

High potential risk of dengue transmission during the hot-dry season in Nha Trang City, Vietnam

Ataru Tsuzuki ^{a,*}, Vu Trong Duoc ^b, Yukiko Higa ^c, Nguyen Thi Yen ^b, Masahiro Takagi ^a

^a Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

^b National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

^c Center for International Collaborative Research, Nagasaki University, Nagasaki, Japan

* Corresponding author at: Institute of Tropical Medicine, Nagasaki University, Nagasaki 852-8523, Japan. Tel.: +81-095-849-7809; fax +81-095-849-7812.

E-mail: atarutsuzuki@hotmail.com (A. Tsuzuki).

Abstract We visited houses and inspected water-holding containers to determine the potential risks of dengue transmission during different seasons. This survey was conducted in 2 neighbourhoods of Nha Trang City in July and December 2006, which correspond to the middle of the hot-dry season and the beginning of the cool-wet season, respectively. We inspected a total of 1,438 wet containers in 196 premises during both survey periods; 20% of the containers were positive for *Aedes aegypti* larvae and 8% for *Ae. aegypti* pupae. Indoor water-holding containers, which were sparsely distributed, exhibited high pupal productivity and efficiency (pupal productivity of a type of container/prevalence of that type of container) in either the first survey conducted in July, or the second, conducted in December. Although rainfall may not influence the number and distribution of water-holding containers in the city, the high average temperature in the first survey period resulted in a higher potential risk of dengue transmission. Our analysis suggests that if intensive source reduction is conducted in summer and containers with high pupal productivity and efficiency are targeted, the risk of dengue transmission in the city could be effectively reduced.

KEY WORDS *Aedes aegypti*; Vietnam; dengue; pupal survey

1. Introduction

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are increasingly important public health problems in the tropics and subtropics, which include many resource-poor countries. No promising vaccine for dengue is currently available. To date, reducing the population density of the mosquito vectors of dengue, predominantly *Aedes aegypti*, has been the only option for controlling dengue virus transmission in the human population (Guzman et al., 2002). Hence, a targeted vector-control strategy that minimizes unnecessary costs is essential for the effective control of this disease. The pupal/demographic survey technique, which targets the most important breeding sites of *Ae. aegypti* in areas endemic for dengue, has recently been introduced, and its advantages have been reported (Barrera et al., 2006; Hammond et al., 2007; Lenhart et al., 2006). The pupal count provides a more reasonable estimate of the number of *Ae. aegypti* adults than the larval count. This is because the pupae have a higher survival rate and it is simpler to identify the species of the emerged adult. Transmission thresholds (the number of *Ae. aegypti* pupae per person above which an outbreak of dengue presumably occurs) were estimated using simulation models developed to provide site- and weather-specific insights into the dynamics of dengue viruses and vectors (Focks et al., 1993a; Focks et al., 1993b). The transmission threshold was estimated as a function of the mean temperature, rate of virus introduction, and immunity of the target human population (Focks et al., 2000). The estimation

of the transmission threshold provides an overall target—an upper limit—of the number of pupae per person present in the environment that ensures that virus introductions would result in little or no transmission.

The pupal/demographic survey is a useful tool to identify which container type(s) should be targeted; however, appropriate time for container management in Vietnam has not yet been determined. Breeding of *Ae. aegypti* is well adapted to the urban environment which provide various types of potential breeding containers. Among these, the number of rain-filled containers that are discarded outdoors may increase during the rainy season. The increase in the number of water-holding containers may also increase the number of *Ae. aegypti* pupae. A high mean temperature promotes the growth of *Ae. aegypti* and the dengue virus (Focks et al., 1993a), and it may result in a lower outbreak threshold, which in turn increases the risk of dengue transmission. It would be desirable to conduct intensive container management during the period with the highest potential risk of dengue transmission.

To identify the effect of rainfall and temperature on the potential risk of dengue transmission, we conducted house-to-house surveys in 2 urban neighborhoods during 2 survey periods (the hot-dry season in June and cool-wet season in December).

2. Materials and Methods

In July (hot-dry season) and December (cool-wet season) 2006, an entomological survey was conducted in Nha Trang City located in the coastal area of central Vietnam. The 2 target neighborhoods inhabited by the local residents were part of 2 urban communes in the city. The study areas (the Bach Dang neighborhood located in the western part of the city and the eighth neighborhood located in the peripheral region in the northern part of the city) were approximately 3 km away from each other. Potable tap water is available throughout the year in both the areas. Unlike the southern part of Vietnam, the Nha Trang area does not experience monsoon during the summer. Therefore, this city has distinct hot-dry (May–October) and cool-wet (November–April) seasons (Fig. 1).

