STUDY OF MOSQUITO FAUNA IN RICE ECOSYSTEMS AROUND HANOI, NORTHERN VIETNAM

Short running head: MOSQUITO FAUNA IN NORTHERN VIETNAM

Shin-ya Ohba[a]*, Nguyen Van Soai[b], Dinh Thi Van Anh[b], Yen T. Nguyen[b], Masahiro Takagi[a]

[a]Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University, Sakamoto, Nagasaki 852-8523, Japan

[b]National Institute of Hygiene and Epidemiology, 1 Yersin Street, Hanoi, Vietnam

*Corresponding author: Shin-ya Ohba

[1]Present address: Biological Laboratory, Faculty of Education, Nagasaki University, Bunkyo, Nagasaki 851-2125, Japan

Phone and Fax: +81-95-819-2393; E-mail: ooba@nagasaki-u.ac.jp

Abstract

Species of the *Culex vishnui* subgroup, *Cx. fuscocephala* and *Cx. gelidus*, which are known Japanese encephalitis (JE) vectors, are distributed in rice agroecosystems in Asian countries. Hence, although ecological studies of rice agroecosystems in northern Vietnam are necessary, very few integrated studies of breeding habitats of mosquitoes, including JE vectors, have been conducted. We carried out a field study and investigated the mosquito fauna in six rice production areas in northern Vietnam during the rainy and dry seasons of 2009. Mosquitoes and potential mosquito predators were collected from aquatic habitats by using larval dippers. We collected 1780 *Culex* individuals (including 254 *Cx. tritaeniorhynchus*; 113 *Cx. vishnui*, 58 *Cx. vishnui* complex., consisting of *Cx. vishnui* and *Cx. pseudovishnui*; 12 *Cx. gelidus*; 1 *Cx. bitaeniorhynchus*; and 1 *Cx. fuscocephala*), 148 *Anopheles* individuals (including 5 *An. vagus*), 1 *Mansonia annulifera*, and 1 *Mimomyia chamberlaini* during the rainy season. During the dry season, we collected 176 *Culex* individuals (including 33 *Cx. vishnui*, 24 *Cx. tritaeniorhynchus*, 8 *Cx. vishnui* complex, and 1 *Cx. gelidus*) and 186 *Anopheles* individuals (including 9 *An. tessellatus*, 2 *An. kochi*, and 2 *An. barbumbrosus*). We found mosquitoes in all aquatic habitats, namely, rice fields, ditches, ponds, wetlands, irrigation canals, and rice nurseries, and *Cx. tritaeniorhynchus* and *Cx. vishnui* complex were found in all the above six areas. Heteroptera such as *Micronecta*, Veliidae, and Pleidae were abundant and widely distributed in both the seasons. The abundance of mosquito larvae was higher in the rice fields, ditches, and ponds during the rainy season than during the dry season. *Cx. tritaeniorhynchus*, *Cx. vishnui* complex, *Cx. fuscocephala*, and *Cx. gelidus* were abundant in rice agroecosystems (rice fields, ditches, ponds, and wetlands) in northern Vietnam, and their abundance was high during the rainy season. These findings deepen

our understanding of mosquito ecology and strengthen mosquito control strategies to be applied in rice ecosystems Vietnam in the future.

Key Words: Agriculture, *Culex vishnui* subgroup, integrated vector management, mosquito control

1. Introduction

Mosquito-borne diseases such as malaria and Japanese encephalitis (JE) are major public health threats in Asian countries. JE has spread throughout Asia and may have originated in the Indo-Malaysian region of Asia (Solomon et al., 2003). JE cases have been reported in India, Nepal, Sri Lanka, Bangladesh, Myanmar, Laos, Cambodia, Thailand, Vietnam, Malaysia, China, Philippines, Indonesia, Korea, Japan, Papua New Guinea, and most recently, in the southern parts of Australia. In Asian countries, *Culex tritaeniorhynchus* Giles, *Cx. vishnui* complex, *Cx. fuscocephala* Theobald, and *Cx. gelidus* Theobald are JE vectors (Gingrich et al., 1992; Reuben et al., 1994; Stoops et al., 2008; Van den Hurk et al., 2009; Van Peenten et al., 1975; Vythilingam et al., 1997). The *Cx. vishnui* subgroup species, viz., *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, and *Cx. vishnui*, are the vectors of JE in Southeast Asia (Van den Hurk et al., 2009), and they breed in a wide range of aquatic habitats (Stoops et al., 2008). The common breeding habitats of these vector mosquitoes are rice fields, furrow pits, puddles, cisterns, and permanent and transient ground pools. Of these, the most important breeding habitat is the rice field ecosystem (Mogi, 1978; Victor and Reuben, 1999), and their population abundance is closely related to rice agroecosystems (Keiser et al., 2005; Lacey and Lacey, 1990; Takagi et al., 1995; Takagi et al., 1997).

