Published in final edited form as:
J Vector Ecol. 2008 June ; 33(1): 76–88.

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

Adriana Troyo[1,2], **Olger Calderón-Arguedas**[2], **Douglas O. Fuller**[3], **Mayra E. Solano**[2], **Adrian Avendaño**[2], **Kristopher L. Arheart**[1], **Dave D. Chadee**[4], and **John C. Beier**[1,5]

1*Global Public Health Program, Department of Epidemiology and Public Health, University of Miami, 12500 SW 152 St. Building A, Miami, FL 33177, U.S.A.*

2*Centro de Investigación en Enfermedades Tropicales, Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica*

3*Department of Geography and Regional Studies, University of Miami, Miami, FL 33146, U.S.A.*

4*Department of Life Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago*

5*Abess Center for Ecosystem Science and Policy, University of Miami, Miami, FL 33146, U.S.A.*

Abstract

Dengue is the most important arboviral disease worldwide and the principal vector-borne disease in Costa Rica. Control of *Aedes aegypti* populations through source reduction is still considered the most effective way of prevention and control, although it has proven ineffective or unsustainable in many areas with a history of mosquito control. In this study, seasonal profiles and productivity of *Aedes aegypti* were analyzed in the city of Puntarenas, Costa Rica, where vector control has been practiced for more than ten years. Households contained more than 80% of larval habitats identified, although presence of habitats was more likely in other locations like lots and streets. In the wet season, habitats in the "other" category, like appliances, small manholes, and miscellaneous containers, were the most frequent habitats observed as well as the most common and productive habitats for *Ae. aegypti*. In the dry season, domestic animal drinking containers were very common, although concrete washtubs contained 79% of *Ae. aegypti* pupae collected. Individually, non-disposable habitats were as likely or more likely to contain mosquito larvae, and large containers were more likely to harbor mosquito larvae than the small ones only in the dry season. Considering various variables in the logistic regressions, predictors for *Ae. aegypti* in a habitat were habitat type ($p<0.001$), setting ($p=0.043$), and disposability ($p=0.022$) in the wet season and habitat capacity in the dry season ($p=0.025$). Overall, traditional *Ae. aegypti* larval indices and pupal indices in Puntarenas were high enough to allow viral transmission during the wet season. In spite of continued vector control, it has not been possible to reduce vector densities below threshold levels in Puntarenas, and the habitat profiles show that non-household locations, as well as non-disposable containers, should be targeted in addition to the standard control activities.

Keywords

Aedes aegypti; container; Breteau index; pupal survey; Costa Rica

INTRODUCTION

Several pathogens that affect human health are transmitted by 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 each year (World Health Organization 2002, Gibbons and 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 mosquitoes 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, Calderón-Arguedas et al. 2007a). *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 and Chadee 1997, Gubler 1998, Calderón-Arguedas et al. 2004). 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 and Kouri 2003, Gubler 2005, Chadee et al. 2005, Calderón-Arguedas et al. 2007b). A recent study suggests that evidence in favor of community-based dengue control programs is weak (Heintze et al. 2007).

Dengue is the most important vector-borne disease in Costa Rica. *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 ten 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 study 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 ten localities of the Greater Puntarenas area (Figure 1), which is a small port city of 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 m 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 Calderón-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 h. 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 (CalderónArguedas et al. 2004). 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 and La Classe 1955, Gonzalez and Darsie 1996, Vargas 1998). The presence of *Ae. aegypti* larvae, as well as the number of *Ae. aegypti* pupae, were specially noted in order to determine pupae per area and pupae per person (Focks and 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 the presence of mosquito larvae, or specifically *Ae. aegypti*, 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 the 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 1). The significance level for all statistical analyses was set at 0.05.

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 three 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 habitats positive for *Ae. aegypti*. Chi-square tests showed a significant association between the presence of one or more larval habitats in a location and the locality it belonged to, location type, and number of people in a house (Table 2). 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 three people and in locations such as lots, streets, and schools. The presence of mosquito larvae or pupae in a location was also associated with location types like large lots and streets (Table 2).

The wet season logistic regression revealed that when all variables were taken together, only locality was a significant predictor for the presence of one or more larval habitats in a location (Table 3). 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).

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 4). 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 5). 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 5). 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 5). 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 6).

