

## Developmental analysis of immature stages of *Sarcophaga* (*Parasarcophaga*) *albiceps* Meigen, 1826 (Diptera: Sarcophagidae) on *Gallus gallus* carcass: Their applications as forensic indicators

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**Abstract :** The blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*) are among the first wave of faunal succession on human and animal cadavers. Thus, their immature stages are used to estimate the post mortem interval (PMI). The larval development might vary seasonally. A study was conducted to investigate the effect of seasonal variation on development of immature stages (larvae and pupa) of *Sarcophaga* (*Parasarcophaga*) *albiceps* on carcass of *Gallus gallus* (n=3). Results showed no significant differences in the average length, average width and calculated average biomass of the immature stages as [F(2,9)=0.0184, p= 0.9817], [F(2,9)=0.2415, p= 0.7903] and [F(2,9)=0.5254, p= 0.6083] respectively. These results might have important implications to forensic entomologists, (p > 0.05). Since one approach of PMI estimation uses larvae collected from crime scene and comparing them with reference data, derived from rearing of larvae. The results indicate that *Sarcophaga* (*Parasarcophaga*) *albiceps* can be utilized as forensic indicator for above said purpose, as there is no significant developmental variation of immature stages seasonally, when reared on *Gallus gallus* carcass.

**Keywords:** Forensic dipterology, India, Sarcophagidae, Forensic entomology, *Sarcophaga* (*Parasarcophaga*) *albiceps*, Avian model.

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### I. Introduction

Nature has provided insects with built-in receptors, to assess, quantify and locate carcasses of both human and animals, forensic entomology uses this precarious property of insects namely, the orders Diptera and Coleoptera, to estimate minimum time since death. Post mortem changes of decaying organisms depend on various factors, Micozzi (1991). Estimating post mortem interval thus becomes a rather difficult task, as time since death increases the normal physiological and pathological tests reliability and accuracy decreases, Bass (1984). Since the normal physiological and pathological tests for estimating PMI yield ambiguous results after 84 hours, Henssage (1995) for both human and non-human carrion.

Therefore to avoid ambiguity in PMI estimates and to obtain more accurate information, other processes are needed to be investigated for establishing correct PMI. It has been found that any physical or biochemical change that is a function of time since death can be utilized for the aforesaid purpose, better known as PMI dependent process. A carcass is a depleting resource, which attracts a variety of scavengers, Putman (1983). Generally to estimate PMI for vertebrate carcass various processes are used, whose principles are based on PMI dependent process, Hall (1990). One of these processes is the immature insect species development model, where immature stages of the insects consume dead human or animal tissue at different stages of their life cycle. The estimated age of an immature insect that feeds on the carrion provides the minimum PMI. Investigators in India, when faced with a need to estimate a portion of the PMI from entomological data from the scene lack this reference data on this model. The current study aims to bridge this gap, for wildlife crimes pertaining to avian model and extrapolation of the data can be used for human model, *Sarcophaga* (*Parasarcophaga*) *albiceps* Meigen, 1826 being the forensic indicator and model organism for immature insect species development on avian carcass. [1]

The family Sarcophagidae has 126 species recorded from India. The adult flies of the largest members of Indian Sarcophagidae are ♂ *Sarcophaga* (*Parasarcophaga*) *albiceps* (Meigen, 1826) their size ranging from 11-18 mm. *S. albiceps* have been known to cause tissue myiasis in bulls and breed in human and rabbit excretions. Adults of this species seem to be active throughout the year and common in this part of India, making them ideal candidates for being forensic indicators for avian and human models. *Sarcophaga* (*Parasarcophaga*) *albiceps*

(Meigen, 1826) is an arthropod, that belongs to class Insecta, order Diptera, family Sarcophagidae and subfamily Sarcophaginae. The genus *Sarcophaga* was first described by Meigen and the subgenus *Parasarcophaga* was first described by Johnston and Tieggs. Nandi had re-described the Indian species as *Parasarcophaga albiceps* but the present accepted valid name is *Sarcophaga (Parasarcophaga) albiceps*. [2-8]

This fly is almost cosmopolitan in distribution, it is found in PALAEARCTIC - Albania, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, Byelorussia, China (Gansu, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Jilin, Liaoning, Neimenggu, Ningxia, Shaanxi, Shandong, Shanghai, Shanxi, Sichuan, Xizang), Croatia, Czech Republic, Finland, France, Germany, Greece, Gruzia, Hungary, Israel, Italy, Japan (Hokkaido, Honshu, Kyushu, Shikoku), Kazakhstan, Latvia, Moldova, Netherlands, North Korea, Norway, Poland, Portugal, Romania, Russia (Central European Territory, East Siberia, Far East, North European Territory, South European Territory, West Siberia), Serbia, Slovakia, South Korea, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom. AFROTROPICAL - Kenya. ORIENTAL - Andaman Is, Bangladesh, Bhutan, Indonesia (Flores, Java, Kalimantan, Lombok, Sulawesi, Sumatra, Timor), China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, Jiangxi, Yunnan, Zhejiang), Japan (Ryukyu Is), Malaysia (West Malaysia), Laccadive Is, Nepal, Pakistan, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Vietnam. AUSTRALASIAN/OCEANIAN - Australia (Queensland), Hawaiian Is (Hawaii, Kauai, Maui, Molokai, Oahu), Indonesia (Irian Jaya, Moloku), Papua New Guinea (Bismarck Arch.), Solomon Is. [2,3-8]

*S. albiceps* has been bred from rabbit and human excrement. They are found to be parasitic on *Nonagria sp.* and cause tissue myiasis in bulls. The male is attracted to *Aristolochia sp.* (Senior-White, Aubertin and Smart, 1940). *S. albiceps* breeds in decaying organic matter and has been observed to lay eggs on mutton in India and fish in Pakistan (Shazia et al., 2006; Singh & Bharti, 2008). Similar observations have been made of this species breeding on faeces in the presence of carrion in Thailand (Bänziger and Pape 2004). Also, *S. albiceps* has been documented causing cutaneous myiasis of buffalo, cows and humans (Castro et al. 2010). Larvae of *S. albiceps* are also economically important organisms as they are facultative predators of a variety of butterfly (Lepidoptera) pupae and Hymenoptera larvae. [9-15]

The base line data generated from this study might also help in stored product entomology, where this fly can potentially identify rotting meat, which is not suitable for human consumption, biocontrol of Lepidoptera and Hymenoptera, myiasis biology and also can be used in spread of enteric disease and its prediction model (Greenberg, 1971). The current study only focuses on the morphotaxonomy, life cycle assessment, seasonal variations and distribution mapping to assess the immature insect development on avian carcass, which might help PMI estimation for avian carcass in the states where *Sarcophaga (Parasarcophaga) albiceps* is distributed.

