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Freshwater Invertebrate Succession and Decompositional Studies on Carrion in British Columbia

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EXECUTIVE SUMMARY

A successional database for invertebrates in pond and stream habitats has been created. This database is of potential use in estimating the time of submergence in water-related death investigations. This work in the aquatic environment is compared with previous work in the terrestrial environment.

SOMMAIRE

Une base de données des successions des invertébrés dans les habitats lenitiques et lotiques a été crée Cette base de données est utile pour évaluer la durée d'immersion dans les enquêtes sur les décès lies à l'eau. Ce travail dans les environnements aquatiques est comparées aux travaux anterieurs sur les environnements terrestres.

ABSTRACT

I examined the development, species, and sequence of invertebrates associated with submerged pig carrion from August 31, 1996 to September 8, 1997 in the Malcolm Knapp Research Forest, Maple Ridge, B.C. An invertebrate successional database was created for pond and stream habitats for potential use in estimating time of submergence in water-related death investigations. Insects in 10 orders, 34 families, and 46 genera were collected from the carcasses and control sites for both pond and stream habitats. In pond habitats, caddisfly larvae, diving beetles, and blow flies predominated, whereas riffle beetles, chironomids, and blow flies were most common in the stream habitats. Succession in the aquatic environments differed from that in terrestrial environments primarily in the absence of most terrestrial species of Diptera and Coleoptera which were unable to colonize submerged carcasses. Decomposition was delayed in the aquatic environment in comparison with terrestrial environments. Inconsistencies were noted between the times decompositional characteristics appeared in this research and times reported in the literature. Scavenging (by mink) increased decompositional rates but limited species diversity on exposed carrion. When invertebrate succession and decompositional rates and descriptions determined by this research were compared with water-related death investigations, many similarities were observed in both the research and the postmortem descriptions. However, investigators attached to police or coroner's services almost never noted the occurrence of aquatic fauna. Moreover, the longer the postmortem interval, the more general the death investigator's observations became, making it difficult to standardize characteristics for each decompositional stage and hence determining time of submergence and death.

One can on/y see what one observes, and one observes only things which are already in the mind.

-ALPHONSE BERTILLON

ACKNOWLEDGMENTS

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1. INTRODUCTION

Nutrient recycling of decomposed organic matter has long been recognized as an important function of aquatic ecosystems. The few studies that have examined animal matter decomposition in fresh water environments have focused on nutrient cycling variables using fish and bird carrion (Richey et a/. 1975; Cederholm and Peterson 1985; Anderson et a/. 1988; Minshall et al. 1991). These studies did not determine if invertebrate succession occurs on carrion as decomposition progresses.

Colonization of a substrate in water is predictable, has been documented over time on various inert substances (Sheldon 1983; Tevesz 1985), and has recently been applied to forensic cases (Moran 1983; Siver et a/. 1994). However, colonization of substrates by aquatic invertebrates depends on many factors, such as size, texture, and position of the object, flow of water, water temperature, current speed, water depth, and presence of aquatic flora and fauna (Sheldon 1983; Peckarshy 1986). Once an organism has located a substrate, the substrate's characteristics will determine whether the organism remains. The substrate may act as an anchoring site, a food resource, or may afford protection (Haskell et a/. 1989). Several factors may be used to determine the time of submergence of an object, such as carrion, including succession of aquatic invertebrates on the habitat, seasonal indicators, and indicator species.

Compared with processes that occur on dry land, immersion in fresh water is thought to alter faunal succession on and decomposition of carrion

(Lord and Burger 1983; Keh 1985; Haskell et a/. 1989; Kashyap and Pillay 1989; Catts 1992; Catts and Goff 1992; Goff 1993). However, these phenomena are little understood in aquatic environments. For example, Payne and King (1972) focused on terrestrial insects which colonized exposed regions of immersed carrion; Simpson and Knight (1985) limited their research to insects living on a body prior to death, Nuorteva (1977) used aquatic organisms in terrestrial situations, and Vance et *al. (1995)* restricted the organisms collected due to experimental design.

The most common and widely accepted application of entomological evidence in criminal investigations is to determine the postmortem interval (PMI): the time from death to discovery of the corpse (Catts and Goff 1992; Schoenly et al. 1996). Although estimating PMIs in terrestrial situations are standardized and widely accepted in courts of law, estimating PMIs in aquatic environments are largely unexplored.

Numerous investigations have involved entomological evidence on wholly or partially submerged corpses (Littlejohn 1925; Holzer 1939; Nuorteva et *a/. 1974; Goff* and Odom 1987; Hawley et *a/. 1989;* Mann *et a/. 1990;* Siver *ef* a/. 1994; Teather 1994). However, these are all case studies involving single time observations, and PMI has rarely been estimated by entomological evidence alone. Neither decompositional studies nor forensic investigations have provided evidence indicating a predictable sequence of invertebrate succession.

An understanding of the decompositional process is fundamental to the application of entomological data in death investigations, yet few studies have

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examined the decompositional rates of human corpses on dry land (Mant 1960; Rodriquez and Bass 1983; Rodriquez and Bass 1985; Simpson and Knight 1985; Mann et a/. 1990) and fewer still in aquatic environments (Tomita 1976; Smith 1986; O'Brien 1994). On land, Goff (1993) defined five decompositional stages in a corpse: fresh, bloat, decay, post decay, and remains. These stages have not been reliably applied to decomposition in aquatic environments.

Carcasses of domestic pigs, Sus scrofa L., are now commonly used in decompositional studies, because they are widely accepted as surrogates for human corpses (Goff 1993). Like humans, pigs are omnivorous and, therefore, possess a similar digestive system and gut fauna. The last stage of digestion in the intestinal tract of both humans and pigs occurs through bacterial action, not by autolytic enzymatic action as occurs in many other animals (Tortora and Anagnostakos 1984). Although the bacteria in pigs and humans are taxonomically different, in both cases they ferment any remaining carbohydrates in the gastrointestinal tract, release H_2, CH_4 and CO_2 gas, characteristically causing sunken corpses to bloat and refloat. A 23 kg (50 lb) pig is approximately equivalent to the size of. an average adult male human torso, the main site of decomposition and insect colonization (Catts and Goff 1992).

Typically, in a terrestrial situation, insects are often the first witnesses to death, arriving in a predictable sequence (Payne 1965; Easton and Smith 1970; Smith 1986). This sequence is governed by a wide range of rapid and complex chemical (Tomita 1976; Fisher 1980; Kelly 1990), biological (Mant 1960; Fisher 1980; Marchenko 1993; O'Brien 1994), and physical changes (Smith 1986;

Mann et a/. 1990) as carrion decomposes from a fresh state to a skeleton. At each stage of decomposition, a corpse is colonized by different species of invertebrates (Chapman and Sankey 1955; Reed 1958; Easton and Smith 1970; Nuorteva 1977; Putman 1978; Erzinclioglu 1983; Smith 1986; Tullis and Goff 1987; Goff 1993; Anderson and VanLaerhoven 1996). When the sequence of colonizing invertebrates is known, an analysis of the fauna on carrion can be used to determine the PMI in human death investigations.

Factors which affect decomposition and colonization of aquatic invertebrates, and hence estimations of PMI include: season of immersion (Polson and Gee 1973), water temperature (Mant 1960; Jaffe 1976; Fisher and Petty 1977; Spitz 1980), water acidity (Mant 1960; Polson and Gee 1973), presence of clothing (Mant 1960; Polson and Gee 1973; Keh 1985), and biotic variables (Polson and Gee 1973), including amount of body fat (Keh 1985), and scavenging (Mant 1960; Picton 1971; Jaffe 1976; Fisher and Petty 1977; Spitz 1980).

My principal objective was to evaluate whether data on aquatic invertebrate development and succession on carrion has the potential to be used in determining time of death or submergence, as an aid in water death investigations. I compared aquatic invertebrate development and succession on free floating pig carcasses in pond and stream habitats, and assessed the relationships between decompositional stages and water temperature and chemistry, scavenging, and clothing.

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2. MATERIALS AND METHODS

2.1 Research Location and Site Preparation

The research was conducted at the University of British Columbia's Malcolm Knapp Research Forest in Maple Ridge, B.C. This forest is contained within the Coastal Western Hemlock biogeoclimatic zone, which includes the majority of Vancouver Island, the Gulf Islands and extends up the Pacific Coast to the Alaska border (Meidinger and Pojar 1991).

Experiments were conducted in still pond water (four sites) and flowing stream water (four sites). Human remains are frequently discovered in both types of habitat (B.C. Coroners Service 1996). The pond sites were fire ponds formed by damming small streams to impound water to be used in the event of forest fires. They ranged in area from 360 to 700 m^2 and had been established for more than eight years. The four stream sites were arrayed at intervals of greater than 10 m along a stream that flowed northward into a lake. Elevations of the eight sites ranged from 175 to 350 m above sea level.

Two weeks prior to commencing the research, heavy metal cages (A&H Custom Fabrications Ltd., Maple Ridge, British Columbia) previously used to house carcasses on dry land (Dillon and Anderson 1995) were placed at each of the eight sites, with care taken to minimize any disturbance to the aquatic fauna. Cages in fire ponds and streams were not secured, unlike previous experiments with carcasses on dry land (Dillon 1997).

Signs were placed around experimental sites to warn any visitors of the danger of encountering bears that had been attracted to carrion. All experimental work was conducted by two persons, and standard precautionary measures were followed (Dillon and Anderson 1996).

