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Abesh Chakraborty
Diptera Section, Zoological Survey of India, Ministry of Environment & Forests and Climate change (Government of India), M Block, New Alipore, Kolkata, India

Goutam Kr. Saha
Dept. of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata, India

Dhriti Banerjee
Diptera Section, Zoological Survey of India, Ministry of Environment & Forests and Climate change (Mo EF& CC, Government of India), M Block, New Alipore, Kolkata, India

Correspondence
Abesh Chakraborty
Diptera Section, Zoological Survey of India, Ministry of Environment & Forests and Climate change (Government of India), M Block, New Alipore, Kolkata, India

Developmental variation of two different variety of Indian blow flies: *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) on dead *Gallus gallus* (Linnaeus, 1758)

Abesh Chakraborty, Goutam Kr. Saha and Dhriti Banerjee

Abstract
The blow flies (Diptera: Calliphoridae) are among the most important flies for forensic dipterology. Thus, their immature stages are generally used for estimation of PMI and allied estimations. Therefore a study was conducted to investigate the effect of different seasonal variation on development in *Gallus gallus* Caracas, to analyze the seasonal variation of growth of two Indian blow flies for three seasons. Results for *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) showed that there was a significant variation in avg. length, and calculated biomass, width was insignificant among the three seasons of immature stages as [F (2, 33) = 3.440649166, p = 0.043932584], [F (2, 33) = 12.62242037, p = 0.00000849], and [F (2, 33) = 6.044256, p=0.0058] and as [F (2, 33) = 3.583334, p = 0.039057], [F (2, 33) = 5.825099, p= 0.006814] and [F (2, 33) = 1.094117, p = 0.346674] respectively.

Keywords: Diptera, calliphoridae, blow fly, developmental analysis, India

Introduction
Blowflies belonging to the family Calliphoridae are of considerable medical and veterinary importance (Lindsay and Scudder, 1956) [1]. The blow flies (Diptera: Calliphoridae) are among the most widely recognized as being among the first wave of faunal succession to arrive on human cadavers (Nuroteva, 1977; Smith, 1986) [2]. Therefore, their life cycles, developmental rate and succession are utilized for estimating PMI, time between death to discovery of corpse and possible corpse movement (Nuroteva, 1977; Anderson, 2000, Gomes *et al*., 2003; 2005) [4-8].
To determine time since death, considerations of the critical factors affecting the rate of decomposition are important. These factors include location of the body, temperature, general climate, time of year, insect activity, animal activity in the area, and the amount of rainfall (Nafte, 2000) [9]. Temperature and other abiotic factors are the most important variables affecting developmental rate (Myskowiak and Doums, 2002) [10].
The adults are frequently found around carcasses, excrements of man and animals and decaying organic matter of fruits and vegetables. The green bottle blow fly better known as the *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) the former is distributed from Madagascar, Mauritius, Reunion, Rodriguez, South Africa, India to Australia & Pacific islands and the later specimen is almost cosmopolitan in distribution (Pape & Thompson, 2016) [11].
The Calliphorids are both a beneficial and harmful, as Calliphorids cause humans and domesticated animals harm by pathogenic myiasis (cutaneous, intestinal, urethral and auricular); pathogenic disease transmission (James, 1947; Zumpt, 1965 and Greenberg, 1971) [12, 13, 14].
Calliphorids are useful to us as Pollinators of Mango (Sung, 2006) [15] and are used in treatment of necrotic ulcers by maggot debriment therapy (MDT) (Mumcuoglu *et al*. 1999; Martelet *et al*. 2009) [16, 17].
The most common contribution of forensic dipterology is the establishment of post mortem interval (PMI) by collecting entomological evidence collected at the scene and Rearing them to adulthood and acquiring lower threshold, to calculate the unknown portion of the PMI (Anderson 2000) [4, 5]. Therefore medium and long range PMI can be estimated, using the developmental, life cycle and ecological data, coupled with ADD calculation to find the lapse time, the principle can also be used in Time of Negligence (TON) studies of human and animal myiasis (Campobasso *et al*. 2001) [18].

The secondary objective of the study is to generate the base line data, viz to aid estimation of ADD/ADH from data collected in controlled settings, and then confirming them in field (Amendt *et al*. 2007) [19]. Therefore, understanding their life cycle and development pattern in different seasons is important.

Materials and Methods

Study Site

The study was conducted in ZSI, Kolkata, 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 of India (ZSI) (n=3) for three seasons, Kolkata premises, and the construct was kept in ambient outdoor conditions inside ZSI premises.

Trapping of cadaveric diptera

A modified version of the malaise trap was utilized to capture 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 some were killed with ether and preserved in 70% alcohol.

Collection of biotic data

The chicken carcass was placed on a raised platform to attract the blow flies, surrounded by water on all sides to discourage ants and 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, during 2013-2014. As the post feed maggots 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 various studies.

Rearing the flies

Immature stages of the blow flies, basically pupae were isolated and utilized for rearing the flies in SMS media, prepared by (w/v): (commercially available soy protein isolates 3.78 g, milk protein concentrates 1.2 g and silicon dioxide 0.0125 g/ 75 ml water). The media was poured in autoclaved culture vials and cooled in Laminar Air flow. [20]

Data analysis

The immature stages (larvae and pupa) and adults were measured to see the rate of development of *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) in the carcass of *Gallus gallus* (n=3), in all the seasons. The length, width and biomass of larvae (n = 3) were calculated from Leica EZ4 HD microscopic measuring 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 *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) were subjected to a one way ANOVA and compared (Sokal and Rohlf, 1981) [21]. Afterwards the biometric data was graphed with the aid of scatter plot (ROD curve) and regression was done according to a modified process of Chen et.al. (2011) [20]. Lastly ANOVA (1-way), followed by post hoc t-test Bonferroni corrected was done and the means and variance were plotted in error bars to see the average variation levels according to Miller (2001) [22].

