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## Cephalopharyngeal skeleton morphometry of *Hypopygiopsis violacea* (Macquart) (Diptera: Calliphoridae) - A preliminary assessment for its application in forensic entomology

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### Abstract

The objective of this study was to compare the consistency of larval body length and cephalopharyngeal skeleton of third instar larvae, *Hypopygiopsis violacea* (Macquart) (Diptera: Calliphoridae) at 0, 7 and 14-day storage intervals. During third instar feeding stage, larvae were killed in hot water, preserved in 70% ethanol and body length was measured. Cephalopharyngeal skeleton was cleared using 10% KOH and mounted on slides using Euparal. Cephalopharyngeal skeleton of third instar *H. violacea* larvae were weakly correlated with their body length ( $p < 0.05$ ). Based on variance within-subjects, the mouthhook-dorsal cornua (MH-DC), mouth hook-ventral cornua (MH-VC) and anterior dorsal process-dorsal cornua (ADP-DC), exhibited more consistent measurement compared to body length. However, cephalopharyngeal skeleton length was influenced by prolonged storage in 70% ethanol ( $p < 0.05$ ) over 14 days. These findings show the potential of using cephalopharyngeal skeleton morphometry in minimum post mortem interval (mPMI) estimation by having more consistent growth parameters than larval body length.

**Keywords:** Forensic entomology, morphometry, post mortem interval, blow fly, cephalopharyngeal skeleton

### 1. Introduction

Entomological evidence is one of the primary indicators being utilized in death investigation to estimate minimum post mortem interval (mPMI). In forensic entomology, mPMI can be determined based on several growth parameters of insects such as larval length<sup>[1]</sup>, width<sup>[2]</sup>, weight<sup>[3]</sup>, larval cuticle hydrocarbons<sup>[4]</sup> and embryonic development of eggs<sup>[5]</sup>. Among these parameters, larval length is the most widely used growth parameter to estimate age of insect found on cadavers<sup>[6]</sup>. This is based on the premise that larval body length correlates with its age during feeding activity and this information can be used to obtain mPMI<sup>[7, 8]</sup>. In forensic entomology, larval body length has been useful to summarize insect growth and expressed in various forms of developmental modelling such as isomegalen diagram, isomorphen diagram<sup>[1]</sup> and thermal summation model using regression analysis<sup>[9]</sup>.

However, there were drawbacks to using larval body length as a mPMI indicator because they reacted differently to various type of preservatives and treatments. These reactions include expansion or shrinkage of larvae<sup>[10-12]</sup>, alteration in curvature, coloration and turgidity<sup>[13]</sup>. Changes in larval lengths during storage could also affect mPMI estimation as reports indicated there were random changes to larval length at different stage of development when preserved in 70-80% ethanol preservatives<sup>[14, 15]</sup>.

After reviewing issues related to larval length as reported above, we investigated the morphometry of cephalopharyngeal skeleton of third instar larvae as a potential reference value in mPMI estimation. Cephalopharyngeal skeleton in dipteran larvae has been one of the most important aspects in forensic entomology, especially for species identification<sup>[16, 17]</sup>. This morphometric study was based on the cephalopharyngeal skeleton of *Hypopygiopsis violacea* Macquart (Diptera: Calliphoridae), a forensically-significant Malaysian species. Larval morphology and their importance in forensic entomology have been described in previous work<sup>[18]</sup>. The main objective of this study was to find a more consistent growth parameter that is unaltered during storage for mPMI estimation. It was hypothesized that cephalopharyngeal skeleton length obtained from actively feeding third instar *H. violacea* larvae correlates with

body length and these parameters react differently when preserved in 70% ethanol for a duration of 0, 7 and 14 days.

## 2. Materials and Methods

This study consists of two replications. The first replication was carried out from 29 January 2016 until 15 February 2016 whilst second replication was conducted from 10 March 2016 until 10 May 2016. The periods include preparation, fly egg sampling, sample processing and data analysis.