In Vietnam, JE has been recognized as an important public health problem since 1951 (Nguyen and Nguyen, 1995; Okuno, 1978). *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui* complex, and *Cx. quinquefasciatus* Say are the JE vectors distributed in this country (Kuwata et al., 2013; Nguyen et al., 1974; Ohba et al., 2011). A previous study (Hasegawa et al., 2008) showed that the proximity of hosts to breeding sites in a rice-cultivating village in northern Vietnam positively affected the abundance of *Cx. gelidus*, but not of *Cx. vishnui* complex.. However, very few integrated studies of the breeding habitats of mosquitoes in rice agrosystems, including JE vectors, have been conducted in Vietnam.

Wetlands, including rice fields, have a wide variety of natural enemies of mosquitoes (Mogi, 2007; Ohba and Nakasuji, 2006; Sunahara et al., 2002). The impact of natural enemies on *Cx. tritaeniorhynchus* larvae was determined in several ecological studies in rice agroecosystems (Mogi, 1993; Mogi and Miyagi, 1990; Takagi et al., 1996). Fish, aquatic insects, and spiders are predators of *Cx. tritaeniorhynchus* (Mogi, 2007; Watanabe et al., 1968), and these predators are expected to contribute to the integrated vector control management.

In the present study, we conducted an ecological study on mosquito fauna and its potential predators associated with rice agrosystems in six rice production areas around Hanoi in northern Vietnam, during the rainy and dry season of 2009.

2. Materials and methods

2.1 Study site

We conducted this study from June 29 to July 1, 2009 (rainy season) and from October 19 to 21, 2009 (dry season, after the rice-harvesting period) in six districts around Hanoi in northern Vietnam (Table S1; Fig. 1). Farmers cultivate rice two times a year in these areas, February to May and June to September. Rice fields and adjoining aquatic habitats such as ponds (water depth > 1 m), rice nurseries, wetlands (water depth < 1 m), ditches (width < 1 m), and irrigation canal (width > 1 m) were selected as potential mosquito breeding sites (Fig. S1). The aquatic habitats for sampling in each district were selected based on the relative area of each aquatic habitat.

2.2 Sampling methods

We monitored the abundance of mosquito larvae and other insects by a dipping method as described in Ohba et al. (2011). The dipper used for collection was 12 cm in diameter and 5 cm deep. We collected one sample from each study plot; each sample consisted of 30 dips made at 30 points at more than 1 m intervals. If it was not possible to collect 30 dips due to the small size of an aquatic habitat, we made 10 or 20 dips and converted them to 30 dips. By using this method alone, we may have missed a number of predatory insects and fish resting on foliage. We stored and studied all the samples at the laboratory of the National Institute of Hygiene and Epidemiology in Hanoi. Insects, excluding mosquito larvae, were identified to the order, family, or genus level using a binocular. Based on the literature (Mogi, 2007; Shaalan and Canyon, 2009), potential predators were classified into invertebrate (Coleoptera, Hemiptera, and Odonata) and vertebrate predators (fish and anuran larvae).

We preserved the collected mosquito larvae in 70% ethanol until identification, and identified all larvae, excluding damaged and/or first- to third-instar larvae, using taxonomic keys (Rattanarithikul et al., 2005a; Rattanarithikul et al., 2006a; Rattanarithikul et al., 2005b; Rattanarithikul et al., 2006b). Pupae were reared until emergence for species identification. Larvae smaller than the fourth instar were identified to the genus level and counted. Because adults emerged from pupae of *Cx. vishnui* and *Cx. pseudovishnui* were difficult to identify certainty, they were categorized as *Culex vishnui* complex.

2.3 Data analysis

Similar to previous studies (Yasuoka and Levins, 2007; Yasuoka et al., 2006), niche width was calculated for each species using the formula: niche width = number of habitat types at a species collection site divided by six, which is the total number of habitat types included in this study. The knowledge of the environmental factors affecting mosquito abundance in main aquatic habitats in rice ecosystems (rice fields, ponds, and ditches) is indispensable for future mosquito management, because rice ecosystems are the most common and widespread wetlands in Vietnamese agroecosystems. We used a general linear model (GLM) with negative binomial ("glm.nb" package) in R version 2.12.1 (R Development Core Team, 2011) to determine mosquito abundance in rice ecosystems. Season (dry or rainy), predator abundance, and dominant aquatic habitats in rice ecosystems (rice fields, ponds, and ditches) (Zuur et al., 2009) were the factors used in the analysis. The forward stepwise method using the stepAIC package was used for model selection. In addition, the occurrence of *Culex* species was analyzed using the GLM with a binomial distribution, including season (dry or rainy) and dominant aquatic habitats (rice fields, ponds, and ditches) as explanatory variables. Because all sampling sites could not be sampled twice (dry and rainy season), each sampling site was not incorporated as random effect in the statistical model. In this analysis, *Culex vishnui* was included in *Cx. vishnui* complex.