Results and Discussions

The adult were inoculated in this same media, but they didn’t start reproducing immediately, mating was initiated around 24 ± 2 hours after inoculation of culture, ovipositing begun after 36 ± 2 hours after inoculation of the culture. In lab condition it’s found that if *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) were reared at following temperature, relative humidity and photoperiod for different seasons on *Gallus gallus* showed varied growth patterns and biomass accumulations. The length, breadth and calculated biomass of larvae all the three instars, pupa and eggs were measured using Leica EZ4 allied software. (Table.1)

Table 1: Seasonal variation of the abiotic factors

Seasons	Avg. Temperature (°C) ± SE (3)	Avg. Relative Humidity (%)	Avg. Photoperiod (Hrs)
Pre monsoon	37.96	35.5	14:10
Monsoon	31.24	55	11:13
Post monsoon	28.68	28	12:12

Developmental pattern analysis of *Chrysomya megacephala* (Fabricius, 1794)

The duration of all stages of larvae was also significantly different ($p<0.05$). However after the feeding phase was over, the maggots started to migrate for finding suitable pupation site inside the containers, most of them settled near the top of the container, after finding suitable pupation media. The length, width and biomass of *Chrysomya megacephala* (Fabricius, 1794) seemed to vary significantly during post monsoon season, when compared with the pre monsoon and monsoon. Therefore the length and width of *Chrysomya megacephala* (Fabricius, 1794) of the premonsoon and monsoon can be utilized for estimating PMI and in allied disciplines (Table. 2 and Fig.1, 2, 3).

Table 2: Shows the seasonal developmental variation of *Chrysomya megacephala* (Fabricius, 1794) here PRM: Pre monsoon, MON: Monsoon and PST: Post monsoon

Stages	Avg. Hours (Mean ± SE)			Cumulative Avg. hrs			Avg. Length (in mm) (Mean ± SE)			Avg. Width (in mm) (Mean ± SE)			Avg. Biomass (in mm³) (Mean ± SE)		
	PRM (Mean ± SE [25.01])	MON (Mean ± SE [25.02])	PST (Mean ± SE [25.57])	PRM	MON	PST	PRM (Mean ± SE [0.215])	MON (Mean ± SE[0.200])	PST (Mean ± SE[0.132])	PRM (Mean ± SE [0.089])	MON (Mean ± SE[0.08])	PST (Mean ± SE[0.037])	PRM (Mean ± SE [0.216])	MON (Mean ± SE [0.175])	PST (Mean ± SE [0.063])
Eggs	7.59	9.14	8.01	7.59	9.14	8.01	1.29	1.23	1.02	0.553	0.493	0.217	0.31	0.23	0.038
1st instar larvae	33.00	33.54	35.00	40.59	43.08	43.01	1.54	1.48	1.17	0.654	0.594	0.275	0.52	0.41	0.069
2nd instar larvae	40.48	41.28	43.12	81.07	84.36	86.13	2.07	2.01	1.37	0.867	0.807	0.383	1.22	1.03	0.160
3rd instar larvae	50.00	53.00	53.00	131.07	137.36	139.13	2.50	2.43	1.63	1.043	0.973	0.458	2.14	1.81	0.270
Pupae	122.00	124.24	128.16	253.07	262.00	267.29	2.10	2.03	1.46	0.883	0.813	0.360	1.36	1.05	0.590