### i. Background information on species.

*H. violacea* is a forensically important calliphorid in Malaysia [18] and reported as among the earliest blow fly to oviposit on monkey carcasses [19, 20]. Morphological characteristics of adult *H. violacea* along with its co-occurring species in Malaysia, *H. fumipennis* (Walker) have been featured in the collection of Malaysian blow flies and they are generally large flies (>15mm) [21]. Larval morphology of both species has also been described [18, 22] whilst developmental stage and growth rate of *H. violacea* in controlled and natural environments have been studied [23, 24].

### ii. Obtaining *H. violacea* eggs.

*H. violacea* eggs were obtained from fish baits placed in a secondary forest at Forensic Science Simulation Site, Faculty of Health Sciences, Universiti Kebangsaan Malaysia (UKM), Bangi. Baits consisting of approximately 300 g of raw yellow stripe scads (*Selaroides leptolepis* Cuvier) were put inside a black plastic container and placed on the ground in an open area. In local natural surroundings, *H. violacea* has been observed to be among the earliest to arrive to feed and oviposit on decaying material. It was easily identified from other blow fly species by its distinctive large size and silvery genae. In this study, baits were left exposed for a period of 2 hrs to allow oviposition by *H. violacea* females. A single batch of eggs oviposited by a female *H. violacea* was collected carefully by using fine-tip forceps and transferred into a plastic container consists of approximately 30 g fresh cow's liver. The eggs were reared overnight at room temperature (18.5 – 35.0 °C) and relative humidity (44 – 93%).

### iii. Effect of 70% ethanol on cephalopharyngeal skeleton and body length study.

On the following day, larvae from (ii) were transferred evenly into 4 separate rearing containers labelled as A – D. Each container consists of 6 fresh cow's liver cubes (~50 g), placed on sawdust and replenished *ad libitum*. After 48 hrs, third instar larvae were killed using near-boiling water (~80°C) for 30 – 40 secs and subsequently preserved in sterile universal containers with 40 ml 70% ethanol. The preserved 3rd instar larvae from each rearing container were further divided into 3 different replicates, representing storage duration of 0 day, 7 days and 14 days. The durations represent the common receiving period of forensic entomology cases at Forensic Entomology Laboratory, Faculty of Health Sciences, UKM (0-7 days) and turnaround time to complete a forensic entomological report (14 days). During each storage duration, larvae were taken out from the preservative solutions and their body length were measured according to body segments in lateral position i.e. from the tip of mouth hook to the posterior spiracle. After measuring the body length, middle segments of the larvae were cut and soaked in 10% KOH for 15 minutes. The gut contents and the remaining tissues near cephalopharyngeal skeleton were removed, washed with

distilled water and mounted on a glass slide with Euparal and a 5 mm rounded coverslip. Measurement of cephalopharyngeal skeleton was conducted immediately after mounting to avoid the possible effect of remaining KOH on the chitin of cephalopharyngeal skeleton [25]. Each cephalopharyngeal skeleton was measured based on 3 landmarks, i.e. mouth hook to dorsal cornua (MH-DC), mouth hook to ventral cornua (MH-VC) and anterodorsal process to dorsal cornua (ADP-DC) (Fig. 1). These streamline measurement landmarks on cephalopharyngeal skeletons were based on Nateeworanart *et al.* (2010). Larval lengths and cephalopharyngeal skeletons were measured using a stereomicroscope fitted with Dino-Lite® digital microscope and Dino Capture 2.0® Software.

### iv Cephalopharyngeal skeleton and body length correlation study.

This experiment was conducted from 12 March 2016 until 15 April 2016. *H. violacea* eggs were obtained as described earlier in step (ii). After 24 hrs, larvae emerged from a single batch of eggs were separated evenly into five rearing containers (n=5), each with 6 cubes of fresh cow's liver (~50 g). Each day, feeding larvae from a container were withdrawn, killed using near-boiling water (~80°C) for 30 – 40 secs and subsequently preserved in sterile universal containers with 40 ml 70% ethanol. Measurement of cephalopharyngeal skeleton and body length as described earlier in step (iii).

### v. Statistical analysis

Statistical analysis was performed using SPSS18®. Effects of storage time intervals on larval body lengths and cephalopharyngeal skeleton lengths were determined by using either one-way between groups analysis of variance (ANOVA) or its non-parametric analogue, Kruskal-Wallis test, depending on the results of normal distributions. Correlations between cephalopharyngeal skeletons length and larval body lengths were analysed by using Kendall's tau-b rank correlation coefficient.

