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早期腐敗對麗蠅產卵之影響

Effects of early decomposition time on blow fly (Diptera: Calliphoridae) oviposition

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口試委員會審定書

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本論文係廖朝盛君(R02632012)在國立臺灣大學昆蟲學研究所完成之碩士論文，於民國 105 年 1 月 7 日承下列考試委員審查通過及口試及格，特此證明

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中文摘要

當麗蠅停在屍體上決定要產卵並拓殖 (colonize) 到屍體上時，同時也啟動了麗蠅的生命週期，生命週期的開始與結束也將會在屍體上完成。人們可以將這些重要的資訊應用在刑事調查的死後間隔時間 (Post-mortem interval, PMI) 之估算。從死亡發生到麗蠅完成其生命週期的屍體腐敗過程可以分為五個階段，屬於前拓值期 (pre-colonization stage) 的暴露期 (exposure phase)、偵測期 (detection phase) 和接受期 (acceptance phase); 屬於後拓值期 (post-colonization stage) 的消耗期 (consumption phase)以及散佈期 (dispersal phase)。先前大部分的研究主要都致力於後拓值期，不同因子對幼蟲和成蟲生長發育的影響 (如溫度、濕度等等…)。然而前拓值期的研究也是很重要，卻十分的缺乏。此研究主要針對大頭金蠅 (*Chrysomya megacephala*) 的接受期進行研究。接受期的定義為麗蠅找到屍體到決定產卵的這段時期，產卵延遲會導致接受期的延長，進而導致 PMI 估計的偏差。在此研究中，我們假設不同腐敗程度的豬肝可能會影響大頭金蠅對產卵的決定。在野外試驗中，當我們使用不同腐敗程度的豬肝當做產卵基質時，結果顯示在不同處理間的卵數具有顯著差異 (新鮮~腐敗 8 天)。而在偏好實驗中，不同的處理間的卵數也具有顯著差異(新鮮、腐敗 2 天、腐敗 4 天、腐敗 6 天、腐敗 8 天)。我們的結果指出不同腐敗程度確實會影響麗蠅的產卵；而當我們提供單一腐敗程度的產卵基質來測試不同腐敗程度的產卵率差異時，結果顯示不同腐敗程度間的產卵率確實是有顯著差異的，然而在測試不同腐敗程度的接受期長短時，卻沒有顯著差異。總結來說：不同的腐敗程度確實會影響大頭金蠅的產卵行為，但並不會影響到決定產卵的時間長短，然而在此研究中產卵延遲至 9.83 ± 1.92 小時後的現象值得更進一步的研究與調查，希望這些研究資料能在未來對 PMI 估算上的校正有所幫助。

關鍵詞：麗蠅、產卵、腐敗程度、接受期、前拓殖期、法醫昆蟲學、大頭金蠅。

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Abstract

When blow flies land on the corpse and decide to lay eggs and colonize, it also starts their biological life cycle which will begin and finish on the corpse. People could use those vital information to estimate the post-mortem interval (PMI) in criminal investigations. The process of decomposition can be divided into five phases, from the death occurs to the finish of life cycle of blow flies. Those phases of exposure phase, detection phase and acceptance phase, belong to the pre-colonization stage; consumption phase and dispersal phase belong to the post-colonization stage. Most previous studies focused on the post-colonization stage, which are the study of effects from different factors (e.g. temperature, humidity…etc.) on the development of larvae or adults. However, the pre-colonization stage is also important, but related research is scarce. In this study we focus on the acceptance phase of *Chrysomya megacephala*, which was defined as the period between the corpse finding and the decision making of oviposition of blow flies. Longer time period caused by the delay of oviposition will further increase the bias in PMI estimation. In this study, we assumed that different degrees of decay of pork liver may affect the oviposition decision of the blow flies *C. megacephala*. In field experiment, our results showed significant differences on egg number among groups of using different decay-aged liver as oviposition media (fresh to 8-day-old). In preference test, the results showed significant differences on egg number among groups of using

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different decay-aged liver (fresh, 2-d old, 4-d old, 6-d old, 8-d old). Our results indicated different decomposition levels do affect the blow fly oviposition; and when we provide media of different decomposition level individually to test the incidence of oviposition, the results also show significant difference among different treatment. However, in the test of duration of acceptance phase, the results show the durations from media discovery to oviposition have no significant different for different decay-aged media. In conclusion, different decay-aged media have significant effects on the oviposition of blow fly *C. megacephala*, but no effect on the duration of acceptance phase. However, the delay of egg laying could be up to 9.83 ± 1.92 hours due to media of different decomposition level and should be considered in the future study. Hopefully, these data could be helpful to the adjustment of PMI estimation in the near future.

Key words: Blow fly、oviposition、decomposition level、acceptance phase、pre-colonization phase、forensic entomology、*Chrysomya megacephala*.

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1. Introduction

1.1 Forensic entomology

Forensic entomology is a discipline which dedicate to use arthropod as an evident in the crime scene. Insect is the important target to collect in the crime scene, which can help the investigator to acquire accurate and precise conclusion in the investigation (Catts and Goff, 1992). In forensic entomology, it divided into three different subdomains, which are urban entomology, stored-product entomology and medico-criminal forensic entomology (literature cited in Catts and Goff, 1992). Forensic entomology in narrow sense usually indicate medico-criminal forensic entomology, so medico-criminal forensic entomology will be referred to as forensic entomology hereinafter.

Insect can provide numerous useful information. The advantage of utilizing necrophagous insects in crime scene investigation are as followed (Catts and Goff, 1992). (1) Insect usually can detect the corpse and arrive the crime scene soon after corpse been exposed. (2) Investigator can understand the condition of the corpse by examining the insects gathering on the corpse, life stages or status of insects and different composition of the fauna. For example, blow flies usually will gather and oviposit on the opening wounds or natural orifices (Bourel *et al.*,

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2003). Another example is that, if some insects showed on the corpse where did not belong to their natural habitat, it may indicate that the corpse had been moved (literature cited in Keh, 1985). (3) Because the corpse represents a limit and temporary resource (Beaver, 1977), many insects will compete for colonizing on the corpse and utilizing the corpse as food resource or oviposition site (Thompson *et al.*, 2013). Along the decomposition of the corpse, it will go through several stages which the body will emitted different chemical cues to attract different insects, the fauna around the corpse in each decomposition stage is predictable. (4) The fauna around the corpse usually can provide us useful and vital information, but usually ignored by the investigators.

The earliest forensic entomology study document is in thirteenth century of China, Sung Tzu's *The Washing away of Wrongs*. One section in the book, the investigator used the evidence of gathering flies on a blood tainted sickle, to find out the real suspect (literature cited in Catts and Goff, 1992). Beside to find out the killer, lethal weapon and reason of death, another vital information that investigator need to figure out is the PMI (Post-Mortem Interval).

The definition of PMI is the interval between the time when victim died to the moment when the corpse been discovered (Catts, 1992). It can be obtained by estimating the maximum PMI and minimum PMI. In traditional pathology, PMI

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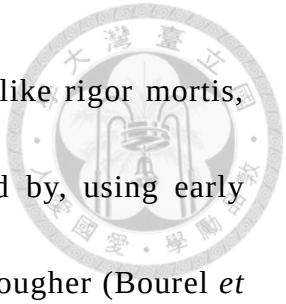

can be estimated by different features showed on the corpse, like rigor mortis, livor mortis or algor mortis, however, along the time passed by, using early postmortem changes on corpses to estimate PMI will become tougher (Bourel *et al.*, 2003; Goff, 2010). In definition, maximum PMI is the time when the victim was last seen alive. Compared with traditional pathology, using insects to estimate minimum PMI may obtain a more precise estimation (Bourel *et al.*, 2003; Greenberg and Kunich, 2002). In traditional pathology, one can only estimate accurately and precisely for the first two or three days after death (Villet *et al.*, 2011). However, to analyze and calculate the age of immature insects (maggots) can precisely estimate the PMI from the first day to several weeks (Amendt *et al.*, 2004). Using insects to estimate minimum PMI becomes an alternative approach. Using accumulated degree hours (ADH) to estimate minimum PMI, by recording the ambient temperature in micro-environment to figure out the larval developmental data with specific species in the early stage of decomposition (Greenberg, 1985; Lord *et al.*, 1986). In the later process of decomposition, the major targets of estimate minimum PMI have shifted from the larval developmental data to the faunal composition (Smith, 1986). The error of minimum PMI acquired from developmental data of larvae can be less than an hour (Gennard, 2007). For applying larval developmental data, three different

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models have been used, which are isomorphen diagrams (Grassberger and Reiter, 2001), isomegalen diagrams (literature cited in Amendt *et al.*, 2011) and thermal summation models (Higley and Haskell, 2010). These developmental models use weights or lengths of larvae to estimate how long do maggots take to development, in order to estimate the minimum PMI.

Along the corpse decomposition, the corpse will go through several different changes. Although the changes of corpse are continuous events which do not have a significant boundary in decomposition process (Goff, 2010), it is easier for data collecting and observation if researchers separate the process into difference stages. Megnin (1894) propose a concept that decomposition of corpses would experience eight stages, each stage has pathological differences, and insects faunal composition presence on the corpse also change in each stage. Nowadays forensic entomologists usually divide decomposition process into four or five stages (Hall and Huntington, 2010). Four stages model in decomposition process are fresh, bloated, decay and remains (Catts and Goff, 1992). Beginning with fresh, which will go through several pathological feature (rigor mortis and livor mortis), no obvious odors emitted from the corpse in this stage, some ants and blow flies will attracted to the corpse several hours after death, depends on the temperature and environmental condition. Microbe continuously metabolize and

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activate, cause huge amount of gas produced inside the abdomen, accompany with slightly odors, numerous blow flies or flesh flies appears, which is the beginning stage of bloated. In the stage of decay, large feeding masses of Dipteran larvae will presence. Larvae will penetrate the skin and cause the body deflated. Some Coleoptera also presence in this stage as predator of larvae. In decay stage, strong and stink odors appears. The last stage is remains (skeletonize). Most of the soft tissues on the corpse has been eaten, left the bones, connective tissues, hair behind. Most of the Diptera larvae already leave the corpse for puparium, only few individuals are left on the remains. Coleoptera are the dominant species in earlier phase of this stage, for consuming those hard tissues. Remain stage can last for years.