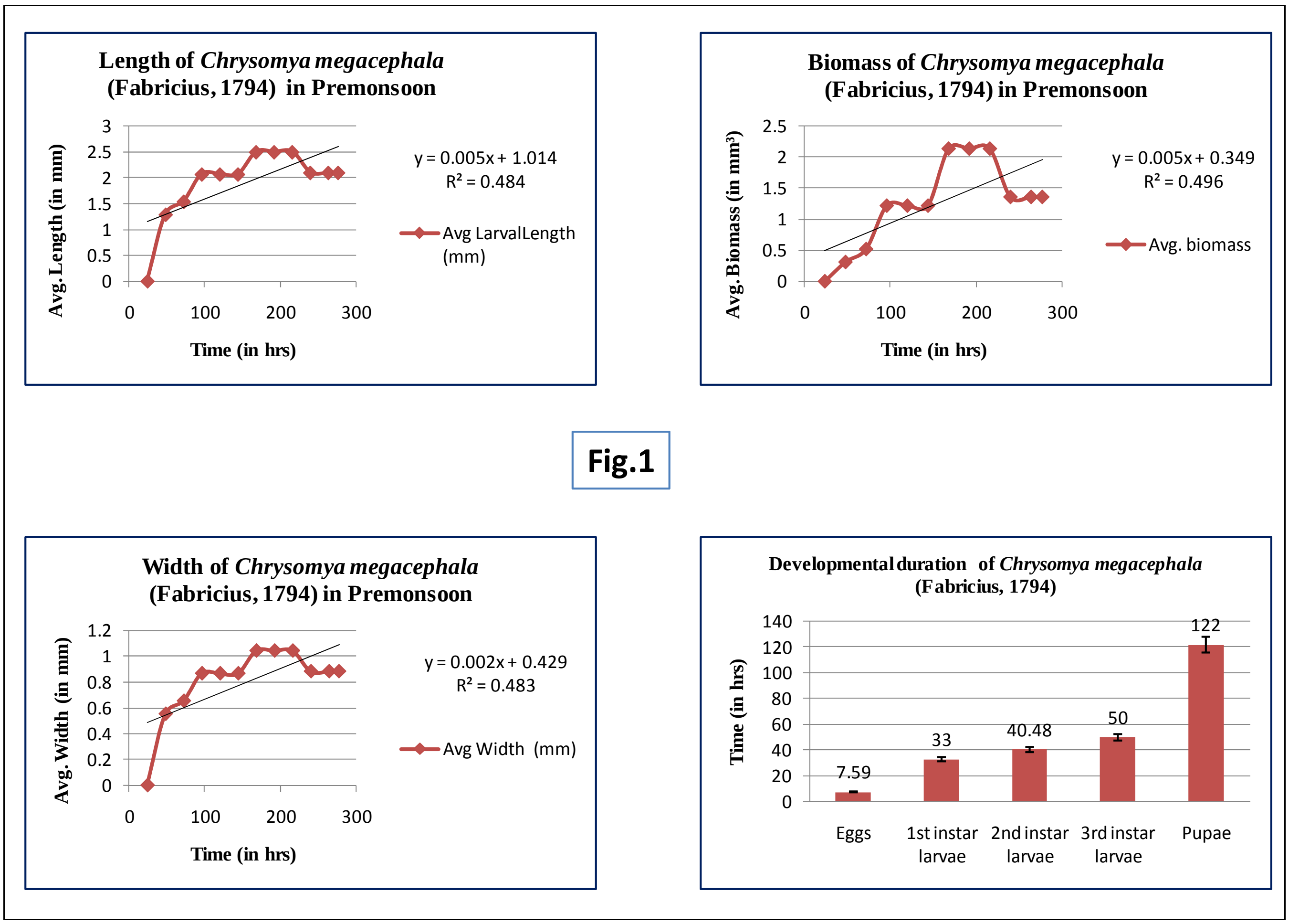
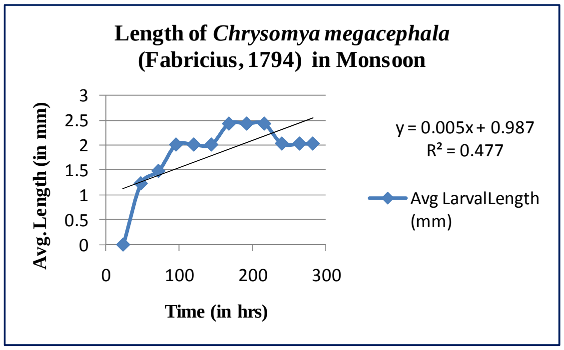
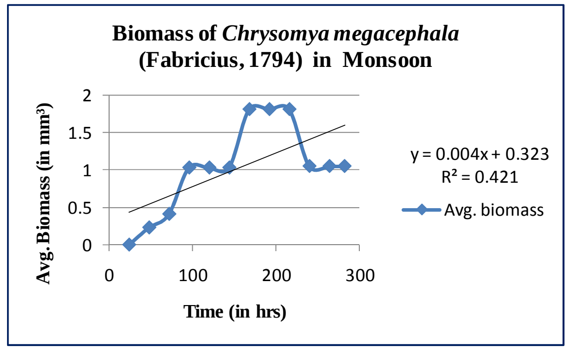
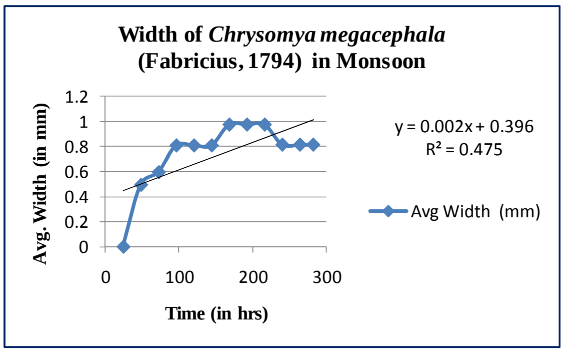


Fig.1


Length of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Length (in mm)
Time (in hrs)
y = 0.005x + 0.987
R² = 0.477
Avg LarvalLength (mm)


Biomass of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Biomass (in mm³)
Time (in hrs)
y = 0.004x + 0.323
R² = 0.421
Avg. biomass


Width of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Width (in mm)
Time (in hrs)
y = 0.002x + 0.396
R² = 0.475
Avg Width (mm)

