Estimating Post-mortem Intervals of Wildlife Carcasses Using Necrophilous Insects

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Abstract: The succession and species composition of necrophilous insects on animal carcasses can be used by wildlife law enforcement officers for estimating post-mortem interval (PMI) at suspicious death scenes. Necrophilous insects infesting wildlife carcasses (Louisiana black bear, white-tailed deer, and alligator) in a woodland habitat were monitored during the spring of 1999 to eventually develop a guide for estimating PMI by wildlife officers based on an insect database. The study was conducted at the Waddill Outdoor Educational Center in East Baton Rouge Parish, Louisiana. An overview of the study is provided herein based on sampling of aerial and ground inhabitating necrophilous insects associated with the carcasses. Sixty-five species of insects were manually collected during the spring study (20 fly species, 33 beetle species, and an assortment of spiders, mites, and other miscellaneous insects of minor forensic importance). On Day 1, adult green blow flies (*Phaenicia coeruleiviridis* [MacQuart]) and black blow flies (*Phormia regina* [Meigen]) were the initial insects associated with the carcasses. Blow fly egg masses were commonly found by Day 2 and were generally located in the mouths and head regions of all carcasses. Soldier flies (*Hermetia illucens* [L.]) became associated with the bear carcass by Day 11 and continued until Day 92 (end of the test). Due to the decomposition of the other carcasses, soldier fly immatures were not found at any other carcass site. Beetles were the second wave of insects associated the carcasses and consisted of both predatory and scavenger species. Skin beetles, which prefer the more advanced decaying carcass stage, were not found on alligators but were collected from all other carcasses. At least some representatives of predator ants species (red imported fire ant, acrobat ant, and carpenter ant) were found from Day 2 until complete decomposition of the wildlife carcasses.

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Forensic entomology is simply defined as the use of entomological information in a public debate or a court of law. The strict medico-legal application of this term involves the use of necrophilous insects collected at a death scene to estimate the post-mortem interval (PMI). In recent years, however, it has been applied more commonly to suspicious or unexplained death scenes involving humans.

Necrophilous insects (i.e., flies and beetles) can also be used to estimate PMI at wildlife death scenes involving poaching or suspicious deaths of endangered species (Shoopman 1997). As an example, an agent with the U.S. Fish and Wildlife Service in Lafayette, Louisiana, recently investigated an unnatural death of a Louisiana black bear in south Louisiana. A sample of blow fly larvae was collected from the bear, and sent to the Forensic Entomology Laboratory at the Louisiana State University (LSU) Agricultural Center in Baton Rouge. The larvae were identified and an estimate of PMI was made. According to the agent, the PMI estimate based on the necrophilous insects was pivotal in obtaining an out-of-court confession from the suspect.

Currently, forensic entomologists are educating police and wildlife law enforcement officers about the use of necrophilous insects in providing another piece to the investigative puzzle. Specifically, officers are trained to obtain and preserve an "entomological picture of the death scene" from an insect-infested cadaver or wildlife carcass.

A cooperative research project was established in 1999 between the Louisiana Department of Wildlife and Fisheries (LDWF) and the LSU Agricultural Center to record the succession and species composition of necrophilous insects on selected wildlife carcasses over the course of 3 seasons, i.e., spring and fall 1999 and winter 2000. Insect specimens from the 3 studies will form a database from which subsequent collections of insects by wildlife officers from unnatural or illegal wildlife death scenes will be compared in estimating PMIs. As a result, a field guide will be developed to assist wildlife officers in estimating a general PMI of the wildlife carcass at the death scene. More specifically, the guide will provide wildlife officers with a working knowledge for collecting and preserving necrophilous insects at a death scene for later transfer to an entomologist for verification of the insect species and refinement of the PMI estimate based on the insect database from this 3-season field study.

Data provided in this publication represent the succession and species composition of necrophilous insects associated with selected high-profile wildlife carcasses during the first 14 days of the 92-day spring study.

Methods

Upon the recommendations of LDWF, the study was conducted during spring 1999 at the Waddill Outdoor Educational Center in the northern part of East Baton Rouge Parish, Louisiana. This 117-hectare woodland habitat represents the typical location where LDWF frequently encounters suspicious deaths of high profile wildlife.

Three types of wildlife were selected by LDWF officers as the most appropriate animal species to include in the field study. These high-profile animals included the

Louisiana black bear (*Euarctos americanus* Gray), white-tailed deer (*Odocoileus virginianus* Rafinesque), and the American alligator *(Alligator mississippiensis).* Swine (*Sus scrofa* Linneaus) were used also as a comparison based on about 20 years of collecting necrophilous insects from swine in selected Louisiana habitats.