3. Results

3.1 Mosquito abundance in each aquatic habitat

In the rainy season, 1780 *Culex* individuals (including 254 *Cx. tritaeniorhynchus*, 113 *Cx. vishnui*, 58 *Cx. vishnui* complex., 12 *Cx. gelidus,* 1 *Cx. bitaeniorhynchus*, and 1 *Cx. fuscocephala*) and 148 *Anopheles* individuals (including 5 *An. vagus*) were collected (Table 1). *Cx. tritaeniorhynchus* occurred in rice fields, ditches, ponds, and wetlands. *Cx. vishnui* was found in rice fields, ditches, and ponds. In contrast, *Cx. gelidus* and *Cx. fuscocephala* were present in ditch habitats, but not in rice fields. The total number of mosquito larvae was higher in ponds, rice fields, and ditches, but a few were present in other aquatic habitats.

In the dry season, we collected 176 *Culex* individuals (including 33 *Cx. vishnui*, 24 *Cx. tritaeniorhynchus*, 8 *Cx. vishnui* complex, and 1 *Cx. gelidus*), 186 *Anopheles* individuals (including 9 *An. tessellatus*, 2 *An. kochi*, and 2 *An. barbumbrosus*), and 1 *Mimomyia chamberlaini* individual (Table 1). The presence of *Cx. vishnui* was confirmed in rice fields, and irrigation canal. *Cx. tritaeniorhynchus* was present in all aquatic habitats. In contrast, *Cx. gelidus* was found in ditches, whereas *Cx. fuscocephala* was not found in any of the habitats. The number of mosquito larvae per site was higher in irrigation canal, wetlands, ponds, and a few mosquito larvae were present in rice fields and ditches, which had high mosquito density during the rainy season.

For each district, *Cx. tritaeniorhynchus* and *Cx. vishnui* were collected from all six rice production areas, and *Cx. tritaeniorhynchus, Cx. vishnui, Cx. fuscocephala*, and *Cx. gelidus* were found in Dan Phuong district (Table 2). In irrigation canal habitat in Cat Que district, we could not confirm the presence of mosquito larvae during the rainy season (period of high stream velocity), but we found 21 *Cx. vishnui* and 6 *Cx. tritaeniorhynchus* individuals among discarded garbage during the dry season (period of low stream velocity) (Fig. 2).

3.2 Mosquito abundance in rice ecosystems

Stepwise GLMs (with negative binomial) revealed that the mosquito abundance in rice fields was affected by the season but not by predators (Season, z = 2.99, P = 0.003; Predator, z = 1.65, P = 0.10). Aquatic habitats were excluded from the first model in the stepwise process.

The occurrence of *Cx. tritaeniorhynchus* showed no correlation with the season but with the type of the aquatic habitat. The occurrence in rice fields was the highest of the three aquatic habitats (Table 3 and 4). For *Cx. vishnui* complex., both the season and type of aquatic habitat were significant; especially, their abundance in rice fields was the highest of the three aquatic habitats. In *Cx. gelidus*, all explanatory variables were not significant, whereas in *Cx. fuscocephala*, the abundance could not be analyzed using GLM because of the small sample size.

3.3 Potential mosquito predators

During the rainy season, we collected 438 Hemiptera, 137 Coleoptera, and 13 Odonata nymphs from all sampled habitats (Table 5). Pooling the samples collected from the six study districts, we showed that Veliidae (total abundance = 158, niche width = 0.50) and *Micronecta* (141 and 0.50, respectively) were widely distributed, but Pleidae (87 and 0.17, respectively) had a narrow distribution. Compared to Hemiptera, Coleoptera (total abundance = 83 for Dytiscidae and 54 for Hydrophilidae) was not highly abundant but were widely distributed (niche width = 0.33–0.50). Similarly, Odonata and fish were less abundant than Hemiptera but occupied a wider niche (0.33–0.67). The number of natural predators per site was the highest in ponds (12.7), followed by rice fields (11.8), ditches (8.3), and irrigation canal (2.0). No predator was found in the wetlands.

In the dry season, 117 Hemiptera, 68 Coleoptera, and 6 Odonata individuals were collected from all sampled habitats (Table 5). Veliidae (total abundance = 33, niche width = 0.67) and *Micronecta* (14 and 0.50, respectively) were abundant, but Pleidae (51 and 0.17, respectively) again showed a narrow distribution. Coleoptera consisted of individuals in Dytiscidae (total abundance = 37, niche width = 0.17 for larvae and 0.33 for adults) and Hydrophilidae (total abundance = 31, niche width = 0.33 for larvae and 0.50 for adults). Odonata consisted of individuals in Anisoptera (total abundance = 6, niche width = 0.50). Fish (7 and 0.33, respectively) were less abundant in the dry season than in the rainy season (12 and 0.67, respectively). The number of natural predators per site was the highest in ponds (total abundance = 9.3), followed by rice fields (4.1), ditches (2.8), rice nurseries (2.0), and wetlands (0.5). No predator was found in the irrigation canal.

4. Discussion

In this study, we showed the presence of *Cx. tritaeniorhynchus, Cx. vishnui* complex., *Cx. fuscocephala*, and *Cx. gelidus* in rice agroecosystems in northern Vietnam (Table 1). The results are similar to reports from other Asian countries (Gingrich et al., 1992; Reuben et al., 1994; Stoops et al., 2008; Takagi et al., 1997; Van Peenten et al., 1975; Vythilingam et al., 1997). *Cx. tritaeniorhynchus* was present in most aquatic habitats, with the largest niche width in both the rainy and dry season, followed by *Cx. vishnui* complex. The findings of these two species are of particular interest because these species are actually the carriers of JE virus in Vietnam (Kuwata et al., 2013).

