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Development of a novel sticky trap for container-breeding mosquitoes and evaluation of its sampling properties to monitor urban populations of *Aedes albopictus*

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Abstract. Collection methods currently used for large-scale sampling of adult *Stegomyia* mosquitoes (Diptera: Culicidae) present several operational limitations, which constitute major drawbacks to the epidemiological surveillance of arboviruses, the evaluation of the impact of control strategies, and the surveillance of the spreading of allochthonous species into non-endemic regions. Here, we describe a new sticky trap designed to capture adult container-breeding mosquitoes and to monitor their population dynamics. We tested the sampling properties of the sticky trap in Rome, Italy, where *Aedes (Stegomyia) albopictus* is common. The results of our observations, and the comparison between sticky trap catches and catches made with the standard oviposition trap, are presented. The sticky trap collected significantly larger numbers of *Ae. albopictus* females than any other Culicidae species representing >90% of the total catches. A maximum of 83 *An. albopictus* females was collected in a single week. A high correlation (Pearson correlation coefficient $r = 0.96$) was found between the number of females and the number of eggs collected by the traps. The functional relationship between the number of eggs and the number of adult females was assessed by major axis regression fitted to $\log(1 + x)$-transformed trap counts as $y = 0.065 + 1.695x$. Trap samples significantly departed from a random distribution; Taylor's power law was fitted to the trap samples to quantify the degree of aggregation in the catches, returning the equations $s^2 = 2.401\ m^{1.325}$ for the sticky trap and $s^2 = 13.068\ m^{1.441}$ for the ovitrap, with s^2 and m denoting the weekly catch variance and mean, respectively, indicating that eggs were significantly more aggregated than mosquitoes ($P < 0.0001$). Taylor's power law parameters were used to estimate the minimum number of sample units necessary to obtain sample estimates with a fixed degree of precision and sensitivity. For the range of densities encountered in our study area during the *Ae. albopictus* breeding season, the sticky trap was more precise and sensitive than the ovitrap. At low population densities ($c. < 0.1$ mosquito/trap), however, the ovitrap was more sensitive at detecting the presence of this species. Overall, our results indicate that our new model of sticky trap can

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be used to sample *Ae. albopictus* females in urban environments, and, possibly, other container-breeding *Stegomyia* mosquitoes (e.g. *Aedes aegypti*). The technical properties of the new trap are discussed with respect to its possible application in monitoring the population dynamics of container-breeding mosquitoes, in studying their bionomics, and in vector surveillance and, possibly, control.

Key words. *Aedes*, *Stegomyia*, container-breeding mosquitoes, monitoring, optimal sampling plans, ovitrap, sticky trap.

Introduction

Container-breeding mosquitoes of the subgenus *Stegomyia*, primarily *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) (Diptera: Culicidae), represent a major threat to health in the tropics as they are the most efficient vectors of significant arboviruses such as yellow fever, dengue and Chikungunya. Moreover, they are significant nuisance pests wherever their distribution extends to temperate regions, as in the case of *Ae. albopictus* in North America and Europe (Gratz, 2004).

Adult mosquito collections provide essential samples for disease surveillance and vector monitoring. Information that is most relevant at the epidemiological level can be gathered by collecting host-seeking females. For a long time, the gold standard for this purpose has involved the collection of mosquitoes that land on human volunteers, but this technique is nowadays considered unethical whenever there is an unacceptable likelihood of exposing the volunteers to the risk of contracting non-preventable and potentially life-threatening diseases. To date, light traps have been widely used to catch crepuscular and nocturnal host-seeking mosquitoes, but they are of little use for day-flying *Stegomyia* (Service, 1993). Collections of host-seeking females of this subgenus are therefore mainly performed using traps baited with host-related semiochemicals or visual stimuli (e.g. Fay–Prince, CDC Wilton or BG-Sentinel traps), which provide reliable samples, but which, in general, are not cost-effective and require a source of power. Alternatively, active collection techniques such as backpack aspirators (Clark *et al.*, 1994; Scott & Morrison, 2003) can be employed to collect all fractions of the adult mosquito population, thus providing indirect indices of mosquito abundance and vector–host contact. Although aspirators are extensively used and catch large numbers of *Ae. aegypti*, they are labour-intensive and require a source of power. Moreover, indices derived from manual collections can be biased by collector efficiency, site of collection (e.g. indoors vs. outdoors), house size, the presence of furniture and duration of sampling.

The absence of an efficient and sensitive collection method for large-scale sampling of adult container-breeding *Stegomyia* represents a major drawback to the epidemiological surveillance of arboviruses, as well as the evaluation of the impact of control strategies, and the surveillance of the spread of allochthonous mosquitoes into non-endemic regions (Scott & Morrison, 2003). The development of new operational techniques to collect adult females of container-breeding *Stegomyia*, and to monitor their densities, is therefore considered a most valuable contribution to the prevention and control of arboviruses, such as dengue (Scott & Morrison, 2003).

Ovitraps represent a sampling technique that is widely used to gather indirect indices of adult abundance of container-breeding species, derived from the number of eggs laid, and to assess their spatial/temporal distribution. Ovitraps are black jars filled with water and provided with a hardboard paddle on which *Stegomyia* females lay eggs (Fay & Eliason, 1966; Service, 1993). The great popularity of ovitraps is attributable to two crucial properties: they are inexpensive and they are simple to assemble and operate. For example, ovitraps have been used to: (a) investigate the ecological parameters of *Ae. aegypti* and *Ae. albopictus* in relation to both eco-climatic factors (Mercado Hernandez *et al.*, 2006) and dengue surveillance (Chen *et al.*, 2005); (b) study the dispersal of dengue vectors (Liew & Curtis, 2005), and (c) evaluate the efficacy of vector control strategies (Vezzani *et al.*, 2004). Although ovitrap collections are of significant operational value, they represent a poor proxy for measuring adult abundance because *Ae. aegypti* and *Ae. albopictus* females scatter the eggs produced during each gonotrophic cycle in many different breeding sites (Rozeboom *et al.*, 1973; Hawley, 1988; Clements, 1999).

The above limitation may be overcome by the use of sticky ovitraps, which are small, dark-coloured jars similar to ovitraps, supplied with adhesive strips on which the mosquitoes become stuck when they land on the trap's internal face. Sticky ovitraps allow a direct count of the actual number of females visiting the trap. Moreover, when used in areas of sympatry of different species of the *Stegomyia* subgenus, whose eggs are morphologically indistinguishable, adults collected by sticky ovitrap are easily identified, whereas ovitraps require that eggs are left to hatch and larvae reared to the fourth instar or adult stage, thus delaying identification for a week or longer (Ritchie *et al.*, 2003). Different designs of sticky ovitraps have been developed and applied in the field. Ordonez Gonzalez *et al.* (2001) tested the efficacy of a sticky ovitrap lined with an adhesive paper strip in mark–release–recapture (MRR) experiments in a dengue-endemic area of Guadelupe, Mexico. Ritchie *et al.* (2003, 2004) tested a similar type of sticky ovitrap supplemented with ovipositional attractants in an *Ae. aegypti*-infested area in Cairns, Australia, and showed an association between the number of adult females captured and the risk of dengue transmission. Sticky ovitraps have also been used for surveillance and behavioural investigations of *Ae. aegypti* and *Aedes polynesiensis* in Moorea, French Polynesia (Russell & Ritchie, 2004) and in the Torres Straits, Australia (Ritchie *et al.*, 2006).

