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Urban mosquito species (Diptera: Culicidae) of dengue endemic communities in the Greater Puntarenas area, Costa Rica

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Abstract

Field studies were conducted to determine the mosquito species richness in the urban area of Greater Puntarenas in Costa Rica. Two cross-sectional entomological surveys were performed in seven localities of Puntarenas: one survey was performed during the wet season and the other during the dry season. The sections evaluated were determined by applying a stratified cluster sampling method using satellite imagery, and a sample of 26 cells (100×100m) was selected for the study. The number of cells per locality was proportional to the area of each locality. The presence of mosquito larvae and pupae in water-filled artificial and natural containers was determined in each cell. Infestation was expressed as a diversity index per type of container (Ii). Eight types of larvae were identified (*Aedes aegypti*, *Culex quinquefasciatus*, *Culex interrogator*, *Culex nigripalpus*, *Culex corniger*, *Culex tarsalis*, *Limatus durhamii* and *Toxorhynchites theobaldi*) and in two cases it was only possible to identify the genus (*Culex* sp. and *Uranotaenia* sp.). *A. aegypti* was the most common species followed by *C. quinquefascitus*. Diversity of wet environments can explain the co-occurrence of various culicid species in some localities. Although *A. aegypti* is the only documented disease vector in the area, *C quinquefasciatus*, *C. nigripalpus*, and the other species of *Culex* could be considered potential vectors of other pathogens. The presence and ecology of all mosquito species should be studied to optimize surveillance and prevention of dengue and to prevent the emergence of other mosquito-transmitted diseases.

Keywords

mosquito; Culicidae; dengue; species richness; Puntarenas; Costa Rica

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Mosquitoes are the most important vectors of pathogenic organisms. Diseases like malaria, dengue fever, yellow fever and West Nile encephalitis are transmitted by culicids (Foster & Walker 2002). In tropical and subtropical countries, dengue is the most important arboviral disease in terms of morbidity and mortality. Some reports estimate dengue incidence at 50 to 100 million cases per year, including 250 000 to 500 000 cases of dengue hemorrhagic fever and approximately 24 000 deaths (Gibbons & Vaughn 2002).

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In Costa Rica, dengue is the most prevalent vector-borne disease, affecting mainly the North Pacific, Atlantic, and Central Pacific regions. In 2005, there were almost 40 000 reported cases (Troyo *et al.* 2006). *Aedes aegypti* Linnaeus 1762, which is the main dengue vector, was eliminated from the country in the late 1950s. However, new dengue cases were reported in September of 1993, a few months after the Ministry of Health reported that *A. aegypti* was once again present throughout the country (Guzmán *et al.* 1998). Since then, the larger cities in the North Pacific, Central Pacific, and Atlantic coasts have been the areas most affected by dengue (Troyo *et al.* 2006).

Puntarenas is one of the cities of Costa Rica where dengue was first reported in 1993 (Guzmán *et al.* 1998). It is the most important city in the Central Pacific Region, and its economy is based almost entirely on tourist activity and fishing (Abarca-Hernández 2008). This city presents some of the common problems of many larger cities in Latin America: poor urban planning, high unemployment rates, poverty, poor solid waste management, among others (INEC 2002). Its urban and environmental conditions make this city an appropriate environment for the presence of *A. aegypti* and consequently dengue.

The heterogeneity of the urban landscape and its relationship with peri-urban areas can support the occurrence of mosquito species other than *A. aegypti* (Vargas 1998). On occasion, some mosquito species can share their larval habitats with *A. aegypti*, but there are other species that have very different and more specific habitats for oviposition and larval development. The species richness is defined as the number of species in a community. Species richness and relative abundance are ecological components that can be used to quantify species diversity (Krohne 1998).

The purpose of this investigation was to determine the species richness in localities of Greater Puntarenas. Results from these analyses may be used to estimate risk of transmission of various mosquito borne diseases and optimize local mosquito prevention and control programs (Impoinvil *et al.* 2007).