2.2 Experimental Procedures

On 31 August 1996, eight pigs (ranging in size from 6.8 to 32 kg) were killed with single shots to the heads from a 15 cm pin gun. Within 2 h of death, the carcasses were transported to the research sites, weighed, partially clothed with T-shirts, underwear or shorts, and socks, and placed in the middle of a cage. The cages protected carcasses from large predators but did not impede the entry of small fish, invertebrates, and small vertebrates (Dillon 1997), or restrict the natural rise and fall of the carcass during decomposition.

Three of four carcasses per habitat were sampled for invertebrates and monitored for temperature, water chemistry, and benthic fauna. The fourth carcass in each habitat was used as a control to assess visually whether the minimal disturbance of weekly sampling disrupted the natural decomposition process. Carcasses were examined two days after death, then approximately every nine days for nine weeks, once a month from December to April and then every two weeks until the eighth of September.

Prior to examining the carcass on each sampling date, photographs were taken of each carcass with a Nikon® F-401X using high speed film, international Standards Organization (ISO) 400. At this time, the percentage of each carcass

that was exposed to the air was estimated by dividing the pig carcass into eighths (Figure 1). Any section of the carcass that was exposed to air was noted and then converted to a percentage.

For one carcass in each habitat, a double channel data logger (SmartReader®1, Young Environmental Systems, Richmond, B.C.) recorded ambient and water temperatures within the cage. A single channel data logger (Hobo@, Hoskins Scientific, Vancouver, B.C.) measured internal temperature with a probe inserted approximately 20 cm into a wound in the torso created by a surgical knife. Once located, probes were not disturbed. All loggers were placed in plastic Ziplock® bags and attached to the tops of the cages. Driorite® was added to each bag to absorb moisture and replaced on every sampling date. Temperatures were recorded every 30 min. Also, ambient air temperature was obtained from an Environment Canada weather station in the Malcolm-Knapp Research Forest. The weather station was located 2 km from the stream experimental sites and approximately 4 km from the most northern pond site.

On each sampling date, all carcasses were thoroughly examined visually for decompositional changes without being disturbed. Observations were recorded and photographed.

One week prior to placement of carcasses on site and at each sampling date, a 250 mL water sample was taken within each cage and at control sites. The control sites were located approximately 5 m away from cages and across

Figure 1. Method of determining the percentage of carcass exposed to air by dividing pig carcass into eighths.
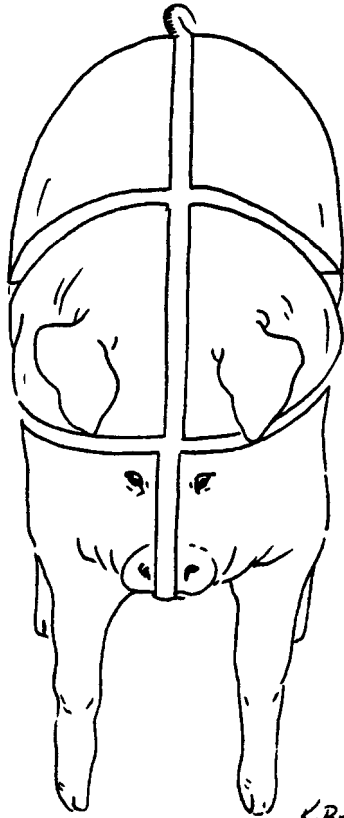
K. Brett.
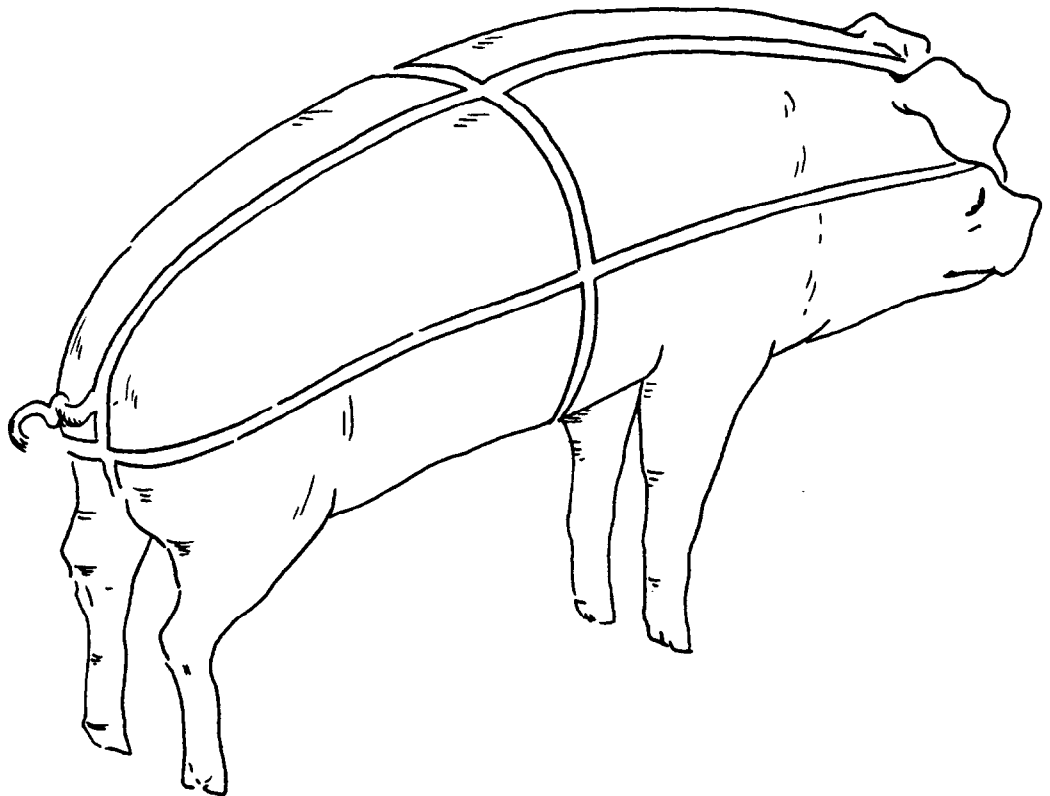
the water intake-outflow pathway in ponds, and 3 m upstream from cages in streams. The water samples were used to determine carbon dioxide content and pH (Anon. 1978). To sample benthic fauna in pond sites, sediment was collected at each water sampling location in a 250 mL plastic bottle. In the stream, bottom fauna were sampled with a surber (Chadwick and Canton 1992). The surber was dropped in three different locations along the stream bed, at least 2 m from the cages, every sampling time. On each sampling date, an aquatic net (Mackay et a/. 1984) lined with muslin, was used to sweep the area within each cage as well as at the control locations for water sampling. The muslin was placed in a Ziplock® bag, taken to the laboratory, and then rinsed with distilled water into a dissecting tray. All invertebrates were preserved in 95 % ethanol and later identified.

The carcass was then removed from the water and placed on the lid of the cage and representatives of each invertebrate species found were collected and preserved in 95 % ethanol (Merritt and Cummins 1996) for later identification. Carcasses were never out of the water for longer than 15 min. Living terrestrial dipteran larvae were collected, and reared to adulthood in the laboratory for identification.

All invertebrates were identified using appropriate keys (Wiggins 1977; Oliver 1983; Pennak 1989; Merritt and Cummins 1996), and compared if possible with known terrestrial specimens from a reference collection (Dillon 1997). Aquatic unknowns were identified by Linda Currie, Fraser Environmental Services, Surrey, British Columbia.

2.3 Statistical Analysis

Ambient air and internal carcass temperatures, ambient and water temperatures, and internal carcass and water temperatures during the fresh stage for pond and stream habitats were compared using linear regression (Minitab®). Linear regression was also used to compare temperature logger data with weather records obtained from the Environment Canada Weather Station in U.B.C. Research Forest.

A chi-square test (Minitab®), $\alpha=0.05$, was performed to determine if some species of insects were carrion associated by comparing their distributions between the cage and control sampling sites for both pond and stream habitats.

2.4 Comparison with Water Death Investigations

To date, postmortem intervals determined in water death investigations tend to be subjective, vague, and based on the investigator's anecdotal descriptions and not on data, and thus are unreliable for legal testimony. I compared the invertebrate succession and decompositional descriptions determined by my research with observations made from 15 fresh water death investigations that occurred in British Columbia in 1996 (B.C. Coroners Service 1996), and for which the PMI was > 72 hours. These cases accounted for 15 % of all water-related death investigations (excluding pending inquiries). Twenty-five cases had a PMI < 72 h or bodies were not recovered. Thirty-eight cases involved insufficient descriptions of the corpses to allow adequate comparisons.

3. RESULTS

3.1 Physical Characteristics

During the first day after death, the maximum internal carcass temperatures in both habitats decreased as the carcasses cooled (Figure 2). Thereafter, neither maximum nor minimum internal carcass temperature differed greatly from water temperatures, which ranged from 10.39 to $14.29^{\circ}C$ in the pond habitat, throughout the fresh stage of decomposition. Water temperature was not obtained from the stream habitat due to failure of the data logger. Mean ambient air temperature was the best available predictor of mean internal body temperature in the stream habitat, but was less predictive in the pond habitat, where water temperature was a somewhat better predictor of internal body temperature (Table 1). Low r^2 values were due to the original data containing outliers and demonstrating heteroscedasticity. Mean temperatures at the weather station were moderately good indicators of the mean ambient air temperatures for both habitats (Table 1).

In the pond habitat, changes in carbon dioxide levels within and outside cages were offset by approximately 7 days (Figure 3). In both habitats, peak CO_2 levels were associated with accumulations of detritus, which in turn were associated with high water levels. In the stream habitat, the water samples from the control sites displayed similar peaks in carbon dioxide levels as those in the pond habitat. However, within the cage carbon dioxide levels rose gradually for 43 days and did not fluctuate with water level or amounts of detritus. For both

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Figure 2. Maximum and minimum ambient air, water, and internal pig carcass temperatures in one pond and one stream habitat for the fresh stage of decomposition.

