pools did not differ significantly between the control and treated zones in terms of the average surface area ($20.0 \pm 1.1 \text{ m}^2$, mean \pm s.e., $n = 50$ per zone; $t_{98} = 0.70$, $p = 0.48$) or average depth of water ($21.8 \pm 0.8 \text{ cm}$; $t_{98} = 1.48$, $p = 0.14$).

The average percentage surface cover of filamentous algae was 16.6% (range of s.e. 16.3–17.0). In 2002, breeding sites did not differ significantly between the control and treated zones in average surface area ($12.5 \pm 0.5 \text{ m}^2$; $t_{98} = 1.87$, $p = 0.06$), whereas pools were *ca.* 2 cm deeper in the control zone ($18.4 \pm 0.7 \text{ cm}$) than in the extraction zone ($16.2 \pm 0.6 \text{ cm}$) at the start of the experiment ($t_{98} = 2.32$, $p = 0.02$). The average percentage cover of filamentous algae was 26.4% (range of s.e. 26.0–26.9).

Prior to the extraction of algae, densities of *An. pseudopunctipennis* larvae were directly proportional to the prevalence of *Spirogyra* in each pool, for the control (figure 2a) and treated zone (figure 2b) in 2001 and 2002 (figure 2c,d, respectively), highlighting the importance of filamentous algae in the reproduction and survival of this species.

(a) Effects of algal extraction on the larval population

Extraction of filamentous algae dramatically affected the density of *An. pseudopunctipennis* larvae during the course of the experiment in both years (table 1). In 2001, sampling prior to extraction of algae indicated no significant differences between treatment and control zones at two weeks prior to treatment (timepoint -2: $F_{1,98} = 1.45$, $p = 0.23$) and immediately prior to treatment (timepoint -1: $F_{1,98} = 0.61$, $p = 0.44$; figure 3a).

Larval densities in the control zone fluctuated between (mean \pm s.e.) 25.7 ± 1.4 and 7.6 ± 0.4 larvae dip⁻¹ during the six-week period following extraction of algae.

By contrast, larval densities in the treated zone fell dramatically, an effect that lasted five weeks before

Table 1. Repeated-measures analysis of the effects of extraction of filamentous algae on population densities of larval and adult *An. pseudopunctipennis* in a 2 year experiment, River Coatán, Chiapas, Mexico.

source of variation	Pillai's trace	F	num. d.f.	den. d.f.	p
larvae					
time	0.897	113.43	14	183	< 0.0001
time × treatment	0.787	48.45	14	183	< 0.0001
time × year	0.417	9.35	14	183	< 0.0001
time × year × treatment	0.324	6.27	14	183	< 0.0001
adults human bait					
time	0.646	9.49	15	78	< 0.0001
time × treatment	0.547	6.29	15	78	< 0.0001
time × year	0.480	4.80	15	78	< 0.0001
time × year × treatment	0.258	1.81	15	78	< 0.0480
adults light traps					
time	0.944	76.00	14	63	< 0.0001
time × treatment	0.820	20.54	14	63	< 0.0001
time × year	0.504	4.57	14	63	< 0.0001
time × year × treatment	0.169	0.92	14	63	0.5460

recovering to control density values (figure 3a). For the period 6–14 weeks post-extraction, the densities of *An. pseudopunctipennis* larvae were almost identical in each zone.

A very similar effect was observed in 2002, with the treated and control zones inverted, except that average population densities were approximately twice as great as the previous year, resulting in a significant interaction of timepoint and year (table 1). As before, sampling prior to extraction of algae indicated no significant differences in mean larval densities between zones at two weeks prior to treatment (timepoint -2: $F_{1,98} = 0.12$, $p = 0.73$) and immediately prior to treatment (timepoint -1: $F_{1,98} = 0.25$, $p = 0.62$; figure 3b). The extraction of algae resulted in a striking decline in the density of *An. pseudopunctipennis* larvae (figure 3b). The treated population did not return to densities similar to those of the control zone until seven weeks post-extraction.