In the each stage of decomposition process that researchers established, it is not hard to see the participation of insects. Insects play an important role in the decomposition process, in early stage after death, decomposition is process by the aerobic microbe from the environment fall on the corpse surface, and the anaerobic microbe which already locate inside the body (Thompson *et al.*, 2013) After hatching, maggots will gather to form maggot masses. Maggot masses will penetrate the epidermis, create an entrance for insects and microbe to invade inside the corpse, enlarge exposure area to the outer environment. The ventilation

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through the entrance make the aerobic microbe and anaerobic microbe to re-distribution on the corpse (Thompson *et al.*, 2013). Corpse condition would change along with the different microbe or insects colonized, create an environment that may attract or inhibit other insect in the succession, which believe is the main reason to the different insect faunal composition through decomposition. Most of the soft tissues on corpse are consumed by the insects, and usually is consumed by dipteran larvae.

Upon all the insects attracted by the corpses, we can divided them into four groups as following (Catts and Goff, 1992).

1. Necrophages: Species that use the corpse as main resources to feed, and also produce offsprings on the corpse. These insects usually can find the corpse in a very short period, like most of the blow flies (Calliphorids), flesh flies (Sacrophagids), and some beetles. The larvae of the blow flies are also an important tool to estimate the PMI.
2. Omnivores: Species that utilize corpses and the faunae around the corpse as food resources. Some insects also use corpse as an oviposit location. Ant, rove beetles and some other coleopterans are belonged to this category. Large numbers of species in this category could retard the process of succession by decreasing the amount of other necrophagous insects (Early and Goff, 1986).

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3. Parasites and predator: Species that are predators or parasitoids to faunal members around the corpse, including some hymenopterans (parasite wasps, yellow jackets, etc.), coleopterans as well as dipteran larvae. Some species can be both necrophage and predator, such as *Chrysomya rufifacies*. First instar larvae of *C. rufifacies* are necrophagous, but begin from second instar, they become as the facultative predators, which may use their spine to strangle and capture other larvae as preys (Byrd and Castner, 2009). Some mites and nematodes are also belonged to this category. Smith (1986) considered species in this group are the secondary important members in forensic entomology.
4. Incidentals: Species that accidentally pass by or live in the place where are overlapping with corpses. They use the corpse as a shelter or extension of habitats, like species of springtails, spiders, centipedes or mites (Goddard and Lago, 1985). Some herbivore insects (Hemiptera) will also present on the corpse by accidentally droping from the plants nearby. Different distributions of insects in different environments may provide the information of movement of body, which are also the vital evidences in the crime scene investigations (Catts and Goff, 1992).

Tomberlin *et al.* (2011b) proposed a framework for study forensic entomology and the insect colonization on the corpse. They use the beginning

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time of insect colonization to separate the process into pre-colonization stage and post-colonization stage. The latter one, the post-colonization stage can be further divided into two phases, started with the insect colonization on the corpse, ended up in the insect dispersal from the remains. First phase of the post-colonization stage is the consumption phase, which is the feeding process of arthropods and its offsprings. When arthropods finish their life cycles from the corpse, or there is no more resources can be utilized on remains, arthropods begin to disperse from the remains, which is the second phase of the post-colonization stage, dispersal phase (Tomberlin *et al.*, 2011b). In post-colonization stage, which is the studies and researches were most dedicated in and have more data been documented. However, pre-colonization phase is usually be ignored by the forensic entomologist (Tomberlin *et al.*, 2011a). The importances of the pre-colonization stage is not less than post-colonization stage because it will affect the time gap between actual death and the time when corpse been colonized by insects. The duration between these two events will strongly influence the accuracy of PMI estimation. Which Tomberlin has suggested that researchers should put more efforts in this field.

The pre-colonization stage begins from the happening of death, The first phase in pre-colonization stage is the exposure phase. In exposure phase, corpse

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will emit some chemical cues to attract arthropods, however, insects haven't receive those chemical cues yet. Then is the beginning of the detection phase. Detection phase contains two steps, they are the activation step and searching step. Activation step started when chemical cues was detected by the arthropods, and the searching step activates when the response of arthropods to search for the actual location of the corpse. Acceptance phase begins when arthropods locate the corpse and have the first contact to the corpse. Arthropods will evaluate the media and make decision to colonize or not. Pre-colonization stage ends up with arthropods' actual colonization of consumption and oviposition. Discussion on the acceptance stage in pre-colonization phase is rare (Tomberlin *et al.*, 2011a). What condition could make blow flies take more time than usual, to evaluate and make decision to oviposit on the media, or make blow flies avoid to lay eggs are interesting questions.

In the death scene investigation, the time when victim dead is the major concern that researchers want to know, and this is what minimum PMI estimate for. Because the time of death is acquired from the time period of maximum PMI and minimum PMI, the longer the period between maximum PMI and minimum PMI, the lower the accuracy we acquire. To estimate the PMI correctly, we are strongly relied on a key assumption which the blow flies can detect the corpse

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and laid eggs on corpse soon after death. So the period between the time when victim die and the time of insect colonization should be as short as possible (Catts and Goff, 1992). But this is often invalid, especially in the indoor, sealed environment or under the extreme weather condition (Amendt *et al.*, 2004; Catts, 1992; Catts and Goff, 1992). In the situations which we mentioned above, insect cannot invade the corpse immediately since the lack of physical mechanism to break through the barriers to the corpse, the decomposition is mostly process by bacteria and fungi only. Microbe that occupied inside and outside of the corpse carry on metabolism, cause the nutrition and water loses. These situations are seldom happened in the natural condition but frequently found in homicide cases which the natural succession patterns were usually changed artificially. So how the primary flies like blow flies will react when the corpse condition does not follow the natural succession pattern? We were curious about the reaction of oviposition behavior of blow flies when they encounter different decay-aged treatment individually. If fresh pork liver is a better medium for oviposition and larval development, will blow flies have higher frequency to lay eggs on the fresh treatment? Will blow flies make a quicker decision when they encountering a more suitable media? On the other hand, if the condition of oviposition media is no longer suitable for oviposition, will the frequency of oviposition decline? Will

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the blow flies take longer time to evaluate the media for oviposition or not? In addition, we were wondering if oviposition performance in the laboratory is similar to that in the field? To answer those questions, we prepared media of different decay-aged pork liver for oviposition and preference test.

1.2 Blow flies biology (*Chrysomya megacephala*)

Among all the insects that attracted by the corpse to feed, to copulate or to oviposit, primary flies like blow flies (Calliphoridae) and flesh flies (Sarcophaga) are always the major members, and theirs larvae can be used to estimate the minimum PMI (Catts and Goff, 1992). Blow flies have the potential to discover the corpse in a very short period of time after the corpse been exposed. For instance, it has been shown that blow flies can arrive the corpse few hours after death in the earlier research (Catts and Goff, 1992). Another research showed that blow flies can arrive the corpse within minutes (Byrd and Castner, 2009). In contrast, there is a problem when using flesh flies to estimate minimum PMI. Flesh flies usually have precocious development (Villet *et al.*, 2011). Usually when the oviposition is about to happen, the well-developed eggs will slide through the oviduct and fertilized when it go through the opening of spermathecal duct, then the fertilized, well-developed eggs will be laid on the media (Smith, 1986). However, sometimes the female adults cannot find a suitable media to oviposit right after the eggs have been well

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developed. In this scenario, eggs been pushed through the oviduct and fertilized, but not yet be laid on the media, cause the eggs started the embryonic development inside the females abdomen. Therefore, it may result in the eggs hatched right after eggs been lay. These may cause the overestimate of PMI (Goff, 2010). Fortunately, the precocious development is not common in Calliphoridae species (Erzinclioglu, 1990).

The experimental subject *C. megacephala* (Fabricius) belongs to Diptera, Calliphoridae, and Chrysomyinae. It originally distributed in Oriental and Australia region (Smith, 1986), but now could be found throughout the South America, South Africa and southern United State. They play as an important role in the forensic entomology and used for minimum PMI estimation (Byrd and Castner, 2009). *Chrysomya megacephala* was also known as the oriental latrine fly, body size are from 8.0–10.0 mm, colors are blue or green with metallic luster (Byrd and Castner, 2009). *Chrysomya megacephala* have red compound eyes, holoptic eye in male which have narrow frons. Female have dichoptic eyes which their frons is much wider. Ommatidia arrange pattern is different from the top to the bottom of the compound eye, form an obvious boundary in the middle of the compound eye, which can easily be recognized (Byrd and Castner, 2009). Gena is yellow and covered with yellow hair. *Chrysomya megacephala* life cycle is about one month in the wild, however, it usually can survive 2 months in the laboratory. Larva stage have three instars. When eggs was laid, it usually would hatch within a day,

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the complete larva stage is 5.4 days, and 5.3 days in pupa stage when it in the condition of 27℃ and 60-70% R.H. (Gabre *et al.*, 2005). Before pupation, it will go through the post-feeding stage. In the post-feeding stage, maggots cease feeding behavior, and disperse from the origin food resources for pupation. Egg batch produced by *C. megacephala* is averaging 223.7 eggs each time. After eclosion from the pupa, *C. megacephala* will take 6.6 days to reach sexual maturation and to be ready to oviposit (Gabre *et al.*, 2005). Mating behavior will happen after ovarian maturation (Norris, 1965). In our anatomical observation, female blow flies ovaries maturation are synchronized, and usually lay whole batch mature eggs when oviposit, this observation also meets Browne's description (Browne, 1993).