## 3. Results

The number of larvae used in this experiment was determined from the total numbers of eggs laid by a single female *H. violacea* adult. From this study, a single female *H. violacea* oviposited 120 - 150 eggs in the 1<sup>st</sup> and 2<sup>nd</sup> study replicates. At room environment (18.5 – 35.0 °C, 44 – 93%RH), it took less than 24 hrs for the larvae to reach 2nd instar and about 72 hrs to reach 3rd instar.

### i. Morphometry of *H. violacea* body length and cephalopharyngeal skeleton.

Measurements of larval body lengths and cephalopharyngeal skeleton parts for both replicates are summarized in Table 1 based on storage intervals of 0, 7 and 14 days. The evaluation of data spread in body and cephalopharyngeal skeleton length was determined from within-groups sample variances ( $s^2$ ). Overall, variances of MH-DC, MH-VC and ADP-DC length were smaller than variance of body length in both study replicates. This indicates measurement of cephalopharyngeal skeleton parts were less dispersed from mean values and more consistent than body length, even when the larvae stored in 70% ethanol for a period of 14 days. In both study replicates, variance of body length ranged between 0.83 - 6.93, MH-DC = 0.006 – 0.014, MH-VC = 0.004 – 0.010 and ADP-DC = 0.004 – 0.012. For larval body length, variance decreased

from 4.380 (day 0) to 1.347 (day 14) in replicate 1 and from 2.664 (day 0) to 0.830 (day 14) in replicate 2.

**ii. Effect of storage on body and cephalopharyngeal skeleton lengths.**

In order to determine whether storage time intervals affected body and cephalopharyngeal skeleton lengths, one-way between group analysis of variance (ANOVA) and its non-parametric analogue, Kruskal-Wallis test, were performed. The analyses were determined based on data normality assumption using Shapiro Wilks test and Levene's homogeneity of variance test for each independent test group (Table 2).

It was discovered that there were varying effects of storage period towards larval body length and cephalopharyngeal skeleton length in both replicates (Fig. 2 & Table 2). In study replicate 1, storage period affected body length. The mean body length increased from 17.98 ± 2.09 mm (day 0) to 19.35 ± 1.16 mm (day 14). There were significant differences in length between day 0 (mean rank= 44.62), day 7 (mean rank=32.47) and day 14 (mean rank=64.31),  $H=22.255$ ,  $df=2$ ,  $N=93$ ,  $p<0.001$ , partial  $\eta^2=0.240$ . Follow up analyses between each group revealed larval body length on day 14 differed significantly from day 0,  $U=291.5$ ,  $z=-2.503$  (corrected for ties),  $p<0.05$ ,  $r=-0.320$ , and day 7,  $U=133.000$ ,  $z=-4.991$  (corrected for ties),  $p<0.001$ ,  $r=-0.629$ . There were also significant differences in MH-DC length between day 0 (mean rank=35.75), day 7 (mean rank=54.64) and day 14 (mean rank=50.00),  $H=8.160$ ,  $df=2$ ,  $N=93$ ,  $p<0.05$ , partial  $\eta^2=0.089$ . It was found that, larval body length on day 0 differed significantly from day 7,  $U=303.500$ ,  $z=-2.486$  (corrected for ties),  $p<0.05$ ,  $r=-0.316$ , and day 14,  $U=304.00$ ,  $z=-2.323$  (corrected for ties),  $p<0.05$ ,  $r=-0.297$ . There was no significant difference between groups for MH-VC lengths,  $H=5.231$ ,  $df=2$ ,  $N=93$ ,  $p=0.073$ , partial  $\eta^2=0.057$ , and ADP-DC lengths,  $H=0.349$ ,  $df=2$ ,  $N=93$ ,  $p=0.840$ , partial  $\eta^2=0.004$ . The results of the second replicate were different where all variables indicated changes between time intervals. Changes were significant in body length between day 0 (mean rank=59.65), day 7 (mean rank = 45.93) and day 14 (mean

