

PIG (*Sus scrofa*) DECOMPOSITION IN LOTIC AND LENTIC AQUATIC SYSTEMS AS TOOL FOR DETERMINATION A POSTMORTEM SUBMERSION INTERVAL IN THE ANDEAN AMAZON, CAQUETÁ, COLOMBIA*

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Abstract

Objectives: Analyze the succession of insects associated with the decomposition of bodies in two aquatic ecosystems in the Andean Amazon. **Scope:** Provide information on the insects associated with bodies that decompose within aquatic habitats and the duration times of each decomposition phase as a tool for the determination of the post-mortem submerged interval (PMSI). **Methodology:** Three carcasses were placed in a stream and three in a lake, the sampling carried out every five hours, from the moment of death to its skeletonization. **Main results:** Total decomposition lasted for 14 days in the stream and 16 days in the lake. Six decomposition stages were observed: submerged fresh, early floating, floating decay, bloated deterioration, floating remains and sunken remains. A total of 1172 organisms were collected on the carcasses in the stream and 1585 in the lake. Trophic relationships identified were: Shredders, collectors, predators, necrophages, sarcosaprophages and incidentals. In the stream, the caddisfly shredders were the most representative organisms in the submerged fresh stage. In the lake, the mayfly shredders, and hemipterous and odonate predators, were the most representative organisms in the early floating stage. The necrophagous and sarcosaprophagous diptera were the most representative organisms in the stages exposed to the surface in both ecosystems. **Conclusions:** The PMSI for both carcasses was similar in the two habitats, each showing six stages of decomposition, confirming that decomposition in lotic and lentic ecosystems is different from that observed for terrestrial environments.

Key words: forensic entomology, aquatic ecosystems, insect colonization, Amazonian.

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DESCOMPOSICIÓN DE CERDOS (*Sus scrofa*) EN SISTEMAS ACUÁTICOS LÓTICOS Y LÉNTICOS COMO HERRAMIENTA PARA LA DETERMINACIÓN DEL INTERVALO DE SUBMERSION POST MORTEM EN LA AMAZONIA ANDINA, CAQUETÁ, COLOMBIA

Resumen

Objetivos: Analizar la sucesión de insectos asociados a la descomposición de cuerpos en dos ecosistemas acuáticos en la Amazonia Andina. **Metodología:** Tres cadáveres fueron ubicados en una quebrada y tres en un lago, los muestreos se realizaron cada cinco horas desde el momento de la muerte hasta su esqueletización. **Resultados:** La descomposición total duró 14 días en la quebrada y 16 en el lago. Seis estadios de descomposición fueron observados: fresco sumergido; flotación temprana; flotación tardía; deterioro de la flotación; restos flotantes y restos sumergidos. En total se colectaron 1172 individuos en la quebrada y 1585 en el lago. Los gremios tróficos identificados fueron: trituradores; colectores; depredadores; necrófagos; sarcosaprófagos e incidentales. En la quebrada, los trituradores tricópteros fueron los más representativos en la etapa fresco sumergido. En el lago los trituradores, moscas de mayo y los depredadores hemípteros y odonatos fueron los más representativos en la etapa de flotación temprana. Los dípteros necrófagos y sarcosaprófagos fueron los más representativos en las etapas donde el cuerpo estuvo expuesto a la superficie en ambos ecosistemas. **Conclusiones:** El ISMP para ambos cadáveres fue similar en los dos hábitats, cada uno evidenció seis etapas de descomposición, lo que confirma que la descomposición en los ecosistemas acuáticos es diferente a la observada para los entornos terrestres.

Palabras clave: entomología forense, ecosistemas acuáticos, colonización insectil, amazonia.

INTRODUCTION

Forensic entomology is the science that studies insects and other arthropods that are associated with corpses. It is often used to estimate the time interval between death and the discovery of the carcass, a period known as Post-mortem interval (PMI), which provides relevant information in legal investigations (PUJOL-LUZ *et al.*, 2008; BYRD & CASTNER, 2010; RAMOS-PASTRANA *et al.*, 2014; RAMOS-PASTRANA & WOLFF, 2017).

The succession of insects on corpses in terrestrial ecosystems has been well studied and is often used for PMI determination; however, the decomposition process and the role of the insects in aquatic ecosystems have received little attention so far (CATTS &

GOFF, 1992; NAWROCKI *et al.*, 1997; BARRIOS & WOLFF, 2011). Only in the 20th century more studies advanced on the use of aquatic insects and other arthropods on the estimation of the post-mortem submerged interval (PMSI), and mainly in countries from the northern hemisphere such as the United States (TOMBERLIN, 1998; CHALONER *et al.*, 2002; WALLACE *et al.*, 2008; MAGNI *et al.*, 2015) and Canada (HOBISCHAK & ANDERSON, 1999, 2002, 2004).

Colonization of a cadaver by aquatic invertebrates depends on abiotic factors, such as the physical and chemical properties of the water, depth and strength of flow, and biotic factors, such as the fauna and flora where the cadaver may be used as a food source or for protection (MERRITT & WALLACE, 2010).

The only study of this kind in the Neotropical Region was that of BARRIOS & WOLFF (2011) in the Colombian Andes. Furthermore, the INSTITUTO NACIONAL DE MEDICINA LEGAL Y CIENCIAS FORENSES (2017) reports that during the last 6 years there were 88 cases of death by drowning in the department of Caquetá, where the bodies were often recovered several days after death.