Pond, Fall 1996

Stream, Fall 1996

Temperature (°C)

Postmortem Interval (days)

14
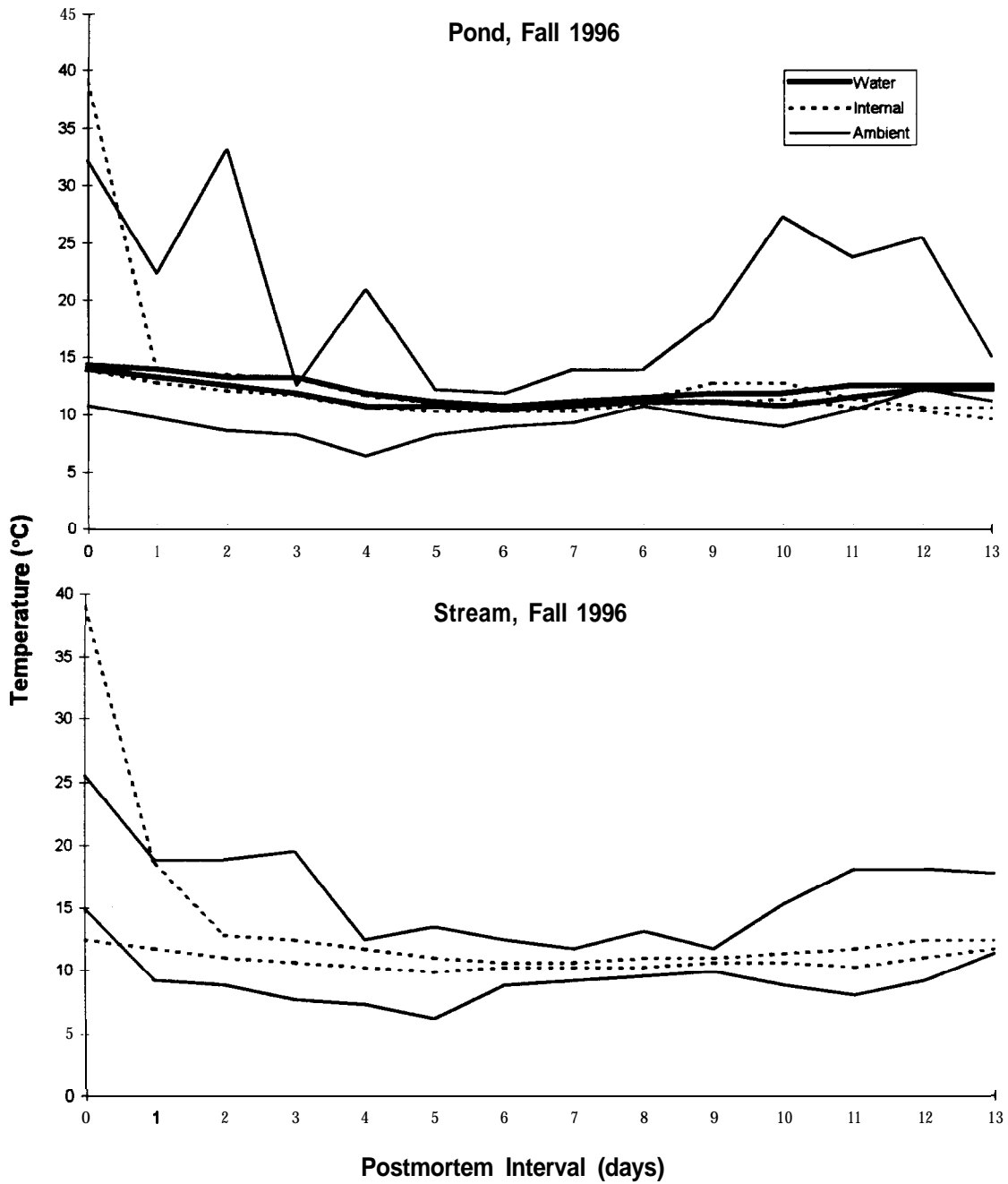
Table 1. Linear regression analysis of relationships between temperatures for the fresh stage of decomposition after the carcass had cooled in pond and stream habitats.

Habitat	Dependent Temperature Variable	Independent Temperature Variable	Temperature	Regression Equation	3	P
Pond	Ambient Air	Internal	Maximum	y = -14.8 + 2.88x	0.223	0.103
			Minimum	y = 14.0 - 0.418x	0.061	0.417
			Mean	γ = -0.9 + 1.34x	0.141	0.207
	Ambient Air	Water	Maximum	y = 2.05 + 0.809x	0.439	0.014
			Minimum	y = 2.03 + 0.644x	0.138	0.211
			Mean	γ = -10.7 +2.12x	0.288	0.059
	Internal	Water	Maximum	y = 2.05 + 0.809x	0.439	0.014
			Minimum	y = 4.71 + 0.541x	0.451	0.004
			Mean	y = 3.73 + 0.650x	0.342	0.036
	Ambient Air	Station	Maximum	y = -10.7+ 1.58x	0.704	0.000
			Minimum	y = 4.47 + 0.466x	0.391	0.022
			Mean	y = -7.37 + 1.48x	0.683	0.000
Stream	Ambient Air	Internal	Maximum	y = 4.42 + 0.913x	0.037	0.028
			Minimum	y = -7.05 + 1.48x	0.428	0.015
			Mean	y = 1.53 + 0.930x	0.458	0.011
	Ambient Air	Station	Maximum	y = 10.1+ 0.2.84x	0.112	0.263
			Minimum	y = 5.78 + 0.280x	0.190	0.137
			Mean	y = 3.66+ 0.576x	0.433	0.014

Figure 3. Mean carbon dioxide levels for control and cage sites for 62 days postmortem in pond and stream habitats.

Pond, Fall 1996

Stream, Fall 1996

- - - - Within Cage--Mean
——— Control--Mean

Carbon Dioxide Levels

Postmortem Interval (days)

17
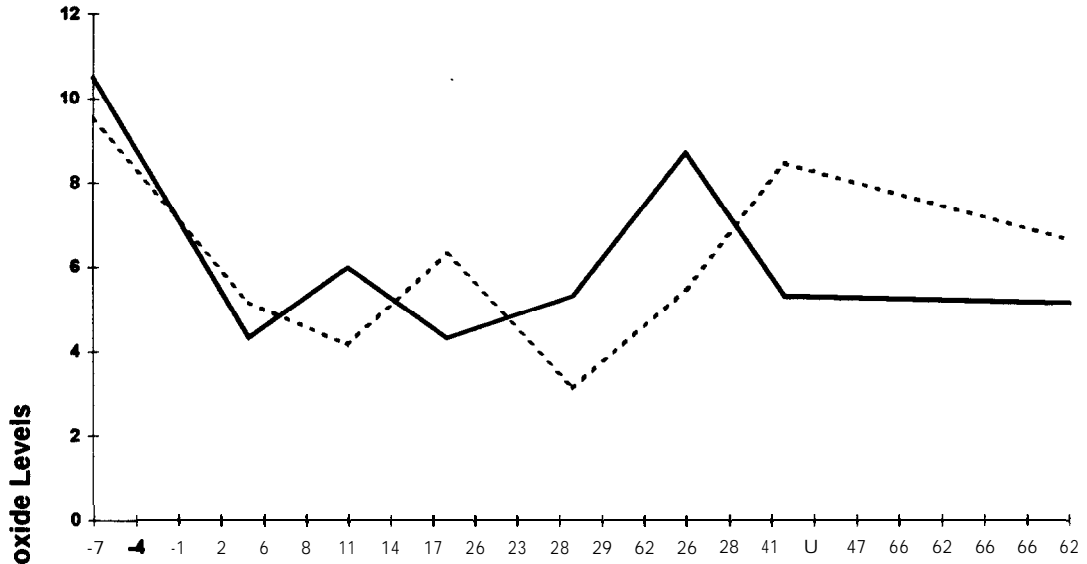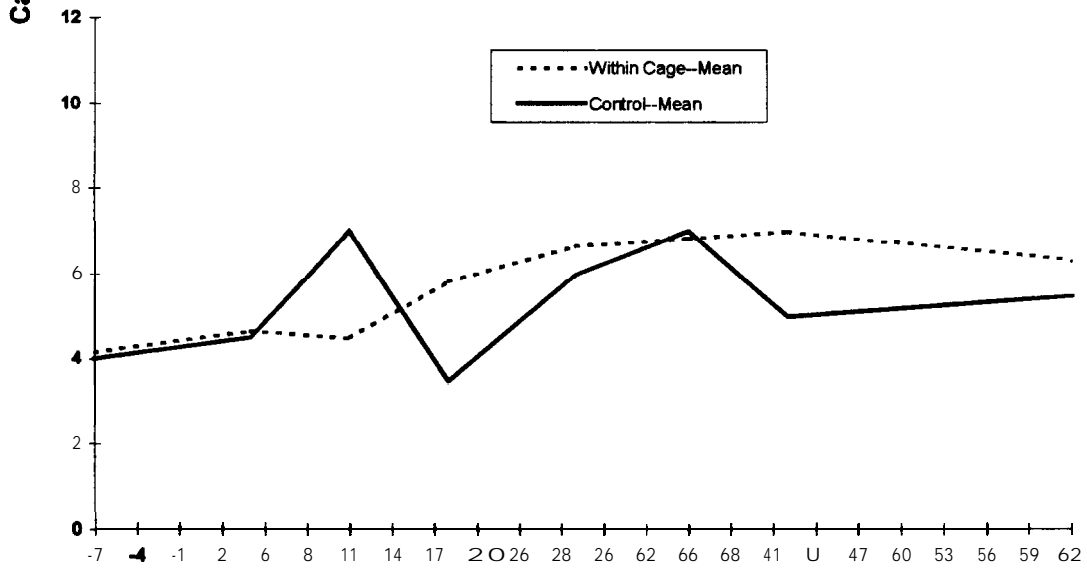
habitats, p   levels were fairly consistent at 5-5.5 for both cage and control sites. In the stream habitat, pH rose on one occasion only (day 28) to 6.5, during a period of heavy accumulation of detritus. The acidity of the water, and the high carbon dioxide levels in both habitats apparently caused saponification, breakdown of the fatty tissues of the carcass.

