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Estimating Filth Fly (Diptera: Calliphoridae) Development in Carrion in Guam

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Abstract–Developmental rates of *Chrysomya megacephala* (F.), *Chrysomya rufifacies* (Macquart), and *Chrysomya nigripes* Aubertin (Diptera: Calliphoridae) were estimated in each of two decomposing pig carcasses (*Sus scrofa,* L.) located in an open field and in a forest on Guam. Significant oviposition occurred within hours of death, and maggot mass formation was observed on both carcasses within the first 24 hr. *C. rufifacies* was the most common fly species collected near the carcasses, followed by *C. megacephala*. *C. rufifacies* was the only fly species reared from samples collected from the carcasses. Fly development rates in the carcass in the sunlit open field were higher than those in the carcass in the shaded forest area. In each habitat, a strong association between maggot length, air temperature, and degree-day accumulation was observed. No carrion beetles or other insect decomposers other than flies were observed. Decomposition on Guam proceeded from fresh bloat to complete skeletonization within a few days and lacked the intermediate steps characteristic of decomposition in more temperate climates. Forensic analysis of outdoor homicide crime scenes on Guam must therefore be completed soon after the time of death to yield useful entomological information. More detailed characterization of the development of forensically important species across an array of conditions is necessary to provide adequate analytical tools for law enforcement agencies in Guam and in Micronesia.

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Introduction

Observations on the species of insects present at homicide scenes and of their stage of development provides forensic scientists with information that may assist them in ascertaining the time and place of death. Intrinsic to the use of entomological evidence is knowledge of the identity, habitat preferences and growth rates of invading necrophilious arthropods. The time required to attain an observed developmental stage can be extrapolated backwards to estimate the approximate time of oviposition and thus the time of death. Numerous studies have been conducted in varied locales to calculate the development rates of various insect species under differing climatic and simulated death conditions (Catts & Haskell 1990, Catts & Goff 1992). Most forensic entomological studies have been conducted in temperate and semi-tropical areas in the USA, Europe, and other developed countries possessing well-developed criminal analysis facilities and trained personnel. Bohart & Gressitt (1951) conducted the most comprehensive survey of forensically important filth flies on the tropical Pacific island of Guam in Micronesia.

The purpose of this study was to identify the major insect groups involved in animal decomposition on Guam, and to compare the current insect fauna with those described in previous insect surveys conducted on Guam in the mid-1900s (Beller 1948, Bohart & Gressitt 1951, Essig 1956). An additional objective was to characterize necrophilious fly infestation and development rates in pig carcasses in hopes of providing local law enforcement officers additional tools for estimating the minimum post mortem interval (PMI) and death locale in Guam homicides.

Materials and Methods

Observations were made on the carcasses of two similarly aged 23 kg domestic pigs *(Sus scrofa* L.*)* located within a fenced field and forested area in east-central Guam at 13.5˚ N 144.8˚ E at 100 m elevation on the University of Guam's Radio Barrigada Agricultural Experiment Station. The two study sites, situated on the elevated limestone plateau that comprises the northern half of the island, were located about 50 m apart in contrasting habitats. The first field site was an open grassy area exposed to continuous sunlight throughout the day. The second site was a dense forest consisting of light underbrush overshadowed by a closed tree canopy so that no direct sunlight penetrated to the forest floor.

The pig carcasses were placed at the sites on 13 July 1999. The pigs had been dispatched with a single shot from a 38-caliber firearm an hour previous to being transported to the Radio Barrigada study sites, with the bullet laterally traversing the head of each pig from ear-to-ear and causing instant death. The carcasses were protected from insect infestation en route to the study sites by a burlap bag covered with double-layer plastic bags. At the study \sites, each carcass was

removed from the bags and secured to the ground by a cover of 2.5 cm mesh chicken wire fastened at the edges by metal stakes. The wire mesh was intended to prevent common scavengers such as feral pigs, dogs, cats, and land crabs from disturbing the carcasses while not inhibiting insect access. Temperature data loggers (Hobo® H8, Onset Computer Corp., Bourne, MA) enclosed in waterproof plastic cases were placed at each site to measure ground, air and internal body temperatures on an hourly basis. A single-channel data logger was buried 10 cm underneath each pig carcass to record soil temperature. A two-channel data logger, mounted on a stake 1 m above ground adjacent to each carcass, recorded air temperature and by means of a remote thermistor probe inserted into the rectum, internal body temperature. Mean daily temperatures were computed from hourly data collected by data loggers at each sampling site. Accumulated degree-days were computed from air temperature measurements at each site using Arnold's (1959) rectangular method and an arbitrary developmental threshold of 10 C.