The number of *Cx. gelidus* was low in ponds in the rainy season and in ditches in the dry season. In Malaysia, the species breeds in ditches, drains, small streams, ponds, temporary ground pools, artificial containers, and rice fields (Gould et al., 1962). In northern Vietnam, this was the most dominant species based on a 10-day-long collection survey of adults in June 2003 (Hasegawa et al., 2008). The number of *Cx. gelidus* adults is positively affected by proximity to the breeding sites and number of cattle hosts (Hasegawa et al., 2008). The abundance of *Cx. gelidus* indicates that our sampling sites as its breeding ground might be far from the cattle hosts.

Cx. fuscocephala was present in ponds in the rainy season and absent in dry season. Our previous study also did not find this species in rice fields in southern Vietnam (Ohba et al., 2011). However, this species was found in rice fields in Sri Lanka (Yasuoka and Levins, 2007; Yasuoka et al., 2006), Indonesia (Stoops et al., 2008), and the Philippines (Mogi and Miyagi, 1990). According to the present study and other reports for Vietnam (Hasegawa et al., 2008; Ohba et al., 2011), *Cx. fuscocephala* may be a rare species in this country's fauna.

Contrary to our previous report for southern Vietnam (Ohba et al., 2011), the abundance of mosquitoes was lower in northern Vietnam during both the seasons. However, JE virus was isolated from *Cx. tritaeniorhynchus* and *Cx. vishnui* complex. collected in Vietnam during the 2006–2008 surveys (Kuwata et al., 2013). Mosquito control efforts in rice ecosystems are necessary to reduce the risk and burden of JE during the rainy season, because mosquito abundance increases in the rainy season in northern Vietnam (Table 3). Hemiptera, including *Micronecta*, Veliidae, and Pleidae, were abundant in both the rainy and dry seasons (Table 5), similar to that in southern Vietnam (Ohba et al., 2011). Hemiptera feed on mosquito larvae (Mogi, 2007; Shaalan and Canyon, 2009). Although we did not confirm whether these hemipterans feed on mosquitoes and no significant relationship was found between their number and the mosquito abundance in this study, it is likely that they eat mosquito larvae. Predatory ability must be determined in each predator species in future studies, since these predators will be expected to contribute to the mosquito integrated vector control management.

Mosquito larvae in the irrigation canal were found only during the dry season (Table 1), which may be explained by the low river flow during a dry season. The residents routinely dispose garbage in the irrigation canal, which then accumulates due to slow stream flow creating pools of stagnant water in the irrigation canal as breeding ground for mosquitoes (Fig. 2). This indicates the need to educate the residents about proper garbage disposal and public health. These results further deepen our understanding of mosquito ecology and strengthen mosquito control strategies to be applied in rice ecosystems in Vietnam in the future.

Acknowledgments

We thank the entomological staff of the National Institute of Hygiene and Epidemiology. This study was partially supported by KAKENHI 19256002, the Japan Society for the Promotion of Science (JSPS), the joint research fund between Nagasaki University and Sumitomo Chemical (Osaka, Japan), and the Ministry of Education, Culture, Sports, Science and Technology (MEXT) to M.T.

References

Gingrich, J.B., Nisalak, A., Latendresse, J.R., Sattabongkot, J., Hoke, C.H., Pomsdhit, J., Chantalakana, C., Satayaphanta, C., Uechiewcharnkit, K., Innis, B.L., 1992. Japanese encephalitis virus in Bangkok: factors influencing vector infections in three suburban communities. J. Med. Entomol. 29, 436-444.

Gould, D.J., Barnett, H.C., Suyemoto, W., 1962. Transmission of Japanese encephalitis virus by *Culex gelidus* Thobald. Trans. R. Soc. Trop. Med. Hyg. 56, 429-435.

Hasegawa, M., Tuno, N., Nguyen, T.Y., Vu, S.N., Takagi, M., 2008. Influence of the distribution of host species on adult abundance of Japanese encephalitis vectors - *Culex vishnui* subgroup and *Culex gelidus* - in a rice-cultivating village in Northern Vietnam. Am. J. Trop. Med. Hyg. 78, 159-168.

Keiser, J., Maltese, M.F., Erlanger, T.E., Bos, R., Tanner, M., Singer, B.H., Utzinger, J., 2005. Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. Acta Trop. 95, 40-57.

Kuwata, R., Nga, P.T., Yen, N.T., Hoshino, K., Isawa, H., Higa, Y., Hoang, N.V., Trang, B.M., Phong, T.V., Sasaki, T., 2013. Surveillance of Japanese encephalitis virus infection in mosquitoes in Vietnam from 2006 to 2008. Am. J. Trop. Med. Hyg. 88, 681-688.

Lacey, L., Lacey, C., 1990. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. J. Am. Mosq. Cont. Assoc. Supplement 2, 1-93.

