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SPECIES DIVERSITY OF MOSQUITOES IN TEMPORARY AND SEMI-PERMANENT BREEDING HABITATS IN PESHAWAR UNIVERSITY CAMPUS, KP PAKISTAN

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Abstract: This study aims to deduce the temporary habitat of mosquitoes, and their seasonal dynamics in Peshawar University campus in selected sites. Monthly sampling of mosquito larvae from six habitats was carriedout from May to December, 2009. The habitats selected for this survey were; bamboo traps, discarded drinks cans, small wastewater channels, mud pots, tyres and a small cemented pond. A total of 2419 adult mosquitoes comprising 1433 females and 985 males belonging to six species viz. *Aedes albopictus* (57.2%), *Aedes unilineatus* (2.5%), *Aedesw-albus* (2.8%), *Armigeres subalbatus* (3.8%), *Culex quinquefasciatus*(33.6%) and one individual of *Anopheles stephensi* were recorded. As for as density and distribution of the recovered species are concerned, *Aedes albopictus* was found to be dominant and frequent, recorded from four out of six habitats. The highest number was recorded in September and October while lowest in the month of May. *Culex quinquefasciatus* was the second most abundant species collected from three habitats with dominant status and moderate distribution. *Aedes unilineatus, Armigeres subalbatus* and *Aedes w-albus* were noted as subdominant species with the former two species infrequent and later sporadic in distribution. Larvae of *Aedes* mosquitoes were found in all habitats except pond water and tyres. Monthly distribution reveals that *Aedes albopictus* and *Culex quinquefasciatus*, during this survey were found through out the study period from May to December. Analysis of variance revealed, that observed mosquito species differs significantly between the months and also among the habitats.

Key Words: Aedes, armigeres, culex, mosquito larvae, habitat, species diversity.

Introduction

As vectors of various diseases, mosquitoes are the deadliest insects on earth. Despite the big campaign against mosquitoes all over the world, these bloodthirsty insects still constitute a severe nuisance for human and domestic animals.

Mosquito exploit almost all forms of lentic water habitats for breeding; where they flourish making the larval mosquito community. Mosquitoes appear in large number after calamitous flooding and the resulting tributaries cause considerable extension of mosquitoes breeding habitats (Jones *et al.*,2004; Rydzanicz and Lonc, 2003). Other factors like global warming, periodic flooding and deforestation also have opened new habitats to mosquitoes which show high plasticity in their breeding behavior and vastly spread their distribution

[Zucker, 1996].

Larval habitats are important determinants of adult distribution and abundance. These are the locations where important life cycle processes take place: oviposition, larval development, adult emergence, resting, swarming and mating. To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species. This requires the study of not only the fluctuations of adult populations, but also the factors affecting larval abundance and distribution. In Pakistan, sufficient attention is not being paid to focus the study of mosquitoes on their breeding biology. More intensive larval ecology studies have been conducted in countries endemic to mosquito borne diseases (Berti, *et al*., 1993; Grillet, 2000; Mangium *et al*., 1996; Rejmankova *et al*., 1999).

While making the first comprehensive survey of mosquitoes from adjoining areas of Peshawar District, Suleman *et al* (1993) reported 31 species largely collected from various permanent habitats. Ali and Rasheed (2009) carried out survey for immature stages of mosquitoes in polluted water of Palosai stream passing near Peshawar University campus and recorded nine species belonging to two genera *Culex* and *Anophelese*.

The objective of this study, a part of series of studies focused on the breeding biology of mosquitoes, was to determine their larval abundance, species composition, and seasonal dynamic in selected habitats mostly temporary in Peshawar University campus.

Materials and Methods

Study Area

This survey of larval mosquito for qualitative and quantitative assessment of mosquito diversity in selected habitats in Peshawar University campus was carried out from May to December 2009.

Peshawar is the provincial capital and the most populous city of Khyber Pakhtunkhwa (KP) with two million inhabitants. Its geographical coordinates are 34° 0' 28" North, 71° 34' 24" East and the total area is 1257 km^2. Elevation above the sea level is 510m (1673 ft). Peshawar is not a monsoon region unlike other parts of Pakistan; still rainfall is received both in winter and in summer resulting in a great variety of larval habitats in this region. Peshawar has a semi-arid climate with very hot summers ranging from May to September and mild winters that starts in mid November and ends in late March. Mean annual temp of the study area ranges from 4°C -18.35°C (Nov – March) to 25-40°C (May – Sept). On record the highest and lowest temperature of this area is 50 °C (122 °F) and −3.9 °C (25 °F) and annual precipitation is recorded as 400 millimetres (16 in). The annual relative humidity ranges between 46-76% in June and August.