Other temperature data were collected using methods described by Catts (1992). These included manual temperature measurements in and around the buccal region and body cavity of the pig carcasses. The thermometer bulb was shaded by a piece of foil to minimize the effect of direct sunlight. Internal buccal temperatures were taken by inserting a thermometer directly into the mouth as long as the integrity of the buccal tissue was maintained. Similarly, body cavity temperatures were recorded from the time that access to the internal tissues was available and extended until all tissues were decomposed. Body cavity temperatures were taken by inserting a thermometer into an area medial to the anterior side of the stomach.

Each site was visited from 14 through 28 July 1999 at about 1500 H each day, at which time manual temperature readings were taken. The sites were visited approximately every other day from 28 July 1999 until 8 August 1999. Rainfall data were obtained from the National Weather Service Station at the Won Pat International Airport, Tiyan, Guam, located approximately 0.4 km west of the study sites.

Adult flies were captured above the carcasses and from nearby vegetation using a sweep net and immediately killed in a jar containing ethyl acetate-saturated gypsum. Collected flies were pinned and identified to species using taxonomic keys in Bohart and Gressitt (1951) and by comparison to specimens maintained in the University of Guam's reference collection. Dr. R.S. Zack (Department of Entomology, Washington State University) confirmed calliphorid and sarcophagid identifications. Voucher specimens of adult flies were deposited in the James Entomological Collection at WSU. Maggots collected from the carcasses were labeled and preserved in vials containing 70% ethyl alcohol. Maggot lengths were measured to the nearest 0.1 mm using hand-held calipers, and data entered into a spreadsheet. Mean body length and standard errors were calculated for each sample, and linear and non-linear regression performed to relate mag-

got length to developmental time and degree-day accumulation (Hintze 2000). Non-linear regression was performed using the Levenberg-Marquardt non-linear least squares algorithm (Nash 1987).

Seven to twelve maggots were randomly collected from throughout each maggot mass in the head and body of each pig carcass at each sampling episode as described by O'Flynn (1983). These maggots were immediately placed in plastic cups containing a layer of canned cat food above a two-cm soil layer and covered with aluminum foil. Cup contents were later transferred in the laboratory into jars containing similar layers of soil and cat food, and covered with cloth mesh secured by rubber bands. The jars were maintained in the laboratory at 28 C until adult flies emerged five to six days later. Adult flies were identified as previously described.

A separate randomly collected maggot sample was collected from each body region as described above during the same sampling episode. These maggots were immediately killed in 70% ethanol and their length subsequently determined in the laboratory using hand calipers. They were identified to species from descriptions of blowfly larvae provided in Bohart & Gressit (1951). These data were used to construct size class frequency histograms for the buccal, body, and rectal regions of the two pig carcasses.

Results

The relative abundance of flies in sweep net samples taken over the carcasses was estimated from the number of a particular species of fly compared to the total number of flies collected at each of the respective sites (Table 1). The most abundant fly species were *Chrysomya rufifacies* (Macquart) followed by *Chrysomya megacephala* (F.). *Chrysomya nigripes* Aubertin was also collected, as was another unidentified calliphorid species. All sarcophagids identified were *Sarcophaga knabi* Parker, which comprised 6% of the total flies collected from the carcasses. Other flies collected included the muscids, *Haematobia exigua* (de Meijere) and *Musca sorbens* Wiedeman, and some unidentified Muscoidea. However, all maggots collected from each pig carcass in the field and reared to maturity in the laboratory were *C. rufifacies*.

Guam has a humid tropical climate with distinct wet and dry seasons. Mean annual temperature is 28 C with relative humidity ranging from 60–100%. The period encompassed by these experiments coincided with the first month of the rainy season, which extends generally from July through December. July has a mean monthly rainfall of about 25 cm (Lander 1994). Rainfall on Guam characteristically occurs in short, intense events during the transition period between wet and dry seasons and during the rainy season. Rainfall patterns during the observation period were typical of the season in that nearly 50% of the total rainfall occurred on a single day, 16 July (Fig. 1), while other significant rainfall episodes occurred about every four to five days.