In this paper, we describe a novel type of sticky ovitrap (hereafter referred to as 'sticky trap') we have developed (patent pending) for container-breeding mosquitoes. Unlike other types of sticky ovitrap, which are coated with adhesive material only on the inner surface of the water container, just above the water interface, our trap is designed to maximize the size of the sticky area, and hence increase the chances of catching mosquitoes when they land on the internal walls. We describe here the sampling properties of the new sticky trap, based on collections made in two study areas in Rome, Italy, where *Ae. albopictus* is abundant, and report the results of a 2-year trial conducted to evaluate the efficacy of the new sticky trap for monitoring the population dynamics of *Ae. albopictus* in comparison with standard ovitrap collections. The results of these trials are discussed in relation to the use of this novel tool for monitoring the population dynamics of container-breeding mosquitoes, for studying their bionomics and for disease and vector surveillance.

Materials and methods

Sticky trap description

Our prototype sticky trap (Fig. 1a) is made of black acrylonitrile butadiene styrene (ABS) and is composed of two elements. The first is a water-holding container, moulded in the shape of an inverted, truncated cone (diameter at base 8.5 cm, diameter at top 12 cm, height 13.5 cm; Fig. 1b). The container has four gullies moulded into its sides to hold the second element, which consists of two panels that intersect perpendicularly (height 19 cm) and which subdivide the upper volume of the trap into four quarters. A round top (diameter 13.5 cm) lies on the two panels, entirely covering the container to provide shelter from sunshine and rain (inverted in Fig. 1c). The walls of the panels in each of the four quarters are lined with transparent plastic sheets (overhead transparency sheets) coated with glue used for rodent and insect control (Zapicol; Zapi Industrie Chimiche SpA, Conselve, Padova, Italy) (Fig. 1d). The glue is formulated to tolerate long periods of heat and humidity while maintaining its adhesive properties. Four small pieces of white plastic (1 × 3 cm), attached to the edges of the sections near the top, hold the coated sheets in place (Fig. 1a, c). Similarly, a round, white plastic base (diameter 16.5 cm) is attached to the base of the trap to increase its stability. The contrast between the black body of the trap and the white base and sheet holders provides a strong visual stimulus to approaching mosquitoes.

Study sites and field sampling

We tested the sampling properties of our new sticky trap in two study areas in Rome. Site 1 was the internal courtyard (about 0.4 ha) of a building located in a residential area. Eight sticky traps (20 traps/ha) were serviced daily from 6 September to 7 October 2003 and on a 10-day schedule from 8 August to 6 September and from 7 October to 6 December 2003. Site 2 was the Zoological Gardens (*Bioparco di Roma*), a 16-ha area located inside a large urban park about 1.5 km from site 1, characterized by high levels of *Ae. albopictus* infestation (Di Luca *et al.*, 2001; Pombi *et al.*, 2003). Sixty sticky traps were deployed throughout the area (3.75 traps/ha) from 24 July 2003 to 30 June 2004 (phase 1: weeks 1–49). From 1 July 2004 to 29 July 2005 (phase 2: weeks 50–105), the number of traps was reduced to 20 (1.25 traps/ha). The population dynamics of *Ae. albopictus* in the area were monitored in parallel for the whole 2-year period by means of weekly collections with 20 ovitraps. During the winter months (weeks 21–37 and 73–89), both traps were serviced every 2–4 weeks. Both types of trap were placed in shaded sites, but were sufficiently exposed to be readily visible.

Sticky traps were serviced as follows: (a) the sticky sheets were removed and mosquitoes were morphologically identified and counted by species and gender immediately in the field; (b) the water container was emptied and filled with approximately 400 mL fresh tap water, and (c) the trap was equipped with new transparent sheets freshly coated with glue. During daily collections at site 1, we marked the positions of stuck mosquitoes on the backs of the adhesive sheets with permanent markers, using a different colour for each day, and renewed the sheets weekly.

Ovitraps were constructed and serviced according to standard practices (Di Luca *et al.*, 2001; Reiter & Nathan, 2001). Small, black plastic pots filled with 400 mL tap water were provided with a masonite paddle (20 × 2 cm) as an oviposition substrate. Ovitraps were serviced at the same time as the sticky traps: the masonite paddle was removed and the ovitrap was emptied, scrubbed and provided with a new clean paddle. Fresh tap water was then poured into the container and the ovitrap was put back in place. Removed paddles were placed in single plastic bags and brought to the laboratory, where the number of eggs was counted under a dissecting microscope. To verify that *Ae. albopictus* was the only mosquito in our study area that laid eggs on the paddles, some eggs were kept and allowed to hatch so that the larvae could be used to identify samples to species. *Aedes albopictus* was the sole species identified in these tests. Traps that were found without water, or that were lost during the trial, were replaced the following week and were discarded from data analysis.

At site 2, a preliminary MRR experiment was carried out in August 2003: 62 female and 20 male *Ae. albopictus* were collected by human landing catches, marked with fluorescent pigments (Day-Glo Color Corp., Cleveland, OH, U.S.A.) and released in the centre of the study area. The sticky sheets that were removed during the weekly service were brought to the laboratory for the following 2 weeks, observed under ultraviolet (UV) light and fluorescent mosquitoes recorded.

Statistical inference

Collection data are presented as the total number of mosquitoes caught and the geometric mean number of *Ae. albopictus* females caught over 10 days (site 1) or each week (site 2). Count data from trapping devices generally exhibit several undesirable properties for the estimation of statistical parameters, of which non-normal distribution and non-constancy of variance represent the most serious departures from the assumptions of