The University campus is located about 10 kilometres North West from the main city on Grand Trunk Road leading toward Torkham, a Town joining the border between Afghanistan and Pakistan. It is situated on 1,050 acres of land, 1,199 ft (365 m) above sea level. It is a residential University with a population of about 35000.

Sampling

Larval and pupa collections were made from May to December 2009 from the following six selected breeding habitats. Some water was introduced to bamboo traps, tires, mud pots and discarded drinks containers. The presence of water in these habitats was ensured throughout the study period.

i) Nine bamboo traps hanged 6 feet high at four different sites
ii) Five discarded drinks cans placed at three sites iii) Two small waste water channel sites
iv) Five mud pots
v) Small pond studied at two sites
vi) Two tires with some water were placed at hanging and horizontal position

The distance between the sites was from 500 meters to one kilometer. The water bodies were surveyed monthly and immature stages comprising larvae and pupae were sampled using dipper, glass dropper and strainer and also the plankton net of appropriate diameter depending on the size of habitat.

Five dips were taken from each habitat with a standard mosquito dipper to collect larvae from relatively large water bodies, while glass pipettes were used for small and shallow water bodies. The samples were taken to the laboratory and reared in disposable plastic containers until adult emergence. The emerged adults were preserved dry over silica gel in eppendorf/ glass tubes prior to identification using appropriate keys (Barraud, 1934; Christophers, 1933).

Data Analysis

Two way analysis of variance (ANOVA) was used to compare the difference in number of mosquito larvae in different habitats found in different months.

Seasonal dynamics of mosquito larvae populations in the sampling site were analyzed by density and distribution. Density is expressed as percentage of specimens of the given species in the whole sample and calculated by the following formula

$$D=I/L \times 100\%$$

where: D- Density, I- number of specimens of each mosquito species, L- number of all specimens.

The following density classes were accepted after Trojan (1992):

Satellite species (D < 1%) Subdominant species (1<D<5%) Dominant species (D > 5%).

Seasonal dynamics of mosquito larvae populations in the sampling sites were analyzed as the percentage of positive sampling sites in which a species was found by using the formula:

$$C = n\,b/N \times 100\%$$

where: C- Distribution, n- number of sites of the species, N- number of all sites. The following distribution classes are adopted (Dzieczkowski, 1972):

C1- Sporadic appearance (Constancy 0-20%)
C2- Infrequent (20.1-40%)
C3- moderate (40.1-60%) C4- frequent (60.1-80%) C5- constant (80.1-100%)

Results and Discussion

Mosquito Species

All studied habitats except tyres were found positive for mosquito immature stages, though the number varied between months and habitat types. Out of the total 2419 adults collected from six sampling habitats, 985 were male and 1433 were females (1:1.5). Taxonomic study reveals six species of mosquitoes belonging to four genera *Culex, Anopheles, Armigeres* and *Aedes.* The former three genera were represented by a single species each, i.e., *Cx. quinquefasciatus*, *An. stephensi* and *Ar. subalbatus* while the later one comprised three species viz, *Ae.albopictus, Ae. unilineatus* and *Ae. w-albus.*

Monthly Distribution of Mosquito Species

The seasonal dynamics of mosquito species during the study period is shown in Fig.1.

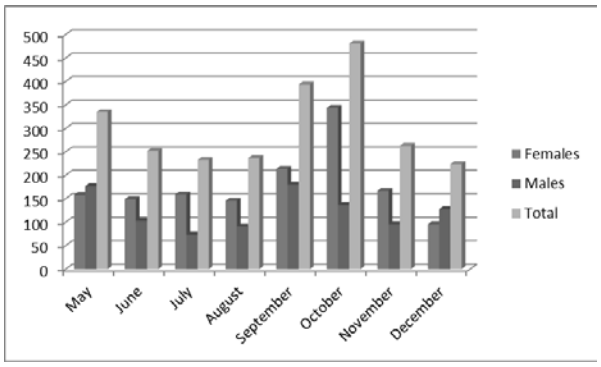

Fig. 1. Monthly fluctuations of mosquito larvae and pupae in Peshawar University campus during the study period (2009).

