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Developmental biology and assessment of post-harvest preference of *Chrysomya megacephala* (Diptera: Calliphoridae) on fish

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Abstract
The ecologically important blow fly, *C. megacephala* (F.) (Diptera: Calliphoridae) which have great importance in forensic, veterinary and medical sciences was attempted to multiply on fish, Indian Mackerel (*Rastrelliger kanagurta*) in the laboratory at 29 ± 2˚C, 60 ± 5% RH. The aim of the study was to understand their biology on fish and evaluate their attraction preference towards post-harvest stages of fish. The flies were multiplied for 5 generations and the biological parameters were; hatching time 0.754 to 0.756 days, larval period 5.1 to 5.5 days, percentage pupation 91-94%, pupal period 5.04 days to 5.9 days, percent adult emergence 89.6 to 92.3%, sex ratio 1:1, male longevity of 24.9 to 26.4 days and female longevity 26.1 to 27.4 days. These parameters were at par with those reared on usual rearing medium beef, while the total developmental time was 11.1 to 11.8 days which was higher than 10.9 days on beef. The results indicated that fish is a suitable medium to rear the flies and development occur at a faster rate. The preference studies indicated that after harvest, the flies prefer 1-2day old fish and underlines the right time to take adequate precautions to prevent post-harvest loss by blow flies.

Keywords: Developmental parameters, developmental time, post-harvest, oviposition, preference assessment

Introduction
Chrysomya megacephala (F.) (Diptera: Calliphoridae), the Oriental latrine fly is a significant species among blow flies with great veterinary, medical, forensic, sanitary and agricultural importance. The species is indigenous to the Oriental and Australian region and are presently distributed world-wide. It is very well habituated to human activities and occurs in rural, urban environments playing a diversified role in nature. The flies multiply on carrions, faeces and other discarded organic materials. Blow flies in general can survive in different habitat and have greater ecological importance as scavengers in decomposition, breaking down vertebrate carcasses and enable nutrient cycling and sanitation. Among the blow flies which colonise on a cadaver within few hours after death, *C. megacephala* is one of the dominant species [28] and becomes forensically important by enabling the determination of post-mortem intervals (PMI) in criminal investigations [14]. Since these flies are closely associated with filthy things in the environment, they have considerable medical importance as they are carriers or vectors of many pathogens and diseases [15]. From the modern agriculture point of view, *C. megacephala* is one of the important pollinator in fruit and vegetable orchards [22]. Recently it has been found that *C. megacephala* is serving as a potential sustainable resource for animal protein, lipids, bio medically important chitosan and biofuel [32].
However, despite all these *C. megacephala* is identified as a serious pest of fish in India [31] and the post-harvest loss in fishing industry by blow flies account up to 40% [1]. It is well known that blow flies are attracted to odoriferous dead animal matter resources for feeding and/or egg laying. This diversified association of *C. megacephala* with environment and humans, necessitates knowledge related to the developmental biology for their effective management. Besides that, information on developmental times of immature stages on alternate substrates will contribute to effective laboratory culture of fly population for research purpose, field augmentations and precise forensic applications. The present study aims to explore their biology on fish and preference towards post- harvest stages of fish. for further mapping of key volatiles responsible for such attractions. The information will be useful in designing a semiochemical based management system for their effective control as pests.

Materials and Methods

C. megacephala flies used in the present study were established in the animal house of Indian Council of Agricultural Research-Central Institute of Fisheries Technology, Cochin (ICAR-CIFT). Initially a few adults were collected from residential complex of CIFT located at Thevara, Cochin (9.9426° N, 76.2986° E) on a fish offal trap. The adults were brought to the animal house of the institute and maintained at 29 ± 2°C, 60 ± 5% R.H and photoperiod of 12:12 (L: D) h. The adults were reared for two generations, identified by an expert and the next generation onwards have designated as F1 generation in the study. The fish used as rearing medium was Indian mackerel (*Rastrelliger kanagurta*), which was procured from local fish markets of Cochin. The adult flies were reared in wooden cages (45 × 45 × 45 cm) with wood sheet base, glass top, three sides covered with cloth of which two sides were having circular whole fitted with sleeves to facilitate serving of the food and cleaning. The front door of the cage was made of acrylic sheet. Adults were provided with fish, sucrose crystals, and water soaked absorbent cotton in petriplates. Feeding was provided *adlibitum.*