Chrysomya megacephala is a dominant necrophagous species in Taiwan, it can be attracted to the corpse soon after exposure. *Chrysomya megacephala* use their third segment of aristate antennae to receive the odor (Erzinclioglu, 1996), and it will use their olfactory sensory to track and search the target in long distant. When *C. megacephala* getting closer, visual ability replaced to ensure the location of the oviposition media (Gomes *et al.*, 2007). Mouth, nostril and anal, these natural orifices are the preliminary targets for gravid female to oviposit. Besides the wounds, those natural orifices are the only way to invade inside the corpse, and usually are soft and moisture enough for first instar larvae to feed. When suitable location has been find, female will stretch their

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ovipositor to reach inside the hole, or folds around the body to oviposit, and start the clock of the succession.

In the recent studies, blow flies *C. megacephala* was found to have gregarious oviposition behavior which have tendency to lay eggs together and form an egg bunch, and gregarious effects do not need to be triggered by all females individuals or by conspecific individual (Yang and Shiao, 2014). They found female adults seldom oviposit when they are the only individual that expose to the oviposition media. However, when they used 1 female with 9 males in the same cage, and the single female could laid eggs.

Many factors will affect blow fly oviposition, including solar exposure, wind, heavy rainfall, temperature, etc. (Amendt *et al.*, 2004). Many study showed that blow fly would not oviposit at night (Payne, 1965), however, nocturnal oviposition was still a controversial issue for many years (Greenberg, 1990; Singh and Bharti, 2001). Besides those environmental factors which will influence blow fly activities and oviposition behavior, oviposition media condition will also affect blow fly oviposition. Yang and Shiao (2012) provide different condition of pork livers as an oviposition media, to test the blow fly of *C. megacephala* and *C. rufifacies* preference, their results indicate that blow fly oviposition behavior will affected by media size, conspecific or heterospecific larvae presence on the media. This study also

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indicated that visual ability plays an important role in locate media location and search for the potential competitors.

There were several researches which focused on the media status affect blow flies oviposition behavior. Blow fly *Calliphora vomitoria* have shown preference on the larger carcass, and oviposition behavior will be inhibited when oviposition media is dehydrate (Erzinclioglu, 1996). The moisture condition of the media is also important in maggots' survivorship. When blow fly oviposit on a dehydrated media, it may cause eggs to dehydrate, change and damage the shape of the chorions, cause them difficult to hatch (Davies, 1950). Dehydration of the media will also cause maggots dehydrated, in addition, if the water content of the media were too high, maggots may drown in the media (Erzinclioglu, 1996). However, most of the blow fly species can recognize the dehydrated layer on the oviposition media (Campobasso *et al.*, 2001).

Previous study showed that when facing nutrient depleted media or very aged (8-day-old) media, blow fly and flesh fly have lower colonization rate compared to that on the fresh media (George *et al.*, 2012), however, there was no significant difference between the specimen number on the aged (4-day-old) and the flesh media. In their study, only aged (4-day-old) and very aged (8-day-old) media had been tested, the tendency of colonization along the different decay-

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aged media had not been tested. The effect of media older than 4-day-old on the oviposition behavior of *C. megacephala* remains unknown. In order to know the preference of *C. megacephala* in different decay-aged media, the sequential data of different decay-aged media is needed. In this study, we are going to use *C. megacephala* to test the tendency of oviposition preference along with different decay-aged media, and also to known the possible duration of time differences in oviposition happened on different decay-aged media (acceptance phase). The changes of time interval in acceptance phase which may break the assumption of "blow flies can reach the corpse and oviposit soon after victim's death" and could influence the minimum PMI estimation.

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2. Materials and methods

2.1 Blow flies used in experiment

Blow flies, *C. megacephala* used in the experiments have already been reared in the laboratory for several years. New individuals were routinely joined into the stock every year, which were captured from the area Agricultural Entomology Building, National Taiwan University. Stock flies were kept in the incubator 27℃, 70% RH, and the photoperiod of 12h L (5 pm to 5 am) : 12h D (5 am to 5 pm). Flies used in the following experimental treatments and replications also follow this set-up, exclude those in the field experiment. The larvae of *C. megacephala* that were reared in the plastic jar with sliced pork liver.

Because the development and nutrient requirement of larvae will influence the adult reproductive performance (Wall, 1993). In addition, according to Shiao and Yeh (2008), when the maggot density raises above 320 maggots per 60 grams medium, the dry weight of the maggots will be significantly lower compared with that under density of 160 maggots per 60 grams medium. Those results indicated that intraspecific competition in the limited food resource will affect the body size of the blow flies, and body size will affect many reproductive results, therefore, it is important to control the larvae growth condition. Thus, in all

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laboratory experiments, the newly emerge blow flies larvae which use in the experiment were reared in the artificial medium, the ingredient of the artificial medium was followed as that in Hung (1995), which are the mixture of fish meal, yeast, agar, and water in proportion of 25 : 10 : 1 : 150. Five hundred eggs were counted and colonized in each 300 grams medium. Larvae will be kept in the artificial medium until they are fully-develop, after that, in post-feeding stage, the container that loaded with artificial media will be placed inside another container with 1 centimeter of sawdust, which can provided shelter for larvae pupation. All the experimental population of adult *C. megacephala* were rear in the artificial medium for lab experiment. After eclosion, adult blow flies were kept in a 30cm x 30cm x 30cm cage which has 3 sides of nylon plastic gauze, 1 acrylic side with entrance with nylon gauze and 2 acrylic side cover the top and bottom of the cage.

In each experiment and replication, twelve pairs of adult male and female blow flies were separated from the experimental population. Water was contain in a covered plastic pudding cup, tissue paper was soak in the water and extent out of the cover of the pudding cup in order to provide water supply for blow flies. Usually we fed adult blow flies with milk pounder and sugar in proportion of 1 : 1. However, *C. megacephala* is kind of anautogenous fly species, which

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needs enough protein to complete their ovary development. In addition, age, egg load and oviposition experience may also influence the oviposition preference and performance (Ueno and Ueno, 2005). To control the ovarian development to the specific stage, the extra protein gain from the milk powder should be avoided. Therefore, we fed adult blow flies with sugar only. To provide necessary protein source for ovarian development, sliced pork liver were provided to the experimental group 2 days after adult emerge. Adult blow flies can laid eggs 4~5 days after protein meal. Gabre et al. (2005) mentioned that adult pre-oviposition period (APOP) of *C. megecephala* which is the time of first oviposition after emergence is about 6.6 days. This agreed with our observation. Wall (1993) concluded that the amount of protein per female blow fly intakes will also influence their reproductive output. Therefore, we provide sliced pork liver at 1st and 2nd day ad libitum (totally 48 hours) and give another 5 days for the adult flies to develop their ovaries. All the female blow flies used in the experiments were 7-days-old with well-developed ovaries and emerged from pupae no longer than 3 week as well as no oviposition experience before.

2.2 Pork liver used in experiment

Pork liver, which been proven as the most attractive oviposition medium to *C. megacephala* (Bunchu *et al.*, 2008), so we decided to use pork liver as our

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main media to test the decay-aged effect (degree of decay) of those media on the blow fly oviposition. Fresh and complete pork liver was purchased from the traditional market near the National Taiwan University. Pork liver was separated from the newly killed swine and purchase in the same day we performed the experiments.

Sixty grams of sliced fresh pork liver was divided immediately from the complete pork liver as experimental fresh treatment after purchase, and the rest of the pork liver was stored in a plastic container with fine mesh coverd on the top. Fine mesh cover can keep the ventilation of the container, make the pork liver expose to the air, also can avoid other insects to invade and colonize the pork liver, especially the flesh flies, which may lower the blow fly fecundity performance if presence on the oviposition media (Yang and Shiao, 2014). The container with the rest of the pork liver and decay-age process was operate in the incubator. The environment setup was 27℃, 70% RH, photoperiod of 12h L : 12h D.

Every day before the experiment, we will slice the amount we need from the remaining pork liver and blend into muddy flesh. Because it can provide homogenization of the decomposition level of oviposition media and the same contact surface in each experiment and replication. Also, if we did not remove the harden layer

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on the surface of the media, it might hinder the oviposit behavior. Sixty grams of muddy flesh were used in each treatment. Because the remaining pork liver was stored in the incubator every day after we slice the amounts we need for experiment. Thus, we can get 60 grams fresh pork liver at the first day we bought, 60 grams of 1-day-old pork liver at the second day, 60 grams of 2-day-old pork liver at the third day after purchase and so on. Those pork liver used in the field experiment did not blend into muddy flesh.

2.3 Experimental set-up

Muddy flesh weighted 60 grams and was filled in the plastic pudding cup. Along with the decay-aged increased, blended pork liver will become more sticky, which make blow flies hard to move on the surface. In order to prevent blow flies to be stuck and drowned in the media, we covered a piece of fine mesh on the media surface (Figure 1). This design enable blow flies to sense, contact and taste the media not to be trapped and easy to move. In addition, it can also uniform the surface texture for all the different decay-aged treatments. Plastic pudding cup will placed in the middle of the bug cage. The cover of the cage would be replaced with a transparent plastic sheet with an artificial hole at the center, which can provide a place for setting up surveillance system setup. Transparent plastic sheet will not block the light to avoid possible influences from the light effects (Figure

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2).