Approximately halfway through the sampling programme each year, the density of larvae in the control breeding sites began to decline. This effect was concurrent with an increase in precipitation and river discharge at the onset of the rainy season that washed away larvae from breeding pools (figure 3a,b).

(b) Effects of algal extraction on the adult population

Both light trapping and the use of human baits indicated a marked reduction in the population of adult *An. pseudopunctipennis* in the treated zone in both years (table 1).

(i) Human bait

In 2001, captures of adult females landing to feed on human baits was initially similar in control and treated zones. However, a significant reduction in the number of captures was observed within one week of the extraction of algae (results pooled for within house and outside house baits) and remained significantly lower than control zone captures until eight weeks post-treatment (figure 3c). Changes in the number of captures in the control zone closely followed the patterns observed in the control zone larval density estimates, with a gradual reduction in captures after week four.

A very similar pattern was observed in 2002, with a rapid reduction in adult captures in the treated zone which lasted for seven weeks until returning to control zone values (figure 3d). Following an initial reduction in adult captures in the control zone concurrent with a brief period of rainfall at night, the numbers of adults caught in the control zone remained relatively static until week 10, whereupon numbers declined, approximately three weeks after the start of the decline in larval densities (figure 3b).

(ii) Light traps

Light trap captures closely resembled the results of human bait captures and confirmed the marked reduction in adult *An. pseudopunctipennis* populations in the treated zone in both years (table 1). This reduction lasted six to seven weeks before returning to control zone values (figure 3e,f). Captures in 2002 were approximately double those of 2001, reflecting the higher larval population density in 2002 compared with the previous year.

(c) Dispersal

The recovery of marked mosquitoes varied from 13 out of 1800 (0.7%) in the first release, 21 out of 1450 (1.4%) in the second release, to 20 out of 500 (4.0%) in the final release. Overall, 92% (48 out of 52) of the marked and recaptured mosquitoes were caught within 4 days of release. Also, 98% (53 out of 54) of marked and recaptured mosquitoes were captured within 1000 m of the point of release (figure 4a–c). The greatest displacement was registered by a single individual, caught at 2500 m from the release point in the first liberation (figure 4a). The slopes of linear regressions differed markedly between different releases but each regression gave good estimates of the observed numbers of mosquitoes captured. In the third release at the central point between the treated and untreated zones (figure 1), only one trap registered captures upstream and so only downstream captures were considered in the regression (figure 4c).

4. DISCUSSION

The mosquito, *An. pseudopunctipennis* breeds in pools containing filamentous algae in the foothills of the mountainous

