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Descriptive Evaluation of Time-Dependent and Seasonal Trends of Flies of Forensic Values on Carcass Decomposition in a Grassland

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Abstract:

Introduction: *Insects found on carcasses especially fly species are forensically important as regards estimation of time of death. Guinea pig carcass (GPC) was studied to determine time-dependent and seasonal trends of flies on carcass decomposition.*

Methods: *One-way access was constructed on a transparent container; to trap flies accessing the GPC therein. Fifteen of the containers for 15 GPCs were used during the dry season, and repeated during the rainy season. At different time intervals, access to one of the GPC was sealed to stop flies from further accessing it. The trapped flies in the containers were collected and preserved with 70% ethanol in specimen bottles according their taxon. Fly larvae found on the GPC were untouched until they developed to adults for collection and identification.*

Results: *Chrysomya albiceps, Chrysomya chloropyga (Calliphoridae) and Musca domestica (Muscidae) were the fly species trapped therein. The arrival time of C. albiceps during the dry and rainy seasons on the GPC was consistent at 3 hours after exposure. On the contrary, the arrival time of C. chloropyga was at day 2, and 9 hours, while that of M. domestica was at day 4 and day 3 for dry and rainy seasons respectively. Chrysomya albiceps was reasonably more abundant and with higher fecundity during the rainy season against the dry season while C. chloropyga and M. domestica did not show meaningful differences for both seasons.*

Conclusion: *Time progression and seasons influenced the flies' arrival time, abundance, and development of their immature ones on the GPC.*

Keywords: *Time progression; Seasonality; Flies; Carcass; Forensic entomology*

1. INTRODUCTION

At death, a carcass becomes a transient ecosystem where flies are noted to be the most visible invertebrate fauna with distinct functional groups [1]. The arrival of the fly species on a carcass at different decomposition stages is either to feed and/or deposit eggs on it for the propagation of new progeny. Other insects and related arthropod species may also arrive for the same purpose or to use the carcass as an annex. Flies that use the carcass for food and egg deposition resource constitute the flies of forensic value and their arrival is predictable except otherwise while other groups add to the ecology of the carcass [2]. The inevitability of flies arrival on carcasses make them a useful silent witness to a dead body [3] because the eggs they deposit and their larval development therein could help to provide an estimated postmortem interval (PMI), which refers to the time since the carcass was last seen alive until its discovery. This information is a vital component of forensic investigation relating to a questionable death [4].

Determination of PMI of a carcass with flies, may either utilize the time of arrival, and developmental stages of the immature flies found on it or by evaluating their succession sequence [5-7]. The arrival, growth, and development of flies on carcasses usually vary but are essential requirements that need to be understood for accurate determination of the carcass PMI [8]. Geographical conditions are major variations when considering the use of flies in estimating the PMI of a body [9,10], therefore it is

sacrosanct to utilize local entomofauna when dealing with flies of forensic value in any real case [11,12].

Studies relating to animal models indicate that Guinea pigs have been used to study carcass decompositions and their associated insects especially flies of forensic value [13,14,15]. Studies have shown that flies that arrive on different types of carcasses are usually within the same taxon but their time of arrival may vary according to the fly species, geographical conditions, and probably the season therein. Basic research on forensic entomology with different animal models [16,17,5,6,18-21] have been studied in Nigeria. However, none of these has considered the time of fly species' arrival concerning the yearly season on carcasses as a priority, so as to use them to evaluate the forensic importance of insects in relation to carcass ecosystem with reasonable certainty of estimating relative time of death. In this study, we intend to evaluate the time at which fly species will arrive on a Guinea pig carcass (GPC-*Cavia porcellus* Linn.), which was placed inside a transparent plastic container with a modified accessibility. The container will be exposed in a grassland in Rivers State, Nigeria during the dry and rainy seasons respectively for comparison of findings. The outcome of the study would be useful to forensic entomologists in the study area, when confronted with the challenges of predicting the minimum postmortem interval at which a carcass would be infested with flies especially in a real-case scenario in grassland ecosystem.