2.3.1 Field experiment

Details of the preparation of pork liver was as mentioned above. Different decay-aged pork livers were prepared from the fresh to the 8-day-old pork liver. We use fresh pork liver in the first day, 1-day-old pork liver in the second day and so on. Each cup of the pork liver is 60 grams in net weight. Plastic pudding cup will be placed in the middle of the wire cage to prevent other animal to interference the experiments. Transparent plastic sheet was covered on the top of the wire cage to avoid the rain but does not block out the sun-light (Figure 3). Experiment devices were placed near the Agricultural Entomology Building of National Taiwan University, from 9 am to 5 pm, 6 replications were conducted. This experiments were carried out in August, November, and December in 2013, and March and April in 2014. The duration for each replication is 9 days. Egg number will be counted and recorded after 5 pm.

2.3.2 Oviposition preference on different decay-aged pork liver

To test the performance of blow flies oviposition preference on different decay-aged pork liver, we need different decay-aged pork liver at the same day. We need to the buy pork liver from traditional market every day, until the day we

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carry out our experiment. Than we can get the sequential decay-aged pork liver from 8-day-old to fresh pork livers in the same day. However, traditional market is always closed on every Monday, so we decided to buy pork liver from the traditional market every two day. Then we can acquired 8-day-old, 6-day-old, 4-day-old, 2-day-old, and fresh pork liver at the same day. Each pork liver in treatments will be ground by a blender, 60 grams muddy flesh was then loaded in a plastic pudding cup, covered with a piece of fine mesh on the top of the media. In order to give blow flies choices, each of loaded plastic pudding cup was labeled with number 8, 6, 4, 2, 0, and then placed all five treatments in the bug cage at the same time. We used the same protocol as in section above to prepare gravid females. Ten gravid female individuals which age are no longer than 3 weeks will be used in each replication and exposed to different decay-aged pork liver for 12 hours in the light, from 5 pm to 5 am, 13 replications were conducted. The eggs they laid will be counted and recorded at the end of the experiment.

2.3.3 Effects of different decay-aged pork liver on blow flies oviposition reaction

Blow flies used in this experiment were as mentioned above. Pork liver were purchased in the first day and separate the fresh treatment at the beginning, the rest of the pork liver will be stored in the incubator with 27℃ and 60-70% R.H.

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We separated 1-day-old pork livers in the second day of the experiment, 2-day-old pork livers in the third day and so on. To avoid the complexity of multiple females eggs laying, which will confuse our experiment results, we decided to use 10 individuals with the proportion of 1 female and 9 males in each group. Different decay-aged pork liver were blended to muddy flesh and filled in plastic pudding cups, from fresh to 11-day-old pork liver, weighted 60 grams. On the top of the surface would covered with fine mesh. Because we think blow flies usually do not have many oviposition medium to choose in the field, to simulate the field situation in the laboratory, we provided single oviposition media with different decay-aged for them to oviposit. *Chrysomya megacephala* would expose to the different decay-aged pork liver in 12 hours from 5 pm to 5 am, 27 replications were conducted. Laid eggs or not and the moment when *C. megacephala* laid eggs will be counted and recorded to calculate the incidence of oviposition. The calculate equation as follow:

$$\text{Incidence of oviposition } (\%) = \frac{\text{total oviposition events}}{total\ replications\ (n)} \times 100\%$$

We also used surveillance system to record the time when oviposition happened, to find out would different decay-aged treatment affect the duration of acceptance phase on *C. megacephala.*

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2.4 Data analysis

Data presented in the first and second experiment is the count data, and it does not follow any certain distribution, so we applied nonparametric analysis. Kruskal-Wallis test was applied in the field experiment, and Dunn's test for the post hoc analysis. To examine how the different decay-aged treatments of oviposition media affect the oviposition behavior in the field, the numbers of eggs on the pork liver were then counted. We also applied Kruskal-Wallis test and Dunn's test post hoc analysis on the second experiment, which we were trying to test the oviposition preference of *C. megacephala* on different decay-aged pork liver at the same time in the laboratory environment. In the third experiment, treatments that blow flies laid eggs were recorded as in category "1", treatment that blow flies did not lay eggs as in category "0". We did not transform our data to applied ANOVA test, because even if the data been transformed by square root transformation or other transformation method, it cannot pass the homogeneity test of variance assumption (Levene's test, p-value < 0.001), indicate that under 95% of confidential level, the variance are not homogeneous, so ANOVA test is meaningless. Therefore, binary logistic regression analysis was applied. We conduct binary logistic regression analysis in this experiment to examine whether the frequency of egg laying showed significantly different in different decay-aged treatments, and Bonferroni test was used for post hoc analysis. In the test for the duration of acceptance phase, data of 10-

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day-old and 11-day-old pork liver have been removed, because less than two sets of data been collected and cannot be analyzed our study. Older decay-aged pork liver have lower incidence of oviposition, in that case, we could not collect enough data to analyze. We use ANOVA analysis. Software SPSS Version 22.0 (IBM Corp, 2013) was used for all the analysis above.

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3. Results

3.1 Field experiment

The results of Kruskal-Wallis test in the field experiments showed $H_c = 26.735 > X^2_{\alpha, df} = 15.507$, *p*-value = 0.001 (N = 6, df = 8, α = 0.05). Statistical values showed there were significantly different among groups (Figure 4). We found that the number of eggs dramatically rose from fresh liver to 2-day-old pork liver, and then quickly descended after 2-day-old pork liver. No eggs has been found in the fresh liver and 8-day-old pork liver. Two-day-old pork liver has the highest number of eggs on it and the highest oviposition frequency, causes the peak in the results. In the post hoc analysis (Dunn's test), the result showed significantly different between the 2-day-old and fresh pork liver. Results of 2-day-old pork liver also significantly different with the 7-day-old and 8-day-old pork liver. There is no significant difference between fresh, 7-day-old and 8-day-old pork liver. Rest of the treatments show no significant difference between each other.

3.2 Oviposition preference on different decay-aged pork liver

To test the oviposition preference of blow fly of *C. megacephala* in different decay-aged treatments. Preference experiments were carried out under the laboratory environment and Kruskal-Wallis test was applied. The results showed

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that $H_c = 18.427 > X^2_{\alpha, df} = 9.488$, *p*-value = 0.001 (N = 13, df = 4, α= 0.05). Statistical values showed significantly different among groups (Figure 5). The highest number of eggs were laid on 2-day-old and 4-day-old treatments. Eggs number on fresh and 6-day-old treatments are relatively lower than that on 2-day-old and 4-day-old pork liver. No egg was laid on the 8-day-old treatment. In the post hoc analysis (Dunn's test), 2-day-old and 4-day-old pork livers have no significant difference with each other, but have significant difference with 8-day-old treatments. Rest of the treatment groups have no significant difference with each other.

3.3 Effects of different decay-aged pork liver on blow flies oviposition reaction

Binary logistic regression analysis was applied. The results show *p*-value < 0.001 (N = 27, α = 0.05). The statistic values showed significantly different among groups (Figure 6). In the post hoc analysis (Bonferroni test), we can found that 2-day-old pork liver have the highest incidence of oviposition among groups (40.74%), and have significant difference with 11-day-old pork liver. In addition, 11-day-old pork liver has significantly low incidence of oviposition among groups (0%). The other combination groups do not have significant difference with each other.

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3.4 Duration of acceptance phase

We use surveillance system to record the time oviposition happened when media of different decomposition levels were provided individually to blow flies in the previous experiments. We applied ANOVA analysis in this experiment. Levene's test *p*-value = 0.580, indicate that under 95% of confidence level, variance is homogeneous. Results showed there are no significantly different among groups, $F = 0.557 < F_{(\nu1, \nu2, \alpha)} = 2.170$ ($\nu1 = 9, \nu2 = 34, \alpha = 0.05$), *p*-value = 0.822 (Figure 7). Mean duration of acceptance phase is 9.83 ± 1.92 hours. The detailed results are given in Table 1.

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4. Discussion

In the field experiment, we applied different decay-aged pork liver in sequential days to test the blow fly reaction. In this experiment, it may encounter raining or other weather condition during the experiment. In addition, the experiments were lasted for 2 years, we didn't consider the weather condition, so the seasonal changes and temperature fluctuation will not be discussed in this study.

We found the oviposition performance of *C. megacephala* reached to the highest in the day 3 with 2-day-old pork liver, which showed significant difference to fresh, 7-day-old and 8-day-old pork liver. Our results agree with the previous study, which indicated the aged pork liver have greater attraction to blow flies than fresh pork liver (Bunchu *et al.*, 2008). However, in their study, only 1-day-old and 3-day-old decay treatments have been tested, it could not provide the complete oviposition information for the continuous process of decomposition. No eggs was laid on 8-day-old pork liver in our study, and the similar results were also shown in George et al. (2012) which the very decayed (8-day-old) treatments had lower oviposition and larviposition preference than fresh treatments, however, they did find both oviposition and larviposition happened in 8-day-old treatments in theirs experiment. Different results of 8-day-old treatment between our study and previous study might cause by different species and the difference in geological region (Amendt *et al.*, 2004), which also indicate that different species and the reaction

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of it while different decay-aged treatments were given should be tested.