rank=27.79),  $H=22.431$ ,  $df=2$ ,  $N=89$ ,  $p<0.001$ , partial  $\eta^2=0.255$ . Larval body length on day 0 differed significantly from day 7,  $U=282.000$ ,  $z=-2.640$  (corrected for ties),  $p<0.05$ ,  $r=-0.338$ , and day 14,  $U=163.00$ ,  $z=-4.114$  (corrected for ties),  $p<0.001$ ,  $r=-0.536$  while day 7 differed significantly from day 14,  $U=209.000$ ,  $z=-3.283$  (corrected for ties),  $p<0.05$ ,  $r=-0.431$ . Cephalopharyngeal skeleton length also showed similar changes throughout preservation periods. Mean length of MH-DC significantly decreased from day 0 (1.855±0.082 mm) until day 7 (1.831±0.092mm) and day 14 (1.714±0.115mm),  $F(2,86)=17.616$ ,  $p<0.001$ ,  $\eta^2=0.290$  while in MH-VC, the mean length significantly fluctuated from day 0 (1.387±0.087), to day 7 (1.406±0.067) and day 14 (1.331±0.098),  $F(2,86)=6.105$ ,  $p<0.05$ ,  $\eta^2=0.125$ . In ADP-DC, the mean length decreased from day 0 (1.327±0.072mm), until day 7 (1.296±0.066mm) and day 14 (1.212±0.093mm),  $F(2,86)=17.152$ ,  $p<0.05$ ,  $\eta^2=0.286$ . Post hoc analyses for MH-DC, MH-VC and ADP-DC groups ( $\alpha=0.05$ ) showed larvae stored in 70% ethanol until day 14 significantly differed from those of day 0 and day 7, indicating the effect of storage duration on cephalopharyngeal skeleton could be observed in a period of 14 days.

**iii. Correlation between body length and cephalopharyngeal skeleton.**

In a separate experiment to investigate whether cephalopharyngeal skeleton segments correlate with body length, correlation tests were carried out. Due to violation of normality assumptions of the data, Kendall's tau-b rank correlation coefficient was employed to assess the dependence of variables. Findings indicate MH-DC length correlates weakly with body length,  $\tau=0.347$ ,  $p<0.05$ , two-tailed,  $N=20$ . Similar positive and weak correlation was also observed between MH-VC length and body length,  $\tau=0.263$ ,  $p<0.05$ , two-tailed,  $N=20$ . However, there was no correlation detected between ADP-DC and body length,  $\tau =0.417$ ,  $p=0.105$ , two-tailed,  $N=20$ . This study revealed MH-DC, MH-VC and ADP-DC lengths were significantly correlated (Table 3).

**Table 1:** Morphometry of *Hypopygiopsis violacea* (in mm) in the first and second replicate based on storage periods (BL: Body length, MH-DC: mouth hook-dorsal cornua, MH-VC: mouth hook-ventral cornua and ADP-DC: anterodorsal process-dorsal cornua).

Parameter	Day	Replicate 1								Replicate 2							
		M	Min	Max	Range	s	SE <sub>M</sub>	s <sup>2</sup>	N	M	Min	Max	Range	s	SE <sub>M</sub>	s <sup>2</sup>	N
BL	0	17.981	13.804	20.995	7.191	2.093	0.382	4.380	30	17.633	14.456	20.397	5.491	1.632	0.293	2.664	31
	7	14.366	14.989	19.916	4.927	1.287	0.228	1.657	31	16.754	14.430	18.903	4.473	1.106	0.202	1.223	30
	14	19.351	17.174	21.680	4.506	1.151	0.208	1.347	32	15.837	14.139	17.849	3.710	0.911	0.172	0.830	28
MH-DC	0	1.750	1.585	1.858	0.273	0.078	0.014	0.006	30	1.855	1.701	2.011	0.310	0.082	0.015	0.007	31
	7	1.836	1.670	2.071	0.396	0.120	0.021	0.014	31	1.831	1.670	2.071	0.401	0.092	0.017	0.009	30
	14	1.808	1.654	1.975	0.321	0.087	0.016	0.008	32	1.714	1.431	1.944	0.513	0.115	0.022	0.013	28
MH-VC	0	1.320	1.210	1.422	0.212	0.063	0.012	0.004	30	1.387	1.140	1.514	0.374	0.087	0.016	0.008	31
	7	1.350	1.255	1.471	0.216	0.075	0.013	0.006	31	1.406	1.289	1.572	0.283	0.067	0.012	0.005	30
	14	1.367	1.193	1.487	0.294	0.074	0.013	0.006	32	1.331	1.104	1.513	0.409	0.098	0.019	0.010	28
ADP-DC	0	1.297	1.133	1.429	0.296	0.088	0.016	0.008	30	1.327	1.188	1.481	0.293	0.072	0.013	0.005	31
	7	1.309	1.124	1.484	0.360	0.107	0.019	0.012	31	1.296	1.170	1.423	0.253	0.066	0.012	0.004	30
	14	1.312	1.178	1.478	0.300	0.075	0.014	0.006	32	1.212	0.985	1.404	0.419	0.093	0.018	0.009	28