## II. Materials And Methods

### II.a.) Study Site

The study was conducted in Kolkata, ZSI, Latitude :22° 30' 51.6888" and Longitude: 88° 19' 30.5256" were recorded by GPS meter. A dead *Gallus gallus* (Linnaeus, 1758) was bought from a market near Zoological Survey (ZSI) of India (n=3) for three seasons, Kolkata premises, and was kept in ambient outdoor conditions, inside the ZSI premises. The data for the abiotic factors was gathered from the meteorological data collected from the Meteorological Department, Alipore, Kolkata. The local climatic regimes of West Bengal is sub divided into three seasons, viz., pre monsoon (March to June), monsoon (July to October) and post monsoon (November to February). During the experiments, the measured average temperature (°C) ranged from 35 – 43, in the pre monsoon season, 37 – 30, in the monsoon season and 35 – 25, in the post monsoon season. Relative humidity (%) ranged from 59 – 45, in the pre monsoon season, 90 - 75, during the monsoon season and 42 - 35, in the post monsoon season. Average precipitation was null during the pre and post monsoon seasons, the range of the monsoon season was found to be 58 – 35. And average wind speed (km/hr) ranged from 31 - 13, in the pre monsoon season, 20 - 12, in the monsoon and 15 – 8, in the post monsoon season. (See table.1).

### II.b.) Collection of the fly specimen

The chicken carcass was placed on a raised platform, surrounded by water on all sides to discourage ants and a malaise trap was used for overhead capture of dipteran specimens. The malaise trap was used to capture the flies in one part of ZSI, Kolkata, in 2013-2014. As the post feed maggots (larvae of 3<sup>rd</sup> instar) starts their migration away from the carcass and into suitable pupation material, in this case a cotton was used to help the larvae to pupate, and then the contents of that piece of cotton was placed into a glass container, to see progression of normal development. From here few immature specimens (n = 4) were collected for taxonomic, LM and ecologic studies.

A modified version of the malaise trap was utilized to capture adults, the schematics and collection of the adult. Different developmental stages were observed on taxonomic and ecologic basis, and the immature

insects were collected with the help of fine forceps for taxonomic studies and were killed with ether and preserved in 70% alcohol. The experiments, taxonomic and morphological identification and quantification were carried out in the above mentioned Laboratory of Diptera Section, Kolkata, India. The meteorological data was collected from the Meteorological Department, Alipore, Kolkata.

The construct was checked regularly and notes on the appearance time of *S. albiceps*, their larvipositing time, larval developmental time, pupal developmental to adult emergence, were photodocumented by the nikkon p520 camera and Leica EZ4 HD.

### **II.c.) Morphological identification of the different developmental stages of *Sarcophaga (P) albiceps***

The morphology of *Sarcophaga (P) albiceps* was observed under light microscope (LM) at each stage in its life cycle. To observe the anatomical feature of the genitalia, both male and female flies were dissected and examined under LM [18]. The male genitalia and the 5<sup>th</sup> sternite, are the most important characteristic feature used to differentiate different flesh fly species. The abdominal segments between 3rd and 4th segments of the flies were dissected on the clean glass slide using a sharp blade, transferred to a mixture of 10% sodium hydroxide and 95% ethanol for 3 days and following procedures were done as detailed in ref.14, 18. The genitalia and ovipositor were observed under Leica M205 Stereozoom dissecting microscope and photographs taken by the allied Leica camera. The growth and development of *Sarcophaga (P) albiceps* initiating from the first instar larvae, to development into second, third instar larvae, pupae, imago and finally emergence of the adult fly (Figs. 1) on dead carrion of the *Gallus gallus* (n=3) is being reported for the first time from India thus establishing the role of *Sarcophaga (P) albiceps* as a forensic indicator for wild life crime and stored products entomology from India.

### **II.d) Developmental data analysis**

The immature stages (larvae and pupa) and adults were measured to see the rate of development of *Sarcophaga (P) albiceps* in the carcass of *Gallus gallus* (n=3), in all the seasons. The length, width and biomass of larvae (n = 5) were calculated from Leica EZ4 HD microscopic measurement data for all three seasons. The time spent was monitored recorded and the percentage of time spent in each stage was calculated. The effects of various seasons on the growth and development (length, width and biomass) of *S. albiceps* were subjected to a one way ANOVA. (Sokal and Rohlf 1981).

## **III. Results**

### **III.a) Morphology-based identification**

#### **III.a.1) Description of the morphology of the male and female adult fly**

##### **III.a.1.1) Male Morpho-description**

The males are generally of variable size ranging from moderately large to large (11-17 mm). The width of the frons about 3/5<sup>th</sup> to that of an eye. Frontal vittae, para-frontal and para-facial, black with silvery pollen on it. Antennae darkish brown. The 1<sup>st</sup> segment of the antennae seems to be blackish brown, 2<sup>nd</sup> segment darkish black and 3<sup>rd</sup> moderately brown with whitish pollen. Vibrissae and arista long and plumose along basal 2/3<sup>rd</sup> of facial ridge. Palpi brownish. Proboscis jet black and stout. Thorax greyish in colour with three longitudinal black stripes. Pro stigmatic and pro pleural bristles well developed and accompanied with short hairs. Pro and mesothoracic spiracles brown; latero-scutellar, apico-scutellar and disco-scutellar bristles were 3, 1, and 1 pair each respectively.

