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Characterization of mosquito breeding sites in the Cape Verde islands with emphasis on major vectors

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Abstract
Characterization of breeding site environment is a crucial step into the understanding of mosquito biology and the dynamics of the transmitted diseases. In Cape Verde a single study addressed this issue, however more than 30 years has passed making it obsolete. In the present study we characterized mosquito breeding sites with emphasis on two major vectors. During mosquito sampling, measurements of physicochemical parameters of standing water were conducted. Aquatic fauna and flora were also characterized. We found a high species richness in the study area. Mosquitoes reproduction was dependent of standing water created by human activities. We did not detect differences between water collections used by mosquitoes to breed when compared to those unused. Our results suggest that mosquito biological control, by the use of freshwater fish, is somewhat effective. Moreover, our results highlight the breeding site management as an important vector control strategy in irrigated areas in Cape Verde.

Keywords: Mosquitoes, Vectors, Habitat, Characterization, Cape Verde Islands.

1. Introduction
Cape Verde is an archipelago composed of 10 islands and located approximately 500 km of the West African coast. It is situated in the Sahel zone, a group of countries with a dry subtropical climate where humidity can reach values below 10%. Cape Verde has a short rainy season (from July to October) and an annual average rainfall of c.a. 225 mm [1]. Agriculture is an important source of income and contributes to approximately 10% of the Gross Domestic Product [2]. Despite significant improvement in this sector [3], previous customs – such as flood irrigation – persist, resulting in standing water that may serve as breeding sites for several arthropods, including mosquitoes.

So far, 11 mosquito species have been identified in Cape Verde. However, *Anopheles arabiensis* (so far the only member of *An. gambiae* complex) and *Aedes aegypti* are the major vector [4]. These two taxa have been recognized as vectors of several infectious agents causing human diseases such as malaria, dengue, yellow fever, and lymphatic filariasis [4]. To control these vectors and diseases, a mixture of several methods is used, including larval insecticide, larvivorous fish, petroleum derivative, drying protection and/or elimination of breeding sites (referred later as breeding site management), indoor residual spraying, and social mobilization [5, 6].

The impact of breeding site environment on mosquito life traits is well documented. Previous studies showed that temperature and larval diet influences developmental time, survival rates as well as competence of *Ae. aegypti* to transmit infectious agents [7-9]. It was shown that development rate of *An. arabiensis* from egg to adult increases from 18 to 32℃ [10]. In addition, it was also shown that the abundance of this specie is associated with the presence of others mosquito species and depend on a variety of physicochemical parameters [11]. Besides the shift in developmental time, breeding sites environment influences male sexual performance [12], fitness cost [13] and vector competence [13, 14] of *An. gambiae s.s.* In *Culex* mosquitoes, temperature influences larval developmental time and survival of adults [15]. Mosquito larvae co-habitation also influences mosquito life traits. For example, co-habitation of *An. gambiae s.s.* and *Cx. quinquefasciatus* in semi-natural natural conditions affects *An. gambiae* body size and could have consequences in diseases transmission [16]. These results together suggest that mosquito larvae habitats has a crucial role in the transmission and dynamics of mosquito-borne diseases and need further investigation. In Cape Verde, so far only a single publication has paid attention to this issue [17] but these results date from more than 30 years ago. In this study we characterize mosquito breeding sites in the Cape Verde

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Islands, with emphasis in the two major vector, *An. gambiae s.l.* and *Ae. aegypti*. We found that these species co-exist in the study area and use similar water collection to breed. We highlight breeding site management as an important vector borne measure, but further studies on the efficacy of introduced freshwater fish in mosquito biological control.