Air and soil temperatures recorded by thermoprobes varied within each carcass and were different between the sunlit carcass in the field and the shaded carcass in the forest. Mean ground temperatures below both carcasses consistently matched or exceeded air temperatures. Mean air temperatures at both sites remained relatively constant, ranging from 25 to 29 C in the field and from 24 to 25 C in the forest. Mean air temperatures in the field consistently exceeded mean air temperatures in the forest by two to three degrees C. Similarly, mean ground temperatures in the field were always greater than those in the forest. Mean ground temperatures at both sites peaked about 4 days following placement of the carcasses at the study sites, with peak ground temperature in the field about twice that of peak ground temperature in the forest.

Temperatures were recorded in the buccal area of the open-field carcass for only three days, and for 7 days in the forest carcass (Fig. 2A) as tissues rapidly decomposed at both sites. Temperatures in the field carcass generally exceeded those in the forest carcass. A similar pattern was observed for body cavity temperature measurements, where temperature in the field carcass was measured for six days compared to nine days for the carcass in the forest (Fig. 2B). The body cavity of the field carcass was breached within 24 hr of exposure, allowing direct access to internal tissues by invading flies. Breaching occurred at about 72 hr in the forest carcass. As observed in the buccal area of each carcass, internal body temperatures in each carcass gradually declined as tissue deteriorated.

Rectal temperatures were recorded by thermoprobes for the duration of the experiment (Fig. 2C). While rectal tissues were intact, temperatures were consistently higher in the open-field carcass. As tissues deteriorated in both carcasses, temperature differences diminished. Even though visual observation suggested that rectal tissue decomposition in the sunlit open-field carcass was more rapid than in the shaded forest carcass, rectal temperatures were similar after 4 days post mortem, and remained consistently similar for the duration of the experiment apart from 9 days post mortem.

Table 1. Classification of insects collected in and near pig carcasses.

	Relative Abundance
Calliphoridae	
Chrysomya megacephala (F.)	19
Chrysomya nigripes Aubertin	8
Chrysomya rufifacies (Macquart)	48
unidentified	2
Sarcophagidae	
Sarcophaga knabi Parker	6
Muscidae	
Haematobia exigua (de Meijere)	13
Musca sorbens Wiedeman	1
unidentified Muscoidea	4

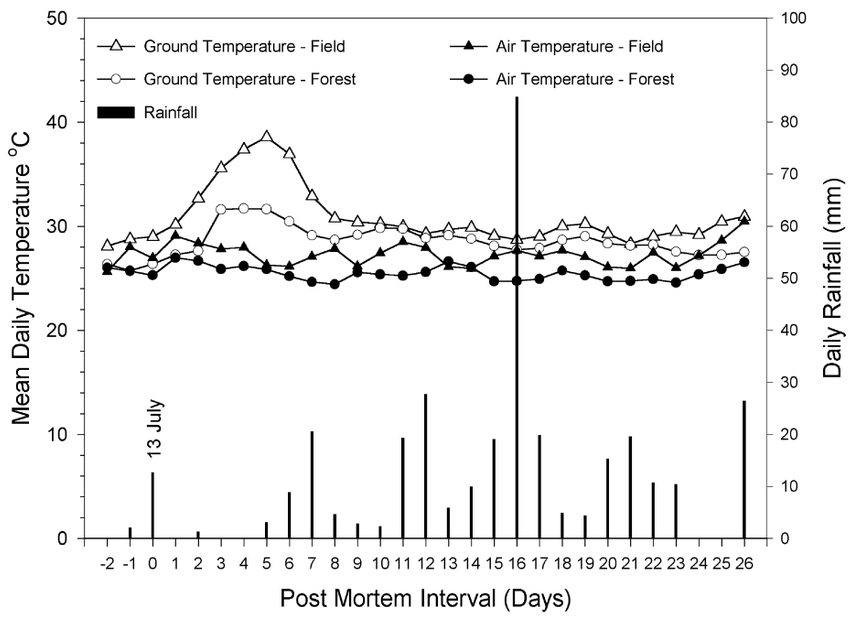

Fig. 1. Daily local rainfall and mean air and soil temperatures at the field and forest study sites.

