

## Brief communication (Original)

# Evaluation of bait attractiveness for forensically important flies in lowland and montane forest in Peninsular Malaysia

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**Background:** Blowflies (order: Diptera, family: Calliphoridae) and house flies (order: Diptera, family: Muscidae) are important indicators to determine postmortem intervals in forensic entomology. Knowledge of the ecology and bionomics of individual species is necessary for the use of these flies as forensic indicators. Baited trapping is often employed to collect flies for such studies. However, the effect of baits on the diversity and abundance of baited trap collections is not known.

**Objective:** To compare the sampling effectiveness of several types of bait in terms of diversity and abundance of forensically important flies.

**Materials and methods:** The effectiveness of *belacan* (fermented shrimp paste), sugar, beef, ox liver, and fish were tested in three locations at different altitudes in Malaysia; Gombak Field Study Station (about 200 m above sea level (a.s.l.); N3°19'28", E101°45'09"), Awana (about 1100 m a.s.l.; N3°24'03" E101°46'59") and Bukit Cincin (about 1700 m a.s.l.; N3°25'58" E101°47'05").

**Results:** Fish consistently attracted more flies and species than other baits. Beef and ox liver produced moderate or inconsistent results. Flies were rarely attracted to preserved baits such as shrimp paste or odorless baits such as sugar. At higher elevations, flies were attracted to a wider variety of baits. Meat may cause a bias towards mated gravid females and produce a biased sample of the true population.

**Conclusion:** Fish produced the most consistent results in terms of highest number of flies collected and wide diversity of species sampled, odor, especially from rotting meat, is important in attracting carrion flies.

**Keywords:** Calliphoridae, Diptera, forensic entomology, Muscidae, sampling methodology

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Blowflies (order: Diptera; family: Calliphoridae) and houseflies (order: Diptera; family: Muscidae) are commonly used for forensic investigations to estimate the post-mortem interval (PMI) of a cadaver [1, 2]. However, fly species have specific rates of development [1, 2], and different habitat types (e.g. forests, urban areas, agricultural areas) have different species assemblages [3-6]. Identifying the species that are unique to certain habitats allows dipterans to be used as a geographical indicator, which is useful in cases where the body has been moved [2]. Collection methods must be able to collect large

numbers of fly species in a habitat and be cost-effective. Unlike the use of animal carcasses as models for cadavers, which are costly, time consuming and lack the replication required for statistical analysis [7], trapping is a relatively simple method used for rapid assessment of biodiversity, distribution, and ecology [8,9,10]. Baited trapping has been conducted in tropical South-East Asia, but the bait used varies between studies and therefore the effects of different baits in field studies are a potential source of bias [5, 6, 11-13].

We conducted a field test of five different types of bait in three different habitats to compare the sampling effectiveness of each bait for forensically important carrion flies in terms of diversity and abundance.

