



Analysis of relationship between anopheles subpictus larval densities and environmental parameters using remotote sensing (rs), a global positioning system (gps) and a...

Anno, Sumiko ; Takagi, Masahiro ; Tsuda, Yoshio ; Yotopranoto, Subagyo ; Dachlan, Yoes Prijatna ; Bendryman, Sri Subekti ; Ono, Masaji ;...

(Citation)

The Kobe journal of the medical sciences, 46(6):231-243

(Issue Date)

2000-12

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(URL)

<https://hdl.handle.net/20.500.14094/E0001062>



**ANALYSIS OF RELATIONSHIP BETWEEN *ANOPHELES SUBPICTUS*
LARVAL DENSITIES AND ENVIRONMENTAL PARAMETERS USING
REMOTE SENSING (RS), A GLOBAL POSITIONING SYSTEM (GPS) AND
A GEOGRAPHIC INFORMATION SYSTEM (GIS)**

Sumiko ANNO*, Masahiro TAKAGI**, Yoshio TSUDA**,
Subagyo YOTOPRANOTO***, Yoes Prijatna DACHLAN****,
Sri Subekti BENDRYMAN****, Masaji ONO*****, and Masato KAWABATA*

* International Center for Medical Research,
Kobe University School of Medicine

** Department of Medical Entomology,
Institute of Tropical Medicine, Nagasaki University,
1-12-4 Sakamoto, Nagasaki 852-8523 Japan

*** Department of Parasitology,
Faculty of Medicine, Airlangga University,
Jl. Dharmahasada 47, Surabaya, Indonesia

**** Department of Helminthology,
Faculty of Veterinary Medicine, Airlangga University,
Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia

***** Environmental Epidemiology Section,
Environmental Health Sciences Division,
National Institute for Environmental Studies,
16-2 Onogawa, Tsukuba-Shi, Ibaraki 305-0053 Japan

KEY WORDS

Remote Sensing (RS); Global Positioning Systems (GPS); Geographic Information
Systems (GIS); *Anopheles subpictus*

Received for publication: September 18, 2000

ABSTRACT

Remote Sensing (RS), a Global Positioning System (GPS) and a Geographic Information System (GIS) were used to analyze relationship between *Anopheles subpictus* larval densities and environmental parameters in the Sekotong district on Lombok Island, Indonesia. Distance from the coast to larval habitats, season and surface water were considered as environmental parameters for determining *An. subpictus* larval densities. Japanese Earth Resources Satellite (JERS) Visible and Near Infrared Radiometer (VNIR) satellite imagery for the area acquired by National Space Development Agency of Japan (NASDA) were used to detect water, which could be used to characterize larval habitats. Data on larval sampling sites obtained from a GPS were entered into a GIS for mapping larval habitats to measure distance between the coast and the larval habitats. A GIS was used for overlaying of data coverages (i.e., water distribution from RS data and larval habitats coupled with data on larval densities) to identify factors that may explain the spatial distribution patterns of larval densities. *An. subpictus* larval densities were significantly associated with season and distance from the coast to larval habitats. The rainy season and the distance from the coast to larval habitats were critical environmental determinants for presence of *An. subpictus* larvae in the study. In this paper, we investigated relationship between *An. subpictus* larval densities and the environmental parameters using RS/GPS/GIS to determine if these tools could be used to predict larval densities.

INTRODUCTION

World Health Organization (WHO) revised a strategy for malaria control at the 31st World Health Assembly in 1978.²⁴⁾ A key element of the WHO strategy was the development of new methods for attacking malaria through vector control, directed at larval populations. Most larval habitats can be described in terms of environmental parameters such as emergent vegetation, precipitation and surface water. Successful larval control could depend on the ability to identify larval habitats and distinguish between high- and low-producing sites in a timely manner.²³⁾

Remote Sensing (RS) is the technique of obtaining information about an object collected by a sensor that is mounted on satellites without actually being in contact with the object. RS systems measure electromagnetic energy generated by electromagnetic radiation from a source through space, and reflected from the object to the sensor. In