2 Materials and Methods
2.1 The study area
This study took place in an irrigated area in Santa Cruz district, Santiago Island (Figure 1). The local population is of approximately 26,600 habitants, living mainly in rural areas. This district covers 112 km² and the population density is of about 240 hab/km² [2]. Santa Cruz is the second most important district of the country and the first in Santiago Island in irrigated agriculture [18]. Santa Cruz, together with Praia and Santa Catarina – both in Santiago Island – is the main focus of for malaria transmission, as it was also for dengue transmission during the first dengue epidemic in Cape Verde [6, 19].
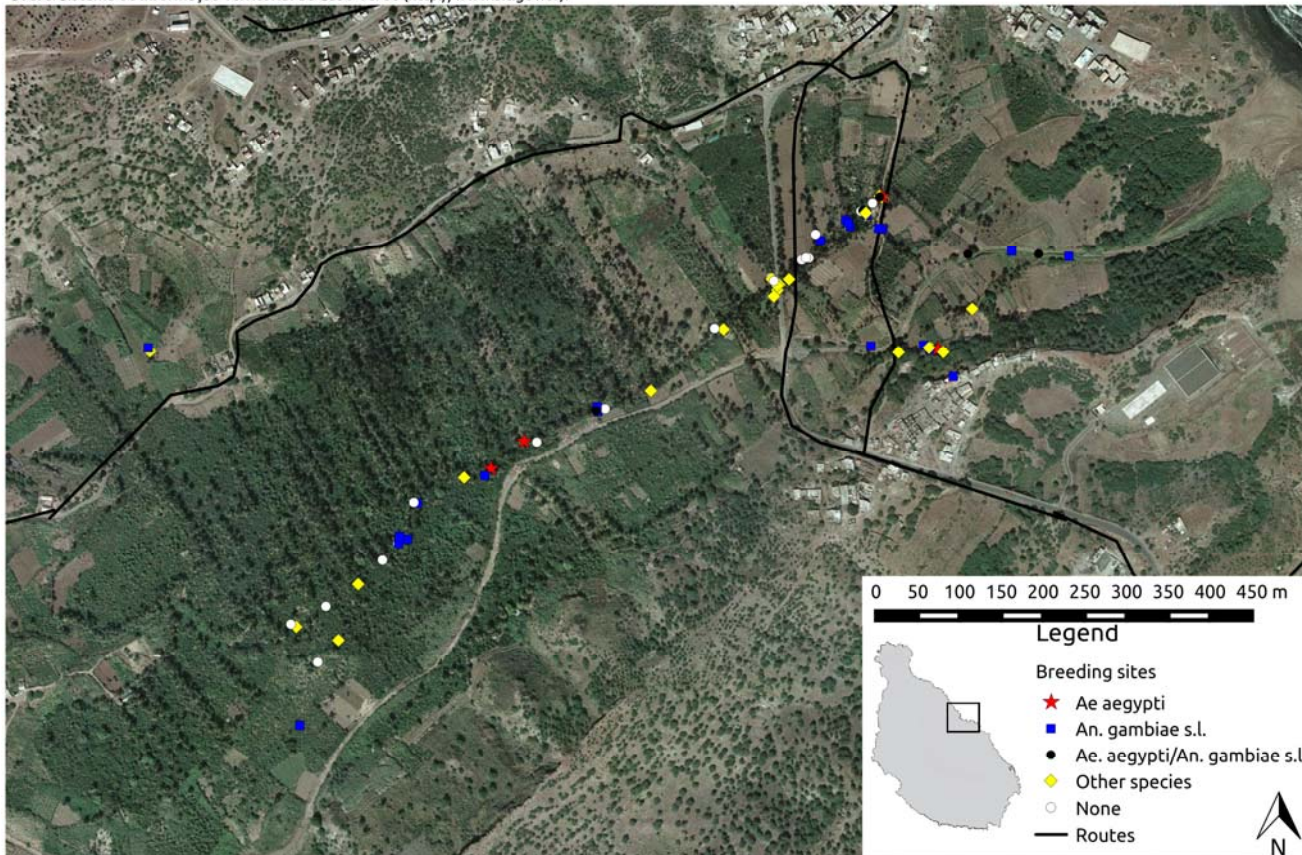

Fig 1: Geographic localization of the study area. Santa Cruz is a district located in the eastern coast of Santiago Island. The coordinates of each breeding sites were taken by using a GPS at the moment of sampling (see Methods). *Ae. aegypti* breeding sites are represented by a red star. A blue squared represent *An. gambiae s.l.* breeding sites while those where both species occurred are represented by a black circle. Breeding sites where these two species did not occurred are represented by yellow diamond while non-productive water collections at the moment of sampling are represented by white circle.