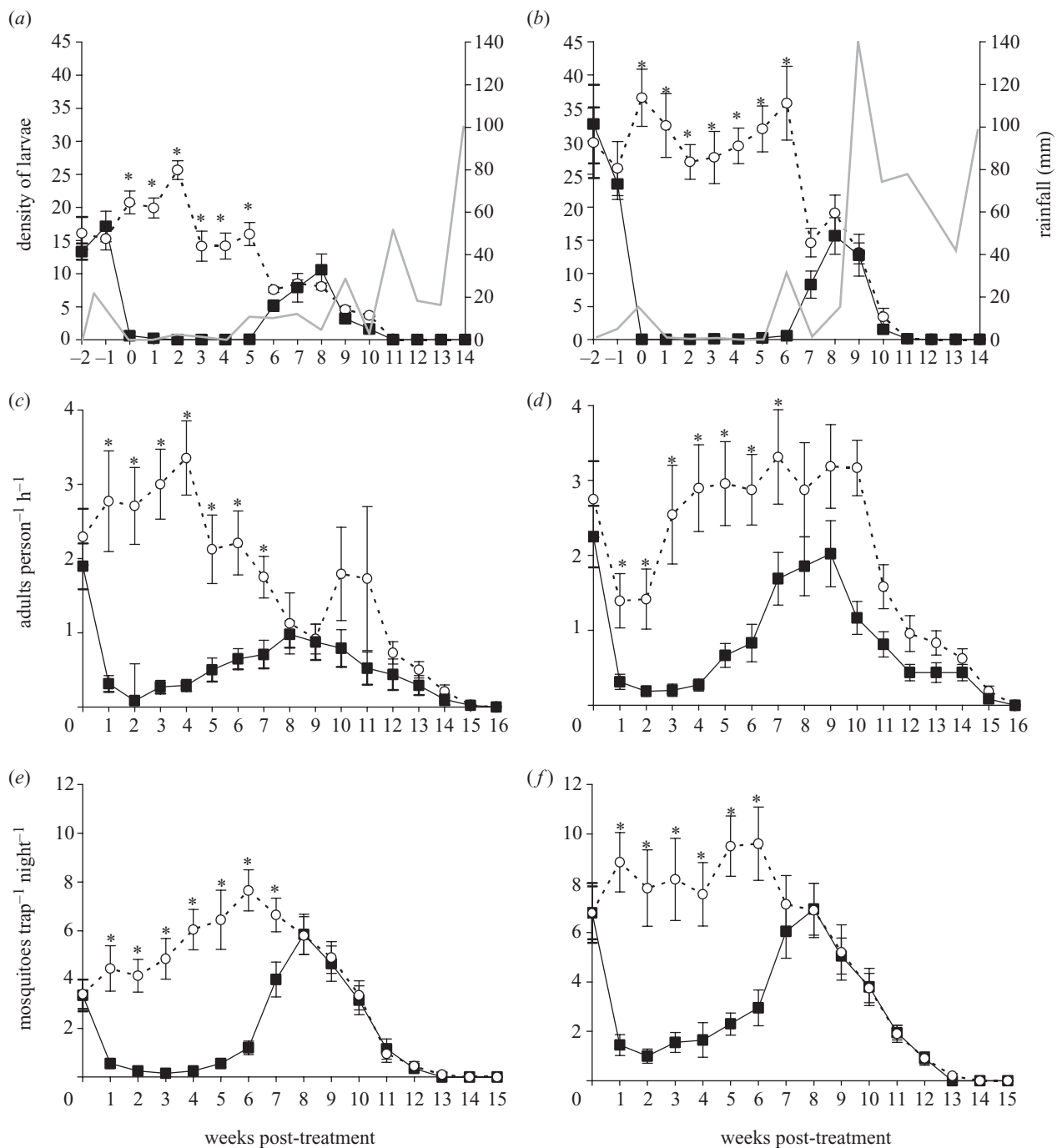


Figure 3. Effect of algal extraction from breeding sites of *An. pseudopunctipennis* on the density of larvae in the treated zone (black squares, solid line) and the control zone (open circles, dashed line) in the field experiment performed in (a) 2001 and (b) 2002. The moment of completion of algal extraction was taken as week zero. Weekly precipitation is shown by the grey line. Adult populations were monitored by human baits in (c) 2001 and (d) 2002, and by light trapping in (e) 2001 and (f) 2002. In all cases, the asterisks above the points indicate significant differences between values observed in the control and treated zones (repeated-measures ANOVA: $p \leq 0.05$).

Mesoamerican region (Rejmankova *et al.* 1993; Manguin *et al.* 1996). Densities of *An. pseudopunctipennis* larvae were directly proportional to the prevalence of filamentous algae in each pool sampled at the beginning of this study, highlighting the importance of algae as larval food and as a refuge against predators (Orr & Resh 1989). Previously, a positive association was observed between the percentage of vegetative cover of the emergent macrophyte *Myriophyllum aquaticum* and abundance of eggs and larvae of *Anopheles* spp. in a

Californian wetland (Orr & Resh 1992), although the relationship was much weaker than that detected in the present study.