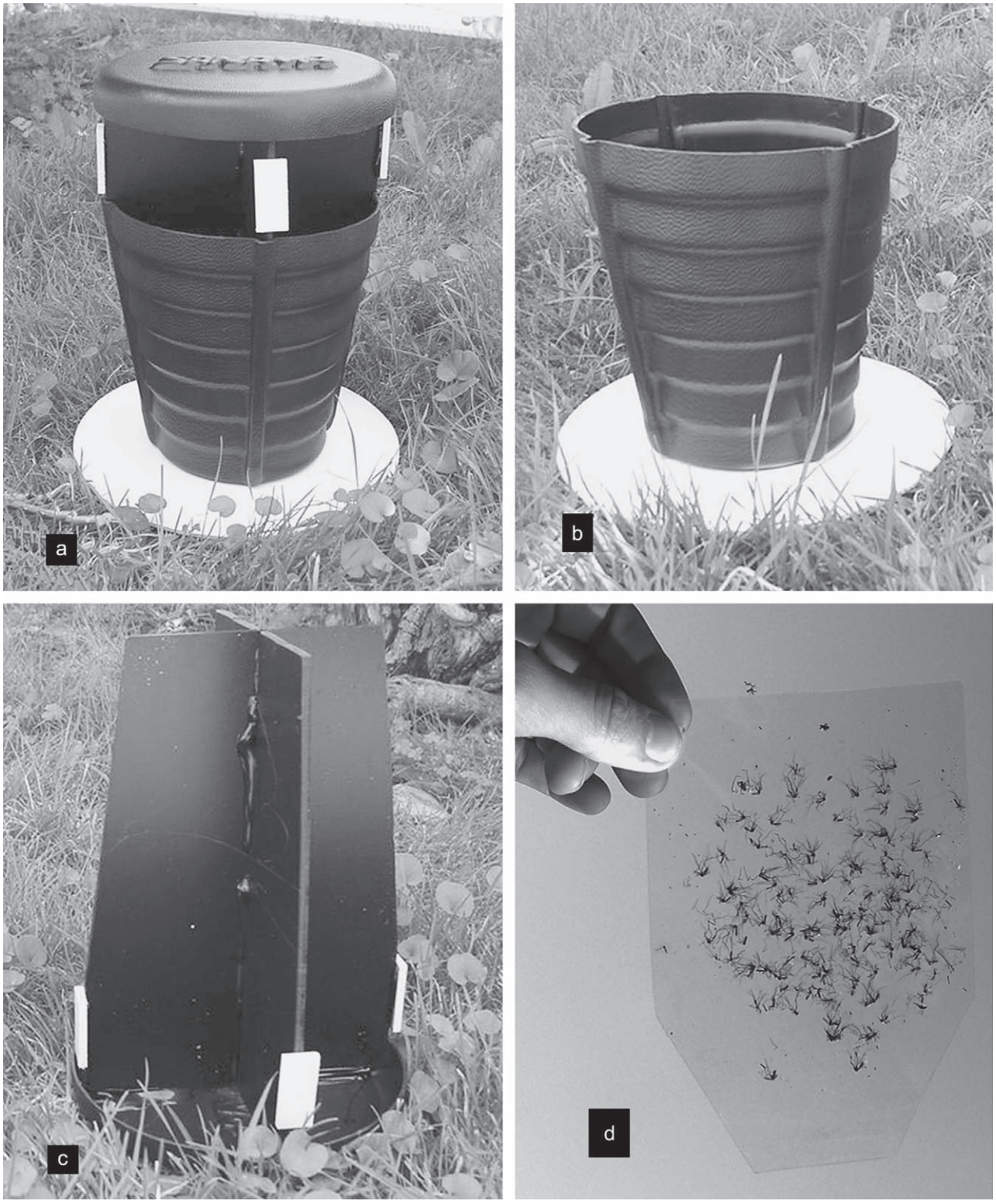

Fig. 1. Photographs showing details of the new sticky trap developed and tested in this study. (a) The trap assembled as operative. (b) Close-up of the water-holding container. (c) The trap's disassembled top and intersecting sections. (d) Close-up of the adhesive plastic sheets.

parametric statistical models. Transformation of trap counts can alleviate the problem of heteroscedasticity in the data. In our case, we assumed a log-normal distribution and transformed trap catches to $\log(1 + x)$ prior to analysis; the addition of 1 to the counts prior to transformation was necessary as a result of the frequent presence of traps containing no eggs or mosquitoes (i.e. zero counts). Then, 10-day and weekly mean catches were calculated from the transformed data. Subsequently, mean catches were back-transformed to a linear scale to give weekly geometric mean catches; these are usually referred to as Williams' means.

One of our aims was to investigate whether the sticky trap provided samples that were consistent with the dynamics of the population of *Ae. albopictus* present in our study area, as inferred from an independent and proven sampling technique (i.e. the ovitrap). Hence, we aimed to verify whether the two sampling techniques gave correlated catches, and assessed the functional relationship of the weekly mean catch of an ovitrap with that of a sticky trap. The degree of correlation between catches of *Ae. albopictus* collected by sticky traps and ovitraps was evaluated by calculating the Pearson product−moment correlation coefficient. Homogeneity of the correlation coefficient between the two sampling phases (which corresponded to two slightly different sampling plans [cf. above]) was tested by calculating a chi-square statistic according to the procedure described in Sokal & Rohlf (1995). As both the sticky trap and ovitrap were subject to sampling error, the functional relationship between these sampling techniques could not be assessed by ordinary regression. We fitted a type 2, or major axis, regression line instead (Sokal & Rohlf, 1995).

Sampling properties of trapping devices, which are evaluated to determine optimal sampling plans, depend on the spatial dispersion of the sampled population. We assessed the dispersion parameters of *Ae. albopictus* using our weekly collections at site 2 by individual sticky traps and ovitraps as sample units, by calculating the variance: mean ratio (VMR) of weekly catches. In the case of a population that is dispersed at random, the VMR = 1; populations regularly distributed in space are characterized by values of VMR < 1, whereas values of VMR > 1 indicate an aggregated distribution. We assessed whether the VMR departed significantly from unity by applying the chi-square test according to Elliott (1977; pp. 40–44). An alternative approach is to assume that trap catches are aggregated, in which case one needs to estimate the degree of aggregation present in the samples. A popular statistical model used to describe the spatial dispersion of an aggregated population is the negative binomial distribution. This is characterized by the parameter k, which can be used as an index of aggregation. In a series of samples, a common k (k_c) can be approximated from the slope of the regression line relating $x' = m^2 - s^2/n$ and $y' = s^2 - m$, where m is the mean and s^2 the variance of samples of size n (Elliott, 1977; pp. 63–66). The regression line passes through the origin and has slope $1/k_c$. This estimation is possible only when there is no relationship between m and $1/k_c$.

A more general relationship used to quantify the degree of aggregation in samples is based on Taylor's power law. This statistical model is an empirical functional relationship that linearly relates on a logarithmic scale the variance (s^2) and the arithmetic mean (m) of samples such that $s^2 = am^b$, where b is an index of aggregation, and a is a constant dependent on the size of the sample unit and environmental conditions (Taylor, 1961). The value of b is independent of mean density and many studies have shown that it is usually a constant characteristic of a species in a particular environment. Uniform, random and aggregated distributions are, respectively, expressed by values of $b < 1$, $b = 1$ and $b > 1$ (Taylor, 1984; Kuno, 1991).

