Possible New Controlling Measures for the pyrethroid-resistant malaria vectors

H. Kawada
Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Abstract

Over the past decade, the dramatic success of long-lasting insecticidal nets (LLINs) in African countries has been countered by the rapid development of pyrethroid resistance in vector mosquitoes. There have been few studies on the efficacy of LLINs in areas where vector mosquitoes are pyrethroid resistant. The use of excito-repellency chemicals might be bio-rational, since such repellency will not induce physiological resistance. However, little is known about the relationship between the mode of insecticide resistance and excito-repellency in pyrethroid-resistant mosquitoes. The goals of our study were to investigate (1) differences in reactions to LLINs between individuals and populations of vector mosquitos in an area where multi-modal pyrethroid resistance has developed, (2) the effect of LLINs on these malaria vectors, and (3) the development of new control techniques to supplement LLINs. Laboratory contact tests for repellency showed that resistant species governed by *kdr* (knockdown resistance) (*Anopheles gambiae* s.s.) lose repellency to pyrethroids, whereas those lacking *kdr* (*An. arabiensis* and *An. funestus* s.s.) maintain high repellency irrespective of whether or not they possess cytochrome P450-related metabolic resistance factors. LLINs were effective against these pyrethroid-resistant malaria vectors, because they limited feeding on humans during bedtime. However, notable time shifts in human blood feeding activity developed in both *An. arabiensis* and *An. funestus* s.s., whereas no such time shift developed in *An. gambiae* s.s. These time shifts might be partially explained by differences in repellency by pyrethroids for these species. LLINs might not be effective because most blood feeding occurs when people are active outside the bed nets. Screening eaves with pyrethroid-impregnated wide-mesh nets was found to be effective in reducing human exposure to malaria vectors. The excito-repellency of pyrethroids that act as a spatial barrier or reduce feeding motivation of mosquitoes might be another countermeasure.

Keywords: vector control, pyrethroid, permethrin, metofluthrin, repellency, LLIN

INTRODUCTION

Chemical substances, whether naturally occurring or artificially synthesized, continue to play an extremely beneficial role in human welfare. Life would be unsustainable without the products of chemical reactions, such as oxygen, water, and various nutrients. Various foods and compounds such as drugs, fibers, and pesticides have been artificially designed and produced in order to protect and improve the quality of human life. Bio-rational and logical approaches by both users and suppliers of chemical substances are required so that humans can continue to benefit from the use of chemicals.

Research on the development of natural pesticides is considered a bio-rational approach because it may equate the adverse environmental effects of chemicals to those of naturally occurring substances. One of the most successful developments in the manufacturing of pesticide chemicals was the discovery of pyrethrum and the subsequent successful synthesis of pyrethroids. For example, one of the first synthesized pyrethroids, allethrin, (Schechter et al., 1949) continues to be used for preventing mosquito bites without any toxicological or operational problems. Devices with the highest popularity and the most long-standing use that incorporate pyrethroids are mosquito coils, mosquito mats, and liquid vaporizers. Pyrethroids belonging to the knockdown agent group, such as allethrin, pyrethrin, and prallethrin, are used in these devices.

The use of pyrethroids in insecticide-treated bed nets (ITNs) is a simple and inexpensive protection measure against malaria and has been shown to reduce morbidity in children less than 5 years old by 50% and global child mortality by 20–30% (Binka et al., 1996; Lengeler et al., 1996; Nevil et al., 1996). However, the impregnation and re-impregnation of ITNs requires technical skills, materials, and human labor, which may not always be available (Lines, 1996). Mosquito nets pre-treated with insecticide were a breakthrough measure to resolve this problem (Guillet et al., 2001). Olyset® Net, made of polyethylene netting material (mesh 11 holes/cm^2) with permethrin (2%) incorporated into the polymer before monofilament yarn extrusion, and the PermaNet®, made of polyester netting material (mesh 25 holes/cm^2) with deltamethrin (55 mg ai/m^2) incorporated in a resin coating of the fibers, are two successful LLINs (long-lasting insecticidal nets) recommended by the WHO.

Operational limitations of LLINs

ITNs or LLINs are effective against malaria vectors only when the vector mosquitoes are endophagous and their feeding time corresponds to the time when people are sleeping inside bed nets. Behavioral changes in vector mosquitoes from endophagous to exophagous and/or the shifting of biting time from midnight to dawn or dusk may reduce their physiological resistance to insecticides as well as the effectiveness of bed nets. Our trial in residential houses in western Kenya where LLINs (Olyset® Nets) were properly used showed that the percentage of human feeding success was reduced to 15.9%–24.6% and concluded that LLINs were effective against three major malaria vectors (*An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s.) during bedtime. The overall activity of the three primary malaria vectors in this study area has not changed from that reported prior to the extensive use of ITNs and LLINs in Africa. However, both *An. arabiensis* and *An. funestus* s.s. had notable human blood feeding activity in the evening and slight activity in the early morning, while *An. gambiae* s.s. did not, indicating that mosquito biting took place when people were active outside of the LLINs (Kawada et al., 2014a; Figures 1, 2).

Another limitation of the effectiveness of LLINs is the fact that they are only effective when people are inside of them. Recently, Iwashita et al. (2010) reported that bed net use by children between 5 and 15 years of age in villages along Lake Victoria in western Kenya was lower than that among other age classes. Bed net use was strongly affected by sleeping arrangement and the availability of suitable locations for hanging bed nets. The ease of hanging a bed net is particularly important for children who often sleep in places such as living rooms where daily net hanging can be difficult and troublesome. Hence, the use of bed nets is sometimes limited to those sleeping in a bedroom (parents and babies). The rest of family members (in particular children older than 5 years) sleep in living rooms without a bed net, resulting in high numbers of *Plasmodium falciparum* cases in these age groups. Children who sleep on the floor are less likely to use LLINs, and *P. falciparum* infection was significantly higher among children who slept on the floor without LLINs (Minakawa et al., 2015) than among those who slept in beds with LLINs.

Eaves (the gaps between the top of the wall and the roof) are common to houses throughout Africa and are thought to be the primary entrance for malaria vectors (Njie et al., 2009). Changes in house design, such as the screening or closing of eaves, may reduce human exposure to malaria vectors (Lindsay et al., 2003). Restructuring houses or physically closing the eaves will be expensive and may reduce the quality of living because of blocked ventilation.

The most important limiting factor of LLIN use is pyrethroid resistance in vector mosquitoes. Insecticide use continues to be the most effective means of controlling malaria, dengue hemorrhagic fever (DHF), and other arthropod-borne diseases. Globally, pyrethroids constitute approximately 81% of the spray utility, of which 68% is used for residual spraying and 24% for space spraying, and 100% of WHO-recommended insecticides for the treatment of LLINs (van der Berg et al., 2012). Among pyrethroids that are used for vector control, 98.7% contain photo-stable pyrethroids such as α-cypermethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, etofenprox, λ-cyhalothrin, and permethrin (Zaim and Jambulingam, 2007). Pyrethroid resistance will be a major problem for the vector control program, since there are currently no suitable chemical substitutes for pyrethroids. Pyrethroid resistance is high in *An. gambiae* s.l. in West and Central Africa. Within Eastern and Austral Africa, *An. gambiae* s.l. populations are mostly susceptible to pyrethroids in Tanzania, Mozambique, and Madagascar, but highly resistant in Uganda, Ethiopia, Kenya, Zambia, Zimbabwe, and South Africa (Corbel and N'Guessan, 2015). The distribution of high allelic frequency of *kdr* (knockdown resistance) mutations (point mutations at the voltage-gated sodium channel, L1014S) in *An. gambiae* s.s. converged in the northern and southern coastal regions as well as the western regions (including highland areas) of Lake Victoria (Kawada et al., 2011b). These regions are some of the focal points identified as high vector transmission regions in Kenya, where more than 50% of the population is exposed to ≥ 40% $PfPR_{2\text{-}10}$ (*P. falciparum* parasite rate corrected to a standard age-range of 2 to < 10 years old) (Noor et al., 2009) and there is high coverage by LLINs or ITNs. For example, the percentage of households with at least one LLIN in Nyanza and Western provinces (> 70%) was higher than that in the other provinces (< 70%) (Kenya HDS Final Report, 2009). Mathias et al. (2011) reported that the East African *kdr* allele (L1014S) concurrently increased in frequency during the past decade in *An. gambiae* s.s. (most of which are homozygous for the *kdr* allele) in western Kenya as household ownership of insecticide-treated bed nets increased regionally.

LLINs have several operational and critical limitations. Therefore, novel, convenient, and sustainable devices, or self-protection measures that can substitute or complement LLINs, are required for more effective prevention of malaria vectors. The present paper describes several attempts to develop new mosquito control techniques using a pyrethroid within the knockdown agent groups (metofluthrin) and a slow-released type I pyrethroid (permethrin) as a spatial and excito-repellent agent.