Two carcasses of each wildlife type were used in the spring study with the exception of the bear. Since the Louisiana black bear is a threatened species, discriminate harvesting of several bears to coincide with the initiation of the field test was not an option. The single bear carcass used in the spring study became available due to an accidental encounter with an automobile along a rural highway in southern Louisiana. Often when accidents occur, they do so at night when blow flies are not actively searching for a source to deposit their eggs (Tessmer and Meek 1995). Thus, there is less chance for the bear carcass to become infested with fly eggs before removal by wildlife officers. The 147-kg bear carcass used in this study did not exhibit prior insect infestations or any traumatic external damage. Upon retrieval, the relatively fresh bear carcass was wrapped in polyethylene to prevent blow fly egg deposition and then transported to a freezer at the LSU School of Veterinary Medicine in Baton Rouge.

Two juvenile alligators (81 and 97 cm long) and 2 adult deer (32 and 41 kg) were harvested by LDWF officers. The carcasses also were wrapped in polyethylene to prevent egg deposition and stored in a walk-in cooler at LDWF headquarters in Baton Rouge. All polyethylene-wrapped bear, deer, and alligator carcasses were transported to the Waddill Outdoor Educational Center on 31 March 1999, i.e., one day prior to the beginning of the spring study on 1 April. The wildlife carcasses remained wrapped in polyethylene at the woodland location for a 24-hour period allowing the external surfaces of the carcasses to approach ambient temperatures. All carcasses were unwrapped on the first day of the study.

Two swine carcasses (*x* 272 kg) were obtained from the LSU Swine Unit in Baton Rouge on the first day of the study (1 April). The freshly terminated animal carcasses were immediately secured in plastic trash bags to prevent fly egg deposition and were transported also to the Waddill Outdoor Educational Center.

Seven sites within 16-ha section of the woodland habitat were selected by LDWF officers as representative locations where carcasses of these high profile wildlife have been commonly found under suspicious circumstances. Each of these sites were separated from one another by at least 91.5 m to reduce the potential for the same adult fly and beetle populations from visiting multi-carcasses during the study period. The carcasses were randomly assigned to the sites by individually drawing site numbers and carcass types.

At each site, hardware screen enclosures, supported by vertical wooden stakes, were placed around each carcass to inhibit disturbance by vertebrate scavengers. Four $5 \times 5 \times 5$ cm stakes measuring 0.9 m high were placed equidistance around each carcass and securely driven into the ground. The 0.9-m wide hardware screen was stretched around the vertical wooden stakes and secured tightly. Necrophilous insects had full access to the carcasses either through the 0.64-cm hardware mesh itself or by entering from the opening above.

Necrophilous insect populations on all 7 carcasses were sampled over the course of decomposition. Sampling occurred daily for the first 2 weeks, every other day for 2 weeks, semi-weekly for 2 weeks and once per week thereafter. Sampling was discontinued on a given carcass when it became skeletonized or reached a dry, mummified condition.

Two sampling methodologies were used to collect a representative sample of each necrophilous insect species present but not to capture every specimen. Flying insects closely associated with the carcass were aerially netted immediately upon arrival at the carcass site. The captured insects were anesthesized using ethyl acetate and subsequently placed into labeled plastic bags.

The second method of sampling involved manually searching the exterior of the carcass as well as beneath the carcass and within the surrounding leaf litter and ground surface out to a meter from the carcass. Again, a representative sample of each necrophilous insect species present at the time of sampling was collected and placed into labeled glass vials containing 80% ethyl alcohol.

Color 35 mm photographic slides were regularly taken of all carcasses on each sampling data to serve as a visual record of the decomposition stage. White index cards, marked with the site number and sampling date, were placed on the carcass prior to photographing for documentation.

Selected meteorological data were collected on each sampling date. Rainfall and ambient temperatures were monitored with the use of a rain gauge and a high/low thermometer. Also the internal temperatures of the carcasses were recorded on each sampling date using a pointed, aluminum-encased thermometer (Taylor 6072, Juarez, Mexico). The entire pointed shaft of the thermometer was embedded within the carcass between the ribs and remained in place until skeletonization.