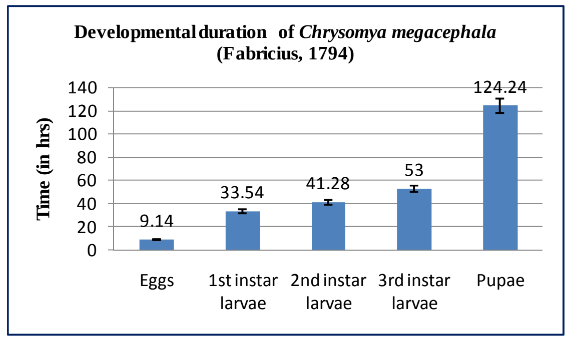
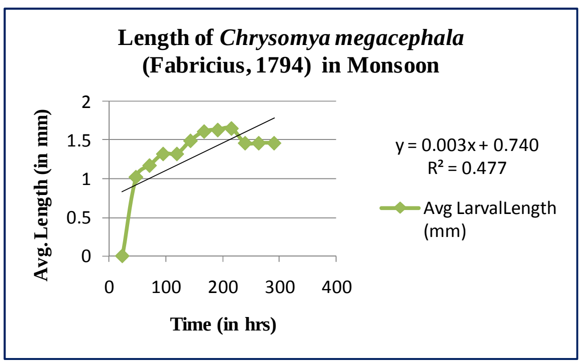
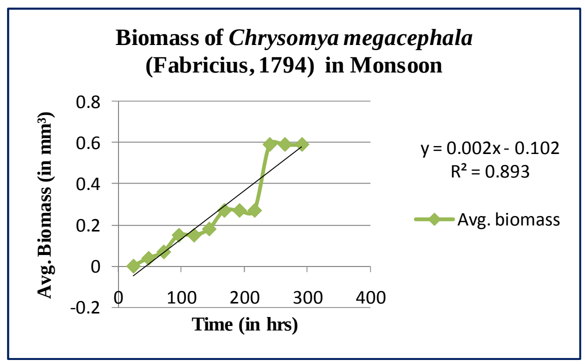
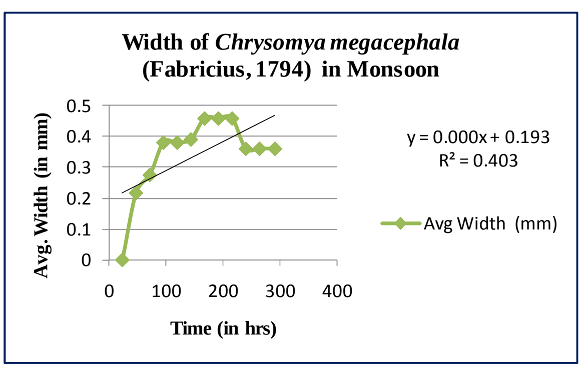

Developmental duration of Chrysomya megacephala (Fabricius, 1794)
Time (in hrs)
9.14
33.54
41.28
53
124.24
Eggs
1st instar larvae
2nd instar larvae
3rd instar larvae
Pupae

Fig.2


Length of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Length (in mm)
Time (in hrs)
y = 0.003x + 0.740
R² = 0.477
Avg LarvalLength (mm)


Biomass of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Biomass (in mm³)
Time (in hrs)
y = 0.002x - 0.102
R² = 0.893
Avg. biomass


Width of Chrysomya megacephala (Fabricius, 1794) in Monsoon
Avg. Width (in mm)
Time (in hrs)
y = 0.000x + 0.193
R² = 0.403
Avg Width (mm)

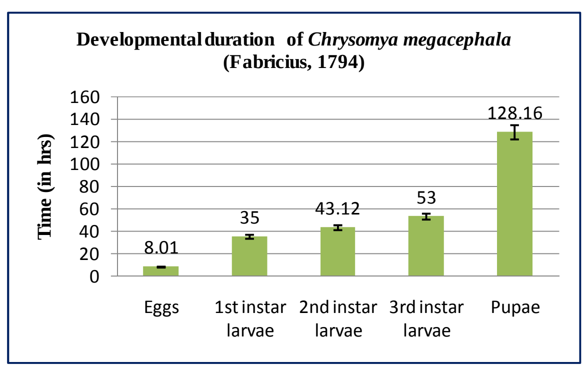

Developmental duration of Chrysomya megacephala (Fabricius, 1794)
Time (in hrs)
8.01
35
43.12
53
128.16
Eggs
1st instar larvae
2nd instar larvae
3rd instar larvae
Pupae

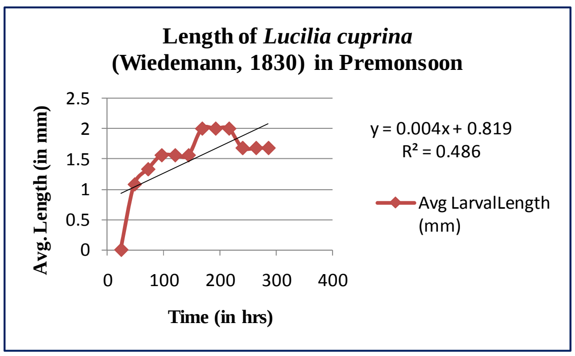
Fig.3

Developmental pattern analysis of *Lucilia cuprina* (Wiedemann, 1830)

The duration of all stages of larvae was also significantly different ($p<0.05$). However after the feeding phase was over, the maggots started to migrate for finding suitable pupation site inside the containers, most of them settled near the top of the container, after finding suitable pupation media. The length, width and biomass of *Lucilia cuprina* (Wiedemann, 1830) seemed to vary significantly during the pre monsoon and monsoon, when compared with post monsoon season. Therefore the length, width and biomass of *Lucilia cuprina* (Wiedemann, 1830) of the post monsoon season can be utilized for estimating PMI and in allied disciplines (Table. 3 and Fig. 4, 5, 6).

Table 3: Shows the seasonal developmental variation of *Lucilia cuprina* (Wiedemann, 1830), here PRM: Pre monsoon, MON: Monsoon and PST: Post monsoon.

Stages	Avg. Hours (Mean ± SE)			Cumulative Avg. hrs			Avg. Length (in mm) (Mean ± SE)			Avg. Width (in mm) (Mean ± SE)			Avg. Biomass (in mm³) (Mean ± SE)		
	PRM (Mean ± SE [25.36])	MON (Mean ± SE [25.45])	PST (Mean ± SE [25.49])	PRM	MON	PST	PRM (Mean ± SE [0.182])	MON (Mean ± SE[0.179])	PST (Mean ± SE[0.222])	PRM (Mean ± SE [0.074])	MON (Mean ± SE[0.069])	PST (Mean ± SE[0.075])	PRM (Mean ± SE [0.122])	MON (Mean ± SE [0.112])	PST (Mean ± SE [0.150])
Eggs	8.26	8.32	8.00	8.26	8.32	8.00	1.08	1.14	1.630	0.403	0.343	0.593	0.140	0.110	0.450
1st instar larvae	34.06	34.32	34.45	42.32	43.04	42.25	1.33	1.39	2.160	0.504	0.444	0.974	0.270	0.222	1.610
2nd instar larvae	43.32	42.24	42.40	86.24	85.28	85.25	1.56	1.72	2.430	0.717	0.657	0.907	0.630	0.580	1.570
3rd instar larvae	52.40	52.00	53.00	138.04	137.28	138.25	2.00	2.15	2.770	0.893	0.823	0.823	1.250	1.140	1.470
Pupae	125.16	127.12	127.20	263.20	264.40	265.45	1.68	1.95	2.270	0.733	0.663	0.753	0.710	0.670	1.010