The egg laid fish pieces were transferred to 1Kg capacity pearl pet containers in which a thin layer of autoclaved saw dust was added. The containers were covered with muslin, secured with rubber bands were used as larval rearing units. Larvae were transferred to fresh container with fish, as the fish was consumed. Pre-pupal stages were transferred to ventilated breadboxes in which autoclaved saw dust was partially filled to facilitate pupation. Pupae were sieved off using a metal sieve (Filterwell-2400 Microns-Mesh size 2.36 mm) and kept in adult rearing cages for emergence.

Hatching time and developmental parameters

Adults from the generation after two successive laboratory rearing on fish was selected and progeny were designated as F1 generation of the study. The developmental parameter assessment was carried out at 29 ± 2°C, 60 ± 5% R.H and photoperiod of 12:12 (L: D) h. Eggs laid on fish meat were collected using a fine brush (Camlin 6s-66) and about 40 eggs were transferred to a Borosil petriplate (9.5 cm diameter) in which a little slurry of fish blood and meat was spread. The hatching of first 25 eggs (each in 4 replicates) was monitored at 30 m. intervals, and repeated three times in each generation. After hatching 25 larvae each were transferred to 500 g capacity Pearlpet containers with little saw dust and cut pieces of fish. Other variables of the study *viz.*, larval period, percentage pupation, pupal period, percentage of adults emerged, sex ratio and longevity of both sexes were recorded for five successive generations on fish and a single generation was studied on beef meat as a control. The experiment had four replicates each time and repeated thrice for each generation and control.

Preference assessment studies

The fish samples of different post-harvest stages were prepared by keeping the fish pieces in cleaned 500 ml. Borosil beakers, secured with the muslin cloth and stored at 27 ± 2°C. To prevent the attack of other flies, the beakers were kept protected with fine meshed fruit bags available in the local market. The samples were designated as raw fish fresh (RFF), raw fish 1 day old (RF 1D), raw fish 2 day old (RF 2D), raw fish 3day old (RF 3D) and raw fish 4day old (RF 4D).

Oviposition preference studies were conducted in rearing cages. The preference study was conducted as a multiple choice test. About 30 g of the fresh and different day old fish samples were placed in Borosil petriplates (10×10 cm) and these were randomly placed in the cages each time to avoid position bias.8-10 day old gravid females (20 Nos.) and males (10 Nos.) of *C. megacephala* were released in each cage and allowed to freely oviposit on their choice of different post-harvest stages of fish meat for 6 hrs. The plates were removed and weight of egg deposited on each sample in petriplate was weighed using a digital electronics balance (Sartorius BP 211D). The experiment was conducted in triplicates and repeated 3 times.

Statistical analysis

The data analysis was performed in IBM SPSS Statistic version 20. One-way ANOVA at 5% level of significance was performed to compare the means of various developmental parameters and multiple choice test of oviposition. Tukey's multiple comparison tests were used for *post-hoc* analysis. The results are expressed as mean ± standard deviation.

Results

Results of the present study indicated a significant difference in certain developmental parameters. The mean hatching time of eggs for F1 to F5 generation which ranged from 0.754 ± 0.001 to 0.756 ± 0.000 days and it did not differ significantly from control (0.754 ± 0.000) ($p>0.05$) (Table1). The larval period of F1 to F3 generation showed a significant increase from 5.304 ± 0.007 to 5.547 ± 0.022 days as compared to control (5.108 ± 0.022 days) ($p<0.05$). However, the same in F4 and F5 generations were homogenous to control ($p>0.05$). The percentage pupation was 91±1 to 94 ± 2% in F1 to F5 generations and it did not differ significantly from control ($p>0.05$) (Table 1). The mean pupal period differed significantly between F3, F4 and F5 generations which were 5.097 ± 0.021 days to 5.911 ± 0.019 days respectively ($p<0.05$) (Table1). The mean % of adult emergence of F1 to F5 did not differ significantly from control ($p>0.05$).