2. Materials and Methods

2.1. Study Area

The study was conducted in a grassland (4º 52 33.40 N and 6º 56 34.56E) in front of the Department of Animal and Environmental Biology (AEB), University of Port Harcourt, Rivers State, Nigeria. The University is located in Choba, Obio-Akpor Local Government Area, Rivers State, Nigeria (4 º 45N and 6 º 50E). Choba is a sub-urban area within the Port Harcourt metropolis in the South-South region of Nigeria. It lies in the tropical rainforest zone with distinct rainy (May-October) and dry seasons (November-April). The temperature of the zone ranges from 26ºC to 37ºC with a relatively high humidity with a bout of break in August (August break) lasting more than a week. [22,23]. The study started from 24th January to 17th March 2023 to depict dry season, while the rainy season study started from 3rd June to 2nd July 2023.

2.2. Experimental Animal and Procedure for Data Collection

The Guinea pig (*Cavia porcellus* Linn.) was selected as an acceptable animal model for the study because of its domestication and fast reproduction cycle, and its omnivorous peculiarity with humans [24]. Thirty adult Guinea pigs carcasses (GPC) (fifteen for dry season and fifteen for rainy season) that weighed approximately 488 ± 1.89 g on average were used for the study. They were obtained from the Animal House, University of Port Harcourt. They were euthanized by a certified veterinarian before been sacrificed by cervical dislocation [25]. Each carcass immediately after death, was placed in a transparent plastic container (40 x 30 cm^2) with a lock lid. Fifteen of these containers were individually half-filled with sterile sand before the placement of the carcass so as to absorb the fluid seepage, and to enhance larval pupation. Circular incisions were made on the sides of the containers to prevent the soil from being wet and to guarantee airy circulation.

The incisions were covered with muslin cloth and fastened with glue to prevent other microfauna from accessing the GPC in the containers. A 5cm diameter perforation was made on each of the container lids. Funnels were individually plugged into the perforated lids, which serve as one-way entrance for the flies to access the GPC in the containers. The 15 containers were simultaneously exposed at the grassland at 8: 00 AM during the dry season of the year. The same processes, procedures, and number GPC and containers were used again for the rainy season for the same year.

At 11: 00 AM of the same day (day 1) of exposure representing 3 hours of post-exposure (3HPE), all the containers were inspected and insects trapped in the containers were collected. This process was repeated at 1: 00 PM, 3: 00 PM, and 5: 00 PM respectively on day 1, which represented 5HPE, 7HPE, and 9HPE respectively. On the second day, the process was repeated once at 8: 00 AM, denoting 24 hours after the GPC was exposed, which represented day 2 of post-exposure (2DPE). The day 2 process was continually repeated on day 3 till day 9DPE. After the inspection at 3HPE, one of the containers was picked and sealed completely by removing the funnel and closing the hole with

cotton wool. The process was repeated at 5HPE, 7HPE, 9HPE, 2DPE till 9DPE) to prevent flies from further accessing the GPC in the container, and to ascertain the species of flies that would emerge therein. Thus, 12 out of the 15 containers were randomly picked in a reduction order from 3HPE to 9DPE and sealed. The three remnant containers (13, 14, and 15) were not sealed until day 12, which marked the end of the study. They were labelled as the control (C1, C2 and C3). No further collection of flies in the sealed containers except for regular inspection to observe larval development if any, and emergence of new adult flies. In order to collect the flies that are trapped in any of the containers, its funnel was removed, sealed with cotton wool, and immediately taken to the insectarium of the Department of AEB for the collection of adult flies. The selected and sealed container was then placed in a mosquito-rearing cage (60 x 50 cm^2), and its lid was removed through the armhole to allow the flies to escape into the cage. A battery-operated suction device was used to collect the flies and placed them in a bottle with screw cap, and preserved with 70% ethanol.

Afterwards, the lid of the sealed container was placed back, removed from mosquito-rearing cage, and returned to the study site for continuation of the study. Any fly species that emerge as adults in the containers thereafter were similarly collected as described earlier. They were morphologically sorted into their taxonomic groups with the aid of a dissecting microscope and identification guide by Lutz et al. [26], and a checklist of insects in Nigeria [27]. A taxonomist at the Insect Museum, Ahmadu Bello University Zaria, Nigeria validated the identity of the flies.

