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Analyses of Dengue Fever and Aedes aegypti (Diptera: Culicidae) Larval Habitats in a Tropical Urban Environment of Costa Rica using Geospatial and Mosquito Surveillance Technologies

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ANALYSES OF DENGUE FEVER AND *Aedes aegypti* (Diptera: Culicidae) LARVAL HABITATS IN A TROPICAL URBAN ENVIRONMENT OF COSTA RICA USING GEOSPATIAL AND MOSQUITO SURVEILLANCE TECHNOLOGIES

By

Adriana Troyo

A DISSERTATION

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ANALYSES OF DENGUE FEVER AND *Aedes aegypti* (DIPTERA: CULICIDAE) LARVAL HABITATS IN A TROPICAL URBAN ENVIRONMENT OF COSTA RICA USING GEOSPATIAL AND MOSQUITO SURVEILLANCE TECHNOLOGIES

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Dengue is currently the most important arboviral disease globally and is usually associated with built environments in tropical areas. Control measures are currently focused on community participation in control of the vector *Aedes aegypti* and larval source reduction. In Costa Rica, dengue fever is a relatively recent re-emerging disease and has become a serious public health problem. Remotely sensed information can facilitate the study of urban mosquito-borne diseases like dengue by providing multiple temporal and spatial resolutions appropriate to investigate urban structure and ecological characteristics associated with infectious disease. Initial studies showed that although dengue is a serious public health problem in Costa Rica, there is a need for interdisciplinary scientific research to guide vector control. Therefore, the dengue situation in Puntarenas, Costa Rica, and applications of remote sensing to study infectious diseases like dengue within urban environments was analyzed. Satellite imagery of high and medium spatial resolution was obtained to evaluate relationships between urban structure and incidence of dengue fever at the locality level. Using the satellite imagery, a geographical sampling method was developed and applied for seasonal entomological field surveys in Puntarenas. Very high resolution imagery from QuickBird was utilized to
determine the relationships between *Ae. aegypti* larval habitat abundance and tree cover or built areas. Results showed that the most relevant *Ae. aegypti* larval habitats in Puntarenas were outdoor miscellaneous containers, cans and plastic food containers that fill with rain water in the wet season, while washtubs were the most productive habitats in the dry season. Dengue incidence and abundance of larval habitats in the urban environment were directly associated with tree cover and inversely associated with built areas. Environmental conditions and urban structure, as well as human behavior were related in different ways to dengue incidence and *Ae. aegypti* larval habitats. Overall, remotely sensed information was useful in developing sampling strategies for field surveys and determining factors within the urban environment that may promote persistence of mosquito larval habitats and increased dengue risk. The geographical methods and relationships revealed will be useful in determining target areas for more efficient vector control.
Dedication:

To my parents and grandparents, my greatest teachers along this journey.
Acknowledgements:

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Introduction

Dengue is a viral disease of great global public health concern that causes more illness and death than any other arbovirus (WHO 2002). Globalization, population growth, and uncontrolled or unplanned urbanization have all been major factors influencing the current pandemic (Kuno 1995). There are four serotypes of dengue (DEN-1, DEN-2, DEN-3, and DEN-4), which are transmitted mainly by *Aedes aegypti*, a mosquito that lives in close association with humans in urban and sub-urban environments. Therefore, dengue is generally considered a disease of urban areas, and its epidemiology is highly related to the biology of the mosquito vector, the environment, and human behavior.

Dengue fever is the most important vector-borne disease in Costa Rica, and *Ae. aegypti* is the mosquito responsible for transmission. *Ae. aegypti* was officially eliminated from the country in 1960, but reinfestations occurred in some areas, and the mosquito was reported throughout the country in 1993. At the end of 1993, transmission of dengue fever was reported in the country (WHO 1994). Since then, there have been more than 150,000 cases reported (Guzman et al. 1998, Ministerio de Salud 2005).

This dissertation focuses on the epidemiology of dengue in the city of Puntarenas, Costa Rica, which is on the Pacific Coast of the country. Puntarenas is one of the cities where dengue was re-introduced in 1993, and its population has been greatly affected by dengue since then. The main objective is to determine characteristics of the urban
structure and environment that affect dengue incidence and vector populations in Puntarenas through an interdisciplinary approach, taking into consideration remotely sensed data and GIS technology, and epidemiological methods in infectious diseases and vector ecology.

The fact that dengue is a disease primarily of urban environments implies that its epidemiology is dependent on many interactions and not only centered on the mosquito vector. However, the vector is the principal target of most control approaches. The structure of the urban environment and specific human activities that promote vector permanence and disease transmission could play an important part that has not been thoroughly evaluated and is generally not considered within specific areas. Integrating remote sensing/GIS in the analysis of urban structure and its association with dengue will open new possibilities in the application of these tools for the study of urban disease epidemiology, specially focusing on the use of high-resolution imagery. At the same time, the identification of factors of the urban structure that promote dengue incidence and/or high mosquito prevalence would allow more focused control measures and the possibility to identify high-risk areas with similar urban structure patterns as the study site.

By identifying the most relevant breeding habitats and their possible link to human behavior and activities, specific habitat types and human behavior could be targeted within adequate source reduction and educational approaches. In this sense, determining the specific types of containers that are more important as larval habitats could help explain the epidemiological patterns of the disease and guide better control activities.
Overall, these methods would help construct new models for prevention and control that incorporate satellite imagery to identify specific high-risk areas for developing more localized strategies and at the same time provide information on vector biology needed to direct these activities more efficiently.
Chapter I

Remote Sensing and its applications in the Epidemiology of Vector-Borne Diseases: The Urban Challenge

Remote sensing is the “science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in contact with the object, area or phenomenon under investigation” (Lillesand et al. 2004). The remotely collected data can be of many forms, but it is common to relate remote sensing to electromagnetic energy sensors that are being operated from airborne and spaceborne platforms. These sensors acquire data on the way various earth surface features emit and reflect electromagnetic energy, and these data are analyzed to provide information about the resources under investigation (Lillesand et al. 2004).

Within epidemiology, disease ecology investigates the biological, physical and anthropogenic links between the environment and disease, and therefore must account for spatial variation in transmission. In addition, transmission commonly requires close contact between susceptibles and infectives and may be linked to landscape and environmental variables (Graham et al. 2004). Therefore, there is great potential for the application of remote sensing techniques in disease epidemiology, which had been suggested as early as 1970 (Cline 1970). Currently, images from space allow an immediate impression of patterns and structures in an area, often with great clarity. They provide contextual and quantitative information that was not possible before development of aerospace remote sensing systems. Measurements of surface water and land
temperatures, vegetation conditions, mineral content of soil and rock, atmospheric conditions, ocean turbidity, cloud height, aerosol distributions, wild fires, upper atmospheric winds, rainfall, global topography, and more are possible from space (Huh & Malone 2001). Current epidemiological applications of remote sensing include mainly mapping exercises, in which statistical associations are demonstrated between ecological variables and processes that can be observed remotely. These are then correlated with disease incidence and prevalence, as well as vector distribution (Hay 1997).

Satellites have very different characteristics and functions. They can be divided into two categories, according to their orbit: low earth orbit/polar orbiting satellites and high earth orbit/equator positioned (geostationary) satellites. Usually, the low earth orbit satellite sensors provide higher spatial resolution, but lower temporal resolution (for example 30 m every 16 days), whereas high orbiting satellites provide lower spatial resolution but at much more frequent intervals (Huh & Malone 2001). The sensors, mainly radiometric imaging devices, can be passive or active. Passive sensors measure natural radiation reflected or emitted from earth features, whereas active sensors illuminate the scene and measure the returning, reflected fragments of the outgoing signal (Huh & Malone 2001).

There are many environmental factors that can be detected from spaceborne platforms for application to infectious diseases. These include surface temperatures, condition of vegetation, soil moisture, standing water, atmospheric water vapor, topography, and mineral and soil types (Huh & Malone 2001). Of increasing relevance is the information on the vegetation that can be extracted from visible and near infrared radiation. High reflectance in the near infrared and low reflectance in the visible
(especially in the red) characteristic of healthy and vigorous vegetation can be used to derive several different vegetation indices. Two of the more commonly used vegetation indices in infectious diseases are the simple vegetation index (\(VI= \text{NIR-RED}\)) and the normalized difference vegetation index (\(\text{NDVI}= \frac{\text{NIR}-\text{RED}}{\text{NIR}+\text{RED}}\)).

Remote sensing offers powerful tools for describing, explaining, and predicting epidemiological phenomena, which can be used to develop or improve surveillance, prevention, and control strategies. Satellite sensor data can be used directly, but are often combined to produce indices, such as the vegetation indices mentioned previously, that are related to ground-based variables relevant to epidemiological events. For instance, satellite data have been correlated with key behavioral or demographic rates of the vectors and intermediate hosts of indirectly transmitted diseases, thereby identifying the conditions that are best for pathogen transmission (Rogers & Randolph 2003). Remote sensing has been applied to many diseases such as cholera, Hantavirus, Q fever, fascioliasis, and schistosomiasis (Lobitz et al. 2000, Glass et al. 2000, Yang et al. 2005, Davis et al. 2002, Zhang et al. 2005, Brooker 2002, Correia et al. 2004), where the information obtained has been used to analyze the environmental characteristics that influence the disease mainly through studying the specific reservoirs and intermediate hosts. In addition, vector-borne disease systems have many applications for remote sensing, considering the environmental factors that can affect and explain the distribution of vector populations and potential for transmission.
Remote sensing in vector-borne diseases of medical importance

Vector-borne diseases usually refer to diseases where an arthropod (mite, tick, louse, mosquito, fly, etc) transports the infectious agent to a susceptible host. Mechanical vectors facilitate contact between the susceptible individual and the infectious agent by simply acting as passive carriers, so the vector transports the agent but is not usually necessary for its transmission (flies, cockroaches). Biological vectors are considered part of the microorganism’s life cycle, and the infectious agent multiplies or goes through development phases before being transmitted to the susceptible host. This category is of more interest and includes arthropods that transmit common human diseases like leishmaniasis, malaria, lymphatic filariasis, dengue, West Nile encephalitis, African trypanosomiasis, Chagas disease, onchocerciasis, and Lyme disease.

Remote sensing methods have been applied to study many of these diseases, and a common approach to remote sensing in epidemiology is classifying images into habitats for disease vectors (Graham et al. 2004). The principal goal of remote sensing in epidemiology is to map the distribution of a disease so that control efforts in endemic situations and intervention strategies in epidemic situations may be most effectively directed (Hay et al. 1997). Climatic and ecological information extracted from remotely sensed data, together with appropriate field studies, can be used to identify and map the potential habitats of disease vectors, predict spatial/temporal alterations in vector populations, monitor quantitative and qualitative alterations in habitat, and plan control programs indicating areas of greater risk (Hay et al. 1997, Thomson & Connor 2000).
Tick-borne diseases

Ticks are arthropods that usually have complex life cycles and can transmit a variety of pathogens to humans and animals while feeding on the animal’s blood. Examples of diseases that can be transmitted by ticks include east coast fever in cattle, Lyme disease, and rickettsiosis. The diseases that affect humans can be considered zoonosis: diseases that primarily affect and are maintained in animal populations but can affect humans if they come in contact with the infectious agent. Some of the different habitats for the tick vectors that can affect humans have been studied, and for instance, risk maps for the tick *Ixodes ricinus* and tick-borne encephalitis have been produced (Hay et al. 1997). Transmission risk of Lyme disease has also been modeled and mapped using remotely sensed data, and vegetation indices have been useful to determine relative tick abundance, vector and reservoir (deer) habitats, and human risk for disease (Dister et al. 1997, Eisen et al. 2005, Beck et al. 2000).

Trypanosomiasis

There are 2 main diseases caused by trypanosomes in humans: African sleeping sickness and Chagas disease. These are two very different diseases caused by different species of trypanosome parasites in Africa and America that are transmitted by *Glossina* (tsetse flies) and Reduviid bugs (kissing bugs) respectively. Both however, are generally considered zoonoses, and the parasite is transmitted by the vector in wild and domestic animal populations.

African sleeping sickness has been studied in various ways using remotely sensed data. The spatial distribution of this disease seems to be best studied by focusing on
vector requirements using data from the National Oceanic Atmospheric Administration (NOAA) series of meteorological satellites. With these tools, researches have concluded that temperatures or NDVI can predict distributions of tsetse flies in different areas of Africa (Bergquist 2001). NDVI seems to integrate a variety of environmental factors of importance to tsetse survival, and associations that have been found include relationships between NDVI and mortality (inverse), fly size, and incidence of trypanosomiasis (Hay et al. 1997).

**Leishmaniasis**

There are many parasites of the genus *Leishmania* that can affect humans causing different forms of the disease (cutaneous, mucocutaneous, visceral leishmaniasis), and all are transmitted by species of sand flies. Most leishmaniasis are zoonotic and maintained in small animals in nature, but others are urban or peri-urban depending on the biology of the vectors and animal reservoirs. Remote sensing has been used to investigate the environmental determinants of the distribution of vector species like *Phlebotomus orientalis*, for which studies show vector presence in areas that have higher temperatures and NDVI using data from NOAA satellite series (Bergquist 2001, Gebre-Michael et al. 2004). In America, Landsat Thematic Mapper (TM) images have been used to classify areas in Brazil and use this classification to study the risk of American visceral leishmaniasis (Thompson et al. 2002).
Onchocerciasis

The worm that causes onchocerciasis or river blindness (*Onchocerca volvulus*) is transmitted by black flies (family Simuliidae). Although this is a worm that infects only humans, the vectors develop in running rivers, where water has high oxygen concentration. Therefore, this vector and the disease are highly affected by the environment. Satellite imagery (Landsat Return Beam Vidicon) and aerial photography have been used to determine the course of rivers for control of black flies in Sudan, and Landsat TM data have been used to map land-cover within countries of West Africa (to help plan development potential for land in controlled areas) (Hay et al. 1997). The greatest use for remote sensing might be in combination with geographical information systems (GIS) to establish buffer zones around potential breeding sites to target control and manage land-use (Hay et al. 1997).

Mosquito-borne diseases

Many diseases that are transmitted by mosquitoes are of great public health concern, and they include diseases like malaria, lymphatic filariasis, dengue fever and dengue hemorrhagic fever, West Nile encephalitis, St Luis encephalitis, yellow fever, and Rift Valley Fever. Of these, malaria has been the mosquito-borne disease where remote sensing has been more widely applied. Lymphatic filariasis is in the process of mapping, and this is carried out mainly by using GIS technology (Bergquist 2001).

The mosquito-borne arboviruses (West Nile, St Luis Encephalitis, Rift Valley, dengue) have been studied using remote sensing data. For instance, color infrared aerial photography was used to map the forested and open wetlands, marshes and residential
areas in Michigan for control of *Aedes* and *Culex* mosquitoes, vectors for St. Louis encephalitis. Known flight range of mosquito species, combined with distance between residential areas and vector habitats served to identify priorities for control (Hay et al. 1997).

GIS technology has been used in dengue research, and risk maps have been produced (Carbajo et al. 2001), but remotely sensed data has been applied only recently. Results from one of these studies used Landsat TM land cover maps and showed that built-up areas seem to influence dengue and constitute the higher risk zones, while forest areas have no influence on the disease (Nakhapakorn & Tripathy 2005). Another study used remotely sensed images (including monthly NDVI) from the Advanced Very High Resolution Radiometer (AVHRR) as additional layers in time-specific ecological niche modeling to predict spatial dynamics of mosquito vectors and dengue cases (Peterson et al. 2005).

Malaria and its anopheline vectors have been studied in various settings using different remote sensing technologies. For example, high NDVI values have been associated to high mosquito producing rice fields in the early growing season (in California), and classification of Landsat scenes have generated land cover maps for use in defining areas of high mosquito production potential, mosquito abundance, and risk for malaria (Hay et al. 1997, Sithiprasasna et al. 2005a, Brooker et al. 2004). In addition, AVHRR data has also shown relationships between high NDVI values and falciparum malaria prevalences (Nihei et al. 2002). Moreover, data from this sensor has been used to develop predictive models for malaria prevalence in East Africa (Omumbo et al. 2005), and land cover maps derived from AVHRR data have even been used to map anopheline
distributions and malaria risk in Europe (Kuhn et al. 2002). Low spatial resolution imagery from NOAA and Meteosat series satellites has also evidenced relationships between satellite sensor data (NDVI and cold cloud duration) and environmental variables associated with malaria transmission (Thomson et al. 1996).

Remote sensing applications and urban vector-borne diseases

Even though remotely sensed data is being used to study the epidemiology of many vector-borne diseases, most of the research to date has been focused on the characteristics of the environment that can affect intermediate host or vector populations in non-urban settings. In some specific areas, transmission of diseases that are generally rural like leishmaniasis and malaria may occur within the urban environment. However, other diseases such as dengue and lymphatic filariasis are usually considered more suited to urban areas, where inadequate housing, drainage systems, water supply, and solid waste collection services increase availability of larval habitats that are suitable for the specific vectors (Kuno 1995). This means that these disease system and some of the characteristics of the vectors and/or reservoirs are influenced by human behavior and structure of the urban environment that could be evidenced through remote sensors.

High-resolution data would be very useful to study factors affecting disease, vector, or reservoir distribution within the urban environments. The identification of small areas within cities, trees, buildings, rooftop characteristics, and distances between residences can be achieved with very high-resolution (<5m) sensors such as IKONOS, OrbView-3, and Quickbird (Lillesand et al. 2004, Correia et al. 2004). City blocks can be detailed at 15 m resolution, while at 20-30 m resolutions allow identification of urban
areas, roads, and airports (Correia et al. 2004). However, the disadvantage of these high-resolution data is their low temporal resolution.

Urban patterns in leishmaniasis can be observed for example when new housing projects are built on the limits between the city and the forest (Correia et al. 2004), or when the sand fly vector is highly anthropophilic (prefers biting humans) or lives in close association with humans and domestic animals (serve as reservoirs). Lymphatic filariasis behaves as an urban disease when transmitted by anthropophilic *Culex* mosquitoes (like *Culex quinquefasciatus*) that are common in some urban areas where breeding sites are numerous. Therefore, identification of characteristics such as urban growth, housing and vegetation types, road structures, drainage systems, flooded areas, and soil moisture availability within the urban area could yield disease patterns.

Malaria in urban environments has been the focus of very few studies. Currently, an estimated 200 million people live in urban settings where they are at risk for contracting the disease (Keiser et al. 2004). Multispectral Thermal Imagery satellite data (5 meter resolution bands) was used in one study to calculate the NDVI, which was shown to be inversely correlated with household density and a significant factor affecting the abundance of potential anopheline larval habitats in a positive manner after controlling for household density (Eisele et al. 2003). Given the possibility of mosquito adaptation to urban environments and subsequent epidemiological differences from the rural settings, it would be important to evaluate the factors within urban ecosystems that influence vector populations and transmission potential.

Dengue is considered primarily disease of urban areas, and two investigations that employed remote sensing in studying the epidemiology of dengue used Landsat TM and
AVHRR data (Nakhapakorn & Tripathy 2005, Sithiprasasna et al. 2005a). By using the multispectral Landsat TM data (30 m spatial resolution), it was possible to show that built-up areas had higher risk for dengue and forest areas indicated low or no risk (Nakhapakorn & Tripathy 2005), which would be generally expected due to the biology of the mosquito vector and disease epidemiology. The high temporal, but low spatial resolution AVHRR data was used to extract monthly NDVI values and include them in ecological models over a large area in Mexico. Since *Ae. aegypti* and dengue are commonly found in many urban areas of the tropics, these studies show that remotely sensed data could contribute to understanding some of the factors of the urban structure that may be associated to or influencing different dengue transmission patterns within the urban setting.

It is interesting to note that there are very few studies in infectious diseases that have used multispectral data with spatial resolution of less than 5 m. These scenes usually cover smaller areas, but the level of detail may prove to be useful when studying variations in disease distributions within cities. Until recently, there did not seem to be any studies directly related to infectious diseases that used IKONOS or Quickbird data (Correia et al. 2004). IKONOS imagery was employed in previous studies to display anopheline distributions (Sithiprasasna et al. 2003) and to construct a household GIS database for health studies (Ali et al. 2004). More advanced applications of IKONOS data have generated land use/land cover classifications for integration with a GIS and studying malaria transmission risk in Thailand (Sithiprasasna et al. 2005b). Similarly, Quickbird data, which has the highest spatial resolution commercially available (0.6 m panchromatic, 2.4 m multispectral) (Lillesand et al. 2004), was used along with Landsat...
ETM+ images to study anopheline mosquito habitats in Korea (Sithiprasasna et al. 2005a). The Quickbird images were used to display spatial data (in form of geographical coverage) and to establish the GIS for the study area.