Therefore, this study aims at analyzing the arthropod succession associated to corpses in two aquatic ecosystems (lentic and lotic) to serve as subsidy for future PMSI determinations in similar areas.

MATERIALS AND METHODS

Study area and sampling

This study was performed in the Centro de Investigaciones César Augusto Estrada Gonzalez “Macagual” of the Universidad de la Amazonia, located in the rural area of the Municipality of Florencia, Caquetá, in the Colombian Amazonian Piedmont (N 1°37' W 075°36'), at approximately 280 m a.s.l., and annual average rainfall of 3,600 mm, average temperature of 27 °C, and average humidity of 85 % (IGAC, 2010).

Due to the ethical limitations of Colombia in the slaughter of animals for experimental purposes and the difficulties due to the large number of samples, six 9 kg white pigs (*Sus scrofa* Linnaeus, 1758) were used as decomposition models; they were slain on December 21st, 2011, at 10:00 am, with a 3 ml Euthanex (Pentobarbital Sodium 390 mg/ml, Diphenylhydantoin Sodium 50 mg/ml) intracardiac lethal injection, previously authorized by the ethics committee of the Universidad de la Amazonia.

Three bodies were left in a lotic ecosystem (a 20 m wide and approximately 70 cm deep stream) and the other three were left in a lentic ecosystem (a lake of 2,500 m² and approximate average depth of 3 m). To guarantee the independence of the data,

the distance between each carcass was of 100 m, each being placed in a separate metal cage (70 x 50 x 50 cm with an approximate 2 cm mesh) with a screen bottom, which allowed for the submersion and flotation of the carcasses as well as access of the arthropods to it. The distance between each ecosystem is approximately 1 km.

From the stage fresh to sunken remains, sampling was done each 5 hours without day-night interruption, air (digital thermohygrometer Thermo), water and carcass rectal temperature (digital thermometer Elan) were recorded, as well as average dissolved oxygen (digital dissolved oxygen meter Schott Duran) and pH (digital pH meter Schott Duran). Rainfall data was taken from a meteorological station located at approximately 1 km from the study site. The arthropods found over the cages, swimming around the body and trapped on the bottom screen were collected using entomological nets and tweezers, larger individuals such as decapods were hand-collected. Immature were preserved in 80 % ethanol, which was changed each 24 h during the first week, and adults were pinned.

Insect succession patterns were evaluated during the decomposition of the six corpses. Each phase was delimited based on physical change of the carcasses (rectal temperature and weight loss) and was related to the presence, development stage and abundance of its associated insects, and physical and chemical variables, according to the criteria established by MERRITT & WALLACE (2010) and BARRIOS & WOLFF (2011). Trophic relationships were based on the trophic relationships proposed by MERRITT & CUMMINS (1996) and BARRIOS & WOLFF (2011) for aquatic species: Shredders, collectors, grazers and predators. Terrestrial species were classified as filter feeders, necrophages, sarcosaprophages and incidentals, as proposed by SMITH (1986) and BARRIOS & WOLFF (2011).

The individuals collected were taxonomically identified to species level, when possible, using the keys proposed by SMITH (1986), ROLDAN (1996), CARVALHO & RIBEIRO (2000), NAVARRETE-HEREDIA *et al.* (2002), FERNÁNDEZ (2003), FERNÁNDEZ & SHARKEY (2006), FLÓREZ & WOLFF (2009), BROWN *et al.* (2009), BROWN *et al.* (2010), and CARVALHO *et al.* (2012), with the use of a stereomicroscope Olympus SZ61. Afterwards, the specimens were deposited in the Entomological Collection of the Universidad de la Amazonia (LEUA; RNC in process).

Data analysis

A multivariate analysis of variance (MANOVA $p < 0.05$) was performed to determine the relationships between insect diversity and physical and chemical variables (water temperature, carcass temperature, dissolved oxygen and water pH), in the different aquatic ecosystems (lake and stream), which represent the sampling universe related to the decomposition specific to each site. These analyses were performed using the EstimateS software version 9.0 for Windows (COLWELL, 2013).

RESULTS

A total of 2757 organisms associated to the submerged carcasses were collected, 1172 in the lotic (12 orders, 40 families, 59 genera and 62 species or morphospecies) (Table 1) and 1585 in the lentic environment (9 orders, 66 families, 98 genera and 106 species or morphospecies) (Table 2). The PMSI, from death to sunken remains, was of 13 days in the lotic and 15 days in the lentic environment. Six decomposition stages were determined, based on MERRITT & WALLACE (2010) and BARRIOS & WOLFF (2011): Submerged fresh, early floating, floating decay, bloated deterioration, floating remains and sunken remains.

Table 1. Succession of arthropods collected in pig carcasses in a lotic system of the Andean Amazon Caquetá, Colombia.