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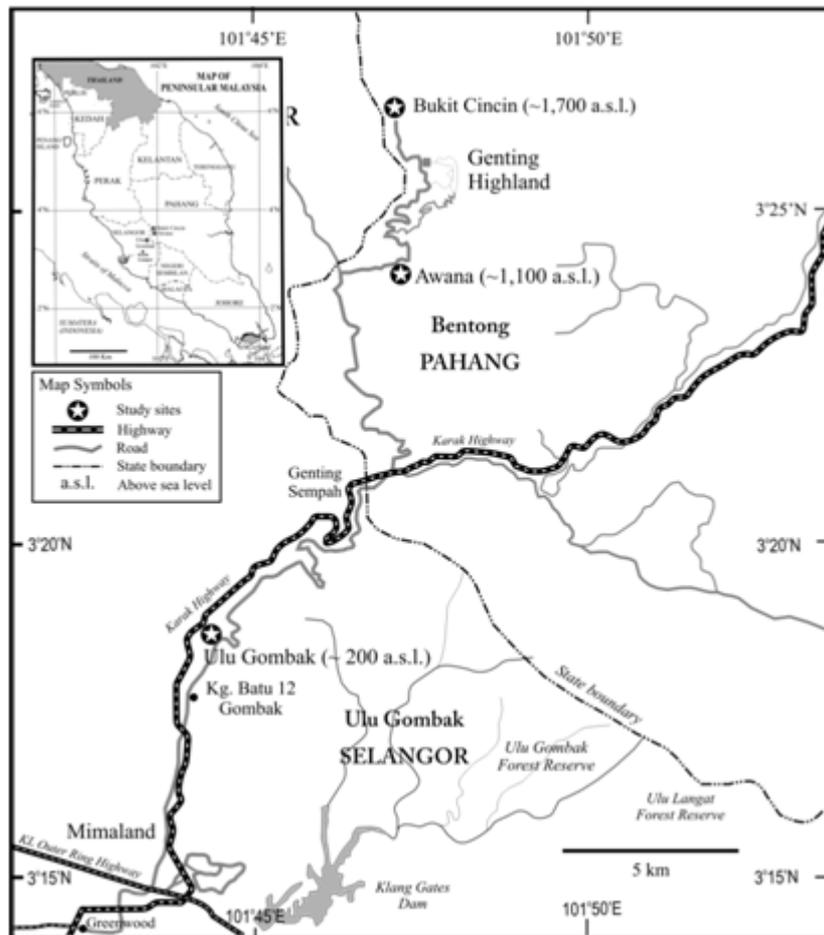
## Materials and methods

The study was conducted between July to August 2009 at three different locations (**Figure 1**); Bukit Cincin, Pahang (N3°25'58" E101°47'05", upper montane forest, about 1700 m above sea level (a.s.l.), mean temperature = 24.2°C, mean humidity = 63.6%), Awana, Pahang (N3°24'03" E101°46'59", open field next to transitional forest, about 1100 m a.s.l., mean temperature = 33.4°C, mean humidity = 60.5%) and Ulu Gombak, Selangor (N 3°19'28" E101°45'09", lowland forest, about 200 m above sea level, mean temperature = 27.7°C, mean humidity = 81.3%). The floristic characteristics of the study sites can be found in Nakashizuka et al. [14].

The trapping procedures used in this study were described by Nazni et al. [6]. Cuboidal metal fly traps [25.0 cm (length) × 25.0 cm (breadth) × 30.0 cm (height)] were used to capture the flies. Baits used in this study were fermented shrimp paste (*belacan*), beef, ox liver, fish (bigeye scads, *Selar*

*crumenophthalmus*), and sugar. The baits weighed 25 g each and placed in separate containers. Twenty-five traps (5 traps for each bait) were set and three replicates were conducted in each study site. Traps were set at the edges of forested areas to minimize the effects of vegetation. The traps were set at 0900 and collected at 0830 on the following day. The flies were identified using published taxonomy keys [15-18].

To compare the effectiveness of each bait in terms of diversity, all samples in a location were pooled. The estimated number of species in a location and 95% confidence intervals were calculated using the Chao 2 estimator. The observed number of species for each bait after 15 samples was then calculated using rarefaction (Mao  $\tau$ ) and the 95% confidence intervals were calculated. Both values were then plotted to show degree of diversity sampled by each bait in each location. All calculations were conducted using EstimateS software [19].



**Figure 1.** Location of (1) Bukit Cincin, Pahang, (2) Awana, Pahang, and (3) Ulu Gombak, Selangor

**Results and discussion**

Fish was the most effective bait measured by raw abundance (Table 1) and consistently high diversity in all locations (Figure 2). The difference between fish and other baits in terms of species richness is significant ( $p < 0.05$ ) in the Gombak and Awana, but not in the Bukit Cincin because of overlapping of 95% confidence intervals. This difference in observed diversities is the result of the large numbers of individuals per sample effort produced by fish (Table 1), as more individuals sampled translates into more species [8]. However, there is no significant difference between the observed species richness of fish and ox liver in Bukit Cincin (Figure 2a). In habitats with few species (Bukit Cincin), a bait that attracts smaller numbers of flies may produce a relatively accurate estimation of the diversity present by random chance, but this may not be the case in habitats of high diversity (Gombak). When the observed number of species is compared with the estimated number of species, in Gombak and Awana most baits aside from fish failed to capture