According to the individual Chi-square tests, the presence of mosquito larvae in a habitat was associated with locality, location type, indoor/outdoor habitat setting, habitat type, and habitat disposability (Table 2); however, presence of larval habitats in a household were not associated with 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 2).

The logistic regression analysis showed that setting and habitat type were the two significant predictors for presence of mosquito larvae in a habitat (Table 3). 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 to be 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 predictors (Table 3). 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 to be 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 to the presence of larval habitats in a location (Table 7), where streets and schools seemed to be the locations more likely to have larval habitats.

The dry season logistic regression revealed that location type and people were significant predictors for the presence of one or more larval habitats in a location (Table 3). 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 of 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 4). 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 5). 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 5) and other large habitats (Table 6).

The presence of mosquito larvae in a habitat was individually associated with habitat type, capacity, and disposability during the dry season (Table 7). 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 with habitat capacity (Table 7), 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 predictor for the presence of mosquito larvae in a habitat (Table 3). 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 predictor for the 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 8). 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 nigripalpus, Culex interrogator, Culex coronator, Culex corniger, Ochlerotatus taeniorhynchus, Toxorhynchites sp., and *Uranotaenia* sp. In addition, *Ae. aegypti* larvae shared the habitat in 29 cases (19% of all habitats positive for *Ae. aegypti*), which were commonly *Cx. quinquefasciatus* (eight habitats) and *L. durhamii* (seven habitats) but also all other species mentioned except *Uranotaenia* sp.

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 require redirecting 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 (Calderón-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 to 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 (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 and Chadee 1997, Focks 2003, Barrera et al. 2006).

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. 2004, 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 and 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 that were 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 that 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. 2006).

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 is 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 and Chadee 1997, Calderón-Arguedas et al. 2004). 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 washtubs during the dry 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, intersectoral, and interinstitutional, 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.

Acknowledgments

We thank the people of Puntarenas and the local Ministry of Health, Lissette Retana, Nelson Mena, Iván Coronado, Adriana Duarte, Julio Rojas, and Christian Fonseca for their extensive efforts in performing the field surveys.

This research was supported by Grant Number P20 RR020770 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. AT, OC, MES, and AA were also supported by University of Costa Rica and projects VI-803-A6-401 and VI 803-A6-039, and JCB by the Abess Center for Ecosystem Science and Policy, University of Miami.

REFERENCES CITED

Baly A, Toledo ME, Boelaert M, Reyes A, Vanlerberghe V, Ceballos E, Carvajal M, Maso R, La Rosa M, Denis O, Van der Stuyft P. Cost effectiveness of *Aedes aegypti* control programmes: participatory versus vertical. Trans. R. Soc. Trop. Med. Hyg 2007;101:578–586. [PubMed: 17368696]

Barrera R, Amador M, Clark GC. Use of the pupal survey technique for measuring *Aedes aegypti* (Diptera: Culicidae) productivity in Puerto Rico. Am. J. Trop. Med. Hyg 2006;74:290–302. [PubMed: 16474086]

Bisset-Lazcano JA, Marquetti MC, Portillo R, Rodríguez MM, Suárez S, Leyva M. Factores ecológicos asociados con la presencia de larvas de *Aedes aegypti* en zonas de alta infestación del municipio Playa, Ciudad de La Habana. Cuba. Rev. Panam. Salud Publica 2006;19:397–384.

Burkot TR, Handzel T, Schmaedick MA, Tufa J, Roberts JM, Graves PM. Productivity of natural and artificial containers for *Aedes polynesiensis* and *Aedes aegypti* in four Amarican Samoan villages. Med. Vet. Entomol 2007;21:22–29. [PubMed: 17373943]

Calderón-Arguedas O, Solano ME, Troyo A. Sitios potenciales para la multiplicación de *Aedes aegypti* (Diptera: Culicidae) en relación con las características de las viviendas de la comunidad "La Carpio", San Jose, Costa Rica, durante la estación seca de 2003. Rev. Costarric. Salud Pública 2003;12:22–30.

Calderón-Arguedas O, Troyo A, Solano ME. Caracterización de los sitios de multiplicación de *Aedes aegypti* (Diptera: Culicidae) en el caserío "La Carpio", San José, Costa Rica durante la estación seca del año 2003. Rev. Biomed 2004;15:73–79.