The extraction of algae brought about a striking decline in the density of *An. pseudopunctipennis* larvae, resulting in a concurrent reduction in the adult population in both years of the study. The treated population did not return to densities similar to those of the untreated control zone until five to seven weeks post-extraction. Approximately halfway through the sampling programme each year, the density of

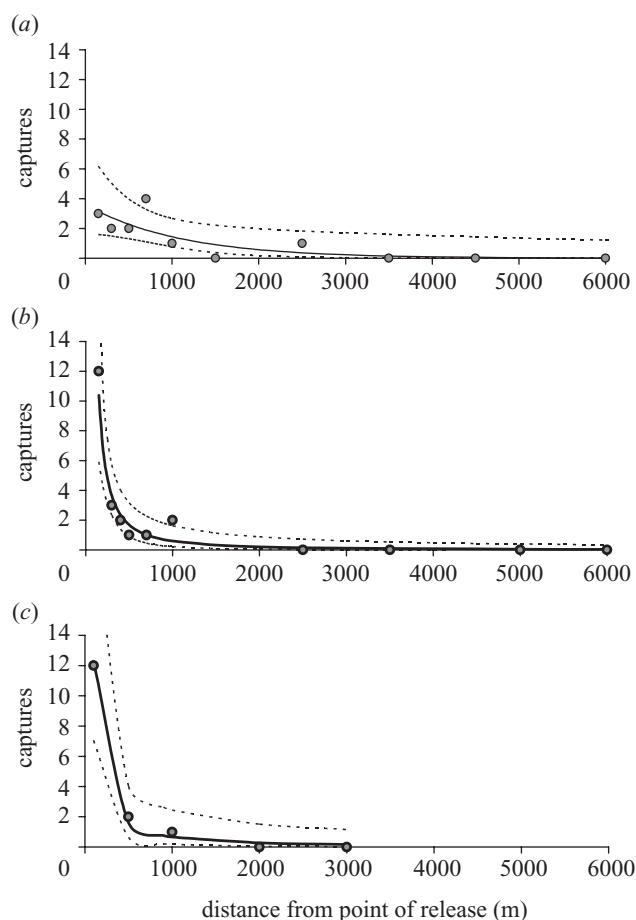


Figure 4. Recapture of marked mosquitoes by light trapping at up to 6000 m from the release point at (a) farthest upstream point 1, (b) farthest downstream point 2 and (c) central point 3 (only analysed upstream because of the prevailing wind direction at moment of release). The solid line indicates the Poisson regression model and 95% confidence limits (dashed lines) performed in GLIM. The location of each release point is shown in figure 1.

larvae in the control breeding sites began to decline. This effect was concurrent with an increase in precipitation and river discharge at the onset of the rainy season that flushed larvae from breeding pools (Berti *et al.* 1993). The effect of algal extraction on the adult *An. pseudopunctipennis* population was estimated using light traps and human baits. Numerous factors affect the efficiency of light traps for mosquito monitoring (Service 1977, 1993), although light trap and human bait catches are usually highly correlated for a number of anopheline species (Lines *et al.* 1991; Mathenge *et al.* 2002) and light traps are often a logistic necessity for monitoring large-scale interventions (Githeko *et al.* 1996). However, light trap captures may be biased to various degrees depending on the mosquito population density and species under study. The fraction of mosquitoes infected by *Plasmodium* sporozoites may also be quite different in light traps and human landing captures (Mbogo *et al.* 1993; Hii *et al.* 2000). However, adult mosquito captures by both light traps and human baits closely followed the pattern of changes that we observed in larval densities.

Annual climatic cycles have a major influence on the insect vector populations and thereby drive seasonal patterns in mosquito borne diseases (Hay *et al.* 2000). In the case of Mesoamerica, *An. pseudopunctipennis* is recognized as the principal malaria vector in the dry season, whereas, during the rainy season, breeding sites suitable for reproduction of *An. albimanus* appear and this becomes the principal vector species (Gómez-Dantés & Rodríguez 1994).