We followed Pitcairn *et al.* (1994), who elaborated two approaches to optimal sampling plans. On one hand, the quantitative analysis of dispersion is useful in determining the pattern and number of samples necessary to obtain mean estimates with a required level of precision. For Taylor's power law model, the minimum number N of samples to be collected to obtain sample estimates of mean density of fixed proportion $\pm$ d (hereafter, sample *precision*) is:

$$N = \left(\frac{Z_{a/2}}{d} \right)^2 a \cdot m^{b-2} \quad (1)$$

where Z is the standard normal distribution value for a given probability α (Buntin, 1994). By contrast, when the density of a population is low, it can be useful to know what sample size N^* is necessary to obtain at least one individual with a given probability of success (hereafter, sample *sensitivity* [Pitcairn *et al.*, 1994]). This is given by:

$$N^* = \frac{\log_e(1-\alpha)}{\log_e P_0} \quad (2)$$

where P_0 is the probability of collecting no individuals; using Taylor's power law, P_0 can be estimated as:

$$P_0 = \left(1 + \frac{am^b - m}{m} \right)^{\frac{-m^2}{am^b - m}} \quad (3)$$

Results

Sticky trap catches

We observed that mosquitoes that landed on the adhesive surface initially remained erect, with only the legs and/or wings attached to the glue, thereby retaining their morpho-taxonomical character in good view and shape. Later, however, mosquitoes fell laterally or collapsed ventrally, and became completely stuck to the adhesive sheets. Despite this, most of the specimens retained visible diagnostic characters even after 10 days, and only a few could not be identified, as reported by Ritchie *et al.* (2003). Although we did not systematically record the gonotrophic stage, we observed that mainly gravid females were collected; however, unfed and freshly fed females were also found. In some cases, eggs extruding from the abdomens of gravid females were observed attached to the sticky surface. Larvae were found in the sticky traps only very rarely. Geckos and lizards were occasionally found stuck to the adhesive sheets and snails were observed resting in the traps.

Site 1. By the end of the 4-month sampling period, the sticky trap had collected 1606 *Ae. albopictus* in 958 trap days. Most of these (85.7%) were females, thus resulting in a mean collection rate of 1.4 females/trap/day. The maximum geometric mean number of females/trap/10-days was 27.4 in August and 44.9 in early September, after which it gradually decreased to 0.2 in early December. The maximum number of females collected in a single trap was 101. Moreover, few *Culex pipiens* ($n = 35$) were caught.

The number of *Ae. albopictus* females collected daily in the eight sticky traps is shown in Fig. 2. The maximum and minimum numbers of females collected were 25 and two, respectively. During daily collections, we sometimes observed whole sets of mosquito legs stuck to the adhesive sheets or areas without glue in spots previously marked for the presence of mosquitoes. We concluded that mosquitoes had shed their legs when trying to fly off from the trap, or, in the second instance, that the bodies of mosquitoes had possibly been eaten by animals visiting the traps.

Site 2. By the end of the 2-year sampling period, the sticky trap had collected 21 978 *Ae. albopictus* in 28 265 trap days. Most of these (91.6%) were females, giving a mean collection rate of 0.71 females/trap/day. The maximum mean numbers of females/trap/week collected were 24.7 (week 5) and 20.2 (week 59) in the 2003–04 and 2004–05 seasons, respectively; between weeks 19 and 41 and weeks 70 and 91 no mosquitoes were collected. The maximum number of females collected in a single trap was 83. A small number of *Cx pipiens* L. females ($n = 546$) and males ($n = 192$), a few *Culiseta longiareolata* (Macquart), *Aedes geniculatus* (Olivier), *Aedes berlandi* Séguy and a few unidentified sandflies were also trapped.

Figure 3 shows the population dynamics of *Ae. albopictus* adult females and eggs as obtained in sticky trap and ovitrap catches, respectively. When traps were serviced every 2–4 weeks, the mean number of mosquitoes or eggs was divided by the corresponding number of weeks separating two successive sampling events. Overall, the sticky trap and the ovitrap yielded, respectively, 0.6 *Ae. albopictus* females/trap/day and 6.1 eggs/ovitrap/day in phase 1, and 1.0 females/trap/day and 9.0 eggs/ovitrap/day in phase 2. Limiting the analysis to the peak seasons (i.e. weeks 1–12 and 50–67 in phases 1 and 2, respectively, which corresponded to periods when $> 95\%$ of the ovitraps were found to have at least one egg), the sticky trap and the ovitrap yielded 2.2 females/trap/day and 13.7 eggs/ovitrap/day, respectively, in phase 1 and 1.9 females/trap/day and 19.4 eggs/ovitrap/day, respectively, in phase 2. The results of the preliminary MRR experiment showed an overall recapture rate of 22.6% (i.e. 14 of the 62 *Ae. albopictus* females marked with fluorescent pigments and released in the centre of site 2 were recaptured within 2 weeks of being released, and 11 of them were caught in the first week). No marked males were recaptured. Fluorescent individuals were found mainly in traps close to the release site, but also in the most distant traps in the study area, about 210 m from the release site.

Relationship between sticky trap and ovitrap catches

The logarithm for the mean number of *Ae. albopictus* females caught during a week at site 2 by the sticky traps was highly correlated with the logarithm for the mean number of eggs found in the ovitraps (Pearson correlation coefficient $r = 0.958$, 95% confidence interval [CI] 0.932–0.974, $n = 66$, Student's $t = 15.3$, $P < 0.0001$) (Fig. 4). The correlation coefficient remained homogeneous between the two sampling phases, at $r = 0.956$ for the first period and $r = 0.968$ for the second period ($\chi^2 = 0.46$, $P = 0.79$). On the logarithmic scale, the slope of the fitted major axis regression line was 1.695 (95% CI 1.555–1.853) and the intercept 0.065 (Fig. 4). The slope of the major axis regression line on the logarithmic scale was significantly different from 1 (its 95% CI did not overlap unity); hence the functional relationship between the two sampling techniques was density-dependent on the linear scale, with ovitraps collecting proportionally more eggs than sticky traps collected mosquitoes at increasing population densities.

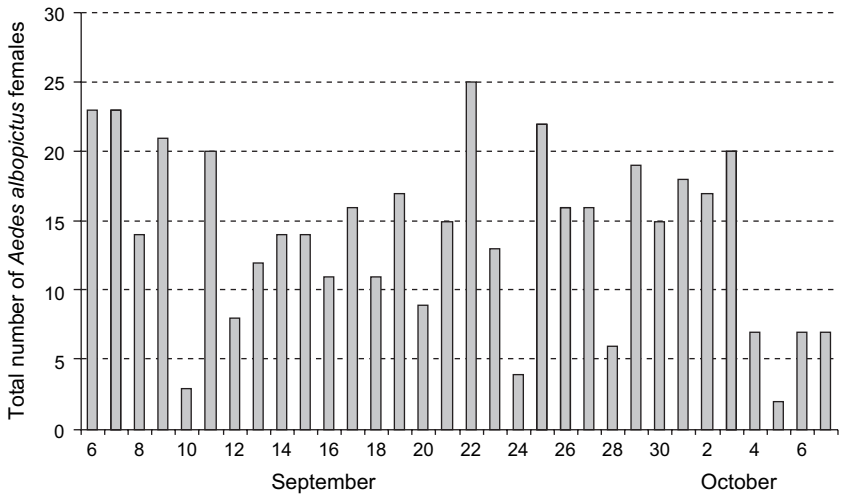

Fig. 2. Total numbers of *Aedes albopictus* females collected daily with eight sticky traps at site 1.

