Background

Forensic entomology investigation is based on the understanding of the distribution and bionomic of the sarcosaprophagous insect community. Although there are some species of flies, namely *Chrysomya megacephala* (Fabricius) and *Ch. rufifacies* (Macquart) (Calliphoridae), that can colonize corpses or carcasses under various environmental conditions (Lee et al. 2004; Sukontason et al. 2005; Syamsa et al. 2017), there are other fly species that only show preferences towards specific environmental conditions. For example, *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* (Meigen) (Calliphoridae) species are commonly found in man-made environments in Europe (Vanin et al. 2008; Pohjoismaki et al. 2010). The phorid flies and the muscid fly *Synthesiomyia nudiseta* (Wulp) (Muscidae) are known to colonize the corpses or carcasses in the building (Omar et al. 1994; Reibe and Madea 2010; Syamsa et al. 2012; Syamsa et al. 2017), while flies *Eristalis* spp. of Syrphidae families tend to frequent corpses or carcasses in a watery environment (Lee et al. 2004; Ahmad et al. 2007; Heo et al. 2008; Magni et al. 2013).

However, there is a dearth of scientific studies related to forensically important flies in an aquatic environment. In this article, we report three forensic cases of the body found in aquatic areas in Kuala Lumpur, Malaysia. We...
also describe the challenges faced by entomologists and forensic investigators when dealing with such cases, particularly to preserve the entomological evidence as well as to determine the postmortem interval (PMI).

**Materials and methods**

All three cases involving bodies found in aquatic environments in Kuala Lumpur, Malaysia (3.10°N, 101.73°E), which experiences uniform temperature throughout the year with an average of 27 °C and annual relative humidity is approximately 80%. Insect specimens were collected from the bodies during autopsies performed at the Forensic Unit, Department of Pathology, Hospital Universiti Kebangsaan Malaysia. Entomological studies were carried out at the Forensic Entomology Laboratory, Faculty of Medicine, Universiti Kebangsaan Malaysia. Eggs and larvae were collected using blunt forceps based on the prescribed method by Amendt et al. (2007). The number of larvae collected was depended on the number of larvae available on the body. All of the larvae were collected if fewer than 100 larvae were observed or and approximately 1 to 10% of the were larvae collected if thousands were available (Amendt et al. 2007).

The specimens were collected in two sets (1) preserved in glass vials containing 70% ethanol, and (2) cultured on beef liver provided ad libitum in plastic containers measuring 5 cm × 5 cm × 4 cm. Ambient temperature and relative humidity during the rearing process were recorded every 30 min by placing a thermohygrometer in the rearing room. Newly emerged adult flies were pinned for identification using identification keys (Kurashashi et al. 1997). Preserved larval samples were prepared according to the method described by Omar et al. (1994). The stage, size, and species of the larvae were subsequently observed under a light microscope for identification based on the keys of Omar (2002). Documentation of adult and larva species was conducted using a Leica EZ4D digital microscope fitted with Leica Application Suite (Leica, Switzerland).

**Case presentation**

**Case 1**

A 40-year-old male body was found in the waterside of an abandoned mine. He was fully clad and was in an active decomposition state. Thousands of blowfly *Ch. megacephala* larvae were found crawling mainly on clothes at the abdominal area (Fig. 1).

**Case 2**

A body of a 43-year-old male was found floating on a river stream of Sungai Klang, Kuala Lumpur. He was fully clad with a pair of long pants and a shirt and was in a bloated stage of decomposition. The entomological evidence was collected during the autopsy, consisting of L3 larvae of *Ch. megacephala* wandering around the clothes. Only five larvae of *Eristalis* spp. were present on the body of the deceased. An attempt to rear the larvae was not successful as it did not reach pupation (Fig. 2).

**Case 3**

The bloated body of an 11-year-old boy was found trapped in a monsoon drain in Cheras, Kuala Lumpur. It was believed that the deceased slipped into the drain while walking in heavy rain, 2 days before the body was found. There were several injury marks on his head, most probably due to the hits that occurred as a result of the strong current taking him in the storm drain. Entomological specimens were collected during an autopsy. Fly eggs were found in abundance especially along the waist area. Thousands of various-sized larvae identified to be blowfly...
Ch. megacephala and Ch. rufifacies were found crawling mainly on the neck and clothes on the upper half part of the body. However, only three Eristalis spp. larvae were noted moving up and down on the lower part of his body (Fig. 3). Unfortunately, the reared larvae were unable to pupate and become adults, hence hindering the species identification.