Clearly an important issue in the experimental design employed in this study is that of the balance between the scale of the experiment and the replication of treatments (Cottenie & De Meester 2003). Replication in this experiment was possible only over time (years) because it was necessary to apply the extraction treatment on a large scale (3 km section of the river) to minimize the influence of adult immigration from surrounding areas. This limitation in design was taken into account by the univariate repeated-measures analyses that we employed. The fact that we had clear *a priori* predictions regarding the direction of the change expected following extraction of algae, combined with the marked similarity of changes in mosquito densities observed in each year of the study, give us confidence that the results of the study were not unduly influenced by the severe limitations on experimental replication.

Virtually all mosquitoes used in mark-release experiments were captured within 1000 m of the point of release. This indicates that dispersal from the untreated zone into the treated zone was unlikely to have had a major influence on the estimates of adult mosquito abundance, despite the long sampling period of the experiment. For quantitative estimates of mosquito dispersal, the number of traps placed at greater distances from the release point should be increased to account for the dilution of marked mosquitoes in space (Service 1993). About half of the studies of mosquito dispersal report recapture of marked mosquitoes no farther than 1 km from the release point (Service 1993), although greater dispersal distances may be achieved by deliberate or accidental exploitation of wind currents (Service 1980, 1997). In this respect, older mosquitoes carrying sporozoites will tend to travel farther than typical members of the population (Gilles & Wilkes 1965; Killeen *et al.* 2000), underlining the need to implement environmental manipulation practices and other vector control interventions on a spatially large (regional) scale (Killeen *et al.* 2003). Clearly, mosquitoes disperse for different reasons depending on their sex, mating and feeding status, and gonotrophic cycle (Service 1997).

The abundance of filamentous algae is positively correlated with the concentration of nitrates in riverside pools (Rejmankova *et al.* 1991). Elevated levels of nitrogen in river water is believed to originate from fertilizer use in the numerous coffee plantations located upstream of the experimental site.

The intimate association between *An. pseudopunctipennis* and riverside pools containing filamentous algae means that remote sensing techniques could prove to be an important tool for identifying the distribution of breeding sites and targeting appropriate control measures (Gubler 1998; Rogers *et al.* 2002). Remote sensing could also be used to identify probable candidates for community-based interventions where villages are sited in proximity to habitats likely to support anopheline breeding. The role of

community participation in vector control in southeast India was pivotal to the success of a programme involving manual extraction of algae from coastal lagoons which represented the principal breeding site of *An. subpictus* (Rajagopalan *et al.* 1991a). Similarly, the extraction of aquatic plants including *Pistia*, *Eichhornia* and *Salvinia* spp. was successful in controlling populations of *Mansonia* spp., the principal vector of human filariasis in this region (Rajagopalan *et al.* 1991b). Such community-based programmes must be considered more attractive than the use of herbicides applied to water courses for control of aquatic vegetation, as occurs in certain regions.

In conclusion, habitat manipulation by extraction of filamentous algae appears to offer a unique opportunity for sustainable control of an important vector of malaria in Mesoamerica. When coordinated at the local community level, this technique may represent a valuable intervention, complimenting insecticide spraying of households, to minimize *Plasmodium* spp. transmission rates.

We thank Joaquin Covarrubias, José L. Espinosa and Eleazar Pérez for technical assistance. The study received financial support from the CONACYT-SIBEJ project 20000502025 and the Plan Tecnológico de Navarra.