Results and Discussion

A total of 390 aerial and soil surface samples were collected from the 7 carcasses monitored during all or part of the 92-day spring study. These samples yielded 65 species of insects and arthropods (i.e., spiders, mites and other less significant specimens). Due to the rapid skeletonization of the white-tailed deer, alligators, and swine within the first 14 days, only the comparative data collected during the first 2 weeks are reported here.

A total of 46 species of necrophilous insects were collected by aerial netting and ground sampling on and near the carcasses by Day 14. However, only 20 necrophilous species are reported here within as determinded by their forensic importance, abundance, and common association with the wildlife carcasses (i.e., 9 fly and 11 beetle species). The transient relationships of fly and beetle life stages associated with the carcasses are shown in Tables 1–4.

White-tailed Deer

The presence and succession of necrophilous insects associated with white-tailed deer carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, are given in Table 1. A total of 9 fly species, 9 beetles, and 2 ant species were collected from the deer carcasses during the first 2 weeks of the study. Adults of the green blow fly (*Phaenicia coerudeiviridis* [MacQuart]) began visiting the carcasses within minutes after the deer carcasses were placed at the sites and unwrapped from the polyetylene on Day 1. The adult flies continued their presence around the deer carcasses for several days. Green blow fly eggs were present by Day 2 as were adults of the black blow fly (*Phormia regina* [Meigen]). Immature stages of the black blow fly were slightly delayed in becoming established (Day 4).

Black carpenter ants (*Camponotus pennsyvanicus* [DeGeer]) were collected in the vicinity of the carcasses beginning on Day 2. Although black carpenter ants were observed transporting blow fly eggs and larvae away from the carcasses, this species generally feeds on a wide variety of food sources and is not a primary necrophilous insect.

As the fly larval mass began to increase in size, several predatory beetle species were found in close association with the carcasses. Rove beetles and clown beetles arrived by Day 3 and remained for more than a week on or near the carcasses. By Day 9, however, when the mass of fly larvae dispersed from the body, the incidence of predatory beetle collections diminished.

American Alligators

The presence and succession of necrophilous insects associated with alligator carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, are given in Table 2. The lowest number of forensically important insect species (15) was recorded from alligators during the study (6 fly species, 8 beetle species, and 1 ant species) compared to the other animal carcasses. No adult blow fly activity was observed on the alligator carcasses immediately following their placement at the field sites (Day 1). Adult blow flies and their egg were not observed until Day 2, whereas, black blow fly adults were not collected until Day 4. Rove beetles (*Philonthus* spp.) and carrion beetles (*Oiceoptoma inaequale* [Fab.]) also were found on the carcasses by Day 2 and 3, respectively, but were noticeably fewer in numbers collected compared to the other carcass types. Clown beetle species and the rove beetle (*Creophilus maxillosus* [L.]) did not appear until several days later. As predators of fly larvae, it is logical for such beetles to not arrive until a substantial mass of larvae developed (i.e., Days 5 and 6).

Domestic Swine

The presence and succession of necrophilous insects associated with swine carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, are given in Table 4. Within the first 14 days, more necrophilous insect species were recovered on and within the immediate vicinity of the two swine carcasses than from any other carcass type used in this study. A total of 21 species were recovered which included 9 fly species, 11 beetle species, and one ant species.

Adult green blow flies and black blow flies began visiting the swine on Day 1 immediately after the carcasses were placed in the woodland habitat. Initial blow fly eggs were deposited on the swine carcasses between Days 1 and 2, with egg deposition continuing for several days. Green blow flies appeared initially to be the predominant species based on the recovery of egg masses and the laboratory rearing and identification of the hatched larvae. The identifications of the laboratory-reared larvae were later correlated with the identifications of field-collected adults and larval stages.

Black blow fly larvae were not collected until Day 4 from the swine carcasses, however, the larvae present were recognized as 3rd instar larvae (i.e., more mature larvae). This stage of development strongly indicates that the black blow fly began depositing eggs late on Day 1 or by Day 2. Apparently, the initial numbers of larvae were below a detectable level in comparison to the domination of the carcass by green blow fly immatures and/or were located in an obscured location, thus, unintentionally missed in the sampling effort.