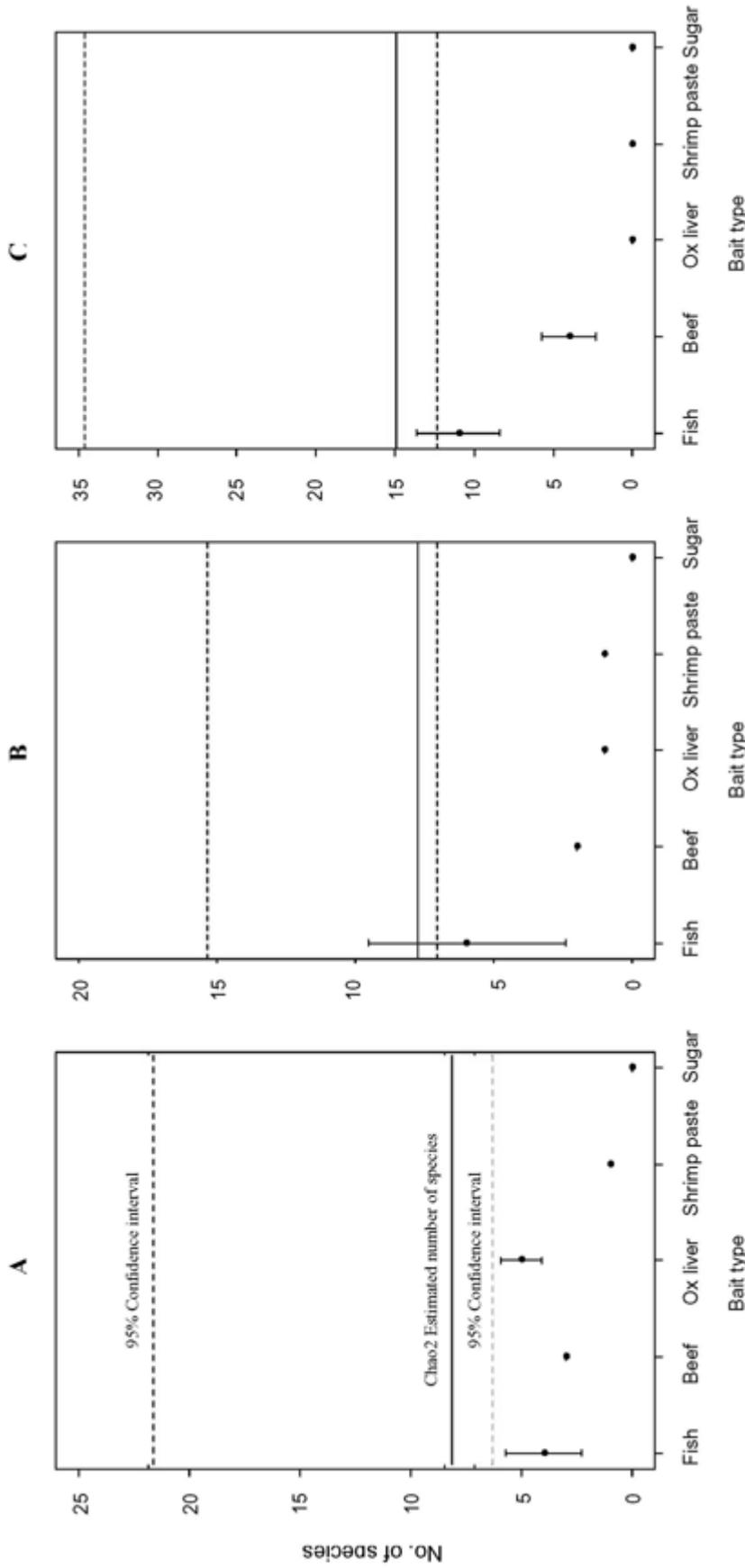
an accurate representation of the diversity of those location.