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICTUS* ANALYSIS

situ data need to be collected to calibrate and interpret remotely sensed data. Remotely sensed images (data) are usually represented as a two-dimensional array of squares (pixels) on the computer screen. Pixel data can be related directly to features on the ground. The information as RS data can be useful for identifying larval, vector and host habitats and generating risk maps that can be used in control programs. The applications of RS to vector-borne diseases were published.^{4, 11, 13, 14, 15, 17, 20)}

A Global Positioning System (GPS) receives radio waves transmitted from the satellites. By receiving signals from multiple satellites, and through a process of triangulation, the GPS can be calculated with a high degree of accuracy and precision. Position is calculated based on distance measures to satellites, which are based on the travel time of the radio signals from the satellites to a GPS. A GPS, which provides accurate locations, is used to map households and other features of interest in malaria surveillance.⁵⁾

A Geographic Information System (GIS) is a computer-based system for automating, storing, manipulating and displaying mapped information and data. It includes spatial data (locations) in the form of geographic coverages (maps) and descriptive data (attributes) in the form of a relational database associated with the mapped features. GIS technologies are increasingly applied to studies of vector-borne diseases. For malaria, a GIS was used for associating RS data with ground-verified habitat information to identify factors that may explain the spatial distribution patterns of disease.^{2, 7, 21)} Therefore, the applications of RS/GPS/GIS can help identify sites with high larval densities and provide a useful tool for malaria control programs.¹⁶⁾

Our team conducted research on *Anopheles subpictus* considered as a main vector for malaria transmission on Lombok Island, Indonesia.²⁶⁾ This study focused on understanding the ecology of *An. subpictus* and identifying the environmental parameters associated with *An. subpictus* larval densities. Findings revealed that stagnant surface water with low (0 to 29%) salinity in coastal areas during the early rainy season (October through December) provides optimal *An. subpictus* larval habitats, and thereby high larval densities are produced. In the early rainy season, the salinity of surface water becomes lower than that in the dry season while water is still stagnant. This situation persists until heavy rain flushes away mosquito larvae. *An. subpictus* larval production begins in October and reaches its peak in December. Other environmental determinants associated with high densities of *An. subpictus* larvae were irrigated rice fields, lagoons, fishponds, mangrove creeks, and river mouths, all of which were characterized by open/surface water.^{6, 9, 26)}

An. subpictus larval abundance and production in the Sekotong district of Lombok

Island may be influenced by field location, season and surface water. A major objective of this study was to investigate relationship between *An. subpictus* larval densities and the environmental parameters using RS/GPS/GIS to determine if these tools could be used to predict the larval densities.

MATERIAL AND METHODS

Study site

Lombok Barat regency, Indonesia, which lies 38 km east of Bali, is a malaria endemic area with around 12% slide positivity rate (SPR); this is the highest SPR in the Nusa Tenggara Barat Province, Indonesia.^{12, 22)} The study site was focused on Sekotong district with approximately 34 km by 26 km consisting of low-lying coastal plain, hilly area and mountains covered with disturbed forest or/and forest (Figure 1). The climate in the region is tropical, with a distinctive rainy season (November through April) and dry season (May through October). There were four villages Sayong, Lendangre and Labuhanpoh located in the northern coastal region and Longlongan located more than 1.5 km away from the coast.

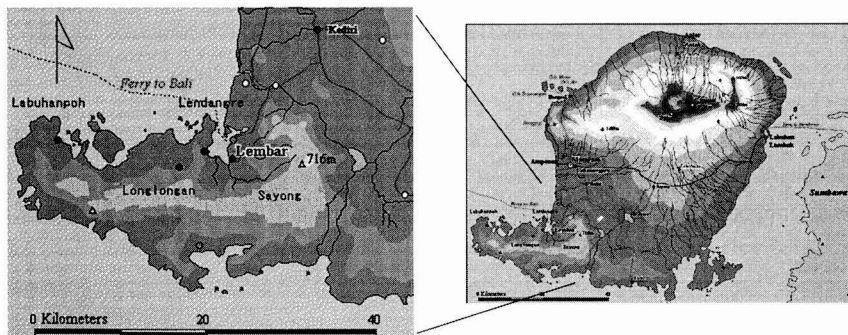


Figure 1. Location of the study area of the western part of Sekotong district on Lombok Island, Indonesia. Each dot represents one of the 4 villages used in the study.