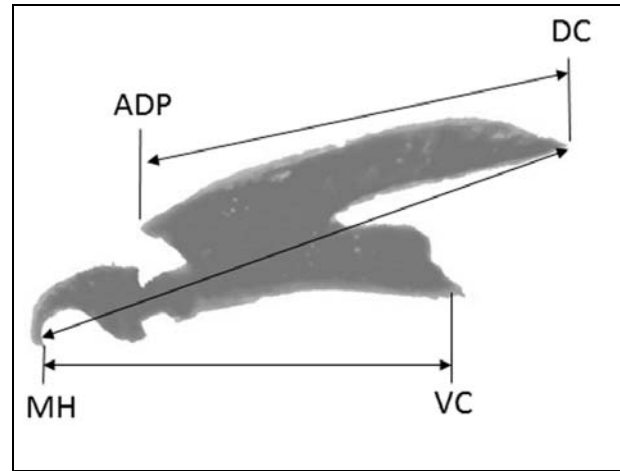
**Table 2:** Results of between groups comparison test using one-way between groups ANOVA (indicated by \*) and Kruskal Wallis (indicated by \*\*) tests. Effect sizes for one-way between groups ANOVA and Kruskal Wallis are represented by  $\eta^2$  values, i.e 0.01=small, 0.059=medium and 0.138=large [29].

Growth Parameter	p value	Effect size	Changes detected between groups
<b>Replicate 1</b>			
Body length**	$p<0.001$	0.240	Day 0 – Day14 Day 7 - Day 14
MH-DC**	$p<0.05$	0.089	Day 0 – Day 7 Day 0 – Day 14

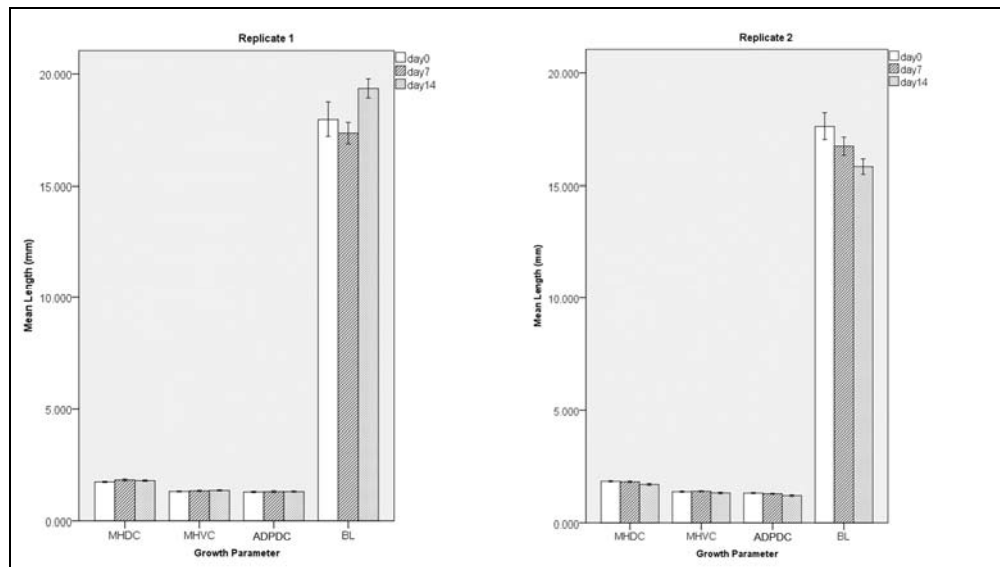
MH-VC**	$p=0.073$	0.057	-
ADP-DC**	$p=0.840$	0.004	-
<b>Replicate 2</b>			
Body length**	$p<0.001$	0.255	Day 0 – Day 7 Day 7 – Day 14 Day 0 – Day 14
MH-DC*	$p<0.001$	0.290	Day 0 – Day 14 Day 7 – Day 14
MH-VC*	$p<0.05$	0.125	Day 0 – Day 14 Day 7 – Day 14
ADP-DC*	$p<0.001$	0.286	Day 0 – Day 14 Day 7 – Day 14