In the oviposition preference test of different decay-aged pork liver for *C. megacephala*, the results shows the 2-day-old and 4-day-old treatments have the highest attraction to *C. megacephala*. In the post hoc analysis, the 4-day-old pork liver did not show significantly different to the 2-day-old pork liver. In addition, the results show that there is no eggs been laid on the 8-day-old pork liver. In our preference test, *C. megacephala* did avoid to lay egg on the 8-day-old pork liver. However, we did not use Y-tube to find out whether olfactory cues play an important role in this process. We simply provided 5 different decay-aged treatments for oviposition selection. The factors that may cause the results of the preference test is still unknown. How *C. megacephala* distinguish the different decay-aged treatments need further investigation. In the previous studies, researchers found the *C. megacephala* could use their visual, olfactory and tactile sensory to locate the place to lay eggs, evaluate the resources from the information which acquired from those sensory system (Tomberlin *et al.*, 2011b). However, in our experiment, we did not test any sensory organs of blow flies that involved in the preference of oviposition process, so we can not reach any conclusion about which sensory organ plays the major role in detecting conditions of different treatments.

However, Kasper *et al*. (2012) studied the different VOCs (volatile organic compounds) emitted from the mouse carcasses under different storage time and weather

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condition using GC-MS analysis. In their studies, researchers found that the concentration and composition of VOCs emitted from mouse carcasses changed with different storage times, from fresh, 10-day-old to 30-day-old treatment. The Sulphur compounds in the VOCs have known to show great attraction to necrophagous insects including blow flies. In addition, their results also showed the concentration of Sulphur compounds changed when storage times or temperatures were different. That indicated the depletion of methionine and cysteine during the decomposition process (Kasper *et al.*, 2012). We can conclude from their results that the proteolysis within decomposition cause the degradation of Sulphur compounds. In another world, changes of the VOCs composition in the decomposition process might be one of the factors that affect the oviposition preference for blow flies on different decay-aged pork liver. In addition, during our pork liver storage process (preparation of decay-aged treatment), some blow flies accidentally invaded into the incubator and lay eggs on the fine mesh covered on the top of the container of liver which were already stored for more than 8 days. Although the blow flies which accidentally invaded into the incubator could only use the odor cues to detect those decay-aged treatments. The oviposition behavior still could be observed. But in our preference test, there is no oviposition behavior happened on that kind of the treatments after 8-day-old. Thus, we believe not only the olfactory system plays in oviposition decision process, but also other sensory system involved for helping blow flies to make

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oviposition decision.

Gregarious oviposition might be a factor that caused the variation in egg numbers on different treatments. Gregarious oviposition is defined as the female individuals which have the tendency to produce collective egg mass aggregately (Aponte *et al.*, 2003). *Chrysomya megacephala* preferred to lay eggs on the media which already have eggs on it (Esser, 1990), and the accumulation of the blow fly individuals also act as an attractant to other blow flies (Norris, 1965). We did not use a single female but use 10 female adults instead in our experiments, we have the following reasons for that: *Chrysomya megacephala* seldom laid eggs when they are along (Yang and Shiao, 2014). Replacing female individuals with male can trigger oviposition behavior as well (Yang and Shiao, 2014). However, we are afraid of the foraging preference of male blow flies will affect aggregation behavior of females and influence their oviposition choices. And the average fecundity of 10 females are not significantly different with that of 50 females (Yang and Shiao, 2014). So we decided to use 10 female adults in our experiments. But the effects from the female gregarious oviposition could not be controlled. However, in our raw data, eggs clutches seldom appeared in a single medium, which indicated that the gregarious oviposition is probably not a major factor that may influence the decision of *C. megacephala.*

Comparison the field experiment and the preference test, no egg has been found on

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the fresh pork liver in the field experiment. But were laid on the fresh pork liver in the preference test. Why there is no egg laid on the fresh pork liver in the field experiment? Bunchu *et al*. (2008) showed that fresh pork liver have attraction to *C. megacephala* than the 3-day-old pork liver and 1-day-old pork liver, only about 60% of *C. megacephala* could response to the fresh media. Although fresh pork liver also can attract blow flies, some researchers believed that the amounts and concentration of Sulphur compounds emitted from the protein resources which attract necrophagous insects are not high enough in the very beginning of decomposition (Kasper *et al.*, 2012; Tomberlin *et al.*, 2011b). In addition, when body was exposed in the nature environment, blow flies should go through the exposure phase, detection phase and acceptance phase, than reach to the oviposition stage (Tomberlin *et al.*, 2011b). Wind speed, wind direction (Bunchu *et al.*, 2008) and the concentration of Sulphur compound may change the duration of exposure phase and detection phase for blow flies(Tomberlin *et al.*, 2011b). However, in our laboratory environment, pork liver was directly provided to the blow flies inside the bug cage. Therefore, in the laboratory environment, we can assume that we shorten the duration of exposure phase and detection phase, but the acceptance phase remains unchanged, which means blow flies have longer time to make their decisions to oviposit or not. However, this argument need further studies to confirm.

Both field experiment and preference test showed no eggs on the 8-day-old pork

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liver. What are the possible reasons? In our experiments, we removed the hardened external layers of the media by homogenizing them in a blender. In addition, the fine mesh covered on the medium provides the same contact surface and uniform texture. Therefore, blow fly sensory system will not be affected by the different contact surface or texture. Previous researches showed that the moisture of the oviposition media could affect the larval development (Erzincliogl u, 1996). If the media was too moist, blow fly larvae may drown to death, in contrast, if the media was too dry, it may damage the egg and cause the low hatching rate. The dehydration of the media will cause the larvae dehydrate, and the water loses will harden the surface of the media make larvae hard to penetrate the surface (Erzincliogl u, 1996). It is possible that blow flies would avoid to lay eggs on the dehydrated media (Erzincliogl u, 1996).

In the third experiments, we provided different oviposition media of different decomposition level individually to test the reaction when *C. megacephala* has no other media to choose, which is similar to the field situation. Since we believe there are not many oviposition media in the field which can be chose by the blow flies in the same time. In addition, what will happen if we simulate field selection situation in the laboratory condition? The results showed most of the treatments do not have significance different with each other, but the incidence of oviposition on 2-day-old pork liver was significantly higher than that on the 11-day-old pork liver. Comparing the field and preference tests,

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the peak of the incidence of oviposition also appeared on the 2-day-old pork liver (40.74%), but significantly low incidence of oviposition was showed on 11-day-old pork liver (0%). Those results of the 8-day-old, 9-day-old and 10-day-old pork livers do not show significantly low incidence of oviposition. In another words, the effect of decomposition media was delayed when single media was provided under laboratory condition. We think this delay may refer to the natural reaction of *C. megacephala*. Under the natural situation, insect with high fecundity such as *C. megacephala*, can continuously reproduce during their life span, they do not really need to choose the very ideal oviposition media to laid their eggs. In addition, scarce and limited resources like corpses should be a highly competitive resource (Beaver, 1977), therefore, blow fly of *C. megacephala* would always lay eggs when they encounter suitable oviposition media except for some extremely unsuitable conditions. However, this argument needs further investigation.

In the test of duration of acceptance phase, when oviposition media of different decomposition levels were provided individually, the results showed no significant difference was found among groups. That indicates under 95% confident interval, the durations of acceptance phase have no significant difference in different decomposition levels. However, the average mean duration is 9.83 ± 1.92 hours, which is relatively long if we compare with the assumption of "oviposit after arrive the corpse in a short

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period". Although most of the data we collected in each treatment are not enough for statistic analysis, data collected in 1–day-old (9.43 ± 2.31 hours) and 2-day-old (9.04 ± 2.40 hours) pork livers should be reliable. In addition, the sex ratio is 1 : 9 (female : male) in this study, this ratio does not followed the natural condition of 1 : 1 (Das and Dasgupta, 1982), whether the sex ratio could induce the early oviposition or not remains unclear. However, the information of the delay oviposition in certain situation should be concerned in further studies and crime investigation.

Different diet that larvae consumed will affect their development, including different animal cadaver or different organs of the same cadaver to different physical conditions (frozen / thawed) of oviposition media (Clark *et al.*, 2006; Day and Wallman, 2006a; b; Ireland and Turner, 2006; Kaneshrajah and Turner, 2004). Decomposed pork liver would also retard the larval development (Richards *et al.*, 2013). Prolonging of developmental time may cause the larvae to increase the encounter risk of predators and parasites. The decision made by a female blow fly to oviposit on a very decayed media would retard the development rate of its offsprings and increase the risk of exposure to predators and parasites might decrease the fitness of its offsprings. Therefore, it is reasonable to believe that blow fly would avoid to lay eggs on the media when it has oviposition choice, which might retard their larval development and lower their fitness.

On the other hand, microbe may also play an important role in attracting or inhibiting

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the blow flies (Burkepile *et al.*, 2006; Thompson *et al.*, 2013; Tomberlin *et al.*, 2012). Usually, we thought the microbe on the corpse behaves as a decomposer, however, if the microbe colonizes the corpse rapidly, it may also behave as a competitor to other consumers (Burkepile *et al.*, 2006). Necrophagous insects such as blow flies usually represent the early visitor and colonizer of the carcass, in these cases, some microbes may played as an attractant to the blow flies (Tomberlin *et al.*, 2012). Nevertheless, we blocked the invasion of insects during the decomposition process in our study, especially the dipterans, therefore, the remaining media would be swarmed by the microbes. Previous research indicated that releasing of the quorum sensing signal by specific bacteria to interact with other conspecific bacteria, may attract some blow fly species that treated this chemical signal as an attractant (Tomberlin *et al.*, 2012). In that study, Tomberlin *et al.* (2012) demonstrated the close connection of that blow fly *Lucilia sericata* detection and the quorum sensing of the bacteria might be associated. In addition, along with the decomposition process, nutrient quality and quantity change, indicate the microenvironment related to the microbe change as will in the perspective of blow fly (Burkepile *et al.*, 2006). Composition of the microbe population was dynamic within this dynamic process (Burkepile *et al.*, 2006). Therefore, it is possible that different microbe associated with the corpse would produce chemical compound which blow flies not prefer, or the chemical cue emit from the medium which can attract *C. megacephala* is degrading.