2.3. Data Collection and Ethical Approval

The fly species trapped and emerged in the containers were recorded as individual seasonal abundance. The first arrival time of the fly species on the containers across different inspection times for the two seasons were recorded and evaluated. The ambient temperature and the relative humidity of the study site were recorded for the first 30 days with a portable digital thermo-hygrometer while the volume of rainfall was measured with a manual rain gage calibrated in millimeter.

All the procedures carried out in this study as regards the animal model for research and its sacrifice, were in line with the Guidance on the Operation of the Animals (Scientific Procedures – Act 1986), EU Directive 2010/63 for the protection of animals used for scientific purposes, and that of Georgina et al. [25] and Abajue et al. [28]. The procedures were approved by the University of Port Harcourt Research Ethics Commission (UPT/CEREMAD/REC/MM88/065).

3. RESULTS

Three fly species of forensic values; *Chrysomya albiceps* (Wied.), *Chrysomya chloropyga* (Wied.), and *Musca domestica* (Linn.) in two families (Calliphoridae and Muscidae), were trapped in the containers that were used to expose the GPC. The first arrival time of *C. albiceps* during the dry and rainy seasons was consistent at 3 hours after post-exposure (3HPE). On the contrary, the first arrival time of *C. chloropyga* was noted at 2-day post-exposure (2DPE) while *M. domestica* first arrived on 4-day (4DPE) during the dry season. During the rainy season, *C. chloropyga* first arrived at 9 hours (9HPE) while *M. domestica* first arrived on 3-day (3DPE). Based on abundance, a total of 324 individual flies were trapped in the containers during the dry season against 466 individual in the rainy season.

The fly species showed higher abundance in the rainy season than the dry season. *Chrysomya albiceps* in particular were more preponderance during the rainy season than the dry season while *Chrysomya chloropyga* and *Musca domestica* did not show variable differences in abundance during the dry and rainy seasons. On species count, *Chrysomya albiceps* was the dominant fly species with 244 individuals during the dry season and 379 during the rainy season. It was followed by *M. domestica* with 67 during the dry season and 71 during the rainy season while *C. chloropyga* has 13 during the dry season and 16 during the rainy season (Table 1).

Other fly species trapped include *Drossophila melanogaster* (Meig.) and *Sarcophaga* sp. in the families (Drossophilidae and Sarcophagidae). The two species did not show consistency in their time of arrival, and were not discrete on the GPC. Table (2) shows the mean temperature, relative humidity, and rainfall data (34.15°C, 47.4% and 0.76mm) for the dry season, which were completely different from that of rainy season (26.42°C, 87.6%, and 19.00mm).

Table 1. Arrival time of Fly Species on the Guinea pig Carcass in the Containers at the University of Port Harcourt, Nigeria

Exclusion Time	No. Containers	Dry Season (Species)				Rainy Season (Species)			
		Ca	*Cc*	*Md*	Total	*Ca*	*Cc*	*Md*	Total
3HPE	15	6	0	0	6	18	0	0	18
5HPE	14	11	0	0	11	7	0	0	7
7HPE	13	9	0	0	9	6	0	0	6
9HPE	12	21	0	0	21	36	3	0	39
2DPE	11	33	3	0	36	73	2	0	75
3DPE	10	54	7	0	61	97	2	4	103
4DPE	9	71	0	3	74	33	1	2	36
5DPE	8	29	3	7	39	22	4	13	39
6DPE	7	5	0	11	16	24	1	6	31
7DPE	6	3	0	17	20	13	0	11	24
8DPE	5	0	0	3	3	5	0	4	9
9DPE	4	1	0	2	3	9	1	14	24
C1	3	0	0	4	4	2	2	9	13
C2	3	0	0	11	11	3	0	5	8
C3	3	1	0	9	10	1	0	3	4
Total		244	13	67	324	349	16	71	436

***Note**: Ca = Chyrsomya albiceps, Cc = Chrysomya chloropyga, Md = Musca domestica*

Table 2. *Mean Temperature, Relative humidity, and Rainfall at the study Site of Decomposing Guinea Pig Carcass in the Containers at the University of Port Harcourt, Nigeria*