REFERENCES

- Attaran, A., Roberts, D., Curtis, C. & Kilama, W. 2000 Balancing risks on the backs of the poor. *Nature Med.* **6**, 729–731.
- Ault, S. K. 1994 Environmental management: a re-emerging vector control strategy. *Am. J. Trop. Med. Hyg.* **50**, 35–49.
- Berti, J., Zimmerman, R. & Amarista, J. 1993 Spatial and temporal distribution of *Anopheles* larvae in two malarious areas in Sucre State, Venezuela. *Mem. Inst. Osw. Cruz.* **88**, 353–362.
- Collins, F. H. & Paskewitz, S. M. 1995 Malaria: current and future prospects for control. *A. Rev. Entomol.* **40**, 195–219.
- Cottenie, K. & De Meester, L. 2003 Comment to Oksanen (2001): reconciling Oksanen (2001) and Hurlbert (1984). *Oikos* **100**, 394–396.
- Curtis, C. F. 2000 The case for de-emphasizing genomics in malaria control. *Science* **29**, 1508.
- Curtis, C. F. & Davies, C. R. 2001 Present use of pesticides for vector and allergen control and future requirements. *Med. Vet. Entomol.* **15**, 231–235.
- Curtis, C. F. & Lines, J. D. 2000 Should DDT be banned by international treaty? *Parasitol. Today* **16**, 119–121.
- Fernández-Salas, I., Roberts, D. R., Rodríguez, M. H. & Marina-Fernández, C. F. 1994a Bionomics of larval populations of *Anopheles pseudopunctipennis* in the Tapachula foothills area, southern Mexico. *J. Am. Mosq. Control Assoc.* **10**, 477–486.
- Fernández-Salas, I., Rodríguez, M. H., Roberts, D. R., Rodríguez, M. C. & Wirtz, R. A. 1994b Bionomics of adult *Anopheles pseudopunctipennis* (Diptera: Culicidae) in the Tapachula foothills area of southern Mexico. *J. Med. Entomol.* **31**, 663–670.
- Gilles, M. T. & Wilkes, T. J. 1965 A study of the age-composition of populations of *Anopheles gambiae* Giles and *A. funestus* Giles in north-eastern Tanzania. *Bull. Entomol. Res.* **56**, 237–262.
- Githeko, A. K., Mbogo, C. N., Curtis, C. F., Lines, J. & Lengeler, C. 1996 Entomological monitoring of large-scale vector-control interventions. *Parasitol. Today* **12**, 127–128.
- Gómez-Dantés, H. & Rodríguez, M. H. 1994 *Paludismo y dengue: de la erradicación a las zonas de riesgo. Cuadernos de Salud*, vol. II. Mexico City, Mexico: Secretaría de Salud.
- Gregg, W. W. & Rose, F. L. 1982 The effects of aquatic macrophytes on stream microenvironment. *Aquat. Bot.* **14**, 309–324.
- Gubler, D. J. 1998 Resurgent vector-borne diseases as a global health problem. *Emerg. Infect. Dis.* **4**, 442–450.
- Hall, T. F. 1972 The influence of plants on anopheline breeding. *Am. J. Trop. Med. Hyg.* **21**, 787–794.