Field evaluation of the effectiveness of metofluthrin-impregnated plastic strips in the spatial repellency of vector mosquitoes

Metofluthrin, (SumiOne®) 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (E:Z ≈ 1:8)(1*R*, 3*R*)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate, is a newly synthesized pyrethroid (Ujihara et al., 2004). Metofluthrin belongs to the group of knockdown agents but has two unique characteristics that none of the conventional pyrethroids possess. Metofluthrin has a high vapor pressure (1.87×10^{-3} Pa at 25°C), which is 2-fold and 100-fold greater than that of *d*-allethrin and permethrin, respectively. Metofluthrin vaporizes at room temperature, while other conventional pyrethroids require heating for vaporization. Another unique characteristic is its high killing efficacy against mosquitoes 28–79 times more effectively than *d*-allethrin (Argueta et al., 2004). These unique characteristics of metofluthrin may lead to the development of new mosquito controlling devices that do not require any external energy for vaporization and have lower costs and longer effective durations.

In preliminary studies, using a simple prototype device with metofluthrin-impregnated multilayer paper strips, the chemical showed promising spatial repellency against some mosquito species (*Anopheles sundaicus* (Rodenwaldt), *An. balabacensis* (Baisas), and *Culex quinquefasciatus* (Say)) in both laboratory and field conditions on Lombok Island, Indonesia (Kawada et al., 2004a, b). In order to increase the effectiveness of metofluthrin, Kawada et al. (2005) manufactured a cylindrical slow-release plastic device that was impregnated with 1000 mg metofluthrin in a 20 g strip. With this device, the authors obtained prolonged duration of activity of > 14 weeks at the rate of 4 strips per beruga (a traditional wall-less outdoor living structure) on Lombok Island (Figures 3 and 4).

The next step in the development of the devices was manufacturing a new plastic strip that would reduce the release rate of metofluthrin to approximately 50% of that of the previous plastic device. The new prototypes of metofluthrin-impregnated plastic strips were evaluated against malaria vector, *An. gambiae* Giles complex, in the Kongo villages of Bagamoyo district in coastal Tanzania (Kawada et al., 2008). The study used 20 houses (10 intervention and 10 control) and was conducted over 124 days. The mosquito density indices of the intervention houses were significantly lower than those of the control houses (98.7% reduction of total mosquito collection over the controls; Table 1). Recently, Kawada et al. (unpublished data) found metofluthrin-impregnated plastic strips to be significantly effective at reducing invasions of a pyrethroid-resistant *An. gambiae* s.s. population, which has developed > 90% homozygous L1014S point mutation at the voltage-gated sodium channel (*kdr*) in western Kenya (Figure 5).

Table 2 lists the environmental factors and the effective duration of the metofluthrin-impregnated plastic strips for the intervention houses in Tanzania (Kawada et al., 2008) and in My Tho city, Tien Giang, Vietnam, where a similar metofluthrin trial was conducted in the same season in 2005 (Kawada et al., 2006). Variables including average temperature and humidity were calculated on an hourly basis from June 20 to August 3, 2006 for Bagamoyo and from June 20 to September 4, 2005 for My Tho. The room temperature was lower and the humidity was higher in Bagamoyo houses compared to the corresponding conditions in the My Tho houses. Although the floor area and the structure volume were larger in My Tho houses, compared to those in Bagamoyo houses, the corrected opening area per total average volume of the houses in Bagamoyo was almost twice that of houses in My Tho, thereby indicating that the Bagamoyo houses are more "open" than the My Tho houses (Kawada et al., 2008).

Metofluthrin-impregnated strips significantly reduced the density index of mosquitoes under several environmental conditions within the intervention houses in Vietnam (*Aedes aegypti*), Indonesia, Tanzania, and Kenya (malaria vectors and *Culex* species). Kawada et al. (2004a, b, 2005, 2006, 2008) reported that mosquitoes were repelled by airborne metofluthrin vapors through the two main modes of pyrethroid action, i.e., knockdown activity and biting inhibition or disruption of orientation toward the host. Of these, the latter may be categorized as a sub-lethal effect that results from neural excitement, which appears to occur at an earlier stage of pyrethroid toxicity (MacIver, 1964; Winney, 1975; Birley et al., 1987). Kawada et al. (2006) reported that both an increase in the average room temperature and a decrease in the open area of the rooms treated with metofluthrin-impregnated strips had an increased spatial repellent effect. Paradoxically, in our recent field test in Malawi, we found that the evaporation rate of metofluthrin was not positively related to room temperature (Kawada et al., unpublished data). The above facts also suggest that the evaporation rate of metofluthrin was much higher in the well-ventilated houses, such as thatched-roofed houses with large open eaves, than in houses with small eave openings, thereby indicating the importance of air movement for removing and accelerating the release of the active ingredient (AI) from the surface of the strips. The corrected opening area/volume ratio in the Bagamoyo houses was nearly twice as high as that of the houses in My Tho city (Table 2). The large and numerous open eaves in typical rural African houses are considered to be important nighttime entrances for *An. gambiae* (Snow, 1987; Lindsay et al., 2003; Pålsson et al., 2004). However, these openings might increase the evaporation rate of metofluthrin and result in high mosquito control efficacy. Therefore, we cannot simply conclude that the large opening area of the Bagamoyo houses negatively affects the spatial repellent efficacy of metofluthrin.

Field trial on house screening with Olyset® Nets in a Kenyan malaria endemic area

There have been several attempts to use LLINs to control other vectors, including *Ae. aegypti* (Curtis et al., 1996; Igarashi, 1997; Jeyalakshmi et al., 2006; Kroeger et al., 2006; Tsunoda et al., 2011) and *Phlebotomus* (Dinesh et al., 2008; Emani et al., 2009; Faiman et al., 2009; Kasili et al., 2010; Das et al., 2010). Olyset® Net and/or PermaNet® LLINs have also been used as curtains (Curtis et al., 1996; Igarashi, 1997; Kroeger et al., 2006; Vanlerberghe et al., 2011a, b) and water container covers (Kroeger et al., 2006; Vanlerberghe et al., 2011a, b; Tsunoda et al., 2011). Eaves are thought to be the most important entrance for malaria vectors. The use of nets to screen ceilings and eaves is likely to provide the greatest benefit in moderating disease transmission (Lindsey et al., 2003; Kirby et al., 2009) and will be more readily accepted if the nets have a coarse mesh size to provide optimum ventilation.

Small-scale trials using Olyset® Net materials were performed in Mbita, Nyanza province, western Kenya in 2010 and 2011. *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. are the main malaria vectors in this area and were recently reported to have developed multimodal pyrethroid resistance (Kawada et al., 2011a). *An. rivulorum*, which is a sibling species in the *An. funestus* complex, is also a minor vector in this area (Kawada et al., 2012a).

Olyset® Nets impregnated with 2% permethrin were used in the study. The net materials were cut and sewed into 7×5 m sheets and ring bands were attached along the diagonal of the nets for hanging under the ceiling (Figure 6). The study was performed in three houses (two intervention, one control) in Nyandago village in Gembe East, Mbita Division in the Suba district of the Nyanza province, western Kenya. The shielding effect of the ceiling nets was evaluated by counting the number of indoor resting mosquitoes collected using a battery-powered aspirator. The Olyset® Net covering the ceiling and the eaves resulted in a significant reduction in the number of resting mosquitoes (a mixed population of *An. funestus* s.s. and *An. arabiensis*) inside houses (Kawada et al., 2012b). In the intervention houses, the number of mosquitoes drastically decreased 1 day after the installation of ceiling nets and lower densities were maintained for the 9 months until the removal of the nets; the mosquito density in the control house remained at a high level during the experimental period (Figure 7). Lindsay et al. (2003) reported that insecticide-treated nets provided marginal protection compared to that of untreated screen nets. The present study, however, emphasizes the necessity of using pyrethroid-impregnated nets as a chemical barrier, although this protection may be partly due to the increased ventilation from the coarse mesh size of Olyset® Nets (Figure 8).

A large-scale field trial of ceiling nets combined with LLINs (Olyset® Net) in 1800 houses in western Kenya was begun in 2001. A significant reduction in the number of mosquitoes (mixed population of *An. funestus* s.s. and *An. arabiensis*) and *P. falciparum* positive cases in the intervention houses was observed for over 12 months after initial intervention (Minakawa et al., unpublished data). Screening ceilings and closing eaves with insecticide-treated nets with a coarse mesh size, such as the Olyset® Net, will be accepted by residents, and is a cost-effective and environmentally safe way to prevent mosquitoes from entering houses.