Louisiana Black Bear

The presence and succession of necrophilous insects associated with the Louisiana black bear carcass in a woodland habitat in East Baton Rouge Parish, Louisiana, are given in Table 3. A pictorial sequence of its decomposition is provided also in Figure 1 (A–D). No adult blow flies were present during the early afternoon sampling period of Day 1. Eggs, very young larvae, and adults of the black blow fly were

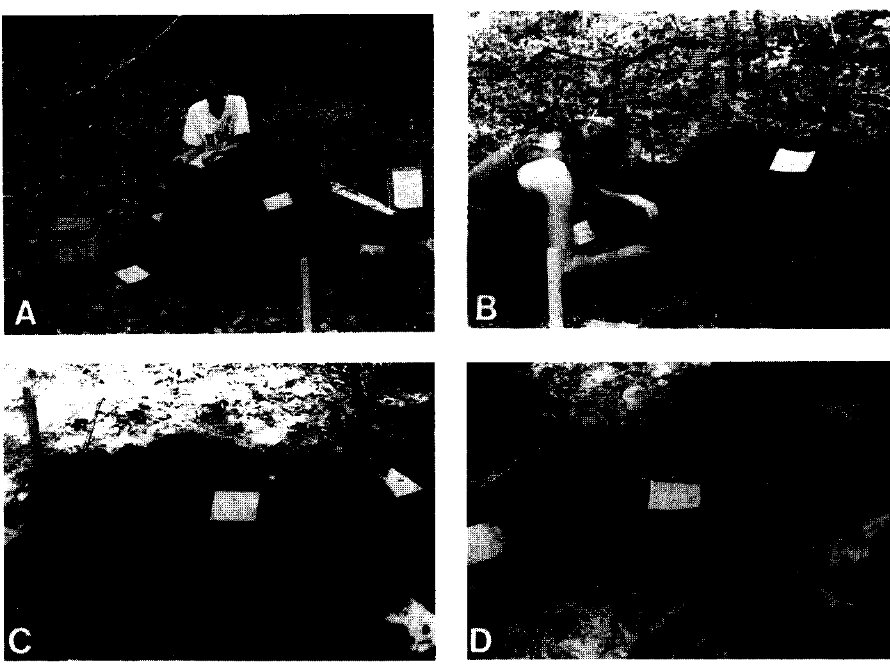

Figure 1. Decomposition sequence of a Louisiana black bear carcass during April 1999 at the Waddill Outdoor Education Center in East Baton Rouge Parish, Louisiana: Days post-exposure: A) fresh carcass (Day 2); B) active decay (Day 9); C) putrid decay (Day 20); and D) dry remains (Day 72).

Table 1. Presence and succession of necrophilous insects associated with white-tailed deer carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, from 1–14 April 1999.[a] A, adult; L, larvae; E, eggs.

		Stages of decomposition													
		Fresh		Bloat		Active decay					Putrid			Dry remains	
		Day													
Species	Common name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diptera															
Calliphoridae eggs	Blow fly eggs		—	E	—				E						
Phaenicia coeruleiviridis	Green blow fly	A	—	—	A&L	—	—	—	L	—	—				L
Phormia regina	Black blow fly		—	A	—	—	—	—	A&L	—	—	—	—	A	
Sarcophaga sp.	Flesh fly								—	A					
Hydrotaea leucostoma	Dump fly				—	—	A	—	—	—	A&L	—	—	—	L
Hermatia illucens	Black soldier fly								—	—	L	—	—		
Fannia scalaris	Latrine fly			A		A		A	—	—	L	—	A&L		L
Piophila casei	Cheese skipper fly			—	A	—		—	—	—	A	—	—	—	
Prochyliza xanthostoma	Cheese skipper fly					A		—	—	—	A	—	—	—	
Sepsis sp.	Black scavenger fly											—	A		
Coleoptera															
Creophilous maxillosus	Rove beetle				A		—	—	A	—	—		A		L
Philonthus spp.	Rove beetles			—	—	—	A	—	—	—	—	A&L	—	—	A
Aleocharinae	Rove beetles			A			A		—	—	—	A	—	—	—
Euspilotus assimilis	Clown beetle			—	—	—	—	A	—	—	—	—			A
Hister abbreviatus	Clown beetle								A		—	A			A
Oiceoptoma inaequale	Carrion beetle				—	—	—	A	—	—					
Necrodes surinamensis	Carrion beetle						A		—	A	—	—	—	L	—
Necrophila americana	Carrion beetle				A				A						
Omosita colon	Sap beetle				A				A		—	—	A	—	—
Hymenoptera															
Camponotus pennsylvanicus	Black carpenter ant		—	A		—	—	A	—	—		A			
Crematogaster sp.	Acrobat ant														A

[a] Based on manual sampling of aerial and ground inhabiting insects.

Table 2. Presence and succession of necrophilous insects associated with alligator carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, from 1–14 April 1999.[a] A, adult; L, larvae; E, eggs.