- Hay, S. I., Myers, M. F., Burke, D. S., Vaughn, D. W., Endy, T., Ananda, N., Shanks, G. D., Snow, R. W. & Rogers, D. J. 2000 Etiology of interepidemic periods of mosquito-borne disease. *Proc. Natl Acad. Sci. USA* **97**, 9335–9339.
- Hay, S. I., Cox, J., Rogers, D. J., Randolph, S. E., Shanks, D. G., Stern, D. I., Myers, M. & Snow, R. W. 2002 Climate change and the resurgence of malaria in the east African highlands. *Nature* **415**, 905–909.
- Hii, J. L. K., Smith, T., Mai, A., Ibam, E. & Alpers, M. P. 2000 Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. *Bull. Entomol. Res.* **90**, 211–219.
- Hoffman, S. L. 2000 Research (genomics) is crucial to attacking malaria. *Science* **290**, 1509.
- Killeen, G. F., McKenzie, F. E., Foy, B. D., Schieffelin, C., Billingsley, P. F. & Beier, J. C. 2000 A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *Am. J. Trop. Med. Hyg.* **62**, 535–544.
- Killeen, G. F., Knols, B. G. & Gu, W. 2003 Taking malaria transmission out of the bottle: implications of mosquito dispersal for vector-control interventions. *Lancet Infect. Dis.* **3**, 297–303.
- Kitron, U. 1987 Malaria, agriculture, and development: lessons from past campaigns. *Int. J. Hlth Serv.* **17**, 295–326.
- Kitron, U. & Spielman, A. 1989 Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States and Italy. *Rev. Infect. Dis.* **11**, 391–406.
- Lines, J. D., Curtis, C. F., Wilkes, T. J. & Njunwa, K. J. 1991 Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bull. Entomol. Res.* **81**, 77–84.
- Longnecker, M. P., Klebanoff, M. A., Zhou, H. & Brock, J. W. 2001 Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. *Lancet* **358**, 110–114.
- Longnecker, M. P., Klebanoff, M. A., Brock, J. W., Zhou, H., Gray, K. A., Needham, L. L. & Wilcox, A. J. 2002 Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. *Am. J. Epidemiol.* **155**, 313–322.
- Manguin, S., Roberts, D. R., Peyton, E. L., Rejmankova, E. & Pecor, J. 1996 Characterization of *Anopheles pseudopunctipennis* larval habitats. *J. Am. Mosq. Control Assoc.* **12**, 619–626.
- Marten, G. 1986 Phytoplankton management for mosquito control: the potential of indigestible green algae. *J. Trop. Med. Hyg.* **89**, 213–222.
- Mathenge, E. M., Killeen, G. F., Oulo, D. O., Irungu, L. W., Ndegwa, P. N. & Knols, B. G. J. 2002 Development of an exposure-free bednet trap for sampling Afrotropical malaria vectors. *Med. Vet. Entomol.* **16**, 67–74.
- Mbogo, C. N. M., Glass, G. E., Forster, D., Kariru, E. W., Githure, J. I., Ouma, J. H. & Beier, J. C. 1993 Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *J. Am. Mosq. Control Assoc.* **9**, 260–263.