Total developmental time or the generation time, from F1 to F5 (11.10 ± 0.019 to 11.80 ± 0.016 days) was showing an increase in each generation, which was significantly higher than control ($p<0.05$) (Table 1). The ratio of the male progenies in each generation did not differ significantly within, and with control ($p>0.05$). However, the female ratio was significantly different in F4 and F5 generation (1.1 ± 0.025) ($p<0.05$) when compared to F1 to F3 and control ($p>0.05$). In all the generations, male female maintained a 1: 1 sex ratio (Table1). The longevity of males obtained in F1 generation was (26.471 ± 0.447) and was longer and significantly different from that of control (25.038 ± 0.117) ($p<0.05$) while F2 to F5 generation was homogenous (Table 1). There was no significant difference in the longevity of females in F1 to F5 generations and in control ($p>0.05$) (Table 1).

In the multiple choice oviposition preference study of *C. megacephala* conducted with different post-harvest stages of fish indicated that the preference within the choice was most significantly higher in 1 day old raw fish (RF 1D) in terms of the mean weight of eggs deposited which was 188.62 ± 11.83 mg. ($p<0.05$) (Fig.1).The weight of eggs on two-day old raw fish (RF 2D) also was significantly different with 163.87 ± 12.5 mg ($p<0.05$) but was homogenous with fresh raw fish (RF F) 159.71 ± 7.87 ($p>0.05$). RF 4D and RF 3D were the least preferred choices in terms of the mean weight of eggs

laid on (135.85 ± 7.98 and 112.49 ± 5.25 respectively) which were homogenous with RF F and was not statistically significant. (p> 0.05).

Discussion

Developmental parameters

Most of the blow fly species belonging to Calliphoridae are anautogenous and females require a quantity of protein intake towards maturation of ovaries [13], apart from serving as a general diet ingredient. In nature blow flies multiply on carcasses, human excrement, garbage etc. which are sources of ephemeral natural proteins. The most common natural protein diet used in laboratory multiplication of *C. megacephala* is beef [10]. Though fish is found to be an attractive medium for blow fly larvae, detailed biology on it has not been studied except a few parameters from studies by Esser [9]. In the present study, the fish which used as rearing medium was excellent source of protein which account up-to19.2% to 20.5% [16]. The obtained results which were all more or less at par with control which indicating that fish is a complete proteinacious medium that can be used as a natural alternate resource to multiply *C. megacephala.* Further, insect development is dependent on environmental temperature and at warmer temperature with high humidity conditions faster development is reported [4] while in vice versa conditions rate of development is low.

The hatching time of eggs in the present study did not differ significantly in the five generations and in control. The mean hatching time was 0.754 to 0.756 days respectively in treatment generations and 0.754 days in control. Siddiki and Zambare [24] reported a hatching time of 18:08 hrs. (0.753 days) on beef liver when reared at 32.5˚C and 21.5% relative humidity (RH). Esser [9] reported a hatching time of 6-15 hrs. (0.25-0.62 days) on salted fish at ambient temperature while Reddy *et al.,* [20] reported an incubation period of 0.48 days on fish meat at 27-29 ˚C with a RH of 60-70%. Studies with different temperature range show variation in the incubation period of *C. megacephala* eggs and such variation might be due to the effect of temperature as reported by Abd Algalil and Zambare [2].