Data collections and processing

Mangrove creeks, lagoons, rivers, fishponds, ponds, irrigated rice fields, channels, swampy fields, wells, springs, and ditches considered as the potential breeding sites for

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICTUS* ANALYSIS

An. subpictus larvae were selected for larval collection within the study area. The number of larval sampling sites was 20 in Sayong, Labuhanpoh and Longlongan and 6 sites in Lendangre. Larvae were collected by 10 dips in each site during 2-month intervals from August, 1994, through July, 1995. Identification and mounting of collected larvae was carried out in the field at the time of each survey. A number of larvae confirmed as anopheline malarial vector was recorded. The GPS (Trimble Japan, Tokyo, Japan) was used to record accurate locations of each sampling site. The data on larval sampling sites obtained from the GPS were entered into Arc View GIS (ESRI, Redlands, CA) for mapping larval habitats and measuring distance between the coast and larval habitats.

Satellite data processing

We used Japanese Earth Resources Satellite (JERS) Visible and Near Infrared Radiometer (VNIR) satellite imagery acquired by National Space Development Agency of Japan (NASDA), which has a spatial resolution of VNIR is 18 X 24 m, with three spectral channels covering the visible (bands 1, 2) and near infrared (band 3) parts of the spectrum, which are used for ground-water exploration.¹⁸⁾ We analyzed dry season JERS-VNIR data from August 13, 1996 and wet season data from November 23, 1995, a selection that was limited by problems with cloud cover. This time lag between dates of larval collections and RS data acquisition can be a problem for studying land use because it can be dynamic in the region; however, it is not a significant problem for studying the relatively stable surface water sites (which comprise most of the larval habitats we studied). Also, during the past five years (from January 1993 through December 1997), year to year variations in rainfall amount/duration and temperature had shown similar trends. The accumulated monthly rainfall in the area had been less than 100 mm in the dry season, gradually increased in the early rainy season and reached its peak (approximately 300 mm) in February. There had been few changes in temperature even though there had been a drop in temperature of between 1 and 2 degrees Celsius in the dry season.¹⁹⁾ Therefore, it could be concluded that there were little changes in natural wetland, creating little influence on RS data except man-made changes (e.g., deforestation). Thus, it is acceptable to compare larval abundance data for the sampling dry/wet seasons with the RS data for dry/wet seasons with regards to the weather patterns.²⁵⁾

To detect surface water from the RS data, we used IMAGINE Subpixel Classifier,TM as a multispectral imagery exploitation tool (ESRI, Redlands, CA). The

IMAGINE Subpixel Classifier processing module enables the classification of subpixel materials in multispectral imagery. Each pixel in a scene contains a mixture of materials (i.e., the pixel's multispectral characteristics). This module can isolate the contribution of a specific material within the mixed pixel, providing the capability to discriminate the material even when it represents only a small fraction of a pixel. The classifier identifies the locations of pixels that contain the material and reports how much of the material is in each pixel, referred to as the material pixel fraction.¹⁾ We selected 4 material pixel fraction classes which report subpixel detections in .20 material pixel fraction increments. Pixels containing between 20% and 39% of the material pixel fractions belong to class .20 – .39. Pixels containing between 40% and 59% of the material pixel fractions belong to class .40 – .59. Pixels containing between 60% and 79% of the material pixel fractions belong to class .60 – .79. Pixels containing between 80% and 100% of the material pixel fractions belong to class .80 – 1.00. We let material pixel fractions less than 20% belong to class .00 – .19 and added the class to the original classes up to 5 classes.