Season of Study	Temperature (°C)	Relative Humidity (%)	Rainfall (mm)
Dry Season	34.15	47.40	0.76
Rainy Season	26.42	87.60	19.00

The interaction between the exclusion times and the seasons contributes to variations in the species abundance. These factors are key determinants of species abundance but their independent effects are more prominent. Different exclusion time validates how long any of the carcasses in a container were able to trap insects during dry and rainy seasons. Thus, the rainy season consistently showed higher insect abundance than the dry season at most exposure times. During the rainy season, insect abundance seems more variable, with sharp peaks and drops, especially at day 3, 6 and control 1 (6DPE and C1). The abundance reaches its highest at these points, indicating that insect activity was influenced by exclusion time during this season. The dry season shows a relatively flatter curve, with less dramatic fluctuations in abundance, indicating a more stable but lower overall insect abundance, which also peaks between days 3 and 6 (Figure 1).

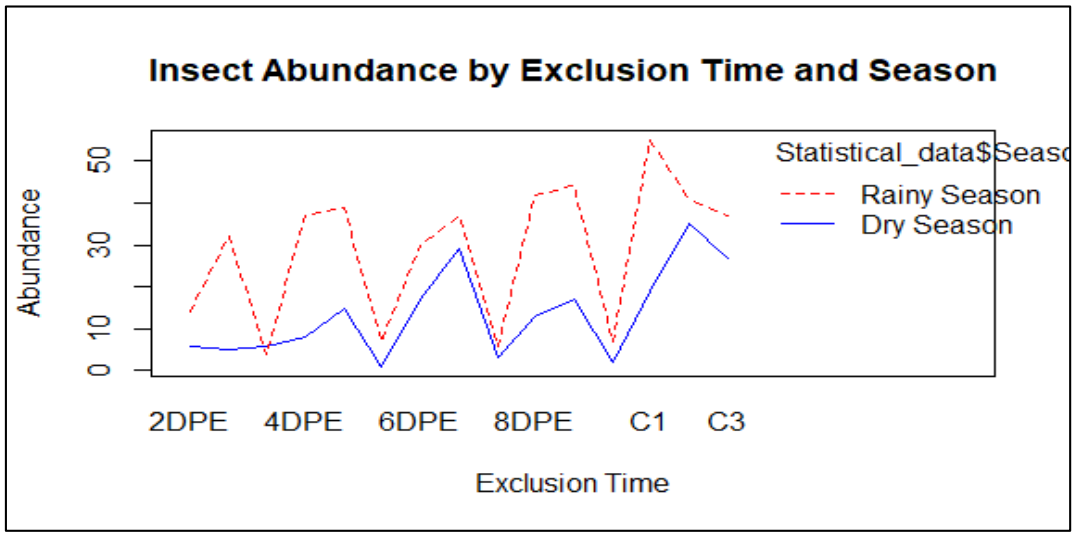

Figure 1. *Insect abundance across different exclusion times for dry and rainy seasons*

The flies were able to lay eggs on the carcasses and their time of adult emergencies vary during the two seasons (Fig 2). During the dry season, *Chyrsomya albiceps* were the first to emerge as adults on day 10 through day 14 from the containers, which represent different hours and days of sealing the containers (time of excluding flies access to the containers). For *M. domestica*, adult emergence from the containers was observed on day 20 through day 22 in the container sealed at day 7 (7DPE) and the control containers (C1-C3) only, while no adult emergence was observed for *C. chloropyga* for the season under review. During the rainy seasons, adults of *C. albiceps* emerged earlier on day 9 through day 15 from all the containers as observed during the dry season. Adults of *C. chloropyga* emerged first on days 10, 11, and 12 from the containers excluded at day 7, day 9 (7DPE, 9DPE), and in all the control containers respectively. Adult *M. domestica* emerged on day 17 through day 19 from the containers excluded at day 6, day 9 (6DPE, 9DPE), and the controls respectively. The difference in the time of emergence of *C. albiceps* for dry and rainy seasons was not far apart but that of *M. domestica* was distinctly far apart (Figs 2 and 3).