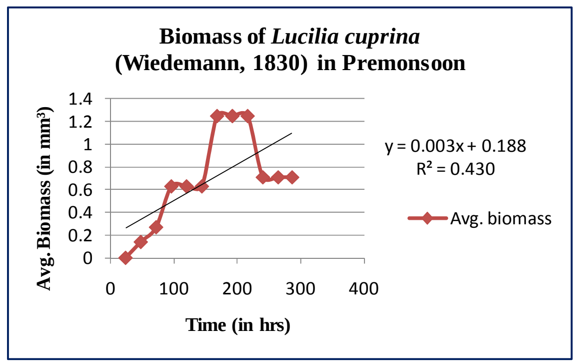
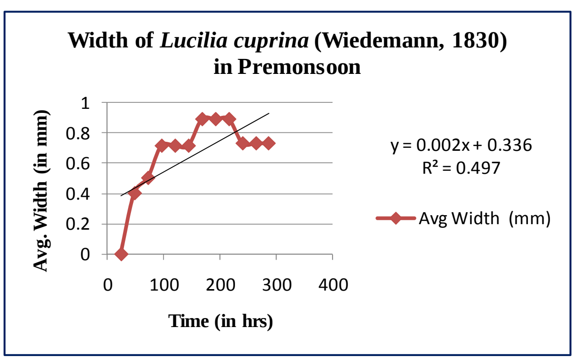
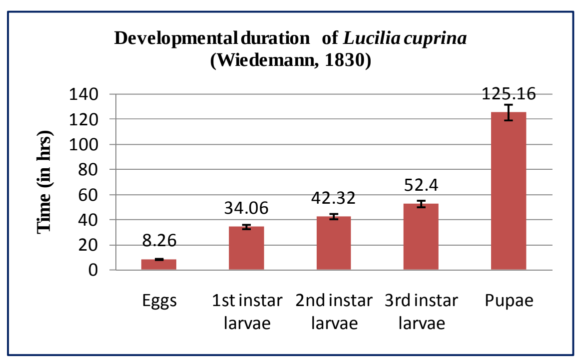
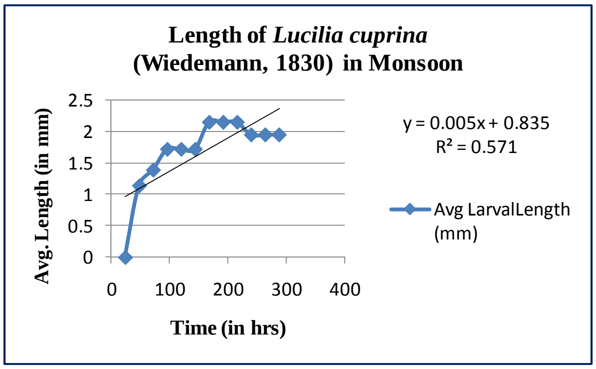


Fig.4






Length of *Lucilia cuprina* (Wiedemann, 1830) in Monsoon
Avg. Length (in mm)
Time (in hrs)
y = 0.005x + 0.835
R² = 0.571
Avg LarvalLength (mm)

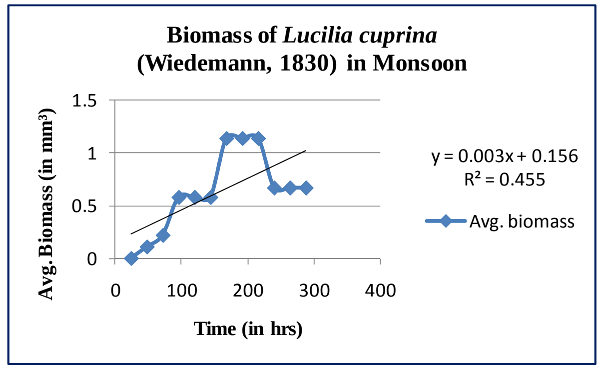
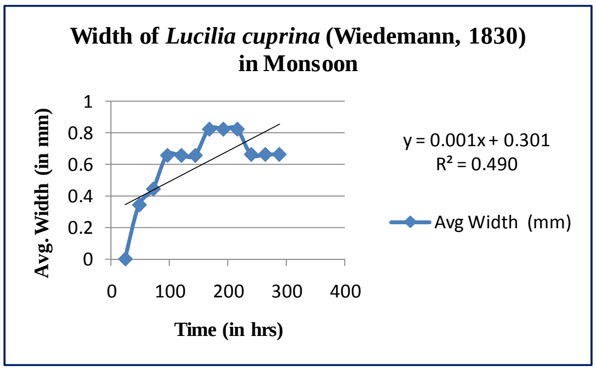
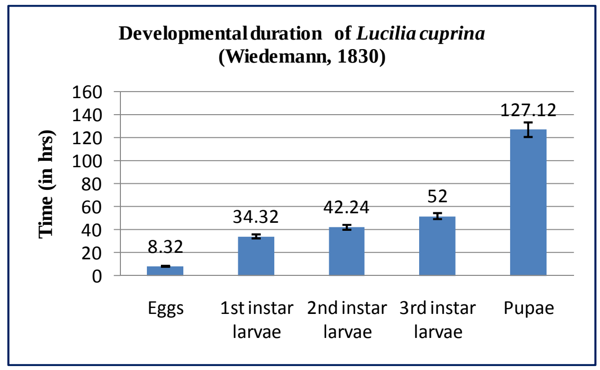
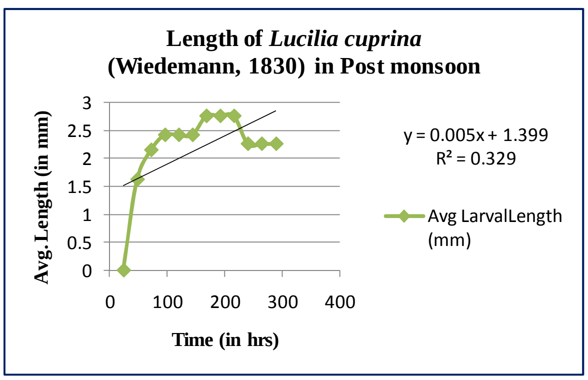