Mogi, M., 1978. Population studies on mosquitoes in the rice field area of Nagasaki, Japan, especially on *Culex tritaeniorhynchus*. Trop. Med. 20, 173-263.

Mogi, M., 1993. Effect of intermittent irrigation on mosquitoes (Diptera: Culicidae) and larvivorous predators in rice fields. J. Med. Entomol. 30, 309-319.

Mogi, M., 2007. Insects and other invertebrate predators. J. Am. Mosq. Control Assoc. 23, 93-109.

Mogi, M., Miyagi, I., 1990. Colonization of rice fields by mosquitoes (Diptera: Culicidae) and larvivorous predators in asynchronous rice cultivation areas in the Philippines. J. Med. Entomol. 27, 530-536.

Nguyen, H., Nguyen, T., 1995. Japanese encephalitis in Vietnam 1985-1993. Southeast Asian J. Trop. Med. Public Health 26, 47-50.

Nguyen, T.K.T., Vien, N.T., Mai, T.T., Nguyen, T.N.X., 1974. Japanese encephalitis vectors: isolation of virus from culicine mosquitoes in the Saigon area. Southeast Asian J. Trop. Med. Public Health 5, 408.

Ohba, S., Nakasuji, F., 2006. Dietary items of predacious aquatic bugs (Nepoidea: Heteroptera) in Japanese wetlands. Limnology 7, 41-43.

Ohba, S., Trang Huynh, T.T., Loan Luu, L., Tran Ngoc, H., Le Hoang, S., Takagi, M., 2011. Mosquitoes and their potential predators in rice agroecosystems of the Mekong Delta, Southern Vietnam. J. Am. Mosq. Control Assoc. 27, 384-392.

Okuno, T., 1978. An epidemiological review of Japanese encephalitis. World Health Statistics Quarterly 31, 120-133.

R Development Core Team, 2011. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Jones, W.J., Coleman, R.E., 2005a. Illustrated keys to the mosquitoes of Thailand II. Genera *Culex* and *Lutzia*. Southeast Asian J. Trop. Med. Public Health 36, Supplment 2, 1-97.

Rattanarithikul, R., Harrison, B.A., Harbach, R.E., Panthusiri, P., Coleman, R.E., 2006a. Illustrated keys to the mosquitoes of Thailand IV. *Anopheles*. Southeast Asian J. Trop. Med. Public Health 37, Supplement 2, 1-128.

Rattanarithikul, R., Harrison, B.A., Panthusiri, P., Coleman, R.E., 2005b. Illustrated keys to the mosquitoes of Thailand I. Background; geographic distribution; Lists of genera, subgenera, and species; and a key to the genera. Southeast Asian J. Trop. Med. Public Health 36, Supplement 1, 1-80.

Rattanarithikul, R., Harrison, B.A., Panthusiri, P., Peyton, E.L., Coleman, R.E., 2006b. Illuistrated keys to the mosquitoes of Thailand III. Genera *Aedeomyia*, *Ficalbia*, *Mimomyia*, *Hodgesia*, *Coquillettidia*, *Mansonia*, and *Uranotaenia*. Southeast Asian J. Trop. Med. Public Health 37, Supplement 1, 1-85.

Reuben, R., Tewari, S., Hiriyan, J., Akiyama, J., 1994. Illustrated keys to species of *Culex* (*Culex*) associated with Japanese encephalitis in Southeast Asia (Diptera: Culicidae). Mosquito Systematics 26, 75-96.

Shaalan, E.A.S., Canyon, D.V., 2009. Aquatic insect predators and mosquito control. Trop. Biomed. 26, 223-261.

Solomon, T., Ni, H., Beasley, D.W.C., Ekkelenkamp, M., Cardosa, M.J., Barrett, A.D.T., 2003. Origin and evolution of Japanese encephalitis virus in southeast Asia. J. Virol. 77, 3091.

Stoops, C.A., Gionar, Y.R., Shinta, Sismadi, P., Rusmiarto, S., Susapto, D., Rachmat, A., Elyazar, I.F., Sukowati, S., 2008. Larval collection records of *Culex* species (Diptera: Culicidae) with an emphasis on Japanese encephalitis vectors in rice fields in Sukabumi, West Java, Indonesia. J. Vector Ecol. 33, 216-217.

Sunahara, T., Ishizaka, K., Mogi, M., 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. J. Vector Ecol. 27, 8-20.

Takagi, M., Sugiyama, A., Maruyama, K., 1995. Effect of rice culturing practices on seasonal occurrence of *Culex tritaeniorhynchus* (Diptera: Culicidae) immatures in three different types of rice-growing areas in central Japan. J. Med. Entomol. 32, 112-118.

Takagi, M., Sugiyama, A., Maruyama, K., 1996. Survival of newly emerged *Culex tritaeniorhynchus* (Diptera: Culicidae) adults in field cages with or without predators. J. Med. Entomol. 33, 698-701.