MATERIAL AND METHODS

Puntarenas is located in the Central Pacific Region of Costa Rica (09°56′55″ N, 84°58′24″ W). It is a peninsula that includes an area of approximately 20km^2, with urban and suburban characteristics (Fig. 1). The elevation of the Greater Puntarenas area is from 0m to 15m. The climate 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). The population of the Greater Puntarenas area is close to 100 000 people that live in approximately 20 000 houses (INEC 2002).

Two cross-sectional larval surveys were performed to determine the species richness of mosquitoes in seven localities of Greater Puntarenas. One of the surveys was performed during the dry season and the other was carried out in the wet season. The sampling method was a two-step cluster sampling process established in conjunction with seasonal surveys of container profiles, according to the methodology described by Troyo and collaborators (2008a). Briefly, imagery from the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) and a QuickBird land cover map were used to create the sampling frame by dividing the entire area into a grid with cells of 100×100m. This cell size was considered optimal since cells would contain 13±6 houses for field surveys. Only the 306 cells that had more than 90% of their area within one specific locality of Puntarenas were included in the sampling frame. This would guarantee that larval habitats found in a grid cell searched could be considered as belonging to one locality.

Twenty-six cells were selected for the detailed evaluation of species richness and identification of the possible larval habitats. The number of cells surveyed per locality was proportional to the area of each locality. Due to the geographical nature of the sampling method, each of the sampled cells contained different types of locations such as houses, streets, parks, schools, and public areas.

During the surveys, wet habitats that contained culicid larvae and/or pupae were registered, and a sample of mosquito larvae and pupae were transported to the Medical Entomology laboratory at the University of Costa Rica. Entomological material was cleared and mounted in Hoyer's medium for microscopic analysis and identification, which was based on standard taxonomic keys (Dyar 1928, Carpenter & La Casse 1955, Clark-Hill & Darsie 1983). This taxonomic information was utilized to determinate the species richness in the Greater Puntarenas area. Data were grouped and organized according to season and locality. The infestation was expressed as a Diversity Index per type of breeding site (Ii) (Bisset *et al.* 1985), which is defined as:

$$Ii=\frac{Ni}{N}$$

Where,

Ni = Number of breeding sites (of the type of interest) positive for the i^{th} species

N = Total number of breeding sites (of the type of interest) positive for any of the species

A database was created in Microsoft Excel© and imported to Statistix 8 software (Analytical Software), where the statistical analyses were performed. Chi-square tests of homogeneity were performed to evaluate the distribution of frequencies relating positive containers per locality with particular taxa (Daniel 2004).

RESULTS

Mosquito larvae were detected in each survey. However, the wet season showed the highest percentages of positive locations (Table 1), most of which were houses. However, public and private buildings, as well as urban open spaces like lots, schools, and streets were also positive for habitats containing mosquito larvae (Fig. 2). The species richness was represented by eight species identified as: *Aedes aegypti*, *Culex quinquefasciatus* Say 1826, *Culex interrogator* Dyar & Knab 1906, *Culex nigripalpus* Theobald 1901, *Culex corniger* Theobald 1901, *Culex tarsalis* Coquillet 1896, *Limatus durhamii* Theobald 1901 and *Toxorhynchites theobaldi* Dyar & Knab 1906 (Table 2). Due to poor condition and insufficient development, the remaining two types of larvae were identified to genus: *Culex* sp. and *Uranotaenia* sp. (Table 2). Overall, *A. aegypti* was the most frequently collected species in the localities of Greater Puntarenas (Tables 3 and 4). *Culex quinquefasciatus* was the second most common in terms of occurrence. These two mosquito species were observed in both seasons, but the others were reported only in one of them (Tables 3 and 4). Carrizal was the locality that showed the highest number of locations positive for mosquito larval habitats, and it was also the locality with the greatest richness of mosquito species (Tables 3 and 4).

The distribution of infestation by species among localities did not show homogeneity in either season (dry season: χ^2=28.71, p=0.53, d.f.=30; wet season: χ^2= 52.12, p=0.55, d.f.=54).