Biomass of *Lucilia cuprina* (Wiedemann, 1830) in Monsoon
Avg. Biomass (in mm³)
Time (in hrs)
y = 0.003x + 0.156
R² = 0.455
Avg. biomass

Fig.5


Width of *Lucilia cuprina* (Wiedemann, 1830) in Monsoon
Avg. Width (in mm)
Time (in hrs)
y = 0.001x + 0.301
R² = 0.490
Avg Width (mm)


Developmental duration of *Lucilia cuprina* (Wiedemann, 1830)
Time (in hrs)
8.32
34.32
42.24
52
127.12
Eggs
1st instar larvae
2nd instar larvae
3rd instar larvae
Pupae


Length of *Lucilia cuprina* (Wiedemann, 1830) in Post monsoon
Avg. Length (in mm)
Time (in hrs)
y = 0.005x + 1.399
R² = 0.329
Avg LarvalLength (mm)

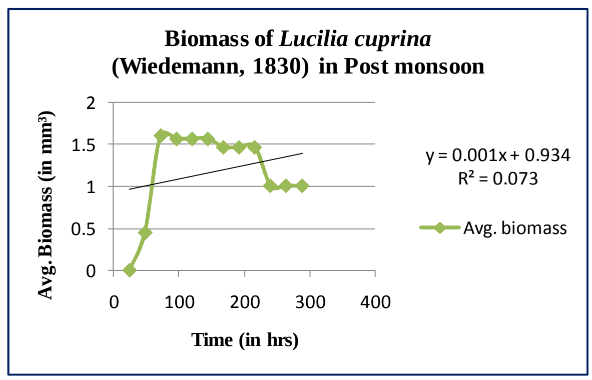
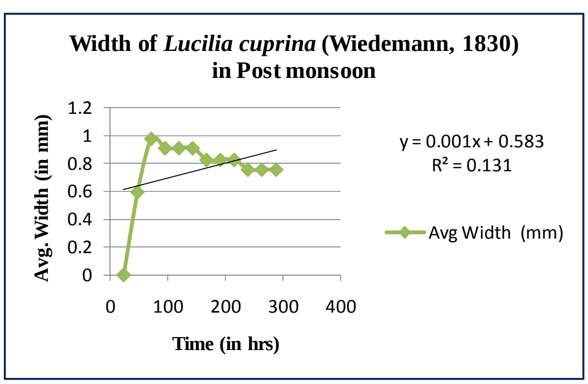
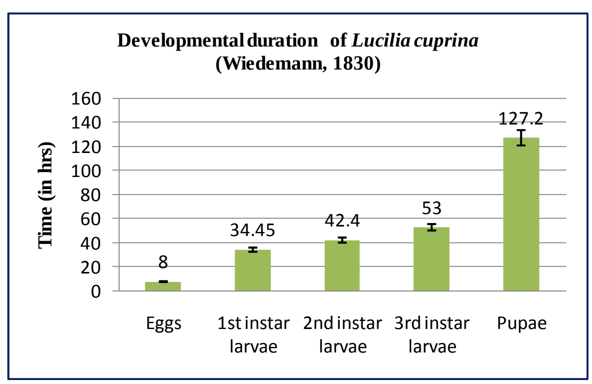

Biomass of *Lucilia cuprina* (Wiedemann, 1830) in Post monsoon
Avg. Biomass (in mm³)
Time (in hrs)
y = 0.001x + 0.934
R² = 0.073
Avg. biomass

Fig.6


Width of *Lucilia cuprina* (Wiedemann, 1830) in Post monsoon
Avg. Width (in mm)
Time (in hrs)
y = 0.001x + 0.583
R² = 0.131
Avg Width (mm)


Developmental duration of *Lucilia cuprina* (Wiedemann, 1830)
Time (in hrs)
8
34.45
42.4
53
127.2
Eggs
1st instar larvae
2nd instar larvae
3rd instar larvae
Pupae

Growth Rate analysis

ANOVA (1-way) was done between the three season's larval length (in mm), width (in mm) and biomass (in mm³), to compare the effect of different seasonal variation in the growth patterns and trajectories of *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830).

Chrysomya megacephala (Fabricius, 1794)

Length

There was a mildly significant effect in the amount of variation in Length (in mm) of the three seasons, level for the three different seasons [F (2, 33) = 3.440649166, p = 0.043932584].