Takagi, M., Suwonkerd, W., Tsuda, Y., Sugiyama, A., Wada, Y., 1997. Effects of rice culture practices on the abundance of *Culex* mosquitoes (Diptera: Culicidae) in northern Thailand. J. Med. Entomol. 34, 272-276.

Van den Hurk, A.F., Ritchie, S.A., Mackenzie, J.S., 2009. Ecology and geographical expansion of Japanese encephalitis virus. Annu. Rev. Entomol. 54, 17-35.

Van Peenten, P.F.D., Joseph, P.L., Atmosoedjono, S., Irsiana, R., Saroso, J.S., 1975. Isolation of Japanese encephalitis virus from mosquitoes near Bogor, West Java, Indonesia. J. Med. Entomol. 12, 573-574.

Victor, T.J., Reuben, R., 1999. Population dynamics of mosquito immatures and the succession in abundance of aquatic insects in rice fields in Madurai, south India. Indian J. Malariol. 36, 19-32.

Vythilingam, I., Oda, K., Mahadevan, S., Abdullah, G., Thim, C.S., Hong, C.C., Vijayamalar, B., Sinniah, M., Igarashi, A., 1997. Abundance, parity, and Japanese encephalitis virus infection of mosquitoes (Diptera: Culicidae) in Sepang District, Malaysia. J. Med. Entomol. 34, 257-262.

Watanabe, M., Wada, Y., Itano, K., Suguri, S., 1968. Studies on predators of larvae of *Culex tritaeniorhyncus summorosus* Dyar. Jpn. J. Sanit. Zool. 19, 35-38.

Yasuoka, J., Levins, R., 2007. Ecology of vector mosquitoes in Sri Lanka - suggestions for future mosquito control in rice ecosystems. Southeast Asian J. Trop. Med. Public Health 38, 646-657.

Yasuoka, J., Levins, R., Mangione, T.W., Spielman, A., 2006. Community-based rice ecosystem management for suppressing vector anophelines in Sri Lanka. Trans. R. Soc. Trop. Med. Hyg. 100, 995-1006.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R: Springer Verlag. pp. 574.