Trophic relationships	Order	Family	Species	SF	EF	FD	BD	FR	SR		
				0	1	2-3	4-5	6-9	10-13		
				J	A	J	A	J	A	J	A
Shredders	Ephemeroptera	Baetidae	<i>Baetis</i> sp.1	X	X	X	X	X			
			<i>Baetis</i> sp.2	X	X	X	X	X			
			<i>Coryphorus</i> sp.	X	X	X	X	X			
		Leptohyphidae	Not identified		X	X			X		
			Leptophlebiidae	<i>Terpides</i> sp.						X	
				<i>Thraulodes</i> sp.		X	X			X	
		<i>Traverella</i> sp.						X	X		
		Not identified		X	X		X	X			
		Trichoptera	Hydropsychidae	<i>Smicridea</i> sp.	X	X					
				<i>Annilipalpia</i> sp.		X					
				Leptoceridae	<i>Atanatolica</i> sp.						X
		Plecoptera	Perlidae	<i>Anacroneria</i> sp.	X						
		Coleoptera	Scydmaenidae	Not identified				X			
				Elmidae	Not identified				X	X	
					Simuliidae	<i>Simulium</i> sp.	X	X	X		X
Collectors	Diptera	Chironomidae	<i>Chironomus</i> sp.	X	X	X	X	X	X		
		Predators	Hemiptera	Gerridae	<i>Eurygerris</i> sp.		X				
<i>Trepobates</i> sp.					X	X					
Hydrometridae	Not identified							X	X		
Mesoveliidae	<i>Mesoveloidea</i> sp.						X				
Naucoridae	Not identified						X				
Notonectidae	Not identified						X				
Veliidae	<i>Rhagovelia</i> sp.1			X	X	X		X			
	<i>Rhagovelia</i> sp.2			X	X						
	<i>Rhagovelia</i> sp.3			X	X	X		X	X		
Coleoptera	Carabidae		<i>Pseudaptinus</i> sp.						X		
			Cicindelidae	<i>Cicindela favergeri</i>						X	
Diptera	Tabanidae		<i>Bolbodimyia</i> sp.				X				
			<i>Chrysops</i> sp.				X				
	Ephydriidae		Not identified				X	X	X		

Trophic relationships	Order	Family	Species	SF		EF		FD		BD		FR		SR		
				0		1		2-3		4-5		6-9		10-13		
				J	A	J	A	J	A	J	A	J	A	J	A	
Necrophages/ Parasites	Hymenoptera	Apidae	<i>Meliponina</i> sp.					X								
		Anthophoridae	Not identified							X		X				
	Odonata	Libellulidae	<i>Origodada</i> sp.						X					X		
		Not identified									X					
	Araneae	Calopterygidae	Not identified				X									
		Ctenidae	Not identified					X								
		Uloboridae	Not identified										X			
	Decapoda	Trichodactylidae	<i>Sylviocarcinus piriformis</i>		X	X	X	X	X	X	X	X	X	X	X	
			<i>Boettcheria</i> sp.				X									
		Diptera	Sarcophagidae	<i>Microcerella</i> sp.						X	X	X				
				<i>Oxysarcodexia</i> sp.						X	X	X				
				<i>Sarcodexia</i> sp.											X	
				<i>Tricharaea</i> sp.					X	X	X					
			Calliphoridae	Not identified									X			
				<i>Chrysomya albiceps</i>					X	X	X					
<i>Cochliomyia macellaria</i>								X	X	X						
<i>Ophyra aenescens</i>								X								
Blattodea	Blattidae	<i>Lamproblatta albipalpus</i>											X			
		<i>Musca domestica</i>					X	X	X							
Sarcosaprophages	Diptera	Muscidae	<i>Cyrtoneurina</i> sp.							X						
			<i>Dasymorellia flavipalpis</i>							X						
			<i>Ophyra aenescens</i>					X	X							
			<i>Phloeoborus punctatoneugorus</i>											X		
Incidentals	Coleoptera	Scolytidae	Not identified										X			
			Laemophloeidae	Not identified										X		
		Diptera	Drosophilidae	Not identified						X						
				<i>Paulistorum</i> sp.						X						
	Syrphidae			<i>Ornidia</i> sp.					X							
	Hemiptera	Aradidae	Not identified						X							
			Orthoptera	Gryllotalpidae	Not identified		X	X					X			

Note: Decomposition stage: SF: Submerged Fresh; EF: Early Floating; FD: Floating Decay; BD: Bloated Deterioration; FR: Floating Remains; SR: Sunken Remains. Insect development stage: J: Juvenile; A: Adult. Numbers below decomposition stage indicate the duration period in days from 0 to 13.

Source: Own elaboration.

Table 2. Succession of arthropods collected in pig carcasses in a lentic system of the Andean Amazon Caquetá, Colombia.