We surveyed wet (water-holding) containers to identify all the potential breeding sites (water-filled containers, surface of stationary water pools, or wells), and estimated the abundance of *Ae. aegypti* larvae and pupae in these containers. The pupae were transported to a laboratory in the city, enumerated, and reared. After this, the species to which the emerged adult belonged was determined. Since it is difficult to access wells for direct collection, funnel traps were used for sample collection and the number of pupae in the wells was estimated (Kay et al., 1992). The productivity (number of pupae in each type of container \times 100/total number of pupae in all containers), prevalence (number of containers of 1 type \times 100/total number of all

types of containers), and efficiency (pupal productivity of a type of container/prevalence of that type of container) of containers has been defined in previous studies (Barrera et al., 2006; Hammond et al., 2007; Morrison et al., 2004), and these previously described methods were applied to determine the potential productivity and efficiency of each container type. The containers were classified into different categories/types on the basis of earlier surveys conducted on dengue-carrying mosquitoes in Vietnam (Tran et al., 1999; Vu et al., 2005).

The threshold estimate was calculated by interpolating the values from a table of reference thresholds (Table 4 in Focks et al.'s study, 2000); this estimate was specific for an average monthly temperature (29.0 °C for the first survey conducted in June and 25.2 °C for the second conducted in December) and a seroprevalence rate of 33% was assumed. Although a seroprevalence survey has not been carried out in Nha Trang City, we assumed 33% immunity to be a sensitive threshold to detect a dengue outbreak in the city on the basis of a study conducted in South Vietnam (Thai et al., 2005). Transmission thresholds for 29.0 °C and 25.2 °C were not listed in the reference table, and the threshold values for these temperatures were calculated linearly. The transmission thresholds were estimated to be approximately 0.38 pupae per person in the first survey and 2.32 pupae per person in the second survey. The number of residents was determined during the first survey period, and it was considered to remain constant between the 2 survey periods. The chi-square test was used to assess the proportional

changes within the groups and a *P* value of 0.05 was considered as statistically significant. The data were entered into EpiData v. 3.1 (EpiData Association, Denmark) and analyzed using Stata™ v. 8.0 (Stata Corp., USA).

3. Results

During both the surveys, 196 residential premises—93 in the Bach Dang neighbourhood and 103 in the eighth neighbourhood—were inspected. In total, 1,438 water-holding containers were found (Table 1). Of these, 284 (20%) were positive for *Ae. aegypti* larvae and 111 (8%) for *Ae. aegypti* pupae. We collected 1,283 *Ae. aegypti* pupae from the 111 containers. Further, 21 *Ae. albopictus* pupae were also detected, but these were excluded from the analyses. The efficiency of the container types (pupal productivity of a type of container/prevalence of that type of container) differed greatly, and could not be predicted by using the values for productivity (Table 1). The efficiency ranged between 0.2 (for flower vases) and 13.9 (for tires), and if all the containers were assumed to be equally efficient, the efficiency would be 1.0. The following container types had a productivity of more than 10.0 (generated more than 10% of the total pupae) and an efficiency of more than 1.0 in either the first or second survey: built-in concrete containers that hold water used for toilet flushing that is collected in small buckets or dippers (toilet concrete basins), small or medium-sized plastic buckets with a capacity less than 100 litres (S/M plastic buckets), large plastic buckets with a capacity of 100 litres or more,

concrete basins, jars, flower vases, wells, and discarded tires (Fig. 2). In the first survey, toilet concrete basins, large plastic buckets, concrete basins, and tires had a high productivity (11.5–25.4). These containers were sparsely distributed in the study areas (0.5–4.7%), and their efficiency was high (2.5–23.0). In the second survey, flower vases, S/M plastic buckets, wells, jars, and toilet concrete basins had a high productivity (14.5–23.5). Among these, flower vases (45.8%) and S/M plastic buckets (30.3%) comprised the majority, and the other containers were sparsely distributed (3.0–6.1%). The efficiency of the sparsely distributed containers such as wells, jars, and toilet concrete basins was high (3.5–5.1), whereas that for the flower vases (0.3) and S/M plastic buckets (0.6) was low. Most of these containers were located indoors or under the eaves, which means that water had been added to them manually and they were not filled by rainfall. The efficiency of containers discarded outdoors was high (1.4–1.5); however, few such containers were found during either of the survey periods and their productivity was low (3.2–4.6). The proportions of the premises containing each container type did not significantly differ between the 2 survey periods ($P > 0.05$ by chi-square test) (Table 2).