When body maggot lengths, determined from samples of living maggots collected from different locations in the carcasses, were assigned to frequency classes, generational differences in the age structure of larvae became apparent (Figs. 3, 4, 5). Small maggots were observed in samples from the buccal area on 14 July and later on 17 July in the field carcass (Fig. 3A). However, the smallest size classes were observed on 14 July and on 19 July in the forest carcass, the latter date being 48 hrs later than the corresponding observation in the field carcass (Fig. 3B). The smallest maggot size classes were never observed in either carcass within the body cavity (Fig. 4A, B). However, there were distinct generational groupings that appeared in the forest carcass. These were evidenced by the appearance of smaller maggot size classes in subsequent samples collected on 18 July, 23 July and 5 August. Generational differences were not apparent among maggot size classes collected from the rectal area of either carcass, although there was a bimodal frequency distribution in the forest carcass collection of 17 July (Fig 5A,B).

Maggot growth was greatest in the buccal area of both carcasses and was significantly correlated to time and degree-day accumulation (Table 2, Fig. 6A). Data suggest that a single maggot generation was collected from the buccal area of both pigs. The growth rate of maggots in the carcass in the field exceeded that of maggots in the carcass in the forest. However, a different scenario was

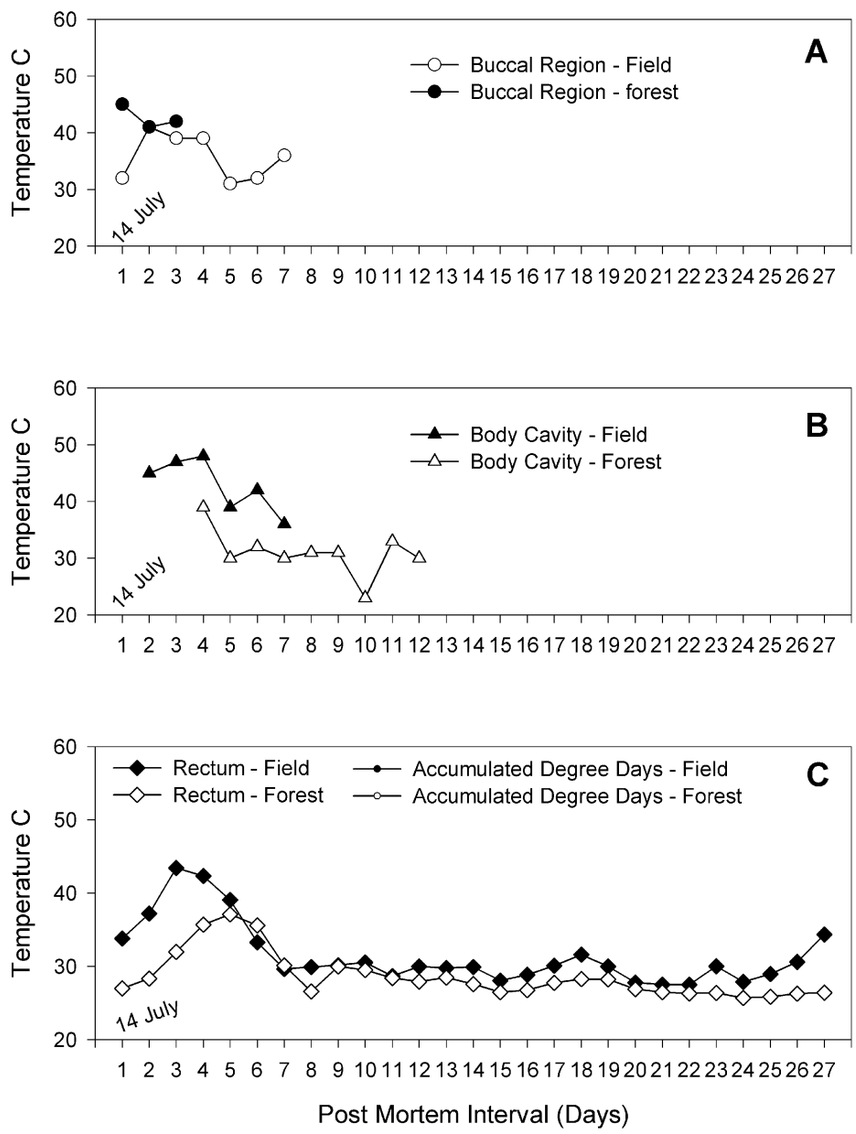

Fig. 2. Mean temperatures of the buccal cavity (A), body (B) and rectum (C) of pigs positioned in an open, sunlit field and in a forested area on Guam.