Monthly distribution of collected mosquitoes shows two peaks first in the month of May then the number declines for two months, second increase in number was observed from the month of August reaching highest level in October followed by the gradual decline again.

Occurrence of immature mosquitoes belonging to various species in different months is shown in Table-1. *Aedes albopictus* and *Culex quinquefasciatus* were collected throughout the study period from May to December. *Ae. unilineatus* and *Ae. w-albus* were also gathered in all the studied months except December. The next frequent species was *Ar. subalbatus* found from May to September. Only one individual belonging to *An. stephensi* was collected from waste water channel in the month of May. *Aedes albopictus* and *Culex quinquefasciatus* were also among the most abundantly occurring mosquito species with 1382 and 814 larvae and pupae of mosquitoes. The rest of the four species were far behind these species in respect of number of immature mosquitoes.

Presence of Mosquito Species for Habitates

Presence of mosquito species for habitates was maximum in bamboo traps with four species, followed by discarded drinks cans and waste water channels with three species each. Mud pots inhabited two species and pond water (semi permanent) was occupied by a single species. Somehow tires could not attract mosquito species for oviposition. *Aedes* species were found in all positive habitats except pond water (Table-2).

Table-3 documented the number of mosquitoes belonging to different species in various breeding sites. Among the total of 2419 individuals, rich collection of mosquitoes was recorded from bamboo traps (826). Second highest number (679) was collected from waste water channel while discarded cans were third in number (638). The least number (139) was observed in

mud pots.

Seasonal Dynamics of Mosquitoes as per Density and Distribution

Seasonal dynamics of mosquito immature populations collected from various habitats were analyzed by density and distribution formulae. As per density, *Ae. albopictus* (57.131) and *Cx. quinquefacitus* (33.650) are within the dominant class; three species *Ae. unilineatus* (2.522), *Ae. w-albus* (2.811) and *Ar. subalbatus* (3.845) are included in the sub-dominant class and only *An. stephensi* (0.0413) is a satellite species (Table 4) accepted after Trojan (1992). According to the distribution criteria only one species *Ae. albopictus* (66.67) can be regarded as frequent (C= 60.1- 80%). *Cx. quinquefacitus* (50.00) was found to be moderate (C=40.1-60%), two species *Ae. unilineatus*(33.33) and *Ar. subalbatus* (33.33) were Infrequent (C=20.1-40%) while *Ae. w-albus* (16.67) and *An. stephensi (*16.67*)* were regarded as sporadic in appearance (Table-4) accepted after Dzieczkowski (1972).

Table 1. Distribution of mosquito's immatures in respective months collected from Peshawar University campus

SPECIES →	***Aedes albopictus***			***Aedes unilineatus***			***Aedesw- albus***			***Ar. subalbatus***			***Cx. quinquefasciatus***			***An. stephensi***			**overall total**		
MONTHS ↓	♀	♂	**Total**	♀	♂	**Total**	♀	♂	**Total**	♀	♂	**Total**	♀	♂	**Total**	♀	♂	**Total**	♀	♂	**Total**
May	88	43	131	0	4	4	3	3	6	33	37	70	34	89	123	0	1	1	158	177	**335**
June	70	71	141	0	5	5	5	4	9	3	3	6	71	20	91				149	103	**252**
July	99	42	141	0	3	3	5	2	7	3	5	8	52	22	74				159	74	**233**
August	75	73	148	0	1	1	0	0	0	2	3	5	69	14	83				146	91	**237**
September	155	110	265	0	0	0	2	2	4	3	1	4	54	67	121				214	180	**394**
October	250	35	285	15	31	46	20	21	41	0	0	0	59	50	109				344	137	**481**
November	78	55	133	0	2	2	1	0	1	0	0	0	88	39	127				167	96	**263**
December	70	68	138	0	0	0	0	0	0	0	0	0	26	60	86				96	128	**224**
TOTAL	**885**	**497**	**1382**	**15**	**46**	**61**	**36**	**32**	**68**	**44**	**49**	**93**	**453**	**361**	**814**	**0**	**1**	**1**	**1433**	**986**	**2419**