The observed number of pupae per person exceeded the transmission threshold in the first survey conducted in July (139%); whereas, the observed number was considerably lower than the threshold in the second survey conducted in December (35%) (Table 3). In the first survey, 128, 25, 21, 58, 100, and 62 pupae were found in 21 toilet concrete basins, 29 wells, 43 jars, 35

large plastic buckets, 10 concrete basins, and 4 tires, respectively. Although these 128 containers constituted only 19% of the total containers studied in the first survey, 78% of the total pupae were found in them. Targeting these containers for container management could have considerably reduced the pupal density below the threshold for disease transmission (i.e., from 139% to 30% of the threshold).

4. Discussion

On comparing the observed number of pupae per person with the transmission thresholds between the 2 survey periods, the risk of a dengue outbreak appeared to be higher during the first survey that was conducted in the hot-dry season in July. The observed number of pupae per person in both the survey periods was nearly the same; however, this number exceeded the disease transmission threshold only during the first survey conducted in July. When the seroprevalence of antibodies against the dengue virus and the rate of virus introduction are constant, the average monthly temperature influences the transmission threshold (Focks et al., 2000; Focks et al., 1993a). There was a large difference in the monthly average temperature between the 2 survey periods, resulting in a remarkable difference in the outbreak thresholds (0.38 in the first survey conducted in June and 2.32 in the second survey conducted in December). The number of dengue patients admitted to hospitals in Nha Trang City usually increases in July, and the number of admissions remains high till November (Fig. 3). The

number of dengue cases does not increase at the beginning of the high-temperature period in the city (May and June); however, previous studies have reported that dengue epidemics take several months to reach a level where they can be recognized as the result of previous conditions (Guzman et al., 2000; Newton et al., 1992). Our results indicated that an extensive reduction in and the management of *Ae. aegypti* breeding sources should be conducted during the high-temperature period in the city (May-August), even when there is no apparent increase in the cases of dengue.

Toilet concrete basins, wells, jars, large plastic buckets, concrete basins, and tires were found to be potentially important breeding containers for *Ae. aegypti* pupae since they had a high pupal productivity and efficiency in the first and/or second survey period. The rainfall in the city during the survey conducted in December was much higher than that during the survey conducted in July; however, our study indicated that the rainfall does not influence the number and distribution of the containers. Most of the residential buildings that were surveyed were adjacent or connected to each other, and outdoor space was absent or limited. In addition, the local government periodically cleaned the streets and public spaces in the urban areas of the city. Therefore, only a few rain-filled containers that had been discarded outdoors were found. Although these results suggest that rainfall does not play an important role in the number and distribution of water-holding containers in our study areas or other areas with similar conditions,

the pupal productivity and efficiency of each container type differed between the survey periods. The pattern of container use (e.g., frequency of water replacement and use of adequate covers) and the timings at which the containers were inspected may have resulted in differences in the pupal productivity. However, targeting all container types that had a high pupal productivity and efficiency in at least 1 of the 2 survey periods could effectively help us manage most of the important breeding sites.

Large-scale longitudinal cohort studies are required to clearly ascertain the temporal and geographical variations in *Ae. aegypti* breeding patterns (Morrison et al., 2004); however, we could not conduct such a study because of resource constraints. Further studies are required to confirm the importance of each container type that harbours mosquito larvae and pupae in the city. This study is the first attempt to ascertain the risk of dengue transmission in different seasons in Vietnam. Our analysis suggests that intensive source reduction in summer targeting containers with high pupal productivity and efficiency will effectively reduce the risk of dengue transmission in the city.

Acknowledgements

We express our deep gratitude to Le Viet Lo, Le Trung Nghia, Ton Nu Van Anh, Bui Cam Nhung, Bui Thanh Phu, in Nha Trang Pasteur Institute for field assistance. We also thank Toshihiko Sunahara and Hitoshi Kawada for valuable suggestions. This work was supported by

the Core University Program sponsored by the Japan Society for the Promotion of Science.