The mean larval period observed in F1 to F3 generations were significantly higher from control while in F4 and F5 generations which was lower. The mean larval period of F1 to F3 generations was 5.30 to 5.53 days and in F4 and F5 generation which was 5.14 days with control having 5.1 days (Table 1). This difference in larval period might be due to the sensitiveness of the larvae to variations in environmental conditions as attributed by Wells and Kurahashi [33]. However, the obtained range of larval period was in accordance with many reports on different natural protein sources at varied temperature and RH. Gabre *et al.,* [10] in their life table studies of *C. megacephala* reported 5.4 days as the mean larval period on beef while Esser [9] reported a larval period of 4-5 days on salt cured dried fish under ambient environmental conditions. Similarly, a larval period of 5.5 days was reported by Reddy *et al.,* [20] on fish meat at 27-29 ˚C and 60-70% RH. The percentage of pupation from F1 to F5 was decreasing from 94-91% but was not significantly different from control and was much higher from the reported result of Gabre *et al.*, [10] which was 85% when reared on beef meet.

The pupal period showed an increasing trend from F1 to F5 generations. The mean values were 5.04, 5.09 and 5.12 days for F1to F3 generations and 5.10 days for control. The most significant increase was observed in F4 and F5 generations from control which was 5.91and 5.87 days respectively. The observed variation may be because of the sensitivity of developing stages to environmental fluctuations as reported by Wells and Kurahashi [33], Bharti *et al.,* [7]. Many studies are there in accordance with obtained result. Rabelo *et al.,* [19] reported a pupal period of 120.0 ± 18.5 hrs. (5 days) when reared on sardine and Esser [9] also observed a pupal period of 4-6 days on salt cured dried fish. Pupal period 5.08 days on beef liver was reported by Abd Algalil and Zambare [2] and there are reports available with lower pupal periods ranging from 3.8 days to 4.7 days [6; 29]. The percentage adult emergence within five generations did not differ significantly which was 89.6 to 92.3% and 92.3% in control. This is higher when compared to the mean adult emergence reported by Gabre *et al.,* [10] on beef which was 85%. Reigada and Godoy [21] reported that, temperature and larval density influences the survival rates of adults and the observed variations would be due to the same effect as experimental groups were with lower densities.

The total time taken for development from egg to adult, the total developmental time or mean generation time was the most influenced and significantly different variable among all under the study. This was showing an increasing trend with 11.10 days to11.80days within the five generations when compared with duration in control, 10.96 days which was significantly higher. Abd Algalil and Zambare [2] reported 11.04 days of total development time for *C. megacephala* at 29 ˚C when reared on beef liver. Gabre *et al.,* [10] reported 11.7 days for total development. Rabelo *et al.,* [19] reported 232 ± 40.0 hrs. (9.66 days) of total developmental time on sardine at 26 ± 2˚C and 75 ± 5% RH. Variations in total developmental time of *C. megacephala* was reported by many researchers in their studies [7, 9] on various protein substrates at different temperature and RH conditions. According to Hu *et al.,* [12] temperature significantly affect the developmental time and size traits in *C. megacephala* and with the increase of which developmental time reduces. Abd Algalil and Zambare [2] and Bansode *et al.,* [6] with their findings support the same effect. The photoperiod also was found to be influential in blow fly development according to Myskowiak and Doums [17]. Hence the accidental fluctuations in temperature and photoperiod that might have occurred during the course of development would have been influential in the observed variations.

Table 1: Developmental parameters of *C. megacephala* on fish

Generation	Developmental parameters									
	Hatching time (Days)	**Larval Period (Days)**	**Pupation (%)**	**Pupal Period (Days)**	**Adult Emergence (%)**	**Generation Time (Days)**	**Male Ratio (%)**	**Female Ratio (%)**	**Longevity Male (Days)**	**Longevity Female (Days)**
F1	0.756 ± 0.000a	5.304 ± 0.007a	94 ± 2a	5.043 ± 0.017a	92.333 ± 1.527a	11.103 ± 0.019a	1.075 ± 0.043a	1.216 ± 0.014a	26.471 ± 0.447a	27.429 ± 0.606a
F2	0.756 ± 0.001a	5.547± 0.022b	92 ± 1a	5.097 ± 0.021b	91 ± 1a	11.400 ± 0.004b	1.083 ± 0.0726a	1.166 ± 0.014a	26.083 ± 0.614ab	26.914 ± 0.738a