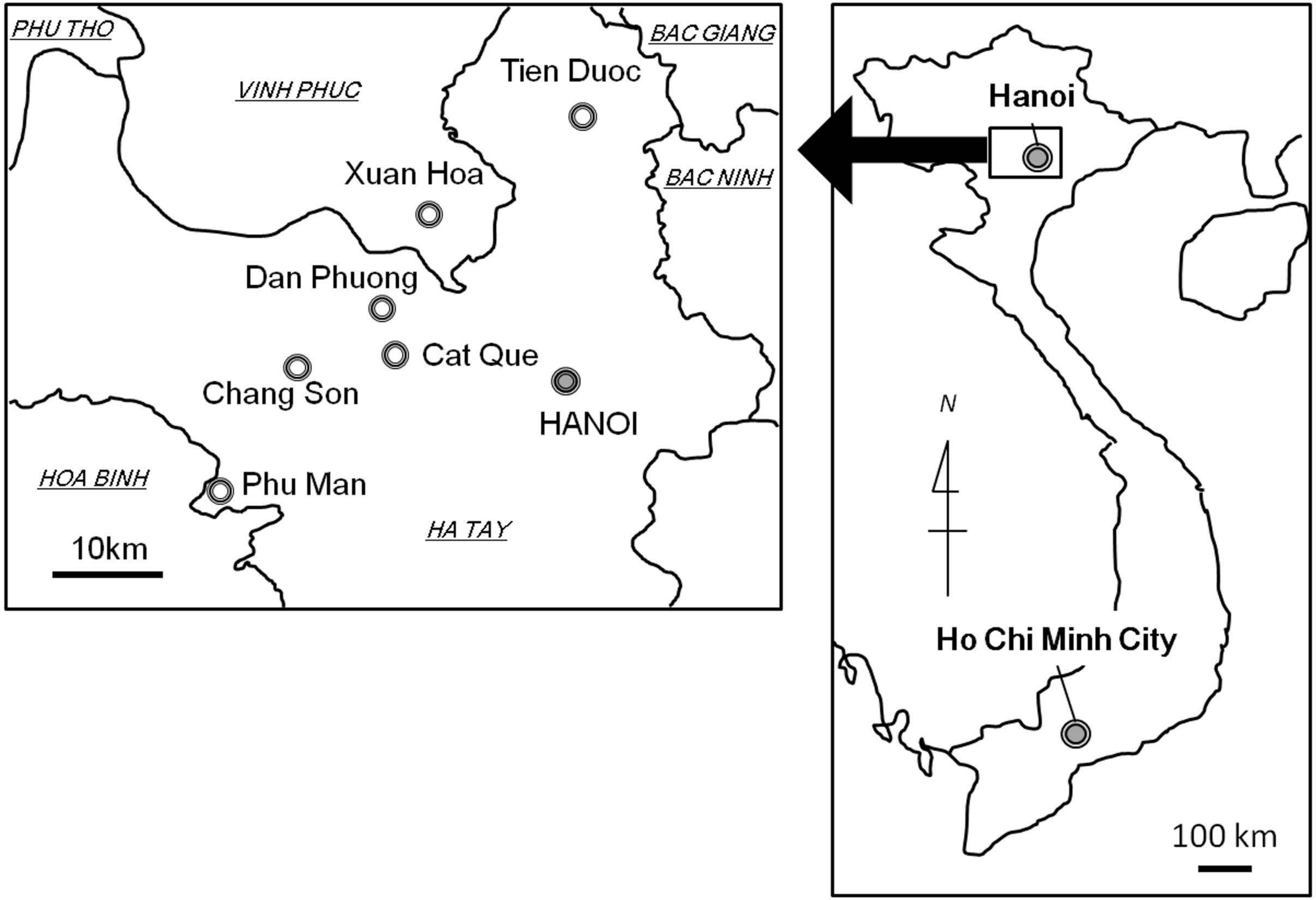
Figure Legend

Fig. 1. Map showing the study sites (districts and towns) in southern Vietnam.

Fig. 2. An irrigation canal in the Cat Que commune in northern Vietnam.

Fig. S1. Aquatic habitats in rice ecosystems in northern Vietnam.

Fig. 1



PHU THO
VINH PHUC
BAC GIANG
Tien Duoc
Xuan Hoa
BAC NINH
Dan Phuong
Cat Que
Chang Son
HANOI
HOA BINH
Phu Man
HA TAY
10km
Hanoi
N
Ho Chi Minh City
100 km

Fig. 2

Fig. S1


Rice field
Pond
Irrigation canal
Wetland
Rice nursery
Ditch

Table 1. List of mosquitoes collected by 30dips in each aquatic habitat in July 2009 (rainy season) and October 2009 (dry season).

Season	Mosquito	Rice field	Ditch	Pond	Wetland	Irrigation canal	Rice nursery	Total	Nitch width
Rainy	***Culex tritaeniorhynchus***	231	2	3	18	-	-	254	0.67
	Culex vishnui	77	11	25	-	-	-	113	0.50
	***Culex vishnui* complex**	58	-	-	-	-	-	58	0.50
	Culex gelidus	-	-	12	-	-	-	12	0.17
	Anopheles vagus	5	-	-	-	-	-	5	0.17
	Culex bitaeniorhynchus	1	-	-	-	-	-	1	0.17
	Culex fuscocephala	-	-	1	-	-	-	1	0.17
	Mimomyia chamberlaini	-	-	1	-	-	-	1	0.17
	Mansonia annulifera	-	-	1	-	-	-	1	0.17
	Culex spp.	537	35	761	8	-	-	1341	0.67
	Anopheles spp.	120	15	8	-	-	-	143	0.50
	Not identified	93	119	1				213	0.50
	Total	1122	182	813	26	0	0	2143	
	No. / site	34.0	16.5	81.3	8.7	0.0	0.0		
Dry	***Culex vishnui***	12	-	-	-	21	-	33	0.50
	Culex tritaeniorhynchus	3	3	9	1	6	2	24	1.00
	Anopheles tessellatus	9	-	-	-	-	-	9	0.17
	***Culex vishnui* complex**	5	-	-	2	1	-	8	0.50
	Anopheles kochi	2	-	-	-	-	-	2	0.17

Anopheles barbumbrosus	-	-	1	-	-	1	2	0.33
Culex gelidus	-	1	-	-	-	-	1	0.17
Anopheles spp.	67	50	46	10	-	-	173	0.67
Culex spp.	9	13	63	24	-	2	111	0.83
Lutzia sp.	-	-	-	-	1	-	1	0.17
Not identified	28	-	1	-	3	-	32	0.50
Total	123	67	120	37	11	5	363	
No. / site	6.8	6.7	12.0	18.5	11.0	5.0		

Table 2. Mosquitoes from 6 rice prduction areas, in northern Vietnam

Species	Area						
	Chang Son	Dan Phuong	Cat Que	Phu Man	Tien Quoc	Xuan Hoa	Total
Cx. tritaeniorhynchus	12	15	53	11	118	5	214
Cx. vishnui	3	24	23	63	15	3	131
Cx. vishnui complex	5	-	5	7	38	-	55
Cx. gelidus	-	12	-	-	-	-	12
Cx. bitaeniorhynchus	-	-	-	-	1	-	1
Cx. fuscocephala	-	1	-	-	-	-	1
An. barbumbrosus	-	-	-	-	-	1	1
An. indefinitus	-	-	-	-	1	-	1
An. kochi	-	-	-	2	-	-	2
An. tessellatus	-	-	-	14	-	-	14
An. vagus	-	-	-	-	1	-	1
Mi. chamberlaini	-	1	-	-	-	-	1
Ma. annulifera	-	1	-	-	-	-	1
Total	20	52	81	81	172	8	414

Table 3. Positive proportion in *Culex* species in rice agrosystems in northern Vietnam.