CONCLUSION

For many decades, pyrethroids belonging to the knockdown agent group have been globally successful as spatial repellents. Spatial repellency will not induce pyrethroid resistance because it results in low fatality rates and selection pressure on the affected insect populations. The discovery of the phenoxybenzyl alcohol moiety accelerated the development of photostable pyrethroids for outdoor use, including their use in agriculture. These "second-generation" pyrethroids have been used worldwide as effective vector control agents using various application techniques, such as residual spraying, ULV (ultra-low volume) spraying, and LLINs. However, photostable and highly effective pyrethroids have accelerated the development of pyrethroid resistance in mosquito populations. Photostable pyrethroids consist of two structurally different types of chemicals depending on the presence of either α-cyano moiety, type I (permethrin, bifenthrin, etofenprox, etc.) or type II (deltamethrin, λ-cyhalothrin, cypermethrin, etc). Olyset® Net is a slow-releasing device composed of plastic fibers impregnated with permethrin—one of the most popular and safe type I pyrethroids. Siegert et al. (2009) reported that the Olyset® Net reduced mosquito landing attempts and elevated their flight frequency, resulting in low mortality. However, mosquito landing attempts on the PermaNet®, containing a type II pyrethroid (deltamethrin) and under the same conditions, were more sustained and caused greater mortality than those on the Olyset® Net. This appears to be important for the effective control of the mosquito population. Highly lethal pyrethroids with less excito-repellency appear to be the most effective at reducing vector mosquito populations, although such highly lethal pyrethroids might accelerate the development of pyrethroid-resistance. However, the excito-repellency of slow-released permethrin might reduce human-vector contact and blood feeding success. For example, there was no difference between the field efficacies of Olyset® Net and PermaNet® as measured by blood feeding rate (Dabire et al., 2006). The positive use of excito-repellency slow-released pyrethroids, therefore, might lead to bio-rational vector control with the maximum reduction of mosquito biting and the minimum risk of resistance. Little is known about the relationship between the mode of insecticide resistance and excito-repellency in pyrethroid-resistant mosquitoes. Recently, Kawada et al. (2014b) reported a different repellent reaction in field-collected *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. from western Kenya. Pyrethroid-resistant *An. gambiae* s.s. populations governed by knockdown resistance (*kdr*), were found to lose repellency to pyrethroids, whereas the other mosquito populations lacking *kdr* (*An. arabiensis*, and *An. funestus* s.s.) maintained high repellency irrespective of their possession of metabolic resistance to pyrethroids (Figure 9). The above finding might inform the development of new personal protection measures using the excito-repellency of pyrethroids, but additional genetic evaluation should be conducted to further support this avenue for research.

Humans have invented insecticides to ensure comfort and achieve ideal conditions. Insecticides should be as effective as possible in order to realize these goals, but development and manufacturing costs should be kept to a minimum (Kawada, 2009). It is, therefore, our duty to use insecticides in the most effective and prudent manner possible in order to maintain their effectiveness and longevity. In order to effectively manage pyrethroid resistance, the establishment of a feasible insecticide management system and a regular monitoring system of pyrethroid susceptibility is essential. Moreover, it is expected that, in the future, new protection measures using excito-repellent type I pyrethroids will be of great interest either as substitutes or as supplements for bio-rational vector control measures. In addition, the use of photo-unstable knockdown agents as spatial repellents will likely increase because they effectively interfere with disease transmission without causing selection pressure to insect populations.

ACKNOWLEDGEMENTS

We thank the staff members of the Thomas Odhiambo campus of the International Center of Insect Physiology and Ecology (ICIPE), Mbita, Kenya; The Kenya Medical Research Institute, Nairobi, Kenya; The National Institute of Infectious Diseases, Tokyo, Japan; The Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; and Sumitomo Chemical Co., Ltd., Hyogo, Japan, for providing technical support and other assistance with this study.

Literature cited

Arugueta, T.B.O., Kawada, H., Sugano, M., Kubota, S., Shono, Y., Tsushima, K., and Takagi, M. (2004). Comparative insecticidal efficacy of a new pyrethroid, metofluthrin, against colonies of Asian *Culex quinquefasciatus* and *Culex pipiens pallens*. Med. Entomol. Zool. *55*, 289-294 http://ci.nii.ac.jp/naid/110003820563#article/en.

Binka, F.N., Kubaje, A., Adjuik, M., Williams, L.A., Lengeler, C., Maude, G.H., Arma, G.E., Kajihra, B., Adiama, J.H., Lengeler, C., et al. (1996). Impact of permethrin treated bed nets in child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. Trop. Med. Int. Health. *1*, 147-154 http://dx.doi.org/10.1111/j.1365-3156.1996.tb00020.x.

Birley, M.H., Mutero, C.M., Turner, I.F., and Chadwick, P.R. (1987). The effectiveness of mosquito coils containing esbiothrin under laboratory and field conditions. Ann. Trop. Med. Parasitol. *81*, 163-171 http://europepmc.org/abstract/med/2891344.

Corbel, V., and N'Guessan, R. (2015). Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: A pragmatic review. In Anopheles Mosquitoes: A Detailed Research, J. Spooner, ed. (New Jersey, USA: Foster Academics), p. 92-149.

Curtis, C.F., Myamba, J., and Wilkes T.J. (1996). Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. Med. Vet. Entomol. *1*, 1-11 http://dx.doi.org/10.1111/j.1365-2915.1996.tb00075.x.

Dabire, R.K., Diabate, A., Baldet, T., Paré-Toé, L., Guiguemde, R.T., Ouédraogo, J.B., and Skovmand, O. (2006). Personal protection of long lasting insecticide-treated nets in areas of *Anopheles gambiae* s.s. resistance to pyrethroids. Malaria J. *5*, 12 http://dx.doi.org/10.1186/1475-2875-5-12.

Das, M.L., Roy, L., Rijal, S., Paudel, I.S., Picado, A., Kroeger, A., Petzold, M., Davies, C., Boelaert, M. (2010). Comparative study of kala-azar vector control measures in eastern Nepal. Acta. Tropica. *113*, 162-166 http://dx.doi.org/10.1016/j.actatropica.2009.10.012.

Dinesh, D.S., Das, P., Picado, A., Davies, C., Speybroeck, N., Ostyn, B., Boelaert, and M., Coosemans, M. (2008). Long-lasting insecticidal nets fail at household level to reduce abundance of sandfly vector *Phlebotomus argentipes* in treated houses in Bihar (India). Trop. Med. Int. Health. *13*, 953-958 http://dx.doi.org/10.1111/j.1365-3156.2008.02096.x.

Emami, M.M., Yazdi, M., and Guillet, P. (2009). Efficacy of Olyset long-lasting bednets to control transmission of cutaneous leishmaniasis in Iran. East Med. Health J. *15*, 1075-1083 http://www.who.int/iris/handle/10665/117735#sthash.lFxikps1.dpuf .

Faiman, R., Cuño, R., and Warburg, A. (2009). Control of phlebotomine sand flies with vertical fine-mesh nets. J. Med. Entomol. *46*, 820-831 http://dx.doi.org/10.1603/033.046.0412.

Guillet, P., Alnwick, D., Cham, M.K., Neira, M., Zaim, M., Heymann, D., and Mukelabai, K. (2001). Long-lasting treated mosquito nets: a breakthrough in malaria prevention. Bull. W.H.O. *79*, 998 http://dx.doi.org/10.1590/S0042-96862001001000017.

Igarashi, A. (1997). Impact of dengue virus infection and its control. FEMS Immnol. Med. Microbiol. *18*, 291-300 http://onlinelibrary.wiley.com/doi/10.1111/j.1574-695X.1997.tb01058.x/pdf.

Iwashita, H., Dida, G., Futami, K., Sonye, G., Kaneko, S., Horio, M., Kawada, H., Maekawa, Y., Aoki, Y, and Minakawa, N. (2010). Sleeping arrangement and house structure affect bed net use in villages along Lake Victoria. Malaria J. *9*, 176 http://dx.doi.org/10.1186/1475-2875-9-176

Jeyalakshmi, T., Shanmugasundaram, R., and Murthy, P.B. (2006). Comparative efficacy and persistency of permethrin in Olyset net and conventionally treated net against *Aedes aegypti* and *Anopheles stephensi*. J. Am. Mosq. Control Assoc. *22*, 107-110 http://dx.doi.org/10.2987/8756-971X(2006)22[107:CEAPOP]2.0.CO;2.

Kasili, S., Kutima, H., Mwandawiro, C., Ngumbi, P.M., Anjili, C.O., and Enayati, A.A. (2010). Laboratory and semi-field evaluation of long-lasting insecticidal nets against leishmaniasis vector, *Phlebotomus* (*Phlebotomus*) *duboscqi* in Kenya. J. Vector Borne Dis. *47*, 1-10 http://repository.seku.ac.ke/handle/123456789/550.

Kawada, H., Maekawa, Y., Tsuda, Y., and Takagi, M. (2004a). Laboratory and field evaluation of spatial repellency with metofluthrin-impregnated paper strip against mosquitoes in Lombok Island, Indonesia. J. Am. Mosq. Control Assoc. *20*, 292-298 http://naosite.lb.nagasaki-u.ac.jp/dspace/handle/10069/16982.

Kawada, H., Maekawa, Y., Tsuda, Y., and Takagi, M. (2004b). Trial of spatial repellency of metofluthrin-impregnated paper strip against *Anopheles* and *Culex* in shelters without walls in Lombok, Indonesia. J. Am. Mosq. Control Assoc. *20*, 434-437 http://naosite.lb.nagasaki-u.ac.jp/dspace/handle/10069/16983.

Kawada, H., Maekawa, Y., and Takagi, M. (2005). Field trial of the spatial repellency of metofluthrin-impregnated plastic strip against mosquitoes in shelters without walls (Beruga) in Lombok, Indonesia. J. Vect. Ecol. *30*, 181-185 http://naosite.lb.nagasaki-u.ac.jp/dspace/handle/10069/16980.