3.2 **Exposure of Carcasses**

Differences in the percentage of the carcasses exposed to air were observed throughout the decompositional stages for each habitat (Figure 4). No similarities were detected for carcasses within the same habitat.   In the stream habitat pig carcasses varied from completely submerged to 50 % exposed; in the pond habitat, carcasses ranged from completely submerged to five eighths exposed.

3.3 **Decomposition**

Because no differences in decompositional rages or invertebrate activity were observed between control (undisturbed) and experimental (disturbed) carcasses in the two habitats, observational data from experimental and control carcasses were combined in analysis of decompositional stages (Figure 5).

The **fresh stage** of decomposition began at death and lasted for 11 to 13 days until the first signs of bloat appeared.   In the pond habitat where full

Figure 4. Percent of carcass exposed to air during decomposition in pond and stream habitats.

Pond Sites

Carcass Exposure (%)

Stream Sites

Postmortem Interval (days)

20
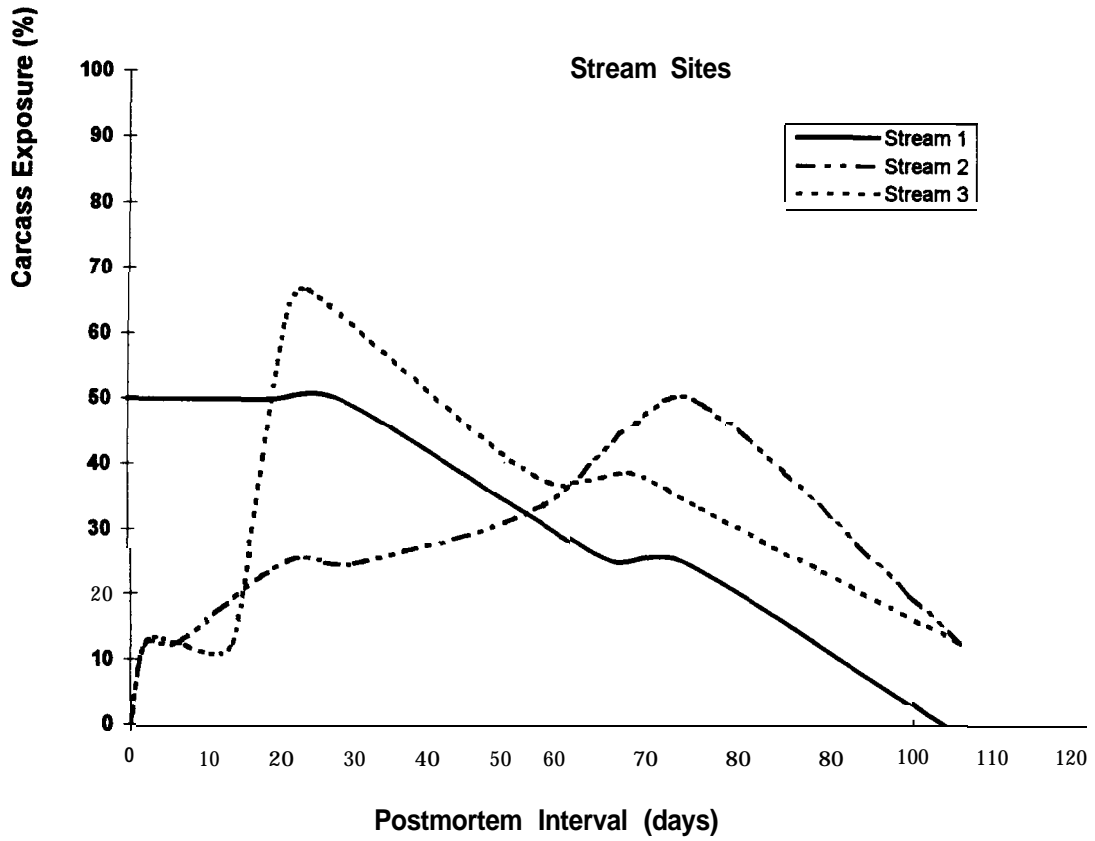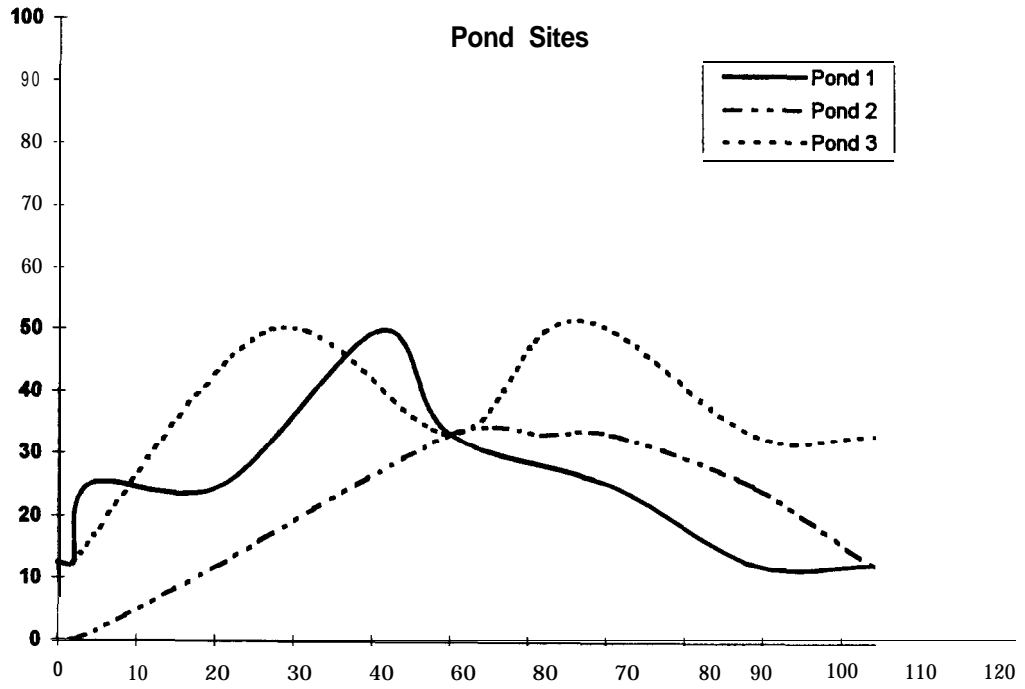
Figure 5. Duration of decay stages for pig carcasses in pond and stream habitats.