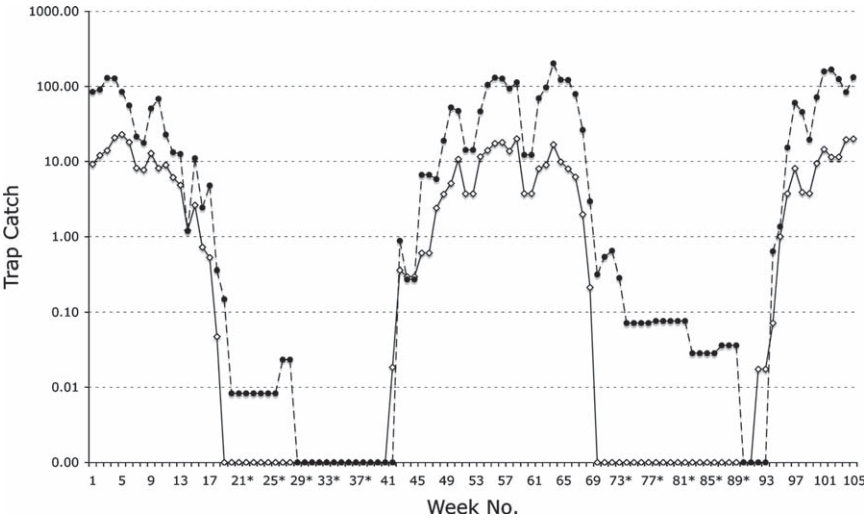

Fig. 3. Population dynamics of *Aedes albopictus* eggs and adult females in the zoological gardens in Rome (site 2) during two successive breeding seasons (August 2003–July 2005). Williams' means of sticky trap (◊) and ovitrap (●) catches are plotted starting from week 1 (1 August 2003). Asterisks at datapoints denote weeks when the traps were in place but were serviced on a 2–4-week schedule; the density for these weeks was interpolated as the mean value for the interval separating two successive sampling events, established from the mean catch of the first week when the traps were serviced.

Dispersion profile and minimum sample size

To assess the sampling properties of both the sticky trap and the ovitrap and to provide a framework for optimal sampling plans for *Ae. albopictus*, the dispersion profiles of sticky trap and ovitrap catches were investigated by calculating the VMR, and optimal sampling plans for each type of trap were quantified according to Taylor's power law.

Dispersion profile. With the exception of four of 55 tests, the VMR of weekly catches by the sticky traps was always significantly > 1, indicating that most of the time the dispersion of *Ae. albopictus* females was not compatible with a random distribution. In the case of the ovitrap, all 64 tests returned a significantly non-random distribution ($P < 0.05$). After applying Bonferroni correction for multiple tests, the numbers of non-significant tests at the 5% experiment-wise error rate increased to seven (12.7%) and four (6.3%) for sticky trap and ovitrap catches, respectively. Weeks when the test returned a dispersion profile compatible with a Poisson distribution covered mean catches in the range of 0.05–1.35 mosquitoes/trap and 0.03–0.78 eggs/trap. Overall, the dispersion of *Ae. albopictus* adult females and eggs among the traps was highly aggregated, except at the lowest range of densities measurable by our sampling plan, when aggregation, even if present, was unlikely to be detected. Thus, we assumed that the negative binomial distribution constituted a good approximation of the dispersion of mosquitoes or eggs among the traps, as repeatedly found in numerous studies of the spatial distribution of insects (Kuno, 1991), and proceeded to calculate the parameter k of this type of distribution. The calculation of a common k, however, was prevented by a significant association between $1/k$ and mean density in the case of sticky trap catches ($F = 4.83$, d.f. $= 1,51$, $P = 0.03$), indicating that for this type of trap the level of aggregation slightly decreases with mean density (slope of regression line relating $1/k$ and mean density, -0.022 ± 0.010 standard error [SE]). Hence, we estimated aggregation and optimal sampling plan parameters from the more general relationship between the variance and the mean of trap catches based on Taylor's power law.

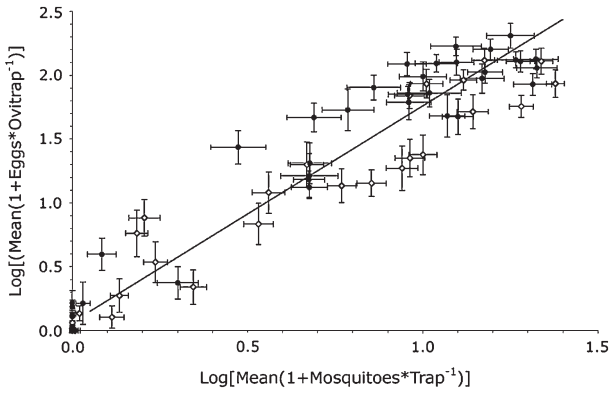

Fig. 4. Correlation between ovitrap and sticky trap mean weekly catches in site 2. ◊ = sampling phase 1; ● = sampling phase 2 (cf. text). Both variates on the abscissa and ordinate are subject to sampling error, which is estimated by standard errors drawn on both axes for each point in the scattergram. The fitted major axis regression is shown as a solid line.

Taylor's power law. The means and variance of weekly trap catches were plotted and regression lines fitted to the datapoints (Fig. 5). The regression coefficient was 1.325 (± 0.027 SE, 95% CI 1.270–1.380) and 1.441 (± 0.034 SE, 95% CI 1.373–1.509) for the sticky trap and ovitrap, respectively. As 95% CIs did not overlap unity, both coefficients were significantly different from 1, at least at $P < 0.05$, indicating an aggregated distribution of catches for both traps. The two coefficients were significantly different from each other ($F = 40.51$, d.f. $= 1115$, $P < 0.0001$), indicating that the degree of aggregation was significantly higher for ovitraps than for sticky traps. Back-transformation of regression intercepts on the linear scale gave the following Taylor's power law equations: $s^2 = 2.401\ m^{1.325}$ for the sticky trap, and $s^2 = 13.068\ m^{1.441}$ for the ovitrap.

Optimal sample plans. We used the estimates of the Taylor's power law coefficients to calculate: (a) the minimum number of sample units necessary to obtain 95% CIs of mean density as $\pm d$ of the sample mean (i.e., trap precision), and (b) the minimum

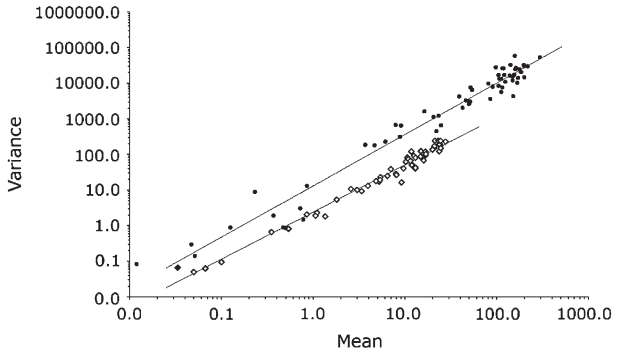

Fig. 5. Fitted Taylor's power law regression lines for *Aedes albopictus* mean weekly catches by the sticky trap (◊) or standard ovitrap (●).

number of sample units necessary to obtain at least one mosquito or egg on 95% of sampling occasions (hereafter trap *sensitivity*), according to formulae derived from optimal sampling theory (see Materials and methods).