The most common habitats containing mosquito larvae were considered permanent and included natural and artificial deposits such as washtubs, roof gutters, puddles, sewers and manholes (Table 4). In this category, gutters and washtubs were frequent sites of larval development. The second most frequent habitat type was the “miscellaneous” category, which included objects like toys, pieces of machinery, cups, shoes, among others, that were usually located outdoors. *A. aegypti* showed the highest diversity indices for most types of containers evaluated (Table 4).

DISCUSSION

Mosquitoes are common dipterans in the Greater Puntarenas area (Table 1). The species richness observed in the present study revealed the coexistence of different taxa in a relatively small geographical area (Table 2).

In this area there are variations in the urban environment between localities that promote the occurrence of different mosquito species. These differences are likely to include urban structure, type of vegetation, natural water deposits, and human behavior, which can favor the selection of oviposition sites by particular species of female mosquitoes. Our results showed that the distribution of the infestation between localities was not homogeneous in any of the surveys performed throughout the year. The Western side of Puntarenas, which includes the localities of Carmen and Centro, constitutes the urban core of the Greater Puntarenas area (Fig. 1). It shows the traditional landscape of coastal city. In these localities, *A. aegypti* and *C. quinquefasciatus* were the most frequent species found. Although both species were observed during the dry season, higher infestation was documented during the wet season (Table 3). Both *A. aegypti* and *C. quinquefasciatus* are very common species in other urban areas of Costa Rica, such as the Central valley (Calderón-Arguedas 2004,Salazar-Chang 2005). In coastal cities of other countries where dengue is endemic, *A. aegypti* and *C. quinquefasciatus*, are also the main urban mosquito species found (Bisset *et al.* 1987).

The majority of the positive habitats identified in the Western section were non-disposable habitats and miscellaneous containers (Table 4), which were located in or around houses. However, there were public spaces and buildings that also contained an important number of larval habitats (Fig. 2). These locations like schools and streets are commonly excluded during entomological surveys, but they can harbor positive water filled containers that help maintain vector populations in endemic areas and can function as a source of mosquitoes that infest nearby areas (Focks *et al*. 1981,Troyo *et al*. 2008b).

In the central portion of the Greater Puntarenas area is Cocal. This locality is an isthmus that links the West side of Puntarenas (peninsula) to the East side (Fig. 1). In this locality the density of houses is low in comparison to other localities in the Greater Puntarenas area. This could explain the very low positivity of mosquito larval habitats in Cocal (Table 1). *A. aegypti* was the only species that was documented in this sector. Similar to observations on the west side of Puntarenas, most of the positive habitats were water filled containers associated with households.

The eastern side of the Greater Puntarenas area includes the localities of Chacarita, Fray Casiano, 20 de Noviembre and Carrizal (Fig. 1). In these localities, the landscape is very heterogeneous, where the occurrence of open spaces, non-paved streets, clusters of houses, and several types of vegetation is common. There is also an important diversity in the type of housing, which includes different types of construction, from beach houses to very poor living ranches. Particularly in localities like 20 de Noviembre and Fray Casiano, socioeconomic conditions are variable and there are a few very poor neighborhoods. In these localities, a high *A. aegypti* infestation was reported, mainly in miscellaneous objects located outside the houses (Table 4). These objects probably collected rained water during the wet season and made them adequate sites for oviposition.