Pond Sites

■ Fresh ❑ Bloat ❑ Decay ▨ Post-Decay ▣ Remains

0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360

Stream Sites

0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
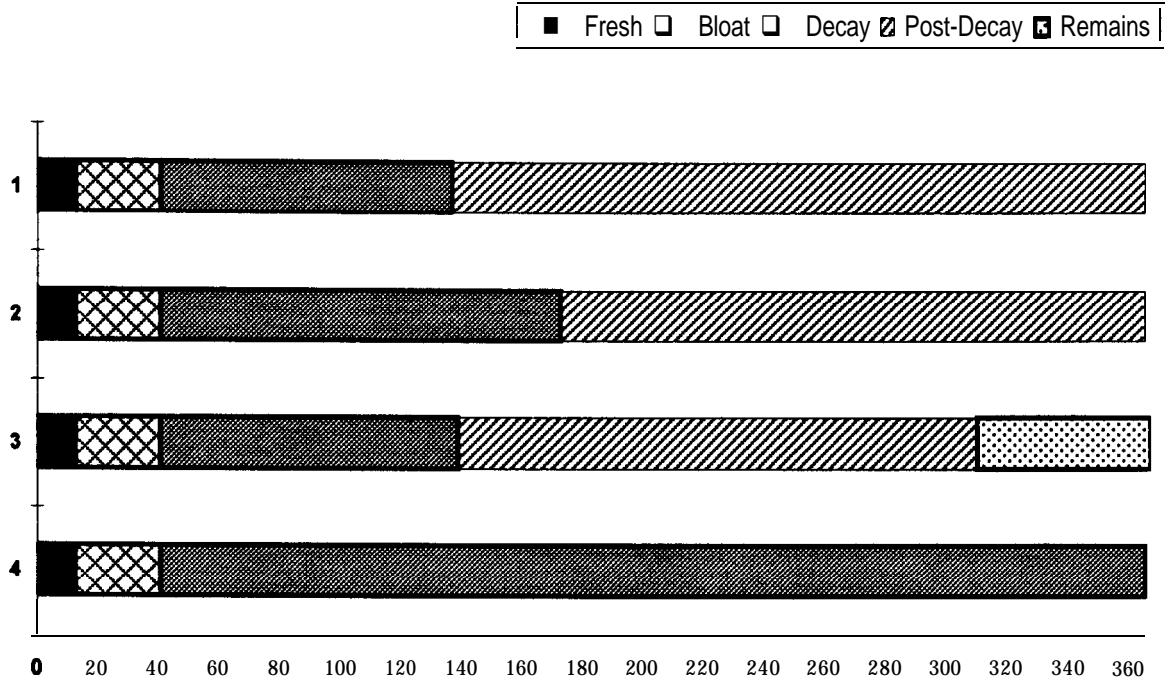
Postmortem Interval (days)
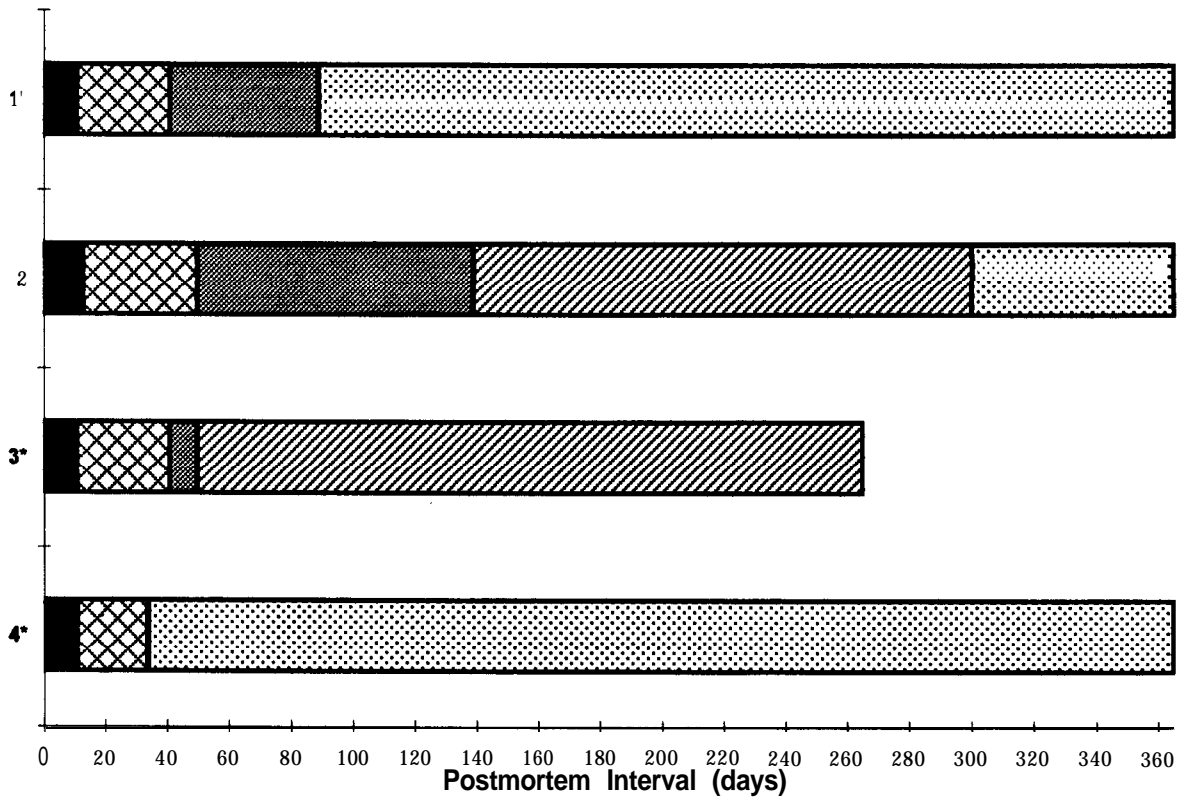
* Carcasses scavenged by mink
Pig 3 (Stream Habitat) was entirely removed by bear at approximately 260 PMI

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submersion was possible, the carcasses floated just below the water surface, with either one ear protruding or the abdomen slightly exposed. Caddisfly larvae appeared by the second day on the facial regions of the carcasses, but not on clothed areas. In the stream habitat, blow flies (Calliphoridae) laid eggs along the edges of clothing on the large portions of the carcass that remained above water. The internal carcass temperature dropped quickly as the body cooled (Figure 2).

Transition to the **bloat stage** in both habitats was marked by distention in the abdomen, which later assumed a fully inflated balloon-like appearance with a putrid odor becoming evident. These characteristics were indications of bacterial activity in the gastrointestinal tract. In the pond habitat, the abdomens protruded greatly, but tight clothing constricted the overall bloating of the carcass. There were fewer caddisfly larvae on the carcasses than in the fresh stage. The bloat stage lasted 28 days. In the stream habitat, bloated carcasses seemed to attract mink, Mustela *vison* Schreb., which scavenged three of the four carcasses during this period. Exposed portions of carrion were predominantly colonized by blow flies and carrion beetles (Silphidae) whereas submerged portions remained uncolonized. Duration of the bloat stage was more variable in the stream habitat, lasting from 23 to 37 days.

The **decay stage** began when a bloated carcass deflated by a slow release of gases through natural orifices or wounds caused by scavenging. Hair and skin flaked off and nails became detached. There was a strong odor associated with the carcass at this stage. Fewer living blow fly larvae were

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observed in both habitats, but dead larvae were not evident. During this stage, adipocere, a waxy substance, was produced by the conversion of fatty tissue into glycerol and an alkali salt (Mortimer 1983). It first resembled pale, rancid butter and later hardened, providing a protective coating for the internal structures. In three carcasses in the pond habitat the decay stage lasted from 98 to 324 days. One carcass remained in the decay stage for more than 324 days, until observations ceased. In the stream habitat, one carcass was in the decay stage for only 9 days, in two others the duration was 48 and 89 days, and for the fourth carcass, which was heavily scavenged by mink, neither the decay stage nor the succeeding post decay stage occurred at all.

By the **post decay** stage, much of the flesh, except for the skin, had been removed by invertebrate scavengers, e.g. crayfish, caddisflies and maggots, if not previously scavenged by mink. Invertebrate populations inhabiting the carrion were very diverse. In the pond habitat, the three carcasses which entered this stage remained in it for 171 to 228 days. Only two carcasses in the stream habitat experienced a post decay period, one for 161 days, and the other for 215 days.

The **sunken remains** stage occurred for only one carcass in the pond habitat, and in three carcasses in the stream habitat. Sunken remains were carcassses that had completely submerged, regardless of the amount of tissue remaining. Benthic fauna including earthworms, snails, and nymphal mayflies and stoneflies started to colonize the nutrient rich area, increasing the diversity of organisms. The odor at this time was less potent than in the previous three

stages. On the scavenged carcasses most of the tissue was removed by mink; therefore, only the bones with greasy decomposed tissue remained at the bottoms of the cages. In the scavenged carcasses, the sunken remains stage was entered on day 89, bypassing the post decay stage. The most heavily scavenged carcass entered the remains stage on day 34, bypassing the two preceding stages.

Scavenging by mink was concentrated on the lower and unclothed portions of carcasses in the stream habitat. On exposed carcasses, invertebrates tended to feed under the clothing where they were protected. Conversely, on submerged carcasses, most invertebrates tended to colonize and feed on the non-clothed portions.

3.4 **Invertebrate Succession**

All invertebrates found on the carcass within the cage and at the control sites in both the pond and stream habitats were identified for a total of 17 orders, 39 families, and 54 genera (Tables 2, 3). Invertebrates (excluding insects) displayed no distinct pattern in times of arrival, or duration of stay. Oligochaetes were present during all decompositional stages including both experimental and control sites for pond and stream habitats. In the pond habitat, bivalves were the only invertebrates which were consistently found on the carcasses. Hydrozetes sp. and *Gyralus* sp. were also present but differences in experimental and control sites were not detected.

Table 2. Succession of invertebrate species (excluding Class Insecta) collected on carcasses and from control sample sites in pond and stream habitats. A=adult.

Decomposition stage	Order and family	Genus and species or common name	Stream cage	Stream control	Pond cage	Pond control
Pre-experiment	**LUMBRICULIDA** Lumbriculidae	Oligcchaetes			A	A
	PELECYPODA Unknown	Biralve			A	
	PODOCOPA Unknown	Seed shrimp				A
Fresh	**GNATHOBDELLIA** Hirudinidae	Leech	A			
	LUMBRICULIDA Lumbriculidae	Oligochaetes				A
	PELECYPODA Unknown	Seed shrimp			A	
Bloat	**LIMNOPHILA** Planorbidae	Gyraulus sp.	A			
	LUMBRICULIDA Lumbriculidae	Oligcchaetes		A	A	A
Decay	**GNATHOBDELLIA** Hirudinidae	Leech		A		
	LUMBRICULIDA Lumbriculidae	Oligochaetes		A	A	A
	ORIBATEI Eremaeidae	Hydrozetes sp.				A
	PODOCOPA Unknown	Seed shrimp				A
	SPIROBDOLIDA Unknown	Millipede			A	
Post Decay	**GNATHOBDELLIA** Hiudinidae	Leech				
	LIMNOPHILA Planorbidae	Gyraulus sp.				A
	LUMBRICULIDA Lumbriculidae	Oligochaetes		A	A	A
	ORIBATEI Eremaeidae	*Hydrozetes* sp.			A	
	PODOCOPA Unknown	Seed shrimp			A	A
Sunken Remains	**LIMNOPHILA** Planorbidae	Gyraulus sp.				A
	LUMBRICULIDA Lumbriculidae	Oligochaetes	A	A	A	

Table 3. Succession of insect species collected on carcasses and from control sample sites in pond and stream habits. A=adult, P=pupae, I=immature, E=eggs.