Wings hayline, with brownish veins; R1 bare ;R 4+5 with a row of 9 short setae located dorsally and extending to atleast the half of basal node upto r-m. Short setae long ventral surface of basal node of R 4+5 ; fifth costal segment a little shorter than the 3<sup>rd</sup>, the former with short spines along basal half of its anterior margin; costal spines stout; epaulet black with short spines; basicostal scale brown; squama white; halter brown.

Legs black in colour; fore femur with a pair of rows of long bristles along posterodorsal surface and a row of bristles along posterior margin of ventral surface. Mid femur with a row of 3 bristles along middle portion of anterolateral surface; Fore tibia with a row of 2-3 short bristles; hind tibia with 2 bristles on middle portion of posterodorsal surface.

Abdomen with silvery grey checkered pattern. The 2<sup>nd</sup> and 3<sup>rd</sup> sternites each with 4 bristles, 4<sup>th</sup> and 5<sup>th</sup> with 2 long bristles. The 5<sup>th</sup> sternite Y-shaped with stout spines laterally and long hairs terminally on arms.

The genital capsule looks oddly golden yellow to brownish yellow in colour. The 1<sup>st</sup> and 2<sup>nd</sup> genital segments reddish-brown in color; inner forceps stout, elongated, without protuberance and spines at inner surface of subapical part, but with tuft of long hairs along basal half; in contrast the outer forceps elongated, somewhat kidney shaped with hairs along distal half. As per ref. 2, 4.(see Fig.1)

### **III.a.1.2) Female Morpho-description**

The females are generally shorter than the males (7 - 12 mm). There is little biometric difference with the males. Apico-scutellar bristles were absent in females. Chaetotaxy of legs same as in males. The 2<sup>nd</sup> and 3<sup>rd</sup> sternites each with 4 bristles, 4<sup>th</sup> and 5<sup>th</sup> with 2 long bristles, 6<sup>th</sup> broader than the rest, 7<sup>th</sup> without hairs but with a row of stout marginal bristles, 8<sup>th</sup> bare, membranous and slightly concave; anal sternite with short hairs; 7<sup>th</sup> tergite with long bristles. As per ref. 2, 4. (see Fig.1)

### **III.a.1.3) Description of the morphology of the developmental stages (larva and pupa) of Sarcophaga (P) albiceps**

Morphological features of the immature stages special attention was given to 3<sup>rd</sup> instar larvae and pupa of *Sarcophaga (P) albiceps*, which revealed distinct morphological parameters of larvae. To identify the larval stages attention was given to the LM of anterior and posterior spiracles and 2<sup>nd</sup> and 3<sup>rd</sup> integument of pupa. The identifying criteria utilized for the identification of *Sarcophaga (P) albiceps* 3<sup>rd</sup> instar larvae and pupae was the presence of spines on the interband area of the thoracic and abdominal segments, which are long and pointed. (see Fig.2)

### **III.b.) Ecology and Life cycle analysis**

The *Sarcophaga (P) albiceps* seems to be larviparous and therefore no eggs were found from the carcass in all the three seasons. *Sarcophaga (P) albiceps* completes its life cycle in 326.11 hrs in premonsoon, 336.38 hrs in monsoon and 343.09 hrs in post monsoon season, the weight of the three carcass being equal for all the seasons. Therefore on average *Sarcophaga (P) albiceps* tends to complete its life cycle in *Gallus gallus* carcass in about 335.43 hrs. We observed the seasonal variation of different phases of life cycle of *Sarcophaga (P) albiceps*, and plotted in bar charts (see Table.2 and Fig.3). Distribution map was created to assess where this data may be utilised in future, this is as per the distribution of *Sarcophaga (P) albiceps*, as per literature and other collection data sources. (see Fig.4)

### **III.c.) Developmental data analysis**

The data of the LM measurements were utilized for analysis of the different developmental stages and adult. The time spent in various stages are averaged and standard error was calculated for the same. The length and width of the various stages were also averaged and standard error was calculated for the same. The whole process was done for all the seasons. The biomass was calculated from the primary LM data of length and width, and putting them in formula for cylinder, biomass was calculated, afterwards this data was averaged and standard error was calculated. The calculated biomass might fit the immature stages better than the adult stages, since the legs and wings are not accounted for in this calculation method. ANOVA (1-way) was done for all the stages averages of (length, width and calculated biomass) for all the seasons.

## **IV. Discussion**

### **IV.a.) Developmental data analysis (Seasonal)**

Each developmental stage, were stopped watched and the averages were calculated. The study was conducted in 2014. The female *Sarcophaga (Parasarcophaga) albiceps*, generally arrives at a carcass after around 24 hours. After their mating with the males, females larviposits.

#### **IV.a.1.) Premonsoon season (PRM)**

From initiation of larviposition to deposition of 1<sup>st</sup> instar larvae it took around 27 hours and 16 minutes, from 1<sup>st</sup> instar larvae to 2<sup>nd</sup> instar larvae it took around 31 hours and 40 minutes, from 2<sup>nd</sup> instar larvae to 3<sup>rd</sup> instar larvae it took around 46 hours and 37 minutes, from 3<sup>rd</sup> instar larvae to pupae it took around 111 hours and 8 minutes and from pupae to adult it took around 110 hours and 10 minutes. Therefore the total life cycle took around 326 hours and 11 minutes to complete its life cycle. (See Graph.1 and Graph.2.)