Figure 2. *Fly Species and their Day of Adult Emergence in the Containers at the University of Port Harcourt, Nigeria during the Dry Season*

Exclusion time	Species and their Days of Adult Emergence													
	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22
3HPE		*Ca*		*Ca*	*Ca*									
5HPE		*Ca*	*Ca*	*Ca*	*Ca*									
7HPE		*Ca*	*Ca*	*Ca*	*Ca*									
9HPE		*Ca*	*Ca*	*Ca*	*Ca*									
2DPE		*Ca*	*Ca*	*Ca*	*Ca*									
3DPE		*Ca*	*Ca*	*Ca*										
4DPE		*Ca*	*Ca*	*Ca*	*Ca*									
5DPE		*Ca*	*Ca*	*Ca*										
6DPE		*Ca*	*Ca*	*Ca*	*Ca*									
7DPE		*Ca*	*Ca*	*Ca*										
												Md		
8DPE		*Ca*	*Ca*	*Ca*	*Ca*	*Ca*								
9DPE		*Ca*	*Ca*	*Ca*		*Ca*								
C1		*Ca*	*Ca*	*Ca*	*Ca*	*Ca*								
													Md	
C2		*Ca*	*Ca*	*Ca*										
														Md
C3		*Ca*	*Ca*	*Ca*	*Ca*	*Ca*								
														Md

Figure 3. *Fly Species and their Day of Adult Emergence in the Containers at the University of Port Harcourt, Nigeria during the Rainy Season*

Exclusion time	Species and their Days of Adult Emergence													
	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22
3HPE	*Ca*	*Ca*		*Ca*										
5HPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*									
7HPE	*Ca*	*Ca*	*Ca*											
9HPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*									
2DPE	*Ca*		*Ca*	*Ca*	*Ca*									
3DPE	*Ca*	*Ca*		*Ca*	*Ca*									
4DPE	*Ca*	*Ca*	*Ca*		*Ca*		*Ca*							
5DPE	*Ca*	*Ca*	*Ca*	*Ca*										
6DPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*									
									Md		*Md*			
7DPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*								
			Cc	*Cc*										
8DPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*								
9DPE	*Ca*	*Ca*	*Ca*	*Ca*	*Ca*									
		Cc	*Cc*											
									Md	*Md*				
C1	*Ca*	*Ca*	*Ca*	*Ca*										
C2	*Ca*	*Ca*			*Ca*									
											Md			
C3	*Ca*	*Ca*	*Ca*	*Ca*										
		Cc	*Cc*	*Cc*										

Note: *HPE = hour of post exclusion, DPE = day of post exclusion, C = control (no exclusion), Ca = Chyrsomya albiceps, Cc = Chrysomya chloropyga, Md = Musca domestica*

4. DISCUSSION

Fly species in the families of Calliphoridae, Muscidae, and Sarcophagidae are among the notable group of insects that often locate carcasses immediately after death in Nigeria [5] but the calliphorids are the dominant group [29]. The three fly species (*Chrysomya albiceps*, *Chrysomya chloropyga*, and *Musca domestica*) are trapped in the containers that enclosed the GPC, and are usually reported as dominant flies associated with different carcasses in southern Nigeria [30]. Their arrival on carcasses is inevitable but the exact time at which they arrive usually varies due to time of the day, season, location, postmortem alteration, and some other factors as outlined by Anderson [31] and Sharma et al. [32].

The arrival time of *Chrysomya albiceps* at 3HPE, which was the earliest arrival time recorded in this study is contrary to previous reports with the earliest time of arrival by Kruger et al. [33]. The contradiction may be attributed to the time of the carcass exposure in this study at 8 am, and the study design. For instance, in this study, the assessment of the GPC in the container was narrowed through a 5cm hole. Again, the container may have affected the rate of the odorous emission of the GPC, while the first inspection of the GPC was delayed till 3HPE.

These reasons may have contributed to the patchiness between the times at which the flies arrived in this study as against the previous studies but depicts that *C. albiceps* can be predicted to arrive on a carcass within 3 hours of post exposure. Interestingly, its consistent arrival after 3 hours of exposure in this study area irrespective of the season overlaps with the time of the day (11: 00 am) when insect activities are higher in the study area.