Decomposition stage	Order and family .	Genus and species/ common name	Habitat			
			stream		Pond	
			cage	control	cage	control
Preaxperiment	COLEOPTERA Elmidae	*Steneimis* sp.		A		
	DIPTERA Chironomidae	*Ploypedilum* sp.			|	|
		Heterotissocladius sp.		|	|	|
	PLECOPTERA Perlodidae	Isoperla sp.				|
Fresh	DIPTERA Chironomidae	*Heterotrissocladius sp.*				|
	HEMIPTERA Genidae	*Aquarius mmigis* (Say)	A			
	TRICHOPTERA Limnephilidae	*Chyranda centralis* (Banks)				
		Ecclisomyia sp.				
Bloat	COLEOPTERA					
	Curculionidae	*Stenopelmus* sp.	A			
	Dryopidae	*Helichus* sp.	A			
	Dytiscidae	*Actilius* sp.			A	
	Elmidae	*Heterlimnius* sp.		A		
	Elmidae	*Stenelmis* sp.			A	
	Hydrophilidae	*Hydrocham* sp.	A			
	Leptodiridae	*Catoptrichus frankenhaussen* (Mannh.)	A		A	
	Staphyiinidae	*Homaeotamus sellatus* (LeConte)	A		A	
	DIPTERA					
	Calliphoridae	*Calliphora vomitoria* (L.)	A/I		A/I	
		Phormia regina (Meigen)			|	
	Chaoboridae	*Mochlonyx* sp.	|			
	Chironomktae	*Heterotrissocladius* sp.			I/P	|
		Polypedilum sp.	|		|	I/P
	Muscidae	undetermined species	A/I		All	
	Sciaridae	*Sciara* sp.			A	
	Sepsidae	*Enicita* sp.			A	
	EPHEMEROPTERA					
	Ephemerellidae	*Serratella* sp.	|	|		
	Leptophlebikiae	*Pamleptophlebia* sp.	|			
	HEMIPTERA Gerridae	*Aquarius remigis* (Say)	A		A	
	HYMENOPTERA					
	Braconidae	undetermined species			A	
	Mymaridae	*Camphmctus cinctus* (Walker)				
	LEPIDOPTERA Noctuidae	*Archanara oblonga* (Grote)				

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Decomposition stage	Order and family	Genus and species/ common name	Habit			
			stream		Pond	
			cage	control	cage	control
	PLECOPTERA					
	Capniidae	*Bolshecapnia* sp.	A			
	Periodidae	Isoperla sp.	I			
	TRICHOPTERA					
	Limnephiiidae	*Chyranda centralis* (Banks)	I			
		Pseudostenophylax sp.				
Decay	COLEOPTERA					
	Dryopidae	*Helichus sp.*		A		
	Elmidae	*Heterlimnius sp.*		A		
		Steneimis sp.		A	A	
	Hydrophiiidae	*Hydrochara* sp.				
	Leptodiridae	*Catoptrichus trankenhaussen* (Mannh.)			A	
	Silphidae	*Nicrophorus* sp.			A	
	COLLEMBOLA					
	isotomidae	Isotomurus tricolor (Packard)			A	
	DIPTERA					
	Caiiiphoridae	*Calliphora vomitoria* (L.)	E/I			
		Phormia regina (Meigen)				
	Chironomidae	*Heterotrissocladius* sp.				
		Polypedilum sp.				
	Empididae	Hemeriodromia sp.		P		
	Tanyderidae	*Protanyderus* sp.				
	EPHEMEROPTERA					
	Ephemereliidae	*Serratella* sp.		I		
	PLECOPTERA					
	Capniidae	*Bolshecapnia* sp.		I/A		
	TRICHOPTERA					
	Limnephiiidae	*Chymnda centralis* (Banks)			I	I
		Limnephilus sp.				
		Pseudostenophylax sp.		C		
		undetermined species				
Post Decay	COLEOPTERA					
	Dytiscidae	undetermined species			A	
	Elmidae	*Heterlimnius sp.*		A		
	Hydrophiiiie	*Hydrochara* sp.			A	A
	DIPTERA					
	Chironomidae	*Chironomus* sp.			I	
		Heterotrissocladius sp.			I/P	I
		Polypedilum sp.			I	I
	Sciomyzidae	*Dictya sp.*	A			
	Siiuiiidae	*Simulium* sp.				
	Tipuiii	*Limnophila sp.*				I
		Prioncera sp.				
	EPHEMEROPTERA					
	Ephemerellidae	*Ephemerella* sp.	I	I	I	
		Serratella sp.	I	I	I	
	Leptophiebiidae	*Paraleptophlebia* sp.		I		
	HEMIPTERA					
	Gerridae	Aquarius *remigis* (Say)			A	

Decomposition stage	Order and family	Genus and species/ common name	Habitat Stream		Pond	
			cage	control	cage	control
	ODONATA					
	Libellulidae	*Libellula* sp.				
	PLECOPTERA					
	Nemouridae	*Prostoia besametsa* (Ricker)				A
	Perlodidae	Isoperla sp.				
	TRICHOPTERA					
	Brachycentridae	*Micmsema* **sp.**				
	Limnephilidae	*Moselyana comosa* (Denning)				
		Chyranda centralis **(Banks)**				
		Limnephilius sp.				
		Pseudostenophytax sp.				
Sunken Remains	**COLEOPTERA**					
	Dytiscidae	undetermined species			A	
	Elmidae	*Heterlimnius* sp.		A		
		Optioservus sp.		A		
	COLLEMBOLA					
	Isotomidae	*Isotomurus tricolor* **(Packard)**	A			
	DIPTERA					
	Chironomidae	*Chironomus* sp.				
		Heterotrissocladius sp.				
		Polypedilum sp.				
	Empididae	*Hemerodromia* sp.		P/A		
	Phoridae	undetermined species				
	EPHEMEROPTERA					
	Ephemerellidae	*Ephemerella* sp.				
		Serratella sp.				
	Leptophiebiidae	*Paraleptophlebia* sp.				
	HEMIPTERA					
	Gerridae	*Aquarius remigis* **(Say)**		A		
	ODONATA					
	Libellulidae	*Libellula sp.*				
	PLECOPTERA					
	Nemouridae	*Prostoia besametsa* (Ricker)	A			
	Perlodidae	*Isoperla sp.*				
	TRICHOPTERA					
	Limnephilidae	*Chyranda centralis* **(Banks)**	I		I/P	
		Limnephilus sp.				
		Pseudostenophytax sp.				
		undetermined species				

Insect specimens in 10 orders, 34 families and 46 genera were recovered (Table 3). Among them were necrophagous species, predators, parasites, and apparently incidental species. Factors including habitat, decompositional stage, scavenging, and presence of clothing caused a diversity of insects and patterns in arrival times and duration of stay.

Differences in the number of species of Diptera, Coleoptera, and Trichoptera were recorded in the two aquatic habitats (Table 4, 5). In the pond habitat, calliphorids dominated the exposed portions of carrion during the entire bloat stage. Also, the Staphylinidae, Silphidae, and Leptodiridae became numerous during this stage. Caddisfly larvae dominated submerged portions of unclothed carrion. Chironomid larvae were also present during the bloat stage, but populations remained constant for the entire experiment with the exception of the month of November. During the decay stage, *Homaeofarsus* sellatus (Staphylinidae) and very few third instar calliphorid larvae remained on exposed, clothed portions of carcasses. *Homaeofarsus* sellatus was still present during the post decay stage. On submerged portions of the carcasses, stonefly larvae were numerous for the first two months, whereas caddisfly larvae and diving beetles remained until the termination of the experiment.

In the stream habitat, calliphorids dominated the exposed portions of a carcass during the fresh and bloat stages. Species in the Leptodiridae and Staphylinidae became evident during the bloat stage. Chironomid larvae were evident during the bloat stage but remained numerous throughout the experiment, feeding both underneath clothes and on unclothed portions of

Table 4. Occurence of insect species according to date and postmortem interval (days) in the pond habitat.

Pond Habitat

	02-Sep (2)	11-Sep (11)	18-Sep (18)	27-Sep (27)	04-Oct (34)	11-Oct (41)	20-Oct (50)	01-Nov (62)	10-Nov (71)	28-Nov (89)	13-Dec (104)	20-Feb (173)	28-Mar (210)	15-Apr (227)	22-Apr (234)	29-Apr (241)	23-May (265)	04-Jun (277)	27-Jun	17-Jul	29-Jul	26-Aug	08-Sep
COLEOPTERA																							
Acilius sp.																							
Cetoptrichus frankenhausen		x x	x	x																			
Dytiscidae					x																		
Homeotarsus sellatus		x	x	x	x	x	x x		x										x	x	x	x	x
Hydrochara sp.				x							x		x										
Nicrophorus sp.		x																			x		
Stenelmis sp.												x		x									
DIPTERA																							
Calliphora vomitoria			x	x	x	x	x																
Chironomus sp.										x													
Ericite sp.						x	x						x										
Heterotrissocladius sp.	x	x x	x x	x x	x x	x						x	x	x		x	x		x	x	x		
Muscidae				x	x																		
Phormia regina	x	x x	x x	x x	x x	x																	
Polypedilum sp.	x	x x	x x	x x	x x	x					x	x	x	x	x	x	x		x		x		
EPHEMEROPTERA																							
Ephemerella sp.		x										x											
Paraleptophlebia sp.																			x x				
Serratella sp.																x		x					
PLECOPTERA																							
Bolshecapnia sp.			x															x					
Isoperla sp.												x	x x		x x	x							
Prostoia besametsa					x																		
TRICHOPTERA																							
Chyranda centralis	x	x	x									x x	x	x	x	x x	x x	x x x	x	x	x		
Limnephilus sp.												x	x			x	x	x x x	x	x	x	x	x
Pseudostenophylax sp.													x	x						x			

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Table 5. Occurence of insect species according to date and postmortem interval (days) in the stream habitat.