Some research on the epidemiology of diseases in urban areas like malaria and dengue has begun, and the application of high-resolution imagery like IKONOS and Quickbird within the urban environment is yet to be analyzed. These very high resolution data could be used to classify smaller structures within cities, identifying objects, roads and paths, housing, vegetation, and their possible association to urban diseases. Application of different classifiers could provide useful maps from which to extract data and analyze relationships to human cases of disease, as well as vector populations and habitats. Urbanization and growth can also be significant to disease dynamics, and it could be analyzed through application of spatial metrics (in packages like FRAGSTATS). These provide quantitative information about the structure and pattern of the urban landscape, and they are able to capture the changing dynamics of urban growth (Herold et al. 2003a).

Concluding remarks

Remote sensing is a tool that can be applied to epidemiology of infectious diseases, especially vector-borne diseases. Application of remotely sensed data in studies has resulted in risk maps, vector distributions maps, as well as associations between disease and environmental variables that observable from satellites. Because of the epidemiology of most diseases and the availability of lower spatial resolution data, many studies have centered on ecological variables of large areas and impact on zoonotic or
rural diseases. However, with the increasing availability of higher resolution data and computer-based tools for GIS and RS analysis, the possibility to study diseases within urban environments is evident.

Urbanization can affect diseases by changing the environment in which humans, vectors and possible reservoirs interact. Overcrowding, high house densities, poor housing conditions, availability of larval habitats could all be contributing to the disease epidemiology. Urban features such as housing and urban mosquito habitats could be mapped for studying diseases like Chagas, dengue, filariosis, leishmaniasis, and malaria in some of the specific areas where they behave as urban or peri-urban diseases. Very high-resolution imagery can be used to classify the environment and look into associations with spatially varying incidence, vector, or reservoir distributions. Spatial metrics could aid in this analysis, by providing better understanding of the landscape, as well as urban growth.
Chapter II

Dengue in Costa Rica: the gap in local scientific research

Globalization has affected the reemergence of infectious diseases, with increased human travel and trade facilitating the introduction of diseases into new areas and the resurgence of diseases that had been eliminated in some places (Harrus & Baneth 2005). However, the increased availability of scientific information from various research groups can help in the development of new and improved disease control measures. The combination of scientific research and reliable field assessments that consider different areas of knowledge and different geographic locations (world regions, countries, and communities) is essential for building greater understanding. This combination can also provide evidence that guides prevention and control measures at the local, national, regional, and global levels.

Dengue is a reemerging and uncontrolled disease. With dengue, there is a need for more scientific research on the local factors that affect the disease system and the relationships among those factors. There is also a need to develop new or improved dengue control approaches (Scientific Working Group 2003). In this chapter, the situation of scientific research on dengue is analyzed for the developing country of Costa Rica. Although dengue has become the most important vector-borne disease in Costa Rica over the last decade, published scientific research dealing with the local situation is scarce.

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This could be due to various factors such as the historical and political context, and limited financial and human resources. Filling this gap in Costa Rica and other developing countries would benefit local control programs and help efforts around the world to develop best practices for dengue prevention and control. Making this new knowledge broadly available could help improve the global dengue situation.

**Dengue and dengue hemorrhagic fever**

A viral disease, dengue is a great global public health concern. It is endemic in more than 100 countries of Africa, Southeast Asia, the Eastern Mediterranean, the Western Pacific, and the Americas. Dengue causes more illness and death than any other arbovirus. Worldwide, there are approximately 2.5 billion people at risk of infection, and the World Health Organization (WHO) estimates that there are about 50 to 100 million cases per year (WHO 2006, PAHO 2006). Although dengue-like symptoms had been reported earlier, the first known pandemic of dengue-like illness began in 1779 (Gubler 1998). Infrequent but often large epidemics occurred from 1780 to 1940. Many tropical urban centers became endemic during this period, and epidemic dengue hemorrhagic fever (DHF) emerged in the 1950s as Asian cities became hyperendemic with co-circulating dengue serotypes (Gubler 2004).

The virus responsible for dengue fever and DHF belongs to the family Flaviviridae (the same family as the yellow fever virus), and four different viral serotypes exist: DEN-1, DEN-2, DEN-3, and DEN-4 (Gubler 2004). Infection with one dengue serotype provides immunity for years, but it does not protect against infection with the
other serotypes (WHO 2006, Gubler 1998). After an infection, a person usually remains asymptomatic or develops self-limiting dengue fever characterized by sudden fever and such symptoms as headache, retroorbital pain, body and joint aches, weakness, and rash. Mild or severe hemorrhagic manifestations can also be present after a first infection, but this is less frequent. Dengue hemorrhagic fever, which is more common in children, can lead to shock from blood loss, and even death. It is generally accepted that having had a prior dengue infection increases the risk of developing DHF upon infection with a different serotype (Gubler 1998).

Dengue virus is transmitted to humans through the bite of infected mosquitoes, and these mosquitoes usually have acquired the virus by ingesting blood of infected and viremic humans (transovarial transmission is also possible). *Aedes aegypti* is considered the main vector, although other *Aedes* species, including *Aedes albopictus*, have been implicated in rural epidemics as well as some urban ones (Gubler 1998, Effler et al. 2005). *Aedes aegypti* is a mosquito that lives in close association with humans in urban and suburban environments. The mosquito preferentially ingests human blood. It breeds in artificial containers such as drums, buckets, tires, flowerpots, and vases (Gubler 1998, Service 1992, Focks & Chadee 1997, Calderon-Arguedas et al. 2004a). Therefore, the epidemiology of dengue is highly related to the biology of the mosquito vector and human behavior, as well as the environment and the virus itself.

*Aedes aegypti* and dengue have a worldwide distribution in the tropics (WHO 2006), and the incidence has increased significantly over the past 25 years (Gubler 2005). In the Region of the Americas, highly effective control campaigns eliminated *Ae. aegypti* from most of Central and South America during the 1950s, but discontinuation of the
control efforts led to reinfestation during the 1970s and 1980s, and the reemergence of
dengue (Gubler 1998). Transport of containers that harbor mosquito larvae (such as tires
and water drums) as well as travel of infected individuals has promoted the introduction
Globalization, population growth, and uncontrolled or unplanned urbanization (where
inadequate housing, water supply, and garbage collection services increase available
larval habitats) have all been major factors influencing the current pandemic (Kuno
1995). These demographic and social changes, as well as a lack of effective mosquito
control, have facilitated the spread and permanence of *Ae. aegypti* and dengue virus in
many areas of the world (Gubler 1998).

There is no effective vaccine for dengue, so vector control is the main approach
for control and prevention. Although insecticide spraying has been used extensively,
larval source reduction (eliminating or cleaning water-filled containers that can harbor
*Ae. aegypti* larvae) is considered the most effective way of reducing and controlling the
mosquito populations (Gubler 1998). This control method has had vertical and
community-based approaches. Most vertical approaches have been unsuccessful due to
poor sustainability, and community-based approaches with extensive health education
and community outreach have been only partially successful. The increasing spread and
incidence of dengue suggests that the current vertical and community-based measures
employed are generally ineffective, are inappropriate, or are being applied incorrectly
(Scientific Working Group 2003).
Scientific interest and opportunities for investigators to obtain funds for research on dengue have improved. Funding agencies such as international organizations and governments have increased the amount of money available for research on dengue in response to its augmenting global incidence and negative impact on public health. The Special Program for Research and Training in Tropical Diseases (TDR), an independent global program of scientific collaboration co-sponsored by the United Nations Children's Fund (UNICEF), the United Nations Development Program (UNDP), the World Bank, and the WHO, has classified dengue as an “emerging or uncontrolled disease,” where research should be directed toward the “acquisition of new knowledge and design of new disease control tools and systems” (TDR 2006a). Currently, research supported by public sector funds is focused mainly on molecular epidemiology, immune pathophysiology, second-generation vaccine discovery, and new or improved approaches to vector control (TDR 2006b). According to the WHO/TDR Scientific Working Group on Insect Vectors and Human Health (2003), vector control objectives could be rationalized by understanding the factors involved (vector, host, virus, environment) and their role in transmission dynamics at the local level.

**Dengue and scientific research in Costa Rica**

Dengue is the most important vector-borne disease in Costa Rica, and *Ae. aegypti* is the mosquito responsible for viral transmission. This mosquito species was eliminated from the country in 1960, but reinfestations occurred in some areas during the 1970s, and *Ae. aegypti* was reported throughout the country in 1993. After more than 30 years of absence, autochthonous cases of dengue fever were reported in the country at the end of
1993 (WHO 1994). Since the reintroduction of *Ae. aegypti* in Costa Rica, there have been more than 140,000 cases reported (Figure 1) (Guzman et al. 1998, Ministerio de Salud 2007, Ministerio de Salud 2005, CCSS 2006a), and all four serotypes have been detected (PAHO 2000).

![Figure 1. Number of dengue cases reported in Costa Rica, 1993 to 2005.](image)

In Costa Rica the Ministry of Health (MOH) is the entity that directs, conducts, regulates, and investigates health development, and the MOH is also in charge of health surveillance (Ministerio de Salud 2006). Dengue control measures and guidelines are generally directed by the MOH, and they include the use of insecticides and larvicides.
during epidemics, environmental care, house-to-house inspections, and educational campaigns urging the community to eliminate larval habitats and protect themselves against mosquito bites (Ministerio de Salud 2005). Associated with the MOH is the Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (Costa Rican Institute of Research and Education in Nutrition and Health) (CRIREN). CRIREN is in charge of national research and education programs in nutrition and health, according to the national policy on nutrition and health (INCIENSA 2006a). Another public institution that works closely with the MOH is the Caja Costarricense de Seguro Social (Costa Rican Social Security System) (CRSS). An autonomous institution, the CRSS is in charge of public health care services, social security, and basic pensions, and it also promotes research on and development of health care (CCSS 2006b). In addition, the general organization of the national health sector includes various ministries and institutions, including universities (specifically the health-related departments and specializations within the universities), the Instituto Nacional de Seguros (National Insurance Institute), Instituto Costarricense de Acueductos y Alcantarillados (Costa Rican Institute of Aqueducts and Sewers), and the private health sector (Ministerio de Salud 2002).

Few studies have assessed the burden of dengue on a country’s economy. Disability-adjusted life years (DALYs) have been estimated for dengue at approximately 427 DALYs per year (2001) per million population in Thailand (Clark et al. 2005), and 658 DALYs per year per million population in Puerto Rico (1984-1994) (Meltzer et al. 1998). This means that the losses from dengue are much greater than those estimated for DHF alone, and similar to those attributed to meningitis, hepatitis, or malaria in Latin America and the Caribbean (Meltzer et al. 1998). In Costa Rica, the CRSS reported
spending approximately US$1.23 million (465,876,441 Costa Rican colones) for dengue care during 2002 and US$1.45 million (605,530,103 colones) in 2003 (CCSS 2006c). Given the number of dengue cases reported in each of those years, the CRSS spent about US$100 per case in 2002 and US$74 per case in 2003 (Ministerio de Salud 2007, CCSS 2006a).

According to a 2002 analysis of the health sector done by the MOH, the overall importance of transmissible diseases has been declining in Costa Rica (Ministerio de Salud 2002), but dengue has been one of the vector-borne diseases of increasing significance (Ministerio de Salud 2007). The analysis recommended maintaining and improving surveillance systems, as well as performing epidemiological studies for implementation and evaluation of national interventions, policies, plans, and projects. In the “National health policy” for 2002-2006 that was produced by the Government of Costa Rica, HIV/AIDS and sexually transmitted diseases were the only infectious diseases for which specific policies were developed (Republica de Costa Rica 2003). However, policies on other infectious diseases were to be integrated into the other general strategies and policies. There was also a policy on “research that responds to established priorities based on the analysis of health conditions and needs of the health sector.” The strategies set for this policy included the development of a strategic agenda for research on health, strengthening of the Consejo Nacional de Investigación en Salud (National Health Research Council), development of mechanisms that guarantee funding for health research that responds to health priorities, and integration of research information and results into the health information system. The “National strategy for integrated dengue control and prevention in Costa Rica” that began in 2004 was intended to strengthen
existing programs, reduce transmission, and develop a comprehensive surveillance system. The strategy specifically includes engaging in and collaborating on entomological, epidemiological, clinical, and social research activities focused on dengue (Ministerio de Salud et al. 2004).

**Scientific research related to dengue in Costa Rica**

Most scientific research in Costa Rica is carried out by public universities and their associated research centers and institutes. While there are many private universities in the country, they produce very little research, compared to the more research-oriented (and generally larger) public institutions. Health-related departments and specializations of public and private universities are official components of the national health sector in Costa Rica. These departments and specializations are responsible for training professionals and technicians in the health fields, but also for developing research projects to strengthen and improve health (Ministerio de Salud 2002). The public universities that are most closely associated with health research and that would be generally expected to engage in research on dengue are the Universidad Nacional (National University) (NAU) and the Universidad de Costa Rica (University of Costa Rica) (UCR). The UCR is the largest public university in Costa Rica, and it is the school that does the most research in the country. UCR staff members also wrote more than 50% of the scientific publications by Costa Rican authors cited in the Science Citation Index during the period of 1999-2001 (Lomonte & Ainsworth 2002).

Research in the area of biomedical sciences is well represented in Costa Rica, and the number of publications has been increasing in recent years (Lomonte & Ainsworth
In addition to research done at public universities, biomedical research is carried out by the MOH (mainly through CRIREN), the CRSS, and other independent research institutes such as the Instituto Nacional de Biodiversidad (National Institute of Biodiversity). The MOH, CRIREN, and CRSS are responsible for many of the scientific publications in the health field. CRIREN has specific research programs in infectious diseases and molecular techniques. These research programs include dengue, as well as dengue diagnostic methods. CRIREN is the national dengue reference center, and it provides laboratory confirmation for suspected dengue cases (INCIENSA 2006b).

There are few scientific articles on dengue in Costa Rica. This is in spite of the country’s capacity for conducting high-quality scientific research, the public health importance of dengue, and the different entities where research is under way or could be developed. This situation is even worse if only scientific research (with a specific methodology, results, and analysis) that has been published in peer-reviewed journals is considered.

The lack of publications is evident when phrases such as “Costa Rica and dengue” or “Costa Rica and Aedes aegypti” are used to search in PubMed, which is the biomedical journal literature search system of the National Library of Medicine, located at the U.S. National Institutes of Health. After searching PubMed, only 11 relevant articles were found. Four of these 11 should not be considered original research pieces. One of the 4 was a general review, and 3 others were general descriptive reports carried in bulletins produced by either the Pan American Health Organization (PAHO) or WHO. Of the 7 original research papers, only the most recently published one (Iturrino-Monge et al. 2006) described the dengue situation in the country in some way (antibody detection in
children). Of the remaining 6 publications, 2 of them only included blood samples from Costa Rica for diagnostic assays (Delgado et al. 2006, Vazquez et al. 1998), one used mosquito samples collected in Costa Rica for detecting insecticide resistance (Coto et al. 2000), one tested a species of copepods from Costa Rica for possible biological control (Schaper 1999), another evaluated insecticides for control (Perich et al. 2003), and the last one dealt with the presence of dengue in bats (Platt et al. 2000). Among the 11 publications, only the most recent one (Iturrino-Monge et al. 2006) included a first author affiliated with a Costa Rican institution. In the 11 pieces found in the PubMed search, there were no articles on public health or population-based epidemiological research, none with descriptive analyses of the vector situation in the country (or specific communities), none on testing for new control approaches, and none on the efficacy of current public health interventions in the country.

Peer-reviewed health journals from Costa Rica and some other Latin American countries that are not included in PubMed can be searched through such other bibliographic databases as REVIST (produced by PAHO’s Latin America and Caribbean Center on Health Sciences Information, includes health journal articles from Costa Rica), SciELO (Scientific Electronic Library Online, produced by the State of São Paulo Science Foundation and the Latin America and Caribbean Center on Health Sciences Information, provides access to a selection of scientific journals from Latin America and the Caribbean), SciELO Costa Rica (one of SciELO’s initiatives under development, includes a selection of scientific journals from Costa Rica), LILACS (Latin American and Caribbean Literature on the Health Sciences, produced by the Latin American and Caribbean Center on Health Sciences Information), and IMBIOMED (Mexican Index of
Latin American Biomedical Journals, provides access to biomedical journals of Latin America and the Caribbean). However, these databases, which include many articles in languages other than English, are frequently overlooked by researchers and health professionals of developed countries (Clark & Castro 2002). Searches in these databases would be expected to yield more scientific publications concerning dengue in Costa Rica, and, in fact, additional articles were found when searches were performed. However, when excluding the 11 pieces already found in the PubMed search, and including only publications in peer-reviewed journals, only 19 new pieces remained. Of these 19, 6 of them were descriptive reports or literature reviews. Of the 13 other papers, 3 of them resulted from a project on biological control efforts using copepods in one community (Schaper et al. 1998, Soto et al. 1999, Cordero-Conejo et al. 2000), and another 3 from an analysis of vector populations in one neighborhood (Calderon-Arguedas et al. 2004a, Calderon-Arguedas et al. 2004b, Calderon-Arguedas et al. 2003). Two were related to clinical symptoms and diagnostic criteria (Suarez-Mastache 1999, Saenz et al. 2001a), 2 to effects of viral infection and the response in cells (Disla et al. 1996, Moya et al. 1996), and the remaining 3 included epidemiological descriptions and evaluation of surveillance (Alvarado-Brown et al. 2005, Recio-Domingo et al. 2002, Saenz et al. 2001b). Therefore, given the results of the literature searches done in databases other than PubMed, it appears that there is some information that may be useful for control activities in Costa Rica, especially in terms of descriptive epidemiological analyses, the analysis of mosquito larval habitats, and the potential for biological control. However, there is still insufficient published original research that is widely available to the scientific community. There are not many original research articles published in peer-reviewed
journals. In addition, the list of published articles shows little diversity of research topics. This is especially true for those areas related to epidemiology and public health, and the appropriateness and efficacy of current prevention and control interventions in Costa Rica.

Another source of scientific research in Costa Rica is students’ university theses. Although the results of some theses are summarized and published in peer-reviewed journals, not all of them are. Complete theses are available in Costa Rica through the universities’ libraries, although accessing them from other countries is more difficult. Therefore, the “unpublished” contents are generally not considered for further global analysis and application. Searches done via the Internet of the databases of the UCR and NAU libraries yielded 10 theses that were directly related to original research on dengue; literature analyses or reviews were excluded. Four of them were theses for the master’s degree in Epidemiology of NAU’s Regional Graduate Program in Veterinary Tropical Sciences (Posgrado Regional en Ciencias Veterinarias Tropicales, Mención en Epidemiología). Six of the theses were from various programs of the UCR (pediatrics, public health, microbiology, nursing). The 10 theses included specific evaluations and descriptions, clinical findings, epidemiological modeling, surveillance strategies, social organization and response, seroprevalence, and viral phylogenetics. This list indicates the increasing interest in various areas of dengue research at these two public universities. However, the continuing dengue problem warrants further evaluation, additional research, and broader presentation of results to the international scientific community.
International organizations such as WHO and PAHO as well as Costa Rican entities such as the MOH, CRIREN, and CRSS have published many documents on dengue, and they have dengue-related information on their Web sites. However, the MOH surveillance information is sometimes not updated for months. The web sites of the WHO, PAHO, CRIREN, MOH, and CRSS list various documents that can give an idea of the dengue problem in Costa Rica. In general, there are 5 types of documents: (1) guidelines for diagnosis, reporting, and control, (2) national programs/policies, (3) reports of surveillance and the local situation, (4) reports of experiences from prevention and control activities, and (5) summaries and results of workshops. However, many of the documents are not available for downloading in full-text format. This makes the complete documents difficult to obtain from distant locations. In addition, the majority of the documents cannot be considered valid scientific research. They do not have a specific initial hypothesis or objectives, a detailed methodology description, or a research results section. In addition, they have not gone through the final validation process of peer review by other scientists.

Possible reasons for the gap in local scientific research

The lack of scientific research on dengue in Costa Rica needs to be viewed in a national context in order to look for possible explanations. Among the possible reasons for the gap are these six: the historical context, limited human resources, limited financial resources, issues with data availability, and difficulties in collaboration. Each of these six will be briefly discussed in the paragraphs below.
**Historical context.** Dengue in Costa Rica reemerged relatively recently, in 1993, after being absent from the country for more than 30 years (WHO 1994). At that time there was probably a lack of knowledge of dengue in the health field, including among scientists and public health officials at universities, research institutes, the MOH, and the CRSS. Moreover, many of those authorities may have considered the existing knowledge on this disease system to be adequate for developing effective control and prevention programs for the country. This could have generated a slow response in local scientific research. In addition, existing research groups already had defined areas of interest in other diseases that were more relevant while dengue was absent. Many of these research group scientists are still active, and they have continued with their areas of research, thus influencing the path that new, younger scientists follow.