References

- Barrera, R., Amador, M., and Clark, G. G., 2006. Use of the pupal survey technique for measuring *Aedes aegypti* (Diptera: Culicidae) productivity in Puerto Rico. *Am. J. Trop. Med. Hyg.* 74, 290-302.
- Focks, D. A., Brenner, R. J., Hayes, J., and Daniels, E., 2000. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am. J. Trop. Med. Hyg.* 62, 11-8.
- Focks, D. A., Haile, D. G., Daniels, E., and Mount, G. A., 1993a. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): analysis of the literature and model development. *J. Med. Entomol.* 30, 1003-17.
- Focks, D. A., Haile, D. G., Daniels, E., and Mount, G. A., 1993b. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): simulation results and validation. *J. Med. Entomol.* 30, 1018-28.
- Guzman, M. G., and Kouri, G., 2002. Dengue: an update. *Lancet Infect. Dis.* 2, 33-42.
- Guzman, M. G., Kouri, G., Valdes, L., Bravo, J., Alvarez, M., Vazques, S., Delgado, I., and Halstead, S. B., 2000. Epidemiologic studies on Dengue in Santiago de Cuba, 1997. *Am. J. Epidemiol.* 152, 793-9; discussion 804.
- Hammond, S. N., Gordon, A. L., Lugo Edell, C., Moreno, G., Kuan, G. M., Lopez, M. M., Lopez, J. D., Delgado, M. A., Valle, S. I., Espinoza, P. M., and Harris, E., 2007. Characterization of *Aedes aegypti* (Diptera: Culicidae) production sites in urban Nicaragua. *J. Med. Entomol.* 44, 851-60.
- Kay, B. H., Cabral, C. P., Araujo, D. B., Ribeiro, Z. M., Braga, P. H., and Sleigh, A. C., 1992. Evaluation of a funnel trap for collecting copepods and immature mosquitoes from wells. *J. Am. Mosq. Control. Assoc.* 8, 372-5.
- Lenhart, A. E., Castillo, C. E., Oviedo, M., and Villegas, E., 2006. Use of the pupal/demographic-survey technique to identify the epidemiologically important types of containers producing *Aedes aegypti* (L.) in a dengue-endemic area of Venezuela. *Ann. Trop. Med. Parasitol.* 100 Suppl 3, 53-9.
- Morrison, A. C., Gray, K., Getis, A., Astete, H., Sihuinchu, M., Focks, D., Watts, D., Stancil, J. D., Olson, J. G., Blair, P., and Scott, T. W., 2004. Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. *J. Med. Entomol.* 41, 1123-42.
- Newton, E. A., and Reiter, P., 1992. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am. J. Trop. Med. Hyg.* 47, 709-20.

- Thai, K. T., Binh, T. Q., Giao, P. T., Phuong, H. L., Hung le, Q., Van Nam, N., Nga, T. T., Groen, J., Nagelkerke, N., and de Vries, P. J., 2005. Seroprevalence of dengue antibodies, annual incidence and risk factors among children in southern Vietnam. *Trop. Med. Int. Health* 10, 379-86.
- Tran, V. P., and Vu, S. N., 1999. Key breeding site of dengue vectors in Hanoi, Vietnam, 1994-1997. *Dengue Bulletin* 23, 67-72.
- Vu, S. N., Nguyen, T. Y., Tran, V. P., Truong, U. N., Le, Q. M., Le, V. L., Le, T. N., Bektas, A., Briscombe, A., Aaskov, J. G., Ryan, P. A., and Kay, B. H., 2005. Elimination of dengue by community programs using Mesocyclops (Copepoda) against *Aedes aegypti* in central Vietnam. *Am. J. Trop. Med. Hyg.* 72, 67-73.

Table 1
Productivity and efficiency of *Ae. aegypti* breeding containers

Container type	Pupae			Number of container	Productivity ^c	Prevalence of container ^d	Efficiency ^e
	n	Avg ^a	Max ^b				
Flower vase	136	0.4	70	622	10.6	43.3	0.2
S/M plastic bucket	197	0.8	116	461	15.4	32.1	0.5
Well	150	21.4	25	61	11.7	4.2	2.8
Jar	204	3.9	75	85	15.9	5.9	2.7
Discarded container	48	1.7	15	40	3.7	2.8	1.3
Ant trap	19	1.1	9	32	1.5	2.2	0.7
Plant saucer	26	6.5	26	7	2.0	0.5	4.2
Toilet concrete basin	250	8.3	56	42	19.5	2.9	6.7
Metal drum	29	4.8	15	9	2.3	0.6	3.6
Large plastic bucket	59	1.6	48	54	4.6	3.8	1.2
Concrete basin	103	8.6	99	20	8.0	1.4	5.8
Tire	62	15.5	22	5	4.8	0.3	13.9
Total	1283	1.6	116	1438	100.0	100.0	1.0

^a Average no. of pupae.