F3	0.756 ± 0.001a	5.538 ± 0.014b	92.333 ± 2.516a	5.128 ± 0.025b	90.666 ± 1.527a	11.423 ± 0.029b	1.108 ± 0.0286a	1.158 ± 0.038ab	25.311 ± 0.506ab	26.801 ± 0.333a
F4	0.754 ± 0.001a	5.140 ± 0.034c	92.333 ± 2.516a	5.911 ± 0.019c	90 ± 2a	11.807 ± 0.016c	1.141± 0.076a	1.1 ± 0.025b	25.339 ± 0.448ab	26.170 ± 0.684a
F5	0.755± 0.002a	5.143 ± 0.018c	91 ± 1a	5.878 ± 0.028c	89.666 ± 0.577a	11.777 ± 0.042c	1.133 ± 0.014a	1.1 ± 0.025b	24.958 ± 0.662b	27.077 ± 0.150a
Control	0.754 ± 0.000a	5.108 ± 0.022c	95 ± 1a	5.101 ± 0.023ab	92.333 ± 1.154a	10.964 ± 0.024d	1.116 ± 0.014a	1.2± 0.000a	25.038 ± 0.117b	27.121 ± 0.178a

*Mean values in columns with different letters are statistically significant (p<0.05)

The male ratio did not differ significantly in generations and with control and was in 1:1 ratio with females in all generations (Table 1). Blow flies in general exhibit 50: 50 male female ratio and the obtained result is in accordance with that. Esser [9] reported 1:1 ratio when reared on unsalted fresh cod, Gabre *et al.,* [10] on beef, and Rabelo *et al.,* [19] on sardine based diet also reported similar sex ratio trend. The female ratio within generations however, showed a significant reduction in F4 and F5 generations (Table1) which was 1.1 ± 0.025 compared to 1.2 ± 0.00 of control and 1.15 to 1.21of other generations. There are no reports available on the reduction in female progenies in biological studies of blow flies. However, this may be due continuous breeding in laboratory where the no. of female progenies in insect populations tends to deteriorate as reported by Ballal *et al.,* [5]. The longevity parameter of males and females deferred significantly with females having longer day's life span. The male longevity obtained in generations were 24.95 to 26.47 days which were not statistically different from control (25.03 days). Among the generations, F1 generation's males had little longer longevity of 26.47 days where as in females, the longevity did not differ significantly in generations and with control as well. The maximum longevity of females was recorded in F1 generation with 27.42 days and the rest had a longevity range of 26.80 to 27.07 days. The observed longevity of male and female is very well in accordance with the report of Gabre *et al.,* [10]. Rabelo *et al.,* [19] reported 37 ± 11 day's longevity for both males and females when reared on sardine based diet and there are even reports with longer longevity [9, 11]. Temperature is the crucial parameter that influence longevity, and variation can also be attributed to the kind of diet and its biochemical compositions as variations in lipid percentage is crucial in determining the mortality rate of blow flies, according to Ujvari *et al.,* [27].

Assessment of preference on post-harvest stages of fish

The multiple choice test conducted with different post-harvest stages of fish and subsequent data analysis revealed that there is significant difference in the preference of *C. megacephala* among different post-harvest stages of fish in terms of oviposition.1dayold fish (RF 1D) was the most preferred choice, followed by 2day old fish (RF 2D). The mean weight of eggs laid on raw fresh fish and raw fish of 3 and 4day old was lower compared to other choices (Fig.1).