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Thus, it is reasonable to think that *C. megacephala* have different behavioral response to different decay-aged treatment may cause by different microbe composition present on the oviposition media.

Another study showed that chemical compound emitted during the decomposition process of corpse itself could provide its information of physiological condition (Birkett *et al.*, 2004). Therefore, both the microbes on the media and the VOCs emitted by the media might provide *C. megacephala* the information of inappropriate media, which cause the reason the *C. megacephala* avoiding to lay eggs on the 8-day-old media in our experiment. The relationships and interactions among bacteria, VOCs, humidity and blow flies need further investigations.

Both ecological and evolution information of the target species are important in forensic entomology (Tomberlin *et al.*, 2011a). When we apply the experimental results to the field, we must be aware of that our experimental design are not always matching with the real situation of our target species in the field. Different ages of the adult blow flies after eclosion may affect the oviposition strategies, and cause different decision making when choosing oviposition media (Tomberlin *et al.*, 2012). And not only the nutritional acquisition of larvae affects adult fecundity, but also the quality and quantity of adult protein intake play important roles on the fecundity (Tomberlin *et al.*, 2012). Interspecific interaction and the individual numbers of conspecific species might also

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affect the fecundity and the decision making of oviposition (Norris, 1965; Yang and Shiao, 2014). To design a better experiment that can correspond to the field situation, we need more investigation data on the biological and ecological information for those blow flies.

Smith (1986) have listed several critical issues that need further research one of them are the “effect of postmortem treatment of the corpse”. Different postmortem treatments usually make different insect succession patterns. Physical condition of the corpse such as it has been burned or immersed in the water also showed different faunal composition. In the case of burned corpse, some case studies showed that the charried corpse would delay the blow fly invasion (Catts and Goff, 1992). However, in the immersed corpse, only a few studies have been carried out. In the homicide cases that happened inside the houses or automobiles which represent the closed environments, insects usually cannot invade the body immediately and delay the colonization of blow flies. In addition, in the situation that no insect invasion in the early decomposition also can be used to simulate the hidden corpse (wrapped, bagged, etc.) and been re-exposed to the environment Therefore, an investigator should not only consider the delay of invasion of insects caused by closed environment, but also possible delayed in time when blow fly oviposit.

In this study, we applied pork liver as the oviposition media, rather than pig carcass, however, the phenomenon of low incidence of oviposition happened on the decay-aged media after 8-days is obvious. Such ecological and biological information might have the

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application potentials in exploring repellent VOCs and the microbial utility. The possible repellent VOCs and the microbe involved in the carcass decomposition need more studies to reveal the mechanism behind these phenomena. In our research, we also found the fresh treatments are not as attractive as we though before, and it need to be considered when dealing with a freshly disposed corpse. The assumption that the blow fly will oviposit right after they arrive the corpse should also be retested and reconsidered. Distribution, population density, proportion of gravid females, age distribution in the field and adult oviposition experience, many of these biological information even the evolutionary information should be surveyed in different geographic regions, due to the different species and environmental conditions may affect behavioral responses of blow flies. We hope this research will be useful for further applications and help opening some new aspects for forensic entomological researches.

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5. Conclusion

We found that decomposition levels do affect blow flies oviposition (especially the *C. megacephala*), in addition, both the highest eggs number laid and the incidence of oviposition did not appear on the fresh treatment. However, when a body was exposed, fresh condition is usually of the case, and that kind of the fresh condition will not as attractive to blow flies as we though before. This study also shows the sequential oviposition data under different decomposition levels and the oviposition preference when different decay-aged pork livers were provided simultaneously. We suggest the factors of water content, microbial composition, emitted VOCs of the oviposition media should be considered as the possible reasons which might trigger or inhibit the blow fly oviposition. However, the detailed mechanism that cause those results here are still unknown. Although we found that 2-day-old pork liver has the highest incidence of oviposition in our research, it does not mean that *C. megacephala* will skip the fresh media without lay eggs in the field. Because we believe insects with such the high fecundity as *C. megacephala* do not have to choose the best oviposition media, except the media is beyond the acceptable condition. In addition, durations in the acceptance phase of female blow fly did not changed by the different decay-aged media, but the acceptance duration or the oviposition delay may up to 9 hours which should be carefully considered in the future applications.

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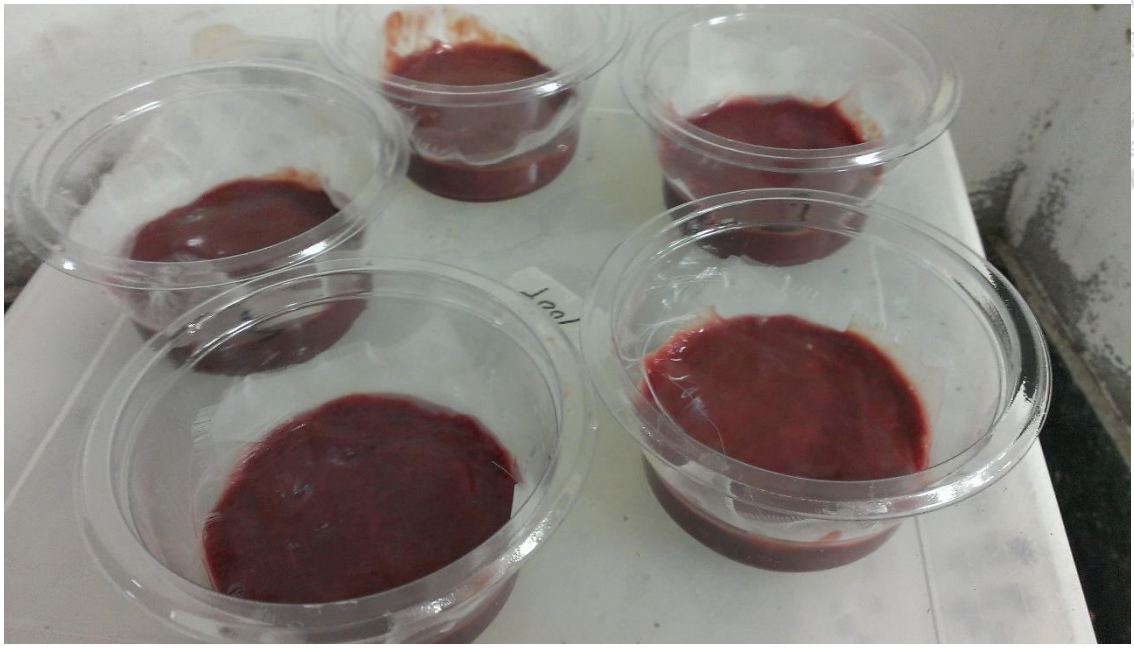

Figure 1. Experimental set-up of oviposition media. According to our observation, pork liver would coat a harden layer on the external surface along the time passed by. Because of the harden coating, texture and decomposition process will be different both in the external and internal pork liver. In order to minimize those effects, each decay-aged pork liver we used was homogenized by blender before experiment. In addition, since the texture of the pork liver was different in each decay-aged treatment, a fine mesh was covered on the top to keep the surface texture uniformed and to prevent the blow fly drowning in the media.

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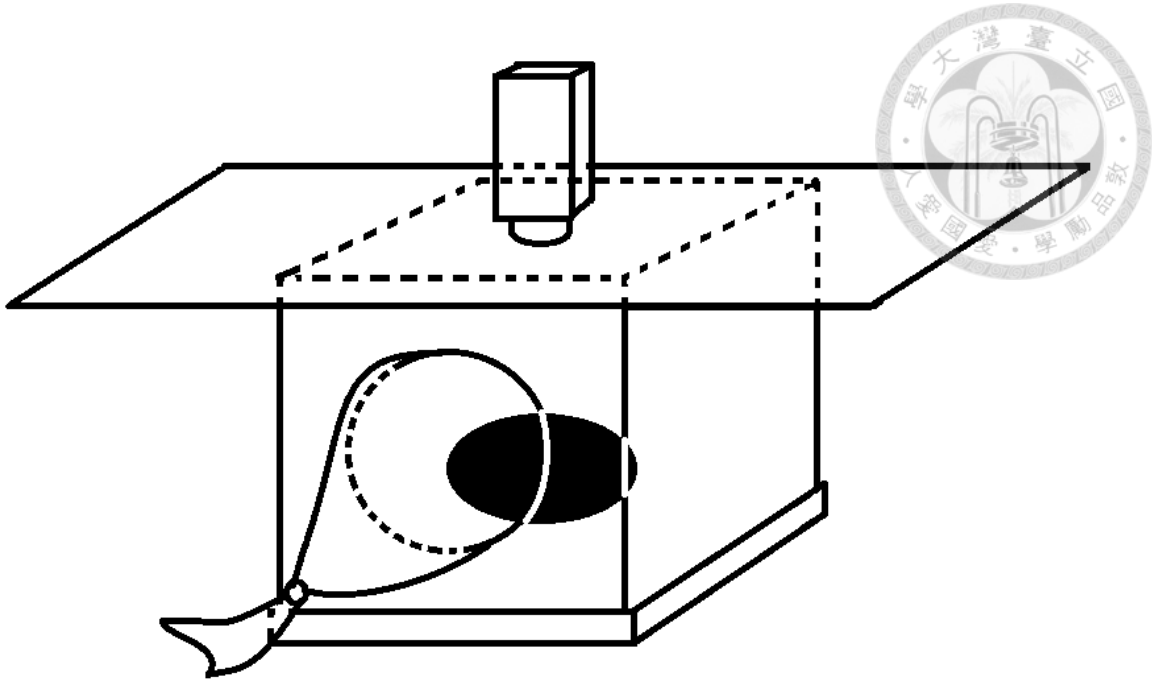

Figure 2. Surveillance system setup. The cover of the bug cage was replaced with a plastic membrane. On the top of the plastic membrane, covered with another transparent plastic sheet to prevent the camera crushing the cover membrane. Both plastic membrane and plastic sheet are transparent and a hole was opened in the center of cover in order to set up a camera. We also opened a hole on the right side of the incubator for the cable connection to the monitor outside the incubator. The hole on the incubator was sealed with aluminum foil to minimize the disturbance of light, temperature and humidity in the incubator.