^b Maximum no. of pupae in an individual container.

^c Productivity = pupae no. x 100/all pupae.

^d Prevalence of container = container no. x 100/all containers.

^e Efficiency = productivity/prevalence of container.

Table 2

Presence of *Ae. aegypti* breeding containers in premises

Container type	% premises with containers	
	1 st survey (July)	2 nd survey (December)
Flower vase	68.9	70.4
S/M plastic bucket	73.5	67.9
Jar	16.3	15.3
Large plastic bucket	13.8	8.2
Discarded container	9.2	6.1
Toilet concrete basin	7.7	9.2
Concrete basin	6.1	4.6
Ant trap	3.6	3.0
Plant saucer	1.0	1.0
Tire	0.5	0.5

Table 3

Observed numbers and transmission thresholds of *Ae. aegypti* pupae per person

	Observed pupae per person ^a	Threshold pupae per person ^b	Ratio ^c
1 st survey (July)	0.53	0.38	1.39
2 nd survey (December)	0.82	2.32	0.35

^a We collected 503 and 780 *Ae. aegypti* pupae in the first and second surveys, respectively. The number of residents were determined as 949 during the first survey period, and it was considered to remain constant between the 2 survey

^b Transmission thresholds in this survey were based on the number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of antibody to dengue during the course of a year after resulting from 12 monthly viral introduction of a single viremic individual. Thresholds were interpolated with the monthly average temperature 29.0°C in the first, July, survey and 25.2°C in the second, December, survey with an assumption of 33% immunity in the human population.