The amounts of the water as the material, which are detected through the RS data, belong to a particular class as previously described. Since pixels containing more than 60% of the material were scarcely identified, we combined and categorized the pixels as containing between 40% and 100% of the material. Thus, we let pixels containing between 40% and 100% of the material belong to class .40 – 1.00. Thus, even though 5 classes were selected at first, we categorized 3 classes. Then, class .00 – .19, .20 – .39, and .40 – 1.00 was assigned for class I, II and III respectively. Because 100% pure pixels for training data were used, material pixel fraction and confidential level were .90 and 1.00 respectively. The accuracy for the distribution of the detected water was assessed by comparing the map of Lombok Island (scale 1:25,000, 1992 edition; Bakosurtanal, Bogor, Indonesia), aerial photographs acquired on August 12, 1993 and ground truth data acquired by field work carried out in July, 1998.

Data on larval sampling sites obtained from a GPS were entered into a GIS for generating larval habitat coverage, which was coupled with database of larval densities. Arc View Spatial Analysis GIS (ESRI, Redlands, CA) was used to overlay coverages (i.e., larval habitats and surface water derived through RS data), and analyze spatial variability of *An. subpictus* larval densities and surface water.³⁾ The output data obtained from this process was used for statistical analysis.

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICTUS* ANALYSIS

Statistical analysis

Larval habitat location (i.e., distance from the coast to larval habitats), season and surface water were considered as critical environmental determinants for presence of *An. subpictus* larvae in this study. We used data on larval densities (i.e., number of larvae per 10 dips) from August, 1994 (dry season) and December, 1994 (rainy season) in order to correspond to the nearest month of RS data assuming the time lag did not influence the RS data with regards to the climate data. Analysis of covariance (ANCOVA) was used to analyze relationships between larval densities and the environmental determinants. We cannot apply standard ANCOVA method based on normal distribution theory because distribution of larval densities was positively skewed as shown in figure 2. In order to compute the ANCOVA, the Generalized Linear Model was chosen as a suitable method for the response variable with the non normal distribution.⁹⁾ The following ANCOVA approach was employed for this analysis: 1) exponential distribution was assumed to be distribution of larval densities; 2) both season (dry or rainy) and class (I, II or III) were expressed as the factor in the model; and 3) distances from the coast to each larval site which were measured using GPS/GIS technologies were expressed as the covariate in the model. A model to estimate the mean of the larval densities was created as follows;

$$\text{Larval densities (mean number of larvae per 10 dips)} = -1/(\mu + \alpha_i + \beta_j + \gamma \times X)$$

Where μ is the mean intercept; α_i is effect of the i -th season ($i=1$ represents dry and $i=2$ represents rainy); β_j is effect of the j -th class ($j=1$ represents class I, $j=2$ represents class II and $j=3$ represents class III); and γ is the slope of X which is observed value of distances from the coast to larval habitats. SAS GENMOD PROCEDURE (SAS Institute, Inc., Cary, NC) was used for the analysis.

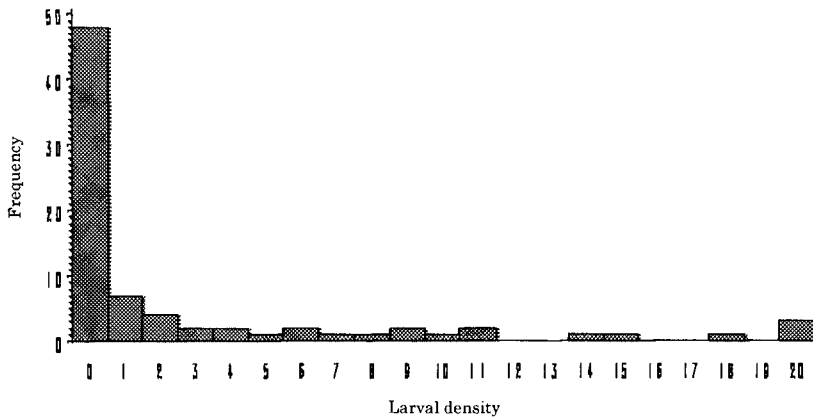


Figure 2. Frequency of observed larval densities (number of *An. subpictus* larvae per 10 dips) for the four test villages. The data represent frequency of *An. subpictus* larval densities collected during a sampling period.