The minimum number of sample units necessary to maintain a fixed level of precision or sensitivity depends upon the mean density of the population to be sampled: at lower population densities, the sampling effort required to keep a certain degree of precision or sensitivity increases monotonically. The functional relationship between mean density and number of sample units is plotted for three levels of precision in Fig. 6a for the sticky trap and Fig. 6b for the ovitrap. As expected, we found that high levels of precision required a large sampling effort at low population densities. For example, Fig. 6a shows that in the case of the sticky trap, 1092 sampling units at a mean density of 0.1 are required to obtain estimates of the sample mean within $\pm$ 20% of the parametric mean with 95% probability. Similarly, Fig. 6b shows that in the case of the ovitrap, 1255 sampling units at a density of one egg per ovitrap are needed to achieve the same level of precision. These are often impractical levels of sampling effort and more feasible targets of accuracy can be arbitrarily established by setting *ad hoc* values for the parameters α and d in equation 1. However, the minimum number of sample units becomes feasible at the range of population densities that are currently observed in medium- to highly infested

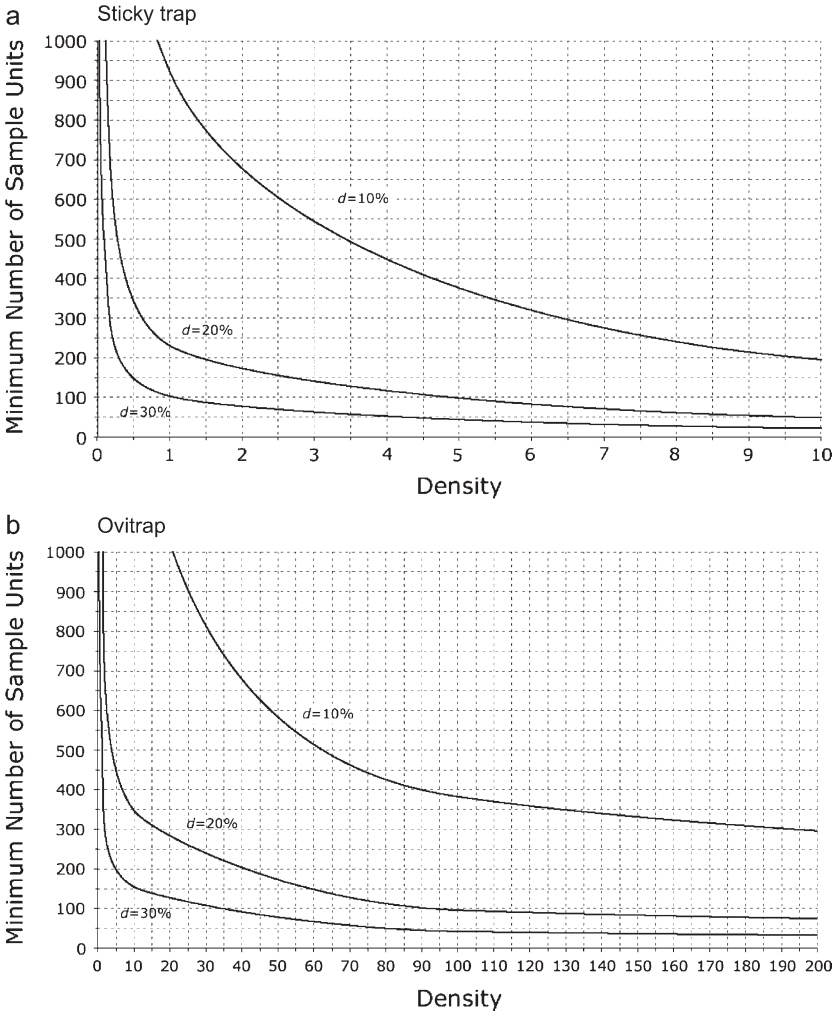

Fig. 6. Trap precision. Relationship between the mean density in the population, expressed as the weekly mean number of *Aedes albopictus* adult females or eggs per trap, and the minimum number of sample units necessary to obtain 95% confidence limits as a fixed proportion $\pm$ d of the sample mean. Three curves are drawn for values of $d = 10\%$, 20% or 30% for both the sticky trap (a) and ovitrap (b).

areas during periods of stable dynamics. At site 2, we frequently recorded catches of *c.* 10 *Ae. albopictus* females per sticky trap and *c.* 100 eggs per ovitrap (Figs 3 and 5). At these population densities, the minimum number of sample units required for a 30% level of precision are $n = 22$, and $n = 42$ units, respectively, representing quite reasonable figures.

Figure 7 shows the relationship between the minimum number of sample units and the mean density for a sampling plan requiring a 95% level of sensitivity. Again, the sampling effort needed is prohibitively large at very low densities (≤ 0.001). At a population density of 0.01, our estimates give $n = 223$ sample units for the sticky trap, and $n = 397$ sample units for the ovitrap. These figures drop to 32 and 72, respectively, at a mean density of 0.1.

However, the units of density are inherently different between the two traps (i.e. one adult female for the sticky trap and one egg for the ovitrap). Thus, in order to compare the sampling properties of the two traps, we calculated calibration curves for the ovitrap, the units of which were standardized according to the functional relationship between the two sampling devices, as assessed by major axis regression (Fig. 4). The resulting calibration curves are plotted in Fig. 8. Figure 8a shows the calibration curve calculated at $d = 30\%$; the plot demonstrates that, at exceedingly low ($\sim < 0.02$) or high ($\sim > 400$) mosquito densities, the ovitrap is comparatively more precise than the sticky trap. Within the range of densities encountered in our study area, however, the sticky trap required a lower number of sample units than the ovitrap for the same degree of precision. The same pattern applies for other degrees of precision, *d*.

At population densities corresponding to a threshold of ~ 0.135 females per sticky trap (i.e. ~ 0.440 eggs per ovitrap), the two sampling techniques require the same number of traps ($n = 25$) to achieve a 95% level of sensitivity (Fig. 8b). At densities higher than this, the sticky trap needs fewer sampling units than the ovitrap to achieve the same degree of sensitivity (i.e. if everything else is equal, the sticky trap is a more sensitive sampling tool than the ovitrap at this range of densities). However, at densities increasingly lower than this threshold, the ovitrap requires a markedly lower number of sampling units.

Discussion

Sampling properties of the sticky trap

Under our ecological conditions, the sticky trap we devised was effective and highly specific for collecting *Ae. albopictus*, which represented 96.8% of the total mosquitoes collected in either site, despite the presence of other mosquito species in Rome (Romi & Sabatinelli, 1997). In fact, only a few *Cx pipiens*, *Cu. longiareolata*, *Ae. berlandi* and *Ae. geniculatus* specimens were captured in the trap. In particular, it is relevant to note that at site 2, *Cx pipiens* represented 3.2% of the mosquitoes collected in the sticky trap, despite the findings of Pombi *et al.* (2003), showing that the larval densities of this species in the sewer catch basins of the area were markedly higher (i.e. 0–33.2 larvae/dip) than those of *Ae. albopictus* (i.e. 0–1.3 larvae/dip).

