Acta Tropica 143 (2015) 89–96

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Seasonal variation in size estimates of *Aedes albopictus* population based on standard mark–release–recapture experiments in an urban area on Reunion Island

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ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
22 December 2014
Accepted 26 December 2014
Available online 13 January 2015

Keywords:
Aedes albopictus
SIT
MRR
Population estimation
Environmental factors
Chikungunya
Dengue

ABSTRACT

The implementation of the sterile insect technique for area-wide vector control requires that natural population density be accurately estimated to determine both the appropriate time to treat and the adequate number of sterile males for release. Herein, we used mark–release–recapture (MRR) to derive seasonal abundance estimates of *Aedes albopictus* population sizes within a delimited geographical area in Reunion Island. Population size of *Ae. albopictus* was estimated through four mark–release–recapture experiments carried out separately in different seasons. Marked males and females were released each time, and recaptured using BG sentinel traps for six consecutive days. Data were used to estimate the population size using a conceptual model that incorporates the variation in daily mortality rates. The likely influence of environmental factors on the magnitude of catches and on population fluctuation was analyzed. A total of 2827 mosquitoes (1914 males and 913 females) were marked and released on four occasions during dry and wet seasons. After release, 138 males (7.21%) and 86 females (9.41%) of the marked specimens were recaptured in subsequent samplings. The effectiveness of the daily captures of wild and released mosquitoes was significantly influenced by meteorological conditions such as temperature, rainfall, wind speed and light intensity. The estimates of *Ae. albopictus* population size obtained with our model estimator ranged from 298 to 1238 males and 604 to 2208 females per ha, with seasonal variability – higher population size in the humid season. The presented results will be essential in designing more effective sterile male release strategies for long-term suppression of wild *Ae. albopictus* populations.

1. Introduction

Aedes albopictus (Diptera: Culicidae) (Skuse, 1894) is currently one of the most efficient urban vectors for Chikungunya as well as a potential vector of dengue virus and other diseases that impose a burden upon human health (Gratz, 2004). Diseases vectored by *Ae. albopictus* mosquitoes are among those undergoing resurgence and redistribution worldwide (Mitchell, 1995; Benedict et al., 2007). In Reunion, whose population suffered from a large Chikungunya epidemic in 2006 (Setbon and Raude, 2008), *Ae. albopictus* is considered as a ubiquitous species able to successfully exploit a large array of habitats. The current control strategy that relies upon elimination of domestic breeding sites and specific use of insecticide treatments in affected areas falls short of successful control, largely a result of selection for resistance (Vontas et al., 2012), but also due to their limited use as a result of the concern for toxicity to non-target organisms (Ponlawat et al., 2005; Wilson et al., 2003). As a result, emphasis has been put on developing alternative or

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http://dx.doi.org/10.1016/j.actatropica.2014.12.011
0001-706X/

complementary strategies to counteract the constant risk of the diseases vectored by *Ae. albopictus*. Nowadays, the interest focuses on the sterile insect methods in which sterile males would be released to reduce conspecific wild populations (Benedict and Robinson, 2003; Alphey et al., 2010). Sterile Insect Technique (SIT) for area-wide integrated pest management is a clean and environmentally friendly method that has the potential to drive vectors to extinction (Hendrichs et al., 2007). It has been applied worldwide to effectively control a variety of insect species of veterinary, human health, and agricultural importance (Klassen and Curtis, 2005; Vreysen et al., 2007). Currently, several SIT feasibility studies are being developed for the release of irradiated males to effectively reduce vector activity and limit insecticide applications in Europe (Bellini et al., 2007), African continent (Malcolm et al., 2009) and in the Islands of the southwest of the Indian Ocean (Oliva et al., 2012). The geographical isolation makes Reunion Island a suitable testing ground for demonstrating the efficacy of SIT against *Ae. albopictus*.

The recently established SIT feasibility program in Reunion Island, has allowed substantial progress to be made toward the understanding of the bio-ecology and behavior of local *Ae. albopictus* populations. In addition, the feasibility of sterile male release technique for effective control of *Ae. albopictus* has been demonstrated by recent laboratory (Boyer et al., 2011, 2012, 2013) and semi-field experiments (Oliva et al., 2012). Similar research efforts directed against wild *Ae. albopictus* populations are yet to be implemented. Such field-testing is a challenge that takes into account specific information on population structure and dynamics. Other studies have focused on modeling aspects of *Ae. albopictus* ecology and behavior so that current methods in vector surveillance and controls, such as larvicide, adulticide, mechanical control and SIT can be successfully implemented and compared (Dumont and Chiroleu, 2010; Anguelov et al., 2011; Dumont and Tchuenche, 2012; Dufourd and Dumont, 2012, 2013; Dufourd et al., 2013). Understanding the dynamics of vector populations makes it easier to design the right control intervention at the right time.