^c Ratio = threshold pupae per person/observed pupae per person

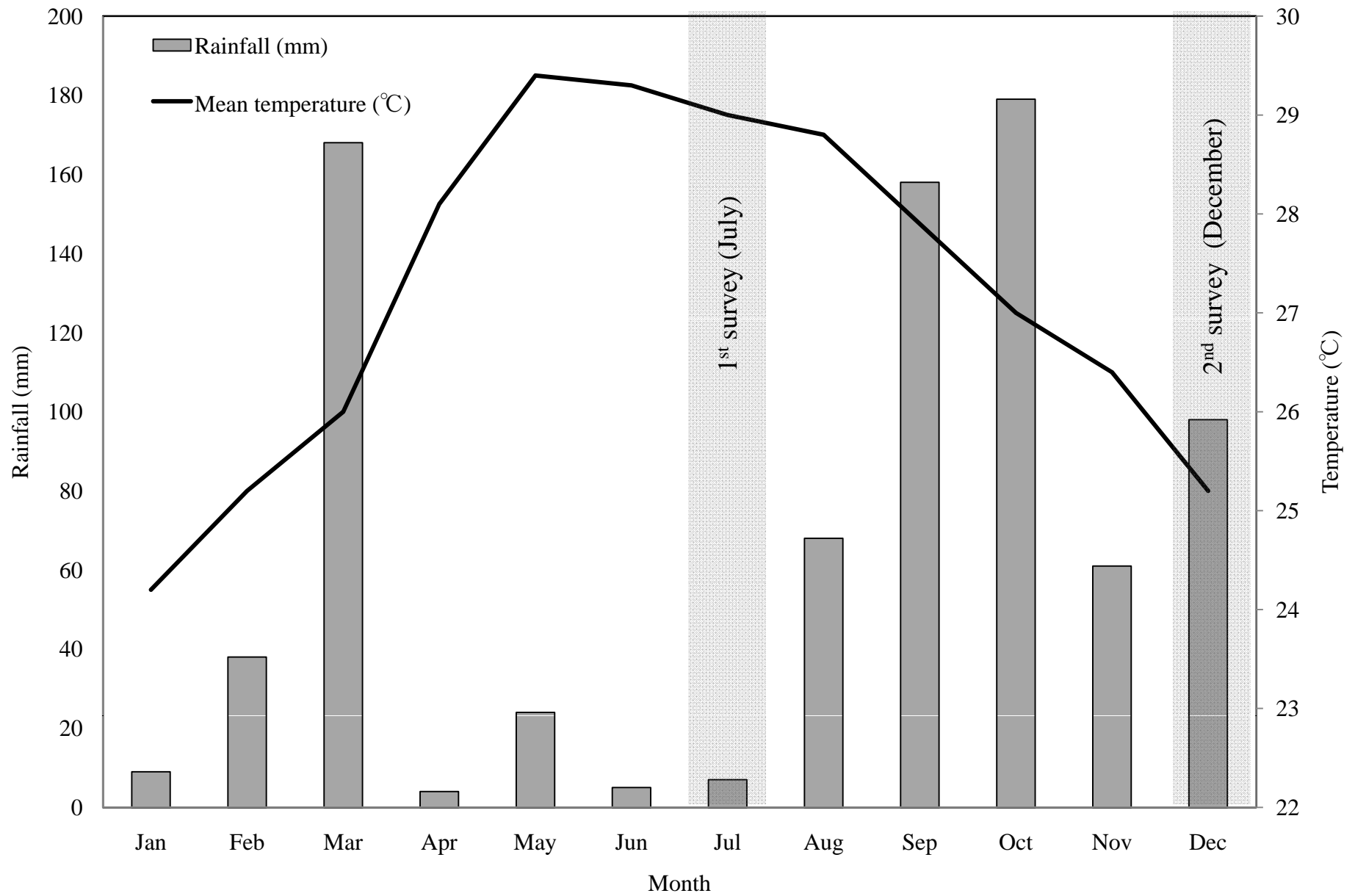


Fig. 1. Monthly rainfall (mm) and mean temperature (°C) in Nha Trang City 2006.