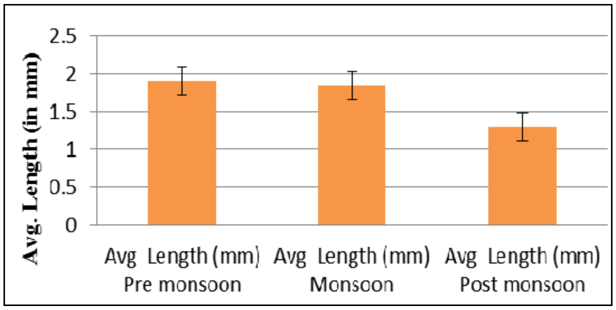


Fig 7: Avg. seasonal variation of length ± SE of immature stages *Chrysomya megacephala* (Fabricius, 1794)

Width

There was a highly significant effect in the amount of variation in width (in mm) of the three seasons, level for the three different seasons [F (2, 33) = 12.62242037, p = 0.00000849].

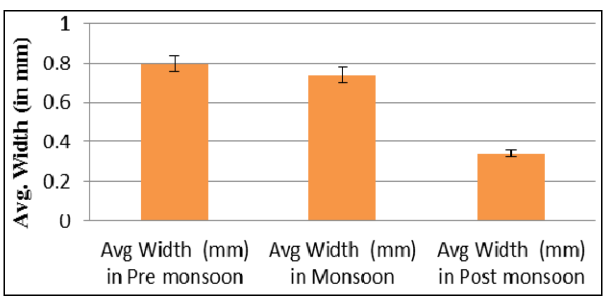


Fig 8: Avg. seasonal variation of width ± SE of immature stages of *Chrysomya megacephala* (Fabricius, 1794)

Biomass

There was a highly significant effect in the amount of variation in width (in mm³) of the three seasons, level for the three different seasons [F (2, 33) = 6.044256, p=0.0058].

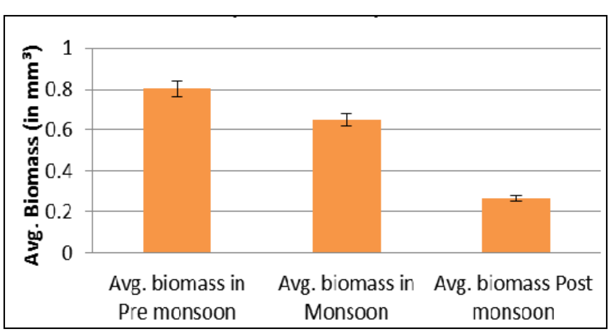


Fig 9: Avg. seasonal variation of biomass ± SE of immature stages of Chrysomya megacephala (Fabricius, 1794)

Lucilia cuprina (Wiedemann, 1830)

Length

There was a mildly significant effect in the amount of variation in Length (in mm) of the three seasons, level for the three different seasons [F (2, 33) = 3.583334, p = 0.039057].

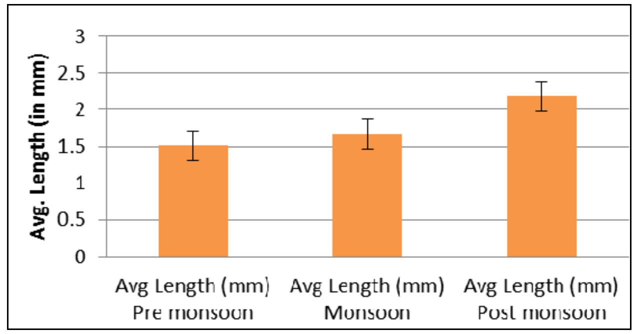


Fig 10: Avg. seasonal variation of length ± SE of immature stages Lucilia cuprina (Wiedemann, 1830)

Width

There was insignificant effect in the amount of variation in width (in mm) of the three seasons, level for the three different seasons [F (2, 33) = 1.094117, p = 0.346674]. (Fig-11)

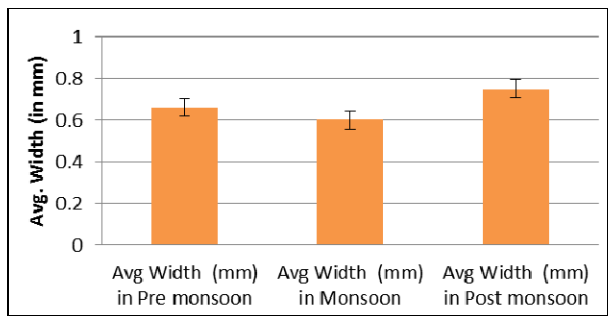


Fig 11: Avg. seasonal variation of length ± SE of immature stages of Lucilia cuprina (Wiedemann, 1830)

Biomass

There was significant effect in the amount of variation in biomass (in mm) of the three seasons, level for the three different seasons [F (2, 33) = 5.825099, p= 0.006814].

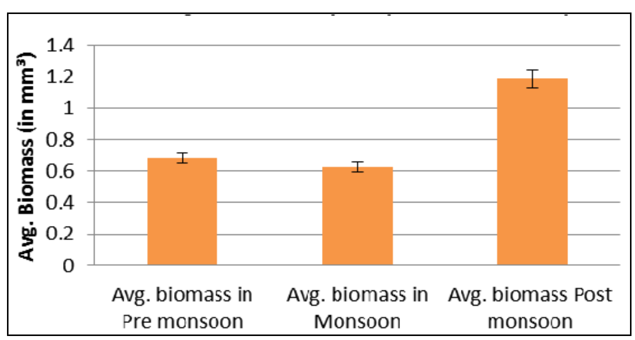


Fig 12: Avg. seasonal variation of length ± SE of immature stages of Lucilia cuprina (Wiedemann, 1830)