**Limited human resources.** The human resources in the MOH, the CRSS, and other public institutions are generally limited, so those persons have little or no time available to conduct research. Outside CRIREN, there are also few real incentives and little support for research efforts, and public institutions must prioritize their activities with the time and personnel that they have available. In addition, the fact that dengue’s reappearance is relatively recent means that there are not many scientists with a long career in dengue research. There are now no scientists, academicians, or health professionals in the country who stand out as established dengue experts, such as by having published more than five original scientific research articles on dengue or *Ae. aegypti*, and with at least one publication in an international journal.
Limited financial resources. It is estimated that the countries of Latin America and the Caribbean account for only around 2% of the worldwide funding and scientific output in the health field (Noronha et al. 2003). Local public funding for research is generally limited in developing countries, and this is the case in Costa Rica. Funding in the MOH and CRSS does not include specific funds for conducting research and writing publications, and activities must be prioritized within their limited budgets. Funding in universities is available specifically for research, but it is still limited and generally goes to research institutes and centers, or to a small number of modest, low-budget projects (in many cases with a budget of less than US$ 1,000 per year, excluding salaries).

Issues with data availability. Health statistics and surveillance data are handled by the CRSS and the MOH, and that information is usually made available to other countries of the Region through bulletins and Web sites. Data on the Web sites is sometimes only updated every few months, so recent data are generally obtained directly from the institutions. Obtaining the data may be difficult for international and even Costa Rican scientists who are not working directly with those institutions. Data on specific individual community control projects and activities is even harder to obtain.

Difficulties in collaborating on research. There are limited research collaborations between the institutions that could be involved in scientific research on dengue in Costa Rica. However, research collaborations could contribute to establishing national priorities to guide research in this specific field. In developing countries, MOH staff members are sometimes asked by researchers from other institutions (from the same country or other
countries) to assist with scientific studies, but those staff members are rarely notified about the resulting publications or credited as coauthors in the resulting articles (Basch 1999). In addition, although many research activities require effort and dedication, the public health sector perceives that there are few measurable direct benefits for the health of people in general (Basch 1999). These issues can determine whether the MOH or other governmental agencies are interested in collaborating with other organizations and individuals on research activities.

**Limited publication of results in scientific journals.** It is possible that more quality research concerning dengue in Costa Rica is being conducted, but the results are not being published in peer-reviewed journals. In fact, the 2004 final report on the “National strategy for integrated dengue control and prevention in Costa Rica” recommended participation in relevant research activities (Ministerio de Salud et al. 2004). However, researchers may not have enough time or incentives to write scientific papers, or they may be more interested in using the research results than in preparing them for publication. In public health institutions, the priority might be to take action based on experiences or results, so there are not many scientific articles resulting from their work. One other possible reason for not publishing results may be the fear of criticism experienced by public health researchers. Other investigators from national and international research institutions may criticize the methodology and comment on the validity of the results presented in publications. In addition, written and televised news reports frequently inform the public about the situation with dengue, with MOH and/or CRSS officials being interviewed or providing information. Therefore, these forms of
communication, along with bulletins and reports, may be considered sufficient for the public by MOH and CRSS government officials. If researchers do not publish scientific papers about the situation in Costa Rica, the country’s situation does not receive serious international feedback.

**Lessons from other countries in the region of the Americas**

From performing literature searches for other countries of Central America, it is evident that the situation with respect to scientific publishing on dengue in those other countries is much like the situation in Costa Rica. However, there are certain nations in Latin America and the Caribbean that have much more experience with dengue research. Two small countries that exemplify this are Cuba and Trinidad and Tobago.

The history of dengue in Cuba is somewhat different from that in Costa Rica. Cuba suffered very severe epidemics of dengue fever and DHF before the 1990s. There was an epidemic in 1977, where approximately 45% of the population was infected with DEN-1. There was an epidemic in 1981, with the DEN-2 serotype and many cases of DHF being reported. After that, dengue was effectively controlled until 1997 (Kouri et al. 1998).

Cuba has a long history of research on dengue, mainly due to the Pedro Kourí Institute of Tropical Medicine (of the Cuban Ministry of Health). This institute contains several PAHO/WHO collaborating centers including the PAHO/WHO Collaborating Center for Virology and the PAHO/WHO Collaborating Center for the Study and Control of Dengue. A search for documents related to dengue and Cuba in PubMed yielded 99 items, with 59 of them being published in 1993 or more recently. A search in LILACS
found 75 items. There were many reviews, and papers in a variety of areas, including
descriptive epidemiology, immunology, diagnostics, vector biology, control, community
organization, and pathology and immunopathology. In addition, a search of the WHO
library located 13 WHO publications, most of which reviewed and analyzed the dengue
situation, experiences, and control programs by using Cuban publications.

Cuban publications have provided scientific evidence for developing guidelines
on dengue and DHF control, as well as for diagnosis and understanding dengue
immunopathology. Cuba is one of the very few places where source reduction efforts
have had a documented success (Focks et al. 2000), and there is also published evidence
for effective intersectoral coordination and control activities (Sanchez et al. 2005). This
research and publications from Cuba are made available in the scientific literature and
have helped in Cuba and around the world to better understand this disease system and
efforts for its control. The information coming from Cuba has also been an important part
of the scientific evidence that has been used to develop WHO guidelines.

Trinidad and Tobago was declared free of *Ae. aegypti* in 1960, but the island of
Trinidad became reinfested very soon after that. However, the island of Tobago remained
free of the mosquito until 1981 (Chadee 2003). Dengue incidence increased during the
1980s and 1990s, and a major outbreak occurred in 1998 (Chadee et al. 1998). A basic
search for scientific articles containing the phrases “dengue and Trinidad”, or “*Aedes* and
Trinidad” in PubMed yielded 75 publications (33 since 1993). A similar search in
MedCarib (Caribbean Health Sciences Literature, produced by the Caribbean Net,
includes health literature from countries of the Caribbean) also listed 75 publications
Most of the 75 papers were related to dengue epidemiology and vector biology, and
included such topics as ecology, dengue transmission dynamics, population genetics, insecticide resistance, epidemiological descriptions, surveillance methods, control approaches and activities, and virology. It is worth noting that many of the articles were linked to the same researchers in Trinidad and Tobago, whose efforts have included collaborations with various international institutions and organizations.

Much of the knowledge on *Ae. aegypti* behavior and dengue transmission in the Americas has come from studies in Trinidad and Tobago. Vector control activities must consider the biology and ecology of the mosquito vector, and scientific evidence is crucial for control approaches. For example, research in Trinidad and Tobago has helped to determine local types of breeding sites that should be targeted for control, thresholds for dengue transmission (which help define vector control targets) (Focks et al. 2000), and new or more adequate methods for entomological surveillance and control (Focks & Chadee 1997, Focks 2003, Chadee 2004). The results from these and other studies of Trinidad and Tobago have been used to support many publications and reviews that are relevant to public health around the world.

Costa Rica could learn from Cuba and Trinidad and Tobago, considering how their research has been critical for the development of local and global dengue control and prevention strategies. The articles published from research conducted in Cuba and Trinidad and Tobago are a great example of interdisciplinary research and intersectoral collaboration. There are currently PAHO/WHO control guidelines that are consistent with this international research, but the growing incidence of dengue in Latin America and the Caribbean suggests that the local factors also need to be studied rigorously and not simply follow approaches that have been established in other geographical areas.
Some prevention and control methods at the local level may be more effective than others depending on the political, social, cultural, educational, environmental, and economical contexts. Therefore, control activities that are based on scientific evidence need to be adapted to the different world regions, countries, and communities.

**Additional benefits and possible negative consequences of local scientific research**

Benefits could come from local scientific research on dengue if that research produced a better understanding of the diseases system, including the local determinants of human and vector behavior, disease severity, and changing dengue epidemiology. In addition, interdisciplinary research could generate evidence concerning other dengue-related issues that are usually not considered during “traditional” infectious disease research. Interdisciplinary methods may reveal the true impact of control activities in the communities, the attitudes of the people toward the disease, and the attitudes of people toward control programs. The information obtained would serve locally to change or improve current approaches. However, this research could have an impact not only on the country where the research was done, but also on other nations in the same world region. Vector control objectives could be rationalized by understanding the factors involved and their role at the local level (Scientific Working Group 2003). Moreover, publication of information about specific experiences would make it possible to evaluate those experiences, and the experiences could provide useful insights for similar control programs in other countries.

Nevertheless, an increased but unbalanced focus on scientific research and publication on dengue in Costa Rica could harm other health programs in the country by
decreasing resources available for other health areas and other diseases. Research might not be widely accepted by government officials from the political point of view if the publication of results promotes criticism from the public toward government-run institutions. This criticism may be a consequence of unexpected “negative” results obtained while investigating public health services administrated by the government, ongoing control programs, and the true effectiveness of control activities. In addition, criticism may come from the general public because of their perception that time and economic resources are not being handled properly. This may happen if the results of publicly funded research do not directly or immediately improve the health situation or have relevance for current health policies and programs. Also, if priority areas are not clearly defined, specific areas of research that should be national or global priorities may be ignored, or there may be redundancy among various research activities, with a resulting waste of human and financial resources.

Conclusions and recommendations

As in other developing tropical countries, dengue has become a serious health problem in Costa Rica, and the situation does not seem to be improving (Fig. 1). Moreover, published scientific research related to dengue in Costa Rica is scarce, especially concerning scientific evidence for local risk factors and control activities. More research is required to support control programs with scientific evidence. In addition, research is needed in the multidisciplinary fields of knowledge that can be related to dengue. This will serve to identify local risk factors and to determine how the behavior of human, vector, and viral populations—and the interactions between them and
the environment—are affecting the disease system. Also, scientific evaluations of current control activities, their true impact on dengue transmission, and the risk for future outbreaks would enhance control and prevention efforts. In turn, this would improve strategies at the community and country level, as well as at the global level, by making the results available through scientific publications.

Health officials, scientists, and research groups in Costa Rica need to strengthen their communications and promote discussion and exchange of ideas concerning the dengue situation. As has been outlined in the national health policy for 2002-2006 (Republica de Costa Rica 2003) and the national strategy for integrated dengue control and prevention (Ministerio de Salud et al. 2004), it is important for the country to set priorities in specific areas of dengue research and to plan for the most relevant investigations. Multidisciplinary and intersectoral participation would improve the research, as dengue epidemiology is closely related to human behavior, the environment, the vector, and the virus. Working within the framework of the scientific research activities and priorities, the collaborating sectors and institutions would need to address the social, political, economical, cultural, and environmental contexts.

Increasing the research collaborations between the public health sector and the academic/research sector in Costa Rica would be beneficial for all those involved. It could lead to an optimization of research funds, human resources, and technological resources (Troyo 2006), as well as a reduction in redundant research efforts. Increased collaboration could also help bridge the gap that often exists between scientific results and public health activities, as well as help promote the acceptance of local scientific evidence and its application in the community (Troyo 2006). Collaboration between local
institutions and international entities could also be promoted. This would increase research resources, training opportunities for scientists, and possibilities for comparing the dengue situation in Costa Rica with that of other countries.

Building capacity and conducting more training in research areas related to dengue are important efforts that could be explored further by government and non-government institutions in Costa Rica. This may result from collaborations with international organizations, academic institutions, and research centers. Scientific expertise needs to be developed to carry out and promote quality health research, and building this capacity is an integral part of the health research systems at the national and global levels (Lansang & Dennis 2004). The growth in expertise could result in more resources being allocated for dengue research, as well as national and international collaboration efforts. Building the interdisciplinary research capacity within the country’s institutions is essential. The country needs dengue research experts in multiple disciplines to produce high quality research in topics like virology, entomology, epidemiology, public health, social sciences, and communication.

Although health authorities in Costa Rica realize the importance of intersectoral and multidisciplinary investigations of dengue (Ministerio de Salud et al. 2004), more efforts are needed to promote timely publication of the research results in scientific journals. This would allow the country to share its knowledge of new approaches for control activities and to receive feedback from fellow scientists. The scientific evidence would be useful in the global efforts to understand dengue and why control methods do not seem effective in many areas of the world. The information would also provide different, Costa Rican (Central American) perspectives to consider when analyzing
evidence-based global guidelines from organizations such as WHO and when assessing
guidelines for the Region of the Americas from PAHO.

The rising incidence of dengue in Costa Rica and in other areas of the world
shows the need for many different efforts to fight this disease. These efforts should
include filling the gaps in local scientific research on dengue. However, this cannot be
done without collaboration among the various sectors related to the health field,
multidisciplinary approaches to understanding and trying to solve disease problems, and
the political will to support scientific research activities.
Chapter III

Urban structure and dengue fever in Puntarenas, Costa Rica

Dengue is the most important arboviral disease in terms of worldwide morbidity and mortality with an estimated 50 to 100 million cases and 12,000 to 24,000 deaths per year (WHO 2002, Gibbons & Vaughn 2002). The principal mosquito vector, *Aedes aegypti*, lives in close association with humans mostly in urban and sub-urban environments (Service 1992). Female *Ae. aegypti* mosquitoes ingest preferentially human blood and commonly lay eggs in water-filled artificial containers such as drums, buckets, tires, flower pots, and vases, which provide habitats for the development of larvae (Service 1992, Focks & Chadee 1997, Gubler 1998, Calderon-Arguedas et al. 2004a).

A number of factors have influenced the recent dissemination of dengue viruses and *Ae. aegypti* throughout the tropics include increasing global trade, migration, and travel, population growth, and uncontrolled or unplanned urbanization (where inadequate housing, water supply, and waste collection services increase available larval habitats) (Kuno 1995). In Costa Rica, dengue is currently the most important vector-borne disease. Although *Ae. aegypti* and dengue were eliminated from the country in 1960, the vector re-infested during the 1980’s, and dengue virus was re-introduced in 1993 (WHO 1994).
Since then, there have been more than 150,000 cases of dengue fever reported (Ministerio de Salud et al. 2004), including almost 38,000 cases in 2005 (Ministerio de Salud 2007).

Remotely sensed data, together with geographical information systems (GIS), have been used in the epidemiology of many vector-borne diseases (Hay et al. 1997, Beck et al. 2000, Thomson & Connor 2000, Bergquist 2001, Correia et al. 2004); however, most of this research has been focused on the environmental characteristics that affect intermediate host or vector populations in ex-urban settings. The study of vector-borne diseases associated with the built environments pose particular challenges owing to urban spatial heterogeneity and structural complexity, complex movement of hosts and vectors, and anthropogenic creation of vector habitats. The recent launch of commercial imaging satellites such as IKONOS and QuickBird offers new opportunities to assess urban habitats for disease vectors by providing very high spatial resolutions (1 m and 0.62 m, respectively) appropriate for identification of city blocks, individual roads, trees, roadways, buildings, and rooftops (Jensen & Cowen 1999). While such imagery produces a fine-scale representation of urban environments, near-nadir observations provided by IKONOS and QuickBird imagery are relatively infrequent compared to other orbital sensors that possess a wide swath and coarse spatial resolution such as the Advanced Very High Resolution Radiometer (AVHRR) and the Moderate Resolution Imaging Spectrometer (MODIS). These latter instruments enable the study of seasonal factors (e.g., humidity, vegetation greenness, temperature) that control various physiological variables related to vector and pathogen phenology (Hay 2000, Goetz et al. 2000, Tatem et al. 2004, Hay et al. 2006).
Only a few studies have used satellite imagery to investigate environmental factors associated with dengue fever. Recent studies involving remote sensing for dengue surveillance have employed coarse spatial resolution data from AVHRR (Peterson et al. 2005, Rogers et al. 2006, Kolivras 2006), as well as medium resolution imagery and thematic maps derived from Landsat Enhanced Thematic Mapper+ (ETM+; 30 m spatial resolution) (Nakhapakorn & Tripathy 2005, van Benthem et al. 2005, Vanwambeke et al. 2006) and SPOT (20 m spatial resolution) (Tran & Raffy 2005). Using Landsat ETM+, several authors studied spatial determinants of dengue infection in specific rural and peri-urban areas (van Benthem et al. 2005, Vanwambeke et al. 2006). For other diseases like malaria, data obtained from very high resolution IKONOS and QuickBird MS bands have been used recently to study disease risk (Sithiprasasna et al. 2005a, Sithiprasasna et al. 2005b) and anopheline larval habitats (Mushinzimana et al. 2006, Jacob et al. 2006).

There are no scientific studies published that have used satellite imagery with very high spatial resolution (<5 m) in multispectral (MS) bands to assess *Ae. aegypti* habitats within urban areas. Moreover, although aerial photography can provide image data appropriate for urban feature extraction, the high costs of aerial missions often prohibit frequent image acquisition and thus reduce the ability to monitor spatiotemporal dynamics of larval habitats within seasons (Moloney et al. 1998). In addition, the very high spatial resolution of the panchromatic band on commercial imaging satellites such as QuickBird coupled with MS information in the visible and near infrared (NIR) bands enables semi-automated identification and mapping of small structures in urban areas that may indicate the presence of habitats and/or incidence of dengue fever.
In this study, relevant spatial and seasonal determinants of dengue incidence were investigated for the Great Puntarenas area, Costa Rica, for the years 2002 to 2004. The approach involved a series of exploratory data analyses of static urban structural features (e.g., houses and other buildings, roads, parks, etc.) as well as dynamically changing variables (e.g., greenness, rainfall) derived through remote sensing and ground data. The choice of variables was informed by a number of factors including the likelihood of obtaining acceptable classification accuracies for relevant urban objects such as trees, houses, and paved surfaces, as well literature on modeling and epidemiological analyses that incorporated static and dynamic spatial variables to explain spatial patterns of dengue incidence and spread (Focks et al. 1993, Nakhapakorn & Tripathy 2005, Tran & Raffy 2005, Kolivras 2006). Thus, the purpose of this study was threefold: 1) to obtain basic spatial information on the urban environment of Puntarenas using satellite and ground-based data, 2) to correlate this information to epidemiological data gathered by local public health authorities, and 3) to explore relationships between specific urban structural metrics and disease parameters to further our understanding of urban features that may favor the spread and persistence of dengue fever in the tropics.

**Methods**

**Study site**

The study focused on the Greater Puntarenas area, Costa Rica, which is located on the Pacific Coast of the country (Figure 2). This area includes the districts of Puntarenas, Chacarita, El Roble, and Barranca, and each district is divided into several localities. The city of Puntarenas is the capital of the province of Puntarenas and is located on a
peninsula and nearby mainland areas that have served as a major tourist destination over the past several decades. The climate in Puntarenas is tropical: mean minimum and maximum daily temperatures are 22°C and 32°C respectively, and there is a marked wet season (May to mid-November) and a dry season (mid-November to April). Within the study area of approximately 20 km², census data indicate the presence of roughly 100,000 people and 20,000 houses, most of which (> 95%) are classified as urban, although there is a marked heterogeneity in their sizes, crowding, and construction quality (INEC 2002). Puntarenas is the site of dengue reintroduction to Costa Rica in 1993 (WHO 1994), and it has been greatly affected by dengue ever since. From 2002 to 2005, more than 7,000 cases of dengue were reported by the Ministry of Health in this area, and most cases reported after 2000 have been caused by the DEN-1 serotype (Ministerio de Salud 2007).
Localities of Greater Puntarenas

Figure 2. Map of the localities of Greater Puntarenas, Costa Rica.

Local data

The weekly number of dengue cases reported in 2002, 2003, and 2004, number of households, estimated population, and line drawings of geographical boundaries for each of the 30 localities in the Greater Puntarenas area were obtained from the local Ministry of Health. Localities are determined by the local health authorities and include sectors of the community with similar population and social structure. Each locality has small
clinics and/or basic health teams which report dengue cases to the central clinic and Ministry of Health. House density (houses per km$^2$) was determined for each locality, and dengue incidence data per 100 population was calculated as:

$$\text{Incidence} = \frac{\text{total number of cases reported (in a year or season)}}{\text{total population}} \times 100$$

The total population was assumed to be constant during both years. To assess local climate and weather conditions, daily observations from the Puntarenas meteorological station were obtained from the National Meteorological Institute, which provided local data on daily rainfall and maximum, minimum, and mean temperatures for the same years.

**Satellite imagery and data**

Seasonality and vegetation greenness was evaluated on a monthly time scale during 2002 using the enhanced vegetation index (EVI) obtained from MODIS (500 m spatial resolution). The EVI provides greater sensitivity to changes in vegetation greenness than other widely used vegetation indices and is appropriate to track canopy phenology in tropical areas where atmospheric and background effects may introduce significant errors, which is expressed as

$$\text{EVI} = G \frac{\text{NIR-red}}{\text{NIR + } C_1 \times \text{red} - C_2 \times \text{blue} + L}$$
where NIR (near infrared), red, and blue correspond to the surface reflectance for the respective band, \( L = 1 \) and is the canopy background adjustment, \( C_1 = 6 \) and \( C_2 = 7.5 \) and are coefficients of the aerosol resistance term, and \( G = 2.5 \) and is the gain factor (Huete et al. 2002). Multitemporal EVI data were obtained from one pixel within a series of co-registered image tiles downloaded from the US Geological Survey EROS Data Center server (http://edcimswww.cr.usgs.gov/pub/imswelcome/). In addition, four scenes from ASTER (15 m spatial resolution) acquired during the dry and wet seasons of 2002 were obtained, and the individual MS bands were georeferenced using a Landsat image of 30 m spatial resolution obtained from the Global Landcover Facility (http://glef.umiacs.umd.edu/index.shtml). The normalized difference vegetation index (NDVI) was calculated from the ASTER MS bands (NDVI = \([\text{NIR}-\text{red}]/[\text{NIR}+\text{red}]\)), and the mean NDVI was extracted for each of the 30 localities using Idrisi Kilimanjaro software (Eastman 2004).