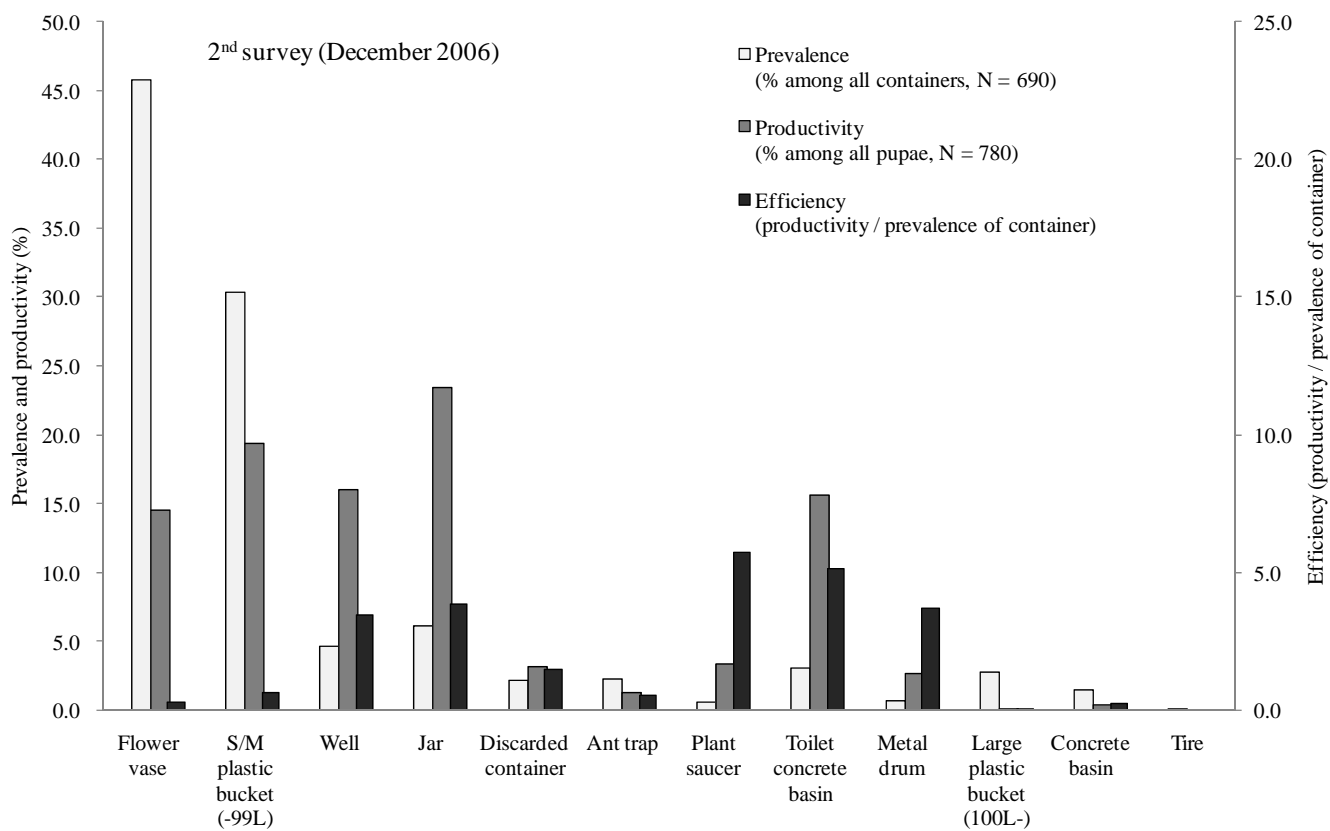
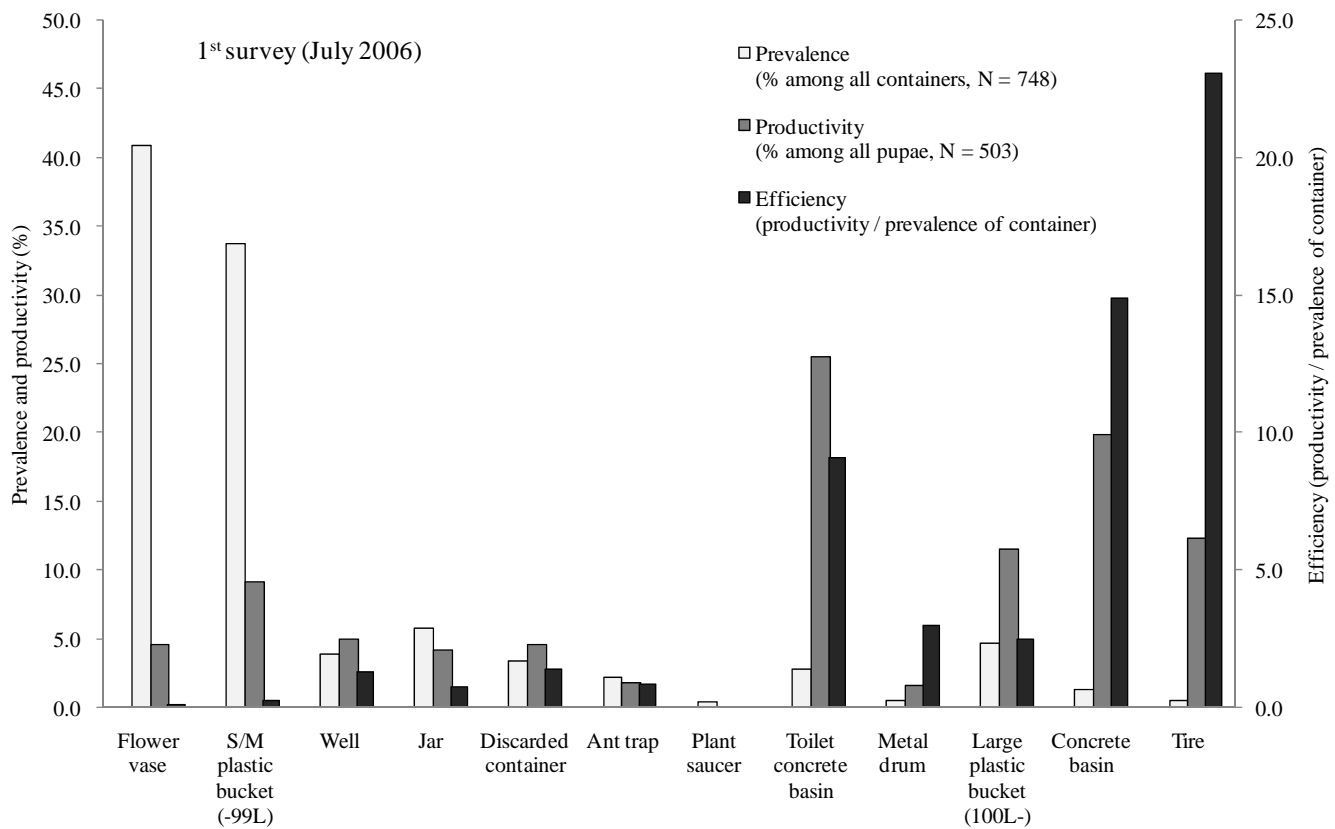


Fig. 2. Prevalence, productivity, and efficiency of *Ae. aegypti* bleeding containers in the 1st survey (upper figure) and the 2nd survey (bottom figure).