Calderón-Arguedas O, Troyo A, Solano ME, Avendaño A. Culicidofauna (Diptera: Culicidae) asociada con contenedores artificiales en una comunidad en riesgo de dengue de San José, Costa Rica. Rev. Biol. Trop. 2007ain press

Calderón-Arguedas O, Troyo A, Solano ME, Avendaño A. Impacto de una iniciativa de participación comunitaria sobre los índices larvales tradicionales para *Aedes aegypti* (Diptera: Culicidae) en una comunidad urbana en riesgo de dengue en San José, Costa Rica. Rev. Biomed 2007b;18:27–36.

Carpenter, SJ.; La Classe, WJ. Mosquitoes of North America. University of California Press; Los Angeles, CA: 1955.

Chadee DD, Williams FLR, Kitron UD. Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998. Trop. Med. Int. Hlth 2005;10:748–754.

Chadee DD. Key premises, a guide to *Aedes aegypti* (Diptera: Culicidae) surveillance and control. Bull. Entomol. Res 2004;94:201–207. [PubMed: 15191621]

Edelman R. Dengue vaccines approach the finish line. Clin. Infect. Dis 2007;15(Suppl 1):S56–60. [PubMed: 17582571]

Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am. J. Trop. Med. Hyg 2000;62:11–18. [PubMed: 10761719]

Focks DA, Chadee DD. Pupal survey: An epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. Am. J. Trop. Med. Hyg 1997;56:159–167. [PubMed: 9080874]

Focks DA, Sackett SR, Bailey DL, Dame DA. Observations on container-breeding mosquitoes in New Orleans, Louisiana, with an estimate of the population density of *Aedes aegypti* (L). Am. J. Trop. Med. Hyg 1981;30:1329–1335. [PubMed: 7325289]

Focks, DA. A Review of Entomological Sampling Methods and Indicators for Dengue Vectors. World Health Organization; Geneva: 2003. Document WHO/TDR/IDE/Den/03.1

Getis A, Morrison AC, Gray K, Scott TW. Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru. Am. J. Trop. Med. Hyg 2003;69:494–505. [PubMed: 14695086]

Gibbons RV, Vaughn DW. Dengue: an escalating problem. Brit. Med. J 2002;324:1563–1566. [PubMed: 12089096]

Gonzalez R, Darsie RF. Clave ilustrada para la determinación genérica de larvas de Culicidae de Colombia y el Nuevo Mundo. Bol. Mus. Ent. Univ. Valle 1996;4:21–37.

Gubler DJ. Dengue and dengue hemorrhagic fever. Clin. Microbiol. Rev 1998;11:480–496. [PubMed: 9665979]

Gubler DJ. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. Rev. Panam. Salud Publica 2005;17:221–224. [PubMed: 15969972]

Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. J. Clin. Virol 2003;27:1–13. [PubMed: 12727523]

Heintze C, Velasco-Garrido M, Kroeger A. What do community-based control programmes achieve? A systematic review of published evaluations. Trans. R. Soc. Trop. Med. Hyg 2007;101:317–325. [PubMed: 17084427]

Impoinvil DE, Ahmad S, Troyo A, Keating J, Githeko AK, Mbogo CM, Kibe L, Githure JI, Gad AM, Hassan AN, Orshan L, Warburg A, Calderón-Arguedas O, Sanchez-Loria VM, Velit-Suarez RV, Chadee DD, Novak RJ, Beier JC. Comparison of mosquito control programs in seven urban sites in Africa, the Middle East, and the Americas. Hlth. Policy 2007;83:196–212.

Kuno G. Review of the factors modulating dengue transmission. Epidemiol. Rev 1995;17:321–335. [PubMed: 8654514]

Maciel-de-Freitas R, Marques WA, Peres RC, Cunha SP, de Oliveira RL. Variation in *Aedes aegypti* (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. Mem. Inst. Oswaldo Cruz 2007;102:489–496. [PubMed: 17612770]

Morrison AC, Astete H, Chapilliquen F, Diaz G, Getis A, Gray K, Scott TW. Evaluation of a sampling methodology for rapid assessment of *Aedes aegypti* infestation levels in Iquitos, Peru. J. Med. Entomol 2004;41:502–510. [PubMed: 15185957]