		Stages of decomposition													
		Fresh		Bloat			Active decay			Putrid			Dry remains		
		Day													
Species	Common name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diptera															
Calliphoridae eggs	Blow fly eggs			——E——											
Phaenicia coeruleiviridis	Green blow fly			——A&L——		-L-		-A&L-	-L-		-A&L-		-L-		
Phormia regina	Black blow fly				—A——			———A&L———							
Hydrotaea leucostoma	Dump fly						——A——			—A&L—	–L–				-A&L-
Fannia scalaris	Latrine fly		–A–		–A–		–A–	–L–			———L———		–L–		–L–
Piophila casei	Cheese skipper fly						–A–		–A–		–A–				
Sepsis sp.	Black scavenger fly									——A——		–A–			
Coleoptera															
Creophilus maxillosus	Rove beetle						——A——								
Philonthus spp.	Rove beetles							————A————					-L-		—A—
Aleocharinae	Rove beetles		–A–								———A———				—A—
Euspilotus assimilis	Clown beetle							————A————							
Hister abbreviatus	Clown beetle								–A–						
Oiceoptoma inaequale	Carrion beetle						————A————				-A&L-				
Necrodes surinamensis	Carrion beetle														—L—
Omosita colon	Sap beetle														—A—
Hymenoptera															
Crematogaster sp.	Acrobat ant		–A–				–A–								

[a] Based on manual sampling of aerial and ground inhabiting insects.

Table 3. Presence and succession of necrophilous insects associated with Louisiana black bear carcass in a woodland habitat in East Baton Rouge Parish, Louisiana, from 1–14 April 1999.[a] A, adult; L, larvae; E, eggs.

		Stages of decomposition													
		Fresh			Bloat					Active decay					
		Day													
Species	Common name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diptera															
Calliphoridae eggs	Blow fly eggs		—	—	E	—	—	—							
Phaenicia coeruleiviridis	Green blow fly		A		—	—	—	A&L	—	—	—	—	L	—	—
Phormia regina	Black blow fly		—	—	—	—	A&L	—	—	—	—	—		L	—
Sarcophaga sp.	Flesh fly								A						
Hydrotaea leucostoma	Dump fly												A		
Hermatia illucens	Black soldier fly									A	—	A&L	—		
Fannia scalaris	Latrine fly										A				
Piophila casei	Cheese skipper fly								—	—	—	A	—	—	—
Coleoptera															
Creophilous maxillosus	Rove beetle					—	—	—	A	—	—	—		L	
Philonthus spp.	Rove beetles			—	—	—	A	—	—	—	A&L	—	A		A
Aleocharinae	Rove beetles						—	—	A	—	—	—		A	
Euspilotus assimilis	Clown beetle			A			—	—	—	—	A	—	—	—	—
Hister abbreviatus	Clown beetle													A	
Oiceoptoma inaequale	Carrion beetle			—	—	A	—	—		A	A&L	L			
Necrodes surinamensis	Carrion beetle							A		—	A	—	—	L	—
Omosita colon	Sap beetle						—	—	A	—	—	—		A	—
Necrobia rufipes	Red-legged ham beetle									A					
Dermestes caninus	Skin beetle									A					
Hymenoptera															
Solenopsis invicta	Red imported fire ant			A			A		—	A		A			

[a] Based on manual sampling of aerial and ground inhabiting insects.

Table 4. Presence and succession of necrophilous insects associated with swine carcasses in a woodland habitat in East Baton Rouge Parish, Louisiana, from 1–14 April 1999.[a] A, adult; L, larvae; E, eggs.

Stages of decomposition: Fresh (days 1–2), Bloat (days 3–4), Active decay (days 5–7), Putrid (days 8–10), Dry remains (days 11–14)