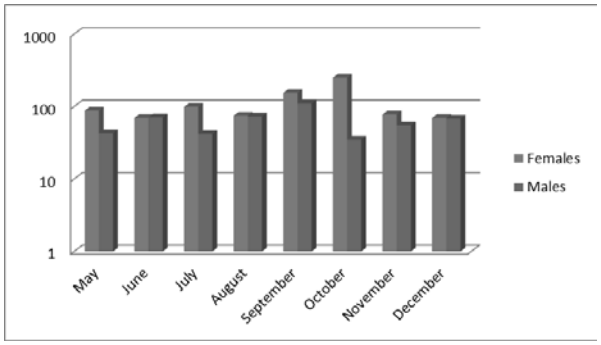

Fig.2. Bar graph showing seasonal dynamics of *Aedes albopictus* collected from Peshawar University campus during the study period (2009).

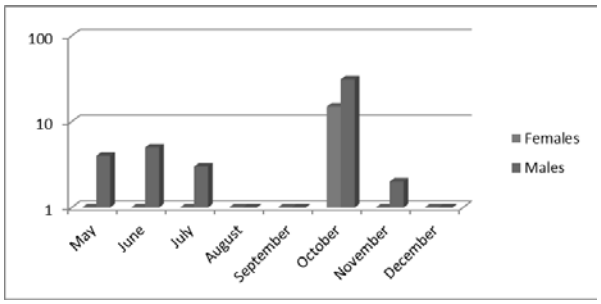

Fig.3. Bar graph showing seasonal dynamics of *Aedes unilineatus* collected from Peshawar University campus during the study period (2009).

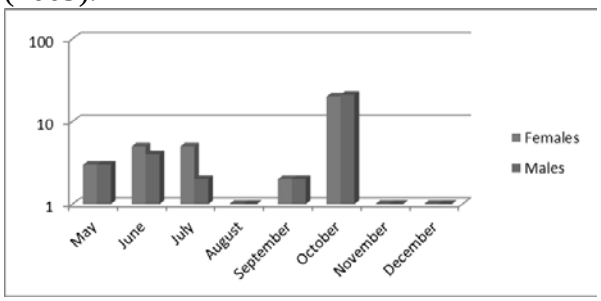

Fig.4. Bar graph showing seasonal dynamics of *Aedes w-albus* collected from Peshawar University campus during the study period (2009).

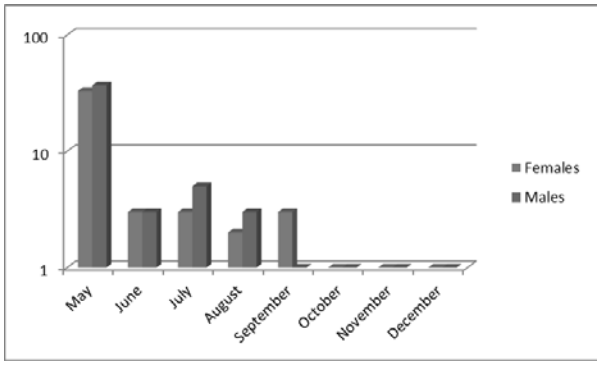

Fig.5. Bar graph showing seasonal dynamics of *Ar. subalbatus* collected from Peshawar University campus during the study period (2009).

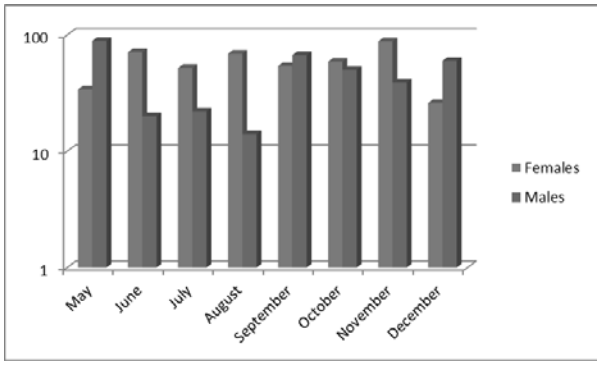

Fig.6. Bar graph showing seasonal dynamics of *Culex. quinquefasciatus* collected from Peshawar University campus during the study period (2009).