Two QuickBird scenes available for March 2002 and October 2003 were mosaicked to produce one single high-resolution image (2.4 m and 0.6 m spatial resolution in the MS and panchromatic bands, respectively), which included the districts of Puntarenas and most of Chacarita (10 localities in total). There were no single scenes available from very high resolution sensors that included a larger area and had acceptable image quality. Moreover, most of the localities are limited by natural barriers including open water and mangroves, so changes in urbanization from 2002 to 2003 are assumed to be minimal. The scenes obtained were individually georeferenced to increase their geospatial accuracy (RMS = 2.9 m and 3.1 m for the 2002 and 2003 scenes, respectively) by using 38 ground control points (GCPs) in total obtained with a hand held global
positioning system (GPS; Garmin GPSmap 76S). Accuracy of each GCP was improved further by taking the mean of three GPS readings acquired at least 5 hours apart and at series road intersections readily visible in the QuickBird panchromatic and MS bands.

Semi-automated land cover maps were produced from the QuickBird scenes by applying supervised image classifiers to each QuickBird image (Figure 3). Classification algorithms included the maximum likelihood (MLC) and back-propagation artificial neural network (BPNN) implemented in Idrisi Kilimanjaro software (Eastman 2004). These classifiers assign each pixel to a specific, predetermined land cover class such as tree, asphalt, building, bare soil, etc., and the resultant classified images were mosaicked. GIS operators were applied to the final classified products to extract data at the locality level. The panchromatic image provided a set of mutually exclusive training and validation points for the automated image classifiers. Once the thematic maps were obtained, accuracy was assessed by using points selected at random from the original panchromatic QuickBird scene. The proportion of built area and tree cover was extracted for the individual localities of Puntarenas, and FRAGSTATS software (McGarigal et al. 2002) was used to extract several spatial metrics of the classified built and tree cover areas. Specific metrics were selected to assess the spatial dispersion and clustering of various urban features such as the total class area, number of patches, patch density, percentage of landscape, percentage of like adjacencies, patch cohesion index, clumpiness index, and connectance index.
Figure 3. Methodology for obtaining the thematic maps from the sets of QuickBird multispectral (MS) bands of Puntarenas, Costa Rica.

The QuickBird images were also classified using the object-oriented classification of eCognition software (Figure 3), which usually improves classification of image objects in built environments (Tarantino 2004, Baatz et al. 2004, Al-Khudhairy et al. 2005, Carleer & Wolff 2006). Segmentation for each image was performed for level 1 at scale parameter = 20, shape factor = 0.3, and compactness = 0.7; a level 2 segmentation was performed at the same scale parameter but using the spectral difference mode. The level 1 scale parameter determined the size of the objects (corresponds to the maximum allowed heterogeneity) (Baatz et al. 2004), while the relatively low shape and high compactness factors (scaled 0-1) favored segmentation of the many small and diverse structures in this urban setting. The level 2 segmentation using spectral difference merged contiguous objects that differed in less than the specified scale parameter (Baatz et al. 2004), allowing for the formation of larger objects but maintaining the smaller ones if the
spectral difference was large. Samples for different objects were selected from the level 2 segmentation of the scenes for a hierarchical classification scheme (Figure 4), and the resulting final maps included the same classes as those obtained with the neural network classification: “water”, “built”, “tree”, “grass/bare soil”, and “paved”. Because of the simplicity of manual correction in e-Cognition software, this was performed on some objects that were evidently misclassified. Once the final thematic maps were obtained, they were imported into Idrisi Kilimanjaro to assess accuracy, produce mosaics, and extract the proportion of built area and tree cover in the same manner as was stated previously.

Figure 4. Hierarchical scheme used for object-oriented classification of QuickBird imagery of Puntarenas, Costa Rica.
There were no vector layers available for the localities in Puntarenas previous to this study. Therefore, polygon topology was built using CartaLinx software (ClarkLabs 1999) by manually digitizing each of the localities from the limits provided by the local Ministry of Health and both ASTER and QuickBird imagery (Figure 2). The resulting layer provided the areas in km² for each locality as well as the polygons required to extract data from the satellite imagery and derived maps. All image processing and GIS operations, unless otherwise stated, were performed using Idrisi Kilimanjaro (Eastman 2004).

Once the thematic maps were obtained, accuracy was assessed by using points selected at random from the original panchromatic QuickBird scene. The proportion of built area and tree cover was extracted for the individual localities of Puntarenas, and FRAGSTATS software was used to extract several spatial metrics of the classified built and tree cover areas in the same manner as was stated previously.

Data analyses

Data on daily rainfall, as well as minimum, maximum, and mean temperatures were re-organized to reflect weekly information to match the temporal aggregation of dengue case data provided by the Ministry of Health. The weekly number of dengue cases for the total Puntarenas area were plotted with the corresponding rainfall and temperature data, and an analysis of cross correlations were performed in each case to determine significant lags between the weather variables and dengue cases. A comparison was also conducted for the monthly EVI data from MODIS and dengue cases. The correlation (Pearson) was analyzed between the mean NDVI data from dry and wet seasons of 2002 and the
corresponding incidence data, as well as case data. Correlations between NDVI and house density were also evaluated.

The accuracies of the classification maps were compared, and Pearson correlations (Statistix software) were determined between dengue incidence and proportional tree cover and built area extracted from the best QuickBird thematic maps. In addition, correlation matrices were created to evaluate linear relationships between metrics of spatial dispersion and clustering of tree cover and built area (extracted with FRAGSTATS). All the statistical analyses used an $\alpha$ of 0.05.

Results

Meteorological influences on timing of dengue incidence

The Ministry of Health in Puntarenas reported dengue cases in both dry and wet seasons for all years analyzed (2002 to 2004). Specifically, 1,434 cases of dengue fever were reported in 2002, 2,017 in 2003, and 442 in 2004. During these three years, the number of cases was higher during the wet season as rainfall increased (Figure 5). The data obtained from the National Meteorological Institute contained missing values, which made the time series analysis of cross correlations difficult for the entire period 2002 to 2004. However, an analysis of cross correlations for 3 specific subperiods was possible, and a significant correlation of 0.73 ($p<0.05$) between rainfall and reported dengue cases with lag of –5 weeks was detected during the period from week 8 to week 31 in 2003.
Figure 5. Weekly number of dengue cases reported and rainfall in Puntarenas, Costa Rica, 2002 to 2004.

The ambient temperatures were also significantly correlated with the number of cases of dengue fever reported, and the use of mean, maximum, or minimum temperatures provided similar results. Overall, the analyses of reported dengue cases and cross correlations for weekly mean, maximum, or minimum temperatures for available sections of 2002 to 2004 resulted in significant negative correlations (values ranged from –0.49 to –0.64; p<0.05 in each case) with lags ranging from –1 to +2 weeks, where high correlations were commonly present when there were no lags.
Seasonal dynamics of vegetation indices

During 2002, the monthly EVI obtained from MODIS for the Puntarenas area was lower during the first and last months of the year, coinciding with the dry season, and it increased later in the usual wet season of the year. The cases of dengue fever also increased, but this was later during the year and several weeks after the initial increase in EVI (Figure 6).

Figure 6. Monthly enhanced vegetation index (EVI) and dengue reported cases in Puntarenas, Costa Rica, 2002.

The mean NDVI calculated from the ASTER MS bands was generally higher in the localities with lower incidence of dengue fever during 2002. This was evident for total reported cases during the year, as well as wet and dry seasons individually. However, the correlation was statistically significant only in the dry season (Pearson $r=-0.40; p=0.03$).
In addition, the NDVI for both seasons was negatively correlated with house density, and this correlation was stronger and more significant during the wet season (Pearson $r=-0.75$; $p<0.0001$).

*Image classification and correlations with dengue incidence*

The acceptable level of accuracy for land cover maps is usually considered to be 85% or greater for products derived from medium resolution satellite data (Wulder et al. 2006). However, multispectral classification of urban areas from very high resolution imagery commonly results in accuracies close to 80% (Sugumaran et al. 2002, Shackelford & Davis 2003a) and may pose difficulties for spectral separation of some water features and asphalt, as well as bare soils and concrete (Sawaya et al. 2003, Herold et al. 2003b). For this reason, the most accurate thematic map resulting from BPNN classification of the 2002/2003 QuickBird image was initially chosen as adequate, with an overall accuracy of 80% and Kappa of 0.74 (Figure 7). In this map, the “built” class had 24% errors of omission and 20% errors of commission, while “tree” class had 7% errors of omission and 10% errors of commission. Changes in urbanization from 2002 to 2003 were assumed to be minimal in this area; therefore, the mosaicked imagery was considered adequate for analyses of dengue case data for both of these years.
The correlation analyses for the incidence of dengue fever using the proportion of built area or tree cover for 2002 at the locality level did not reveal statistical significance (Pearson r=-0.16; p=0.66 and r=-0.27; p=0.45; respectively). However, a similar analysis for the BPNN thematic map with the 2003 dengue case data showed a significant negative relationship with built area (Pearson r=-0.74; p=0.01) and positive relationship with tree cover (Pearson r=0.75; p=0.01). In addition, the “built” class metrics obtained using FRAGSTATS did not show correlations with dengue incidence (Table 1), but the clumpiness index, patch cohesion index, and percentage of like adjacencies from the “tree” class did correlate with dengue fever in 2003 (Table 2).
Table 1. Correlation matrix of dengue incidence in 2002 and 2003, proportional built area, and FRAGTATS metrics extracted for the “built” class of the BPNN map.

<table>
<thead>
<tr>
<th></th>
<th>DI 02</th>
<th>DI 03</th>
<th>CAb</th>
<th>CPYb</th>
<th>COHb</th>
<th>CONb</th>
<th>NPb</th>
<th>PLAb</th>
<th>PLNb</th>
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DI: dengue incidence, CAb: class area, CPYb: clumpiness index, COHb: patch cohesion index, CONb: connectance index, NPb: number of patches, PLAb: percentage of like adjacencies, PLNb: percentage of landscape, PB: proportion of built area.

Table 2. Correlation matrix of dengue incidence in 2002 and 2003, proportional tree area, and FRAGTATS metrics extracted for the “tree” class of the BPNN map.

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</table>

DI: dengue incidence, CAt: class area, CPYt: clumpiness index, COHt: patch cohesion index, CONt: connectance index, NPt: number of patches, PLAt: percentage of like adjacencies, PLNt percentage of landscape, TC: proportion of tree cover.
The object-oriented classification applied to the QuickBird MS bands using eCognition software resulted in a more accurate thematic map of the Puntarenas area, where the improvement was evident in the classification of built areas (Figure 7). This map had an overall accuracy of 86% and Kappa of 0.81. The “built” class had 20% errors of omission and 11% errors of commission, while “tree” class had 14% errors of omission and 8% errors of commission. The correlations between the incidence of dengue fever during 2002 and the proportion of built area or tree cover obtained with eCognition were not statistically significant (Pearson r=0.23; p=0.52 and r=-0.46; p=0.19; respectively). Although tree cover from this map was not significantly correlated (at 95% level) to dengue incidence for 2003 (Pearson r=0.54; p=0.10), a moderate positive relationship was still observed. Furthermore, the correlation of dengue incidence in 2003 with the proportion of built area was significant (Pearson r=-0.73; p=0.02) and confirms a negative relationship between both variables. Of the FRAGSTATS metrics, only the patch cohesion index for “tree” (Table 3), and the clumpiness index and percentage of like adjacencies for “built” (Table 4) showed a significant correlation with dengue incidence in 2003.
Table 3. Correlation matrix of dengue incidence in 2002 and 2003, proportional built area, and FRAGTATS metrics extracted for the “built” class of the eCognition map.

<table>
<thead>
<tr>
<th></th>
<th>DI 02</th>
<th>DI 03</th>
<th>CAb</th>
<th>CPYb</th>
<th>COHb</th>
<th>CONb</th>
<th>NPb</th>
<th>PLAb</th>
<th>PLNb</th>
</tr>
</thead>
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<td></td>
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<td></td>
</tr>
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<tr>
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</tr>
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<tr>
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<tr>
<td>PLNb</td>
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<td>0.77</td>
<td>0.78</td>
<td>-0.08</td>
<td>0.20</td>
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<td></td>
</tr>
<tr>
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<td>0.00</td>
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<td>0.82</td>
<td>0.58</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>PB</td>
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<td>0.84</td>
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</tr>
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<td>0.78</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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</table>

DI: dengue incidence, CAb: class area, CPYb: clumpiness index, COHb: patch cohesion index, CONb: connectance index, NPb: number of patches, PLAb: percentage of like adjacencies, PLNb: percentage of landscape, PB: proportion of built area.

Table 4. Correlation matrix of dengue incidence in 2002 and 2003, proportional tree area, and FRAGTATS metrics extracted for the “tree” class of the eCognition map.

<table>
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<th></th>
<th>DI 02</th>
<th>DI 03</th>
<th>Cat</th>
<th>CPYt</th>
<th>COHt</th>
<th>CONt</th>
<th>NPt</th>
<th>PLAt</th>
<th>PLNt</th>
</tr>
</thead>
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<tr>
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<td></td>
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<td></td>
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</tr>
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<td></td>
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<td></td>
</tr>
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</tr>
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<tr>
<td>p-value</td>
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<td>0.08</td>
<td>0.50</td>
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<tr>
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<td>0.27</td>
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<td></td>
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<tr>
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<td>&lt;0.01</td>
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<td>1.00</td>
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<td>0.35</td>
<td>0.79</td>
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<tr>
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<td>0.86</td>
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<td>-0.86</td>
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<td>&lt;0.01</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.63</td>
</tr>
</tbody>
</table>

DI: dengue incidence, Cat: class area, CPYt: clumpiness index, COHt: patch cohesion index, CONt: connectance index, NPt: number of patches, PLAt: percentage of like adjacencies, PLNt percentage of landscape, TC: proportion of tree cover.
Discussion

The reports of dengue fever cases in Puntarenas showed differences between the years 2002, 2003, and 2004, but clear seasonality within each year. The variation in the number of dengue cases between years could be explained by an earlier start of the rainy season and more total rainfall during 2003 (Ministerio de Salud 2007), as well as herd immunity to the circulating serotype (DEN-1) and improved vector control during 2004 in response to the high incidence during 2003. Seasonality is consistent with results described for other urban areas and suggests that *Ae. aegypti* larvae may be found mostly in outdoor containers that collect rain water (Focks & Chadee 1997, Chadee 2003, Morrison et al. 2004a, Morrison et al. 2006). The increased availability and persistence of larval habitats during the wet season would lead to increases in mosquito densities. An increase in virus transmission following rainfall in Puntarenas is also supported by the lag of –5 weeks observed between cases of dengue fever and rainfall in 2003. Previous studies have reported a similar lag of approximately one month between significant rainfall and dengue cases (Nakhapakorn & Tripathy 2005). This lag may be explained as a sequence of events or a causal chain in which mosquito habitats and densities increase, mosquitoes become infected followed by an extrinsic viral replication phase in the mosquito, infectious mosquitoes transmit the virus to susceptible humans, an incubation period human infection follows, which results in a high proportion of infected people developing symptoms and reporting to local health centers.

Monthly data from MODIS showed that increases in EVI were followed by increases in cases of dengue fever in the Puntarenas area. Vegetation indices (like NDVI
and EVI) can reflect temporal fluctuations in rainfall, due to the changes in photosynthetic activity and in response to soil moisture variations (Thomson et al. 1996). Temporal variations in NDVI were reported as highly predictive for cases of dengue in Mexico (Peterson et al. 2005) and have been studied in more detail for other mosquito-borne diseases such as malaria (Hay et al. 1998, Patz et al. 1998, Gemperli et al. 2006, Liu & Chen 2006). These relationships are usually difficult to assess within small areas using satellite imagery due to the low temporal resolution of high spatial resolution sensors such as QuickBird. In spite of this, the results obtained with the EVI data from MODIS for the Puntarenas area were in concordance with what was observed for weekly rainfall data.

The relationship between dengue fever incidence and NDVI was not clear in Puntarenas, even though the negative correlation between house density and NDVI was strong. The distribution of mosquito-borne diseases has been related spatially to vegetation indices, primarily NDVI. Vector densities and disease are usually higher in areas with high NDVI for diseases like yellow fever and malaria, where common vectors and vector habitats are present in forest, rural, or crop areas with green vegetation (Nihei et al. 2002, Sithiprasasna et al. 2003, Rogers et al. 2006). In the case of dengue, an inverse relationship between disease and NDVI would be expected seeing as NDVI and house density are inversely correlated (Eisele et al. 2003) and the main vector, Ae. aegypti, is closely associated with humans and urban environments (Service 1992). However, this relationship was not detected in the urban environment of Puntarenas. Recent mapping suggests that other variables related to temperature are more important
than vegetation indices in determining dengue distributions at a global scale (Rogers et al. 2006), but more studies are needed to confirm this pattern at a finer scale.

The only significant negative correlation between NDVI and the incidence of dengue fever observed in Puntarenas during the dry season of 2002 may reflect seasonal differences regarding the most productive types of larval habitats. It is possible that for the dry months, localities in Puntarenas with low NDVI (reflecting different vegetation types, less vigorous vegetation, photosynthetic activity, and/or soil moisture) and high house densities presented more dengue cases due to higher human densities that may facilitate virus transmission, as well as specific types and indoor location of the relevant and more productive Ae. aegypti larval habitats. The type of larval habitats may have been containers that are manually filled with water, such as flowerpots, vases, laundry wash tubs, and water storage containers (drums and large buckets). These types of larval habitats are common during the dry season in other urban areas of Costa Rica (Calderon-Arguedas et al. 2004b). In contrast, the spatial distribution of dengue fever and higher vector densities within the localities in Puntarenas during the wet season may have been more affected by other factors related to human activity and housing, as well as the effects of rainfall and vegetation on the productivity and type of relevant larval habitats.

Compared to the NDVI obtained from ASTER, the image classification of QuickBird scenes using both BPNN and object-oriented approaches evidenced more detailed relationships between the incidence of dengue fever and urban structure in Puntarenas. Since NDVI is a measure of photosynthetic activity and vegetation “greenness”(Thomson et al. 1996), it usually results in high values (e.g., >0.5) for all types of photosynthetically active vegetation including trees, grasses, and small shrubs,
while other surfaces such as concrete, tin roofs, soils, and impervious surfaces display low values (e.g., <0.3). Therefore, a mean value for a locality like the one used in this study may capture only a small portion of detailed differences for each type of surface, making it difficult to discern relationships especially during periods of low incidence and using small sample sizes. On the other hand, because the very high spatial resolution of satellite sensors like QuickBird allows identification of small objects within the highly heterogeneous urban environment (Tarantino 2004), the QuickBird imagery of Puntarenas permitted an acceptable separation of built and tree classes from those areas where \textit{Ae. aegypti} would be rarely present such as uncovered roads, large water bodies, and sections grass or bare soil.

In Puntarenas, localities with less built area and more tree cover seemed to have more virus transmission during 2003. However, the relationships were not significant for either map in 2002, which may be an effect of the small sample size in addition to fewer dengue cases, different distribution and/or types of mosquito larval habitats, and different vector control activities taking place. In general, previous studies that have employed satellite imagery have shown that dengue is related to urban or built up areas as opposed to forested areas (Nakhapakorn & Tripathy 2005), but tree cover may play a different role within cities. Given the negative relationship between dengue cases and NDVI during periods of low incidence (dry season) in Puntarenas, a negative relationship with tree cover and positive relationship with built area were expected. However, the inverse was true within this urban setting. This relationship may be similar to what has been suggested for the number of potential anopheline larval sites and household density in urban areas of Kenya (Keating 2003), where the number of habitats first increases with
house density but then decreases when density is very high. Even though these results should be viewed with caution due to the small sample size and imperfect accuracies of the thematic maps obtained, these findings in the urban environment could be explained by increased mosquito densities and availability of larval habitats outside households or built areas and in locations exposed to rainfall.