Trophic relationships	Order	Family	Species	SF	EF	FD	BD	FR	SR	
				0-1	2-3	4-5	6-9	10-12	13-15	
				J	A	J	A	J	A	J
Shredders	Ephemeroptera	Leptophlebiidae	<i>Thraulodes</i> sp.		X					
		Oligoneuriidae	<i>Lachlania</i> sp.			X				
		Polymitarcyidae	<i>Campsurus</i> sp.				X			
		Caenidae	Not identified		X					
Collectors	Coleoptera	Hydroscaphidae	Not identified						X	
	Diptera	Chironomidae	<i>Chironomus</i> sp.	X	X	X		X		
	Coleoptera	Hydrophilidae	Not identified					X		
		Elmidae	Not identified					X	X	
Microsporidae		Not identified			X		X			
Predators	Coleoptera	Carabidae	<i>Brachinus</i> sp.1					X		
			<i>Brachinus</i> sp.2					X		
			<i>Broscus</i> sp.					X		
			<i>Calosoma</i> sp.						X	
			<i>Camptodontus</i> sp.		X	X		X	X	
			<i>Colliuris</i> sp.					X		
			<i>Pheropsophus</i> sp.					X	X	
			<i>Pseudomorpha</i> sp.					X	X	
			<i>Semiardistomis</i> sp.			X		X	X	
			<i>Stenocrepus</i> sp.					X	X	
			Histeridae	<i>Hister</i> sp.					X	X
			Staphylinidae	<i>Philonthus</i> sp.					X	X
			Dytiscidae	Not identified		X	X			X
		Elateridae	Not identified						X	
		Hydrophilidae	Not identified		X	X			X	X
		Meloidae	Not identified					X		
		Noteridae	Not identified		X	X		X	X	X
		Pselaphidae	Not identified			X				
		Dryopidae	Not identified						X	
		Diptera	Dolichopodidae	<i>Dolichopus</i> sp.					X	X
			Tabanidae	<i>Bolbodimyia</i> sp.			X	X	X	
				<i>Chrysops</i> sp.			X	X	X	X
		Ephyridae	Not identified					X	X	
Hemiptera	Belostomatidae	<i>Belostoma micantulum</i>		X	X		X			
		<i>Lethocerus</i> sp.			X	X				
		<i>Centrocorisa kollari</i>		X			X			
		<i>Centrocorisa</i> sp.			X					
		Gerridae	<i>Brachymetra</i> sp.		X	X	X			
		<i>Eurygerris</i> sp.		X						
		<i>Gerris</i> sp.			X					
		<i>Limnogonus</i> sp.		X	X					
		<i>Trepobates</i> sp.		X	X					
		Naucoridae	<i>Heleocoris</i> sp.					X		

Trophic relationships	Order	Family	Species	SF	EF	FD	BD	FR	SR	
				0-1	2-3	4-5	6-9	10-12	13-15	
				J A	J A	J A	J A	J A	J A	
			<i>Pelocoris</i> sp.	X	X					
		Nepidae	<i>Curicta</i> sp.	X			X			
		Notonectidae	<i>Buenoa</i> sp.1	X	X					
			<i>Buenoa</i> sp.2		X					
			<i>Buenoa</i> sp.3			X		X	X	
			<i>Notonecta</i> sp.	X	X	X				
				<i>Crematogaster</i> sp.			X	X		
			<i>Dinoponera</i> sp.					X		
			<i>Odontomachus</i> sp.					X		
			<i>Solenopsis</i> sp.		X					
		Sphecidae	Not identified						X	
		Vespidae	<i>Hypalastoroides</i> sp.			X	X	X		
			<i>Mischocyttarus</i> sp.						X	
			<i>Vespa</i> sp.						X	
	Odonata	Aeshnidae	Not identified						X	
		Coenagrionidae	<i>Argia</i> sp.	X	X	X				
		Libellulidae	<i>Erythrodiplax</i> sp.		X					
			Not identified	X	X	X		X		X
			<i>Miathyria simplex</i>						X	
			<i>Pantala flavescens</i>							X
Necrophages/ Parasites	Coleoptera	Cleridae	<i>Necrobia rufipes</i>						X	
		Scarabaeidae	<i>Dichotomus</i> sp.			X				
		Diptera	Calliphoridae	<i>Chrysomya albiceps</i>			X	X	X	X
				<i>Cochliomyia macellaria</i>		X	X	X	X	X
			Dolichopodidae	<i>Dolichopus</i> sp.					X	
			Drosophilidae	<i>Leucophenga</i> sp.					X	X
			Dryomyzidae	Not identified					X	
			Phoridae	<i>Physoptera</i> sp.			X	X	X	
			Piophilidae	Not identified				X		
			Sarcophagidae	<i>Blaesoxipha</i> sp.						X
				Not identified				X	X	
				<i>Microcerella</i> sp.					X	X
				<i>Peckia</i> sp.					X	X
				<i>Tricharaea</i> sp.			X	X	X	X
				Sphaeroceridae	<i>Coproica</i> sp.		X	X	X	X
					<i>Copromyza</i> sp.		X			
				Ulidiidae	<i>Chaetopsis</i> sp.			X		X
		Blattodea	Blattidae	Not identified		X	X	X	X	
	Sarcosaprophages	Diptera	Muscidae	<i>Ophyra aenes-cens</i>		X	X	X	X	
	Incidentals	Coleoptera	Bostrichidae	Not identified					X	

Trophic relationships	Order	Family	Species	SF		EF		FD		BD		FR		SR	
				0-1		2-3		4-5		6-9		10-12		13-15	
				J	A	J	A	J	A	J	A	J	A	J	A
		Curculionidae	Not identified			X		X							X
		Hebriidae	Not identified					X							
		Melolonthidae	Not identified												X
		Nosodendriidae	Not identified									X			
	Hemiptera	Pentatomidae	Not identified					X							
	Diptera	Drosophilidae	<i>Leucophenga</i> sp.												X
		Micropozidae	<i>Taeniaptera</i> sp.					X				X			
		Milichiidae	Not identified					X	X		X				
			Not identified												X
			Not identified												X
		Mycetophilidae	Not identified												X
		Psychodidae	Not identified									X			
		Syrphidae	<i>Copestylum</i> sp.						X		X	X			
	Hemiptera	Berytidae	Not identified								X				
		Cicadellidae	Not identified								X		X		
			Not identified								X				
		Miridae	Not identified						X						
		Alydidae	Not identified												X
			Not identified									X		X	
	Hymenoptera	Apidae	Not identified						X	X					
		Colletidae	Not identified								X				
		Halictidae	Not identified						X	X		X			
		Sierolomorphi- dae	Not identified												X
	Lepidoptera	Ind.	Not identified			X	X		X	X	X	X	X	X	X
	Odonata	Libellulidae	Not identified												X
	Orthoptera	Acrididae	Not identified						X						