Kawada, H., Iwasaki, T., Loan, L.L., Tien, T.K., Mai, N.T.N., Shono, Y., Katayama, Y., and Takagi, M. (2006). Field evaluation of spatial repellency of metofluthrin-impregnated latticework plastic strips against *Aedes aegypti* (L.) and analysis of environmental factors affecting its efficacy in My Tho city, Tien Giang, Vietnam. Am. J. Trop. Med. Hyg. *75*, 1153-1157 http://www.ajtmh.org/content/75/6/1153.short .

Kawada, H., Temu, E.A., Minjas, J.N., Matsumoto, O., Iwasaki, T., and Takagi, M. (2008). Field evaluation of spatial repellency of metofluthrin-impregnated plastic strips against *Anopheles gambiae* complex in Bagamoyo, coastal Tanzania. J. Am. Mosq. Control Assoc. *24*, 404-409 http://dx.doi.org/10.2987/5743.1 .

Kawada, H. (2009). An Inconvenient Truth of Pyrethroid - Does it have a promising future? -. In Advances in Human Vector Control (ACS Symposium Book 1014), J. Clark, J.R. Bloomquist, and H. Kawada, eds. (New York, USA: American Chemical Society), p. 171-190 http://www.tm.nagasaki-u.ac.jp/medical/PDF/ACS%20Symposium-2009-1014.pdf.

Kawada, H., Dida, G.O., Ohashi, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Maekawa, Y., Mwatele, C., Njenga, S.M., et al. (2011a). Multimodal pyrethroid resistance in malaria vectors *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles funestus* s.s. in western Kenya. PLoS One. *6*, e22574 http://dx.doi.org/10.1371/journal.pone.0022574.

Kawada, H., Futami, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Mwatele, C., Njenga, S.M., Mwandawiro, C., Minakawa, N., et al. (2011b). Distribution of a knockdown resistance mutation (L1014S) in *Anopheles gambiae* s.s. and *Anopheles arabiensis* in Western and Southern Kenya. PLoS One. *6*, e24323 http://dx.doi.org/10.1371/journal.pone.0024323.

Kawada, H., Dida, G.O., Sonye, G., Njenga, S.M., Mwandawiro, C., and Minakawa, N. (2012a). Reconsideration of *Anopheles rivulorum* as a vector of *Plasmodium falciparum* in western Kenya: some evidence from biting time, blood preference, sporozoite positive rate, and pyrethroid resistance. Parasit. Vect. *5*:,230 http://dx.doi.org/ 10.1186/1756-3305-5-230.

Kawada, H., Dida, G.O., Ohashi, K., Sonye, G., Maekawa, Y., Njenga, S.M., Mwandawiro, C., Minakawa, N., and Takagi, M. (2012b). Preliminary evaluation of the insecticide-impregnated ceiling nets with coarse mesh size as a barrier against the invasion of malaria vectors. Jpn J. Infect. Dis. *65*, 243-246 http://doi.org/10.7883/yoken.65.243.

Kawada, H., Ohashi, K., Dida, G.O., Sonye, G., Njenga, S.M., Mwandawiro, C., and Minakawa, N. (2014a). Preventive effect of permethrin-impregnated long-lasting insecticidal nets on the blood feeding of three major pyrethroid-resistant malaria vectors in western Kenya. Parasit. Vect. *7*, 383 http://dx.doi.org/10.1186/1756-3305-7-383.

Kawada, H., Ohashi, K., Dida, G.O., Sonye, G., Njenga, S.M., Mwandawiro, C., and Minakawa, N. (2014b). Insecticidal and repellent activities of pyrethroids to the three major pyrethroid-resistant malaria vectors in western Kenya. Parasit. Vect. *7*, 208 http://dx.doi.org/ 10.1186/1756-3305-7-208.

Kirby, M.J., Ameh, D., Bottomley, C., Green, C., Jawara, M., Milligan, P.J., Snell, P.C., Conway, D.J., and Lindsay, S.W. (2009). Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. Lancet. *374*, 998-1009 http://dx.doi.org/10.1016/S0140-6736(09)60871-0.

Kroeger, A., Lenhart, A., Ochoa, M., Villegas, E., Levy, M., Alexander, N., and McCall, P.J. (2006). Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. Br. Med. J. *332*, 1247-1252 http://dx.doi.org/10.1136/bmj.332.7552.1247.

Lengeler, C., Cattani, J., and De Savigny, D, eds. (1996) Net gain. A new method for preventing malaria deaths (International Development Research Center (Canada), W.H.O.) pp. 189.

Lindsay, S.W., Jawara, M., Paine, K., Pinder, M., Walraven, G.E.L., and Emerson, P.M. (2003). Changes in house design reduce exposure to malaria mosquitoes. Trop. Med. Int. Health. *8*, 512-517 http://dx.doi.org/10.1046/j.1365-3156.2003.01059.x.

Lines, J. (1996). Mosquito nets and insecticides for nets treatment: a discussion of existing and potential distribution systems in Africa. Trop. Med. Int. Health. *1*, 616-632 http://dx.doi.org/ 10.1111/j.1365-3156.1996.tb00087.x.

MacIver, D.R. (1964). Mosquito coils Part II. Studies on the action of mosquito coil smoke on mosquitoes. Pyrethrum Post. *7*, 7-14.

Mathias, D.K., Ochomo, E., Atieli, F., Ombok, M., Bayoh, M.N., Olang, G., Muhia, D., Kamau, L., Vulule, J.M., Hamel, et al. (2011). Spatial and temporal variation in the *kdr* allele L1014S in *Anopheles gambiae* s.s. and phenotypic variability in susceptibility to insecticides in Western Kenya. Malaria J. *14*, 10 http://dx.doi.org/10.1186/1475-2875-10-10.

Minakawa, N., Kongere, J.O., Dida, G.O., Ikeda, E., Hu, J., Minagawa, K., Futami, K., Kawada, H., Njenga, S.M., and Larson, P.S. (2015). Sleeping on the floor decreases insecticide treated bed net use and increases risk of malaria in children under 5 years of age in Mbita District, Kenya. Parasitology. Aug 18:1-7 [Epub ahead of print] http://dx.doi.org/10.1017/S0031182015000955.

Nevill, C.G., Some, E.S., Mung'ala, V.O., Mutemi, W., New, L., Marsh, K., Lengeler, C., and Snow, R.W. (1996). Insecticide-treated bed nets reduce mortality and severe morbidity from among children on the Kenyan Coast. Trop. Med. Int. Health. *1*, 139-146 http://dx.doi.org/10.1111/j.1365-3156.1996.tb00019.x.

Njie, M., Dilger, E., Lindsay, S.W., and Kirby, M.J. (2009). Importance of eaves to house entry by anopheline, but not culicine, mosquitoes. J. Med. Entomol. *46*, 505-510 http://dx.doi.org/10.1603/033.046.0314.

Noor, A.M., Gething, P.W., Alegana, V.A., Patil, A.P., Hay, S.I., Muchiri, E., Juma, E., and Snow, R.W. (2009). The risks of malaria infection in Kenya in 2009. BMC Infect. Dis *9*, 180 http://dx.doi.org/10.1186/1471-2334-9-180.

Okara, R.M., Sinka, M.E., Minakawa, N., Mbogo, C.M., Hay, S.I., and Snow, R.W. (2010). Distribution of the main malaria vectors in Kenya. Malaria J. *9*, 69 http://dx.doi.org/ 10.1186/1475-2875-9-69.

Pålsson, K., Jaenson, T.G.T., Dias, F., Laugen, A.T., and Björkman, A. (2004). Endophilic *Anopheles* mosquitoes in Guinea Bissau, West Africa, in relation to human housing conditions. J. Med. Entomol. *41*, 746-752 http://dx.doi.org/10.1603/0022-2585-41.4.746.

Schechter, M.S., Green, N., and Laforge, F.B. (1949). Constituents of pyrethrum flowers XXIII. Cynerolone and the synthesis of related cyclopentenolones. J. Am. Chem. Soc. 71, 3165-3173 http://dx.doi.org/10.1021/ja01177a065.

Siegert, P.Y., Walker, E., and Miller, J.R. (2009). Differential behavioral responses of *Anopheles gambiae* (Diptera: Culicidae) modulate mortality caused by pyrethroid-treated bednets. J. Econ. Entomol. *102*, 2061-2071 http://dx.doi.org/10.1603/029.102.0607.

Snow, W.F. (1987). Studies of house-entering habits of mosquitoes in The Gambia, West Africa: experiments with prefabricated huts with varied wall apertures. Med. Vet. Entomol. *1*, 9-21 http://dx.doi.org/10.1111/j.1365-2915.1987.tb00318.x.

Tsunoda, T., Kawada, H., Trang, H.T.T., Loan, L.L., San, L.H., Huu, T.N., Huong, V.T.Q., Hasebe, F., Tsuzuki, A., and Takagi, M. (2011). Field trial on a novel control method for the dengue vector, *Aedes aegypti* by the systematic use of Olyset® Net and pyriproxyfen in Southern Vietnam. Parasit. Vect. *6*, 6 http://dx.doi.org/ 10.1186/1756-3305-6-6.