Statistical analysis

The present results were statistically significant for all pre monsoon and monsoon seasons length, width and biomass for *Chrysomya megacephala* (Fabricius, 1794) and post monsoon length and biomass *Lucilia cuprina* (Wiedemann, 1830), therefore a post hoc test computed. For this purpose we selected the T-test post hoc: two samples assuming equal variances and the significance levels were Bonferroni corrected. This test is designed to compare each of our immature stages biometric data on same rearing media but on different seasons and each other. This test is generally designed to compare the pre monsoon, monsoon and post monsoon in *Gallus gallus* carcass. The biometric data of the immature stages, were run in a three way analysis, viz. for *Chrysomya megacephala* (Fabricius, 1794) Length, width, biomass (pre monsoon and monsoon, monsoon and post monsoon, pre monsoon and post monsoon) and *Lucilia cuprina* (Wiedemann, 1830) Length and biomass (pre monsoon and monsoon, monsoon and post monsoon, pre monsoon and post monsoon) respectively. ANOVA (1- way) was conducted, as results were significant post hoc T-test two tail was conducted and Bonferroni corrected.

Length of *Chrysomya megacephala* (Fabricius, 1794)

The pre monsoon and monsoon data showed, [p (T<=t) = 0.416841738, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was no significant difference between the data sets. The pre monsoon and post monsoon data showed, [*p* (T<=t) = 0.009880947, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. The monsoon and post monsoon data showed, [p (T<=t) = 0.015469323, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was mildly significant difference between the data sets. For the purpose of PMI estimations pre monsoon and monsoon data seems to be better, therefore this data can be utilized for PMI estimations (Fig.7).

Width of *Chrysomya megacephala* (Fabricius, 1794)

The pre monsoon and monsoon data showed, [p (T<=t) = 0.304048471, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was no significant difference between the data sets. The pre monsoon and post monsoon data showed, [p (T<=t) = 0.00000281, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. The monsoon and post monsoon data showed, [p (T<=t) = 0.0000078, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was mildly significant difference between the data sets. For the purpose of PMI estimations pre monsoon and monsoon data seems to be better, therefore this data might be utilized for PMI estimations (Fig.8).

Biomass of *Chrysomya megacephala* (Fabricius, 1794)

The pre monsoon and monsoon data showed, [p (T<=t) = 0.206194473, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was no significant difference between the data sets. The pre monsoon and post monsoon data showed, [p (T<=t) = 0.001091552, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. The monsoon and post monsoon data showed, [p (T<=t) = 0.0044678, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. For the purpose of PMI estimations pre monsoon and monsoon data seems to be better, therefore this data might be utilized for PMI estimations (Fig. 9).

Length of *Lucilia cuprina* (Wiedemann, 1830)

The pre monsoon and monsoon data showed, [p (T<=t) = 0.259655805, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was no significant difference between the data sets. The pre monsoon and post monsoon data showed, [p (T<=t) = 0.010392497, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. The monsoon and post monsoon data showed, [p (T<=t) = 0.039326848, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was mildly significant difference between the data sets. For the purpose of PMI estimations pre monsoon and monsoon data seems to be better, therefore this data might be utilized for PMI estimations (Fig.10).

Biomass of *Lucilia cuprina* (Wiedemann, 1830)

The pre monsoon and monsoon data showed, [p (T<=t) = 0.366207, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was no significant difference between the data sets. The pre monsoon and post monsoon data showed, [p (T<=t) = 0.007616, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. The monsoon and post monsoon data showed, [*p* (T<=t) = 0.003167, (α = 0.05/3 = 0.0167)], Bonferroni correction was done for post hoc t test which showed that there was significant difference between the data sets. For the purpose of PMI estimations pre monsoon and monsoon data seems to be better, therefore this data might be utilized for PMI estimations (Fig-12)

Conclusion

The developmental pattern analysis (p<0.05), showed that *Chrysomya megacephala* (Fabricius, 1794), have better growth in warm and moist weather and *Lucilia cuprina* (Wiedemann, 1830), to have better growth in cold weather.

Therefore after rearing of both *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830), through the *Gallus gallus* carrion for three seasons, running the data through various tests it's found that *Chrysomya megacephala* (Fabricius, 1794), growth rate analysis (p<0.05), is more suited for warm and moist climate for better growth and it was found that length, width and biomass all can be utilized, but length might be the better option for estimating PMI and TON, biomass might be utilized for effective study in pre monsoon and monsoon for better results. *Lucilia cuprina* (Wiedemann, 1830), growth rate analysis revealed that it is suited for a more dry and cold climate for better growth and length might yield better results for PMI and TON, biomass might be utilized for effect study in post monsoon for better results.

The analysis of the biometric data revealed that the immature stages varied except for width of *Chrysomya megacephala* (Fabricius, 1794), so further analysis was done and Bonferroni correction (α < 0.0167) was made of the above data following post hoc T-test assuming equal variances, results shows that pre monsoon and monsoon can be utilized for PMI and allied disciplines and length to be the best tool for PMI estimation if immature development model is utilized, for *Lucilia cuprina* (Wiedemann, 1830), results shows that post monsoon to be the better time for their utilization and length and width might be utilized for best results.

The development shows that the two species have partitioned the cadaveric resources season wise, which is reflected in their development. The current developmental data might also be utilized for climatic studies; as decreases of duration of life cycle stages and overall duration with increase in temperature; this data suggest that *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830) are temperature sensitive and therefore their developmental data can be used not only in PMI or allied studies but also, to monitor climatic changes namely temperature. The current data shows that *Chrysomya megacephala* (Fabricius, 1794) is better in pre monsoon and monsoon and *Lucilia cuprina* (Wiedemann, 1830) is better in post monsoon season as per their developmental trajectory shows, and these species can be utilized as forensic indicators. Though further work is needed for their establishment as climatic indicators.

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