Although more people are present in densely built-up areas within cities, there may be less dengue fever reported in some densely built-up areas due to better housing types, smaller back yards, less tree cover, and the presence of more commercial and business buildings. Houses and buildings with better construction that have windows, screens, and possibly air conditioning provide an initial barrier for mosquito and host contact. In addition, water storage for personal use may not be needed as much in these densely built-up locations. Thus, there are probably fewer containers exposed to rainfall that would be suitable for the development of mosquito larvae. Several studies have shown that outdoor habitats protected from direct sunlight are more likely to contain larvae of Ae. aegypti (Vezzani et al. 2005, Bisset-Lazcano et al. 2006, Barrera et al. 2006a). For this reason, more suitable habitats for Ae. aegypti larvae have also been associated with tree cover (Vezzani et al. 2005, Bisset-Lazcano et al. 2006, Barrera et al. 2006a), where shade provided by trees in open areas such as back yards and parks may protect habitats from heating and direct sunlight that may reduce rates of evaporation.

The correlation matrices that included metrics obtained with FRAGSTATS software (McGarigal et al. 2002) showed that characteristics of tree cover and built areas, such as their dispersion and within class relationships, may be useful for determining possible relationships between urban structure and the spatial distribution of dengue fever
within cities. FRAGSTATS metrics provided more detail as to the spatial structure of built and tree categories, and those metrics extracted from the object-oriented classification provided more robust metric values than those obtained from the other image classification methods. Even though some of the variables were correlated to dengue incidence in 2003, most of these were also strongly correlated to the proportion of tree cover or built area. Thus, the application of FRAGSTATS variables like clumpiness index, patch cohesion index, and percentage of like adjacencies warrant further evaluation to determine the most useful variables and their possible relationships with dengue fever.

The overall classification accuracy of the QuickBird imagery using BPNN was acceptable, image segmentation increased accuracy in concordance with previous reports (Thomas et al. 2003, Carleer & Wolff 2006). The individual classes were more uniform and better defined after the object-oriented classification, and most of the speckle within patches was reduced (Figure 7). It is possible that better relationships and different relevant variables may be revealed by improving classification accuracy even further through inclusion of more detailed information on texture, shape, and relationship to neighbors and sub-objects (Shackelford & Davis 2003a, Shackelford & Davis 2003b, Tarantino 2004), as well as additional spectral information and increase in sample size (Roessner et al. 2001).

Although bivariate linear regression and selection of multiple regression models have been used previously (Eisele et al. 2003, Nakhapakorn & Tripathy 2005), the small number of localities is a limitation of our study that could be addressed in future analyses. Regression and correlation require independence for one of the variables, which in this
study would mean each locality. Even though localities are spatially bounded and independence was assumed, most of the boundaries between localities cannot be considered barriers to mosquito or human dispersal, as they have no discernable influence on allowing movement of infected and susceptible populations. However, dispersal of vector populations may be somewhat limited, since some studies suggest that *Ae. aegypti* females frequently do not travel more than 100 to 200 meters (Muir & Kay 1998, Harrington et al. 2005, Russel et al. 2005), and busy roads may act as barriers to their movement (Russel et al. 2005). Since one of the purposes of this study was to identify relationships that may explain variation in dengue incidence, spatial autocorrelation was not considered, although tests for autocorrelation may reveal that it should be accounted for in developing statistical and deterministic spatial models for prediction of dengue fever or vector distributions.

It is important to mention that the possible relationships discussed above between tree cover, built areas, and incidence of dengue fever assume that virus transmission is taking place in the locality of residence, and that it is associated to vector densities and *Ae. aegypti* larval habitats. Also, differences in human behavior, education and control activities in the localities may have been coincidently associated to dengue fever and pose additional explanations for the relationships obtained. It should be noted that mosquito movement is usually not widespread and that transmission, as well as productive mosquito habitats, are frequently clustered at finer levels such as specific houses or neighborhoods (Strickman & Kittayapong 2002, Chadee 2004). Nevertheless, the locality level served as an initial exploration into the possible relationships between
dengue fever and structures of the urban environment that are observable using very high-resolution satellite imagery.

These results should be in the interest of local authorities and urban planners who may seek specific alternatives for dengue prevention and control in sites where vegetation, tree cover, and open areas are frequent. In addition, these approaches may serve to develop risk maps and identify locations within cities that may be targeted for enhanced prevention and control activities. Variables related to tree cover and built area would be helpful inputs when building more robust models and risk maps to make rapid predictions and locate priority zones for control activities, such as insecticide spraying, education, and source reduction campaigns, especially in areas where prompt action is required and limited or no detailed epidemiological and entomological data is available. In this sense, remotely sensed imagery provide a means to guide precision vector control similar to how it has been used to support precision agriculture (Beeri & Peled 2006), which targets areas within agricultural zones that require specific inputs (e.g., fertilizers, pesticides, herbicides) at critical times of the seasonal cycle.

Conclusions

In the urban environment of Puntarenas, it was possible to detect relationships between dengue incidence and local weather data, as well as remotely sensed information extracted from various sources. While weather data and vegetation indices gave insight into temporal patterns of dengue fever at coarse-to-high resolution, the spatial associations using NDVI were not clear especially during the wet season and in a year (2002) when dengue incidence was low. Although dengue fever has been inversely
related to vegetation and directly associated to urban areas at coarse spatial scales (Nakhapakorn & Tripathy 2005), the opposite seems true when analyzing dengue fever distribution at a local scale within the urban environment.

Advanced classification algorithms applied to high-resolution satellite imagery provided useful maps and information for the analysis of dengue fever within urban Puntarenas. Although remotely sensed data may not be useful to detect directly the usually small habitats for Ae. aegypti larvae (Moloney et al. 1998), the information on built environment and tree cover used in this study offered variables that may capture certain emergent properties of urban structure favoring mosquito habitats and disease transmission. Furthermore, these may be introduced into complex models and maps to identify locations of higher dengue incidence and take the appropriate actions. Many of the limitations for the use of GIS and remote sensing in the epidemiology of vector borne diseases in urban environments are currently being addressed with commercial satellites and competition, but some still remain due in part to the limited availability of very high resolution data in some tropical areas, low temporal resolution, lower classification accuracy of detailed thematic maps, and the level of expertise required to manage and develop them adequately.
CHAPTER IV

A geographical sampling method for surveys of mosquito larvae in an urban area using high-resolution satellite imagery

Field evaluations for studying the epidemiology of mosquito-borne diseases in urban areas are commonly performed in locations where densities of mosquitoes and their habitats are known to be high. In addition, surveys are often restricted to sampling of households and buildings during surveys (Morrison et al. 2004a, Chadee 2003). In *Aedes aegypti* surveillance, houses are usually sampled during pupal/demographic surveys, and houses are a main component of two traditional larval indices: House (or Premises) index (HI) and Breteau index (BI) (Focks & Chadee 1997, Focks 2003, Chadee 2004). In all cases, the resulting sampling frame may exclude locations within the complex urban environment such as streets, public buildings, parks, and schools that may provide valuable information about mosquito diversity and types of larval habitats. Therefore, in the case of diseases that are usually considered “urban” like dengue fever and dengue hemorrhagic fever, productive habitats may be overlooked during standard household surveys and bias the results. Sampling strategies for selecting mosquito collection sites may need to include non-residential locations in field surveys, such as those required for studying dengue and other vector-borne diseases of urban environments (Morrison et al. 2006, Barrera et al. 2006a).

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Geographical information systems (GIS) and remote sensing offer powerful tools for describing, illustrating, explaining, and predicting epidemiological phenomena, which can be used to develop or improve surveillance, prevention, and control strategies (Rogers & Randolph 2003). However, these technologies have been used to study vector-borne diseases mostly in non-urban areas and at very broad scales (Hay et al. 1997, Hay et al. 2000, Beck et al. 2000, Rogers et al. 2006). Data currently available from sensors like the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER, 15 m spatial resolution) and QuickBird (0.6 m panchromatic and 2.4 m multispectral spatial resolution) are useful for studying factors that affect diseases within the heterogeneous urban environment. In this report, a sampling strategy is described for the Great Puntarenas area, Costa Rica. This method was developed for sampling specific mosquito larval habitats using GIS technology and high-resolution satellite imagery from ASTER and QuickBird.

Materials and methods

The study site included 10 localities of the Greater Puntarenas area, a city on the Pacific coast of Costa Rica where dengue fever is currently endemic. Puntarenas is the site of dengue reintroduction to Costa Rica in 1993 (WHO 1994), and no detailed entomological or georeferenced data in the form of GIS layers were available at the beginning of this study. High-resolution satellite images were obtained for the Greater Puntarenas area to develop the sampling strategy. Only two QuickBird scenes from March 2002 (dry season) and October 2003 (wet season) were available at very high resolution, each including a different section of the study site. Multispectral bands (blue,
green, red, and near infrared) and the panchromatic band were obtained. In addition, ASTER imagery was available for those same years. All the GIS operations were performed using Idrisi Kilimanjaro software (Eastman 2004).

A classified land cover map generated from the mosaicked 2002 and 2003 multispectral QuickBird imagery by using the back propagation artificial neural network (ANN) in Idrisi Kilimanjaro (Eastman 2004) was selected for the analyses. Training sites for “water”, “tree”, “grass/bare soil”, “built”, and “paved” classes were developed using polygons digitized from visual interpretation of the 0.6 m QuickBird panchromatic band. The ANN algorithm produced a land cover classification with an overall accuracy of 80% and Kappa of 0.74, which was more accurate than those produced by other classification algorithms evaluated such as maximum likelihood. The “built” class had 24% errors of omission and 20% errors of commission, while “tree” class had 7% errors of omission and 10% errors of commission. Most of the Greater Puntarenas area is limited by natural barriers including open water and mangroves, so changes in land cover classes caused by urbanization from 2002 to 2003 were assumed to be minimal.

The “built” class from the land cover map provided patches of pixels that represented individual houses and small buildings in Puntarenas. Since ASTER imagery was already available, grids of different sizes were obtained from it and were used to estimate the mean number of houses/small buildings per cell extracted from the land cover map. According to the mean number of houses/small buildings per area, an optimal grid cell area that would be operationally adequate was estimated at 10,000 m² (Fig. 8). At this cell size, the number of houses per cell was approximately normally distributed and contained 13±6 houses (Shapiro-Wilk normality test W=0.976, p=0.738). A smaller
cell size would not contain enough houses and would require traveling long distances frequently. Therefore, a cell size of 100 by 100 m was considered large enough for a team of 2 people to search in half a day (approximately 3 hours at 15 minutes per house).

Figure 8. Grid cell sizes from ASTER and mean house numbers estimated from QuickBird for Puntarenas, Costa Rica.

A final grid was created using the multispectral Quickbird imagery, and cells were grouped according to each of the 10 localities of Puntarenas included in the cover map. This final grid contained cells 42 by 42 pixels (100.8 x 100.8 m), and only the 355 cells that had more than 90% of their area within one specific locality of Puntarenas were
included in the sampling frame. This would allow a stratified sampling method (below) and guarantee that every larval habitat found in a grid cell searched could be considered as belonging to one locality.

Cells were numbered and a stratified random sample was selected from each locality, which was proportional to the total number of cells. Localities in Puntarenas have been geographically determined by the Ministry of Health. These localities, which correspond to proximate groups of houses where the people usually share socioeconomic characters, are to be serviced by a local health clinic. The stratified sampling method was performed to ensure at least one sample set of each of the 10 localities, which would improve representativeness of the total sample. The random sample consisted of 36 cells, approximately 10% of the total 355 cells (Fig. 9). This number of grid cells was selected such that the time taken to collect the field data would not exceed 3 weeks, since it was necessary for the entomological data to be analyzed within approximately homogeneous external environmental conditions of each season.

Figure 9. Sampling frame developed for the Greater Puntarenas area, Costa Rica, showing the random sample of cells (10%) selected for the entomological field studies.

To initially assess the representativeness of the selected sample grid cells, the QuickBird land cover map was used to extract the proportion of tree area (“tree” class
Kappa = 0.91) in individual grid cells, as well as in the total area of the localities. Tree cover was evaluated because larval habitats have been associated with shade and especially vegetation (Barrera et al. 2006a, Vezzani et al. 2005, Bisset-Lazcano et al. 2005). For each locality, the mean percentage of tree cover in the selected sample cells was compared to the mean percentage of tree cover in the total cells and the percentage of tree cover in the total area of the locality.

The resulting grid with the selected cells was overlaid on the QuickBird panchromatic image for identification and visualization of the location and limits of the specific cells. The maximum and minimum coordinates for the selected cells also served to determine their position while the teams were in the field with global positioning system (GPS) units. By displaying the cells on the QuickBird panchromatic image, printing the images, and taking them to the field, small features that serve as visual limits for the survey cells like roads, houses, and trees can be identified (Fig. 10).
The first entomological survey that applied the sampling method was performed during the wet season 2006 (July and August). The area within each of the selected grid cells was searched for all potential larval habitats, most of which were the traditional “wet containers” (places or objects that held water for more than one day and seemed able to maintain this condition for more than 48 hours). Within the grid cells, numbers
were assigned to each “location”, which was any legally limited section of land that may or may not include a house or building (such as parks, streets and sidewalks, households, lots, churches, construction sites, buildings, parking lots, and schools). In the cases where the limit of the grid cell fell on the footprint of a house or building, only the structures on the North and West boundaries of the cell were considered completely (the structures on the South and East boundaries were not evaluated). This method would cancel out the additional and missing portions of the properties in the limits of the cell. When there were houses in the grid cells, the number of persons living in the house was noted.

All possible habitats were characterized according to their location (household or non-household, and private or public area), type, and size. When present, all pupae and a sample of the larvae were collected and processed as has been done in other areas of Costa Rica (Calderon-Arguedas et al. 2004a, Calderon-Arguedas et al. 2004b). The specimens were transported in glass vials with 70% ethanol to the Medical Arthropodology Laboratory, University of Costa Rica, where they were cleared in lactophenol, mounted in Hoyer’s medium, and identified. The presence of *Ae. aegypti* larvae, as well as the number of *Ae. aegypti* pupae was noted in order to determine the Container Index, Location index, Breteau Location Index, pupae per area and pupae per person. These larval indices are analogous to traditional Container, House (or Premise), and Breteau Indices (Focks 2003) but considering all household and non-household locations in their calculation:
Container Index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 potential habitats.

Location index: Number of locations positive for *Ae. aegypti* larvae and/or pupae per 100 locations.

Breteau Location Index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 locations.

**Results**

The initial assessment of the selected sample grid cells showed representativeness in terms of tree cover for most of the localities. In 8 of 10 localities the difference between the estimated percentage of tree cover (from sample cells) and the real percentage of tree cover was less than 3% (Table 5). The proportions of tree cover and built area are being used for detailed analyses of urban structure and dengue, which will be published elsewhere.
Table 5. Entomological observations for households, non-household sites, and total area of the sample cells surveyed in Puntarenas, Costa Rica.

<table>
<thead>
<tr>
<th>Locality</th>
<th>% tree of total area</th>
<th>% tree mean of total cells</th>
<th>% tree mean of sample</th>
<th>Standard Error*</th>
<th>Difference †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrio El Carmen</td>
<td>22.62</td>
<td>25.15</td>
<td>22.15</td>
<td>5.51</td>
<td>-3.00</td>
</tr>
<tr>
<td>El Centro</td>
<td>15.36</td>
<td>16.35</td>
<td>17.75</td>
<td>3.84</td>
<td>1.40</td>
</tr>
<tr>
<td>El Cocal</td>
<td>18.82</td>
<td>19.07</td>
<td>29.74</td>
<td>5.80</td>
<td>10.67</td>
</tr>
<tr>
<td>Veinte de Noviembre</td>
<td>41.46</td>
<td>40.60</td>
<td>49.31</td>
<td>8.05</td>
<td>8.71</td>
</tr>
<tr>
<td>Chacarita</td>
<td>26.51</td>
<td>20.82</td>
<td>18.84</td>
<td>5.96</td>
<td>-1.98</td>
</tr>
<tr>
<td>Fray Casiano</td>
<td>41.82</td>
<td>38.28</td>
<td>38.55</td>
<td>4.77</td>
<td>0.27</td>
</tr>
<tr>
<td>San Luis</td>
<td>50.53</td>
<td>48.09</td>
<td>47.85</td>
<td>9.54</td>
<td>-0.24</td>
</tr>
<tr>
<td>Carrizal</td>
<td>40.37</td>
<td>41.38</td>
<td>42.48</td>
<td>9.58</td>
<td>1.10</td>
</tr>
<tr>
<td>El Huerto</td>
<td>54.82</td>
<td>54.38</td>
<td>56.75</td>
<td>19.61</td>
<td>2.37</td>
</tr>
<tr>
<td>Linda Vista</td>
<td>54.36</td>
<td>50.21</td>
<td>48.47</td>
<td>12.46</td>
<td>-1.74</td>
</tr>
<tr>
<td>Total area</td>
<td>33.60</td>
<td>32.97</td>
<td>35.08</td>
<td>3.20</td>
<td>2.11</td>
</tr>
</tbody>
</table>

* Standard error corrected for finite populations: 

\[
\left[ \frac{\text{population standard deviation}}{\sqrt{n}} \right] \left[ \frac{(\text{total cells}-n)}{(\text{total cells}-1)} \right]^{\frac{1}{2}}
\]

† Difference = % tree mean sample – % tree mean total cells

During the wet season survey, a total of 581 locations were searched for mosquito larval habitats. The locations mainly included houses but also many non-household locations such as empty lots, streets, parks, soccer fields, public schools, churches, offices, and commercial structures (Table 6). Twenty-six locations that were not categorized as houses harbored one or more positive containers, which represent 26.3% of all larvae-positive locations. Of 830 potential habitats observed, 20.6% were found in non-household locations (9.3% were in public areas), and 16.7% had mosquito larvae and/or pupae. Of mosquito-positive habitats, 29.5% were not in or around houses, and most of these habitats were observed in empty private lots. Most of the positive habitats (78%) contained immature stages of *Ae. aegypti*, and the second most abundant species was *Culex quinquefasciatus* (in 15.8% of positive containers). If only the houses were searched for mosquito larval habitats, 41 positive habitats would have been overlooked.
(25 containing larvae and/or pupae of *Ae. aegypti* and a total 85 *Ae. aegypti* pupae). Table 6 also presents the entomological indices when the total area in the cells is considered as opposed to only the houses. Results for the complete entomological and house surveys by locality, container profiles for wet and dry seasons, mosquito diversity, and associations with urban structure in the Greater Puntarenas area will be published elsewhere.

Table 6. Entomological observations for households, non-household locations, and total area of the sample cells surveyed in Puntarenas, Costa Rica.

<table>
<thead>
<tr>
<th></th>
<th>Households (%)</th>
<th>Non-household locations (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Private</td>
<td>Public</td>
<td>Total</td>
</tr>
<tr>
<td>No. locations evaluated</td>
<td>463 (79.7)</td>
<td>80 (13.8)</td>
<td>38 (6.5)</td>
</tr>
<tr>
<td>No. locations with larvae-positive habitats</td>
<td>73 (73.7)</td>
<td>16 (16.2)</td>
<td>10 (10.1)</td>
</tr>
<tr>
<td>No. potential larval habitats</td>
<td>659 (79.4)</td>
<td>94 (11.3)</td>
<td>77 (9.3)</td>
</tr>
<tr>
<td>No. larvae-positive habitats</td>
<td>98 (70.5)</td>
<td>28 (20.1)</td>
<td>13 (9.4)</td>
</tr>
<tr>
<td>No. <em>Ae. aegypti</em> pupae</td>
<td>445 (84.0)</td>
<td>36 (6.8)</td>
<td>49 (9.2)</td>
</tr>
<tr>
<td><em>Ae. aegypti</em> pupae per person</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Container Index</td>
<td>12.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Location Index</td>
<td>14.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Breteau Location Index</td>
<td>18.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Households + total non-household locations.

**Discussion**

These results support the geographical sampling strategy reported and show that it would yield slightly different and more exact entomological indices than a traditional household survey performed in the same areas. This research shows that in the urban ecosystem of Puntarenas an important portion of the habitats containing mosquito larvae was not in or around houses. As has been reported previously (Morrison et al. 2006, Mahadev et al. 2004), these locations should be considered when studying mosquito ecology or diversity in urban areas, as well as for directing source reduction activities in
dengue prevention and control. As in all studies, the main objective and resources available will determine the best sampling method, although calculating the entomological indices “geographically” would be more accurate than using only household surveys for entomological surveillance in most cases. There would be more detailed information available to direct control strategies, by providing, for example, key mosquito habitats in public areas that are not the direct responsibility of the household owners and may need to be eliminated by public health officials or the local municipality.

In spite of the cited advantages of this method during research and in areas where recent data is not available, it may not be the most suitable for continuous entomological surveillance. This method required GIS knowledge and high-resolution satellite imagery to determine the optimal cell size using the built structures per area and to accurately detect cell boundaries in the field. The optimal methodology would include imagery that is temporarily accurate, and this depends on how fast the urban landscape is changing in the study site. Recent satellite imagery can be costly, especially for programs in developing countries (QuickBird imagery cost is approximately USD $1,300 for the minimum area of 12 km²). Multispectral data from medium resolution sensors like Landsat and ASTER may provide an alternative that is less expensive, but their resolution does not allow for identification of individual houses, small roads, and buildings. While both ASTER and QuickBird data were used in this study, QuickBird imagery may be used to create the grids and calculate houses per area when coarser resolution imagery is not available. In some cases, aerial photography or local vector layers are available at the house level, and these can be substituted for the satellite image layer, depending on the final objective of the research and surveys. However, if an urban area is not under
constant change and once the optimal cell size has been determined, imagery may be obtained every two or three years, and this geographical method may be considered more useful for confirmation and quality control than constant surveillance.