2.2 Mosquito sampling and identification
Between July and October, standing water, tanks and containers were inspected and data about latitude, longitude, and height were recorded using GPS (Garmin, etrex 10). To classify standing water for mosquito presence, we sampled them using a dipper (250 ml) accordingly to their size. If larvae and/or pupae were present, we proceeded as follows. Small containers were emptied with a 1.5 ml pipette. Mosquito larvae and pupae from tanks and large containers were collected with dipper. Larvae and pupae were maintained in vials filled with water up to 75% to total volume. *Cx. tigripes*, the only predatory mosquito species occurring in Cape Verde [4, 20, 21], was kept apart in vials. All mosquitoes were transported to the National Center for Health Development in the capital Praia, Santiago Island, for preparation. Mortality rate of larvae and pupae ate the laboratory was low and not quantified. For identification, L3 and L4 larvae mosquito larvae were maintained in alcohol 70%. Pupae were stored until adults emerged and anesthetized at low temperatures (-4 ºC). Morphological identification was performed as described by Ribeiro and colleagues [17], but additional keys were also used [22-25].

2.3 Breeding site characterization
Five physicochemical parameters of water collection were measured suing a multiparameter (Waterproof Multiparameter PCS Testr 35): i) temperature, ii) pH, iii) sodium chloride contect, iv) total dissolved solids, and v) conductivity. To

avoid bias, we cleaned the multiparameter with fresh water after taking each measurement. Aquatic fauna (e.g. freshwater fish and toads) and flora were recorded as presence/absence data.

2.4 Data analysis
Based on mosquito presence, we classified sites in four groups: i) non-productive water (without mosquito larvae and pupae), ii) *An. gambiae s.l.* breeding sites, iii) *Ae. aegypti* breeding sites, and iv) breeding sites in which these species did not occur. The number of mosquito collected was calculated as frequency and percentage. To access differences in frequency we used test of equality of proportions, assuming that none species was rare (we expect no differences in species frequency). Mean values were compared using non-parametric tests [26, 27]. Bonferroni correction were implemented when comparing more than two groups. Mosquito larvae and pupae associations were accessed using Jaccard index and the significance of the association tested by using χ^2 test [28-31]. We also computed Bray-Curtis index which differs from the previous by taking species abundance in account. All statistical analysis were performed in R v. 3.0.2 [32], using 95% of confidence interval ($\alpha=0.05$).

3. Results
3.1 Mosquito presence and abundance
During this study, 77 sites were sampled and 1,128 larvae and pupae collected. Eight mosquito species were morphologically identified (Table 1). *An. pretoriensis* and *Cx. pipiens s.l.* were the most abundant species, representing slightly more than 65% of the total. *An. gambiae s.l.* and *Ae. aegypti* were equally distributed (12%). *Cx. bitaeniorhynchus*, *Cx. perexiguus*, *Cx. tigripes* and *Culex* sp. were found in low densities (Table 1) together representing less than 10% of the total number collected.

The number of larvae and pupae varied significantly in the course of this study (χ^2=293.68; p-value<0.001). The number of sites sampled per month correlates with the number of mosquito of mosquito larvae and pupae collected (r=0.943; p-value=0.005). We found a similar pattern in the percentage of *An. gambiae s.l.* collected per month (Figure 2). *Ae. aegypti* varied differently, as this species was found in low densities over the period of this study, with a peak in August, when approximately 80% were collected.
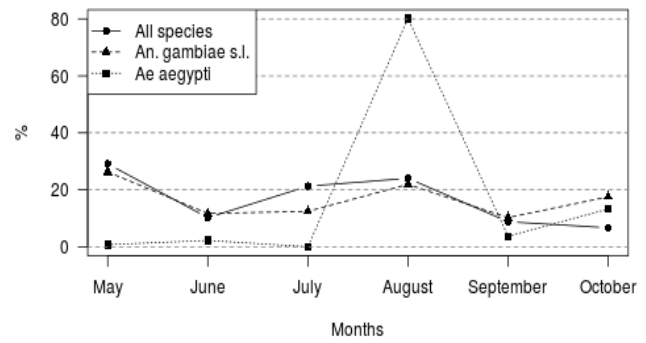

Fig 2: Number of mosquitoes collected over the period of the study. The number of mosquito collected per month was divided by the total collected over the period of this study. The collection rate of *An. gambiae s.l.* follow the collection rate of all mosquito collected during this study. However the collection rate of *Ae. aegypti* showed a different pattern. More than 80% of this specie was collected only in August.