	02-Sep (2)	11-Sep (11)	18-Sep (18)	27-Sep (27)	04-Oct (34)	11-Oct (41)	20-Oct (50)	01-Nov (62)	10-Nov (71)	28-Nov (89)	13-Dec (104)	20-Feb (173)	29-Mar (210)	15-Apr (227)	22-Apr (234)	29-Apr (241)	23-May (265)	04-Jun (287)	27-Jun (300)	17-Jul (320)	29-Jul (332)	28-Aug (360)	08-Sep (371)
Stream Habitat																							
COLEOPTERA																							
Catops sp.		X																					
Catoptrichus frankenhaussen					X																		
Homaeotarsus sellatus		X	X				X																
Hydrochara sp.		X																					
DIPTERA																							
Calliphora vomitoria	X		X	X		X	X	X	X	X													
Chironomus sp.		X																					
Heterotrissocladius sp.				X												X							
Muscidae				X																			
Phormia regina				X	X	X																	
Polypedilum sp.	X			X								X			X		X	X	X			X	
EPHEMEROPTERA																							
Ephemerella sp.												X		X	X		X						
Paraleptophlebia sp.		X	X											X	X		X	X	X				
Serratella sp.			X											X	X	X	X	X	X				
PLECOPTERA																							
Bolshecapnia sp.				X																			
Isoperla sp.																		X	X	X	X		
Prostoia besametsa																			X				

submerged carrion. During the spring, either post decay or sunken remains stages, xmayfly and stonefly larvae dominated submerged portions of carrion.

In the pond habitat, Chyranda *centralis, Helichus* sp., *Calliphora vomitotia, Homeotarsus sellatus Catoptrichus frankenhaussen, Aquarius remigis,* and and *Phormia regina* were all found to be carrion associated using statistical analysis (Table 6). However, *Calliphora vomitoria, Chironomus* sp., and *Homeotarsus sellatus* were determined to be significantly carrion associated in the stream habitat.

3.5 Comparison with Water Death Investigations

Table 7 summarizes the observations that i consider to be potentially useful in forensic investigations. Many similarities were apparent between these observations and the 15 forensic investigations for which there was sufficient documentation to allow a comparison to be made (Table 8). However, differences were noted in the amount of time needed to observe hair shedding and skin slippage. It appeared that the longer the corpse was submerged in water, the more vague the description in the coroners files would be. In only one case did the coroners records include any observations of aquatic or terrestrial invertebrates.

Table 6. Chi-square analysis to determine carrion association of selected species in pond and stream habitats.

Species	Pond x^2	P	Stream x^2	P
Calliphora vomitoria	12.494	0.000	8.258	0.004
Chironomus sp.	**0.119**	0.155	6. 900	0.009 *
Phormia regina	4.381	0.000 *	1.022	
Muscidae	1.011		2.091	0.148
Heterotrissocladius sp.	0.154	0.895	0.088	0.767
Polypedilum sp.	1.287	0.257	9.684	0.731
Acilius sp.	2.028	0.155	NF	
Catop basilaris	2.028	0.155	NF	*
Catoptrichus frankenhaussen	7.358	0.007	1.022	
Dytiscidae	2.983	0.086	NF	
Helichus sp.	18.254	0.000	NF	
Homaeotarsus sellatus	10.748	0.001	4.381	0.037 *
Hydrochara sp.	0.207	0.649	1.020	
Nicrophorus sp.	3.101	0.079 *	NF	
stenelmis sp.	1.001		2.091	0.148
Isoperla sp.	0.119	0.731	3.696	0.055
Leuctra sp.	NF		2.091	0.148
Serratella sp.	2.028	0.155	0.088	0.767
Emphemerella sp.	0.000	1 .000	0.605	0.340
Paraleptophlebia sp.	0.340	0.560	0.605	0.437
Chyranda centralis	40.500	0.000	0.138	0.710
Limnephilus sp.	2.044	0.153	0.000	1 .000
Platycentropus sp.	3.084	0.080	NF	
Aquarius remigis	5.807	0.016	3.067	0.080

• Note Chi-square approximation invalid, 2 cells counts less than 1
    NF - species not found in habitat
    df = 1 for all comparisons

Table 7. Summary of observations that could be of use in forensic investigations in pond and stream habitats.

P.M.I. (weeks)	Stage	Pond	Stream
o-1 .5	Fresh	carcasses 3/4 to 7/8 submerged Trichoptera (caddisflies) ,50	carcasses partially exposed eggs laid along edge of clothing
1.5-2.5	Bloat	eggs of *Calliphora vomitoria* present chironomids, caddisflies, Silphidae present clothing starting to restrict bloat	exposed skin pale in color submerged skin brownish clothing starting to restrict bloat lard-like substance on head no organism visible near natural orifices Staphylinidae present mink scavenging on carcass #4
3-5		Staphylinidae adults present under clothing Silphidae (~5), Calliphoridae and Muscidae present maximum bloat exposed skin hardened	no flies near scavenged areas flies landing and laying eggs on exposed skin Staphylinidae and other Coleoptera present carcasses at maximum bloat scavenged carcass entirely submerged
8-7	Decay	hair falling off, skin sloughing off decreasing bloat white powdery substance deposited on skin lard-like substance on abdomen starting to harden waxy substance soaked through clothing and starting to harden	hair falling off very few dipteran larvae present under clothing, leaves, bars of the cage no evidence of pupae substantial flaking of the skin hoof became detached decrease in bloat
9-10		noticeable decrease in bloat more noticeable odor most exposed skin hairless no living terrestrial dipteran larvae	exposed skin hardened and hairless if maggots present, still surviving carcasses had withered appearance new scavenging by mink
12-15		*1/4* to *1/3* exposed orange-like color to the exposed, hardened skin (mummification)	scavenged carcass completely submerged exposed skin hardened, orange-like color (mummification) very few organisms visible no dipteran larvae visible

P.M.I. (weeks)	Stage	Pond	Stream
26	Post Decay	1/8 exposed blubbery appearance to submerged non clothed areas of the carcass (previously shredded) stonefly and mayfly larvae present within blubbery substance all tissue on feet removed, no bone visible head and feet skeletonized (both submerged)	exposed skin still orange in color stomach still somewhat bloated, soft to the touch new scavenging 1/4 to 1/8 exposed
30-34		blue-grey-white fuzz-like substance covered the unclothed submerged skin more scavenging occurred increased diversity of invertebrates visible Tanyderidae collected near carcasses very small area of carcass still exposed	no organisms visible disarticulation of one scavenged carcass few stonefly larvae present equilibrium of carcass below surface, but rises when rolled extreme odor
37-38		abdomen slightly exposed many caddisfly larvae, few large ones red colored substance covered one carcass maggots on recent scavenging wound on one carcass	only disarticulated bones and adipocere tissue remaining on scavenged carcasses very few invertebrates mouse gnawing
40-43	Sunken Remains	very putrid odor caddisflies removing flesh from bone	smell decreasing typical benthic fauna second scavenged carcass submerged
46-48		gnawing on feet at sediment/water interface by caddisfly larvae caddisflies and diving beetles present	increase in species of benthic fauna

Table 8. Comparison of 1998 water death investigations in British Columbia with this research.

Case#	Coroners Region	Circumstances	Corpse Description Upon Recovery	P.M.I. (days)	Season	Consistency
96-001	Fraser	deceased fell from rock into water and drowned body was found submerged in the centre of the river slough	moderately decomposed, skin slippage and marbling within subcutaneous vessels is prominent over the upper half of the body dark red purge is draining from mouth and nose	5	summer	discoloration, and marbling were consistent with research
96-002	Fraser	body was found floating in river	sand within the airways livor: cannot assess; rigor passed advanced decompositional change with prominent gas production in the abdominal cavity and soft tissues, skin discoloration (green-black-brown), foul odor, skin slippage, washerwoman hands and feet, post mortem animal activity on torso, silt is present diiusely over the body and within the clothing	5	summer	silt and washer woman traits consitent with research, however skin slippage, discoloration, postmortem animal action, bloating and odor didn't occur in 5 days in research
96-003	Vancouver	body was found floating in river under a log boom some scalp lacerations concrete block tape to hand	bloating and distortion of the facial features and some protuberance of the tongue between the teeth immersion changes were present over the chest and thorax with bloating and skin slippage	7	summer	tongue protuberance was the only characteristic consistent with research
96-004	Fraser	deceased was duck hunting, boat capsized, body was found 9 days later by search team	livor: pink, anterior, and posterior tixed rigor: passed postmortem patchy skin loss on the dorsum of the right hand and punctuated postmortem abrasions on face, mild decompositional change of all tissues	9	fall	loss of skin occurred earlier than observed in research

Case#	Coroners Region	Circumstances	Corpse Description Upon Recovery	P.M.I. (days)	Season	Consistency
96-005	Fraser	body found floating down river	advanced decomposition, silt-like material present in upper airway, liior: cannot assess, rigor: passed advanced decomposition with skin slippage, marked skin discoloration foul odor, gas formation, most prominent in the abdominal cavity and scrotum, thin layer of silt over body	-10	summer	silt, discoloration, and Moating consistent with research
96-006	Kelowna	body found floating face down in a glacial pothole, wearing shirt, jeans and running shoes upper (dorsal) head, back and buttocks exposed - legs submerged	no liidy or rigidii, chest down and the lower extremities show only early signs of decomposition stomach hugely filled with gas upper torso and head have been exposed to the elements for a longer period of time and showed marked bloating, loss of skin and skin discoloration	13	fall	bloating and discoloration consistent, however loss of skin occurred earlier than observed in research
96-007	Fraser	deceased was occupant of a vehide which became immersed in water steep underwater embankment van found at 213 ft	pinkish-purple colouration of the face, silty	13	fall	both silting and coloration consistent with research
96-008	Fraser	decomposed, clothed body of a male was found floating in the middle arm of the river, the body was lodged against a log breakwater	silt and densely encased mud adherent to clothing and body surfaces, hair shedding from the scalp, hands showed extreme maceration with washerwoman effects with degloving of the left fingers and fingernails, right hand showed similar change but loosely adherent palmar skin was still present over the thumb and middle fingers, fine irregular defects in the skin of the dorsum of the hands, forearms, and particularly the left	-30	summer	silt washer woman traits, skin slippage, discoloration, and postmortem animal action were consistent with research, although hair shedding occurred earlier on humans than pig models