Ujihara, K., Mori, T., Iwasaki, T., Sugano, M., Shono, Y., and Matsuo, N. (2004). Metofluthrin: A potent new synthetic pyrethroid with high vapor activity against mosquitoes. Biosci. Biotechnol. Biochem. *68*, 170-174 http://www.tandfonline.com/doi/abs/10.1271/bbb.68.170.

van den Berg, H., Zaim, M., Yadav, R.S., Soares, A., Ameneshewa, B., Mnzava, A., Hii, J., Dash, A.P., and Ejov, M. (2012). Global trends in the use of insecticides for vector-borne disease control. Environ Health Perspect. *120*, 577-582 http://dx.doi.org/10.1289/ehp.1104340..

Vanlerberghe, V., Villegas, E., Oviedo, M., Baly, A., Lenhart, A., McCall, P.J, and van der Stuyft, P. (2011a). Evaluation of the effectiveness of insecticide treated materials for household level dengue vector control. PLoS Negl. Trop. Dis. *5*, e994 http://dx.doi.org/10.1371/journal.pntd.0000994.

Vanlerberghe, V., Villegas, E., Jirarojwatana, S., Santana, N., Trongtorkit, Y., Jirarojwatana, R., Srisupap, W., Lefèvre, P., and van der Stuyft, P. (2011b). Determinants of uptake, short-term and continued use of insecticide-treated curtains and jar covers for dengue control. Trop. Med. Int. Health. *16*, 162-173 http://dx.doi.org/10.1111/j.1365-3156.2010.02668.x.

Winney, R. (1975). Pyrethrins and pyrethroids in coils–a review. Pyrethrum Post. *13*, 17-22.

Zaim, M., and Jambulingam, P. (2007). Global insecticide use for vector-borne disease control (WHO/CDS/WHOPES/GCDPP/2002.2., World Health Organization) http://apps.who.int/iris/bitstream/10665/44220/1/9789241598781_eng.pdf?ua=1.

Figure legends

Figure 1.
Indoor activity pattern of female *Anopheles gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. trapped by CDC miniature traps equipped with a collection bottle rotator in houses using LLINs (Olyset® Net). Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$), and bars indicate the 95% confidence limits for the total number of mosquitoes collected during each time period (Kawada et al., 2014a).

Figure 2.
The number of female mosquitoes collected at bedtime (10:00 PM–6:00 AM) and active time (4:00 PM–10:00 PM and 6:00 AM–8:00 AM) using a CDC miniature trap equipped with a collection bottle rotator (Kawada et al., 2014a).

Figure 3.
Field test scene showing the metofluthrin strips in a beruga where Lombok people spend every evening prior to going to bed. Arrows indicate metofluthrin strips (Kawada et al., 2005).

Figure 4.
Changes in the total number of mosquitoes (*Anopheles sundaicus*, *An. balabacensis*, and *Culex quinquefasciatus*) collected per hour during the trial of metofluthrin-impregnated plastic strips on Lombok Island, Indonesia (Kawada et al., 2005).

Figure 5.
The number of female *Anopheles gambiae* s.s. (pyrethroid-resistant wild population that has developed > 90% homozygous L1014S point mutation in the voltage-gated sodium channel) collected in the metofluthrin treated houses (1 strip/10 m^2 and 2 strips/10 m^2 at 1 week after intervention) in western Kenya. The different letters indicate significant differences between the Generalized Linear Mixed Model interventions (Kawada et al., unpublished data).

Figure 6.
Ceiling net using Olyset® Net materials (upper left) and an outline sketch of the ceiling net installed in a house (upper right). Intervention scene with a ceiling net (bottom left & right) (Kawada et al., 2012b).

Figure 7.
Differences between the numbers of mosquitoes (mixed population of *Anopheles funestus* s.s. and *An. arabiensis*) collected in the ceiling net intervention houses (NYAND 8, 11) and those collected in the control house (NYAND 6). The red arrow indicates the day of intervention (Kawada et al., 2012b).

Figure 8.
The average number of mosquitoes collected before intervention of permethrin-impregnated ceiling nets, after intervention, after removal of the permethrin-impregnated ceiling nets, after intervention of permethrin-untreated ceiling nets, and after re-intervention with new permethrin-impregnated ceiling nets. Bars indicate 95% confidence limits. The same letters indicate no significant difference from Tukey's HSD test ($P = 0.05$) of the square root of the ratio of the number of mosquitoes collected in the intervention house to the number collected in the control house, converted into Arcsin (Kawada et al., 2012b).

Figure 9.
Cumulative flying time of female *Anopheles gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. during a 3-min exposure to an Olyset® Net, 0.75% permethrin-impregnated paper, or

untreated net material, using a modified WHO cone bioassay. Letters indicate the significance levels form a Kruskal-Wallis test; bars indicate the SEs.

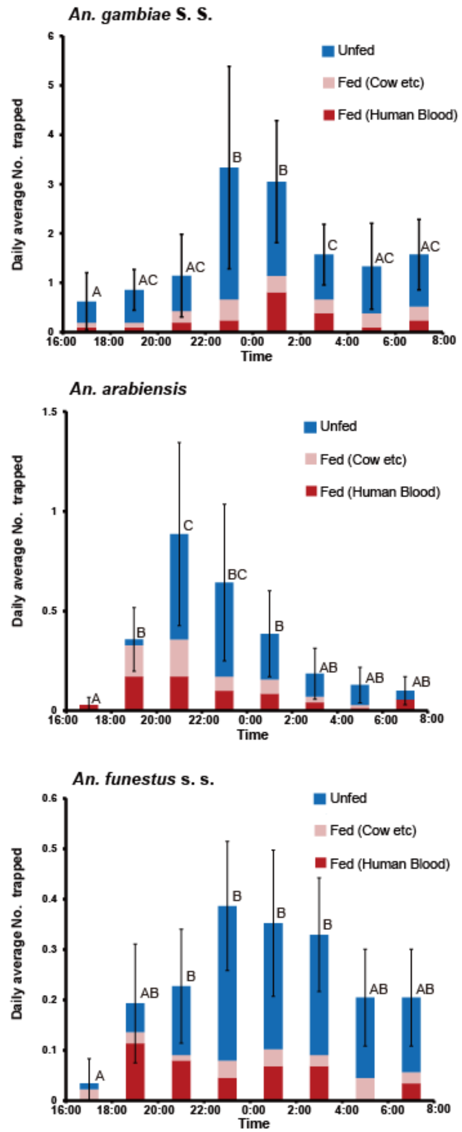
Table 1. Changes in the *Anopheles gambiae* s.l mosquito density index after intervention of metofluthrin-impregnated plastic strips in Bagamoyo, Tanzania (Kawada et al., 2008).

Days after Intervention	Mosquito Density Index (No. of female mosquitoes/house/day)	
	Intervention (95% CI)	Control (95% CI)
20	0.2 (0.4)	2.0 (1.0)
34	0.2 (0.4)	11.4 (5.6)
61	0.0 (-)	8.0 (4.2)
89	0.0 (-)	7.2 (4.4)
124	0.0 (-)	2.4 (2.3)

Table 2. Environmental factors and properties of the metofluthrin-impregnated plastic strips within intervention houses for two trial sites and their respective target mosquitoes (Kawada et al., 2006, 2008).

Environmental factors and strip properties	My Tho (2005)[2] *Aedes aegypti*		Bagamoyo (2006)[2] *Anopheles gambiae* s.l.	
Average temperature (°C)[1]	29.1	(0.8)	24.8	(0.7)
Average humidity (% RH)[1]	70.1	(5.1)	75.3	(3.9)
Total floor area (m^2/house)	32.1	(10.5)	22.0	(14.1)
Total volume (m^3/house)	129.3	(59.4)	58.7	(45.7)
Total opening area (m^2/house)	6.6	(5.0)	5.7	(4.3)
Corrected Opening Area/Volume	0.051		0.098	
No. of metofluthrin strips/m^2	0.31		0.52	
Amount of metofluthrin (mg/m^2)	191		320	
Effective Duration of metofluthrin strips (weeks)	8		> 18	

[1]June 20 - August 3, 2006 in Bagamoyo; June 20 - September 4, 2005 in My Tho
[2]Figures in parenthesis are standard deviations



An. gambiae S. S.
Unfed
Fed (Cow etc)
Fed (Human Blood)
Daily average No. trapped
Time
An. arabiensis
Unfed
Fed (Cow etc)
Fed (Human Blood)
Daily average No. trapped
Time
An. funestus s. s.
Unfed
Fed (Cow etc)
Fed (Human Blood)
Daily average No. trapped
Time