In this chapter, remote sensing and GIS technology provided useful tools to develop a sampling frame for field studies within urban Puntarenas. Although a common approach in entomological studies, sampling areas known to have high mosquito densities may result in significant selection bias. The sampling methodology applied in Puntarenas builds on the strategy proposed by Keating et al. for sampling malaria vectors (Keating et al. 2003), which used coarser resolution satellite imagery. However, the method presented in this report shows that detail provided by high-resolution satellite imagery allows more precise calculations of optimal cell size, as well as useful information for pinpointing specific locations in urban areas and planning operations previous to the site visit. Although high-resolution satellite imagery and GIS were used to evaluate urban areas and randomly select sections aimed at obtaining data on mosquito larval habitats, this method could be applied to sample other interactions and disease systems in urban and peri-urban environments such as malaria, lymphatic filariasis, Chagas disease, and leishmaniasis. Although no entomological data were available in Puntarenas, it is possible that the selection of the cells and cell size would vary if information on vector densities, larval indices, and disease incidence is available, even though the main geographical method and principle will still be applicable. These strategies would reduce bias and provide information from the field that is both practical to obtain and representative. By selecting a geographical approach to sampling in urban
environments that guarantees inclusion of all vector habitats, significant improvements could be made to strategies for prevention and control of vector-borne diseases.
Chapter V

Seasonal profiles of *Aedes aegypti* (Diptera: Culicidae) larval habitats in an urban area of Costa Rica with history of mosquito control

Several pathogens that affect human health are transmitted by culicid mosquitoes. Mosquito-borne pathogens include parasites such as *Plasmodium* and *Wuchereria bancrofti*, as well as many viruses like West Nile, Yellow Fever, and Dengue. Dengue is the most important arboviral disease in terms of worldwide morbidity and mortality, affecting more than 50 million people per year (WHO 2002, Gibbons & Vaughn 2002). Although different control strategies are in place for mosquito-borne diseases, vector control is still considered an essential component of most disease control programs (Impoinvil et al. 2007, Ottesen 2006).

The life cycle of mosquito vectors requires that larvae and pupae develop in habitats containing water, the location, physical, and chemical properties of which may vary depending on mosquito species and local ecology (Shililu et al. 2003, Muturi et al. 2007, Calderon-Arguedas et al. in press). *Aedes aegypti*, the principal vector of dengue viruses, is closely associated with human environments in endemic areas, where indoor and outdoor artificial containers like drums, tires, buckets, flowerpots, and vases make adequate habitats for larval development (Focks et al. 1981, Service 1992, Focks &

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Chadee 1997, Gubler 1998, Calderon-Arguedas et al. 2004a). Although there are various promising trials underway (Edelman 2007), there is still no effective vaccine available for dengue; thus, prevention and control is currently targeted at avoiding human contact with mosquitoes, reduction of adult mosquito populations, and elimination of mosquito larval habitats (Gubler 1998). In addition, human behavior is one of the important factors influencing the epidemiology of dengue fever; therefore, local vector habitat profiles and control strategies will depend on the specific socioeconomic context and behavioral characteristics of the population (Service 1992). However, successful vector control requires detailed local knowledge and frequently fails due to poor sustainability and breakdown of public health infrastructure (Guzman & Kouri 2003, Gubler 2005, Chadee et al. 2005, Calderon-Arguedas et al. 2007). A recent study suggests that evidence in favor of community-based dengue control programs is weak (Heintze et al. 2007).

In Costa Rica, dengue is the most important vector-borne disease. *Aedes aegypti*, the main vector, was eliminated from the country in 1960, but frequent reinfestations occurred during the 1970s and 1980s (WHO 1994). After vector reintroduction, transmission of dengue fever was reported in 1993 in the cities of Puntarenas and Liberia (WHO 1994), and it later spread to other regions of the country. Even though dengue is a public health problem in Costa Rica, there is currently little scientific research available to guide and to evaluate local control efforts (Troyo et al. 2006), which have been continuous in areas like Puntarenas.

In Puntarenas City, Costa Rica, *Ae. aegypti* control has been practiced for more than 10 years. The organization of vector control in Puntarenas has developed into an integrated and inter-institutional approach, with a high level of inter-sector collaboration.
Currently, the techniques used to combat dengue in Puntarenas include epidemiological and entomological surveillance, environmental management, public education, and chemical control (Impoinvil et al. 2007).

The purpose of this analysis was to characterize the most prevalent and productive mosquito larval habitats in wet and dry seasons and determine characteristics associated with the presence of larval habitats and *Ae. aegypti* positivity in Puntarenas. By identifying the most prevalent and productive types of mosquito breeding sites and their distribution, these characteristics can be linked to specific human activities, which is critical for identifying, focusing, and improving current mosquito control efforts in areas with a history of vector control.

**Materials and methods**

This study was performed in 10 localities of Greater Puntarenas area (Fig. 11), which is a small port city (approximately 50,000 people) located on a peninsula in the Pacific coast of Costa Rica (Impoinvil et al. 2007). Localities in Puntarenas are geographical areas determined by the local Ministry of Health that share environmental and social characteristics. The climate in Puntarenas is tropical, with marked wet (May to mid-November) and dry (mid-November to April) seasons and average minimum and maximum daily temperatures of 22° C and 32° C, respectively. Cases of dengue fever and vector control activities have been continuous in Puntarenas ever since dengue transmission was reported in 1993 (WHO 1994).
Cross-sectional entomological larval surveys were performed during wet and dry seasons (last week of July and first week of August, 2006, and last week of January and first week of February, 2007). The geographical method detailed in Troyo et al. (2008) was applied to select the locations and perform the surveys. Briefly, grids that covered the study area were constructed using high-resolution satellite imagery (ASTER and QuickBird), and a cell size of 100 by 100 meters that contained 13±6 houses was considered appropriate for the larval surveys. A stratified random sample of 36 cells (10% from each locality) was selected where all the locations included would be searched for mosquito larval habitats.

A “location” was any legally limited section of land that may or may not include a house or building (such as parks, streets and sidewalks, households, lots, churches, construction sites, buildings, parking lots, small businesses, and schools). The categories used for location types were household, school, empty lot (small), large lot, street, field/stadium, large building, small business, and other. In each selected cell, locations were also categorized according to the entity responsible into public (usually owned by government) such as streets, government offices, parks, and schools or private (owned by individuals or private organizations) such as houses, commercial buildings, and lots. In addition, the availability of piped water, number of persons living in a house, and a
category for house construction quality were noted when grid cells included houses. House construction quality was evaluated according to Calderon-Arguedas et al. (2003), which can be associated with socioeconomic status and can affect presence of larval habitats (Kuno 1995), where “1+” is the poorest construction quality, and “4+” is the best construction quality.

All the locations surveyed in a sample cell were searched during each season for potential larval habitats, most of which were the traditional “wet containers” (places or objects that held water for more than one day and seemed able to maintain this condition for more than 48 hours). Larval habitats were characterized according to their setting (indoor or outdoor), type (can/small plastic food container, bucket, tire, drum, concrete laundry wash tub, roof gutter, domestic animal drinking container, flower pot, vase, sewer, coconut, bottle, other), and capacity (small: <2 liters, medium: 2 to 7 liters, large: >7 liters). In addition, permanent habitats were noted, which were those habitats that could not be easily moved, discarded, or tipped over and would need special treatment to be eliminated such as concrete washtubs, gutters, septic tanks, small manholes, puddles, and sewers.

The presence or absence of mosquito immature stages was noted for each habitat and when present, all pupae and a sample of the larvae were collected and processed, as described for previous surveys in Costa Rica (Calderon-Arguedas et al. 2004b). The specimens were transported in glass vials with 70% ethanol to the Medical Arthropodology Laboratory, University of Costa Rica, where they were cleared in lactophenol, mounted in Hoyer’s medium, and identified (Carpenter & La Classe 1955, Gonzalez & Darsie 1996, Vargas 1998). The presence of *Ae. aegypti* larvae, as well as
the number of *Ae. aegypti* pupae was especially noted in order to determine pupae per area and pupae per person (Focks & Chadee 1997) as well as the Container Index, Location index (Premises index), and Breteau Location Index (Focks 2003, Troyo et al. 2008):

**Container Index**: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 potential habitats.

**Location index (Premises index)**: Number of locations positive for *Ae. aegypti* larvae and/or pupae per 100 locations.

**Breteau Location Index**: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 locations.

**Analyses**

Field data were entered in EpiInfo 3.3.2 and initial analyses were performed in the same software. Chi-square tests of association were applied to determine the significance of the relationship between presence of mosquito larvae or *Ae. aegypti* larvae in a location (or house) and each of the following discrete variables: locality, location type, entity responsible, house construction quality, and number of people in a household. In the same manner, Chi-square tests were applied to determine the significance of the association between presence of mosquito larvae or *Ae. aegypti* larvae in a habitat and locality, location type, indoor/outdoor setting, habitat type, habitat capacity, and habitat disposability. The analyses were performed by season. Finally, seasonal logistic multiple regression models were analyzed using SAS 9.1 software to determine the significant
predictive variables for the presence of one or more larval habitats in a location, the presence of mosquito larvae in a habitat, and the presence of *Ae. aegypti* in a habitat (Table 7). The significance level for all statistical analyses was set at 0.05.

Table 7. Variables included in the seasonal logistic regression models.

<table>
<thead>
<tr>
<th>Outcome variable (season)</th>
<th>Predictor variables</th>
<th>Exclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>One or more larval habitats in a location (wet and dry seasons)</td>
<td>Locality, Private, People, Location type</td>
<td>Localities: El Huerto, Linda Vista. Location types: school, field/stadium, large building, other; large and small lots were considered “lots”.</td>
</tr>
<tr>
<td>Mosquito larvae in a habitat (wet)</td>
<td>Locality, Location type, Setting, Habitat type, Disposability, Capacity</td>
<td>Location types: field/stadium, large building, other; large and small lots were considered “lots”.</td>
</tr>
<tr>
<td><em>Ae. aegypti</em> in a habitat (wet)</td>
<td>Locality, Location type, Setting, Habitat type, Disposability, Capacity</td>
<td>Localities: Linda Vista. Location types: field/stadium, large building, other; large and small lots were considered “lots”.</td>
</tr>
<tr>
<td><em>Ae. aegypti</em> in a habitat (dry)</td>
<td>Locality, Setting, Disposability, Capacity</td>
<td>Localities: El Huerto, Linda Vista, San Luis.</td>
</tr>
</tbody>
</table>

*Categories excluded due to lack of sufficient data for the multiple logistic regression analyses.*
Results

Of the 36 selected cells, two were eliminated due to problems related to access in the locality of Linda Vista. Although it was not possible to gain entrance to all of the locations within a cell, more than 70% of the selected locations were evaluated in each locality (60% or more per cell). During the wet season, 581 locations were identified of which 476 (82%) were evaluated. In the dry season, 626 locations were identified, and 508 (81%) were evaluated. Some of the summarized results for the wet season have been published to support the sampling method developed for these surveys (Troyo et al. 2008). Overall, 99.5% of houses had piped water, and 99% and 98% of houses reported uninterrupted services during wet and dry seasons, respectively. In addition, there were on average 3 persons per household, and most houses had good construction quality: 33% were classified as 4+, 38% as 3+, 25% as 2+, and only 4% as 1+.

Wet season

In the wet season, 99 locations had one or more habitats positive for mosquito larvae and 82 of them (83%) contained one or more larval habitat positive with *Ae. aegypti*. Chi-square tests showed a significant association between the presence of one or more larval habitat in a location and the locality it belonged to, location type, and number of people in a house (Table 8). Locations that had larval habitats seemed less likely to be from Carmen or Centro. Also, larval habitats were more common in houses with more than 3 people and in locations such as lots, streets, and schools. Presence of mosquito
larvae or pupae in a location was also associated to location types like large lots and streets (Table 8).

Table 8. Variables and results of the independent Chi-square tests of association applied to the Puntarenas wet season data.

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variables</th>
<th>$\chi^2$</th>
<th>DF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval habitat(s) in a location</td>
<td>Locality</td>
<td>50.13</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>20.69</td>
<td>8</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>People in a house</td>
<td>5.81</td>
<td>1</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Construction quality</td>
<td>0.85</td>
<td>3</td>
<td>0.838</td>
</tr>
<tr>
<td>Mosquito larvae/pupae in a location</td>
<td>Locality</td>
<td>13.64</td>
<td>9</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>17.32</td>
<td>8</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>Construction quality</td>
<td>1.96</td>
<td>3</td>
<td>0.580</td>
</tr>
<tr>
<td>Mosquito larvae/pupae in a habitat</td>
<td>Locality</td>
<td>28.55</td>
<td>9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>19.27</td>
<td>8</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Habitat setting</td>
<td>4.72</td>
<td>1</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>82.53</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Habitat capacity</td>
<td>2.88</td>
<td>2</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>Habitat disposability</td>
<td>4.00</td>
<td>1</td>
<td>0.045</td>
</tr>
<tr>
<td>Ae. aegypti in a habitat</td>
<td>Locality</td>
<td>27.27</td>
<td>9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>3.46</td>
<td>8</td>
<td>0.902</td>
</tr>
<tr>
<td></td>
<td>Habitat setting</td>
<td>2.02</td>
<td>1</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>65.96</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Habitat capacity</td>
<td>2.82</td>
<td>2</td>
<td>0.245</td>
</tr>
<tr>
<td></td>
<td>Habitat disposability</td>
<td>0.31</td>
<td>1</td>
<td>0.575</td>
</tr>
</tbody>
</table>

The wet season logistic regression revealed that when all variables were taken together, only locality was a significant independent predictor for the presence of one or more larval habitat in a location (Table 9). For instance, locations in San Luis were 15.4 times more likely to contain larval habitats than locations in Cocal (OR: 15.4, CI: 3.8-63.3, p<0.001), 10.8 times more likely than locations in Carmen (OR: 10.8, CI: 3.0-39.6, p<0.001), and 6.6 times more likely than locations in Centro (OR: 6.6, CI: 1.8-24.4,
p=0.005). Also, locations in Carrizal were 6.7 times more likely to have larval habitats than those in Cocal (OR: 6.7, CI: 2.5-17.9, p<0.001), 4.7 times more likely than those in Carmen (OR: 4.7, CI: 2.1-10.6, p<0.001), and 2.9 times more likely than locations in Centro (OR: 2.9, CI: 1.3-6.5, p=0.013).

Table 9. Logistic regression analyses and predictors for presence of larval habitats in a location, presence of mosquito larvae in a habitat, and presence or *Ae. aegypti* in a habitat.

<table>
<thead>
<tr>
<th>Outcome variable (season)</th>
<th>Predictor variables</th>
<th>Wald $\chi^2$</th>
<th>DF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>One or more larval habitats in a location (wet)</td>
<td>Locality</td>
<td>37.50</td>
<td>7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Private</td>
<td>0.65</td>
<td>1</td>
<td>0.422</td>
</tr>
<tr>
<td></td>
<td>People</td>
<td>0.55</td>
<td>1</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>4.33</td>
<td>3</td>
<td>0.228</td>
</tr>
<tr>
<td>One or more larval habitats in a location (dry)</td>
<td>Locality</td>
<td>10.85</td>
<td>7</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Private</td>
<td>0.78</td>
<td>1</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>People</td>
<td>9.66</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>8.01</td>
<td>3</td>
<td>0.046</td>
</tr>
<tr>
<td>Mosquito larvae in a habitat (wet)</td>
<td>Locality</td>
<td>16.45</td>
<td>9</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>7.32</td>
<td>4</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>Setting</td>
<td>5.77</td>
<td>1</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>43.35</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Disposability</td>
<td>0.02</td>
<td>1</td>
<td>0.885</td>
</tr>
<tr>
<td></td>
<td>Capacity</td>
<td>1.45</td>
<td>2</td>
<td>0.485</td>
</tr>
<tr>
<td>Mosquito larvae in a habitat (dry)</td>
<td>Locality</td>
<td>10.75</td>
<td>6</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>Setting</td>
<td>0.26</td>
<td>1</td>
<td>0.607</td>
</tr>
<tr>
<td></td>
<td>Disposability</td>
<td>0.06</td>
<td>1</td>
<td>0.801</td>
</tr>
<tr>
<td></td>
<td>Capacity</td>
<td>9.70</td>
<td>2</td>
<td>0.008</td>
</tr>
<tr>
<td><em>Ae. aegypti</em> in a habitat (wet)</td>
<td>Locality</td>
<td>12.47</td>
<td>8</td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>0.31</td>
<td>4</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>Setting</td>
<td>4.11</td>
<td>1</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>35.86</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Disposability</td>
<td>5.29</td>
<td>1</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Capacity</td>
<td>2.66</td>
<td>2</td>
<td>0.265</td>
</tr>
<tr>
<td><em>Ae. aegypti</em> in a habitat (dry)</td>
<td>Locality</td>
<td>11.22</td>
<td>6</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>Setting</td>
<td>0.74</td>
<td>1</td>
<td>0.389</td>
</tr>
<tr>
<td></td>
<td>Disposability</td>
<td>0.54</td>
<td>1</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>Capacity</td>
<td>7.38</td>
<td>2</td>
<td>0.025</td>
</tr>
</tbody>
</table>
There were 829 larval habitats identified in the wet season surveys with 139 habitats (17%) positive for mosquito larvae and/or pupae and 109 (78% of positive habitats) harboring *Ae. aegypti*. Most larval habitats identified in the wet season were in households (80%), and the same was true for habitats containing *Ae. aegypti* (Table 10). Most habitats (91%) and most of *Ae. aegypti* positive habitats (94%) were located outdoors. Many of the larval habitats observed in the wet season were small cans and plastic food containers (22%), but there were also numerous domestic animal drinking containers noted (15%) as well as those habitats in the “other” category (27%), which included abandoned appliances, lids, toys, fountains, small manholes, and miscellaneous containers (Table 11). Furthermore, many of the habitats positive for *Ae. aegypti* larvae in the wet season were also small cans and plastic food containers (19%), but the ones belonging to the “other” category were the most relevant (38%) (Table 11). Of all *Ae. aegypti* positive habitats in the wet season, 83% were considered disposable. According to the number of *Ae. aegypti* pupae collected, the most productive habitats in the wet
season were those in the “other” category like appliances and small manholes followed by drums (Table 11). Overall, large and medium habitats were more productive, even though the small habitats also accounted for a large portion (28%) of the pupae collected (Table 12).

Table 11. Seasonal distribution of larval habitats identified according to habitat type.

<table>
<thead>
<tr>
<th>Habitat type</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larval habitats (%)</td>
<td>Habitats with <em>Ae. aegypti</em> pupae (%)</td>
</tr>
<tr>
<td>Small can/plastic</td>
<td>183 (22)</td>
<td>21 (19)</td>
</tr>
<tr>
<td>Bucket</td>
<td>97 (12)</td>
<td>13 (12)</td>
</tr>
<tr>
<td>Tire</td>
<td>17 (2)</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Drum</td>
<td>29 (4)</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Washhtub</td>
<td>56 (7)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Gutter</td>
<td>11 (1)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Animal water</td>
<td>123 (15)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Flower pot</td>
<td>10 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Vase</td>
<td>9 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Coconut</td>
<td>19 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Sewer</td>
<td>4 (0.5)</td>
<td>0</td>
</tr>
<tr>
<td>Bottle</td>
<td>48 (6)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>223 (27)</td>
<td>42 (38)</td>
</tr>
<tr>
<td>Total</td>
<td>829 (100)</td>
<td>109 (100)</td>
</tr>
</tbody>
</table>
Table 12. Seasonal distribution of habitats containing *Ae. aegypti* larvae and/or pupae according to habitat capacity.