**Table 3:** Non-parametric analysis using Kendall’s tau-b ( $\tau$ ) correlation coefficient and  $p$  value of relationships between cephalopharyngeal skeleton and body lengths of *H. violacea*,

Growth variables	MH-DC	MH-VC	ADP-DC	BL
MH-DC				
MH-VC	$\tau=0.695$ $p<0.001$			
ADP-DC	$\tau=0.705$ $p<0.001$	$\tau=0.484$ $p<0.05$		
BL	$\tau=0.347$ $p<0.05$	$\tau=0.442$ $p<0.001$	$\tau=0.263$ $p=0.105$	



**Fig 1:** Measurement landmarks of a third instar *H. violacea* cephalopharyngeal skeleton by using a stereomicroscope fitted with digital camera. MH – mouth hook; DC – dorsal cornua; VC – ventral cornua and ADP – anterior dorsal process. The landmarks were adapted from previous research with a change on the ADP [29]. For this sample, MH-DC = 1.782 mm, MH-VC = 1.356 mm and ADP-DC = 1.343 mm.



**Fig 2:** Effect of storage period on larval body mean length (BL) and cephalopharyngeal skeleton mean length (MHDC: mouth hook-dorsal cornua, MHVC: mouth hook-ventral cornua and ADPDC: anterodorsal process-dorsal cornua) in Replicate 1 and 2. Bars=  $\pm 2$  standard error of mean.

**4. Discussion**

The study on the morphometry of *H. violacea* cephalopharyngeal skeleton was aimed to determine whether this measurement could potentially be a better reference for insect growth than larval body length. Other than profiling the cephalopharyngeal skeleton morphometry of the third instar

larvae, this study examined the consistency of these parameters against the larval body length. Based on the value of within-subject variances, it was found that cephalopharyngeal parts, represented by MH-DC, MH-VC and ADP-DC, were less exposed to variation compared to body length when preserved in 70% ethanol. The variance of

the body length was also found to be decreasing throughout storage periods indicating lesser variation was detected when the larvae were stored longer in 70% ethanol.

Larval length measurement has been cited for potential sources of errors in mPMI estimation, especially when the larval structures were affected by the chemical used to preserve the larvae<sup>[10-13]</sup>. The preservative solutions being used in this research was 70% ethanol as it was the 'universal' choice of solution to preserve larvae collected from corpses<sup>[7]</sup>. Alternatively, Kahle's solution could also be used as preservative solution but may also provide different effects towards larval body length<sup>[13]</sup>. Another variable that might change the length of larvae is the storage duration<sup>[14, 15]</sup>. From this research, it was discovered that prolonged duration of storage in 70% ethanol up to 14 days altered the body length and cephalopharyngeal skeleton length of *H. violacea* third instar larvae. Although cephalopharyngeal skeleton could give more consistent measurement compared to larval body length, it could still be affected by the storage duration in 70% ethanol. Based on the results, it was recommended that the best duration to use *H. violacea* cephalopharyngeal skeleton for mPMI analysis was within 7 days after preservation. Another point to note is that the effect of preservative solutions on blow fly larvae might be species specific even the general appearance of larvae of two different species was similar<sup>[27]</sup>. We suggest more studies to be conducted using different species and different stage of development to learn the benefits of utilizing morphometry of cephalopharyngeal skeletons in mPMI estimation.