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In contrast with the Western side of Greater Puntarenas, in the Eastern section most of the positive habitats identified were non-disposable. Within the non-disposable category, gutters were very efficient for maintaining *A. aegypti* larval populations. Gutters represent a real problem, especially since most of the ones observed in Puntarenas were constructed with plastic materials that are easily deformed by environmental conditions like temperature changes. This promotes the accumulation of water and organic debris from vegetation and dust, which makes the conditions ideal for larval development. Additionally, the height of roof gutters does not allow for their continued surveillance by household inhabitants. Similarly to what was observed for the other areas of Puntarenas, other non-disposable deposits like washtubs were important larval habitats in the eastern section of Greater Puntarenas, particularly in the dry season. Washtubs are used by the population to store water in order to facilitate the household labors and not necessarily due to problems with piped water service (Troyo *et al.* 2008b). These containers were the most common type with *A. aegypti* larvae in the dry season, and they are probably responsible for maintaining *A. aegypti* populations during drier months. In a study performed in the locality of "El Progreso", Honduras, washtubs were also important deposits that promoted the development of *A. aegypti* larvae (Leonstini *et al.* 1993). Together, washtubs and gutters could explain the permanence of *A. aegypti* during the entire year in the Puntarenas area, which may allow the occurrence of dengue cases in both dry and wet seasons.

In the eastern localities of Puntarenas, as was described for the western localities, many positive containers were associated with houses, but an important number was also associated with non-household settings like lots, streets and public spaces. A previous study in the Puntarenas area determined that the eastern localities have higher NDVI (normalized difference vegetation index) values, which could be explained by the abundance of trees and open spaces covered by grasses and small vegetation (Troyo *et al.* 2009).

Other culicid species like *L. durhamii* were present in some of the open spaces evaluated such as parks, streets, and lots of the eastern section of Puntarenas. The larvae of *L. durhamii* resemble those of *A. aegypti* when observed by the naked eye, and this can complicate the calculation of aedic indices during dengue surveillance when identification is performed without magnifying devices. In addition, *L. durhamii* larvae have shown a facultative predatory activity when nutritional resources are scarce (Lopes 1999), and this is probably the reason why this species was mostly found sharing the habitat with other mosquito species like *A. aegypti*, *C. quinquefasciatus* and other species of *Culex* (Table 3).

In terms of diversity, Carrizal was the locality that showed the highest mosquito diversity (Tables 1 and 2). In Carrizal, the landscape includes small clusters of houses as well as open spaces, where the occurrence of natural water deposits like small lakes, swamps, and streams is common. In this locality, *A. aegypti* was observed only during the wet seasons, when the general abundance of this species is estimated to be very high. In addition to *A. aegypti* and *C. quinquefasciatus*, there were other species observed in Carrizal: *C. coronator*, *C. tarsalis*, *C. nigripalpus*, *C. interrogator*, *C.* sp., *L. durhamii*, *T. theobaldi* and *Uranotaenia* sp. Most of these species had been documented in a study performed in the irrigation project of Arenal-Tempisque, located in communities of the Guanacaste Province (Vargas & Vargas 2003). *C. coronator*, *C. corniger*, *L. durhamii*, *T. theobaldi* and *Uranotaenia* have also been reported in the Central valley of Costa Rica (Calderón-Arguedas *et al.* 2004,Salazar-Chang 2005).

The analysis of the diversity per type of breeding site demonstrates that *A. aegypti* show high values for the diversity index in nearly all categories of containers (Table 4). In these sense *A. aegypti* is the dominant culicid in the area, and the treatment or elimination of these water filled containers are required for its control. The other species of mosquitoes show low values of the diversity index for particular types of containers (Table 4). With the exception of *C. quinquefasciatus*, these species are usually associated with ecological environments different from those found in urban areas but can occasionally be found in urban ecosystems like the one studied. Therefore, having different species of mosquitoes coexisting in the urban environment suggests that different strategies are needed to control nuisance mosquitoes and potential vectors of human diseases.

The role of *A. aegypti* as the vector of dengue has been documented in the Greater Puntarenas area. A previous study demonstrated that rainfall is associated with increases in dengue cases in Puntarenas. Also, the abundance of *A. aegypti* usually increases in the wet season, which is reflected in the higher values of the traditional larval indices for *A. aegypti* (Troyo *et al.* 2008b).