Note: Decomposition stage: SF: Submerged Fresh; EF: Early Floating; FD: Floating Decay; BD: Bloated Deterioration; FR: Floating Remains; SR: Sunken Remains. Insect development stage: J: Juvenile; A: Adult. Numbers below decomposition stage indicate the duration period in days from 0 to 15.

Source: Own elaboration.

Submerged fresh (day 0 in the stream and 0-1 in the lake)

In both habitats, the carcasses remained completely submerged and death early phenomena were evident, such as stiffness of the extremities. Furthermore, there was a decrease in body temperature, which reached the water temperature not long after death (15 hours in the stream, 24 hours in the lake) (Figure 1, A, B). The most abundant organisms in the stream were the shredders (67 %, n= 91), (Tables 1, 3), while in the lake were the collectors (64 %, n= 210), (Tables 2, 3). Predators were the second most important group in both habitats, 19 % (n= 26) from the total in the stream and 30 % (n= 98) from the total in the lake, (Tables 1, 2, 3). Furthermore, *Sylviocarcinus piriformis* Pretzmann, 1968 (Decapoda: Trichodactylidae), 4 % (n= 5) from the total, was found throughout the whole decomposition process of the stream (Tables 1, 3).

Table 3. Composition of species and abundance during of decomposition in the two aquatic systems (lotic and lentic) of the Andean Amazon Caquetá, Colombia.

Decomposition stage	Stream	Insects suc- cession	Lake	Insects succession
	Trophic relationships		Trophic relationships	
Submerged fresh	Shredders	91 (67)	Shredders	13 (4)
	Colectors	14 (10)	Colectors	210 (64)
	Predators	26 (19)	Predators	98 (30)
	Necrophages and Parasites	5 (4)	Necrophages and Pa- rasites	1 (0.3)
	Sarcosaprophages	0	Sarcosaprophages	0
	Incidentals	0	Incidentals	6 (1,9)
Early floating	Shredders	158 (68)	Shredders	6 (4)
	Colectors	6 (3)	Colectors	6 (4)
	Predators	55 (24)	Predators	69 (47)
	Necrophages and Parasites	10 (4)	Necrophages and Pa- rasites	23 (16)
	Sarcosaprophages	1 (0.4)	Sarcosaprophages	9 (6)
	Incidentals	2 (0.9)	Incidentals	33 (23)
Floating decay	Shredders	127 (47)	Shredders	0
	Colectors	11 (4)	Colectors	0
	Predators	29 (11)	Predators	22 (6)
	Necrophages and Parasites	91 (34)	Necrophages and Pa- rasites	311 (81)
	Sarcosaprophages	9 (3)	Sarcosaprophages	14 (4)
	Incidentals	3 (1)	Incidentals	36 (10)
Bloated deterioration	Shredders	24 (17)	Shredders	6 (1)
	Colectors	5 (3.5)	Colectors	1 (0.1)
	Predators	5 (3.5)	Predators	125 (23)
	Necrophages and Parasites	97 (69)	Necrophages and Pa- rasites	266 (50)
	Sarcosaprophages	10 (7)	Sarcosaprophages	11 (2)
	Incidentals	0	Incidentals	128 (24)
Floating remains	Shredders	89 (24)	Shredders	2 (1)
	Colectors	9 (2.5)	Colectors	3 (2)
	Predators	15 (4)	Predators	68 (41)
	Necrophages and Parasites	249 (67)	Necrophages and Pa- rasites	34 (21)
	Sarcosaprophages	3 (1)	Sarcosaprophages	3 (2)
	Incidentals	6 (1.6)	Incidentals	55 (33)
	Shredders	0	Shredders	1 (4)

Decomposition stage	Stream	Insects succession	Lake	Insects succession
	Trophic relationships		Trophic relationships	
Sunken remains	Colectors	2 (10)	Colectors	0
	Predators	13 (59)	Predators	19 (74)
	Necrophages and Parasites	7 (32)	Necrophages and Parasites	2 (8)
	Sarcosaprophages	0	Sarcosaprophages	0
	Incidentals	0	Incidentals	4 (15)

Note: The values are the number of individuals, followed by the frequency (% in parentheses) in relation to the total number of individuals registered in each decomposition phase.

Source: Own elaboration.