The odor of rotting meat was highly attractive to Calliphorid flies, and fish that was left to rot for several days were more attractive to flies compared with fresh fish. The poor performance of beef and ox liver in this study may be the result of the absence of strong odors and loss of blood and body fluids resulted from defrosting these baits. Bunchu et al. [20] noted that *Chrysomya megacephala* was attracted to pig viscera more than fish in a wind tunnel test, however the distances in the field were much further than wind tunnel and fish may produce an odor plume that travels further than one produced by organ meat. Consistent with findings by Bunchu et al. [20] and Heo et al. [11], dried food products such as shrimp paste did not fare well with flies, possibly because of preservatives being present. Odorless baits such as sugar did not have any effect on Calliphorids or Muscids, but it did attract small numbers of Sarcophagids and Platystomatids at higher elevations.

**Table 1.** Number of adult flies obtained from fly traps containing different baits (n = 15)

Species	Study sites/ baits/ sample size (n)														
	Bukit Cincin					Awana					Gombak				
	F	O	B	F	S	F	O	B	F	S	F	O	B	F	S
<b>Family: Calliphoridae</b>															
<i>Calliphora fulviceps</i>		2													
<i>Chrysomya chani</i>															6
<i>Ch. deflexa</i>									1				2		24
<i>Ch. megacephala</i>	2	21	4	68				5	30				3		309
<i>Ch. nigripes</i>															1
<i>Ch. pinguis</i>		8	5												
<i>Ch. rufifacies</i>															6
<i>Ch. villeneuve</i>									1						15
<i>Hemipyrellia ligurriens</i>															1
<i>Lucilia porphyrina</i>		14	11	16			1						2		2
<i>L. sinensis</i>		1											1		
<b>Family: Muscidae</b>															
<i>Musca</i> sp.						1			1						
<i>Neomyia</i> sp.				1											1
<i>Ophyra chalcogaster</i>								1	4						6
<i>O. spinigera</i>				2											32
<i>Synthesiomyia nudiseta</i>									1						
<b>Total flies (%)</b>	2	46	20	87	-	1	1	6	38	-	-	-	8	403	-
	(1.3)	(29.7)	(12.9)	(56.1)		(2.2)	(2.2)	(13.0)	(82.6)				(2.0)	(98.1)	
<b>Grand total (%)</b>			155	(100)				46	(100)				411	(100)	

F = fermented shrimp (*belacan*), O = ox liver, B = beef, F = fish, S = sugar



**Figure 2.** The estimated number of species present compared to the observed number of species for Bukit Cincin (A), Awana (B), and Gombak (C). The solid line represents the Chao 2 estimate for the number of species present in each location, while the dotted lines represent the 95% confidence intervals. Black error bars represent the 95% confidence interval for the observed number of species for each bait. Dots without error bars represent samples that are too small for the calculation of 95% confidence interval.

The majority of the flies captured were female, similarly reported by Omar et al. [5]. Stoffolano et al. [21] showed that male and virgin female *Phormia regina* tend to be more attracted to fecal material, while mated females prefer rotting meat. The studies showed that meat attracted substantially higher number of females [22, 23]. The higher protein requirements of female *Ch. megacephala* were believed to be the cause of this behavioral difference between sexes [23]. Studies on baited trapping using meat should acknowledge this inherent bias in the sampling method.

In conclusion, fish produced the most consistent results in terms of number of flies collected and the proportion of species diversity sampled; however, conclusions based on such studies should be treated with caution as this bait tends to produce bias when used to sample a fly population. Odor produced from rotting meat appears to play a very important role in attracting carrion flies.

#### Acknowledgements

The authors thank the Medical Entomology Unit, Institute for Medical Research, Kuala Lumpur for providing the fly traps, Mr. Lucas Low Van Lun for helping with the format of this paper, an anonymous reviewer and Mr. Gary Sing Kong Wah for their helpful comments and University of Malaya for funding this research (Project No. RG009/09SUS). The authors have no conflicts of interest to report.

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