Although *C. quinquefasciatus* has not been associated with the transmission of any vector-borne disease in Puntarenas, it has been demonstrated that *C. quinquefasciatus* can feed from both birds and humans, an ideal behavior for the transmission of West Nile Virus (Zinser *et al.* 2004). Therefore, the high prevalence of this species in the area may increase the risk of West Nile Virus transmission. Of the other culicid species found in Puntarenas, *C. nigripalpus* and *C. tarsalis* have also been associated with transmission of West Nile virus (Mores *et al.* 2007, Reisen *et al.* 2006), as well as other viral encephalitis, specifically Saint Louis encephalitis for *C. ningripalpus* and Eastern equine encephalitis for *C. tarsalis* (Kramer 2001). However, there is currently no information about the possible role that these species may play in the transmission of these arbovirosis in Costa Rica.

T. theobaldi, is a predator species in the larval stage, and the fact that the adults do not ingest blood have made it a candidate for biological control of other mosquito species (Lopes *et al.* 1993). Since *T. theobaldi* and *L. durhamii* show predatory activity, their role as ecological modulators of other culicid populations warrants further investigation.

The analysis of each vector as a particular vector of pathogens in Puntarenas and in the rest of the country requires additional evaluation. Although *A. aegypti* is currently the main focus of health authorities, these biodiversity results in Greater Puntarenas demonstrate that the current mosquito control programs should include surveillance of all culicid species present in order to understand the potential risk for transmission of additional mosquito-borne diseases in the area. Moreover, it should be clear that the larval habitats that are targeted during control of *A. aegypti* will not consistently and uniformly affect the abundance of other culicids. When the abundance of *A. aegypti* is low, reinfestation by other mosquito species and nuisance biting can be perceived by people as a fail in the *A. aegypti* control. Therefore all species present and the different strategies required should be considered when developing mosquito control measures.

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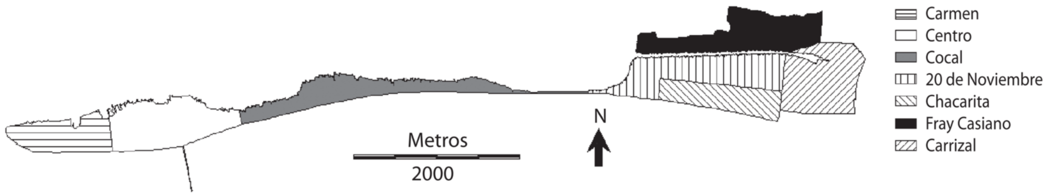

Fig. 1.
Localities of the Greater Puntarenas area, Costa Rica.