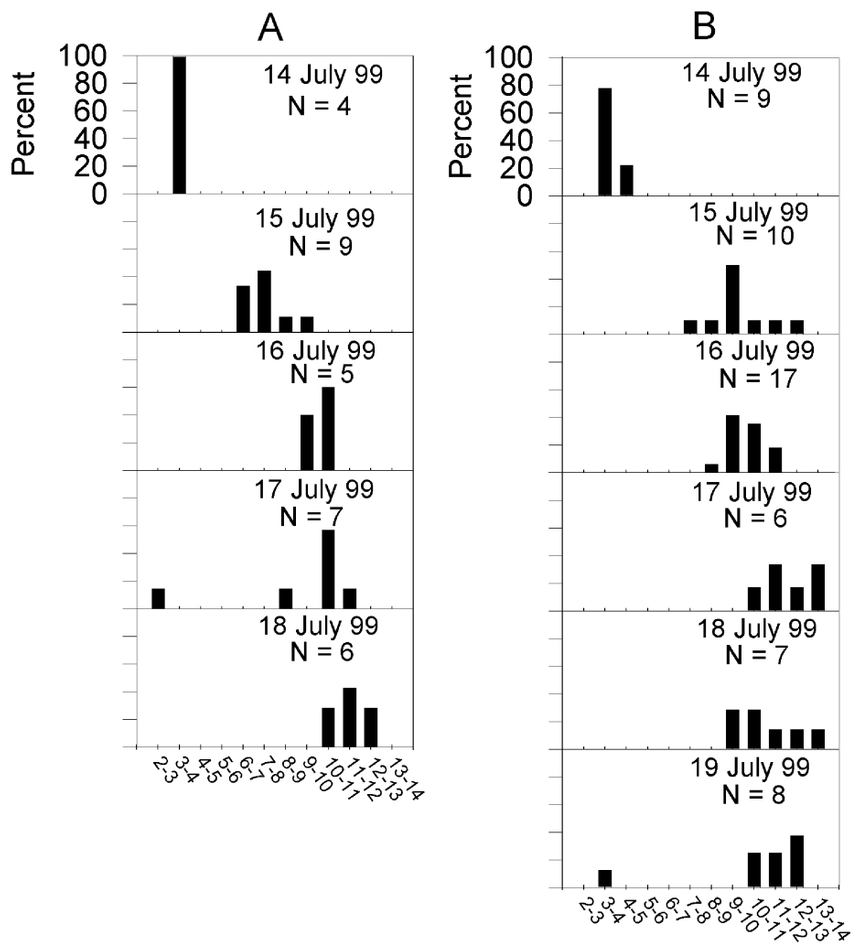

Fig. 3. Size class distribution of maggots collected over time from the maggot mass located in the buccal cavity of the pig carcass in the sunlit field (A) and under a forest canopy (B). The proportion of individuals in each length class (mm) is indicated on the Y-axis with size class intervals located on the X-axis. Sample size (N) is shown for each histogram.

obtained from observations on maggots collected from the body cavity of the two carcasses (Table 2, Fig. 6B). Small maggot size classes were not present in samples taken from the open-field carcass. Rather, mean length of these maggots decreased from the initial sample on 14 July until the last sample taken on 18 July at which time body tissues were completely decomposed. Size decreases likely resulted from physiological changes in mature maggots as they ceased to feed