On the contrary, the arrival time of *C. chloropyga* varied considerably for dry and rainy seasons. Its time of arrival during the dry season slightly contradicted that of the rainy season but suggests preference for a carcass that has undergone high level of autolysis irrespective of the season. Also, the arrival of *M. domestica* at a later stage of the carcasses for both seasons suggests its preference for an advanced decomposing carcasses in agreement with the reports of Grzywacz et al. [34] and Al-Zahrani [35]. Thus, in this study, a 3HPE was the least predictable arrival time of *C. albiceps* on the decomposing carcasses, which is the most dominant fly species of forensic importance in the southern Nigeria [18] irrespective of the season.

The interaction between the exclusion time and the seasons contribute to the variations of the species abundance but their independent effects are more prominent. Different exclusion times which represent how long the carcasses were allowed to trap insects showed reasonable variations with a higher insect abundance in the rainy season than the dry season though occasioned by *C. albiceps*.

Also, the species abundance had sharp peaks and drops, reaching its highest between day 4 and day 6 for both seasons but the dry season showed a relatively flatter curve, less dramatic fluctuations, and with a more stable and lower abundance. These interactions according to Anderson [31], Tembe and Makaratirwa [12] are part of the factors that influence carcass decomposition. In principle, covering the GPC with containers and narrowing accessibility of the carcass by flies are alterations that are away from the natural processes of carcass decomposition and accessibility by insects. Hence, the flies arrival and activity on the GPC were influenced by the time of exclusion for both seasons. In essence, the interaction could suggest that the time at which the fly species arrive on the carcasses is more variable in the rainy season. This may be linked to a higher humidity and temperature at 11 am of the day when the insect activities were notably higher. This is also an indication that the time of insects' activity in different geographical areas vary as reported in Mohr and Tomberlin [36].

In relation to the factors that could drive various diel activities in insects, Gbarakoro et al. [37] outlined the factors that could determine the early arrival of insect to floral vegetation in southern Nigeria. Also, the higher abundance of *C. albiceps* in particular was very much influenced by seasonal changes with a reasonable increase in abundance during the rainy season, which is similar to the report of Dao et al. [38]. On the other hand, *C. chloropyga* and *M. domestica*, exhibited relative stability across the seasons with no major fluctuations in their counts.

Therefore, environmental factors such as precipitation which probably impacted that temperature and humidity variables may have benefited *C. albiceps* during the rainy season

whereas *C. chloropyga* and *M. domestica* may either be less dependent on or more resilient to seasonal variations in the study area.

The first observed emergence of *C. albiceps* as a young adult from the carcasses on days 10 and 9 for dry and rainy seasons respectively is in agreement with the report of Abajue et al. [39]. However, the emergence of *C. chloropyga* on day 11 during the rainy season and the emergence of *M. domestica* on day 20 and 17 for dry and rainy seasons respectively were against the emergence day earlier reported for the species. These observed differences may be attributed to experimental designs.

In this present study, fly species that accessed the carcasses in the containers did not leave hence providing the gravid females the opportunity to lay eggs on the carcasses without disruption. This is contrary to Abajue et al. [39] where fly larvae were randomly collected on outdoor carcasses that were not excluded, and reared in the indoor to adult stage. It is therefore assumed that the larvae of *M. domestica* would have been missed during the earlier collections when the larvae of *Chrysomya* species dominated the carcasses.

5. CONCLUSION

The findings in this study, are indications that environmental seasonality and time progression are influential factors that impacted the arrival time, abundance, and reproductive cycles of the three fly species on the GPC. The arrival of these fly species, and laying of eggs on the carcasses that metamorphosed into larvae therein, and their emergence as young adult flies would provide crucial forensic information about PMI of carcasses that died mysteriously. For instance, the presence of these fly larvae on carcasses without their pupae or puparial casings suggests a PMI of less than 8 days.

The emergence of the fly species at different days on the carcasses also suggest that oviposition is dependent on their arrival time, and the carcasses quality at time of arrival. This study has open up a new dimension that aims at predicting the arrival time of forensic flies on carcasses in the study area.

We therefore suggest for further studies to replicate the study design and incorporate suitable statistical tools to its evaluation devoid of descriptive intuition.

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