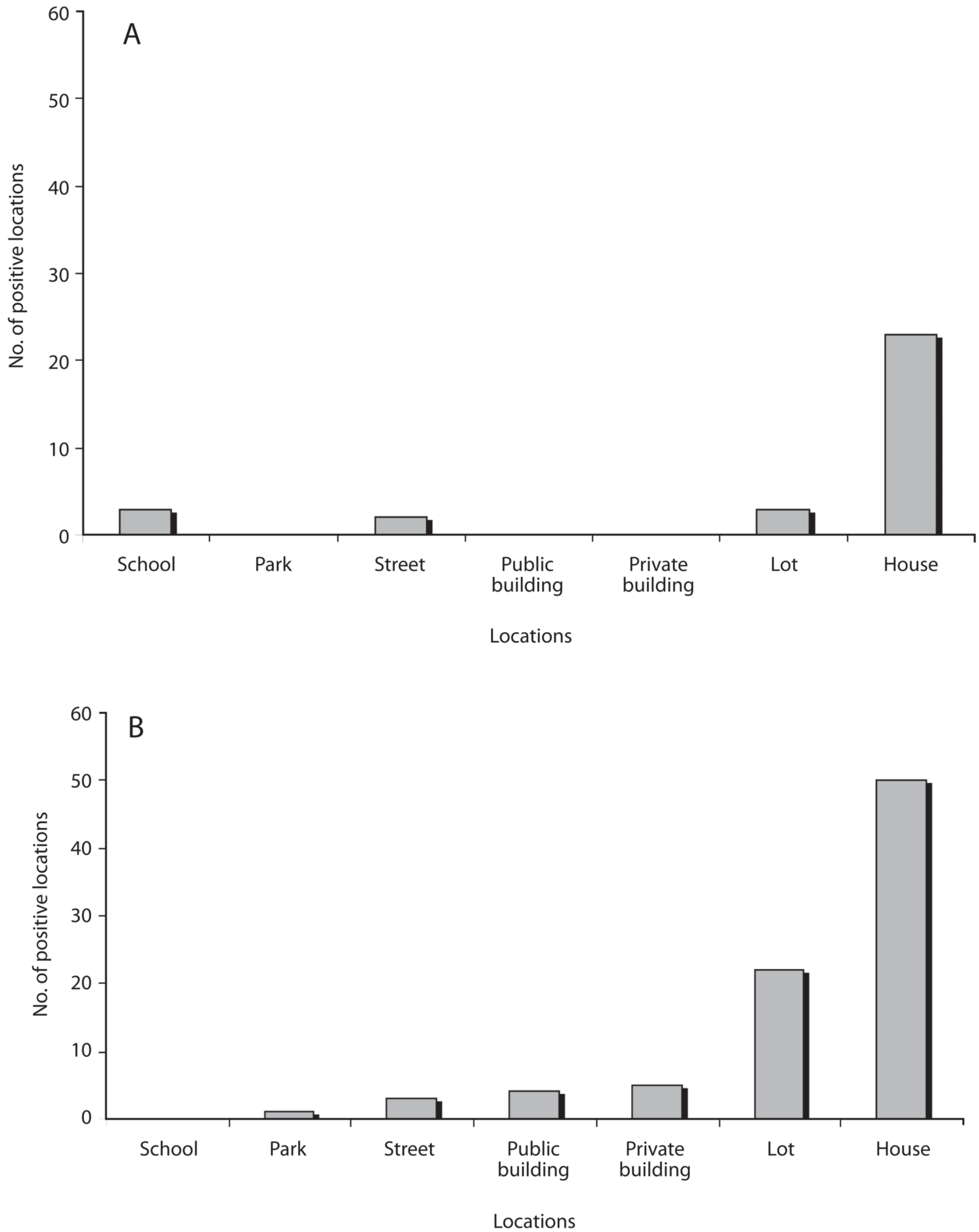

Fig. 2.
Types of locations positive for mosquito larvae. a: dry season; b: wet season.

TABLE 1

Positive locations per locality for mosquito larvae according to season

	Dry season		Wet season	
Locality	**Frequency**	**%**	**Frequency**	**%**
Carmen	6*/101**	5.9	9/69	13.0
Centro	2/67	2.9	13/58	22.4
Cocal	0/40	0.0	7/35	20.0
Chacarita	5/39	12.8	7/47	14.9
Fray Casiano	6/90	6.7	10/48	20.8
20 de Noviembre	5/44	11.4	6/54	11.1
Carrizal	6/52	11.5	15/43	34.9
Total	30/433	6.9	67/354	18.9

* number of positive locations.

** total number of locations surveyed.

TABLE 2

Species of mosquito larvae identified according to season

Season	Genus	Sub-genus	Species
Dry	*Aedes*	(*Stegomyia*)	*A. aegypti*
	Culex	(*Culex*)	*C. quinquefasciatus*
		(*Culex*)	*C. tarsalis*
		(*Culex*)	*C. coronator*
		(*Culex*)	*C. nigripalpus*
		(*Culex*)	*C. interrrogator*
		(*Culex*)	*Culex* sp.
Wet	*Aedes*	(*Stegomyia*)	*A. aegypti*
	Culex	(*Culex*)	*C. quinquefasciatus*
		(*Culex*)	*C. nigripalpus*
		(*Culex*)	*C. interrogator*
		(*Culex*)	*C. corniger*
		(*Culex*)	*C. coronator*
		(*Culex*)	*Culex* sp.
	Toxorrhynchites	(*Linchiella*)	*T. theobaldi*
	Limatus		*L. durhamii*
	Uranotaenia	(*Uranotaenia*)	*Uranotaenia* sp.