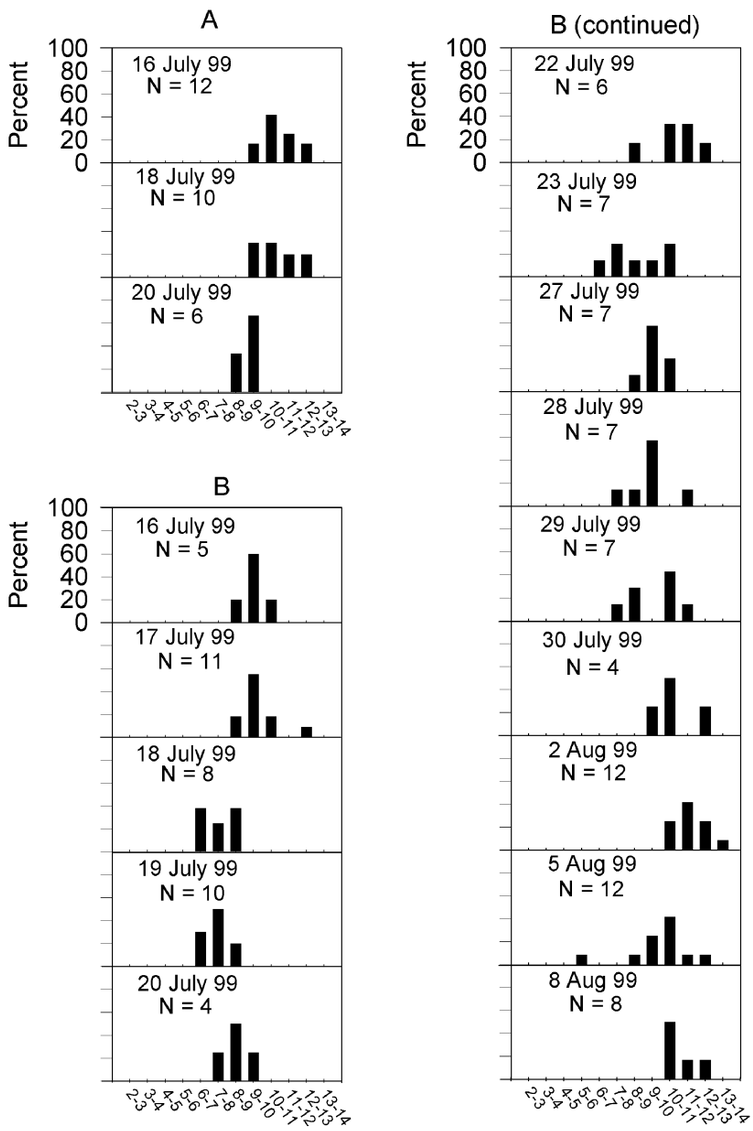

Fig. 4. Size class distribution of maggots collected over time from the maggot mass located in the body cavity of the pig carcass in the sunlit field (A) and under a forest canopy (B). The proportion of individuals in each length class (mm) is indicated on the Y-axis with size class intervals located on the X-axis. Sample size (N) is shown for each histogram.

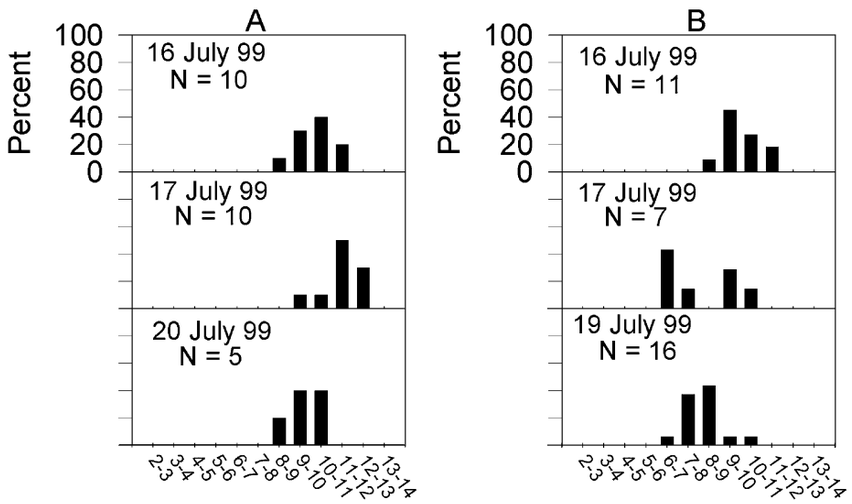

Fig. 5. Size class distribution of maggots collected over time from the maggot mass located in the rectal area of the pig carcass in the sunlit field (A) and under a forest canopy (B). The proportion of individuals in each length class (mm) is indicated on the Y-axis with size class intervals located on the X-axis. Sample size (N) is shown for each histogram.

prior to initiating pupation. In contrast, mean maggot length in collections from the body cavity of the forest carcass increased over time and maggots persisted in the tissues until 26 days post mortem (8 August), although a similar length decrease in mature maggots may be reflected in the slope of the regression line. Maggot length data from the forest carcass suggest that multiple generations of flies infested the carcass over the course of observation, while a single generation may be inferred from samples in the buccal area. All tissues in the buccal area had been decomposed by the end of day 5 post mortem in the sunlit field carcass, and by day 6 post mortem in the shaded forest carcass.