Fig. 1

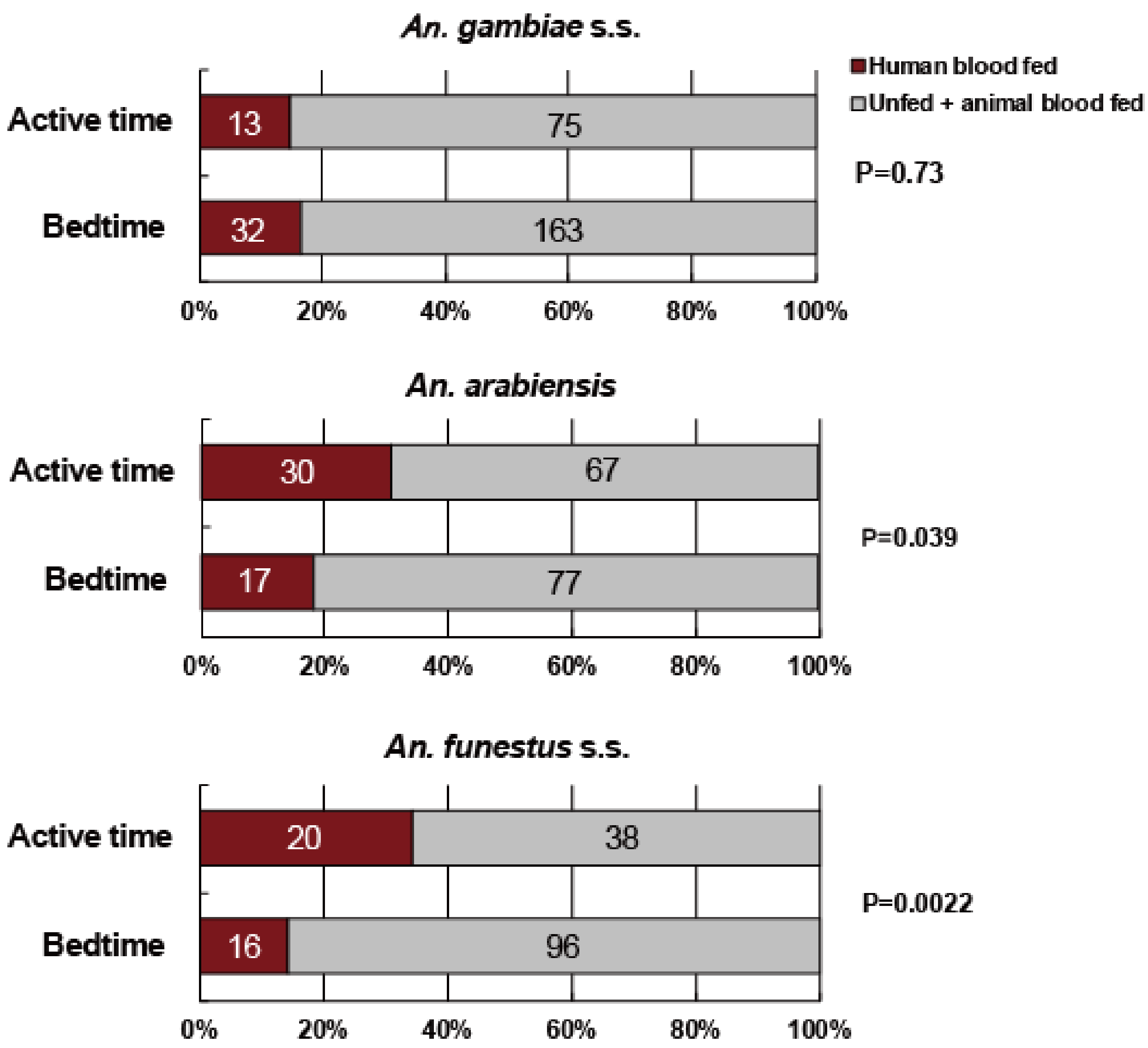

An. gambiae s.s.
Human blood fed
Unfed + animal blood fed
Active time
13
75
Bedtime
32
163
P=0.73
0%
20%
40%
60%
80%
100%
An. arabiensis
Active time
30
67
Bedtime
17
77
P=0.039
0%
20%
40%
60%
80%
100%
An. funestus s.s.
Active time
20
38
Bedtime
16
96
P=0.0022
0%
20%
40%
60%
80%
100%