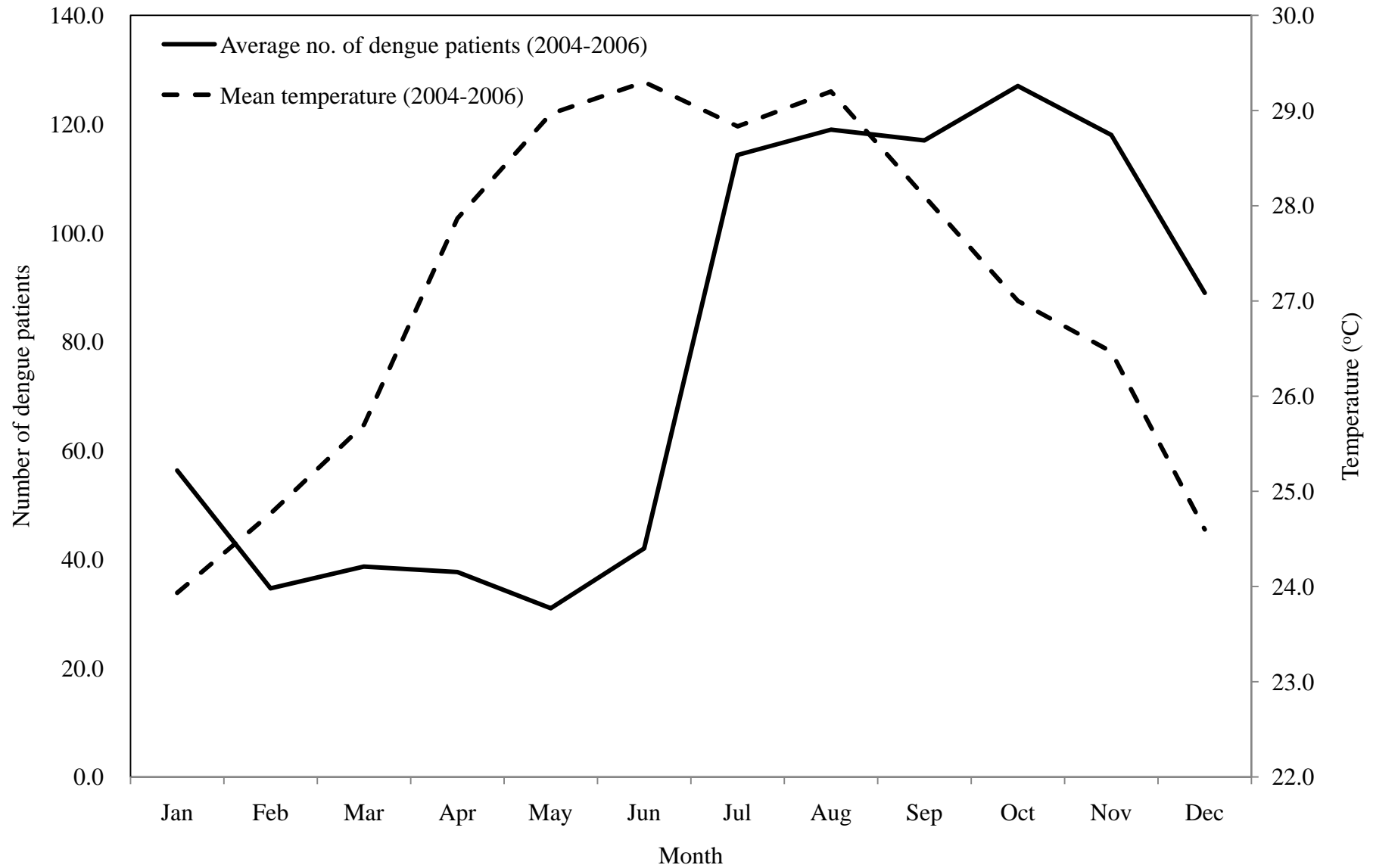


Fig. 3. Mean temperature and average number of dengue patients including dengue fever (DF) and dengue hemorrhagic fever (DHF) cases admitted to hospitals in Nha Trang City between 2004 and 2006.