#### **IV.a.2.) Monsoon season (M)**

From initiation of larviposition to deposition of 1<sup>st</sup> instar larvae it took around 28 hours, from 1<sup>st</sup> instar larvae to 2<sup>nd</sup> instar larvae it took around 32 hours and 30 minutes, from 2<sup>nd</sup> instar larvae to 3<sup>rd</sup> instar larvae it took around 47 hours and 38 minutes, from 3<sup>rd</sup> instar larvae to pupae it took around 115 hours and 23 minutes and from pupae to adult it took around 113 hours and 47 minutes. Therefore the total life cycle took around 336 hours and 38 minutes to complete its life cycle. (See Graph.1 and Graph.2.)

#### **IV.a.3.) Postmonsoon season (PSM)**

From initiation of larviposition to deposition of 1<sup>st</sup> instar larvae it took around 28 hours and 50 minutes, from 1<sup>st</sup> instar larvae to 2<sup>nd</sup> instar larvae it took around 33 hours and 28 minutes, from 2<sup>nd</sup> instar larvae to 3<sup>rd</sup>

instar larvae it took around 48 hours and 23 minutes, from 3<sup>rd</sup> instar larvae to pupae it took around 117 hours and 20 minutes and from pupae to adult it took around 115 hours and 48 minutes. Therefore the total life cycle took around 343 hours and 9 minutes to complete its life cycle. (See Graph.1 and Graph.2.)

#### **IV.a.4.) Average annual developmental time**

Therefore on average from initiation of larviposition to deposition of 1<sup>st</sup> instar larvae it took around 28 hours and 28 minutes, from 1<sup>st</sup> instar larvae to 2<sup>nd</sup> instar larvae it took around 32 hours and 32 minutes, from 2<sup>nd</sup> instar larvae to 3<sup>rd</sup> instar larvae it took around 47 hours and 32 minutes, from 3<sup>rd</sup> instar larvae to pupae it took around 114 hours and 50 minutes and from pupae to adult it took around 113 hours and 1 minute. Therefore the total life cycle took around 335 hours and 43 minutes on average to complete its life cycle. With the average prevalent abiotic conditions. (see Table.1 and Graph.1.)

#### **IV.b. Developmental data analysis (Biometric)**

One approach in PMI estimation involves killing the larvae collected from crime scene and comparing the measured length with reference data derived from rearing of larvae in the laboratory where they are most commonly fed the liver (Williams and Richardson, 1984; Anderson, 2000; Grassberg et al., 2003) or ground beef (Wells and La Motte, 1995) of various mammalian species. In the present study *Gallus gallus* has been utilized as bait to attract *Sarcophaga (Parasarcophaga) albiceps* for three seasons (n = 3). The few of the immature specimens from stages were separated from carcass (n = 4) and light microscopy was done for both taxonomic and biometric studies. The length and width were recorded in mm and the biomass was calculated of immature specimens were calculated using the formula of volume of cylinder from the website [http://www.onlineconversion.com/object\\_volume\\_cylinder\\_tank.htm](http://www.onlineconversion.com/object_volume_cylinder_tank.htm), the volume was calculated for the immature stages, and recorded in mm<sup>3</sup>. The adults were not included in the biometric study as they serve almost no purpose in this immature stage biometric study based PMI estimation. Standard deviation (s) and standard error was calculated for length, width and biomass (Huth et al., 1994).

##### **IV.b.1.) Premonsoon season (PRM)**

In the premonsoon season the length of the 1<sup>st</sup> instar larvae was found to be  $0.3817 \pm 0.0085$  (s = 0.0170). The length of the 2<sup>nd</sup> instar larvae was found to be  $1.012 \pm 0.1121$  (s = 0.2243). The length of the 3<sup>rd</sup> instar larvae was found to be  $1.8845 \pm 0.1513$  (s = 0.3026). The length of the pupae was found to be  $1.3751 \pm 0.0483$  (s = 0.0967). The width of the 1<sup>st</sup> instar larvae was found to be  $0.0775 \pm 0.0057$  (s = 0.0114). The width of the 2<sup>nd</sup> instar larvae was found to be  $0.2445 \pm 0.0318$  (s = 0.0637). The width of the 3<sup>rd</sup> instar larvae was found to be  $0.5017 \pm 0.0360$  (s = 0.0712). The width of the pupae was found to be  $0.5242 \pm 0.0145$  (s = 0.0291). The biomass of the 1<sup>st</sup> instar larvae was found to be  $0.0017 \pm 0.0002$  (s = 0.0004). The biomass of the 2<sup>nd</sup> instar larvae was found to be  $0.0538 \pm 0.0154$  (s = 0.0309). The biomass of the 3<sup>rd</sup> instar larvae was found to be  $0.3882 \pm 0.0776$  (s = 0.1552). The biomass of the pupae was found to be  $0.2985 \pm 0.0237$  (s = 0.0476). (see Table.2)

##### **IV.b.2.) Monsoon season (M)**

In the premonsoon season the length of the 1<sup>st</sup> instar larvae was found to be  $0.3517 \pm 0.0028$  (s = 0.0057). The length of the 2<sup>nd</sup> instar larvae was found to be  $0.9130 \pm 0.0988$  (s = 0.1977). The length of the 3<sup>rd</sup> instar larvae was found to be  $1.6875 \pm 0.0447$  (s = 0.0895). The length of the pupae was found to be  $1.3750 \pm 0.0368$  (s = 0.0736). The width of the 1<sup>st</sup> instar larvae was found to be  $0.0475 \pm 0.0237$  (s = 0.0057). The width of the 2<sup>nd</sup> instar larvae was found to be  $0.1950 \pm 0.0975$  (s = 0.0794). The width of the 3<sup>rd</sup> instar larvae was found to be  $0.3060 \pm 0.0455$  (s = 0.0910). The width of the pupae was found to be  $0.4115 \pm 0.0564$  (s = 0.1129). The biomass of the 1<sup>st</sup> instar larvae was found to be  $0.0005 \pm 0.0000$  (s = 0.0001). The biomass of the 2<sup>nd</sup> instar larvae was found to be  $0.0336 \pm 0.0152$  (s = 0.0305). The biomass of the 3<sup>rd</sup> instar larvae was found to be  $0.1353 \pm 0.0424$  (s = 0.0849). The biomass of the pupae was found to be  $0.1621 \pm 0.0483$  (s = 0.0966). (see Table.2)