<table>
<thead>
<tr>
<th>Capacity category</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ae. aegypti</em> (%)</td>
<td><em>Ae. aegypti</em> pupae (%)</td>
</tr>
<tr>
<td>Small</td>
<td>43 (39)</td>
<td>147 (28)</td>
</tr>
<tr>
<td>Medium</td>
<td>40 (37)</td>
<td>190 (36)</td>
</tr>
<tr>
<td>Large</td>
<td>26 (24)</td>
<td>193 (36)</td>
</tr>
<tr>
<td>Total</td>
<td>109 (100)</td>
<td>530 (100)</td>
</tr>
</tbody>
</table>

According to the individual Chi-square tests, the presence of mosquito larvae in a habitat was associated to locality, location type, indoor/outdoor habitat setting, habitat type, and habitat disposability (Table 8); however, presence of larval habitats in a household were not associated to its construction quality. Larval habitats that were identified from Fray Casiano, El Huerto, and Carrizal, seemed more likely to be positive, as well as those found in locations like streets or large lots, and habitats located outdoors. Tires, sewers, and roof gutters were habitat types associated to positivity when compared to types such as coconuts, bottles and domestic animal drinking containers. In addition, non-disposable habitats (like concrete washtubs, sewers, gutters, manholes, etc.) were also more likely to contain mosquito larvae than disposable containers. Considering specifically *Ae. aegypti*, positivity of the habitats was associated with locality (El Huerto and Centro) and habitat type (tires, gutters, and drums) (Table 8).

The logistic regression analysis showed that setting and habitat type were the two significant independent predictors for presence of mosquito larvae in a habitat (Table 9). Habitats located outdoors were 3.4 times more likely to be positive than those indoors (OR: 3.4, CI: 1.3-9.3, p=0.016). Some habitat types were more likely to be positive for
larvae, for example tires were 5.2 times more likely to contain mosquito larvae than buckets (OR: 5.2, CI: 1.6-17.2, p=0.006), drums were 3.5 times more likely positive than cans/plastic food containers (OR: 3.5, CI: 1.1-10.5, p=0.028) and 4.3 times more likely than concrete washtubs (OR: 4.3, CI: 1.01-18.1, p=0.049), and habitats in the “other” category were 3.4 times more likely to be positive than washtubs (OR: 3.4, CI: 1.2-10.0, p=0.024).

Regarding positivity exclusively by *Ae. aegypti*, logistic regression showed setting, habitat type, and disposability to be significant independent predictors (Table 9). Similar to the analyses for mosquito larvae, outdoor habitats were 2.9 times more likely to contain *Ae. aegypti* than indoor habitats (OR: 2.9, CI: 1.04-8.2, p=0.043), and drums were 4.1 times more likely positive than cans/plastic food containers (OR: 4.1, CI: 1.3-12.9, p=0.016). In addition, disposable containers were 2.7 times more likely to contain *Ae. aegypti* than non-disposable habitats (OR: 2.7, CI: 1.2-6.3, p=0.022).

**Dry season**

In the dry season, only 26 of the 508 locations had habitats with mosquito larvae, and 20 locations (77% of positive locations) had one or more larval habitats that specifically harbored *Ae. aegypti*. According to the individual Chi-square tests, only location type was associated significantly with the presence of larval habitats in a location (Table 13), where streets and schools seemed to be the locations more likely to have larval habitats.
Table 13. Variables and results of the independent Chi-square tests of association applied to the Puntarenas dry season data.

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variables</th>
<th>$\chi^2$</th>
<th>DF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval habitat(s) in a location</td>
<td>Locality</td>
<td>9.58</td>
<td>9</td>
<td>0.386</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>46.69</td>
<td>8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>People in a house</td>
<td>0.68</td>
<td>1</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>Construction quality</td>
<td>0.50</td>
<td>3</td>
<td>0.918</td>
</tr>
<tr>
<td>Mosquito larvae/pupae in a location</td>
<td>Locality</td>
<td>14.35</td>
<td>9</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>6.83</td>
<td>8</td>
<td>0.555</td>
</tr>
<tr>
<td></td>
<td>Construction quality</td>
<td>1.70</td>
<td>3</td>
<td>0.638</td>
</tr>
<tr>
<td>Mosquito larvae/pupae in a habitat</td>
<td>Locality</td>
<td>13.42</td>
<td>9</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>6.95</td>
<td>7</td>
<td>0.434</td>
</tr>
<tr>
<td></td>
<td>Habitat setting</td>
<td>0.13</td>
<td>1</td>
<td>0.449*</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>35.41</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Habitat capacity</td>
<td>13.39</td>
<td>2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Habitat disposability</td>
<td>4.19</td>
<td>1</td>
<td>0.041</td>
</tr>
<tr>
<td>Ae. aegypti in a habitat</td>
<td>Locality</td>
<td>13.05</td>
<td>9</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>Location type</td>
<td>3.11</td>
<td>7</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td>Habitat setting</td>
<td>0.84</td>
<td>1</td>
<td>0.265*</td>
</tr>
<tr>
<td></td>
<td>Habitat type</td>
<td>14.14</td>
<td>8</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>Habitat capacity</td>
<td>6.66</td>
<td>2</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Habitat disposability</td>
<td>0.31</td>
<td>1</td>
<td>0.576</td>
</tr>
</tbody>
</table>

*Expected value of a cell was <5 and Fisher exact test was used

The dry season logistic regression revealed that location type and people were significant independent predictors for the presence of one or more larval habitats in a location (Table 9). Streets were 14 times more likely to contain larval habitats than households (OR: 14.0, CI: 1.8-166.6, p=0.037), and locations with people, such as most households, were 4.6 times more likely to have larval habitats than uninhabited locations (OR: 4.6, CI: 1.8-12.1, p=0.002).
A total 461 wet habitats were identified in the dry season: 27 (6%) were positive for mosquito larvae and/or pupae, and 21 (78% of positive habitats) contained *Ae. aegypti*. Most larval habitats identified in the dry season were found in houses (83%), as well as the majority of the habitats (95%) that harbored *Ae. aegypti* (Table 10). Eighty-seven percent of larval habitats and 81% of *Ae. aegypti* positive habitats identified were located outdoors. Even though many of the habitats found during the dry season were drinking containers for domestic animals and fowl (32%) and those in the “other” category (30%), the concrete washtubs (29%) were the most important in terms of *Ae. aegypti* positivity (Table 11). Of all the habitats that contained *Ae. aegypti* larvae and/or pupae in the dry season, 57% were classified as disposable. However, the habitats with the most productivity were washtubs (Table 11) and other large habitats (Table 12).

The presence of mosquito larvae in a habitat was individually associated with habitat type, capacity, and disposability during the dry season (Table 13). Water drums, sewers, and tires were more likely to contain mosquito larvae than the other types of containers, as were non-disposable habitats. Both the presence of mosquito larvae and specifically of *Ae. aegypti* were associated to habitat capacity (Table 13), where medium and large habitats were related to the presence of larvae and/or pupae.

The logistic regression analysis showed that in the dry season, capacity was the significant independent predictor for the presence of mosquito larvae in a habitat (Table 9). Large habitats were 7.4 times more likely to be positive than small ones (OR: 7.4, CI: 2.0-27.9, p=0.003), and habitats with medium capacity were 5.3 times more likely than small ones (OR: 5.3, CI: 1.6-17.2, p=0.005). In addition, capacity was also a significant
independent predictor for presence of *Ae. aegypti* in a habitat, where medium capacity habitats were 5.2 times more likely to contain *Ae. aegypti* than small habitats (OR: 5.2, CI: 1.6-17.3, p=0.007).

The overall entomological and pupal indices for Puntarenas were higher in the wet season than in the dry season (Table 14). Furthermore, 37% of all positive larval habitats identified in urban Puntarenas contained mosquito species different from *Ae. aegypti*. The other species identified in larval habitats were *Culex quinquefasciatus*, *Limatus durhamii*, *Culex nigrivelatus*, *Culex interrogator*, *Culex coronator*, *Culex corniger*, *Ochlerotatus taeniorhynchus*, *Toxorhynchites* sp., and *Uranotaenia* sp. (Table 15). In addition, *Ae. aegypti* larvae shared the habitat in 29 cases (19% of all habitats positive for *Ae. aegypti*), which were commonly *Cx. quinquefasciatus* (8 habitats) and *L. durhamii* (7 habitats) but also with all other species mentioned except *Uranotaenia* sp.

Table 14. Seasonal *Aedes aegypti* larval and pupal indices from locations evaluated in Puntarenas, Costa Rica.

<table>
<thead>
<tr>
<th></th>
<th>Container Index</th>
<th>Location Index</th>
<th>Breteau Location Index</th>
<th>Pupae per person</th>
<th>Pupae per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet season</td>
<td>13.2</td>
<td>17.2</td>
<td>22.9</td>
<td>0.36</td>
<td>15.6</td>
</tr>
<tr>
<td>Dry season</td>
<td>4.6</td>
<td>3.9</td>
<td>4.13</td>
<td>0.09</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Table 15. Frequency of total habitats containing mosquito species different from *Aedes aegypti* during both wet and dry season surveys in Puntarenas, Costa Rica.

<table>
<thead>
<tr>
<th>Habitat type</th>
<th>Culex quinquefasciatus</th>
<th>Limatus durhamii</th>
<th>Culex nigripalpus</th>
<th>Culex interrogator</th>
<th>Culex coronator</th>
<th>Toxorhynchites sp.</th>
<th>Ochlerotatus taeniorhynchus sp.</th>
<th>Uranotaenia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small can/plastic</td>
<td>2</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Drum</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Washtub</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gutter</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animal water</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Flower pot</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sewer</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Coconut</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>19</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>16</td>
<td>15</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Discussion

Puntarenas is one of the cities of Costa Rica that has been greatly affected by dengue. Ever since dengue cases were reported in 1993 (WHO 1994), the local authorities in Puntarenas have been battling the disease with the use of insecticides and larvicides, as well as education and community involvement in the removal of artificial containers that serve as larval habitats (Impoinvil et al. 2007). However, this study shows that larval habitats are still common in this city, and many of the usual control campaigns may need to re-direct their actions. According to the categorization of houses using construction quality applied during this study, socioeconomic conditions seem relatively
good in Puntarenas when compared to other areas of Costa Rica with high *Ae. aegypti* indices and where many houses are in very poor condition (Calderon-Arguedas et al. 2003). In addition, the statistical tests utilized did not reveal any association of mosquito habitats to house construction quality. Thus, dengue and *Ae. aegypti* persistence in this area is probably more associated with other variables which may include meteorological, cultural, behavioral, and environmental conditions.

Even though mosquito control efforts have been ongoing for more than ten years, results from this study show that larval habitats are still common in Puntarenas, and *Ae. aegypti* larval indices are high enough to maintain dengue transmission, especially in the wet season. The Breteau index in the wet season was much higher than 5, generally considered a threshold for viral transmission (Macdonald 1956, Focks 2003), but it was lower during the dry season. Larval indices in general were relatively low in the dry season but may have been higher in specific neighborhoods and localities where dengue transmission may have been occurring at low levels. Moreover, traditional *Ae. aegypti* larval indices sometimes do not correlate well with adult populations and dengue transmission, and pupal surveys are preferred in most cases (Focks & Chadee 1997, Focks 2003, Barrera et al. 2006a).

According to the threshold levels determined by Focks et al. (2000), the number of pupae per person in Puntarenas, where mean temperature is close to 28°C, may have been high enough to support viral transmission in the wet season but probably not in the dry season, even though the local Ministry of Health reports dengue transmission in both seasons. The use of pupal surveys in routine surveillance and source reduction programs has been under evaluation (Morrison et al. 2004b, Sanchez et al. 2006), and accurately
determining pupal indices posed some problems in the environment of Puntarenas. Many of the most common and productive habitats were large non-disposable or permanent habitats like roof gutters, small manholes, and large concrete washtubs that usually contain large amounts of organic debris and cannot be drained easily to collect and count all pupae. In addition, the presence of more than one mosquito species in a habitat was common in Puntarenas, which made exhaustive collections necessary, and identifying *Ae. aegypti* pupae a tedious process. In this sense, studies in Thailand have determined that filtering every container and complete counts requires great effort and may not be a practical method for routine surveillance (Strickman & Kittayapong 2003). Thus, pupal surveys in Puntarenas may serve as research tools and for periodic determination of productivity but do not seem to be an efficient method for routine entomological surveillance.

The various mosquito species identified sharing habitats with *Ae. aegypti* reaffirm the need for well-trained entomological surveillance teams in endemic areas. Entomological surveillance requires determination of the most relevant larval habitats, larval indices, and periodic pupal surveys, which will need personnel that can identify *Ae. aegypti* larvae and pupae to determine these indices correctly. In Puntarenas, this task would not be easy since other larvae with relatively short siphons like *L. durhamii*, *Cx. corniger*, *Uranotaenia*, and *O. taeniorhynchus*, may resemble *Ae. aegypti* to the unaided eye. In these cases, it has been suggested that microscopic confirmation may be necessary as opposed to simple identification by the relative size of the siphon and larval movement (Getis et al. 2003, Bisset-Lazcano et al. 2003).
In spite of public education and source reduction campaigns, the numerous larval habitats identified in households shows that people may not be taking all the actions necessary to eliminate mosquito larval habitats. Education probably has had an impact since control efforts seem to be more effective in houses than in other areas like schools or lots, and this may be due to source reduction being targeted specifically at households. Moreover, households are the most frequent type of location and therefore account for most habitats in Puntarenas. However, households were less likely to contain larval habitats than other locations, such as lots in the wet season and streets in the dry season. It was notable that in the wet season lots contain habitats that probably fill with rainwater and are less likely to be eliminated than those in households, but in the dry season these habitats may dry up frequently making houses almost the only source of *Ae. aegypti*. Furthermore, when accounting for locality in the logistic regression model, location type and people were not significant predictors for mosquito habitats in the wet season, which reflects the likelihood of finding larval habitats in all location types. Therefore, this suggests that past education campaigns may be changing the profile of mosquito habitats, and community-based approaches may be improved if public spaces are targeted in addition to households at the start and during the wet season.

With the application of the pupal survey, large containers like drums, buckets, and washtubs have been considered to account for most adult *Ae. aegypti* in some areas (Chadee 2004, Burkot et al. 2007, Maciel-de-Freitas et al. 2007). However, this was not the case in Puntarenas, where differences in productivity between large and medium containers were not apparent, and small containers still accounted for more than a quarter of the pupae collected. Focks and Chadee (1997) also identified small containers as the
most important targets for source reduction in Trinidad, followed by water storage containers. Thus, targeting small, as well as large and medium containers is still vital for vector control in Puntarenas during the wet season, since eliminating mainly large containers would only account for 36% of the *Ae. aegypti* population.

In contrast, results suggest that during the dry season the habitats that maintain the *Ae. aegypti* population are mainly large, concrete washtubs, and other non-disposable habitats which are also frequent. Although drums are considered highly productive habitats (Chadee 2004, Burkot et al. 2007, Maciel-de-Freitas et al. 2007), these large containers were not as frequent in Puntarenas, probably due to adequate piped water service. However, it is common for people in Costa Rica to have at least one washtub that is filled with water to facilitate washing clothes and/or to store water in areas with regular water service interruptions. These habitats make good sites for *Ae. aegypti* larvae to develop if they are not emptied and cleaned frequently. According to our surveys, water storage in Puntarenas in washtubs or drums does not seem to be due to problems with piped water service, and keeping washtubs filled with water is probably a common cultural practice in so far as people generally do not regard these containers as sources of dengue vectors. Containers like drums and buckets used to store water become common larval habitats in areas where availability of piped water is a problem (Norman et al. 1991, Focks & Chadee 1997, Calderon-Arguedas et al. 2004b). These washtubs and large containers can hold enough water during the dry season to prevent desiccation and serve as productive mosquito larval habitats. Overall, the most productive types of containers that probably maintain the mosquito population in Puntarenas during the dry season seem less diverse than in the wet season and could be targeted specifically to washtubs and
other large habitats to reduce *Ae. aegypti* levels to a minimum and thus hinder the increase in mosquito densities that occurs during the following wet season.

Although containers that hold drinking water for domestic animals and small cans or plastic food containers were very frequent larval habitats in Puntarenas, most of them did not contain larvae, and non-disposable or permanent habitats were more likely to contain mosquito larvae. This finding may be the result of the ongoing control campaigns that prompt the population to discard containers with water and change animal drinking water frequently, as well as improve local garbage collection services. However, non-disposable habitats like roof gutters, washtubs, and sewers may not be targeted directly in these campaigns as they require special education and treatments that may include removing debris, frequent draining and washing, filling in crevices, using adequate covers, or applying larvicides. Some actions may call for direct involvement of health authorities, and even though community–based approaches are more cost-effective (Baly et al. 2007), focusing on vertical actions carried out by local authorities that would complement current source reduction practices may be the next steps to improve mosquito control activities in areas that have undergone control activities for a long time such as Puntarenas.

In general, mosquito control efforts in Puntarenas have probably aided in the reduction of *Ae. aegypti* densities and dengue cases over the past decade, given that households were not more likely to contain mosquito larval habitats during this study. However, other factors that also reduce transmission may include an increase in immunity to the DEN-1 serotype, which has been circulating in the area for the past five years. In spite of ongoing vector control, it was common to find wet habitats, as well as
those containing *Ae. aegypti* and other mosquito larvae in Puntarenas, especially during the wet season. It is possible that vector populations may be reduced further in Puntarenas by continuing current community participation focused on households and disposable containers but also targeting new non-household settings like streets and lots and implementing ways to eliminate larvae in non-disposable containers (emphasizing on washtubs during the wet season). This will probably require changes in human behavior and the combined efforts of the public and the vector control personnel.

As has been reported in other areas, vector control is sometimes not effective against dengue outbreaks (Chadee et al. 2005), and reducing mosquito levels below transmission thresholds may not be possible with the way control approaches are currently applied. Puntarenas is an example of a city where organization of vector control is community-based, inter-sectoral, and inter-institutional, but these efforts have not achieved a reduction of mosquito densities (in terms of larval and pupal indices) below transmission thresholds. The analyses performed suggest specific characteristics of the locations that make them more likely to contain mosquito habitats, as well as properties of the habitats that make them more likely to contain larvae. Although these likelihoods may not reflect adult mosquito or habitat abundance, they may predict shifts in habitat profiles, reflect the impact of past control activities, and propose directions for improvement of vector control.
Chapter VI

Urban structure and *Aedes aegypti* larval habitats in Puntarenas, Costa Rica

In the past 30 years, remote sensing has been increasingly applied to study infectious diseases, and especially those transmitted by arthropod vectors (Hay et al. 1997, Beck et al. 2000, Thomson & Connor 2000, Bergquist 2001, Correia et al. 2004). Modeling and mapping of infectious diseases and vector distributions has been the focus of ongoing research for applications in public health (Goetz et al. 2000, Hay 2000, Hay et al. 2006, Rogers et al. 2006, Jacob et al. 2006). In addition, land cover maps and information obtained from satellite imagery has revealed factors associated with increased risk of infection and/or densities of vectors like *Anopheles* and *Aedes aegypti* (Peterson et al. 2005, Sithiprasasna et al. 2005a, Sithiprasasna et al. 2005b, Rogers et al. 2006, Mushinzimana et al. 2006, Jacob et al. 2006).

*Aedes aegypti* is the principal vector of the viruses that cause dengue fever and dengue hemorrhagic fever. Dengue is the most important arboviral disease worldwide, with more than 50 million cases reported every year (WHO 2002, Gibbons & Vaughn 2002). In Costa Rica, dengue is the most important vector-borne disease: the Ministry of Health reported almost 38,000 cases in 2005 and more than 24,000 cases in 2007 (Ministerio de Salud 2007). Vector management with community participation and larval source reduction is still considered the most effective way to reduce vector densities and
control the disease (Gubler 1998, Gubler 2005), although the resources available in many developing countries may limit adequate entomological surveillance (Impoinvil et al. 2007).

In previous studies, the use of remote sensing for determining dengue risk or mosquito densities has been questioned due to limited spatial and spectral resolution to identify the small man-made containers that usually act as habitats where *Ae. aegypti* larvae develop (Moloney et al. 1998). However, recent studies have successfully obtained remotely sensed information of medium and low spatial resolution like AVHRR, Landsat, SPOT, and ASTER that relates to mosquito habitats and disease (Peterson et al. 2005, Nakhapakorn & Tripathy 2005, Tran & Raffy 2005, van Benthem et al. 2005, Vanwambeke et al. 2006, Rogers et al. 2006, Kolivras 2006, Fuller et al. under review). Moreover, information obtained from high-resolution sensors such as IKONOS and QuickBird has been associated with the abundance of anopheline larval habitats (Mushinzimana et al. 2006, Jacob et al. 2006) and incidence of dengue in urban environments (Troyo et al. under review a).

In this study, very high-resolution satellite imagery from QuickBird was analyzed to determine the relationships that urban structure, determined by tree cover and built area, may have with the abundance of mosquito larval habitats and *Ae. aegypti* container index in one of the cities in Costa Rica that has been greatly affected by dengue fever.
Materials and Methods

Study site

The site of this study Puntarenas City, Costa Rica, has been described in detail previously (Troyo et al. 2008, Troyo et al. under review a). Puntarenas is a port city located on the Pacific coast of Costa Rica, with approximately 100,000 habitants in an urban area of roughly 20 km² (INEC 2002). Climate is tropical, with marked wet (May to mid-November) and dry (mid-November to April) seasons. *Aedes aegypti* larval habitats are common in this city, and dengue fever has been a public health problem since 1993. More than 5,500 cases of dengue fever were reported by the Ministry of Health in the Puntarenas area from 2005 to 2007 (Ministry of Health 2007).