3.2 Productivity of breeding habitats
Only five different breeding habitats were found (Table 1), i.e. irrigation ditches (57.14%; n=44), thanks (22.08%, n=17), ponds (11.69%; n=9), leaves (7.79%; n=6), and a single drum (1.30%). In this study we took the number of mosquito larvae and pupae collected per breeding habitat as a proxy of their productivity. We found a significant correlation between habitat productivity and their frequency (r=959; p-value=0.009).

Only *An. pretoriensis* occurred in all breeding habitats (Table 1). Half of the mosquitoes were collected from irrigation ditches, with *An. pretoriensis* (33.95%) and *Cx. pipiens s.l.* (24.54%) being the most frequent. In thanks we collected 32% of mosquito larvae and pupae, with *Cx. pipiens s.l.* (45.98%) being the most frequent. Surprisingly, this species was only found in three different breeding habitat (Table 1). *An. gambiae s.l.* and *Ae. aegypti* were found in four different breeding habitat, but differed in the fact that *An. gambiae s.l.* did not occur in drums, while *Ae aegypti* was not in leaves, because only anophelines occurred in that habitat. Leaves (1.77%) and drums (2.84%) were the less productive breeding habitats (Table 1).

Table 1: Variation of the number (and percentage) of mosquito larvae and pupae collected per breeding habitat. The percentage of mosquito per breeding habitat was computed by dividing the number of a given specie over the total collected on that habitat.

| Species | Breeding habitats | | | | | | | | | | Total | |
| | Drums | | Leaves | | Irrigation ditches | | Tanks | | Pounds | | | |
	N	%	N	%	N	%	N	%	N	%	N	%
An. pretoriensis	1	3,12	14	70,00	202	33,95	151	41,83	61	50,83	429	38,03
An. gambiae s.l.	0	0,00	6	30,00	64	10,76	25	6,93	42	35,00	137	12,15
Ae. aegypti	31	96,88	0	0,00	86	14,45	9	2,49	10	8,33	136	12,06
Cx. bitaeniorhynchus	0	0,00	0	0,00	8	1,34	5	1,39	2	1,67	15	1,33
Cx. perexiguus	0	0,00	0	0,00	15	2,52	0	0,00	2	1,67	17	1,51
Cx. pipiens s.l.	0	0,00	0	0,00	146	24,54	166	45,98	3	2,50	315	27,93
Cx. tigripes	0	0,00	0	0,00	25	4,20	5	1,39	0	0,00	30	2,66
Culex sp.	0	0,00	0	0,00	49	8,24	0	0,00	0	0,00	49	4,34
Total	32	2,84	20	1,77	595	52,75	361	32,00	120	10,64	1128	100,00

3.3 Mosquito larval associations
Associations between mosquito species are detailed in Table 2. Five significant associations (χ^2 test; p-value<0.05) were found: *An. pretoriensis* vs. *An. gambiae s.l.* (J=0.556), *Cx. pipiens s.l.* vs. *Cx. tigripes* (J=0.400), *An. pretoriensis* vs. *Ae.*

aegypti (J=0.238), *Ae. aegypti* vs. *Cx. bitaeniorhynchus* (J=0.231), and *Cx. perexyguus* vs. *Cx. pipiens s.l.* (J=0.190). Only *Culex* sp. was not found in any significant association. We also computed Bray-Curtis index and found a similar pattern when compared to the Jaccard index results (Figure 3).

Table 2: Mosquito association in the study area determined by larvae and pupae presence and absence. The p-values were calculated by χ^2 test as described in the literature [28]. Legend: ANP -*An. pretoriensis*, ANG - *An. gambiae s.l.*, AEGY - *Ae. aegypti*, BITAE - *Cx. bitaeniorynchus*, PERE – *Cx. perexiguus*, PIP - *Cx. pipiens s.l.*, TIGR - *Cx. tigripes*.