RESULTS

There were significant correlation ($p < 0.05$) between larval densities and season; and between larval densities and distance from the coast to larval habitats (Table I). This suggests that season is an important factor and distance from the coast to larval habitats is an important covariate when explaining the association between larval densities and environmental determinants. Analysis of class, represented as the surface water was not statistically significant. The amounts of the water detected through the remotely sensed image data did not influence larval densities in this analysis. This result may be attributed to poor image data, that is, since cloud cover could limit detection of water as the material from the RS data, we let the material pixel fraction covered with clouds belong to class I. The data gained from analyzing spatial relationships between water pattern and larval densities was not sufficient for statistical analysis.

It is important to evaluate how a combination of environmental determinants influences larval densities. Table II shows the value of parameter estimates of the ANCOVA model. We can calculate the mean of larval densities using these parameters in table II. For example, if applied to the rainy season, class II and 100 m from the coast to the model, the result would be 2.6 ($= -1/(-0.025-0.000 -0.057 -0.003 \times 100)$).

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICTUS* ANALYSIS

Figure 3 represents a comparison between observed larval densities (in case of the combination of the rainy season, class I and observed value of distances) and estimated larval densities (when applied to the rainy season, class I and observed value of distances to the model). The figure shows both larval densities decreased with distance from the coast to larval habitats. Larval habitats located approximately 1,500 m away from the coast would have extremely low larval densities. This implies that sites located more than 1,500 m away from the coast are out of the flight range (1,500 m) of *An. subpictus*, which inhabits coastal areas.¹⁰⁾

Table I. Result of ANCOVA. Class represents surface water detected through RS data.

Factor	DF	Deviance	Chi Square	P-value
Season	1	2378.90	8.56	<0.0001
Class	2	2478.90	3.96	0.138
Distance	1	2483.84	108.91	0.003

Table II. Result of parameter estimates. Pixels containing between 0% and 19% of water belong to class I. Pixels containing between 20% and 39% of water belong to class II. Pixels containing between 40% and 100% of water belong to class III.

Factor	Level	Estimate	Standard Error
Season	Dry	-0.212	0.0868
	Rainy	0.000	0.0000
Class	I	0.083	0.0499
	II	-0.057	0.1066
	III	0.000	0.0000
Distance		-0.003	0.0004

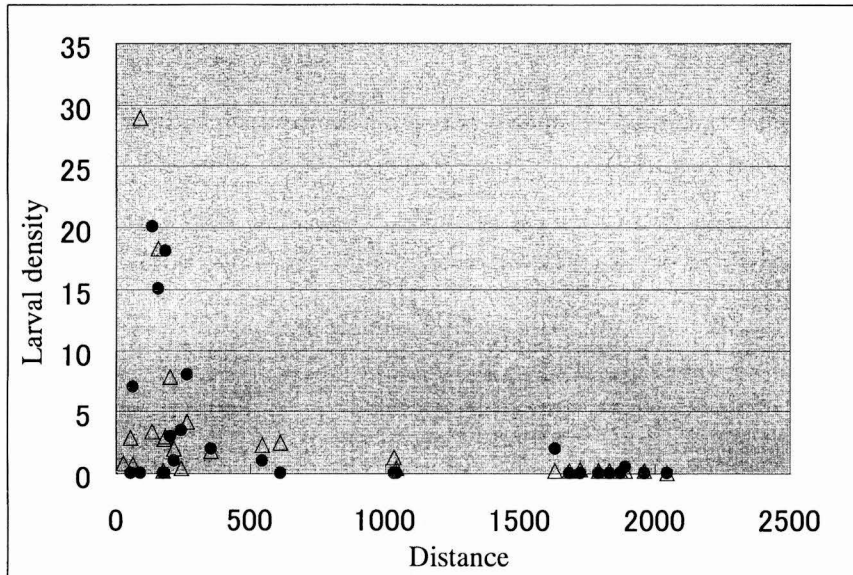


Figure 3. A comparison between observed larval densities (●) (in case of the combination of the rainy season, class I and observed value of distances) and estimated larval densities (△) (when applied to the rainy season, class I and observed value of distances to the model).