##### **IV.b.3.) Postmonsoon season (PSM)**

In the premonsoon season the length of the 1<sup>st</sup> instar larvae was found to be  $0.3720 \pm 0.0043$  (s = 0.0087). The length of the 2<sup>nd</sup> instar larvae was found to be  $1.0132 \pm 0.0706$  (s = 0.1413). The length of the 3<sup>rd</sup> instar larvae was found to be  $1.7450 \pm 0.1245$  (s = 0.2490). The length of the pupae was found to be  $1.3650 \pm 0.0500$  (s = 0.1001). The width of the 1<sup>st</sup> instar larvae was found to be  $0.0116 \pm 0.0058$  (s = 0.0116). The width of the 2<sup>nd</sup> instar larvae was found to be  $0.2312 \pm 0.1156$  (s = 0.0680). The width of the 3<sup>rd</sup> instar larvae was found to be  $0.4892 \pm 0.0358$  (s = 0.0717). The width of the pupae was found to be  $0.5217 \pm 0.0140$  (s = 0.0280). The biomass of the 1<sup>st</sup> instar larvae was found to be  $0.0017 \pm 0.0002$  (s = 0.0004). The biomass of the 2<sup>nd</sup> instar larvae was found to be  $0.0473 \pm 0.0149$  (s = 0.0299). The biomass of the 3<sup>rd</sup> instar larvae was found to

be  $0.3344 \pm 0.0573$  ( $s = 0.1146$ ). The biomass of the pupae was found to be  $0.2934 \pm 0.0234$  ( $s = 0.0469$ ). (see Table.2)

The three seasons data were averaged for length, width and calculated biomass of the immature stages, the results showed no significant differences in the average length, average width and calculated average biomass of the immature stages as  $[F(2,9) = 0.0184, p = 0.9817]$ ,  $[F(2,9) = 0.2415, p = 0.7903]$  and  $[F(2,9) = 0.5254, p = 0.6083]$  respectively, the averages were plotted in error bars  $\pm$  standard error, slight intersection between the error bars meant that average length, average width and calculated average biomass, had little variations, which might be significantly different seasonally on *Gallus gallus* carcass. These results might have important implications to forensic entomologists, ( $p > 0.05$ ). And the scatter plot data was made from time and the averages of the three seasons data were averaged for length, width and calculated biomass of the immature stages, to see how it varied over time. (See Graph.3)

## V. Figures and Tables

Table.1.)

Seasons	Average temperature	Average relative humidity (in %)	Average precipitation	Average wind speed
	(in ° C)		(in %)	(in km/hr)
Pre monsoon	39.4	51.94	0	25.67
Monsoon	33.54	82.47	43.4	15.87
Post monsoon	30.06	38.31	0	11.5

Fig.1) Morphology of adult *Sarcophaga (Parasarcophaga) albiceps* male and female

**A: Side view adult male genital capsule and 5<sup>th</sup> sternite:** The genitalia of an adult male was dissected and processed as mentioned in material and methods, viewed under LM. c:cercus; ep:epandrium; prg:pregonite; j:juxta; v:vesica; s:surstylus; pht: phallic tube.

**B. Female Genitalia:** The female genitalia was dissected out and processed as mentioned in materials and methods. Sternites 6,7 and 8, signum, epiproct, cerci and genital tergites 1 and 2 are shown in the figure. ster-6-8: sternites 6-8; sig: Signum; epi: Epiproct; cer: Cerci; gen-ter-1, 2: Genital tergites 1-2.

**C: The 5<sup>th</sup> sternite:** The U-shaped 5<sup>th</sup> sternite was dissected out for better visualization, This is also the conformatory test for identifying the species as shown by LM.

**D. Left fore wing of an adult male:** The fore wing was visualized under LM and different parts were noted. Sc: Sub Costal vein; C: Costal vein; R<sub>1</sub>: anterior branch of radius; R<sub>2+3</sub>: radial vein; M: media; r-m: radial-medial; bm-cu: basal-medial-cubital; CuA: anterior cubital.

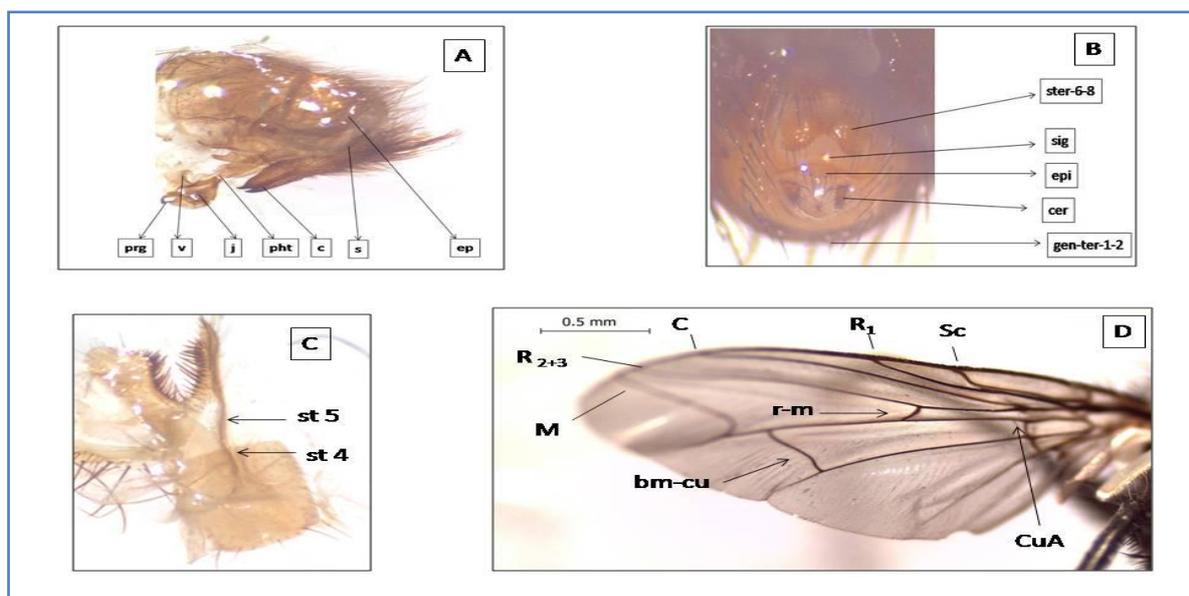


Fig.2) Morphology of immature stages (3<sup>rd</sup> instar larvae) of *Sarcophaga (Parasarcophaga) albiceps*