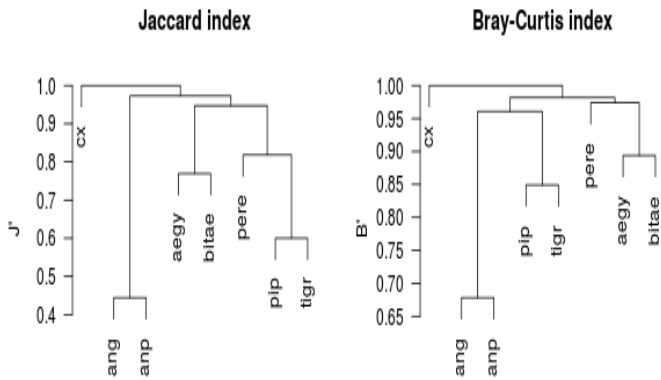|	ANP	ANG	AEGY	BITAE	PERE	PIP	TIGR	*Culex* sp.
ANP	**1**	**p<0.001**	**p=0.039**	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**
ANG	0,556	1	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
AEGY	0,238	0,167	1	p=0.007	p>0.05	p>0.05	p>0.05	p>0.05
BITAE	0,073	0,03	0,231	1	p>0.05	p>0.05	p>0.05	p>0.05
PERE	0,071	0,061	0,133	0,125	1	p=0.02	p>0.05	p>0.05
PIP	0,224	0,111	0,231	0,091	0,19	1	p<0.001	p>0.05
TIGR	0,091	0,027	0,053	0,091	0,182	0,4	1	p>0.05
Culex sp.	0,024	0,032	0	0,2	0	0,048	0,111	1

Fig 3: Jaccard and Bray-Curtis index as computed using the same data set. Dissimilarity was calculated by using 1-*X* where *X* is whether Jaccard index or Bray-Curtis index. Dissimilarity coefficient was used to compute hierarchical cluster analysis. Legend: anp - *An. pretoriensis*, ang - *An. gambiae s.l.*, aegy - *Ae. aegypti*, bitae - *Cx. bitaeniorynchus*, pip - *Cx. pipiens s.l.*, tigr - *Cx. tigripes*, cx - *Culex* sp.

3.4 Breeding site characterization

Between all water collections sampled during this study (n=77), 74.03% (n=57) had at least one larvae and/or pupae. Of these, 52.63% (n=30) were breeding sites of *An. gambiae s.l.*, 21.05% (n=12) breeding sites of *Ae. aegypti* and 36.84% (n=21) were breeding sites in which *An. gambiae s.l.* and *Ae. aegypti* did not occur. Sodium chloride content correlated with total dissolved solids (r_h=0.99; p-value<0.001) and conductivity (r_h=0.99; p-value<0.001), therefore only data from sodium chloride content are present here. We found no differences in the mean value of physicochemical parameters per breeding sites (Table 3; K-W test; p-value>0.05). None of the non-productive breeding sites had aquatic plants. These were found in just 6.49% of sampled water collections sampled in the course of this study. We did not found differences in the proportion of aquatic plants per breeding sites (χ^2 test: p-value=0.394). Half of sampled sites (χ^2 test; p-value=0.362) had potential predators and these were not equality distributed among breeding habitats (χ^2 test; p-value=0.049). Higher proportions were found in *An. gambiae s.l.* breeding sites (Table 3).

Table 3: Physicochemical characterization and distribution of aquatic plants and potential predators among mosquitoes breeding sites. Temperature, pH and sodium chloride content are represented as mean, standard deviation (SD) and range. Distribution of aquatic fauna and flora was represented as percentages. No differences were found between the mean value of temperature (p-value=0.061), pH (p-value=0.555) and sodium chloride (p-value=0.735) content. P-value were computed by using Kruskal-Wallis rank sum test [26]. Differences in frequencies were computed by using one sample proportion test [26, 27] and are represented by different letters. Differences in proportions between groups were also computed by using proportion test. The proportion of breeding sites having aquatic plants were not significantly different between groups (p-value=0.394) but the proportion of potential predator was different (p-value=0.049).