It was also found from this study that *H. violacea* cephalopharyngeal skeleton (MH-DC and MH-VC) correlated with body length and this indicates the potential of utilizing cephalopharyngeal skeleton length to chart the larval growth of this species. However, both cephalopharyngeal skeleton and body length were vulnerable to violation of normality assumptions and must be treated carefully. Although there were positive relationships between cephalopharyngeal skeleton and body length, we suggest future research to increase sample size and add more regular intervals for sampling to improve the strength between variables. Previous reports on dipteran larvae show that during feeding activity, cephalopharyngeal skeleton parts were correlated<sup>[28]</sup> but the utilization of this information in forensic entomology has not been recorded elsewhere. The importance of geometric morphometry of cephalopharyngeal skeleton in forensic blow flies has been highlighted<sup>[17]</sup> but it was not extended to the development of larvae for mPMI estimation whilst the use of larval length or weight to determine growth of insects could lead to error in mPMI estimation if not evaluated correctly<sup>[9]</sup>.

As an effort to minimize potential errors of mPMI estimation using larval body length that provides inconsistent measurement, we proposed cephalopharyngeal skeleton morphometry to be considered an alternative for estimating the age of larvae. Cephalopharyngeal skeleton can be represented by the lengths of MH-DC, MH-VC or ADP-DC. However, the application of measurement using cephalopharyngeal skeleton must also consider the effect of being stored in prolonged duration in 70% ethanol as it might affect the length.

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### 6. References

- Grassberger M, Reiter C. Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. *Forensic Science International*. 2001; 120(1-2):32-36. doi:10.1016/S0379-0738(01)00413-3.
- Day DM, Wallman JF. Width as an alternative measurement to length for post-mortem interval estimations using *Calliphora augur* (Diptera: Calliphoridae) larvae. *Forensic Science International*. 2006; 159(2-3):158-167. doi:10.1016/j.forsciint.2005.07.009.
- Wells JD, LaMotte LR. Estimating maggot age from weight using inverse prediction. *Journal of Forensic Sciences*. 1995; 40(4):585-590.
- Zhu GH, Ye GY, Hu C, Xu XH, Li K. Development changes of cuticular hydrocarbons in *Chrysomya rufifacies* larvae: Potential for determining larval age. *Medical and Veterinary Entomology*. 2006; 20(4):438-444. doi:10.1111/j.1365-2915.2006.00651.x.
- Bourel B, Callet B, Hédouin V, Gosset D. Flies eggs: A new method for the estimation of short-term post-mortem interval? *Forensic Science International*. 2003; 135(1):27-34. doi:10.1016/S0379-0738(03)00157-9.
- Amendt J, Richards CS, Campobasso CP, Zehner R, Hall MJR. Forensic entomology: Applications and limitations. *Forensic Science, Medicine & Pathology*. 2011; 7(4):379-392. doi:10.1007/s12024-010-9209-2.
- Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJR. Best practice in forensic entomology--standards and guidelines. *International Journal of Legal Medicine*. 2007; 121(2):90-104. doi:10.1007/s00414-006-0086-x.
- Sharma R, Garg RK, Gaur JR. Various methods for the estimation of the post mortem interval from Calliphoridae: A review. *Egyptian Journal of Forensic Sciences*. 2015; 5(1):1-12.
- Baqué M, Amendt J. Strengthen forensic entomology in court-the need for data exploration and the validation of a generalised additive mixed model. *International Journal of Legal Medicine*. 2013; 127(1):213-223. doi:10.1007/s00414-012-0675-9.
- Adams ZJO, Hall MJR. Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. *Forensic Science International*. 2003; 138(1-3):50-61. doi:10.1016/j.forsciint.2003.08.010.
- Midgley JM, Villet MH. Effect of the killing method on post-mortem change in length of larvae of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) stored in 70% ethanol. *International Journal of Legal Medicine*. 2009; 123(2):103-108. doi:10.1007/s00414-008-0260-4.
- Tantawi TI, Greenberg B. The effect of killing and preservative solutions on estimates of maggot age in forensic cases. *Journal of Forensic Sciences*. 1993; 38(3):702-707.
- Rosilawati R, Omar B, Syamsa R, Lee HL, Nazni WA. Effects of preservatives and killing methods on