Species	Common name	Days 1–14
Diptera		
Calliphoridae eggs	Blow fly eggs	——E——
Phaenicia coeruleiviridis	Green blow fly	–A– ————A&L———— –A– –L–
Phormia regina	Black blow fly	——A—— ——A&L—— -L- -A&L- ——A——
Sarcophaga sp.	Flesh fly	–A– –A– ——A—— –A–
Hydrotaea leucostoma	Dump fly	–A– ——A—— -A&L- -L- -A&L- -L- -A&L- -L-
Hermatia illucens	Black soldier fly	–L– –A–
Fannia scalaris	Latrine fly	–A– –A– –A– ——A&L—— -L- -A&L- -L-
Piophila casei	Cheese skipper fly	——A—— –A– ——A——
Prochyliza xanthostoma	Cheese skipper fly	——A—— –A–
Sepsis sp.	Black scavenger fly	–A– ——A—— –A–
Coleoptera		
Creophilous maxillosus	Rove beetle	————A———— –L–
Philonthus spp.	Rove beetles	————A———— –A&L– ————A————
Aleocharinae	Rove beetles	——A—— ——A—— –A–
Euspilotus assimilis	Clown beetle	————A————
Hister abbreviatus	Clown beetle	–A– –A–
Oiceoptoma inaequale	Carrion beetle	————A———— ——L——
Necrodes surinamensis	Carrion beetle	–A– –A&L– ——L—— –L–
Necrophila americana	Carrion beetle	–A– –A– –A–
Omosita colon	Sap beetle	——A—— ——A—— ——A—— ——A——
Necrobia rufipes	Red-legged ham beetle	——A—— –A–
Dermestes caninus	Skin beetle	–A–
Hymenoptera		
Camponotus pennsylvanicus	Black carpenter ant	–A– –A–

[a]Based on manual sampling of aerial and ground inhabiting insects.

collected during the sampling period on Day 2. The young larvae were returned to the laboratory and reared to a larger size for positive identification. By Day 4, however, late 2nd and 3rd instar larvae were collected, reared, and identified as the green blow fly. The larval size at the time of collection indicates that the eggs of this species were present on Day 2, in other words, the eggs were unintentionally omitted from the sample due either to their obscured location on the carcass compared to other eggs masses and/or were too few in number when the egg sample was collected.

Blow fly adults continued to visit the bear carcass up to day 12. Cheese skipper adults (*Piophila casei* L.), preferring the active decay stage, were collected beginning on Day 8. Black soldier fly adults *(Hermatia illucens)* typically appear when the carcass is in the active stage of decomposition. The first adult samples of this species were collected on Day 9.

Representatives of predatory beetles of fly larvae (i. e., rove, clown, and carrion beetles) were first observed on Day 3 and were present throughout the remaining 14-day period. The greatest number of beetle species present on the bear carcass during the first 2 weeks occurred between Days 6–9.

General Comments

Several observations were made over the course of the field study relating to the necrophilous insect activity on the carcass and the decomposition process. First, the duration of insect activity on the carcass as a whole directly corresponds to the carcass size, and ultimately the number of fly larvae it can support. For example, the predacious rove beetle *(C. maxillosus)* which feeds on adult blow flies and their larvae, were collected for only 2 days from the young alligators. In comparison, *C. maxillosus* was found 6–7 days on all larger carcass types.

Second, the larger the carcass the longer it takes for the carcass to skeletonize. Similarly, the longer a carcass is infested with fly larvae the more beetle species are present serving as predators of fly larvae and scavengers of carrion.

Third, selected necrophilous insects were commonly found on all carcasses. However, the number of days they were associated with the carcasses varied. For example, the cheese skipper fly was collected at all carcass types; however, it was collected from the alligators for only 3 days versus a minimum of 7 days for all other animal types.

Lastly, the horizontal bars in Table 1 – 4 do not reflect relative abundance of predatory beetle and blow fly populations despite the fact that their numbers did dramatically decline once entering advanced and putrid stages of decomposition. This decline was expected and supported by the decreasing number of beetles and blow flies collected on and around the vicinity of each carcass.

Summary

The succession and species composition of necrophilous insects on a wildlife carcass often follows a predictable pattern relative to the different decomposition stages. Entomologists rely on this established pattern of insect association with de-

caying remains in order to estimate PMIs. Adult blow flies are capable of locating a fresh carcass within minutes of death and begin depositing eggs, thereby setting the "biological clock" in motion. It is the responsibility of the forensic entomologist to determine how far the "hands of the biological clock" have moved when estimating a PMI.

This field study is the first such research conducted in the southeastern states that relates to necrophilous insect activity on selected high-profile wildlife carcasses. The entomological data directly applies to environmental conditions typically found in Louisiana and along the upper gulf coast area. The data obtained will be of particular importance if and when the information is required in a litigated case against an accused poacher in a court of law.

In addition, these data eventually will be used to develop a field guide to assist wildlife law enforcement officers in estimating a general PMI of unnatural or illegal deaths of wildlife. More importantly, the guide will educate the wildlife officer on how to collect a credible insect sample from a carcass and how to preserve the insect specimens for later processing by a professional entomologist.

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