**A: Anal division:** Posterior view of 3<sup>rd</sup> instar larvae of *Sarcophaga (Parasarcophaga) albiceps*, p1-p6: papillae (1,2,3,4,5,6).

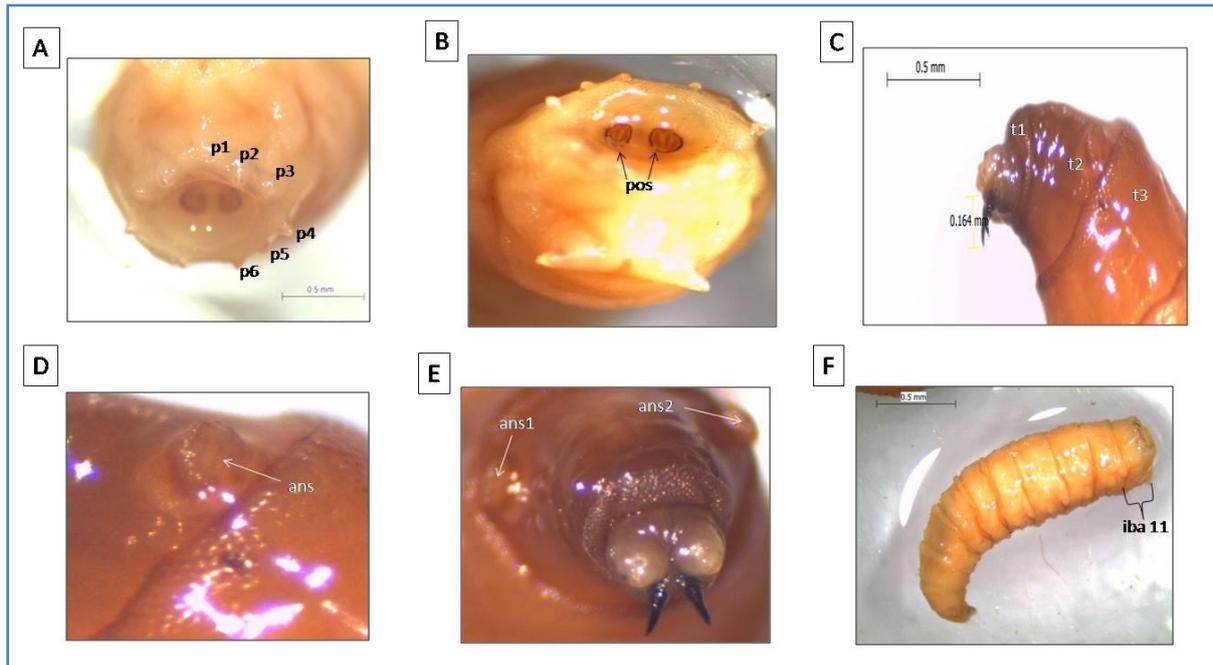
**B: Anal division:** Posterior view of 3<sup>rd</sup> instar larvae of *Sarcophaga (Parasarcophaga) albiceps*, pos: posterior spiracles (1,2).

**C: Anterior of body:** Ventral view of post feed 3<sup>rd</sup> instar larvae of *Sarcophaga (Parasarcophaga) albiceps*, t1-t3: thoracic segments (1,2,3).

**D: Anterior spiracle.**

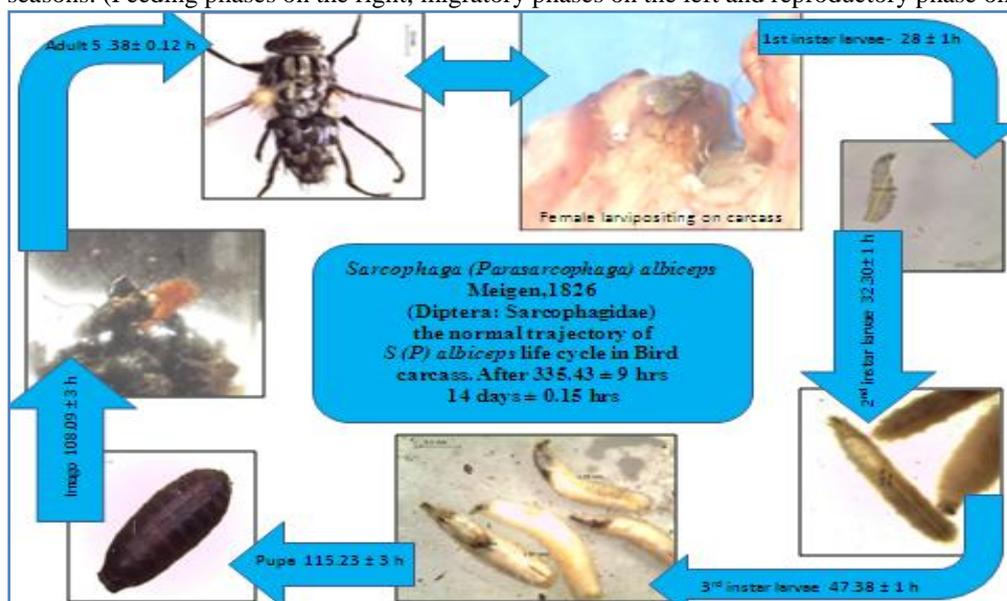
**E: Anterior of body:** Anterior end of body of post feed 3<sup>rd</sup> instar larvae of *Sarcophaga (Parasarcophaga) albiceps*, ans1,2: anterior spiracles (1,2).