- morphological features of a forensic fly, *Chrysomya megacephala* (Fabricius, 1794) larva. Tropical Biomedicine. 2014; 31(4):785-791.
14. Midgley JM, Villet MH. Development of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. International Journal of Legal Medicine. 2009; 123(4):285-292. doi:10.1007/s00414-008-0280-0.
  15. Richards CS, Rowlinson CC, Hall MJR. Effects of storage temperature on the change in size of *Calliphora vicina* larvae during preservation in 80% ethanol. International Journal of Legal Medicine. 2013; 127(1):231-241.
  16. Bunchu N, Thaipakdee C, Vitta A, Sanit S, Sukontason K, Sukontason KL. Morphology and developmental rate of the blow fly, *Hemipyrellia ligurriens* (Diptera: Calliphoridae): Forensic entomology applications. Journal of Parasitology Research. 2012: 1-10. doi:10.1155/2012/371243.
  17. Nuñez JA, Liria J. Cephalopharyngeal geometric morphometrics in three blowfly species (Diptera: Calliphoridae). Journal of Entomology and Zoology Studies. 2016; 4(1):338-341.
  18. Ahmad Firdaus MS, Marwi MA, Syamsa RA, Zuha RM, Ikhwan Z, Omar B. Morphological descriptions of second and third instar larvae of *Hypopygiopsis violacea* Macquart (Diptera: Calliphoridae), a forensically important fly in Malaysia. Tropical Biomedicine. 2010; 27(1):134-137.
  19. Omar B, Marwi MA, Oothuman P, Othman HF. Observations on the behaviour of immatures and adults of some Malaysian sarcosaprophagous flies. Tropical Biomedicine. 1994; 11:149-153.
  20. Ahmad NW, Lee HL, Chen CD. Comparative insect fauna succession on indoor and outdoor monkey: Carrions in a semi-forested area in Malaysia. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(2):S232-S238. doi:10.1016/S2221-1691(11)60161-5.
  21. Kurahashi H, Benjaphong N, Omar B. Blow flies (Insecta: Diptera: Calliphoridae) of Malaysia and Singapore. Raffles Bulletin of Zoology. 1997; 5:1-88.
  22. Heo CC, Latif B, Silahuddin SA, Nazni WA, Omar B. Morphological descriptions on the larvae of *Hypopygiopsis fumipennis* (Walker, 1856) (Diptera: Calliphoridae). Tropical Biomedicine. 2015; 32(1):151-159.
  23. Chen CD, Nazni WA, Lee HL, Jeffery J, Wan Norjuliana WM, Abdullah AG *et al.* Larval growth parameters and growth rates of forensically important flies, *Hypopygiopsis violacea* Macquart, 1835 and *Chrysomya rufifacies* Macquart, 1842. Proceedings of the 3rd ASEAN Congress of Tropical Medicine and Parasitology. 2008; 3:97-100.
  24. Mohd Zachery K, Adibah MS, Wan Nurainie WI. Developmental stages of *Hypopygiopsis violacea* (Family: Calliphoridae), a forensically important blowfly on rat carcass. Borneo Journal of Resource Science and Technology. 2015; 5(1):26-33.
  25. Gong P, Wang J, Liu B, Ru G, Feng J. Dissolution of chitin in aqueous KOH. Cellulose. 2016; 23(3):1705-1711. doi:10.1007/s10570-016-0932-z
  26. Nateeworanart S, Bunchu N, Vitta A. Cephalopharyngeal skeleton measurement of *Chrysomya megacephala*, third instar larvae, collected from Naresuan University, Phitsanuk Province. Journal of the Medical Technologist Association of Thailand. 2010; 38(1):3100-3105.
  27. Day DM, Wallman JF. Effect of preservative solutions on preservation of *Calliphora augur* and *Lucilia cuprina* larvae (Diptera: Calliphoridae) with implications for post-mortem interval estimates. Forensic Science International. 2008; 179(1):1-10. doi:10.1016/j.forsciint.2008.04.006.
  28. Pettitt FL. Distinguishing larval instars of the vegetable leafminer, *Liriomyza sativae* (Diptera: Agromyzidae). Florida Entomologist. 1990; 73(2):280-286.
  29. Allen P, Bennett K. SPSS for the Health & Behavioural Sciences. Edn 1, Thomson Learning, Victoria, 2008, 84.