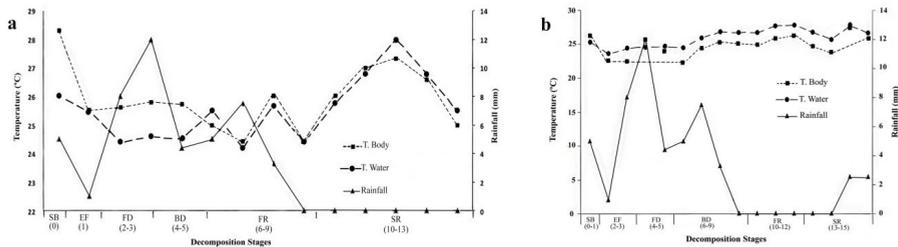


Figure 1. Rainfall, body and water temperature. SF: Submerged Fresh; EF: Early Floating; FD: Floating Decay; BD: Bloated Deterioration; FR: Floating Remains; SR: Sunken Remains. A. Stream; B. Lake. Source: Own elaboration.

Early floating (day 1 in the stream and 2-3 in the lake)

This stage started when the carcasses began to float, and characterized by the distended abdomen presenting a reddish and greenish-blue coloration, shedding of the skin, release of viscous fluids through the anus and mouth, and a decomposition odour. In the stream, it was characterized by the still dominating shredders, the necrophages displacing the collectors, the first appearance of the sarcosaprophages (Table 3), and by the presence of incidentals and predators (Table 1). In the lake, this stage was characterized by the presence of aquatic insects (Table 2), with predators and incidentals being the most abundant and displacing the collectors; the sarcosaprophages also appear for the first time in this habitat (Table 3).

Floating decay (days 2-3 in the stream and 4-5 in the lake)

In this stage, the carcasses are still floating, the skin on the head and extremities starts to shed as on the abdomen, the abdomen starts to deflate and presents blisters with a greenish fluid, the greenish-blue coloration becomes darker, and the flaccidity on the extremities and the decomposition odour become more evident. In the stream the

still dominating shredders, followed by the necrophages (Table 1). In the lake, the necrophages were the most abundant, followed by the incidentals (Table 2); in both habitats the predators were the third most abundant. The presence of flying insects, indicating the start of the colonization by the terrestrial carrion fauna (Table 3).

Bloated deterioration (days 4-5 in the stream and 6-9 in the lake)

This stage started when the blood, body fluids and faeces left the abdomen and the carcasses deflated completely, the decomposition odour diminished and the disarticulation started, with the carcasses losing their original shape. The necrophages were the most abundant in both habitats, representing 50 % of the total in the lake and 69 % in the stream; in the lake the incidentals were the second most abundant group, followed by the predators; in the stream the filter feeders were the second most abundant group, followed by the sarcosaprophages (Table 3).

Floating remains (days 6-9 in the stream and 10-12 in the lake)

This stage started when the head bones and the phalanges from the lower extremities got loose, ending when the remains were completely submerged. The exposed areas presented a waxy aspect, rancid odour and soft texture, conditions similar to corpses in the saponification stage. In the stream, the necrophages, followed by the filter-feeders and predators, were the most abundant groups, while in the lake the predators, incidentals and necrophages were the most abundant (Table 3).

Sunken remains (days 10-13 in the stream and 13-15 in the lake)

This stage started when the remains were completely submerged, and a great part of the carcasses were composed of bones, cartilage and skin that remained at the bottom of the cages. Sampling ceased at this stage. During this stage the fauna was mostly aquatic, and the most abundant organisms in both habitats were the predators with 59 % in the stream and 74 % in the lake, followed by the necrophages 32 % in the stream and the incidentals 15 % in the lake (Table 3).

Ecological succession

In the lotic ecosystem, the decapod *S. piriiformis* and the midge *Chironomus* sp. were present during the whole decomposition process; *Coryphorus* sp. (Ephemeroptera: Leptohyphidae), *Baetis* sp.1 and sp.2, were present from the freshly submerged to the floating remains stage; *Rhagovelia* sp.3 (Hemiptera: Veliidae) and *Simulium* sp. (Diptera: Simuliidae) were present in all stages except in the bloated deterioration stage; *Rhagovelia* sp.1 was present from the freshly submerged until the floating decay stage, absent during the bloated deterioration but reappearing again during the floating

remains stage; the blowflies *C. albiceps* and *C. macellaria*, and flesh flies *Oxysarcodexia* sp., *Tricharaea* sp. and *Peckia* sp. were present from the floating decay until the floating remains stage; the *Trepobates* sp. (Hemiptera: Gerridae) and *Rhagovelia* sp.2 and *Smicridea* sp. (Trichoptera: Hydropsychidae) were present from the freshly submerged until the floating decay stage; the hemipteran *Euguerris* sp. and the stonefly *Anacroneuria* sp. only occurred in the freshly submerged stage; the caddisfly *Atanotolica* sp. was only present in the floating remains stage (Table 1).

In the lentic ecosystem, *Brachymetra* sp. (Hemiptera: Gerridae), *Notonecta* sp. (Hemiptera: Notonectidae), and *Argia* sp. (Odonata: Coenagrionidae) were present since the freshly submerged until the floating decay stage. *O. aenescens*, *Chrysops* sp. (Diptera: Tabanidae) and *Coproica* sp. (Diptera: Sphaeroceridae) were present from the early floating until the floating remains stage and *C. macellaria*, *C. albiceps*, *Tricharaea* sp. and *Physoptera* sp. (Diptera: Phoridae) were present from the floating decay until the floating remains stage. *Thraulodes* sp. (Ephemeroptera: Leptophlebitidae) and *Limnogonus* sp. (Hemiptera: Gerridae), *Trepobates* sp., *Pelocoris* sp. and *Buenoa* sp.1 (Hemiptera: Naucoridae) were exclusive of the submerged fresh and early floating stages, while the *Lachlania* sp. (Ephemeroptera: Oligoneuriidae) and *Campsurus* sp., *Centrocorisa* sp. and *Buenoa* sp.2., and *Erythrodiplax* sp. were exclusive of the early floating stage (Table 2).