**F: Anal division of body ventral view:** Post feed 3<sup>rd</sup> instar larvae of *Sarcophaga (Parasarcophaga) albiceps*, shows iba11 : inter band area 11. There are a total of 12 iba's in *Sarcophaga (Parasarcophaga) albiceps*, not marked.



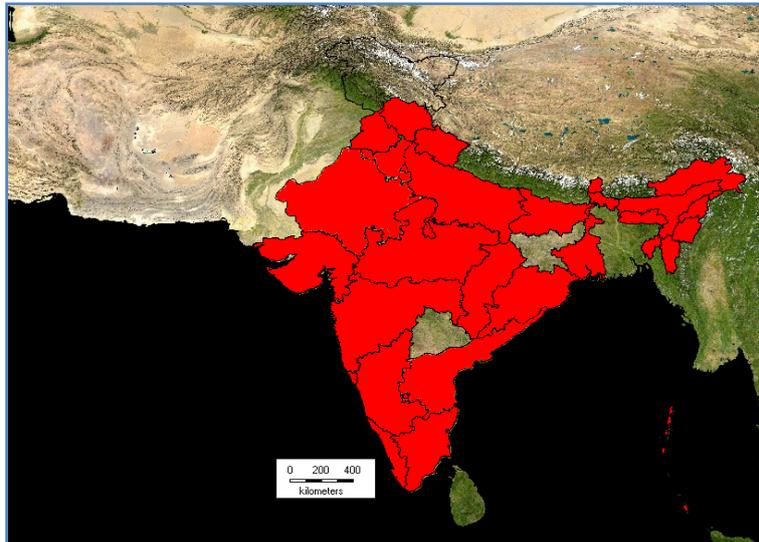
**Fig.3) Life cycle of *Sarcophaga (Parasarcophaga) albiceps*.**

The figure represents the life cycle starting from adult, to different instars, pupae and then imago in a cyclical way, time taken between the phases are given with average times  $\pm$  SE on the *Gallus gallus* carcass for the three seasons. (Feeding phases on the right, migratory phases on the left and reproductive phase on top).

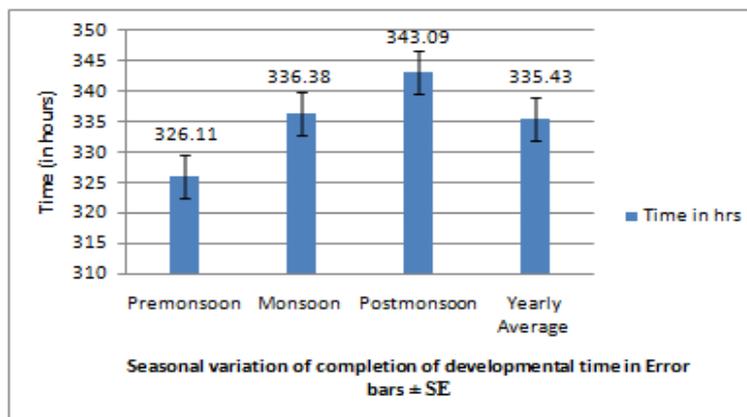


**Fig.4.) Distribution map of *Sarcophaga (Parasarcophaga) albiceps* in India**

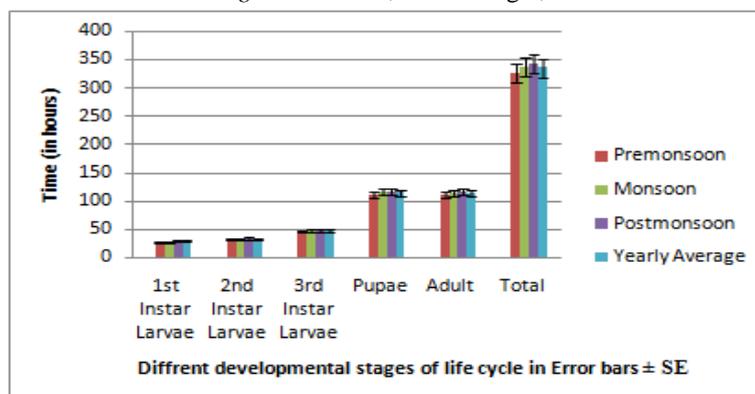
*Sarcophaga (Parasarcophaga) albiceps* are marked in the colour red in the physical map of India. Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Manipur, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal, Andaman and Nicobar, Daman Diu, Pondicherry are marked in light red in the physical map of India. [3]



**Graph.1.)** The graph shows the time  $\pm$  SE, for developmental period of *Sarcophaga (Parasarcophaga) albiceps* for completion of life cycle in the different seasons and overall annual average on *Gallus gallus* carcass, West Bengal, India.