			Species			
Season	Site	No. total sites	*Cx. tritaeniorhynchus*	*Cx. vishnui* complex	*Cx. gelidus*	*Cx. fuscocephala*
Rainy	Rice field	33	33.3	60.6	-	-
	Ditch	11	9.1	18.2	-	-
	Pond	10	10.0	20.0	10.0	10.0
Dry	Rice field	18	5.6	22.2	-	-
	Ditch	10	20.0	-	10.0	-
	Pond	10	20.0	-	-	-

Table 4. GLM results for the mosquito abundance in rice agroecosystems.

Species	Source	Parameter estimate	S.E.	*Z*	*P*	
Cx. tritaeniorhynchus	(Intercept)	-3.06	0.727	-4.21	0.000	***
	Season/rainy	0.42	0.574	0.73	0.464	
	Aquatic/pond	0.00	0.843	0.00	1.000	
	Aquatic/rice field	**1.60**	**0.682**	**2.35**	**0.019**	*****
Cx. vishnui complex	(Intercept)	-4.67	0.933	-5.01	0.000	***
	Season/rainy	**1.84**	**0.642**	**2.86**	**0.004**	******
	Aquatic/pond	0.00	1.027	0.00	1.000	
	Aquatic/rice field	**3.31**	**0.797**	**4.15**	**0.000**	*******
Cx. gelidus	(Intercept)	-3.56	1.241	-2.87	0.004	**
	Season/rainy	-0.62	1.430	-0.43	0.665	
	Aquatic/pond	0.00	1.430	0.00	1.000	
	Aquatic/rice field	-19.08	8,324.000	0.00	0.998	

Bold type factors are significant ($P < 0.05$).

Table 5. List of potential mosquito predators collected by 30dips in each aquatic habitat in July 2009 (rainy season) and October 2009 (dry season).

Season	Order	Family or genus	Rice field	Ditch	Pond	Wetland	Irrigation canal	Rice nursery	Total	Nitch width
Rainy	Hemiptera	Geriidae	-	-	1	-	-	-	1	0.17
		Veliidae	153	4	1	-	-	-	158	0.50
		Anisops spp.	13	1	7	-	-	-	21	0.50
		Notonecta spp.	3	9	13	-	-	-	25	0.50
		Pleidae	-	-	87	-	-	-	87	0.17
		Micronecta spp.	71	63	7	-	-	-	141	0.50
		Diplonychus spp.	1	-	3	-	-	-	4	0.33
		Sigara spp.	1	-	-	-	-	-	1	0.17
	Coleoptera	Hydrophilidae adult	30	-	-	-	-	2	32	0.33
		Hydrophilidae larva	21	1	-	-	-	-	22	0.33
		Dytiscidae adult	61	1	2	-	-	-	64	0.50
		Dytiscidae larva	12	6	1	-	-	-	19	0.50
	Odonata	Zygoptera	4	1	-	-	-	-	5	0.33
		Anisoptera	3	4	1	-	-	-	8	0.50
	Vertebrate	Fish	5	1	4	-	2	-	12	0.67
		Tadpole	11	-	-	-	-	-	11	0.17
		Total	389	91	127	0	2	2		
		No. / site	11.8	8.3	12.7	0.0	2.0	2.0		
Dry	Hemiptera	Geriidae	-	-	1	-	-	-	1	0.17
		Veliidae	21	8	3	1	-	-	33	0.67
		Anisops spp.	-	-	6	-	-	-	6	0.17

	Pleidae	-	-	51	-	-	-	51	0.17
	Micronecta spp.	6	1	7	-	-	-	14	0.50
	Diplonychus spp.	-	-	7	-	-	-	7	0.17
	Sigara spp.	5	-	-	-	-	-	5	0.17
Coleoptera	Hydrophilidae adult	3	-	4	-	-	2	9	0.50
	Hydrophilidae larva	21	1	-	-	-	-	22	0.33
	Dytiscidae adult	-	16	6	-	-	-	22	0.33
	Dytiscidae larva	15	-	-	-	-	-	15	0.17
Odonata	Anisoptera	3	1	2	-	-	-	6	0.50
Vertebrate	Fish	-	1	6	-	-	-	7	0.33
	Total	74	28	93	1	0	2		
	No. / site	4.1	2.8	9.3	0.5	0.0	2.0		

Table S1. Mosquito sampling site in dry and rainy 2009 in northern Vietnam

Season	Commune	Rice field	Ditch	Pond	Wetland	Irrigation canal	Rice nursery	Total
Rainy season	Chang Son	6	2	2	1	-	-	11
	Dan Phuong	5	2	2	-	-	1	10
	Cat Que	6	3	-	1	1	-	11
	Phu Man	5	1	2	-	-	-	8
	Tien Quoc	5	1	3	-	-	-	9
	Xuan Hoa	6	2	1	1	-	-	10
		33	11	10	3	1	1	59

Season	Commune	Rice field	Ditch	Pond	Wetland	River	Rice nursery	Total
Dry season	Chang Son	-	1	2	1	-	-	4
	Dan Phuong	-	2	2	-	-	1	5
	Cat Que	4	3	-	-	1	-	8
	Phu Man	5	1	2	-	-	-	8
	Tien Quoc	5	1	3	-	-	-	9
	Xuan Hoa	4	2	1	1	-	-	8
		18	10	10	2	1	1	42