Environmental factors

During the first 24 hours after death, the temperature of the bodies decreased until reaching the water temperature (25.5 °C in the stream and 24.5 °C in the lake) (Figure 1, A, B). In the stream, starting from the floating decay until the bloated deterioration stage, the body temperature was higher than the water temperature; after that period, and until the end of the decomposition process, the temperatures of body and water were more homogeneously high (27 °C and 28 °C), which coincided with the decrease in rainfall (0 mm) (Figure 1, A). In the lake, the floating decay stage presented the highest body temperature (25 °C) of all stages in that habitat, surpassing the water temperature, even though it was during the highest rainfall (25 mm); during the remaining stages the water temperature was above the carcass temperature (Figure 1, B).

The pH remained stable and neutral (pH=6) in the stream from the submerged fresh stage until the first day of the floating remains stage, however, it decreased during the floating remains stage (pH=5.5) and then was more alkaline from the last day of that stage until the end of the decomposition (pH=7.7) (Figure 2, A). In the lake it remained neutral (pH=6) during the whole decomposition process (Figure 2, B).

The dissolved oxygen in the stream was directly influenced by rainfall and inversely to body and environmental temperature. Lower rainfall had lower O₂ levels; noteworthy

are the ninth day of the end of the floating remains stage and the sunken remains stage where the lowest levels of O₂ (11 mg/l) and rainfall (0 mm) were observed, while the body and water temperatures were relatively high (27 °C and 28 °C) (Figure 3, A). In the lake, the O₂ levels were directly proportional to body temperature, reaching the lowest level (4 mg/l) in the sunken remains stage, except the bloated deterioration stage where the O₂ level was inversely proportional to the temperature (Figure 3, B).

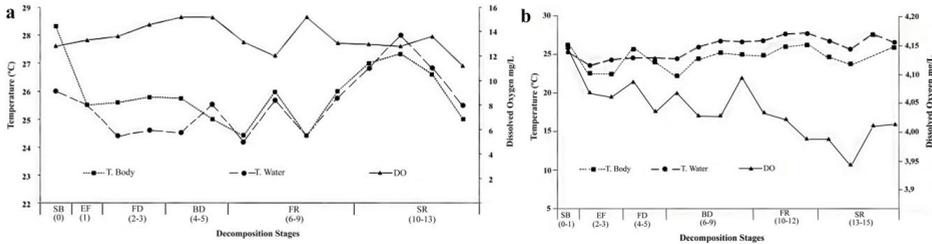


Figure 2. pH, body and water temperature. SF: Submerged Fresh; EF: Early Floating; FD: Floating Decay; BD: Bloated Deterioration; FR: Floating Remains; SR: Sunken Remains. A. Stream; B. Lake. Source: Own elaboration.

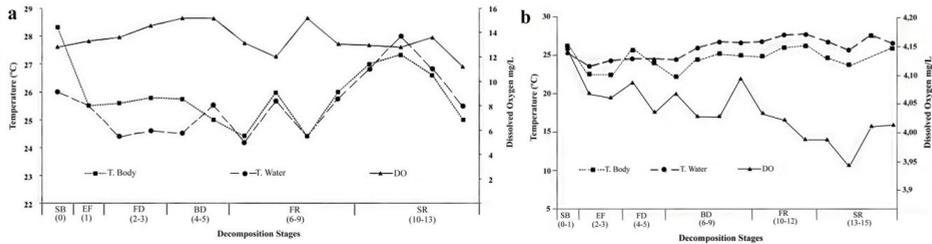


Figure 3. Dissolved oxygen, body and water temperature. SF: Submerged Fresh; EF: Early Floating; FD: Floating Decay; BD: Bloated Deterioration; FR: Floating Remains; SR: Sunken Remains. A. Stream; B. Lake. Source: Own elaboration.

DISCUSSION

Six decomposition stages were observed in both ecosystems, which is in agreement with other studies from North America (HOBISCHAK, 1998; MERRITT & WALLACE, 2010) and Colombia (BARRIOS & WOLFF, 2011), corroborating that corpse decomposition in aquatic ecosystems is different from decomposition in terrestrial ecosystems where there are only five stages (SMITH, 1986; RAMOS-PASTRANA & WOLFF, 2011; RAMOS-PASTRANA *et al.*, 2014).

Complete corpse decomposition in Amazonian aquatic ecosystems is faster, 13 days in a stream and 15 days in a lake, and contrasts with other regions in Colombia as

demonstrated by BARRIOS & WOLFF (2011), 80 days in a stream and 74 in a lake, and countries in other latitudes as shown by HOBISCHACK (1998), 336 days in a stream and lake. The difference surpasses 100 %, which can be related to the different climate conditions, mainly the temperature, of the Andes and northern countries when compared to the Amazon region. Furthermore, the stream, due to the constant inundations during this study, acted as a mechanical factor removing soft tissue from the carcasses, accelerating the decomposition as already noted by KEIPER & CASAMATTA (2001) and ANDERSON (2010). Thus, this demonstrates that corpse decomposition depends on several factors (CAMPOBASSO *et al.*, 2001). Determining the total decomposition time, and the duration of each stage, in aquatic environments acts as a guide for the PMSI determination in these habitats (ANDERSON & HOBISCHACK, 2004; ANDERSON, 2010).