It was relatively easy to assess the taxon and gender of captured specimens directly by eye in the field, even without the help of a magnifying glass. However, we anticipate that in areas where *Stegomyia* mosquitoes of similar morphology are present (e.g. *Ae. aegypti*, *Ae. albopictus*, *Ae. scutellaris*), species identification under a dissecting microscope is advisable. In fact, we already have records showing that, in different geographical areas (i.e. central Thailand, northern Queensland, Burkina Faso and Venezuela), the sticky trap captures container-breeding mosquitoes other than *Ae. albopictus*, such as *Ae. aegypti,* the major vector of yellow fever and dengue, and *Culex quinquefasciatus*, a vector of other arboviruses and filariae (Facchinelli *et al.*, 2005, unpublished data; Valerio *et al.*, 2005, unpublished data; Costantini *et al.*, 2006 unpublished data).

The number of mosquitoes on the sticky sheets was easily counted directly in the field; however, it is possible that the catch was sometimes underestimated as a result of small geckos, lizards and snails having eaten the bodies of captured mosquitoes, leaving sets of legs on the glue, as shown by the recurrent observation of whole sets of mosquito legs without bodies stuck on the adhesive sheets.

As expected, the majority of females collected were gravid, although we frequently also noticed the presence of unfed and,

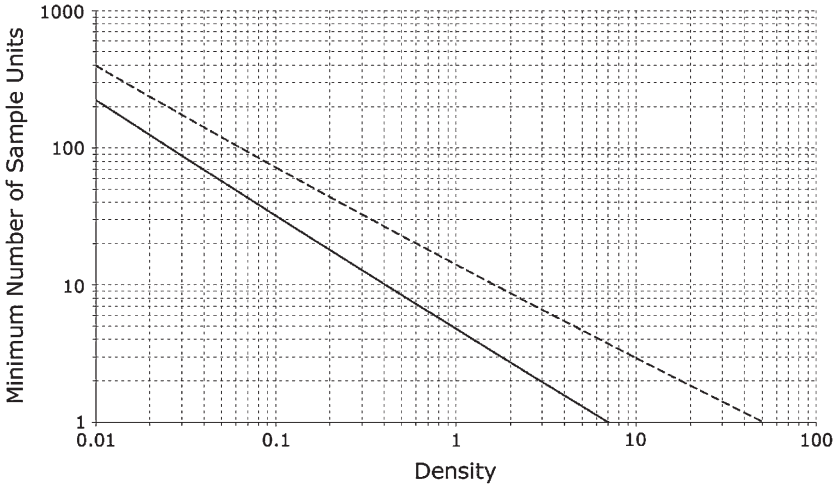

Fig. 7. Trap sensitivity. Relationship between the mean density in the population, expressed as the weekly mean number of *Aedes albopictus* adult females or eggs per trap, and the minimum number of sample units necessary to obtain at least one adult female *Ae. albopictus* with the sticky trap (solid line), or *Ae. albopictus* egg with the ovitrap (dotted line), on 95% of sampling occasions.

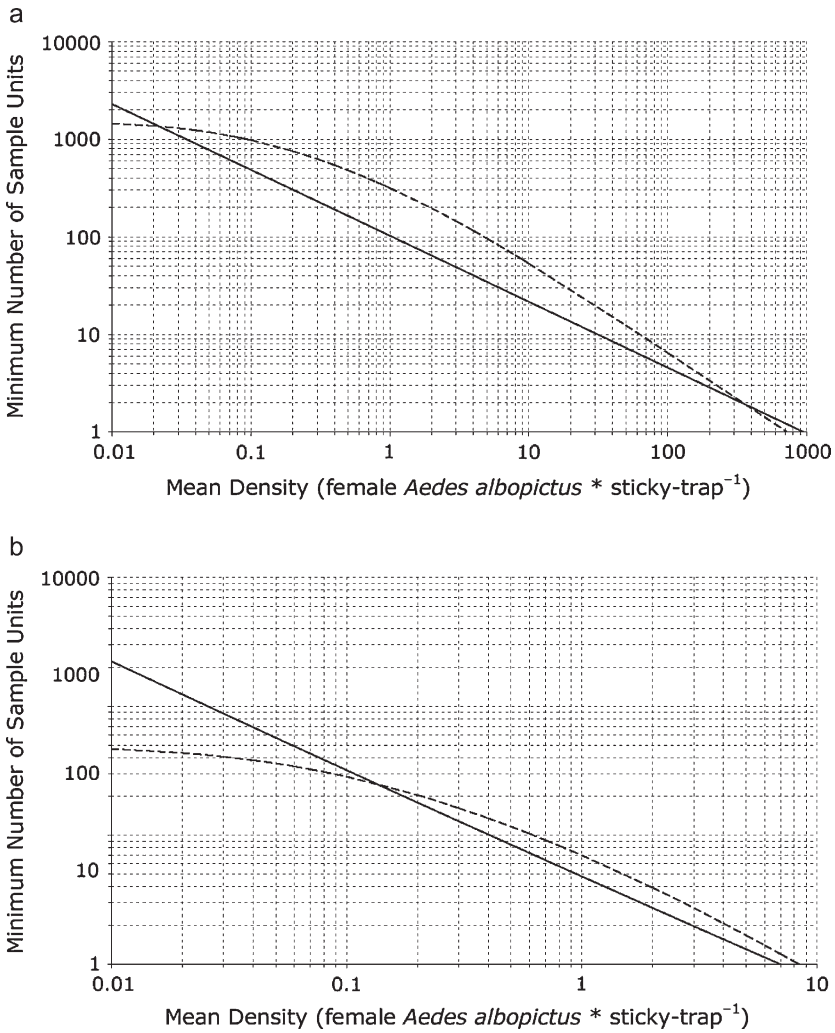

Fig. 8. Standardized calibration curves for 95% trap sample precision (a) and sensitivity (b). In (a), the curves are calculated for $d = 30\%$. Solid lines refer to the sticky trap, and are identical to those plotted in Figs 6a and 7 for this sampling device. Dotted lines refer to the calibration curves for the ovitrap.

more rarely, blood-fed females, suggesting that the traps were exploited not only by ovipositing females, but also by other fractions of the mosquito population, probably in response to cues associated with resting sites. This hypothesis is supported by the repeated presence of males in the traps, although they represented $< 10\%$ of the total number of *Ae. albopictus* collected.

Both the 10-day and weekly schedules of trap servicing were adequate under our conditions. The lid on the top of the trap protected the mosquitoes on the sticky sheets from rain and sunshine, thereby preserving both captured specimens and glue in good condition. However, the optimal frequency of trap servicing might depend on local ecological and environmental conditions. We anticipate that, under certain conditions, debris might accumulate on the sticky sheets, hindering the identification of captured mosquitoes and the adhesive properties of the glue. Moreover, as we occasionally found some larvae breeding in the trap, a weekly servicing would be advisable under climatic conditions favourable for rapid larval development. We also hypothesize that rains stronger than those experienced during our trial might wet and damage the captured specimens. A larger lid could be provided in these cases to protect the adhesive surfaces from heavy rains.