Discussion

Goff (1993) divided decomposition into five general stages: fresh, bloated, decay, post-decay, and skeletal. Transition from one stage to the other may not be distinct since defining characteristics may overlap or vary dependent on local conditions. Though decomposition is generally more rapid in the tropics than in temperate climates, decomposition rates can vary between different locations based on microhabitat differences (Goff 1993). In the present study, initial arthropod invasion occurred during the fresh stage, and continued through bloat, with the fresh stage beginning and ending nearly simultaneously for both carcasses. During the bloated stage each carcass swelled with gases within 24 hours of

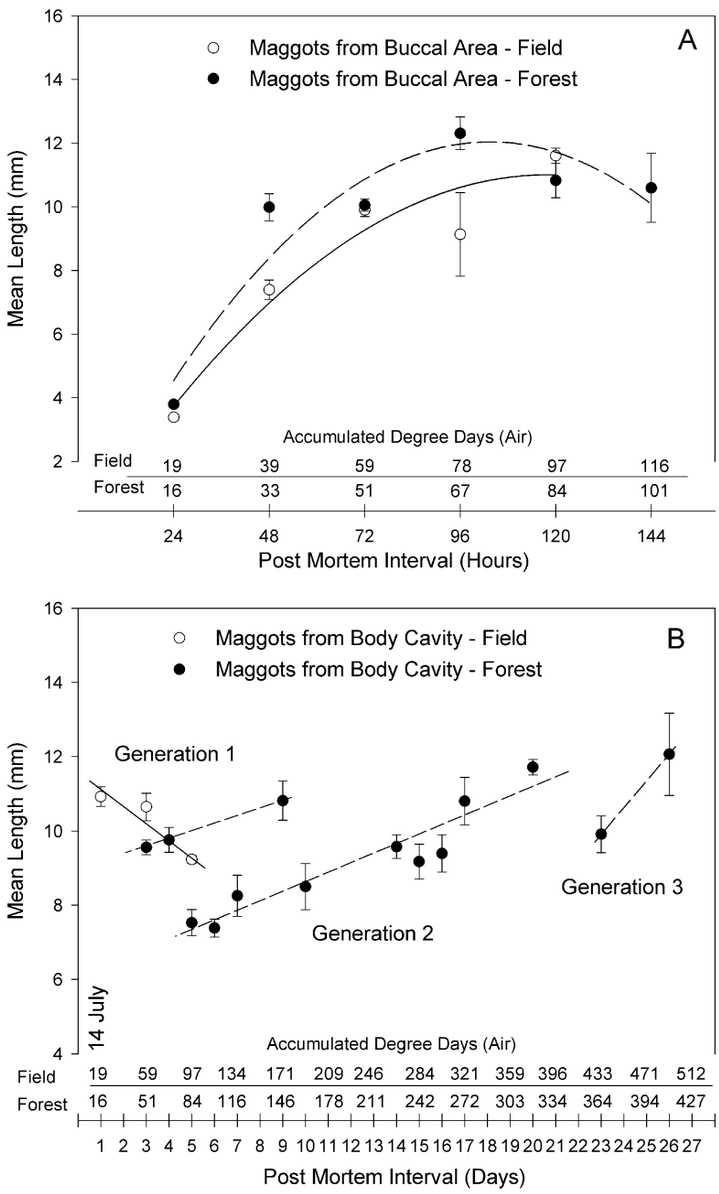

Fig. 6. Maggot length (mean + SE) in the buccal area (A) and body cavity (B) of pig carcasses located in a sunlit field and in a shaded forest on Guam. Lines shown in the figure were fitted by regressing maggot length onto the post mortem interval as described in the text.

death. The bloated state continued until day 2 (15 July) for the sunlit field carcass and until day 3 (16 July) for the shaded forest carcass. The bloated phase in each carcass terminated in cutaneous ruptures that allowed fly access to internal tissues. The carcass in the field exhibited a 10 cm-long rupture in the abdomen, while the carcass in the shaded forest ruptured in the gastrointestinal region, forcing the intestines and part of the stomach outside the body. The decay stage for both carcasses began as the carcass deflated, and rapidly ended as tissues were removed by fly maggots, leaving only bone and skin. No mummification was observed in either carcass, as decomposition proceeded rapidly from the decay stage to skeletal stage.

Calliphorid and sarcophagid flies were the exclusive decomposers observed infesting the pig carcasses in this study. There were no carrion beetles collected, which is consistent with previous insect surveys on Guam (Beller 1948, Bohart & Gressitt 1951, Essig 1956). Carrion beetles have been described infesting domestic pig carcasses in arthropod succession studies in the Hawaiian Islands (Richards & Goff 1997).