Muturi EJ, Shilulu JI, Gu W, Jacob BG, Githure JI, Novak RJ. Larval habitat dynamics and diversity of *Culex* mosquitoes in rice agro-ecosystem in Mwea, Kenya. Am. J. Trop. Med. Hyg 2007;76:95–102. [PubMed: 17255236]

Norman G, Theodre A, Joseph A. An insular outbreak of dengue fever in a rural south Indian village. J. Commun. Dis 1991;23:185–190. [PubMed: 1812164]

Ottesen EA. Lymphatic filariasis: Treatment, control and elimination. Adv. Parasitol 2006;61:395–441. [PubMed: 16735170]

Sanchez L, Vanlerberghe V, Alfonso L, Marquetti M, Guzman MG, Bisset J, van der Stuyft P. *Aedes aegypti* larval I ndices and risk for dengue epidemics. Emerg. Infect. Dis 2006;12:800–806. [PubMed: 16704841]

Service MW. Importance of ecology in *Aedes aegypti* control. Southeast Asian J. Trop. Med. Publ. Hlth 1992;23:681–689.

Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, Ghebregziabiher A, Sintasath D, Bretas G, Mbogo C, Githure J, Brantly E, Novak R, Beier JC. Larval habitat diversity and ecology of anopheline larvae in Eritrea. J. Med. Entomol 2003;40:921–929. [PubMed: 14765671]

Strickman D, Kittayapong P. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. Am. J. Trop. Med. Hyg 2003;68:209–217. [PubMed: 12641413]

Troyo A, Fuller DO, Calderón-Arguedas O, Beier JC. A geographical sampling method for surveys of mosquito larvae in an urban area using high-resolution satellite imagery. J. Vector Ecol 2008;33*add pages*

Troyo A, Porcelain SL, Calderón-Arguedas O, Chadee DD, Beier JC. Dengue in Costa Rica: the gap in local scientific research. Pan. Am. J. Publ. Hlth 2006;20:350–360.

Vargas, M. El mosquito, un enemigo peligroso (Diptera: Culicidae). Editorial Universidad de Costa Rica; San José, Costa Rica: 1998.

World Health Organization. Outbreak of classic dengue, Costa Rica. Wkly. Epidemiol. Rec 1994;69:85–86. [PubMed: 8003402]

World Health Organization. Report by the Secretariat. Fifty-fifth World Health Assembly; Geneva: 2002. Dengue prevention and control. [http://www.who.int/gb/ebwha/pdf_files/WHA55/ea5519.pdf]

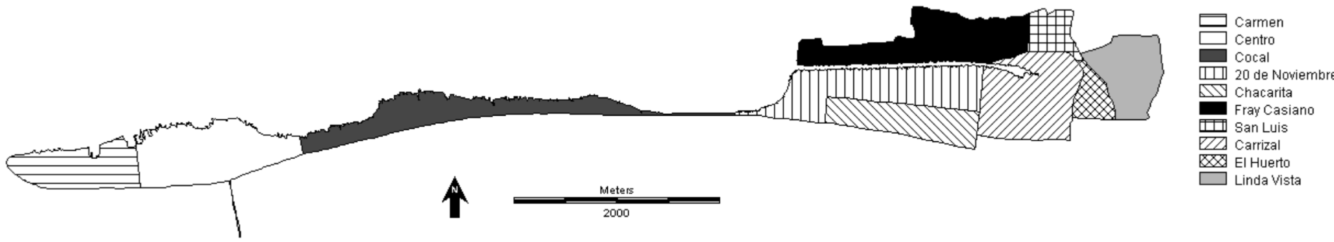

Figure 1.
Map of the localities of Puntarenas surveyed.

Table 1

Variables included in the seasonal logistic regression models.

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

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

Table 2

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

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

Table 3

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.