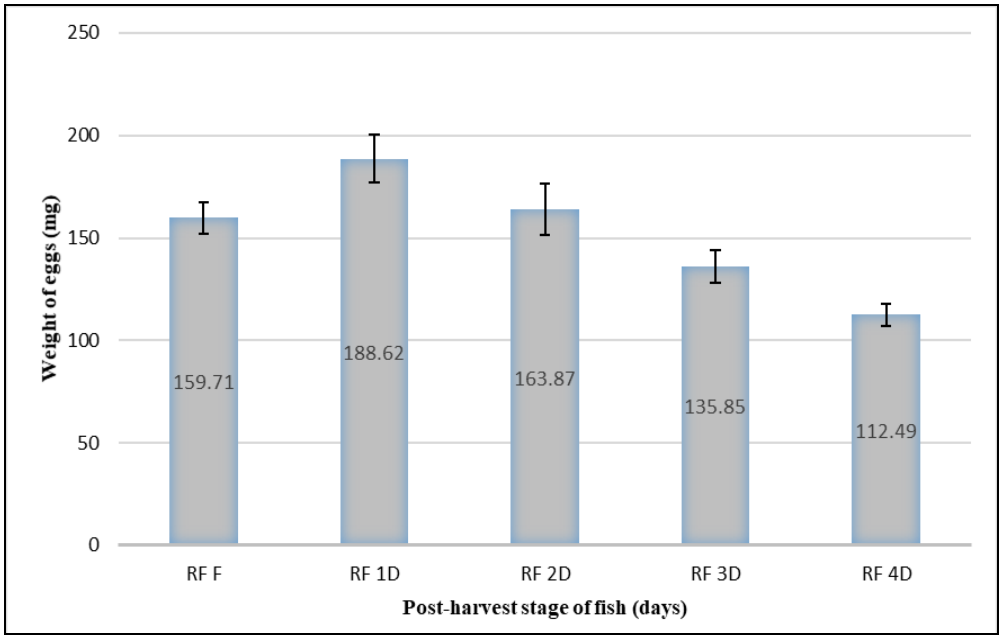

Fig 1: Preference of *C. megacephala* on post-harvest stage of fish

Yang and Shiao [34] conducted a similar study of *C. megacephala* with decaying pork liver of 2 and 4 days old as a dual choice test separately, with fresh liver. They found that 2day old decay meat was the most preferred over other choices and discussed that each species of carrion fly has a favourable time of attraction and appearance. According to that, in the present study 1day old fish might be in the right time of attraction and preference was indicated. Similarly, a study conducted by Zhu *et al.,* [35] on another blow fly species *Cochliomyia macellaria* reported significantly higher number of eggs on 3day old rotten chicken liver compared to 3day old rotten beef liver and fresh chicken, beef livers. Gas chromatography mass spectrometry (GCMS) analysis of the samples followed by electroantennography (EAG) and oviposition preference bioassays revealed that seven volatile compounds present in the rotten liver was responsible for the attraction and oviposition preference of the flies.

Fish, like any other vertebrate undergo post-mortem changes

after harvest. The decomposition of vertebrate tissue has five stages *viz.*, fresh, bloat, active and advance, decay and dry remains and is coupled with chemical processes of autolysis and putrefaction. Unlike other vertebrate tissues, fish contains high amount of poly unsaturated fatty acids-PUFA [3] which accelerates the rate of spoilage or decomposition fast in fish. Break down of proteins, nucleic acids, lipids and carbohydrates in vertebrate tissues generate lot of volatile organic compounds [8]. Statheropaulos *et al.,* [25] opined that the volatile organic compounds released during decomposition process attract the carrion insect. This fact can be attributed to the present ovipositional preference shown by *C. megacephala* which might be coupled with their high capacity of odour detection and vision.

Conclusion

The present study provides a detailed biology of *C. megacephala* on fish at a relatively high temperature common of tropics and all developmental variables except fecundity is accounted. The obtained developmental data on immature stages, viability of adults and its close range with usual rearing substrates, underlines that fish is a perfect natural medium for rearing the species for varietal purposes. The critical comparison of the developmental parameters with other natural protein substrates and temperatures would enable the forensic entomologists to critically apply the developmental data in investigations of PMI. The data obtained from the timing of preferred oviposition indicate a time bound progressive state of spoilage in fish. This clearly provide an indication to plan necessary precautions to prevent the post-harvest loss of fish by blow fly damage.

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