DISCUSSION

The relationships between *An. subpictus* larvae densities and environmental determinants in the Sekotong district on Lombok Island were analyzed using RS/GPS/GIS tools in the study. *An. subpictus* larval densities were significantly associated with season and distance from the coast to larval habitats. The rainy season and the distance from the coast to larval habitats were critical environmental determinants for presence of *An. subpictus* larvae in the study. The result of our study revealed that the effect of class represented as surface water was not statistically significant. Yet, surface water is necessary for *An. subpictus* larval habitats and can be considered as one of the environmental determinants when analyzing associations between larval densities and environmental determinants. Due to cloud cover, there was limited detection of water, which led to reduced data to be used for statistical analysis. This factor may have influenced the final results.

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICIUS* ANALYSIS

The success of further studies requires more advanced remote sensing techniques for solving a problem caused by clouds. Improved cloud-penetrating radar needs to be considered for further studies of this kind. More, high spatial resolution of satellite sensors adequate for monitoring small sites will help for further studies. The RS technique, RS data and RS/GPS/GIS tools will enable us to predict *An. subpictus* larval densities. These factors will make an RS/GPS/GIS approach to the application of environmental management measurement for vector control of larval abundance more effective. Therefore, this approach will be able to be used for predicting potential disease outbreaks, targeting vector-borne disease surveillance and intervention programs.

ACKNOWLEDGMENTS

We thank Dr. Ratna and Mr. Budhi (Nusa Tenggara Barat, Mataram, Indonesia) for help with collecting data and assistance with the field study. Thanks also to Dr. Yamagata (the National Institute for Environmental Studies) for valuable comments and Mr. Omori (Science University of Tokyo) for assistance with statistical analysis.

FINANCIAL SUPPORT

This work was supported by Japan Society for Promotion of Science (JSPS), National Space Development Agency of Japan (NASDA) and the National Institute for Environmental Studies (NIES).

REFERENCES

1. Applied Analysis Inc. 1998. IMAGINE Subpixel Classifier™ User's Guide. Applied Analysis Inc., Billerica, MA.
2. Beck, L.R., Rodriguez, M.H., Dister, S.W., Rodriguez, A.D., Rejmankova, E., Ulloa, A., Meza, R.A., Roberts, D.R., Paris, J.F., Spanner, M.A., Washino, R.K., Hacker, C., and Legters, L.J. 1994. Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *American Journal of Tropical Medicine and Hygiene*. 51:271-280.
3. Environmental Systems Research Institute (ESRI), Inc. 1997. ArcView Spatial Analyst User's Guide. Redlands, Environmental Systems Research Institute, Inc., CA.
4. Hay, S.I., Snow, R.W., and Rogers, P.J. 1998. From Predicting Mosquito Habitat to Malaria Seasons Using Remotely Sensed Data: Practice, Problem and Perspectives.