Outcome variable (season)	Predictor variables	Wald χ^2	DF	p
One or more larval habitats in a location (wet)	Locality	37.50	7	<0.001
	Private	0.65	1	0.422
	People	0.55	1	0.456
	Location type	4.33	3	0.228
One or more larval habitats in a location (dry)	Locality	10.85	7	0.145
	Private	0.78	1	0.377
	People	9.66	1	0.002
	Location type	8.01	3	0.046
Mosquito larvae in a habitat (wet)	Locality	16.45	9	0.058
	Location type	7.32	4	0.120
	Setting	5.77	1	0.016
	Habitat type	43.35	10	<0.001
	Disposability	0.02	1	0.885
	Capacity	1.45	2	0.485
Mosquito larvae in a habitat (dry)	Locality	10.75	6	0.096
	Setting	0.26	1	0.607
	Disposability	0.06	1	0.801
	Capacity	9.70	2	0.008
Ae. aegypti in a habitat (wet)	Locality	12.47	8	0.131
	Location type	0.31	4	0.989
	Setting	4.11	1	0.043
	Habitat type	35.86	10	<0.001
	Disposability	5.29	1	0.022
	Capacity	2.66	2	0.265
Ae. aegypti in a habitat (dry)	Locality	11.22	6	0.082
	Setting	0.74	1	0.389
	Disposability	0.54	1	0.461
	Capacity	7.38	2	0.025

Table 4

Seasonal distribution of larval habitats identified according to location type.

Location type	Wet season		Dry season	
	Larval habitats (%)	Habitats with *Ae. aegypti* (%)	Larval habitats (%)	Habitats with *Ae. aegypti* (%)
Household	659 (80)	84 (77)	383 (83)	20 (95)
School	37 (4)	6 (6)	21 (5)	1 (5)
Empty lot (small)	58 (7)	6 (6)	17 (4)	0
Large lot	31 (4)	7 (6)	4 (1)	0
Street	16 (2)	2 (2)	14 (3)	0
Large building	14 (2)	2 (2)	11 (2)	0
Field/stadium	6 (1)	1 (1)	6 (1)	0
Small business	7 (1)	1 (1)	0	0
Other	1 (0.1)	0	5 (1)	0
Total	829 (100)	109 (100)	461 (100)	21 (100)

Table 5

Seasonal distribution of larval habitats identified according to habitat type.

Habitat type	Wet season			Dry season		
	Larval habitats (%)	**Habitats with *Ae. aegypti* (%)**	***Ae. aegypti* pupae (%)**	**Larval habitats (%)**	**Habitats with *Ae. aegypti* (%)**	***Ae. aegypti* pupae (%)**
Small can/plastic	183 (22)	21 (19)	55 (10)	15 (3)	3 (14)	1 (0.7)
Bucket	97 (12)	13 (12)	49 (9)	29 (6)	1 (5)	0 (0)
Tire	17 (2)	9 (8)	7 (1)	4 (1)	1 (5)	3 (2)
Drum	29 (4)	8 (7)	91 (17)	10 (2)	3 (14)	7 (5)
Washtub	56 (7)	5 (5)	44 (8)	75 (16)	6 (29)	104 (79)
Gutter	11 (1)	4 (4)	49 (9)	0	0	0
Animal water	123 (15)	2 (2)	2 (0.4)	147 (32)	0	0
Flower pot	10 (1)	2 (2)	11 (2)	10 (2)	1 (5)	0
Vase	9 (1)	2 (2)	0	11 (2)	1 (5)	0
Coconut	19 (2)	1 (1)	5 (1)	3 (1)	0	0
Sewer	4 (0.5)	0	0	8 (2)	0	0
Bottle	48 (6)	0	0	9 (2)	0	0
Other	223 (27)	42 (38)	217 (41)	140 (30)	5 (24)	16 (12)
Total	829 (100)	109 (100)	530 (100)	461 (100)	21 (100)	131 (100)

Table 6

Seasonal distribution of habitats containing *Ae. aegypti* larvae and/or pupae according to habitat capacity.

Capacity	**Wet season**		**Dry season**	
	Habitats with *Ae. aegypti* (%)	***Ae. aegypti* pupae (%)**	**Habitats with *Ae. aegypti* (%)**	***Ae. aegypti* pupae (%)**
Small	43 (39)	147 (28)	5 (24)	2 (2)
Medium	40 (37)	190 (36)	11 (52)	9 (7)
Large	26 (24)	193 (36)	5 (24)	120 (92)
Total	109 (100)	530 (100)	21 (100)	131 (100)

Table 7

Variables and results of the independent Chi-square tests of association applied to the Puntarenas dry season data

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

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

Table 8

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

	Container Index	Location Index	Breteau Location Index	Pupae per person	Pupae per hectare
Wet season	13.2	17.2	22.9	0.36	15.6
Dry season	4.6	3.9	4.13	0.09	3.9

NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 9

Frequency of habitats containing other mosquito species in Puntarenas, Costa Rica.

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