Fig. 2

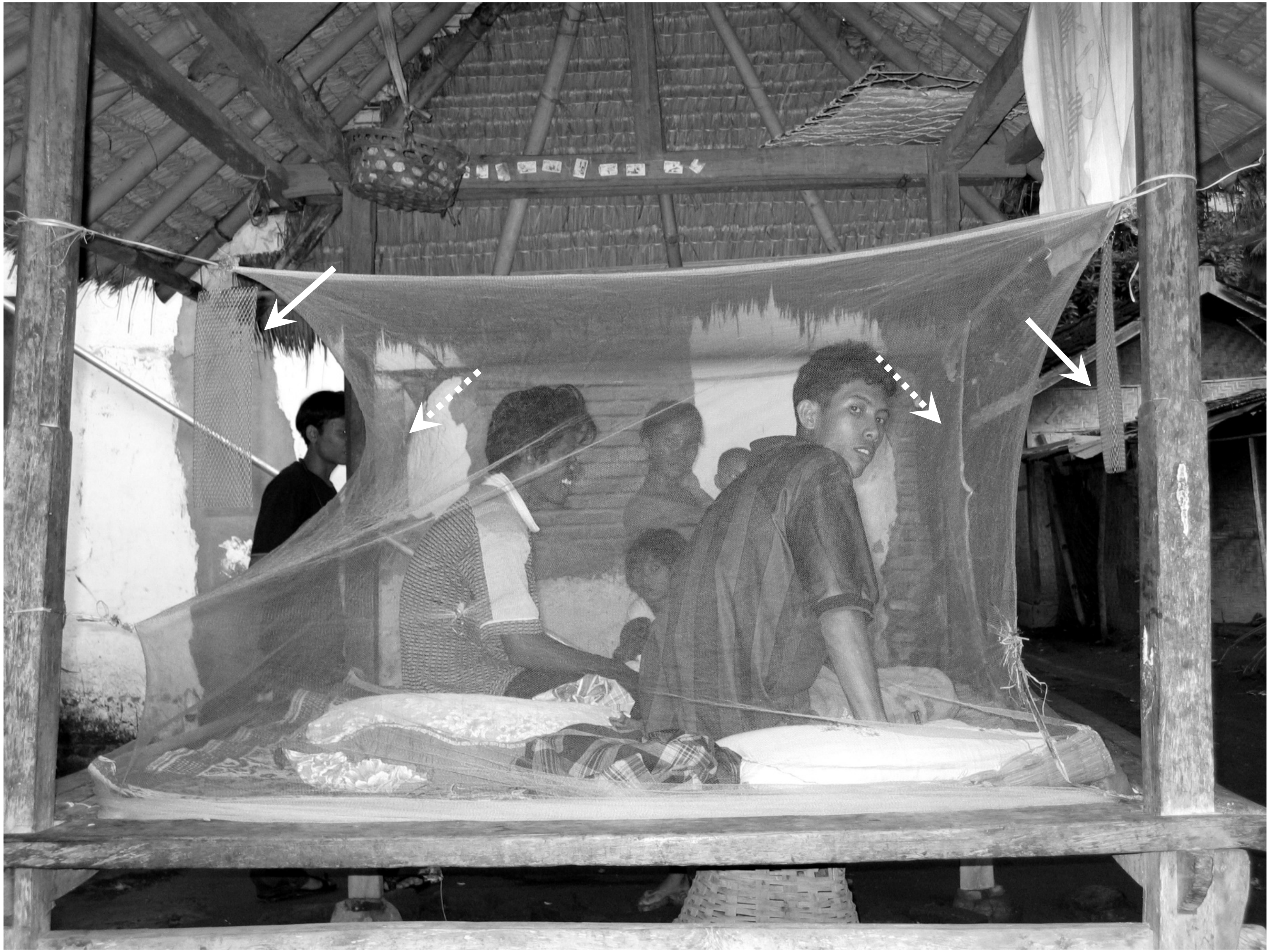


Fig. 3

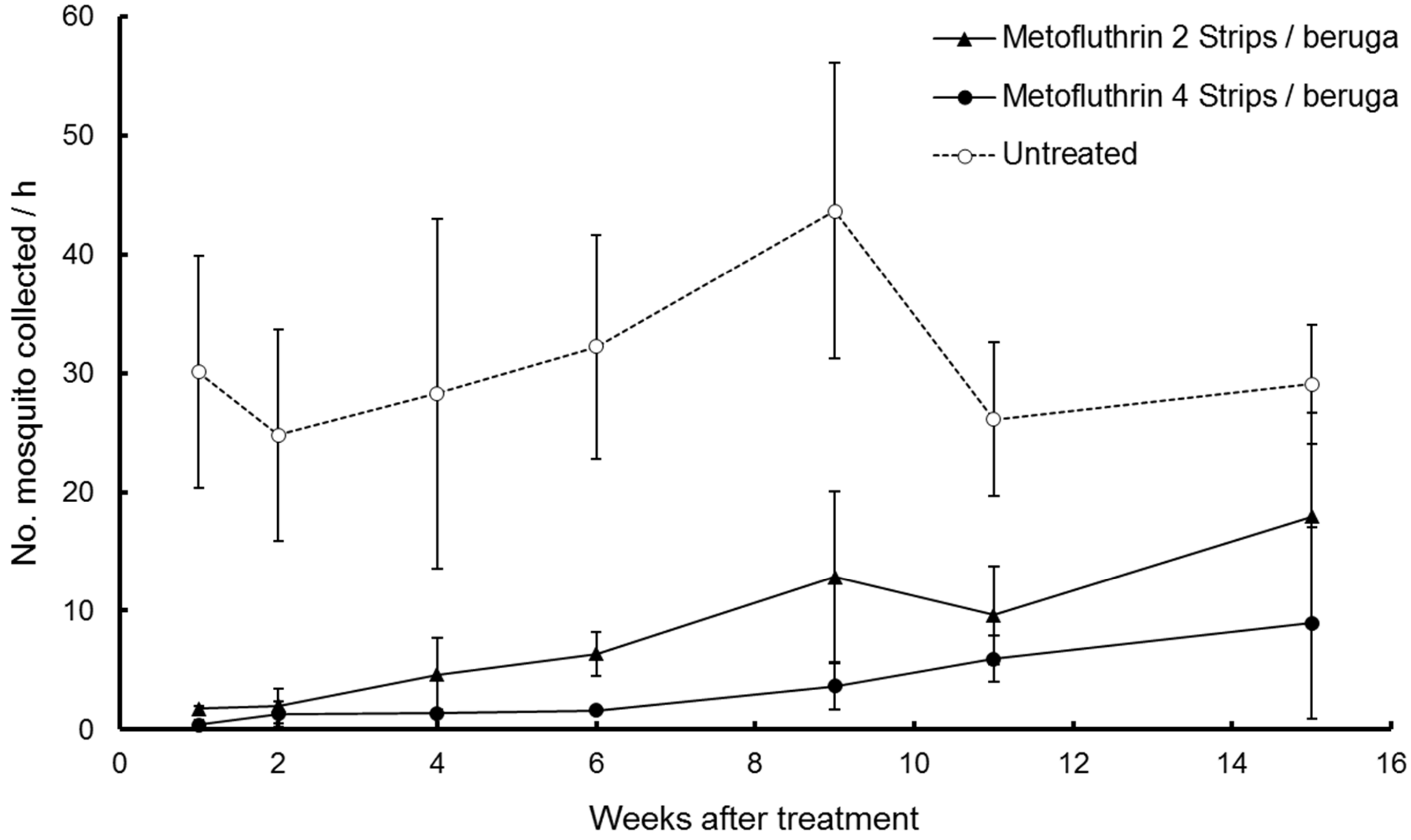

Metofluthrin 2 Strips / beruga
Metofluthrin 4 Strips / beruga
Untreated
No. mosquito collected / h
Weeks after treatment
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16

Fig. 4

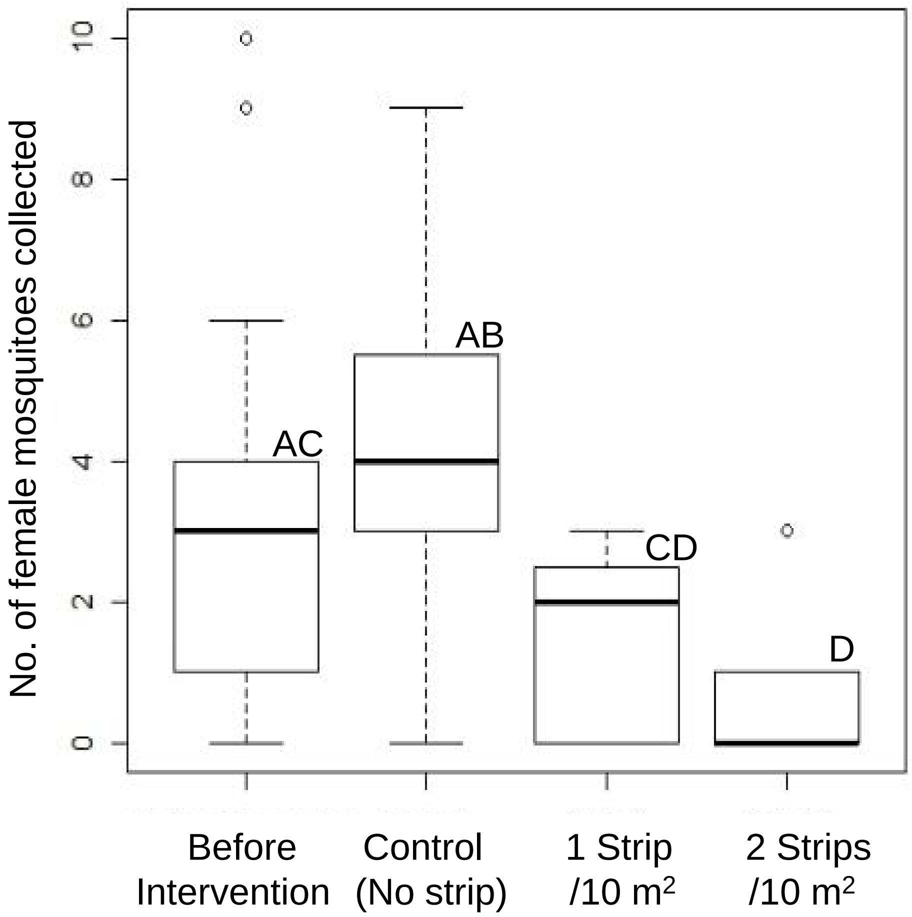

No. of female mosquitoes collected
0
2
4
6
8
10
AC
AB
CD
D
Before Intervention
Control (No strip)
1 Strip /10 m2
2 Strips /10 m2