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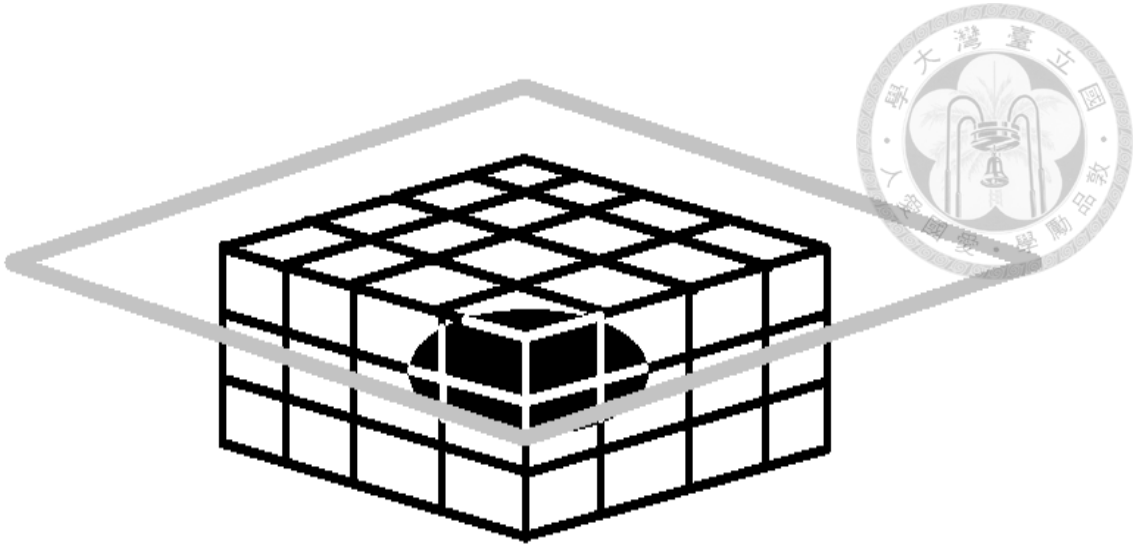

Figure 3. Field experimental set-up. A cup of pork liver weighted 60 grams (without cup) was placed inside a wire cage, which can prevent the possible interference from other vertebrates. A transparent plastic sheet was covered on the top of the wire cage to avoid rainfall and not block the exposure of sunlight. Experiments lasted for 8 hours, from 9 am to 5 pm. Replacing the cup of pork liver inside the wire cage with different decay-aged treatments every day.

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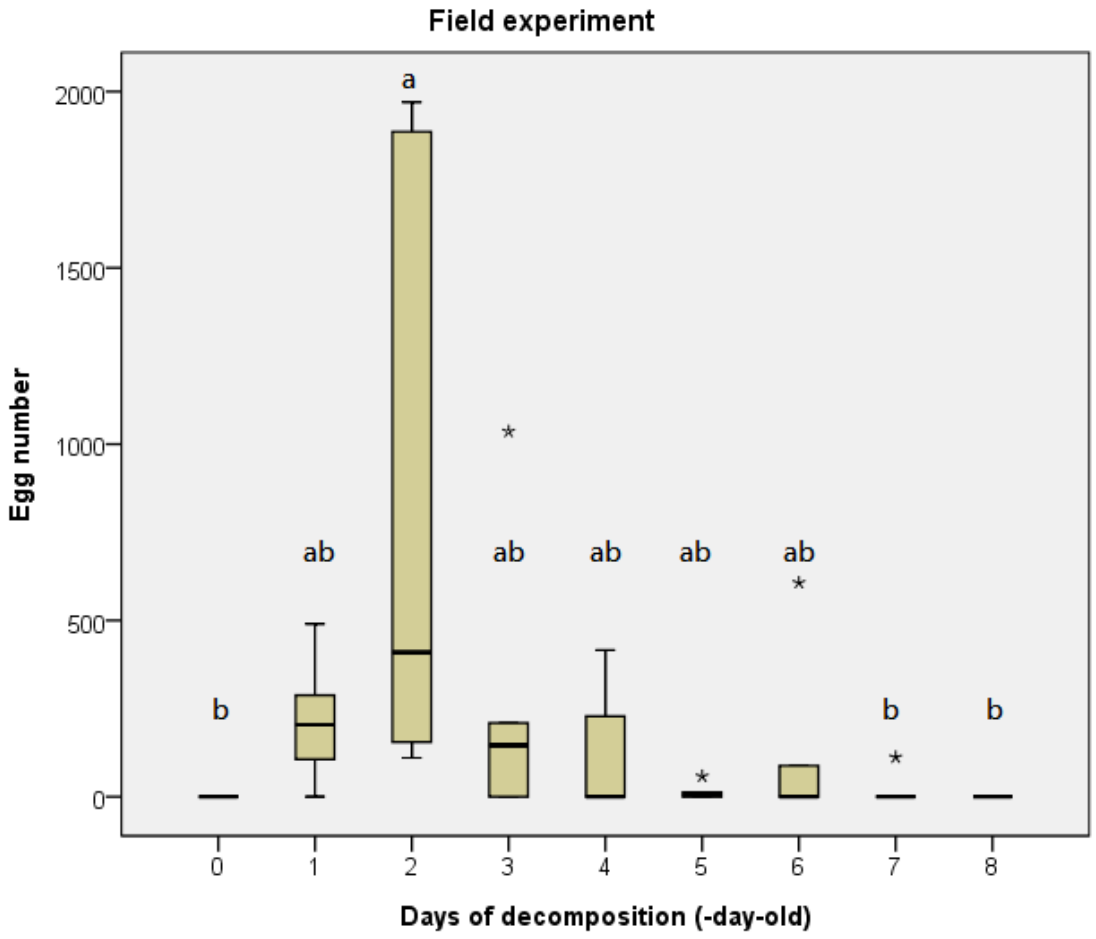

Figure 4. The results of field experiments. To test the effects of different decay-aged pork livers on blow fly oviposition. Six replications were conducted (N=6), *p*-value = 0.001. Two-day-old pork liver have the highest number of eggs, and no egg was laid on the fresh and 8-day-old pork liver. Letter a, b and ab represent the statistically different groups in each treatment. Two-day-old pork livers (a) show significant difference with fresh pork liver (b), 7-day-old pork livers (b) and 8-day-old pork livers (b). The rest of the groups (ab) show no significant difference in the post analysis.

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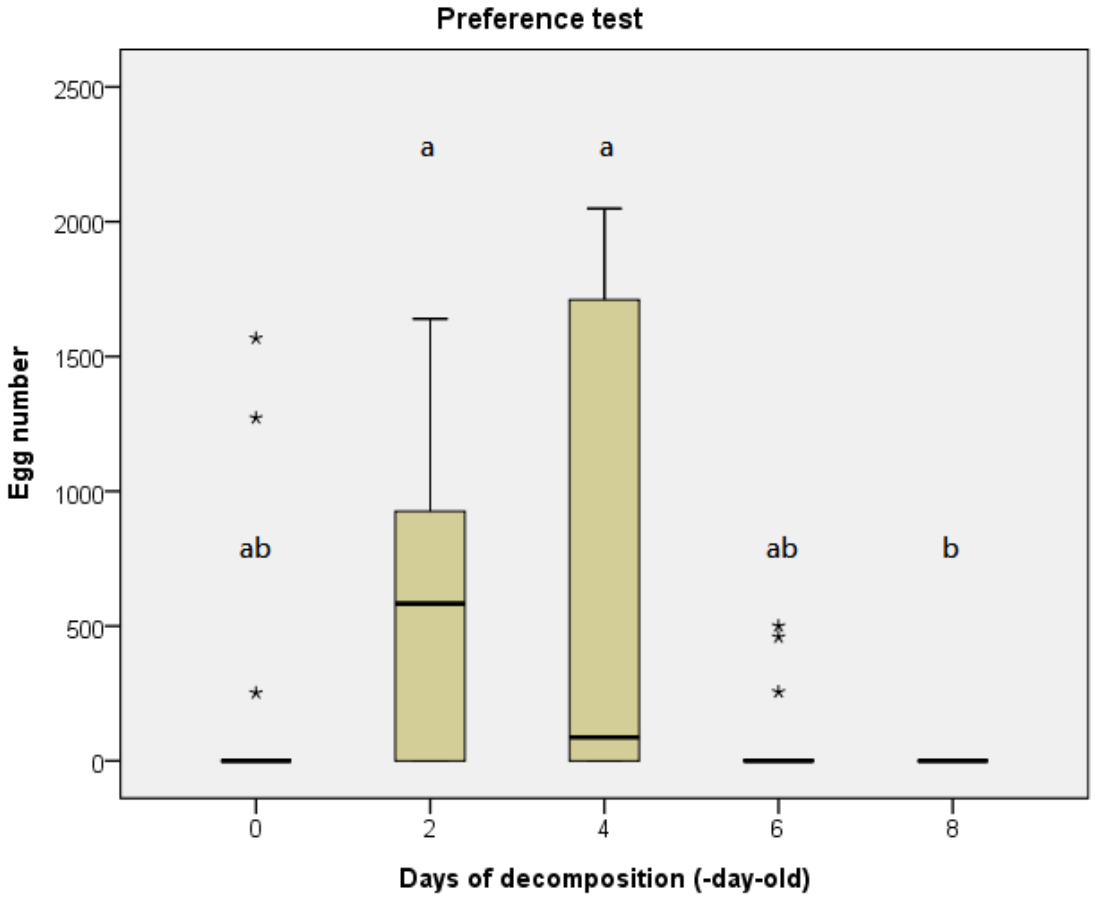