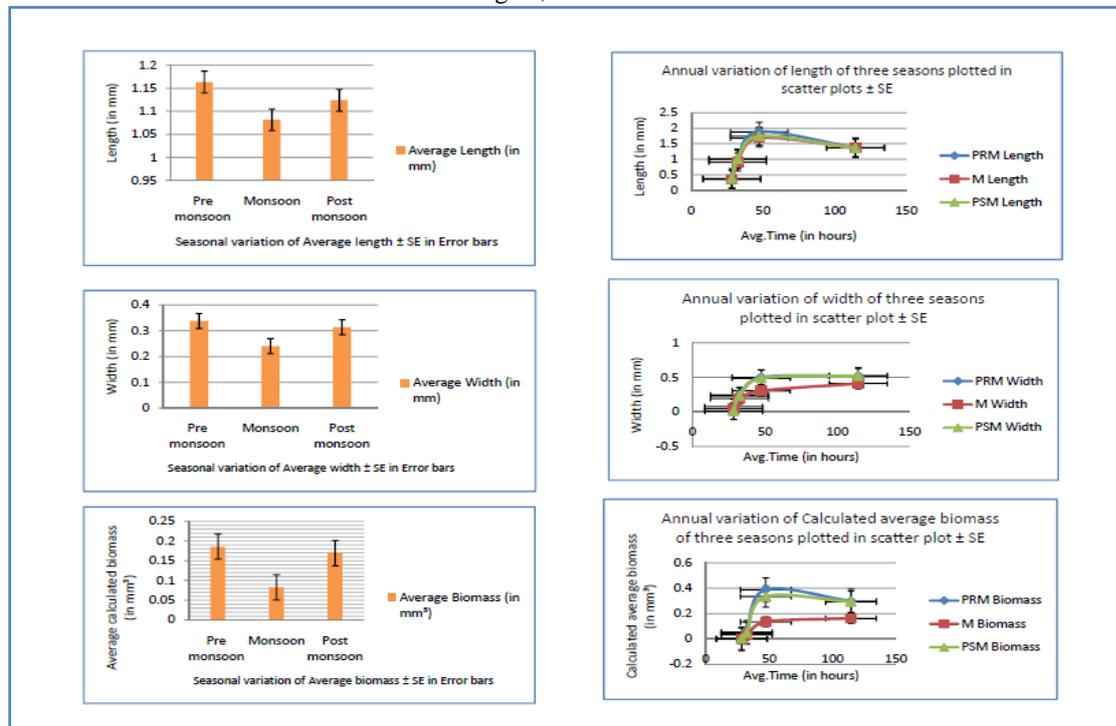
**Graph.2.)** Graph showing the time  $\pm$  SE, taken by various stages during development period of *Sarcophaga (Parasarcophaga) albiceps* for completion of life cycle in the different seasons and overall annual average on *Gallus gallus* carcass, West Bengal, India.



**Table.2.)** Shows the average of time, length, width and calculated biomass ± their respective SE

Stages	Avg. Hours (Mean ± SE)	Avg. Length (in mm) (Mean ± SE)			Avg. Width (in mm) (Mean ± SE)			Avg. Calculated Biomass (in mm <sup>3</sup> ) (Mean ± SE)		
		PRM	M	PSM	PRM	M	PSM	PRM	M	PSM
1 <sup>st</sup> instar larvae	28.28 ± 0.3914	0.3817 ± 0.0085	0.3517 ± 0.0028	0.3720 ± 0.0043	0.0775 ± 0.0057	0.0475 ± 0.0237	0.0116 ± 0.0058	0.0017 ± 0.0002	0.0005 ± 0.0000	0.0017 ± 0.0002
2 <sup>nd</sup> instar larvae	32.32 ± 0.5435	1.0120 ± 0.1121	0.9130 ± 0.0988	1.0132 ± 0.0706	0.2455 ± 0.0318	0.1950 ± 0.0975	0.2312 ± 0.1156	0.0538 ± 0.0154	0.0336 ± 0.0152	0.0478 ± 0.0149
3 <sup>rd</sup> instar larvae	47.32 ± 0.5382	1.8845 ± 0.1513	1.6875 ± 0.0447	1.7450 ± 0.1245	0.5017 ± 0.0360	0.3060 ± 0.0455	0.4892 ± 0.0358	0.3882 ± 0.0776	0.1353 ± 0.0424	0.3344 ± 0.0573
Pupae	114.50 ± 1.8058	1.3751 ± 0.0483	1.3750 ± 0.0368	1.3650 ± 0.0500	0.5242 ± 0.0145	0.4115 ± 0.0564	0.5217 ± 0.0140	0.2985 ± 0.0237	0.1621 ± 0.0483	0.2934 ± 0.0234

**Graph.3)** Show developmental trajectory on the right side as average biometric measurement data's are plotted against average time for three seasons ± SE. On the left are the graphs from ANOVA (1-way) data's averages of lengths, width and biomass.



## VI. Conclusion

The systematic analysis of dipteran colonies on carcass can be a valuable forensic tool in the determination of PMI (Keh, 1985). Many variables that generally influence the rate of development for insects are not usually incorporated into the insect development model, for estimating PMI. This is largely due to the lack of base line data of relevant to the non human model implied (Villet et al., 2010). However in the current scheme of models, which uses aging of blow fly larvae, are produced by extrapolation from analysis of the development rates of larvae, generally reared on single medium: liver (Levot et al., 1979; Byrd and Butler, 1996) or ground beef (Anderson, 2000; Grassberger et al., 2003) for human model, so for avian model *Gallus gallus* was utilized for the purpose of rearing and cultivating reference data for the highly thermophilic variety that is prevalent in India and its extrapolated data might be utilized for human model also.

Since the average length, width and calculated biomass, did not show any significant difference, therefore this current work may be used as reference data for *S. albiceps* as a forensic indicator, for future studies on length and width based PMI estimation. In this study, biomass of immature stages was calculated for the same purpose as length and width. More study are need to be undertaken. Perhaps in the future a similar study on different substrates might be conducted, to assess significant variation on different substrate on a spacio-temporal basis.

In conclusion, larvae of *Sarcophaga (Parasarcophaga) albiceps* seemed to target the head region as the site of entry in to the carcass. The present results suggest that *Sarcophaga (Parasarcophaga) albiceps* show no significant variances in length and width, therefore this can be utilized as a reference data for PMI estimations. The present result also suggests some limitation in the current scheme of forensic application of data that derive from a type of animal tissue, other than that on which the larvae has been feeding on. The other being that mostly till date blow fly larval length and width have been utilized for PMI estimations, current paper also includes pupa and the forensic indicator being flesh fly instead of blow fly, for generation of base line data. Also another parameter is added to the arsenal of forensic entomologist, the volume of larvae and pupae are taken into consideration as its a product of length and width, wich might be useful in PMI estimation and other allied disciplines.

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