**Discussion**

Observations on the number of larvae presence on corpses found that its frequency was relatively low (Fig. 3) compared to the number of larvae that could be found on a corpse on land in the outdoor environment (Heo et al. 2007; Heo et al. 2008). Apart from the factors of the river current that may take away some of the entomological evidence, the reduced numbers of larvae on corpses may also be due to the technique of pulling the body ashore by the forensic officers which may cause most of the larvae or eggs on the body to be dislodged into the water. Therefore, training and improvements in the aspect of handling corpses found in aquatic environments should be conducted to preserve the entomological evidence as best as possible.

The presence of Ch. megacephala immature stages in all three cases strengthened the fact that it is the most forensically dominant fly species in Malaysia. This species can survive and compete successfully in various types of habitats under tropical climates (Lee et al. 2004; Syamsa et al. 2017), including aquatic environments as highlighted in this study. Similar observation was also reported by Sukontason et al. (2005) in Thailand where they recorded the presence of flies Ch. megacephala and Ch. rufifacies on corpses found in water reservoir areas. In Italy, among other flies recorded infesting bodies in aquatic environments were Ca. vicina and Ch. albiceps (Wiedemann) (Calliphoridae), Fannia sp. (Fannidae), Ophyra sp. (Muscidae), and Syritta pipiens Linnaeus (Syrphidae) (Magni et al. 2013).

One of the notable findings of this study was the presence of Eristalis spp. larvae in the second and third cases. The presence of Eristalis spp. of the Syrphidae family, also known as rattail maggot due to its unique morphology, indicates that this species is of forensic importance in Malaysia. This finding was also supported by Lee et al. (2004) and Ahmad et al. (2007), who also stated that this species could be an indicator of the death location as its immature stages require an aquatic environment to live and thrive. In the USA, Lindgren et al. (2015) conducted a year-long case study on simulated cadavers, observing the presence of Eristalis arbustorum larvae on one of the cadavers that were partially submerged in a grave filled with rainwater. Similarly, Archer and Ranson (2005) recorded the occurrence of this genus on decomposing bodies found in freshwater at Victoria, Australia. All of these studies agree that the preferences of this particular species on corpses associated with aquatic environments could be useful in forensic investigation, particularly to determine whether the body has been moved from one place to another.

Typically, corpses and carcasses in aquatic environments such as ponds or swamps will experience a series of submerging and floating phases (Mann et al. 1990; Heo et al. 2008; Magni et al. 2013; Ramos-Pastrana et al. 2019; Dalal et al. 2020). During this floating phase, forensically important flies will colonize the corpse and can be used for PMI estimation (Mann et al. 1990). However, the accuracy of PMI estimations is highly dependent on insect biology, including environmental preferences and constraints (Introna et al. 2011). Therefore, the PMI for all these cases could not be estimated due to the lack of information about the decomposition process for submerged bodies, especially in the aquatic environment in Malaysia. This is because most of the studies in the field of forensics emphasized on the terrestrial environment, with only 15% of research involving exposure to the aquatic environment (Merritt and Wallace 2010).

For case 1 of the current study, the body was in an active decomposition stage, while for both case 2 and case 3, the bodies were in the bloated stage of decomposition. The decomposition process may undergo modifications due to the corpse being exposed to low water temperature, which will slow down the decomposition process and affect the colonisation and the development of forensically important flies. Heo et al. (2008) conducted a study on the faunal distribution of pig carcasses placed in ponds. After a while, it was found that the pig carcass sank to the bottom of the pond before returning to float on the surface on the third day. Blowfly Ch. megacephala and Ch. rufifacies, on the other hand, were

![Fig. 3](Image) Various sizes of fly larvae were seen infesting the body of case 3.
found to only start laying eggs on corpses on the fourth day. This indicates that the process of insect colonization of corpses found in aquatic environments can be delayed up to 4 days. More experimental studies should be conducted to ascertain the insect preference and behavior related to this specific condition. Heo et al. (2008) also observed that the activity of flies on pig carcasses in aquatic environments was not as active as the activity of flies observed on carcasses placed on land (Heo et al. 2007). Therefore, extra precaution is required when analyzing entomological evidence collected from an aquatic environment to avoid misinterpretation and errors in estimating PMI.

Haskell et al. (1989) stated that several factors influence the process of colonization of insects in watery areas including the size and position of the corpse, the depth of water, and the speed of water flow. According to Magni et al. (2013), the determination of PMI for submerged corpses should take into account several important parameters such as the process of limb disintegration experienced by corpses, adipocere formation, and collection techniques of entomological specimens in aquatic environments. In addition to the ambient temperature, the water temperature at the time of submergence plays a vital role in the development of fly larvae (Myskowiak and Doums 2002; Ames and Turner 2003). Hence, the knowledge of the biology of forensically important flies at low temperatures is critical to assist forensic entomologists in determining accurate PMI estimation. This requires expertise from various disciplines to ensure the reliability of the evidence and information obtained.