		Non productive	*An. gambiae s.l.*	*Ae. aegypti*	Other species
Temperature	Mean	26,68	28,49	28,2	26,68
	SD	1,88	2,86	2,41	1,94
	Range	23.9 - 29.7	24.2 - 34.5	24.2 - 32.8	23.2 - 31.2
pH	Mean	7,5	7,8	7,53	7,52
	SD	0,79	0,95	0,33	0,78
	Range	6.40 - 9.57	6.77 - 11.57	7.07 - 8.33	6.27 - 9.50
Sodium chloride	Mean	0,14	0,12	0,12	0,13
	SD	0,07	0,05	0,07	0,07
	Range	0.04 – 0.33	0.05 – 0.19	0.05 – 0.32	0.01 – 0.33
Aquatic plants (%)	Present	0 (a)	13.33 (a)	8.13 (a)	4.76 (a)
	Absent	100 (b)	86.67 (b)	91.67 (b)	95.24 (b)
Potential predators (%)	Present	40 (a)	56.67 (a)	25 (a)	33.33 (a)
	Absent	60 (a)	43.33 (a)	75 (a)	66.67 (a)

In marked contrast, physicochemical parameters of water - but not pH (K-W=9.26; p-value=0.06) – varied significantly per breeding habitats (Figure 4). Mean temperature values of irrigation ditches (26.77 ºC±2.33) was lower than values from pounds (29.89 ºC±2.96; p-value=0.05) and leaves (29.23ºC±0.62; p-value=0.029).The mean value of sodium chloride content of leaves (0.57g/litter±0.33) was significantly different from those of irrigation ditches (1.44g/litter±0.60; p-value=0.008).
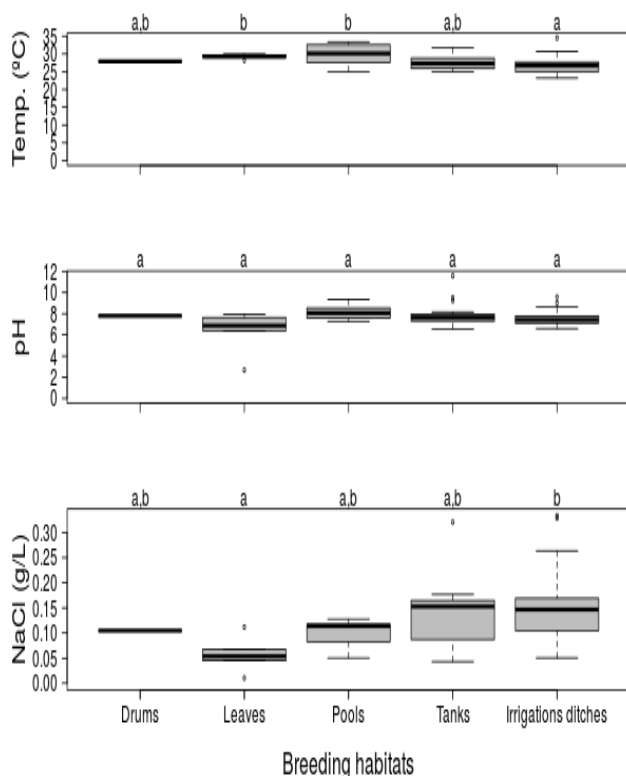
Fig 4: The variation of temperature (top), pH (center) and sodium chloride content (bottom) among mosquito breeding habitat sampled in the course of this study. The mean values between groups were compared using pairwise Wilcoxon rank sum test [26]. Significant differences are represented as letters on top.

4 Discussion

In the present study we characterized mosquito breeding sites in an irrigated area of Santiago Island, Cape Verde Islands. We focus our analysis in two major mosquito vectors that has been associated with transmission of infectious agents causing human diseases [4]. Few water collections were sampled in the course of this study. Although this study was conducted in an irrigated area, the dry subtropical climate of Cape Verde does not favor the presence of surface freshwater bodies [1], explaining the limited number of water collections and mosquito breeding sites.

We found a high species richness in our study area, compared to the number of species found in the country as a whole. In Cape Verde, 11 mosquito species have been documented so far [4, 33], and in this study we identified eight of these by using morphology (Table 1). This high number is presumably due to i) availability of plants' sap and blood sources, and ii) inefficacy or non-existence of vector control measures. In a similar study in Santa Cruz in which 345 water collections were sampled, only few mosquito species were identified and at low densities [34]. So in Cape Verde irrigation areas can be seen as sentinel sites for research n vector biology. In another survey conducted in 2007 in the islands of Maio, Santiago, Fogo, and Brava, 79 mosquito breeding sites were identified and 1104 adult mosquitoes emerged from larvae collected[21], similar to ours.