Additionally, implicit in the optimal spatial release of sterile insects is the necessity of estimating the carrying capacity for the wild populations in the natural environment and the associated spatial movement through dispersal ability (Bellini et al., 2010; Brown et al., 2014), among other relevant features. Indeed, one of the critical parameters for SIT deployment is to find threshold values for the population density that determine the appropriate time to treat and the number of sterilized males that must be released to achieve a desired goal (Jeffery et al., 2009). This problem has been addressed for *Aedes* spp. and other mosquito species through the use of mark–release–recapture (MRR) technique (Gillies, 1961; Costantini et al., 1996; Reiter, 2007; Valerio et al., 2010; Marini et al., 2010; Cianci et al., 2013). Moreover, conceptual models have been proposed that can provide reasonably good predictions for species-specific dispersion and size within a geographic area (reviewed by Seber, 1986). Here, MRR experiments were carried out with the aim of providing up-to-date estimates of sizes of wild *Ae. albopictus* populations that are necessary for successful SIT control. While adding important information to existing data on the behavior and life history traits of wild local populations (Boyer et al., 2011, 2012; Delatte et al., 2009, 2010; Jacquet et al., 2012; Lacroix et al., 2009a), the presented study will also be useful in the calculation of required sterile insect release rates for future control programs against *Ae. albopictus* in Reunion Island.

2. Materials and methods

2.1. Released mosquitoes

Ae. albopictus adults (Diptera: Culicidae) (Skuse, 1894) used for MRR experiments were obtained from eggs collected in ovitraps within the study zone (Ligne Paradis – Saint-Pierre, 21°18′56′′ South; 55°29′13′′ East) in 2010. Ovitraps made up of black plastic containers, half filled with rainwater and 5% sugar-cane solution were placed in the field for three consecutive days. Wild females laid eggs in each ovitrap on a brown scratched germination paper (Seedburo Equipment Company, Des Plaines, IL, USA) placed within the internal surface of the trap. Larvae that hatched from ovitrap-collected eggs were reared in the laboratory to adulthood in plastic trays ($38 \times 28 \times 8\,cm^3$) containing 1 L of tap water. They were fed with a mix of rabbit/fish food on a daily basis. Pupae were collected and transferred into individual vials ($25\,cm^3$), where adults emerged. Upon emergence, male and female adults were kept separately in two different holding cages ($30 \times 30 \times 30\,cm^3$). They were maintained under controlled conditions at $27 \pm 1\,°C$ and 80% R.H. in a 12:12 photo-period in climatic chambers, and fed with a 10% sugar solution until the time to release. Released males and females were less than 48h-old.

On the morning of each release session, males and females that had not been blood-fed were separately marked with red fluorescent dust (Day Glo Rocket Red A-16, Switzer Brothers, Cleveland, OH) by aspirating batches of ten adult mosquitoes into individual dusted paper cups. The cups were carefully agitated for homogeneous marking of the insects. Subsequently they were kept undisturbed for 10 min after which, all dead mosquitoes were counted and excluded from the total number of individuals for release. In preliminary experiments, the possible effect of marking on mosquito survival was tested on 20 replicates by comparing mortality of marked and unmarked control individuals kept separately under laboratory conditions for 15 days. No significant difference in mortality was recorded between marked and unmarked mosquitoes. Mosquitoes intended for release were from the same larval cohorts kept into their respective paper cups, which were later transported to the release sites.

2.2. Mosquito release and sampling

This study was carried out within the CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) campus located about 5 km north of the city centre of Saint-Pierre and 500 m from the nearest human population. The site has a surface area of 5.32 ha and is partially isolated from the surroundings by large highways and a largely vegetated (crop culture), non-residential area. Considering that in a previous experiment in La Reunion, very few marked mosquitoes were recaptured beyond 50 m from the release point (Lacroix et al., 2009a), this relative isolation is expected to minimize mosquito loss by emigration during MRR experiments.

Release and sampling was performed using the protocol detailed in Lacroix et al. (2009a,b); Lacroix et al. (2009a,b). Briefly, a limited release of approximately 369–1030 marked mosquitoes (including males and females in various ratios) was performed at a single, central position of the study location. The mosquito intended for release were taken from the *Ae. albopictus* samples that emerged from larvae cohorts collected in the urban aquatic habitats at various intervals. This technical constraint prevented us from being able to release exactly the same number and ratio of mosquitoes. This was performed 1 h before sunset when *Ae. albopictus* activity is reduced (Wilkinson et al., 2014), in order to minimize bias in the 'escape' dispersal during release. Four MRR experiments were performed in different seasons in order to gauge seasonal variation in population densities: the first release begun in mid-summer (weeks 11–13) in March 2010, the second and third experiments were in June (weeks 22–25) and September, (week 35–37), in the beginning and at the end of austral winter, respectively, and the last experiment took place in November–December during the period of greatest *Ae. albopictus* activity (weeks 47–49). The recapture sites