A laboratory study by Singh and Bala (2011) on larval Ch. megacephala and Ch. rufifacies found that the survival rates were inversely proportional to the period the larval had been immersed in the water. The stage of larvae found on the corpse when it underwent a sinking phase is also an important factor that determines the rate of larval survival. The lowest survival rate was observed in young 10-h-old larvae which were unable to withstand immersion periods of more than 2 h. For instar III larvae, the immersion period of more than 5 h was sufficient to provide 100% mortality on larval survival (Singh and Bala 2011). In addition to the larval stage, the immersion factors on pupa stage survival were also studied (Reigada et al. 2011). The pupa age factor plays an essential role in determining survival with longer soaking periods giving lower survival rates. Magni et al. (2021) studied the survival and eclosion of Ca. vomitoria (Linnaeus) (Calliphoridae) and L. sericata in-puparial forms after submersion in various types of water. Both species were shown to have a higher survival rate in tap water than in river or salt water. Whereas the eclosion time after submersion was influenced by the age of in-puparial forms when immersed, the types of water, and the duration of submersion. All of these studies are very useful in the investigation of cases of corpses found in the aquatic environment, especially for cases where the corpse undergoes a submerged phase after the larvae develop and enter the pupa stage.

Conclusions
The present study provides additional knowledge in the context of Malaysian forensic entomology investigations and the distribution of forensically important flies, especially in aquatic areas. Eristalis spp. has shown exclusive preference on corpses found in aquatic areas and its occurrence habitat other than this may indicate the movement of the body after death. However, descriptions with regard to their potential use in forensic entomology are lacking. Therefore, a study on this genus is crucial to create a deeper understanding of carrion ecology, especially related to forensic entomology work in aquatic areas.

Abbreviation
PMI: Postmortem interval.

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Authors’ contributions
Abdullah SR collected and analyzed the entomological specimens, and was a major contributor in writing the manuscript. Swarih MS and Shahrom AW supervised and assisted the specimen collection process in the mortuary. All authors have read and approved the submitted manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
This project obtained ethical approval from Universiti Kebangsaan Malaysia Ethics Committee with the reference number FSK/BIO/MED/2011/BAHARUDIN/27-JANUARY/356-FEBRUARY-2011-FEBRUARY-2013.

Consent for publication
Written informed consent for publication was obtained from the relative of the deceased.

Competing interests
The authors declare that they have no competing interests.

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References
Ahmad FMS, Marwi MA, Jeffery J, Hamid NAA, Zuha RM, Omar B (2007) Review of forensic entomology cases from Kuala Lumpur Hospital and Hospital Universiti Kebangsaan Malaysia. 2002. J Trop Med Parasitol 30:51–54.
Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc-HN, Hall MJR (2007) Best practice in forensic entomology - standards and guidelines. Int J Leg Med 121(2):90–104
Arnes C, Turner B (2003) Low temperature episodes in development of blowflies: implications for postmortem interval estimation. Med Vet Entomol 17(2):178–186
Archer MS, Ranson DL (2005) 3. Potential contamination of forensic entomology samples collected in the mortuary. Med Sci Law 45(1):89–91
Dalai J, Sharma S, Bhardwaj T, Dhattarwal SK, Verma K (2020) Seasonal study of the decomposition pattern and insects on a submerged pig cadaver. J Forensic Leg Med 74:102023
Haskell NH, McShaffrey DG, Hawley DA, Williams RE, Pless J (1989) Use of aquatic insects in determining submersion interval. J Forensic Sci 34(3):622–632
Heo CC, Marwi MA, Ahmad Firdaus MS, Jeffery J, Omar B (2007) A preliminary study of insect succession on a pig carcass in a palm oil plantation in Malaysia. Trop Biomed 24(2):23–27
Heo CC, Marwi MA, Jeffery J, Omar B (2008) Insect succession on a decomposing piglet carcass placed in a man-made freshwater pond in Malaysia. Trop Biomed 25(1):23–29
Introna F, De Donno A, Santoro V, Pecchio F et al (2011) The bodies of two missing children in an enclosed underground environment. Forensic Sci Int 207:e40–e47
Kurahashi H, Benjaphong N, Omar B (1997) Blow flies (Insecta: Diptera: Calliphoridae) from a human corpse in a high-rise building in Malaysia: a case report. Trop Biomed 29(1):107–112
Syamsa RA, Omar B, Ahmad FMS, Shahrom AW et al (2012) An occurrence of Synthesiomyia nudiseta (Wulp)(Diptera: Muscidae) from a human corpse in an enclosed underground environment in Kuala Lumpur. J Forens Legal Med 45:41–46
Vanin S, Tasinato P, Ducolin G, Terranova C, Zancaner S, Montisci M et al (2008) Use of Lucilia species for forensic investigations in Southern Europe. Forensic Sci Int 177(1):37–41

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