Fig. 5

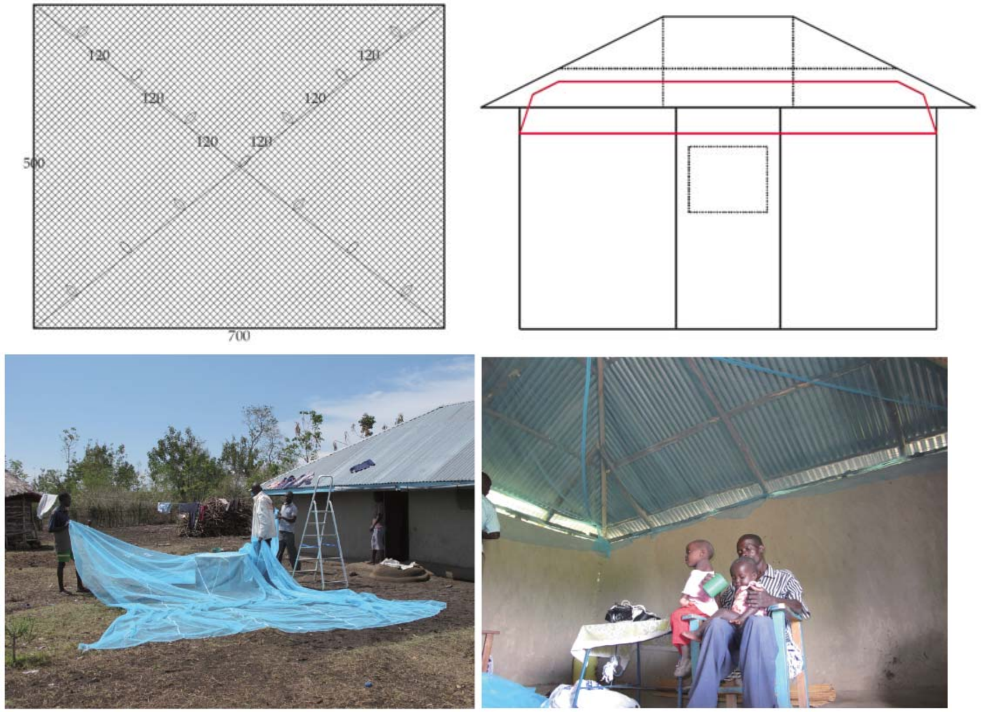


Fig. 6

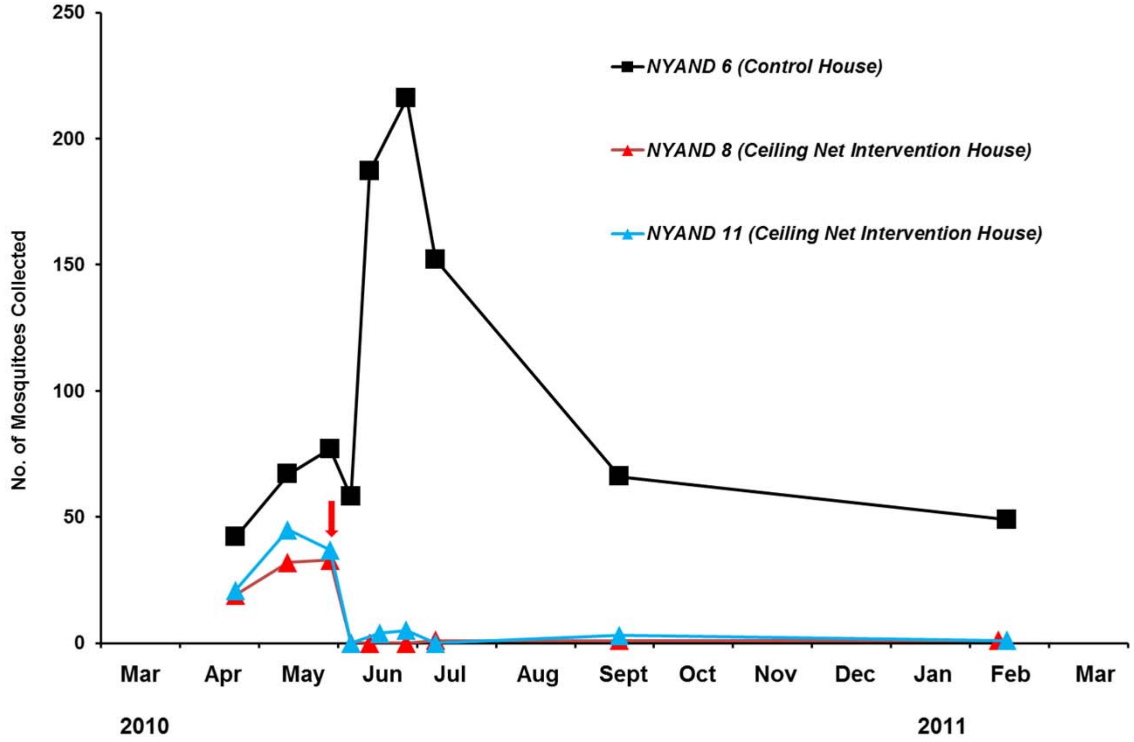
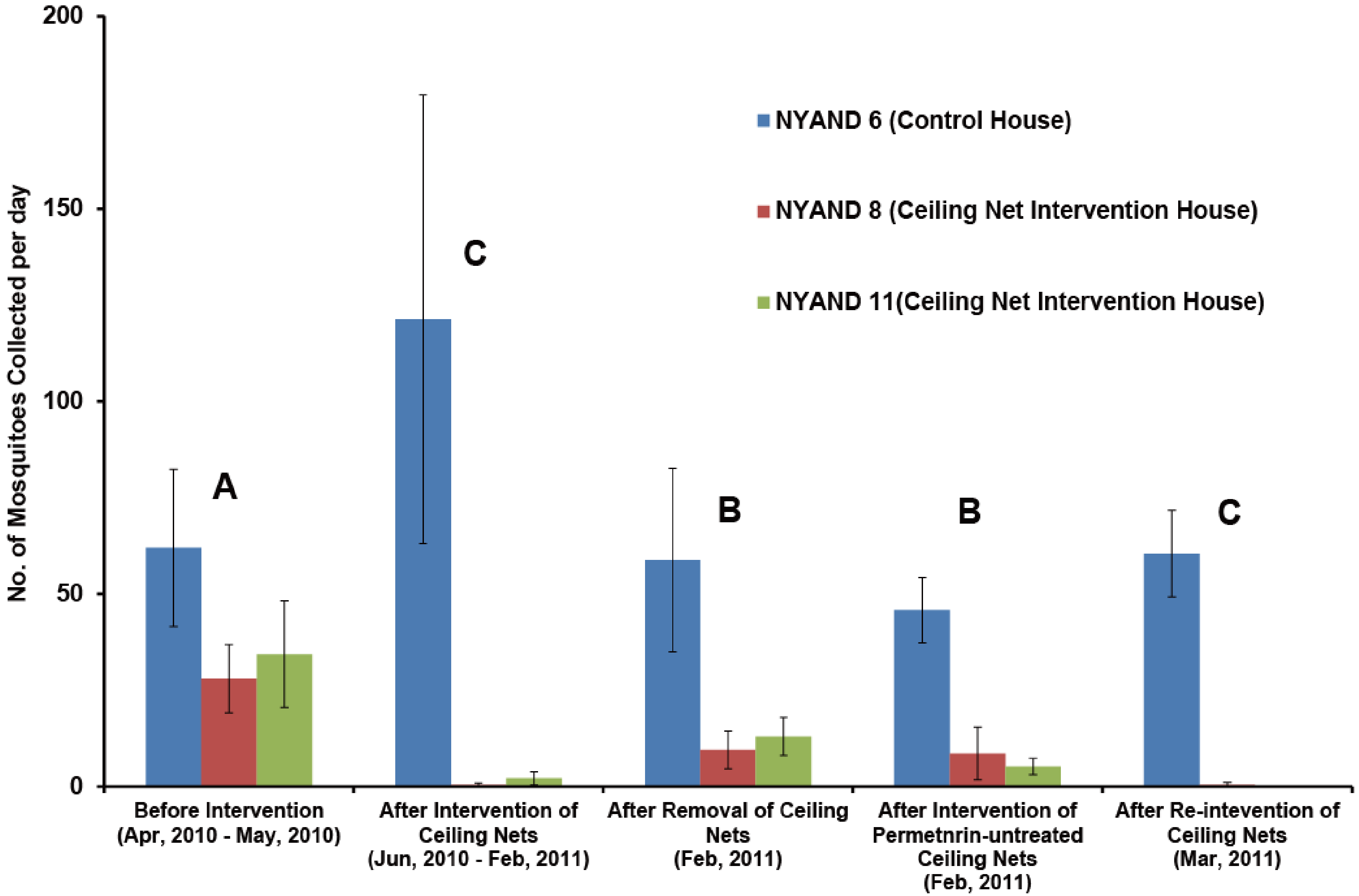
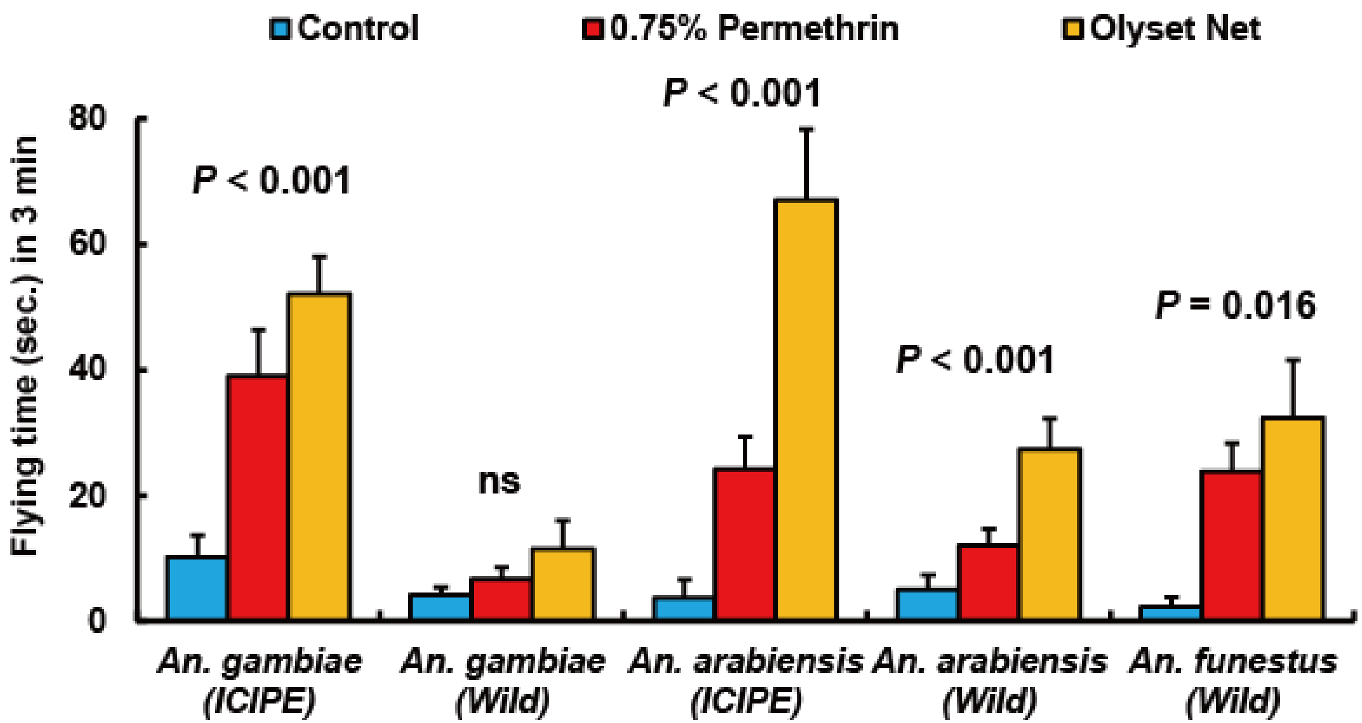

250
200
150
100
50
0
No. of Mosquitoes Collected
NYAND 6 (Control House)
NYAND 8 (Ceiling Net Intervention House)
NYAND 11 (Ceiling Net Intervention House)
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
2010
2011

Fig. 7



No. of Mosquitoes Collected per day
200
150
100
50
0
NYAND 6 (Control House)
NYAND 8 (Ceiling Net Intervention House)
NYAND 11(Ceiling Net Intervention House)
A
C
B
B
C
Before Intervention (Apr, 2010 - May, 2010)
After Intervention of Ceiling Nets (Jun, 2010 - Feb, 2011)
After Removal of Ceiling Nets (Feb, 2011)
After Intervention of Permetnrin-untreated Ceiling Nets (Feb, 2011)
After Re-intevention of Ceiling Nets (Mar, 2011)

Fig. 8



Control
0.75% Permethrin
Olyset Net
P < 0.001
P < 0.001
P < 0.001
P = 0.016
ns
Flying time (sec.) in 3 min
80
60
40
20
0
An. gambiae (ICIPE)
An. gambiae (Wild)
An. arabiensis (ICIPE)
An. arabiensis (Wild)
An. funestus (Wild)

Fig. 9