- Parasitology Today. 14:306-313.
5. Hightower, A.W., Ombok, M., Otieno, R., Odhiambo, R., Oloo, A.J., Lal, A.A., Nahlen, B.L., and Hawley, W.A. 1998. A geographic information system applied to a malaria field study in western Kenya. *American Journal of Tropical Medicine and Hygiene*. 58:266-272.
 6. Ikesyouji, T. 1993. *The Interface between Mosquitoes and Humans*, 1st ed. Tokyo University Press, Tokyo.
 7. Kitron, U. 1998. Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *Journal of Medical Entomology*. 35:435-445.
 8. McCullagh, P. and Nelder, J.A. 1983. *Generalized Linear Models*. 1st ed. Chapman and Hall, New York.
 9. Miyagi, I., Toma, T., Mogi, M., Martono, Yotopranoto, S., Arifin, Z., and Dachlan, Y.P. 1994. Mosquito species (diptera: culicidae) from Lombok Island, Indonesia. *Mosquito Systematics*. 26:19-24.
 10. Nagpal, B.N. and Sharma, V.P. 1995. *Indian Anophelines*. 1st ed. Science Publishers Inc., North Lebanon, NH.
 11. Pope, K.O., Rejmankova, E., Savage, H.M., Arredondo-Jimenez, J.I., Rodriguez, M.H., and Roberts, D.R. 1994. Remote sensing of tropical wetlands for malaria control in Chiapas, Mexico. *Ecological Applications*. 4:81-90.
 12. Propinsi Nusa Tenggara Barat (NTB) 1996. *Annual Malaria Incidence Puskesmas (Annual Malaria Incidence of Health Centers): Report of a NTB Task Group*. NTB, Indonesia.
 13. Rejmankova, E., Savage, H.M., Rejmanek, M., Roberts, D.R., and Arredondo-Jimenez, J.I. 1991. Multivariate analysis of relationships between habitats, environmental factors and occurrence of anopheline mosquito larvae *Anopheles albimanus* and *An. pseudopunctipennis* in southern Chiapas, Mexico. *Journal of Applied Ecology*. 28:827-841.
 14. Rejmankova, E., Savage, H.M., Rodriguez, M.H., Roberts, D.R., and Rejmanek, M. 1992. Aquatic vegetation as a basis for classification of *Anopheles albimanus* Wiedemann (Diptera: Culicidae) larval habitats. *Environmental Entomology*. 21:598-603.
 15. Rejmankova, E., Roberts, D.R., Pawley, A., Manguin, S., and Polanco, J. 1995. Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. *American Journal of Tropical Medicine and Hygiene*. 53:482-488.
 16. Roberts, D.R. and Rodriguez, M.H. 1994. The environment, remote sensing and malaria control. *Annals New York Academy Sciences*. 740:396-402.

USE OF RS/GPS/GIS FOR *ANOPHELES SUBPICTUS* ANALYSIS

17. Rodriguez, A.D., Rodriguez, M.H., Meza, R.A., Hernandez, J.E., Rejmankova, E., Savage, H.M., Roberts, D.R., Pope, K.O., and Legters, L. 1993. Dynamics of population densities and vegetation associations of *Anopheles albimanus* larvae in a coastal area of southern Chiapas, Mexico. *Journal of the American Mosquito Control Association*. 9:46-58.
18. Salomonson, V.V. 1983. Water Resources Assessment. p.1497-1500. *In* Manual of Remote Sensing. 2nd ed. American Society of Photogrammetry, VA.
19. Selaparang Meteorological Organization. 1998. Weather Report. Selaparang Meteorological Organization, Mataram, Indonesia.
20. Sharma, V.P., Dhiman, R.C., Ansari, M.A., Nagpal, B.N., Srivastava, A., Manavalon, P., Adiga, S., Radhakrishnan, K., and Chandrasekhar, M.G. 1996. Study on the feasibility of delineating mosquito-genic conditions in and around Delhi using Indian Remote sensing Satellite Data. *Indian Journal of Malariology*. 33:107-125.
21. Sharma, V.P. and Srivastava, A. 1997. Role of geographic information system in malaria control. *Indian Journal of Medical Research*. 106:198-204.
22. Soedarto, Martono, Yotopranoto, S., and Arifin, Z. 1994. Studies on Anophelines Fauna and Species Specific Breeding Habitats in Lombok Barat, Nusa Tenggara Barat Province, Indonesia 1992-1993: Report of a Malaria Study Group. NTB, Indonesia.
23. Wood, B., Washino, R., Beck, L., Hibbard, K., Pitcairn, M., Roberts, D., Rejmankova, E., Paris, J., Hacker, C., Salute, J., Sebesta, P., and Legters, L. 1992. Distinguishing high and low anopheline-producing rice fields using remote sensing and GIS technologies. *Preventive Veterinary Medicine*. 11:277-288.
24. World Health Organization. 1978. Malaria control strategy. A31/19. Report by the Director General. WHO, Geneva.
25. Yasuoka, Y. 1999. Personal communications. Institute of Industrial Science, University of Tokyo, Tokyo, Japan.
26. Yotopranoto, S., Takagi, M., Tsuda, Y., Subekti, S., Kanbara, H., and Dachlan, Y.P. 1996. Malaria vector situation at coastal villages in Lombok Island, Indonesia. Report. Tropical Disease Research Center, Airlangga University, Surabaya, Indonesia and Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan.