IMPACT OF ISOLATED DIETS ON THE GROWTH RATE OF *CHRYSOMYA MEGACEPHALA* (DIPTERA)

D. Alexander, Anulin Christudhas* and Manu Thomas Mathai

* Department of Zoology, Madras Christian College, Tambaram, Chennai-600059, INDIA.
E-mail: anulindhas@yahoo.com

[Alexander, D., Christudhas, A. & Mathai, M. T. 2015. Impact of isolated diets on the growth rate of *Chrysomya megacephala* (Diptera). Munis Entomology & Zoology, 10 (2): 377-383**]**

ABSTRACT: The association of *C. megacephala* with decomposing carrion is of particular interest in the field of forensic entomology. The impact of different isolated diets influence the larval weight and length including head capsule which accounts on its fine articulated cephalopharyngeal sclerites. The larval feeding duration highly influence the duration of life cycle in *C. megacephala*.

KEY WORDS: Forensic Entomology, Carrion, Blow flies, Morphometrics.

The most important dipteran group associated with animal carrion both in terms of number as well as the role that they play in decomposition, are the green and blue bottles commonly known as carrion blowflies, these necrophages and sarcophages species feeding on corpse tissue include the genera *Chrysomya, Lucilia, Calliphora* and *Sarcophaga*, which are cosmopolitan in distribution (Putman, 1977). The association of *Chrysomya megacephala* (Fabricius) with the decomposing carrion is of particular interest in the field of forensic entomology (Keh, 1985; Catts & Goff, 1992). Age determination of these insects usually is the basis for making post mortem interval estimations (Marchenko, 1980; Nainis et al., 1982). As decomposition progresses, the insect and other vertebrates that colonize corpses, can provide valuable information concerning the time and manner of death.

Early historical documented accounts on forensic entomology are almost nonexistent. A documented account appeared first in thirteenth century (McKnight, 1981). The age of the maggots and the development of *C. megacephala* determines the period of corpse exposure (Goff et al., 1986). "A manual of forensic entomology" (Smith, 1986) provides a series of 19 cases submitted by European Forensic Entomologist and "Entomology and Death" (Catts & Haskell, 1991), is a procedural guide attempting to familiarize death investigations as well as entomologists with procedures and analysis in handling entomological data in death investigations.

In the ecosystem, the insect reproductive biology is governed by both biotic and abiotic factors. The primary necessity for reproduction is the abundant availability of food materials, from which the larvae and adults derive energy for their growth and development. Almost all organic materials in nature serve as food for insects (Brues, 1946). The quality and quantity of the food is also a primary factor which influence the biology of the insects (Shahein, 1986).

A decomposing carrion is a highly temporary habitat available for a limited duration of time, compared to other habitats hence, the biology of blowflies are well synchronized with the host availability. In *C. megacephala*, the life cycle, fecundity, size and weight of the life stages are under the influence of the host availability (Bhuvaneswari & Daniel, 1994). Though *C. megacephala* feed, breed

and oviposit randomly on the carrion, noticeable aggregation was recorded on certain regions like brain, clotted blood and decomposing meat.

The association of *C. megacephala* with the decomposing carrion is of particular interest in the field of forensic entomology apart from its economic importance as an effective pollinator of mangoes (Hu et al., 1995). The length of the oldest maggots recovered from the corpse often provide an accurate estimate of the time of death hence, the study is to understand the impact of three isolated diets on the size and weight of the pupal forms along with the adult emergence and the details in the structure of cephalopharyngeal sclerites, including the recording of the size of the head capsule.

MATERIAL AND METHODS

The live adult individuals of *C. megacephala* were trapped using rotten beef, etherized, and reared under captivity en mass on beef diet. The individuals were grouped to 10 at the sex ratio (3:1) of female to male from mass culture as the males are promiscus and reared on the isolated diets like bovine brain tissue, blood clot and decomposing meat under controlled conditions. The development of *C. megacephala* was examined at 30°C.

Following emergence, the larvae were reared in batches of 15 to 25 and observed at different feeding durations with 24hrs extended feeding range up to 288hrs. After each feeding duration, 30 larvae were randomly removed from the different cultures for morphometrics. The pupae were undisturbed and observed till their emergence, based on which the number of viable pupae on different diet was calculated. Statistical analysis was computed to determine the mean and standard deviation of the various parameters of the larvae, pupae and adult.

RESULTS

The studies on the impact of the larval feeding duration in the biology of *C. megacephala* reveal that it produced variations in the size of the larvae and pupae with differential feeding time. Under normal conditions the emerging larvae pupate after a period of 72hrs of feeding. This study reveals that the feeding by larvae less than 72hrs fails to pupate. The larvae after 144hrs of feeding starts shrinking in its total body length.

The focus on the influence of the isolated food materials: the brain, the blood and the meat on the rate of larval growth and the time of pupal formation after different larval feeding durations reveals that the rate of growth of larvae is comparatively greater in the brain tissues, where the larvae attained a length of 8.520.1031mm when compared to the other two meals, the blood and the meat, where the length are 7.980.0258mm and 8.230.1204mm respectively. The comparative growth of the larval forms on the three isolated food materials up to a feeding duration of 288hrs is tabulated (Table 1). Correspondingly the pupation with the different larval feeding duration was also recorded (Table 1 & 2). The pupation occurred very late on the brain diet, only after 120hrs of feeding but, in the case of meat the pupation occurs immediately after 48hrs of feeding. In all this diets the length of the pupae gradually increase towards the maximum feeding duration almost all larvae pupated after 120-240hrs of feeding on the diets blood and meat. The maximum recorded pupal length was when fed with the brain diet (9.940.0376mm).

The first instar was muscoid shaped and composed of 12 segments. The cephalic segment pocess a pair of terminal organs, a pair of multi-branched oral

hooks situated at mid-dorsal region of the mouth and three oral grooves at each side of the mouth. Each oral hook contains 3-4 rows of single, curved spines with sharp tips. The anterior spiracle is not apparent in this instar. The prominent features of the caudal segment are a pair of posterior spiracular discs and a pair of circular, deep depressions ventral to the spiracular disc. Each spiracular disc contains two straight spiracular slits that coalesce ventrally and are interspaced with bundles of relatively thin and multi-branched spiracular hairs (Plate 1).

The general morphology of the second instar larva is very similar to that of the first instar. The dorsal and terminal organs still remain with minimal change, but the ventral organ and grooves are more extensive and well developed. The labium appears as a trilobed structure. The ventral organ appears as C-shaped with four short spines appearing on the anterior side of the inner curvature of the organ. Prothoracic anterior spiracles become well developed. The caudal segment bears the posterior spiracular discs having two separated straight slits encircled by four multi-branched spiracular hairs. The button or cedysial scar appears as a hole ventro-medially on the posterior spiracular discs.

Cephalopharyngeal skeleton: In *Cyclorrhapha*, the typical mouthparts undergo atrophy in correlation with the reduction of the head, the maxillae and labium are scarcely recognizable other than papillae representing their palp. In *C. megacephala* there is a very characteristic framework of articulated sclerites, known as cephalopharyngeal skeleton (Thompson, 1938) Plate 2. This is a secondary development and is composed in the mature larva of the following principle sclerites. The most anterior are the mouth-hooks or mandibular sclerites which articulate basally with hypostomal or intermediate sclerites. The latter is H-shaped, its halves being joined by a transverse bar: the hypostomal sclerites receive the opening of the salivary duct. Behind this sclerite is the much larger basal or pharyngeal sclerite. The latter is formed of two lateral, vertical lamellae which units ventrally forming a trough in which is lodged the pharynx. In many species, circular dentate sclerite units the bases of the mandibular sclerites: various other small accessory sclerites are frequently present, notably in carnivorous species.

In saprophagous larvae (Keilin, 1915) the floor of the pharyngeal sclerite is beset with longitudinal ridges which project into the cavity of the pharynx: larvae feeding on living animal or vegetable tissues are devoid of pharyngeal ridges or, if latter be present (as in *Pegomyia*) they are reduced. Furthermore, in phytophagous larvae the mandibular sclerites are usually toothed, and in carnivorous larvae they are sharply pointed: in the parasitic forms the buccal armature undergoes marked reduction. The size of the larval head capsule of *C. megacephala* is measured in mm on three different diets (Table 3). The size of the head capsule is influenced by the duration of feeding.

DISCUSSION

No other order of insects presents so great a diversity of larval habits as Diptera. Only four families have the majority of their species phytophagous in the larval stage: cocidomyidae, tephritidae, agromyzidae and chloropidae. While the mycetophilidae and platypezidae are fungivorous. The saprophagous habit is largely in evidence among the anthomyidae. Other notable scavengers are the bibionidae, sepsidae, phoridae, heleomyzidae and scatophaginae. Next to the parasitic hymenoptera, the dipteral constitute the most important natural controlling agency over the increase of other insects.

The trapping materials used: vinegar and brown-sugar mixture and the decomposing meat, the maximum attraction of the flies were noticed only on the decomposing meat. The attractivity of decomposing fish, human faeces and fermenting banana as bait to female calliphoridae and sarcophagidae in different stages of ovarian development was that the former undergoing intense vitellogenesis was attracted to fish and mature flies with eggs are attracted to banana and the latter at the beginning of vitellogenesis found fish and faeces most attractive (D'Almedia & Lima, 1995).

The duration of the larval feeding influence the size at different life stages and the duration of the lifecycle of *C. megacephala*. Correspondingly there is variation in the internal reproductive organs of the adults. Food can influence the growth in terms of the body length of the adults depending upon the nutritive value. In *C. megacephala* the sexual dimorphism is not well expressed.

The mating behavior in *C. megacephala* shows that the males are promiscuous, mating with several females and the mating takes place in "male above female position. The pheromones play an important role in sexual attraction like lepidopteran (Tinbergen, 1989). The position of contact during mating behavior is venter-venter in *C. megacephala* and it varies in other orders of insects (Richards, 1927).

Dipterans of forensic importance can be powerful tool in investigation of homicide and other deaths; particularly care is taken to collect specimens and record information. Many of direct observation of blowflies on or in corpse are reported by medical pathologists. With rare exceptions, experimental studies having forensic implications are carried out on carcasses rather than on corpses. Not only an animal size and species produce results that differ in some respects from those one would expect. If the experiments were carried out on man, the condition of the experiment may differ from those observed in forensic investigations. Efforts to adopt entomological data obtained for economic, medical or other purposes may in some cases be only partially successful, since critical elements are either unexplored or unreported.

The larval morphological description using SEM suggest that when first instar larvae are collected from a corpse, they should be reared to the second instar in order to easily differentiate between *C. megacephala* and *C. rufifacies*. However, rearing the third instar is required to definitely separate these two species from other closely related blowflies that may be found in a corpse, up to 42 calliphorid species are recorded (Tumrasvin et al., 1979).Thus the SEM results are beneficial for specific identification of larva in forensic investigations. Similarly low levels of variation between species of the same genus are diagnosed by sequencing the mitochondrial DNA (Harvey et al., 2003).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Zoology, Madras Christian College, Tambaram, for permitting to conduct the research.

LITERATURE CITED

Bhuvaneswari, & Daniel, M. 1994. Impact of larval feeding duration on the viable pupal production in *C. megacephala* F. (Diptera:Insecta). Proceedings of the IX All India Symposium on Invertebrate Reproduction, Sivakasi.

Brues, C. T. 1946. Insect dietary. An account of the food habits of insects. Harvard University Press, Cambridge.

Catts, E. P. & Goff, M. L. 1992. Forensic entomology in criminal investigations. Annual Review of Entomology, 37: 253-272.

Catts, E. P. & Haskell, N. H. 1991. Entomology and death: a procedural guide. Joyce's print shop, Clemson South Carolina.

D'Almedia, J. M. & Lima, S. F. 1995. Attractiveness of different baits and its relationship with phases of ovarian development in Calliphoridae and Sarcophagidae (Insecta:Diptera). Revista Brasileira de Zoologia, 11 (2): 177-186.

Goff, M. L., Odom, C. B. & Early, M. 1986. Estimation of postmortem interval by entomological techniques: a case study from Oahu, Hawaii. Bulletin of the Society Vector Ecology, 11: 242-246.

Harvey, M. L., Dadour, I. R. & Gaudieri, S. 2003. Mitochondrial DNA cytochrome oxidase I gene: Potential for distinction between immature stages of some forensically imporant fly species Diptera in western Australia. Forensic Science International, 131: 134-139.

Hu, T., Len, C. H. & Lee, B. S. 1995. The laboratory rearing and radiation effects of gamma ray on the pupae of *C. megacephala* Fabricius. Chinese Journal of Entomology, 152: 103-111.

Keh, B. 1985. Scope and Applications of Forensic Entomology. Annual Review of Entomology, 30: 137-154.

Keilin, D. 1915. Recherchessur les larves de Diptere scyclorhaphes. Bulletin Scientifique de la Belgique, 49: 15-198.

Marchenko, M. J. 1980. Classifying of cadaveric entomofauna. Biology of flies: The forensic medical role. Sudebno-MeditsinskaiaEkspertiza, 23: 17-20.

McKnight, B. E. 1981. The washing away of wrongs: forensic medicine in thirteen-century China. University of Michigan Press.

Nainis, I. V. J., Marchenko, M. I. & Kazak, A. N. 1982. A calculation method for estimating by entomofauna the period during which the body had remained in the place where it was found. Sudebno-MeditsinskaiaEkspertiza, 25: 21-23.

Putman, R. J. 1977. Dynamics of the blow fly, *Calliphora erythrocephala* within carrion. Journal of Animal Ecology, 46: 853-866.

Richards, O. W. 1927. Sexual selection and allied problems in the insects. Biological Reviews and Biological Proceedings of the Cambridge Philosophical Society, 298-360.

Shahein, A. 1986. Effect of intra specific competition between larvae of *Calliphora erythrocephala* Meig. Calliphoridae: Diptera. Beitragezur Entomologie, 36 (2): 233-235.

Smith, K. G. V. 1986. A manual of forensic entomology. British Museum, London.

Thompson, W. R. 1938. A dipterous parasite of mycetophylids. Parasitology, 30: 176-180.

Tinbergen, N. 1989. The study of instinct. Oxford University Press, London.

Tumrasvin, W., Kurahashi, H. & Kano, R. 1979. Studies on medically important flies in Thailand 7. 42 species of Calliphorid flies including the taxonomic keys (Diptera: Calliphoridae). Bulletin of Tokyo Medical and Dental University, 26 (4): 242-272.

Table 1. Larval and pupal length (in mm) of *Chrysomya megacephala* on three different diets.

Duration (Hours)		24	48	72	96	120	144	168	192	216	240	264	288
Larvae	Brain	8.52±0.1031	9.10±0.0079	12.72±0.1923	15.29±0.1884	17.17±0.052	18.58±0.0698	16.39±0.0554	15.61±0.0831	14.47±0.0676	13.38±0.0554	12.92±0.0397	-
	Blood	7.98±0.0258	8.61±0.046	12.4±0.5431	14.12±0.1032	15.8±0.079	15.99±0.0952	14.19±0.0722	13.77±0.0641	12.57±0.5594	11.55±0.0353	-	-
	Meat	8.23±0.1204	8.76±0.0389	12.64±0.114	14.8±0.079	16.64±0.0962	17.88±0.0609	15.81±0.134	14.6±0.0715	13.47±0.0622	12.5±0.0721	-	-
Pupae	Brain	-	-	-	-	8.95±0.0376	9.04±0.0316	9.28±0.032	9.42±0.0808	9.63±0.0349	9.78±0.038	9.82±0.0316	9.94±0.0376
	Blood	-	-	-	8.07±0.0469	8.64±0.0316	8.83±0.0414	8.98±0.1283	9.1±0.038	9.28±0.032	9.45±0.0414	9.36±0.0303	-
	Meat	-	-	8.21±0.0685	8.52±0.0622	8.87±0.0303	8.95±0.0376	9.16±0.0378	9.28±0.032	9.45±0.0414	9.66±0.0808	9.78±0.0469	-

Table 2. Larval and pupal weight (in grams) of *Chrysomya megacephala* on three different diets.

Duration (Hours)		24	48	72	96	120	144	168	192	216	240	264	288
Larvae	Brain	0.009±0.0003	0.0193±0.0019	0.0345±0.0019	0.0572±0.0015	0.0769±0.003	0.1032±0.0001	0.0913±0.0005	0.0877±0.0012	0.086±0.0005	0.0855±0.0007	0.0812±0.0007	-
	Blood	0.0061±0.0005	0.0079±0.0002	0.0314±0.0037	0.0456±0.0069	0.0635±0.0008	0.0875±0.0008	0.0723±0.0005	0.0613±0.0005	0.0594±0.0003	0.058±0.0007	-	-
	Meat	0.0077±0.0003	0.0128±0.0003	0.0321±0.0032	0.0498±0.0017	0.0687±0.0042	0.0942±0.0026	0.083±0.0008	0.0815±0.0008	0.078±0.0016	0.072±0.0003	-	-
Pupae	Brain	-	-	-	-	0.0348±0.0004	0.0386±0.0006	0.0415±0.0003	0.0452±0.0002	0.0487±0.0004	0.0491±0.0003	0.0506±0.0005	0.0515±0.0004
	Blood	-	-	-	0.0316±0.0003	0.0329±0.0007	0.0352±0.0005	0.0389±0.0003	0.0419±0.0003	0.0446±0.0002	0.0462±0.0001	0.0478±0.0003	-
	Meat	-	-	0.0302±0.0001	0.0326±0.0001	0.0336±0.0002	0.0373±0.0003	0.0405±0.0004	0.0443±0.0004	0.0467±0.0004	0.0487±0.0004	0.0492±0.0008	-

Table 3. Larval head capsule (in mm) of *Chrysomya megacephala* on three different diets.

Duration (Hours)	Brain	Blood	Meat
24	0.6±0.0158	0.48±0.0258	0.58±0.0207
48	0.69±0.087	0.56±0.0559	0.65±0.036
72	0.75±0.0396	0.67±0.0414	0.74±0.0371
96	0.8±0.0291	0.71±0.024	0.79±0.0286
120	0.86±0.0602	0.78±0.0568	0.82±0.0349
144	0.9±0.0595	0.82±0.043	0.87±0.0396
168	0.82±0.0316	0.79±0.0303	0.77±0.0316
192	0.76±0.024	0.7±0.037	0.73±0.0316
216	0.7±0.0255	0.61±0.0316	0.63±0.0158
240	0.65±0.0414	0.59±0.024	0.6±0.0192
264	0.54±0.0319	-	-

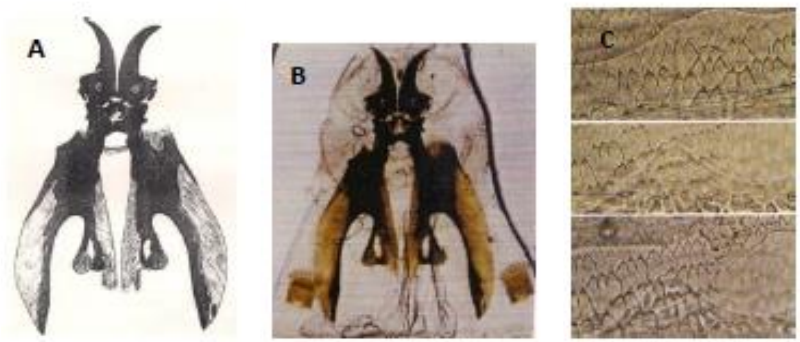

Plate 1. Cephalopharyngeal skeleton of *C. megacephala* after larval treatment with KOH for 15 minutes. A) Cephalopharyngeal skeleton B) showing mandibular sclerite, dentate sclerite, hypostomal sclerite, pharyngeal sclerite and Lateral organ C) Cuticular armature at the region of cephalic capsule.