Comparison of sticky trap and ovitrap catches

At site 2, the mean number of *Ae. albopictus* females collected by the sticky traps was highly correlated with the mean number of eggs collected by the ovitraps over a wide range of densities. Despite the operational differences between the two sampling phases (the sampling plan changed from a sticky trap : ovitrap ratio of 3 : 1 to one of 1 : 1 between phases; see Materials and methods), the degree of correlation between the two sampling tools remained homogenously high. A linear relationship between the mean number of eggs collected by the ovitrap and the mean number of females collected by the sticky trap was fitted on the logarithmic scale (Fig. 4). This model allows for the description of the density-dependent relationship between the mean number of females/sticky trap and the

mean number of eggs/ovitrap under our experimental conditions. From the log-regression model, it can be estimated that when the sticky trap collects one or 10 *Ae. albopictus* females/trap, the ovitrap is expected to catch 2.8 or 66.6 eggs/ovitrap, respectively. Thus, no single multiplication factor on the linear scale can relate these two variates.

Both types of trap yielded highly heterogeneous catches, as demonstrated by the high VMRs, indicating that some traps caught greater numbers of mosquitoes or eggs, whereas others were markedly less productive. Taylor's power law was fitted to the trap samples, providing evidence that the degree of aggregation was significantly higher in the case of eggs per ovitrap compared with mosquitoes per sticky trap. Given the aggregated pattern of the trap samples, we used the coefficients of Taylor's power law regression lines (Fig. 5) to develop optimal sampling plans (equations 1−3). The usefulness of this conceptual framework is that optimal sampling plans can set a priori levels of statistical *precision* required from sample estimates (e.g. when levels of infestation must be compared among locations, or before and after a vector control intervention). For example, suppose that a vector control operation is planned in an area where *Ae. albopictus* densities are expected to average 10 adult females per sticky trap. Vector control managers may wish to verify that they can obtain a reduction of at least 50% in mosquito densities, and want to be able to detect whether their control intervention is successful in attaining this target or otherwise. Thus, the sampling plan required to evaluate the impact of the control operation must be set so that there is sufficiently high statistical power (say, 95% probability) to be able to detect a significant difference between the mean densities before and after treatment. One way to do so is to calculate (using equation 1) the number of sampling units that will return estimates of mean density before and after treatment whose 95% CIs do not overlap. With a fixed precision of 30%, the sample estimate of mean density will lie in the range of 7–13 before treatment, and 3.5–6.5 post-treatment if the control operation attains its target. By putting the relevant parameters in equation 1 (i.e. $d = 0.3$, $\alpha = 0.95$, $m = 10$ before the control intervention, and $m = 5$ after the control intervention), one can calculate a priori the minimum number of sample units required to attain the level of statistical precision needed to test this hypothesis. In this fictitious case, n should be 22 before and 35 after the control operation.

Under certain operational conditions it is more appropriate to estimate the minimum number of samples required to give a positive record with a known level of probability. This is often the case in surveillance campaigns where only the presence of a potential invader species needs to be ascertained, not its density. In these conditions, the invader will often be present at very low densities during the early stages of colonization. Thus, good sensitivity of the sampling plan, rather than precision, is required in this context.

Our results show that, at population densities higher than 0.135 females/trap, our novel sticky trap is more sensitive than the ovitrap in detecting the presence of *Ae. albopictus* (Fig. 8b), (i.e. a lower number of sticky traps than ovitraps is needed to catch at least one mosquito [vs. one egg] on 95% of sampling occasions). At densities below this threshold, the ovitrap is more sensitive than the sticky trap. This reversal is presumably the outcome of the different sampling properties and unknown relative efficacy of the two trapping devices, whereby the density-dependent functional relationship relating the two sampling devices and differences in dispersion profiles contribute to shape the observed pattern.

Conclusions

Overall, our results indicate that our new model of sticky trap can be used effectively to catch large numbers of *Ae. albopictus* adults, and as an alternative sampling tool to the standard ovitrap for monitoring the population dynamics of *Ae. albopictus* and, possibly, other container-breeding mosquitoes, particularly those belonging to the subgenus *Stegomyia (*e.g. *Ae. aegypti*). Moreover, our results indicate that the sticky trap is more sensitive than the ovitrap for detecting the presence of *Ae. albopictus* during its reproductive season in Rome, although the opposite was shown at very low densities. It will therefore be important to determine the sensitivity of the sticky trap before using it in surveillance activities under other ecological situations.

The sticky trap has several operational advantages over the ovitrap: (a) in areas of sympatry of *Aedes* whose eggs are morphologically indistinguishable, specimens captured with the sticky trap can be easily identified, whereas the ovitrap requires that eggs are kept for the identification of larvae or adults, which represents a major constraint in quarantine surveillance operations, where the immediate identification of introduced pests is desirable; (b) specimens collected by the sticky trap can be counted relatively easily, eliminating the need for egg-counting under a microscope, and (c) the sticky trap overcomes the inherent limitation of trapping devices that collect eggs rather than adult mosquitoes: the latter do not allow a simple extrapolation of the number of ovipositing females from the number of eggs collected. However, the efficiency of the sticky trap is likely to be affected by the availability and abundance of alternative breeding sites in the surveyed area, as has already been demonstrated with the ovitrap (Focks, 2003).

Compared with other types of sampling device targeted at trapping host-seeking adult mosquitoes (e.g. the Fay−Prince, CDC Wilton and BG-Sentinel traps), the sticky trap is cheap, easy to manipulate and does not need a source of power. However, it should be stressed that as the sticky trap collects mainly gravid females, it could be employed for the surveillance of container-breeding mosquito pests and vectors, and/or the monitoring of the impact of mosquito control tactics, but not for the estimation of man−vector contact. Compared with backpack aspirators which are used periodically (e.g. for a few minutes/week in each sampling premise) to actively collect mosquitoes, the sticky trap operates for the whole sampling period and is not biased by the operator's skill level.

The sticky trap also represents an attractive tool for ecological and epidemiological research. The specimens collected by the sticky trap can be used for studies on dispersal and longevity: the results of our preliminary MRR experiment confirm that specimens dusted with fluorescent powders can be detected relatively easily by observing the sticky sheets under UV light, as has already been shown with a different type of sticky trap

(Russell *et al.*, 2005). It is interesting to note that, whereas we obtained a recapture rate of 22.6%, Russell *et al.* (2005) reported a recapture rate after 15 days of only 3.4% for *Ae. aegypti*, although their trial was carried out in a study area of comparable size and with a much higher density of traps (9.2 traps/ha vs. 3.8 traps/ha. Differences in ecological conditions and target species may account for these results. Although we did not carry out such specific tests in our study, we anticipate that the specimens collected by our sticky trap could also be: (a) assayed for the presence of pathogens, such as the dengue virus (Bangs *et al.*, 2001; Ritchie *et al.*, 2004); (b) genotyped for insecticide resistance alleles (Rodriguez *et al.*, 2001), and (c) analysed for the origin of the bloodmeal in the case of specimens captured before the completion of bloodmeal digestion. Finally, we suggest that, in areas where alternative breeding habitats or resting sites are scarce, our sticky trap may potentially be used as a mass trapping device for vector control and integrated pest management.

Further studies are in progress to evaluate the sampling capacity of the sticky trap in *Ae. aegypti*-infested areas (where this species represents the main dengue vector), in either the presence or absence of oviposition attractants, which might increase the sticky trap catch (Reiter *et al.*, 1991; Ritchie, 2001).

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