KNIGHT (1997) concluded that submerged bodies take twice the time to decompose than bodies left in terrestrial environments, which was corroborated by BARRIOS & WOLFF (2011) in their study in the Colombian Andes, but contrasts with the current observations. In the Amazonian Piedmont, the decomposition in the stream lasted for 13 days and in the lake lasted for 15 days in the current study, while in the study of RAMOS-PASTRANA & WOLFF (2011) and RAMOS-PASTRANA *et al.* (2014) it lasted 38 and 54 days, respectively. KNIGHT (1997) suggests that aquatic ecosystems act as inhibitors of necrophagous insects, which only act sporadically while the bodies are exposed to the air, a fact corroborated in the current study where this exposed time was not enough for the terrestrial organisms to complete their life cycle on the carcasses.

HASKELL *et al.* (1989) proposed that midges (Diptera: Chironomidae) and caddisflies (Trichoptera) could be used to determine PMSI. According to HOBISCHACK & ANDERSON (2002), in habitats such as water tanks, *Polypedilum* sp. (Chironomidae) occurred during the whole year except in November, January, and February, *Heterotrissocladius* sp. (Chironomidae) occurred in carrion from September until October and from February until July, and *Chyranda centralis* Banks, 1990 (Trichoptera: Limnephilidae) occurred during all months except August, November, December and January. In the current study, *Chironomus* sp. occurred in all the decomposition stages except in the sunken remains stage, a situation that HASKELL *et al.* (1989) considered as enough information to determine the PMSI. However, HOBISCHACK & ANDERSON (2002) state that this is not enough to determine such time intervals.

It is important to establish the trophic relationships involved in the corpse decomposition in these environments, since not all organisms associated to the corpse are considered to act on its decomposition. As currently observed, in these aquatic environments there are other factors that determine the duration of the decomposition, such as temperature and the mechanical action of the water current. Thus, it

was essential to know the role of each species associated to the carcass: Shredders, collectors, predators, necrophages, sarcosaprophages and incidentals. The corpse can be used by these arthropods as a food source, shelter, and a supply of microhabitat and substrate SORG *et al.* (1997), which explains the presence of *Baetis* sp.1, *Baetis* sp.2, *Coryphorus* sp. (shredders) and *Chironomus* sp. (collector) that were present on the carcasses during the whole decomposition process, except on the sunken remains stage, and *Simulium* sp. (collector) that was present in the submerged fresh, early floating, floating decay and floating remains stages.

Colonization time by insects and other arthropods, the succession model and the decomposition rate are affected by factors such as geographical locality, corpse exposure, season, and habitat where the corpse is located (PAYNE, 1965; HOBISCHAK *et al.*, 2006). For bodies that decompose in aquatic ecosystems, there are two classes of variables that affect its decomposition rate, the ones associated to body, clothing and type of trauma, and those related to the aquatic medium, such as temperature, rainfall and available oxygen (MERRITT & WALLACE, 2010). In the current study, the body and water temperature were inversely proportional to rainfall, the highest temperatures occurring when rainfall was at its lowest in the final stages of the decomposition, which is in agreement with the findings of BARRIOS & WOLFF (2011) where the lowest temperatures occurred with the highest rainfall levels.

The O₂ availability was constant and relatively high in the stream, while in the lake it decreased with the increase in temperature, which is in agreement with the observations of MERRITT & WALLACE (2010). The pH levels were constant in both ecosystems, pH 6 for the lake and 7.8 for the stream, with some fluctuation in the stream in the last days of the decomposition, a characteristic variation for this variable in ecosystems of the Colombian Amazon Region (SINCHI-COLOMBIA, 2007).

Although the number of biomodels used as replicates was not high, this study represents a significant advance in forensic entomology for Colombia and the Neotropical region, since it presents updated databases on the decomposition process of corpses in aquatic environments (lotic and lentic). It is also the first report on the succession and trophic roles of arthropods associated to pig carcasses in fresh water ecosystems of a rural area of the department of Caquetá in the Colombian Amazonian Piedmont. These findings will be of great value in determining the PMSI in future criminal investigations that involve corpses decomposing in such environments.

CONCLUSIONS

The post-mortem submerged interval (PMSI) for both carcasses was similar in the two habitats, each showing six stages of decomposition, confirming that decomposition in lotic and lentic ecosystems is different from that observed for terrestrial environments.

Mainly benthic arthropods, with trophic relationships of collectors and shredders, are indicators of the submersion stages, while *C. albiceps* and *C. macellaria*, and flesh flies *Oxysarcodexia* sp., *Tricharaea* sp. and *Peckia* sp. are indicators of the floatation stages, therefore freshwater arthropods can be used to estimate PMSI. Studies of the succession of insects associated to decaying bodies in special microenvironments in Colombia are very significant for their contribution to the development of forensic science in the country, as well as a potential forensic tool in cases of human corpses found in lotic and lentic ecosystems.

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