The carcass in the sunlit field decomposed most rapidly, with a single generation of maggots consuming the entire carcass in four days. Decomposition of the carcass in the forest appears to have supported three generations of *C. rufifacies* maggots. Mature *C. rufifacies* maggots may have cannibalized conspecifics at each site and preyed on maggots of other species (Fuller 1934, Goodbrod & Goff 1990), which would contribute to the observation of no other fly species being reared from collected maggot samples.

Mean air temperature on Guam is relatively uniform, with a seasonal difference between mean summer and winter temperature of only 2 C (National Weather Service, Tiyan, Guam). Air temperature in the sunlit field was only 1.8C higher than that of the shaded forest. However, this relatively small difference in air temperature resulted in a difference of 85 degree-days between the two habitats during the study period, and was likely associated with the higher growth rate observed in flies the field carcass (Fig. 6B) and the more rapid decomposition of that carcass. Ground temperature under the sunlit carcass in the field averaged 1.2 C higher than ground temperature under the shaded carcass in the forest. An exception was the period from day 1 post mortem to day 9 post mortem, when heat contributed by the maggot mass likely contributed to the elevated ground temperatures of near 40 C for the sunlit carcass and 33 C for the shaded forest carcass. The nearly 2:1 difference between ground temperatures under the two carcasses may have been due to a significantly larger subcutaneous maggot population in the sunlit carcass in the field that was neither visible nor adequately sampled in this study.

The decomposition rate in the sunlit field site was much greater than the eight days reported at a similarly exposed site at 650 m elevation in Hawaii by Richards and Goff (1997). They reported carrion beetles as well as *C. rufifacies* and *C. megacephala* as the primary decomposers at the Hawaii site. Estimated

development rates for *C. rufifacies* in this study exceeded those reported by Byrd & Butler (1997), who estimated a development period from egg to adult ranging from 190 to 162 hr under mean cyclic temperature regimes of 15.6, 21.1, 26.7, and 35.0 C in the laboratory. They concluded that *C. rufifacies*' predictable developmental time and low larval length variation should permit accurate and repeatable estimates of post mortem interval. The substantial differences in decomposition rates between carcasses in our study sites, and between our study and that of Richards & Goff (1997) in Hawaii, however, suggest that relatively small differences in temperature, perhaps in interaction with other factors at a given site, combine to produce results unique to each site. Forensic analysis of entomological factors at a given locality in Micronesia, and in other humid tropical regions, will require a more controlled evaluation of arthropod succession and development across the range of local micro-environmental conditions. Furthermore, the present study indicates that post mortem interval based on fly maggot growth rates may be difficult to assess due to overlapping generations, rapid developmental rates and swift tissue deterioration.

Table 2. Linear and non-linear models and associated sample sizes (N) and coefficients of determination (r^2) for regressions of the length of maggots collected in pig carcasses in the field and forest onto post mortem interval (HOURS, DAYS) and degree-day accumulation with a 10 C activity threshold (DD_{10}).

Regression Model	N	r^2
Maggots from Buccal Area – Field		
$Y = -0.46 + 0.19*HOURS - (8.25*10^{-4})* HOURS^2$	5	0.96
$Y = -0.36 + 0.24*DD_{10} - (1.23*10^{-3})* DD_{10}^2$	5	0.96
Maggots from Buccal Area – Field		
$Y = -0.46 + 0.19*HOURS - (8.25*10^{-4})* HOURS^2$	4	0.94
$Y = -0.36 + 0.24*DD_{10} - (1.23*10^{-3})* DD_{10}^2$	4	0.94
Maggots from body Cavity – Field		
$Y = 11.96 - 0.85*DAYS$	1	0.93
$Y = 11.93 - 0.04*DD_{10}$	1	0.93
Maggots from body Cavity – Forest – Generation 1		
$Y = 8.79 - 0.63*DAYS$	1	0.93
$Y = 8.84 - 0.04*DD_{10}$	1	0.93
Maggots from body Cavity – Forest – Generation 2		
$Y = 6.54 - 0.50*DAYS$	7	0.95
$Y = 6.53 - 0.03*DD_{10}$	7	0.94
Maggots from body Cavity – Forest – Generation 3		
$Y = 7.73 - 2.17*DAYS$	2	1.0
$Y = 7.91 - 0.12*DD_{10}$	2	1.0

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