Table 2. Larval habitats and species of mosquito collected from Peshawar University campus during the study period (2009)

Habitat type	Mosquito species							
	Ae. albopictus	*Ae. unilineatus*	*Ae. albus*	*w-*	*Ar. Subalbatus*	*Cx. quinquifasciatus*	*An. stephensi*	Total
Bamboo traps Discarded drinks	+	+	+		+			4
cans	+	+			+			3
Pond water						+		1
Small waste water								
channels	+					+	+	3
Mud pots	+					+		2
Tyres								0

Table 3. Monthly distribution of mosquito species collected from different habitats in Peshawar University campus during the study period (2009)

	Bamboo traps				**Discarded drinks Cans**			**Pond Water**	**Wastewater channels**			**Mud Pots**		**Tyres**	
MONTHS	***Ae. albopictus***	***Ae. unilineatus***	***Ae. w-albus***	***Ar. subalbatus***	***Ae. albopictus***	***Ae. unilineatus***	***Ar. subalbatus***	***Cx. quinquefasciatus***	***Ae. albopictus***	***Cx. quinquefasciatus***	***An. stephensi***	***Ae. albopictus***	***Cx. quinquefasciatus***		**TOTAL**
MAY	52	3	6	65	30	1	5	18	49	95	1	0	10	0	**335**
JUNE	57	5	9	6	83	0	0	25	1	54		0	12	0	**252**
JULY	55	2	7	7	86	1	1	9	0	43		0	22	0	**233**
AUGUST	58	1	0	5	83	0	0	12	6	55		1	16	0	**237**
SEPTEMBER	141	0	4	4	120	0	0	22	3	90		1	9	0	**394**
OCTOBER	161	46	41	0	122	0	0	22	2	51		0	36	0	**481**
NOVEMBER	55	2	1	0	65	0	0	17	13	97		0	13	0	**263**
DECEMBER	33	0	0	0	41	0	0	12	64	55		0	19	0	**224**
TOTAL	**612**	**59**	**68**	**87**	**630**	**2**	**6**	**137**	**138**	**540**	**1**	**2**	**137**	**0**	**2419**

Table 4. Density and distribution of observed mosquito species collected from different habitats in Peshawar University campus during 2009

Species	Females	Males	Total	Density	Density status	Distribution	Distribution status
		D=I /L x 100%				C = n b/N x100%	
Ae.albopictus	885	497	1382	57.131	Dominant	66.67	Frequent
Ae.unilineatus	15	46	61	2.522	Subdominant	33.33	Infrequent
Ae.w-albus	36	32	68	2.811	Subdominant	16.67	Sporadic appearance
Ar.subalbatus	44	49	93	3.845	Subdominant	33.33	Infrequent
Cx.quinquefacitus	453	361	814	33.650	Dominant	50.00	Moderate
An.stephensi	0	1	1	0.0413	Satellite species	16.67	Sporadic appearance
Total	1433	986	2419				

In recent years, mosquitoes gained remarkable significance because of increasing rate of man- mortality in tropical and sub tropical regions of the world due to mosquito borne diseases. Despite improvements in the prevention and treatment of these diseases mosquitoes still constitute to be a severe nuisance for humans and domestic animals in many rural, suburban and urban areas around the world (Jones *et al.*,2004; Rydzanicz and Lonc, 2003). To control mosquitoes, the understanding of larval ecology is essential irrespective of the suggested measures targeted at the adult or larval stages.

This study was mainly confined to the immature stages of mosquitoes in temporary habitats or habitats with relatively small water bodies. As compared to large water bodies the limiting factors regarding food and environment are more severe in these habitats, therefore, only the limited number of species is expected. Among the six species recorded in the present study, three belong to the genus *Aedes* and one each belong to *Armegeris, Culex* and *Anophelese*. The mosquitoes of the former two genera primarily breed in small water bodies while the latter two are largely cosmopolitan regarding habitat types but mostly breed in permanent habitats.