- Numerical Algorithms Group 1993 *The GLIM system: release 4 manual* (ed. B. Francis, M. Green & C. Payne). Oxford: Clarendon.
- Orr, B. K. & Resh, V. H. 1989 Experimental test of the influence of aquatic macrophyte cover on the survival of *Anopheles* larvae. *J. Am. Mosq. Control Assoc.* **5**, 579–585.
- Orr, B. K. & Resh, V. H. 1992 Influence of *Myriophyllum aquaticum* cover on *Anopheles* mosquito abundance, oviposition, and larval microhabitat. *Oecologia* **90**, 474–482.
- Rajagopalan, P. K., Das, P. K. & Panicker, K. N. 1991a Income generation and *Anopheles* control in some south Indian villages. In *Control of disease vectors in the community* (ed. C. F. Curtis), pp. 128–129. London: Wolfe Publishing.
- Rajagopalan, P. K., Das, P. K. & Panicker, K. N. 1991b Community participation in the control of vectors of *Brugia malayi* in Shertallia, Kerala, India. In *Control of disease vectors in the community* (ed. C. F. Curtis), pp. 129–132. London: Wolfe Publishing.
- Rejmankova, E. H., Savage, M., Rejmanek, M., Arredondo-Jiménez, J. I. & Roberts, D. R. 1991 Multivariate analysis of relationships between habitats, environmental factors and occurrence of anopheline mosquito larvae *Anopheles albimanus* and *An. pseudopunctipennis* in southern Chiapas, Mexico. *J. Appl. Ecol.* **28**, 827–841.
- Rejmankova, E. H., Roberts, D. R., Harbach, R. E., Pecor, J., Peyton, E. L., Manguin, S., Krieg, R., Polanco, J. & Legters, L. 1993 Environmental and regional determinants of *Anopheles* (Diptera: Culicidae) larval distribution in Belize, Central America. *Environ. Entomol.* **22**, 978–992.
- Rogers, D. J. & Randolph, S. E. 2000 The global spread of malaria in a future, warmer world. *Science* **289**, 1763–1766.
- Rogers, D. J., Randolph, S. E., Snow, R. W. & Hay, S. I. 2002 Satellite imagery in the study and forecast of malaria. *Nature* **415**, 710–715.
- Rose, R. I. 2001 Pesticides and public health: integrated methods of mosquito management. *Emerg. Infect. Dis.* **7**, 17–23.
- Savage, H. M., Rejmankova, E., Arredondo-Jiménez, J. I., Roberts, D. R. & Rodríguez, M. H. 1990 Limnological and botanical characterization of larval habitats for two primary malarial vectors, *Anopheles pseudopunctipennis* and *Anopheles albimanus*, in coastal areas of Chiapas state, Mexico. *J. Am. Mosq. Control Assoc.* **6**, 612–620.
- Service, M. W. 1977 A critical review of procedures for sampling populations of adult mosquitoes. *Bull. Entomol. Res.* **67**, 343–382.
- Service, M. W. 1980 Effects of wind on the behaviour and distribution of mosquitoes and blackflies. *Int. J. Biometeorol.* **24**, 347–353.
- Service, M. W. 1989 The importance of ecological studies on malaria vectors. *Bull. Soc. Vector Ecol.* **14**, 26–38.
- Service, M. W. 1993 *Mosquito ecology. Field sampling methods*, 2nd edn. Amsterdam, The Netherlands: Elsevier.
- Service, M. W. 1995 Can we control mosquitoes without pesticides? A summary. *J. Am. Mosq. Control Assoc.* **11**, 290–293.
- Service, M. W. 1997 Mosquito (Diptera: Culicidae) dispersal: the long and short of it. *J. Med. Entomol.* **34**, 579–588.
- Shiff, C. 2002 Integrated approach to malaria control. *Clin. Microbiol. Rev.* **15**, 278–298.
- Sina, B. J. & Aultman, K. 2001 Resisting resistance. *Trends Parasitol.* **17**, 305–306.
- Smock, L. A. & Stoneburner, D. L. 1980 The response of macroinvertebrates to aquatic macrophyte decomposition. *Oikos* **35**, 397–403.
- Southwood, T. R. E. & Henderson, P. 2000 *Ecological methods*, 3rd edn. Oxford: Blackwell.
- Taylor, N. J. 1979 A rapid efficient area sampler for estimating absolute abundance of floodwater mosquito larvae. *Environ. Entomol.* **8**, 1004–1006.
- Ulloa, A., Arredondo-Jiménez, J. I., Rodríguez, M. H. & Fernández-Salas, I. 2002 Mark-recapture studies of host selection by *Anopheles* (*Anopheles*) *vestitipennis*. *J. Am. Mosq. Control Assoc.* **18**, 32–35.
- Utzinger, J., Tozan, Y. & Singer, B. H. 2001 Efficacy and cost effectiveness of environmental management for malaria control. *Trop. Med. Int. Hlth* **6**, 677–687.
- Utzinger, J., Tanner, M., Kammen, D. M., Killeen, G. F. & Singer, B. H. 2002a Integrated programme is key to malaria control. *Nature* **419**, 431.
- Utzinger, J., Tozan, Y., Doumani, F. & Singer, B. H. 2002b The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. *Trop. Med. Int. Hlth* **7**, 657–677.
- WHO 1975 *Manual on practical entomology in malaria, part II. Methods and techniques*. Geneva: World Health Organization.
- WHO 1982 *Manual on environmental management for mosquito control, with special emphasis on malaria vectors, report no. 66*. Geneva: World Health Organization.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.