Figure 5. The results of preference test of *C. megacephala* oviposition behavior among different decay-aged treatments inside a bug cage (30 cm × 30 cm × 30 cm). Thirteen replications were conducted (N = 13), *p*-value = 0.001. Ten adult females were used in each replication. Letter a and b represent the statistically different groups in each treatment. Four-day-old pork livers (a) have the highest number of eggs. Eight-day-old pork livers (b) have no egg. The rest of the treatment groups have no significant different between each other.

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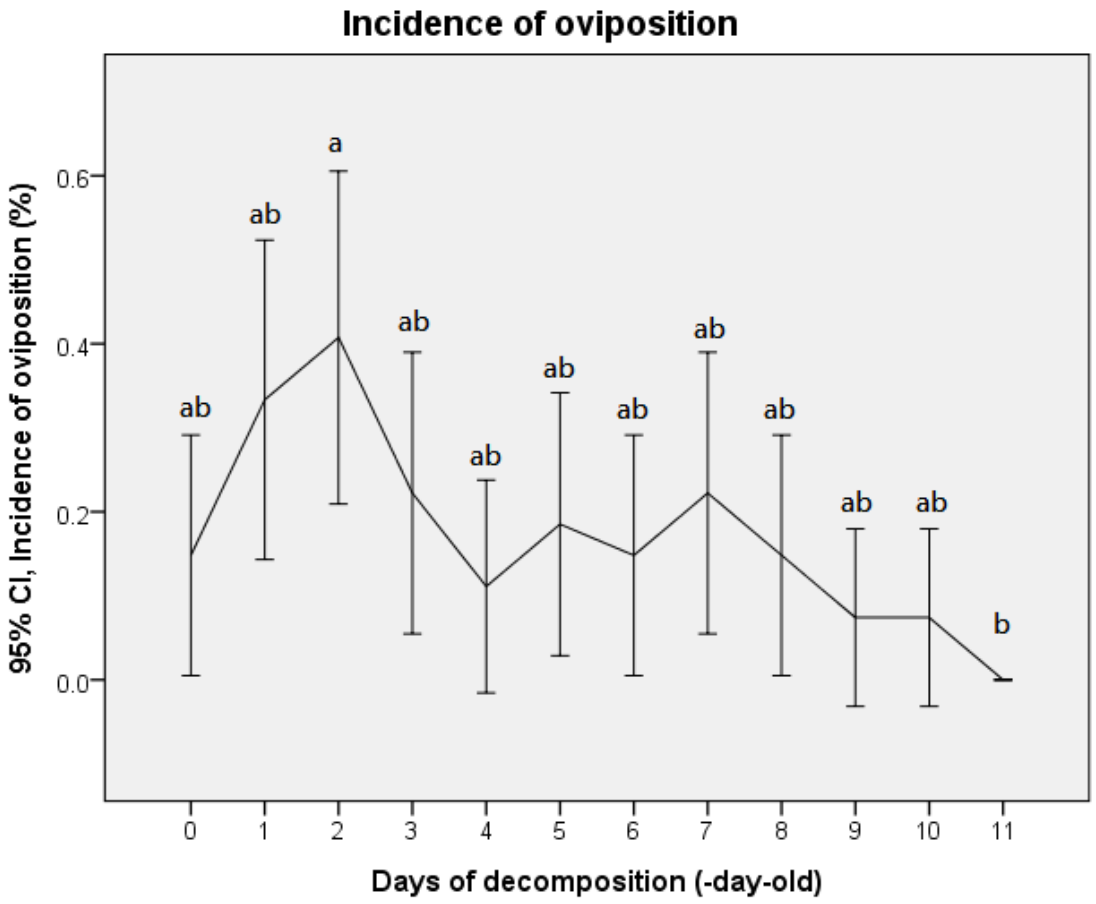

Figure 6. The results of the incidence of oviposition of *C. megacephala* when single medium of different decomposition levels was provided in a bug cage (30 cm × 30 cm × 30 cm). Binary logistic regression analysis were applied. Twenty-seven replications were conducted, p-value < 0.001. Different letters represent statistically different groups in post hoc analysis (Bonferroni test). 2-day-old pork livers (a) have significant difference with 11-day-old pork livers (b). The rest of the treatment groups have no significant difference between each other.

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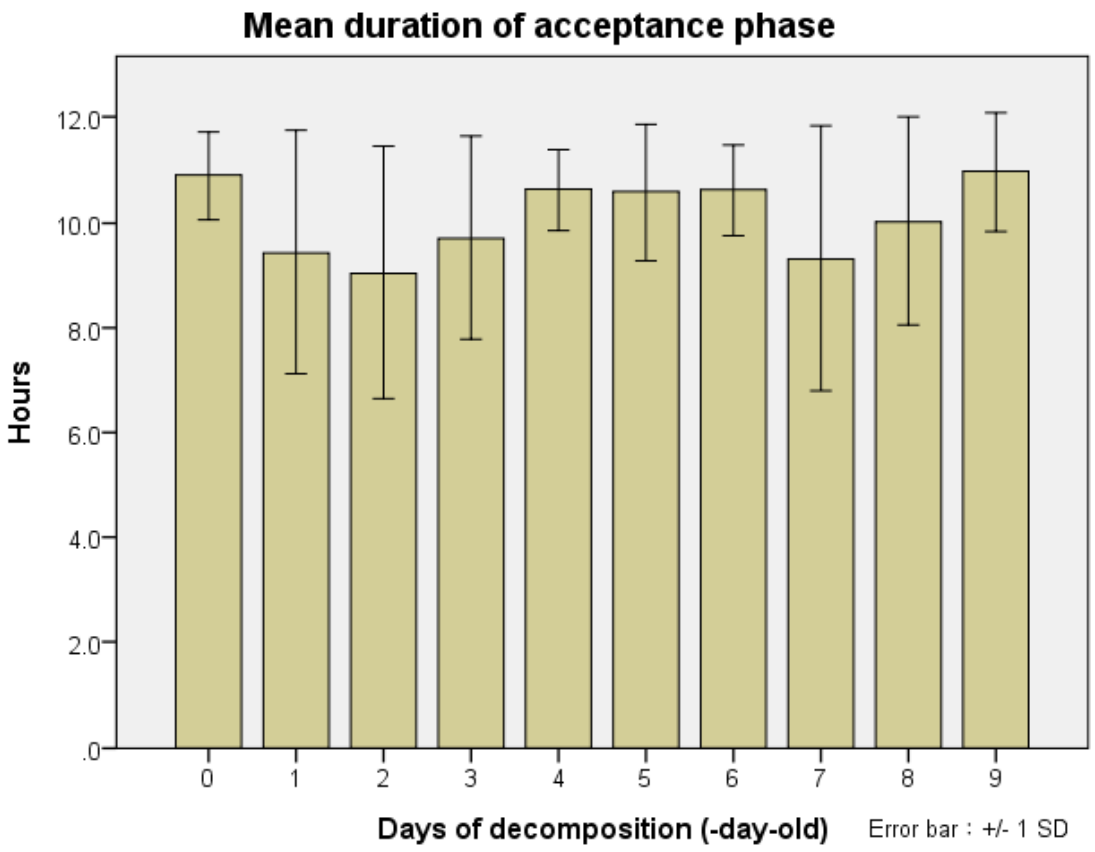

Figure 7. The results of duration of acceptance phase when different decay-aged oviposition media were provided respectively. *p*-value = 0.822 > 0.05, which indicate that there are no significant difference among each treatments under 95% confidence interval.

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Table 1. ANOVA table of duration of acceptance phase, the results show there are no significant difference among groups. (Abbrev: DL = decomposition level (decay-days), N = data collected, SD = standard deviation, SE = standard error, CI = confidential interval, LL = lower limit, UL = upper limit)

DL	N	Mean	SD	SE	95% CI	
					LL	UL
0	3	10.8867	0.82136	0.47421	8.8463	12.9270
1	8	9.4325	2.31044	0.81686	7.5009	11.3641
2	10	9.0430	2.39716	0.75805	7.3282	10.7578
3	4	9.7075	1.92609	0.96305	6.6427	12.7723
4	2	10.6150	0.75660	0.53500	3.8172	17.4128
5	5	10.5680	1.28741	0.57575	8.9695	12.1665
6	3	10.6100	0.85159	0.49166	8.4945	12.7255
7	4	9.3150	2.51577	1.25789	5.3118	13.3182
8	3	10.0267	1.97257	1.13886	5.1265	14.9268
9	2	10.9600	1.11723	0.79000	0.9221	20.9979
Sum	44	9.8305	1.92059	0.28954	9.2465	10.4144

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