TABLE 3

Number of containers positive for each mosquito species according to locality and season

Season	Locality	Species										
		Ae	Cq	Cc	Ct	Cn	Cco	Ci	Cx	Ld	Ur	Tt
Dry	Carmen	4 (20.0*)	1 (10.0)						1 (25.0)			
	Centro	2 (10.0)										
	Cocal											
	Chacarita	5 (25.0)	1 (10.0)									
	Fray Casiano	4 (20.0)	3 (30.0)									
	20 de Nov.	5 (25.0)	2 (20.0)									
	Carrizal		3 (30.0)	1 (100.0)	1 (100.0)	1 (100.0)		2 (100.0)	3 (75.0)			
	Total	20 (100.0)	10 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)		2 (100.0)	4 (100.0)			
Wet	Carmen	9 (11.8)	1 (7.1)									
	Centro	14 (18.4)										
	Cocal	8 (10.5)										
	Chacarita	8 (10.5)	1 (7.1)			3 (33.3)		1 (50.0)				
	Fray Casiano	9 (11.9)	2 (14.3)			1 (11.1)				2 (18.2)		
	20 de Nov.	6 (7.9)	2 (14.3)			1 (11.1)	1 (100.0)					
	Carrizal	22 (28.9)	8 (57.1)	1 (100.0)		4 (44.4)		1 (50.0)	2 (100.0)	9 (81.8)	1 (100.0)	2 (100.0)
	Total	76 (100.0)	14 (100.0)	1 (100.0)		9 (100.0)	1 (100.0)	2 (100.0)	2 (100.0)	11 (100.0)	1 (100.0)	2 (100.0)

Ae: A. aegypti; **Cq**: C. quinquefasciatus; **Cc**: C. coronator; **Ct**: C. tarsalis; **Cn:** C. nigripalpus; **Cco**: C. corniger, **Ci**: C. interrogator, **Cx**: Culex sp.; **Ld**: Limatus durhamii; **Ur**: Uranotaenia sp.; **Tt**: Toxorhynchites theobaldi.

* percents.

TABLE 4

Diversity index (Ii) per type of container identified

		Species										
Season	**Container**	**Ae**	**Cq**	**Cc**	**Ct**	**Cn**	**Cco**	**Ci**	**Cx**	**Ld**	**Ur**	**Txt**
Dry	Buckets	0.50 (1/2)*							0.50 (1/2)			
	Drums	0.80 (4/5)	0.20 (1/5)						0.20 (1/5)			
	Cans	0.75 (3/4)	0.25 (1/4)									
	Permanent	0.56 (9/16)	0.43 (7/16)	0.06 (1/16)	0.06(1/16)	0.06(1/16)		0.12 (2/16)	0.12 (2/16)			
	Miscellaneous	0.75 (3/4)										
Wet	Buckets	1.00 (3/3)										
	Drums	1.00 (7/7)	0.14 (1/7)									
	Tires	1.00 (2/2)										
	Cans	1.00 (15/15)	0.13 (2/15)					0.06 (1/15)		0.40 (6/15)		0.13(2/15)
	Permanent	0.86 (24/28)	0.25 (7/28)	0.04 (1/28)		0.25 (7/28)	0.03 (1/28)	0.03 (1/28)	0.03 (1/28)		0.03 (1/28)	
	Drinking Waters	1.00 (1/1)	1.0 (1/1)									
	Miscellaneous	0.93 (27/29)	0.10 (3/29)			0.07 (2/29)				0.21 (6/29)		

Ae: Ae. aegypti; **Cq**: C. quinquefasciatus; **Cc**: C. coronator; **Ct**: C. tarsalis; **Cn:** C. nigripalpus; **Cco**: C. corniger, **Ci** C. interrogator, **Cx**: Culex sp.; **Ld**: Limatus durhamii; **Ur**: Uranotaenia sp.; **Tt**: Toxorhynchites theobaldi.

*absolute frequency.