Aedes albopictus appeared to the most dominant and frequently found species during the survey. It was observed in most of the habitats studied, but showed special preference to bamboo glasses and discarded drinks cans. Suleman *et al*. (1993) reported it from the tree holes while studying a variety of potential breeding habitats of Peshawar region. Bamboo glasses and discarded cans are comparable to the tree holes regarding quantity of water. This species is found to be playing a major role in the transmission of dengue in some countries of South East Asia (Gratz, 2004; Hammon,1966; Runnick, 1967; Smith, 1956; Stephenson et al., 2003)

Culex quinquefasciatus is the second most abundant and frequent species found in three types of habitats. This species primarily breed in polluted water therefore high number observed in waste water

channels. Mukhtar *et al*., (2003) found the highest number of this species while studying role of waste water irrigation in mosquito breeding in Southern Punjab. Suleman *et al*., (1993) and Ali & Rasheed, (2009) also recorded this species in abundant number from various habitats in Peshawar. It is the main source of biting in this region like most of other parts of the world. Though, generally requiring large water bodies for breeding, it also inhabits container habitats with little water. Mwangangi *et al*., (2009) carried out survey on immature mosquitoes in different habitats including some temporary ones like puddles and tyre tracks. They reported *Culex quinquifasciatus* from all habitats. This species is the major vector of bancroftrian filariasis in South East Asia, sub tropical countries in the Middle East [Service,1996]. Despite the wide distribution of *Culex quinquifasciatus* in Pakistan the disease of filariasis is not prevailing in this country, though a case of this disease has been reported by Aslamkhan in Behari colony (inhabitants migrated from Bangla Desh) (Aslam Khan and Pervez, 1981).

Ar. subalbatus larvae were collected from bamboo traps and discarded drinks cans showing its preference to container habitats. Suleman *et al*., (1993) (*loc.cit*) reported it from tree holes while Ali and Rasheed (*loc.cit*) recorded it in polluted water of a stream but the present survey showed its absence in waste water channels. Similarly, *Ae. unilineatus* was also collected from the above mentioned habitats while *Ae. w-albus* was found in bamboo traps only. A single larva of *An. stephensi* from waste water channels was recovered. Previously, Mukhtar *et al*., (2003) and Ali & Rasheed, (2009) also recovered it from polluted water supporting the view about waste water as preferential habitat of this species. Although its presence is negligible in the present study, it has a very wide distribution, occurring from Iran and other countries in the Middle East across Pakistan, India, to Mayamar, Thailand and China (*loc.cit*). It is the main vector of urban malaria in much of the Indian subcontinent. Its adults bite humans both indoor and outdoor and stay mainly indoor afterwards [Service,1996]. *An. stephensi* reported to be primarily responsible for the transmission of malaria in KP province of Pakistan.

Kumar and Vam, (1992) studied breeding habitats of mosquitoes in nine categories including fountains, tanks, tires, barrels and tins in Panaji, India. They reported six species including *Cx. quinquefasciatus, An. stephensi* and *Ae. albopictus,* same observation also made in the present study. Similarly *Cx. quinquefasciatus, Ar. subalbatus* and *Ae. w-albus* are also recorded by Aditya *et al*., (2006) while surveying mosquitoes from different habitats including some temporary ones in Darjeeling Himalayas, India. Another survey conducted on container breeding mosquitoes by Thete and Shinde, (2013) in India reported four species of mosquitoes, including *Ae. albopictus* and *Cx. quinquefasciatus.* Similarly, *Ae. albopictus* was found in a variety of temporary and semi-permanent breeding habitats in Malasia (Saleeza *et al*., 2011) showing the capability of this species to breed in a wide range of container types.

Monthly distribution of collected mosquitoes shows two peaks first in the month of May and second in the month of October. Rydzanicz and Lonc, (2003)

reported almost similar pattern of distribtion in Wroclaw, Poland during the survey of mosquito larvae in different months of the year. Rainfall and temperature may be the contributing factors for changes in the observed species variation, through time and habitat.

Conclusion

Some trend of habitat preference was observed in this study. *Ae.w-albus* was collected only from bamboo glasses while *Ar. subalbatus* and *Ae. unilineatus* were collected from bamboo glasses and discarded drinks cans showing strong inclination towards temporary habitats. One species each belonging to the genera *Culex* and *Anopheles* were collected from waste water and pond water but none of other habitats, (with relatively less water) indicating their preference for large water bodies rather than small bodies of water.

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