Entomological data

Two cross-sectional entomological field surveys were performed in the study site during the wet season (July-August) of 2006 and dry season (January-February) of 2007. The geographical sampling method for the field surveys and the resulting larval habitat profiles have been described and analyzed (Troyo et al. 2008, Troyo et al. under review b). Briefly, a grid (cell size of 100 by 100 meters) was overlaid on QuickBird panchromatic satellite imagery from 2002 and 2003, and a 10% random sample of cells stratified by locality was selected for entomological surveys. Within 34 selected cells, locations (such as households, parks, schools, streets, buildings, lots, etc.) were identified and searched for all possible larval habitats (wet containers). All mosquito pupae and a
sample of larvae were collected when present, and *Ae. aegypti* larvae and pupae were confirmed in the laboratory.

**Satellite imagery and image classification**

QuickBird multispectral (bands 1 to 4) and panchromatic scenes for September 2006 (wet season) and February 2007 (dry season) were acquired to match the seasonal entomological surveys. Imagery was cloud-free, and it did not require atmospheric correction previous to the analyses. All the multispectral bands were georeferenced to match QuickBird imagery from 2002 and 2003 that had been used to develop the sampling frame for field surveys (Troyo et al. 2008). Georeferencing was performed with the resample module in Idrisi Andes (Eastman 2006) using 52 ground control points at road intersections, which were identified on the panchromatic 2002/2003 imagery. The final RMS for the dry season multispectral bands was 1.8 m and 1.6 m for the wet season bands.

Land cover maps for wet and dry seasons were obtained from the QuickBird multispectral bands using the image classifier in eCognition software as has been described for very-high resolution imagery of Puntarenas (Troyo et al. 2008, Troyo et al. under review a). Previous analyses have also shown that object segmentation improved the accuracy of land cover maps (Troyo et al. under review a). Therefore, segmentation for each image was performed for level 1 at scale parameter = 20, shape factor = 0.3, and compactness = 0.7; a level 2 segmentation was performed at the same scale parameter but using the spectral difference mode. The level 1 scale parameter determined the size and maximum allowed heterogeneity for segmentation of structures in the urban setting.
(Baatz et al. 2004). The level 2 segmentation merged contiguous objects that differed in less than the specified scale parameter (Baatz et al. 2004). After level 2 segmentation, samples were selected for a hierarchical classification scheme, and the resulting land cover maps included the classes “water”, “built”, “tree”, “grass/bare soil”, and “paved”.

The land cover maps for wet and dry seasons were imported into Idrisi Andes software to evaluate the classification accuracy and for data extraction. Accuracy was assessed by using points selected without bias from the original panchromatic QuickBird scenes. A total 21,355 points were selected for assessment of the wet season images and 16,691 for the dry season images, where each class contained a number of points that was proportional to its area in the land cover maps. Clustering (randomness) of the points was evaluated by obtaining a nearest neighbor statistic in ArcGIS (NN = observed mean distance /expected mean distance). For the wet season image, the points were considered moderately clustered or dispersed (NN = 0.48, Z = -2.43, p = 0.015), while for the dry season image, the points were not clustered (NN = 1.15, Z = 0.64, p = 0.522). Overall, the wet season image was 85.9% accurate, with a Kappa of 0.81. The “tree” class in the land cover map of the wet season had 13.7% errors of omission and 20.7% errors of commission (Kappa = 0.81), and the “built” class had 6.4% errors of omission and 5.7% errors of commission (Kappa = 0.91). The dry season image had an accuracy of 90.6% and Kappa of 0.86. The “tree” class in the land cover map of the dry season had 11.6% errors of omission and 5.6% errors of commission (Kappa = 0.84), and the “built” class had 10.9% errors of omission and 2.9% errors of commission (Kappa = 0.86).
Statistical analyses

For each season, the proportion of tree cover and built area was extracted from the 34 cells of each land cover map that were evaluated during seasonal entomological surveys (Troyo et al. 2008, Troyo et al. under review b). In addition, information from the field surveys was grouped for each of the cells sampled, and the Ae. aegypti container index and pupae per person were calculated per cell. Distribution of the data was evaluated and seasonal multiple linear regression models were analyzed for the outcome variables 1) number of larval habitats, 2) Ae. aegypti container index, and 3) pupae per person using the independent variables proportion of tree cover or built area extracted from the QuickBird imagery. All statistical analyses were performed using SAS 9.1 software, and the significance level was set at 0.05.

Results

During the wet season, multiple regression models showed that the tree cover and built area were able to significantly explain the variation in total larval habitats, and tree cover explained variations in Ae. aegypti container index. On their own, tree cover (TREE) and built area (BUILT) did not explain the variation unless the models included a correction variable by the number of location evaluated (LOCE) in the cell (Table 16). The LOCE corresponds to the total number of locations (households, parks, streets, lots, fields, buildings, etc.) where access was granted and were searched in their entirety for mosquito larval habitats during the field surveys. The variations in Ae. aegypti pupae per person were evaluated only during the wet season, as the pupae collected per cell during the dry season were insufficient for an adequate analyses. In the dry season, neither tree
cover nor built area significantly explained the variations in total larval habitats or *Ae. aegypti* container index (Table 16). The resulting significant models for the wet season were:

\[
\text{# larval habitats} = -10.187 + 35.933(\text{TREE}) + 1.716(\text{LOCE})
\]
\[R^2 = 0.650, F = 28.75, p<0.001\]  

\[
\text{# larval habitats} = 10.314 – 30.465(\text{BUILT}) + 1.632(\text{LOCE})
\]
\[R^2 = 0.613, F = 24.55, p<0.001\]

\[
\text{*Ae. aegypti* container index} = 36.544 – 38.295(\text{TREE}) – 0.550(\text{LOCE})
\]
\[R^2 = 0.188, F = 3.59, p=0.040\]
Table 16. Parameter estimates for the multiple linear regression models analyzed during wet and dry seasons in Puntarenas, Costa Rica.

<table>
<thead>
<tr>
<th>Model: dependent variable</th>
<th>Independent variables</th>
<th>DF</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>t value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. # larval habitats (wet)</td>
<td>Intercept</td>
<td>1</td>
<td>-10.187</td>
<td>5.256</td>
<td>-1.94</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>TREE</td>
<td>1</td>
<td>35.933</td>
<td>10.442</td>
<td>3.44</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>LOCE</td>
<td>1</td>
<td>1.716</td>
<td>0.238</td>
<td>7.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2. # larval habitats (wet)</td>
<td>Intercept</td>
<td>1</td>
<td>10.314</td>
<td>5.000</td>
<td>2.06</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>BUILT</td>
<td>1</td>
<td>-30.465</td>
<td>10.925</td>
<td>-2.79</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>LOCE</td>
<td>1</td>
<td>1.632</td>
<td>0.248</td>
<td>6.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3. container index (wet)</td>
<td>Intercept</td>
<td>1</td>
<td>36.544</td>
<td>7.982</td>
<td>4.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>TREE</td>
<td>1</td>
<td>-38.295</td>
<td>15.860</td>
<td>-2.41</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>LOCE</td>
<td>1</td>
<td>-0.550</td>
<td>0.362</td>
<td>-1.52</td>
<td>0.138</td>
</tr>
<tr>
<td>4. container index (wet)</td>
<td>Intercept</td>
<td>1</td>
<td>17.032</td>
<td>7.628</td>
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<tr>
<td>5. Pupae per person (wet)</td>
<td>Intercept</td>
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<td>6.677</td>
<td>2.861</td>
<td>2.33</td>
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<tr>
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<tr>
<td>6. Pupae per person (wet)</td>
<td>Intercept</td>
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<td>2.558</td>
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<td>9. container index (dry)</td>
<td>Intercept</td>
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<td>4.240</td>
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<td>10. container index (dry)</td>
<td>Intercept</td>
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<td>0.113</td>
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*TREE: tree cover, BUILT: built area, LOCE: locations evaluated.
Discussion

Results show that remotely sensed information can be used to explain and predict the abundance of mosquito habitats in urban environments such as Puntarenas, especially in the wet season when vector densities increase and most dengue cases occur in most tropical endemic areas (Focks & Chadee 1997, Chadee 2003, Morrison et al. 2004a, Morrison et al. 2006, Troyo et al. under review a). The statistical multiple linear regression models using tree cover and built area were able to explain more than 60% of the variation in the number of larval habitats identified in the cells surveyed during the wet season. The positive relationship between the number of larval habitats and tree cover suggests that, in areas with a similar number of locations evaluated, more tree cover means greater numbers of larval habitats. On the other hand, cells with a high proportion of built area have fewer larval habitats.

If larval habitats are considered sources of *Ae. aegypti* female mosquitoes and indirect indicators of potential viral transmission, these results would agree with previous observations that suggest dengue fever is directly correlated to tree cover and indirectly correlated to built area (Troyo et al. under review a). Specifically within the urban environments, areas with more tree cover in the wet season harbor more larval habitats, which increase vector densities, dengue transmission, and reports of dengue fever cases in those areas. The most relevant *Ae. aegypti* larval habitats in Puntarenas during the wet season have been identified as outdoor miscellaneous containers, cans, and plastic food containers that usually fill with rain water (Troyo et al. under review b). Moreover, tree cover has been associated with mosquito larval habitats in several studies, seeing as trees can conceal containers and protect them from direct sunlight and desiccation (Vezzani et
In addition, households with larger back yards usually have more tree cover, and these non-built areas permit more outdoor surfaces where containers can accumulate and fill with rain. Therefore, areas within the urban environment with greater tree cover probably contain numerous *Ae. aegypti* and other mosquito larval habitats in the wet season and should be targeted strongly during larval source reduction campaigns.

In contrast with results from very high resolution QuickBird imagery (Troyo et al. under review a), the relationship between incidence of dengue fever and built up areas has revealed positive relationships when evaluated at coarser spatial scales (Nakhapakorn & Tripathy 2005, Fuller et al. under review). Using Landsat ETM+ imagery (30 m spatial resolution), Nakhapakorn and Tripathy (2005) showed that built-up areas constituted areas of higher dengue risk while tree covered or forested areas were at the lowest risk of dengue fever. In addition, Fuller et al. (under review) analyzed ASTER imagery (15 m resolution) and suggested that the relationship between built area and dengue incidence may be linear over a specific range of percent built-up, and further increase in built-up area does not increase the number of mosquito habitats.

Although the relationship between total larval habitats and tree cover was positive, the container index for *Ae. aegypti* was inversely associated with tree cover. This may suggest that, since fewer habitats are available for females to lay their eggs, they oviposit in most of the containers they can find, making the proportion of habitats containing *Ae. aegypti* higher in areas with less tree cover. However, considering the two opposite associations found with tree cover in this study, the areas at most risk for higher *Ae. aegypti* densities would be those with an intermediate level of tree cover and built
area. Thus, in the wet season, these areas would contain numerous larval habitats, a large proportion of them containing *Ae. aegypti*, which would imply higher densities of vectors and dengue transmission. In their study, Fuller et al. (under review) also suggest that moderately built-up areas with a certain amount of tree cover are most likely to experience higher dengue incidence.

Although the effects of tree cover and built areas on larval habitats were evident in the wet season, no association was clear in the dry season. This result was probably due to the difference in the most relevant larval habitats in Puntarenas. During the dry season, the most important larval habitats are non-disposable containers like washtubs and those used for water storage that are usually filled with water by people (Troyo et al. under review b). It is possible that the distribution of *Ae aegypti* and other mosquito larval habitats is more associated with human behavior than environmental conditions or urban structure (Troyo et al. under review b).

Satellite imagery has provided information that significantly explains and can be used to predict the abundance of mosquito larval habitats in this urban environment during the wet season. With models such as the ones provided here, urban areas or specific neighborhoods that are difficult to access due to social conflicts, rough terrain, or limited resources could be evaluated using remotely sensed data from satellites or aerial photography and targeted for the most efficient vector control interventions. Applicability extends to areas outside Costa Rica to include other dengue endemic areas around the world, like the South Pacific. In addition, risk maps can be created to determine hot spots for higher densities of mosquito larval habitats that may include vectors of malaria, dengue, lymphatic filariasis, and other diseases that may be present in urban areas.
Conclusions

Although dengue is a serious public health problem in Costa Rica, it is evident that insufficient local scientific research exists to combat the disease and mosquito vector (Troyo et al. 2006). Local research is necessary to evaluate local changes in vector ecology and behavior that may be due to control measures, and also to direct new control measures to improve effectiveness and efficiency of vector control programs. Therefore, strengthening of intersectoral, interinstitutional, and interdisciplinary efforts are required to stop increases in dengue incidence and address the social, political, economic, cultural, and environmental contexts of the disease.

In Puntarenas, one of the areas of Costa Rica greatly affected by dengue since 1993, mosquito larval habitats were still common and so it can be inferred that vector control has not been able to reduce *Aedes aegypti* densities below dengue transmission thresholds. The most common types of larval habitats were not the most productive habitats according to the pupal surveys, and this supports studies that indicate differences between larval and pupal indices (Troyo et al. under review b). Although a Breteau index of 5 has been considered a threshold for dengue transmission (Macdonald 1956), current research indicates that pupal surveys provide better approximations of transmission risk (Focks & Chadee 1997). Therefore, results from Puntarenas support the validity of the pupal survey, although issues that should be improved and addressed include the need for
technical expertise to identify pupae and the difficulty of collections from some large and permanent containers.

The low pupal indices estimated for Puntarenas suggest that dengue transmission thresholds in this city may be lower than those previously established in other tropical endemic areas. The local Ministry of Health continuously reported dengue transmission in Puntarenas (Ministerio de Salud 2007), in spite of the low pupae per person estimates (Focks et al. 2000, Troyo et al. under review b). Moreover, it is likely that dengue transmission thresholds in Puntarenas are much lower than expected, considering this area probably has high dengue seroprevalence due to underreporting and asymptomatic dengue cases (Porter et al. 2005, Iturrino-Monge et al. 2006). It is possible that areas with low overall pupal indices like Puntarenas, which in some cases may fall below previous threshold estimates, contain several pockets of transmission where pupal indices and vector densities are high. Therefore, determining transmission thresholds in the larger areas may require an estimation of the number and extent of neighborhoods or groups of blocks with higher expected vector densities, which may be facilitated by determining key variables and creating maps using geospatial technologies.

The new geographical sampling method described was applied successfully in Puntarenas and may be used to improve representativeness and accuracy of samples in different urban environments and for other diseases such as Chagas disease, filariasis, leishmaniasis and urban malaria. In Puntarenas, it allowed the use of high-resolution satellite imagery to determine optimal cell size and sample cells remotely, as well as to identify of the area to be surveyed once in the field (Troyo et al. 2008). The information that resulted from seasonal field surveys accurately identified the most abundant
mosquito larval habitats, the most abundant \textit{Ae. aegypti} habitats, and the most productive types of containers (Troyo et al. under review b). Therefore, results evidenced the effects of ongoing vector control and suggested new directions for mosquito control that would achieve the greatest vector reductions.

Satellite imagery of low, medium, and very high spatial resolutions (MODIS, ASTER and QuickBird) supplied information in the form of vegetation indices and key variables to evaluate temporal and spatial patterns of dengue incidence and distributions of mosquito larval habitats in the urban environment. Moreover, this is the first study to link field data and satellite imagery with a spatial resolution of <5 meters to study the epidemiology of dengue and \textit{Ae. aegypti} habitats within the urban environment. This very high resolution imagery provided new opportunities to assess urban habitats for disease and mosquito vectors by providing spatial resolutions appropriate for identification of individual structures and key variables like tree cover and built area within the urban environment.

The relationships revealed between urban structure and dengue fever, which closely agree with the associations between urban structure and mosquito larval habitats, should be in the interest of local authorities and urban planners who can seek specific alternatives for dengue prevention and control. For instance, in localities with higher tree cover and dengue incidence, like Fray Casiano and Carrizal, locations were also more likely to contain larval habitats, and habitats were more likely to contain mosquito larvae than locations like Centro and Cocal, which also had had lower dengue incidence (Troyo et al. under review a, Troyo et al. under review b). Therefore, the different analyses performed in Puntarenas agree that dengue incidence is linked to mosquito habitats, and
that both are related to the structure of the built environment at least in terms of tree
cover and proportion of built area.

Vector control in Puntarenas can be improved by targeting the most productive
containers like washtubs in the dry season, as well as areas or neighborhoods with higher
dengue risk, which can be determined with geospatial models and maps using the key
variables identified. Educating the community on alternate mosquito control methods
such as drainage, use of larvicides, and cleaning for large non-disposable larval habitats
like washtubs may decrease vector densities. In addition, continuing current mosquito
control practices like community involvement and education in dengue prevention and
vector control, can be enhanced by also targeting the actions to streets, schools, and lots.
Thus, larval habitat reduction in public areas, as well as application of larvicides or
elimination of non-disposable habitats will require more hands-on action by local health
authorities in the Ministry of Health and CCSS (Caja Costarricense del Seguro Social)
and the local municipality.

Integration of several disciplines in the efforts to combat dengue and its vector
like education, sociology, engineering, epidemiology and public health, medical
entomology, medicine, microbiology, geography, economics, and law, needs to be
enhanced. In addition, ministries other than the Ministry of Health (like those of
environment, science and technology, economics, interior and police, education, and
planning), public organizations and institutions (like universities, water and sewage,
research centers, and the government’s legislative branch), and the local municipality
should be involved. This would improve community education and mobilization, disease
and vector surveillance, mosquito control, local research, and law implementation, which would attack the diverse angles and complexity of the dengue situation.

The interdisciplinary methods for Puntarenas described may be applicable in other urban regions of Costa Rica, as well as in tropical endemic areas around the world to target mosquito control. Meteorological conditions like temperature and seasonal rainfall fluctuations in endemic areas may affect mosquito larval habitats in a similar manner as has been observed for Puntarenas, where numerous outdoor containers collect rain water, and the number of larval habitats and dengue cases increase during the wet season (Focks & Chadee 1997, Chadee 2003, Morrison et al. 2004a, Nakhapakorn & Tripathy 2005, Morrison et al. 2006, Vezzani et al. 2005). This is true even in some areas where research and vector control has been conducted, but *Ae. aegypti* and virus transmission are still present or have resurged like Cuba, Puerto Rico, Singapore, and Thailand (Strickman & Kittayapong 2002, Strickman & Kittayapong 2003, Bisset-Lazcano et al. 2006, Barrera et al. 2006a, Barrera et al. 2006b, Sanchez et al. 2006, Ooi et al. 2006). Thus, these geospatial approaches in the epidemiology of dengue and the results described for Puntarenas will serve to direct and target control in this area, as well as develop risk maps of similar areas to identify locations within cities that may be targeted for enhanced dengue prevention and vector control activities.

Although urbanization patterns and disease epidemiology in other regions probably differ from the situation in Puntarenas, geospatial technologies may reveal significant disease relationships and associations with remotely sensed information in different endemic areas. The key variables identified can be integrated to geospatial models and maps to locate neighborhoods of increased risk and develop early warning
systems that would integrate temporal and spatial variables like meteorological conditions, social characteristics, disease and vector surveillance information, and urbanization patterns and structure. The tools can be developed locally, but various local instruments could be integrated and scaled up to a national or even regional level. Thus, approaches and methods similar to the ones described here can be utilized to determine associations and key variables for improved surveillance and vector control even though the most productive habitats for *Ae. aegypti*, human behavior, construction materials, and the process of urbanization will vary between areas.

In addition, these methods can be applied for other diseases, especially considering the limited use of very high resolution imagery to study vector diseases in urban areas, which seems limited to malaria (Sithiprasasna et al. 2003, Sithiprasasna et al. 2005a, Sithiprasasna et al. 2005b, Jacob et al. 2007). Nevertheless, obstacles such as the limited availability of very high resolution data in some tropical areas, lower classification accuracy in urban areas, and the level of expertise required to manage geographical information need to be minimized in order to develop true interdisciplinarity in this area of research.

The approaches applied in these studies provided new interdisciplinary methods to study dengue and identified key variables that may be capturing emergent properties of urban structure, which favor mosquito habitats and disease transmission. Also, it provides a detailed evaluation of relationships and highlights a great potential for incrementing the complexity and usefulness of interdisciplinary approaches to study infectious diseases in urban environments.
Even though dengue is an escalating problem, interdisciplinary and intersectoral approaches are being evaluated for surveillance, treatment, prevention, and control (Sanchez et al. 2005, Impoinvil et al. 2007). In addition, vaccines are also under evaluation and may be available to the population in the future (Edelman 2007). Integrating disciplines, institutions, research, environmental context, and the community will provide more complete knowledge and different alternatives to combat this complex disease. Therefore, with fluent communication and strong commitment from all those involved, there will be better research and surveillance (epidemiological and entomological), as well as more effective and efficient vector management to ultimately control the disease.
References