In this study we collected 1,128 mosquito larvae and pupae of which more than 65% were *An. pretoriensis* and *Cx. pipiens s.l.* (Table 1). Similar results were obtained in previous studies [21, 33]. Despite *An. gambiae s.l.* and *Ae. aegypti* having similar abundance (~12%), *An. gambiae s.l.* was found in every month

and in a similar abundance, while *Ae. aegypti* was almost exclusively found in August (Table 1). The high collection rate of *Ae. aegypti* correlates with the first seasonal rainfall and this collaborates with studies conducted in Thailand [35], Argentina [36], Brazil [37, 38], and Saudi Arabia [39].

This is the first time that *Cx. perexiguus* was found in Santa Cruz district (Table 1). This species was first found in São Domingos and Cidade Velha [21], also in Santiago Island, which is approximately 15 and 30 km way from Santa Cruz. Data currently available do not support any hypothesis that could explain the occurrence and distribution of this species, but limited sampling effort seems to be the main reason for it not previously being recorded in Santa Cruz. However, introduction – perhaps from São Domingos – may also be possible. We also found unidentified *Culex* sp., perhaps *Cx. tritaeniorhynchus*, a species only recently identified in the Cape Verde archipelago [33]. The low density of *Culex bitaeniorhynchus* (syn. *Cx. ethiopicus*), *Cx. perexiguus* and *Cx. tigripes* is in accordance with previous studies in Cape Verde [21, 33].

We only found five different – mainly human-made – breeding habitats in our study area, of which irrigation ditches and tanks were the most common (Table 1). Flood irrigation is a common practice in our study area, requiring structures to store and transport water. Although *An. pretoriensis* was found in all breeding habitats, only a single specimen was found in the only drum we sampled. Studies conducted in and around human dwellings found that drums are the type of water container most frequently used by mosquitoes to breed, even when it is not the most common breeding habitat available [34, 40].

We found that *An. pretoriensis* is the most frequent species in irrigation ditches, while *Cx. pipiens s.l.* was mostly found in tanks (Table 1). Accordingly, we found that the latter only occurs in three different breeding habitats, while *An. gambiae s.l.* and *Ae. aegypti* were found in four different types of water container. In Cape Verde, *An. gambiae s.l.* as well as *Ae. aegypti* use a wide range of water containers to breed. Apart from the ones identified in this study (Table 1), in Cape Verde *An. gambiae s.l.* breeding sites include pounds and short-term fresh and brackish surface of water during the rainy season [17]. *Ae. aegypti* also breed in barrels, pots (for flowers or otherwise) and other domestic containers [17, 40].

The first mosquito species similarity index was given so far by Ribeiro and colleagues [17]. These authors used Cole index, which is biased towards species frequency [28, 29, 31]. Therefore, we used Jaccard index, which is easy to compute and measures the extent in which two species have habitat in common. As this index do not take in account species abundance, we also computed Bray-Curtis index which takes [28-31]. We found a similar pattern when compared to the first (Figure 3), suggesting consistency in our results.

Unidentified *Culex* sp. was the only taxon not found associated significantly. Both *An. pretoriensis* and *Cx. pipiens s.l.* - the most common species – associated significantly with other two species, as well as *Ae. aegypti*. This suggests that mosquito larvae association do not depend only on species abundance. Accordingly, we did not find significant association between *An. pretoriensis* and *Cx. pipiens s.l.* (Table 2). Oviposition behavior of gravid females and breeding sites preferences may be the cause of this difference in species association [41-43]. Our previous results suggests that these species prefers to breed in different habitat (see above), explaining the lack of association (Table 2).

An. gambiae s.l. and *Ae. aegypti* shared almost 17% of their breeding sites (J=0.167). In the previous study conducted in Cape Verde in which data of mosquito associations were accessed (Ribeiro *et al.* 1980), these two species were not observed together. High association level between anopheline species have previously reported [17]. The co-occurrence of *An. gambiae s.s.* and *Cx. quinquefasciatus* has been suggest to impact mainly the body size of *An. gambiae* and probably malaria and filariasis transmission [16]. The co-occurrence of *An. gambiae s.l.* and *Ae. aegypti* in Santa Cruz could also impact their life traits and disease transmission capacities. We found a significant association between *Cx. pipiens s.l.* and *Cx. tigripes* (J=0.400; p-value<0.001), a predatory species. Laboratory studies suggests that this species prefers *Ae. aegypti* larvae rather than those of *Culex* or *Anopheles* species, possible due to the high spontaneous movement of *Ae. aegypti* larvae [44, 45]. The lack of significant association between *Cx. tigripes* and *An. gambiae* (J=0.027) and *Ae. aegypti* (J=0.053) can indicate that this predatory species is controlling these two species, but not *Cx. pipiens s.l.* Alternatively, it may indicate that these species have distinct breeding site preference but it seems to not be the case (see Table 1). In practical perspectives, these associations of different species (e.g. *An. gambiae s.l.* and *Ae. aegypti*) is of major interest and suggest that breeding site management is an important vector control measure in Cape Verde.

More than 70% (n=57) of sites sampled during this study had at least one larva or pupa. Of these, 52.63% were *An. gambiae s.l.* breeding sites and 21.05% breeding sites of *Ae. aegypti*. These two species did not occur in 36.84% of breeding sites. Although five physicochemical parameters were analyzed in this study, we only shown three because sodium chloride content, total dissolved solids and conductivity correlate. This is the first study in Cape Verde in which temperature of mosquito breeding sites were measured. Water temperature influences many aspects if mosquito life traits and even vector competence [7, 15, 16]. However none of the parameters were measured differs among different breeding habitats we sampled in the course of this study (Table 3).

Our temperature values ranged from 23.2 to 34.5 ºC, similar too values found by other authors [46-49]. We also assessed the pH value of breeding sites. Our values were slight lower than those observed previously in the Cape Verde Islands for *An. gambiae s.l.* and *Ae. aegypti*, respectively (see Ribeiro *et al.* 1980; Table 3). It is important to note that *Ae. aegypti* and *Ochlerotatus taeniorhynchus* complete larval development in pH value ranging from 4 to 11 [50], values similar to ours. In another study, in anopheline larval habitats, pH values ranged from 5.4 to 10[49], also falling within our range. Ribeiro and colleagues [17] reported the sodium chloride content of five breeding sites of *An. gambiae s.l.* (3.84±2.49g/litter) and one of *Ae. aegypti* (0.29g/litter). In our study, sodium chloride content was lower for both species breeding sites: *An. gambiae s.l.* (0.12±0.05g/litter) and *Ae. aegypti* (0.12±0.07g/litter).

Only few sites sampled had aquatic plants (Table 3). The proportion of breeding sites of *An. gambiae s.l.* with aquatic plants was similar to those found in the past, but not for *Ae. aegypti*. Ribeiro and colleagues [17] only found *Ae. aegypti* in freshwater with little organic matter dissolved and without any vegetation. Unexpectedly, potential predators (toads and freshwater fish) were found at half of our sites, including in *An. gambiae s.l.* breeding sites (Table 3). *Amietophrynus regularis* (syn. *Bufo regularis*) is an amphibian know to occur in Cape Verde Islands [51], probably introduced from Guinea

Bissau to control mosquitoes [52]. Although *Gambusia affinis* has been said to occur in Cape Verde [6], the only introduced freshwater fish identified in Cape Verde so far was *Poecilia reticulata* (cf. Lucek and colleagues [53]). However, these authors did not study any sites in Santiago Island, where the majority of mosquito studies - including ours - have taken place. Lower proportions of *Ae. aegypti* breeding sites were found positive for potential predators, but no significant differences were found. However, as no experiments were carried out to ascertain that they indeed act as predators, they are here referred to as potential predators only. Since biological control has been recommended in Cape Verde [6], further studies on introduced freshwater fish are needed, as the efficacy of this approach in Cape Verde is as yet unknown.

The findings of this study suggest that the large variety in species found is largely due to the use of flood irrigation, which requires human-made structures to store and transport water. However, our results emphasize breeding site management as an important vector control measure since several species can the controlled at once. Our findings also suggest that biological control may not be an effective vector control measure, since we found potential predators (including freshwater fish) in half of mosquito breeding sites in our study area. These results underline the need for studies on the effectiveness of freshwater fish on mosquito control.

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