Coroners Case# Region	Circumstances	Corpse Description Upon Recovery	P.M.I. (days)	Season	Consistency
		anterior shoulder - consistent with postmortem animal action, skin surface generally are mottled greenish-brown with blotchy areas of surface black pigmentation, the abdomen and scrotum are distended with gas			
96-009 Fraser	body was located in river caught in fast moving current partially clothed - pants, belt, long johns, bare feet	condition of body was consistent with being submerged for a prolonged period, mixed air, fluid and silt in stomach and lungs, postmortem animal action, skin surfaces show red decomposition torso is thick set through the chest gaseous extension of the abdomen loss of scalp hair, the ears are lost from animal action, nose and soft tissues of mouth are still present hands show degloving of the palmar surfaces, animal action is also evident behind the right shoulder with a deep cavity extending into the axilla, left arm still intact, legs show fine abrasion of skin overlying the knee caps, erosions of soft tissues with lost nails involving the great toes	37	spring	silt, postmortem animal action, and nail detachment were consistent, however hair shedding and skin slippage were not
96-010 Kamloops	body discovered snagged against a marker buoy in the middle of the river	moderately advanced decomposition moderate odor of putrification	38	spring	too vague to comment odor consistent with research

Case#	Coroners Region	Circumstances	Corpse Description Upon Recovery	P.M.I. (days)	Season	Consistency
96-011	Kamloops	body found floating face down approximately 3 km west of airport; river was cold, high and fast flowing	no rigidity of the upper and lower limbs, skin colour was green with marked sloughing of the skin, and bloating of the soft tissues, the skin had a generalized discoloration, malodorous fluids leaking from most orifices, tongue appeared protuberant, generalized marbling of the skin	52	summer	all descriptions were consistent with research
96-012	Kelowna	deceased fell through ice on the the river, body was found many kilometers downstream	generalized maceration of the skin and the scalp has lost most of the hair, extremities and trunk showed no other abnormalities except marked green discoloration of the anterior aspect of the abdomen and lower aspects of the chest well established decomposition with associated putrefactive odor	63	spring	hair shedding, loss of skin, and noticeable odor all consistent with research
96-013	Kelowna	deceased slipped and fell into river remains found 4 km from point last seen	mandible and skull	-90	summer/ fall	not consistent with research
96-014	Fraser	body was located in the river under bridge, approx 5 ft under the surface of the water, held in place by some logs jammed under the bridge, fully clothed	advanced decompositional change, foul odor, skin discoloration, adipocere formation, most prominent on thorax and left limbs loss of soft tissue on the right palm to tendons of anterior wrist, and base of thenar eminence to tendons, over left knuckles to bone, livor: cannot assess, rigor: passed	91	spring	foul odor, skin discoloration, adipocere, and postmortem animal scavenging all consistent with research
96-015	Fraser	body was discovered in a pool of water under a pile of logs, branches and debris	advanced decomposition, fully clothed the upper part of body, above the level of the pelvis, was almost completely skeletonized with liquefying adipocere and heavy infestation with maggots	-180	winter	adipocere consistent with research, however skeletonization and appearance of maggots not consistent with research

4. DISCUSSION

4.1 Physical Characteristics

The nearly equal water and internal carcass temperatures (Figure 2) are in contrast to the very high temperatures in carcasses on land, caused by the presence of maggot masses (Goff 1993). No such masses ever formed in this study. Because water temperatures were predictive of internal carcass temperatures (Table 1), they may be used in estimating the rate of development of terrestrial dipteran larval on the carcass and thus could aid in determination of PMIs. However, forensic investigators must consider the amount of moisture in the environment. If high moisture levels and low temperatures occur, larval development may be retarded (i.e. larvae may remain as third instars for months), which will affect estimation of time of submergence or death. Also there is an extremely high mortality rate of pre-pupal larvae; therefore pupal cases are usually not recovered from clothing or carcasses in water. In human death investigations larvae taken from corpses in water have suffered >95 % mortality, when reared in the laboratory (Anderson, pers. comm.). Unlike terrestrial cases where evidence can be found around a carcass months after death, dead larvae or pupal cases are apparently rapidly washed away by rain or fast flowing water. The absence of diurnal changes in water or internal temperatures is also in contrast with the terrestrial situation (Anderson and VanLaerhoven 1996).

An increase in carbon dioxide as a byproduct of decomposition was expected near the carcasses; however, no such increase was observed (Figure 3). Algae and bacteria became abundant, giving the carcasses a fuzzy appearance. It is likely that increased populations of photosynthetic algae prevented the carbon dioxide from accumulating to expected levels (Golterman 1975). The pH was determined to be 5-5.5 in both habitats. The acidity was enough to facilitate the observed saponification of the carcass (Mant and Furbank 1957).

4.2 Exposure of Carcass

Differences in exposure of carcasses in the stream habitat may have been attributed in part to the depth of water. Some of the carcasses in the stream habitat were not entirely submerged due to the shallow water in September 1996. Size of pig may also have influenced exposure. Pig size varied from 6.8-32 kg, and size has a direct relationship with CO_2 production which causes refloat (Knight 1997). My observations of variable exposure of carcasses were similar to those of O'Brien (1994) on three carcasses, two which floated when placed in water, and one which sank and remained submerged for 3 months. The removal of tissues by scavengers increased decomposition rate, causing scavenged carcasses to submerge faster than unscavenged carcasses.

4.3 Decomposition

Traditionally, the rate of decomposition in both above-ground and buried carrion has been determined by measuring loss of weight over time (Hewadikaram and Goff 1991; Anderson and VanLaerhoven 1996; VanLaerhoven 1997). Loss of weight has been attributed to release of body fluids, maggot migration, and decomposition (Hewadikaram and Goff 1991). However, in an aquatic environment, weight loss measurements would be confounded by water taken up by a carcass. This phenomenon was also observed in terrestrial carcasses due to clothing taking up water (Dillon 1997). Therefore, observational determination of decompositional stages can be very important and can serve as a guide in determining time of submergence or death.

Decomposition was delayed in both aquatic habitats compared with that in terrestrial habitats in the same season and geographic location (Dillon 1997). These delays may have been due to cool water temperatures and the absence of maggot masses. Durations were 11 to 13 days in fresh, 23 to 37 days in bloat, 0 to 324 days in decay, 0 to 228 days in post decay and 0 to 331 days in sunken remains stages, compared with approximately 7 days in the fresh stage, 18 days in bloat, 11 days in decay, and 116 in post decay (Dillon 1997) in the terrestrial habitat.

Scavenging by mink was observed on all carcasses in the stream habitat, and was very severe on one carcass. Decomposition of carrion during the fall in a shaded terrestrial environment was also primarily propelled by scavenging

(Dillon 1997). Scavenging apparently increased the rate of decomposition and limited the diversity and number of invertebrates (until the sunken remains stage), as in terrestrial environments (Dillon 1997). Because of the accelerated decomposition in scavenged carcasses and the complete bypassing of the decay stage in one carcass, and both the decay and post-decay stages in another (Figure 5), great care should be exercised in interpreting decomposition in forensic investigations that involve scavenged carcasses in aquatic habitats.

The presence or absence of clothing may also influence the interpretation of the role of associated invertebrates on submerged or exposed carrion. For example, on the submerged portions of carcasses, clothing prevented feeding by invertebrates such as crayfish and caddisfly larvae, while on exposed portions of carrion it provided shelter for insects such as dipteran larvae. However in the stream habitat, stonefly larvae and chironomids fed underneath the clothing which provided shelter.

4.4 **Invertebrate Succession**

My observations (Tables 4, 5) suggest that there is a predictable succession of invertebrates that colonize carrion in aquatic habitats. However discretion must be used when evaluating succession for the use of determining time of submergence or death. Differences in species found in the two habitats were due to environmental conditions and the preferences of the organism. For example, the habitat itself may influence the species present, such as in poorly oxygenated pond water or in oxygen-rich stream water. The opportunity to

exploit the presence of carrion may be a secondary determinant of the species that are present.

Calliphora *vomitoria, Enicita* sp., and *Phormia regina* were found on shaded, unclothed, exposed portions of carcasses in the bloat stage. Later colonization by *Catops basilaris,* and *Nicrophorus* sp. was similar to that in a terrestrial habitat (Dillon 1997): however, the species of Staphylinidae differ on land.

Insect species were different and less diverse than those observed by Payne and King (1972), who also found maggots migrating off the partially-submerged carcasses. They also found more insect species on the carcasses in water than on land and predicted an even richer fauna including truly aquatic insects if experiments were done in natural conditions rather than in a tank. In contrast, I found numbers of insect species and families were significantly lower in the aquatic habitats (Table 3) than in terrestrial habitats in the same forests (Dillon 1997).

Haskell *et a*/. (1989) predicted that the occurrence of chironomid midges and caddisflies could be used to determine the duration of submergence. These species were determined to be carrion associated (Table 6) and should be used to aid in determining time of submergence or death. However, in the pond habitat (Table 4), I found the midge, *Polypedilum* sp. to be present throughout the year except for November, January and February, and another midge, *Heterotrissocladius* sp. to be present on carrion in September, October, and February to July. The caddisfly Chyranda centralis was present on carrion in

September and October, and from February to July. Therefore, these observations alone would be grossly insufficient in accurately indicating the duration of submergence. More accurate determinations might be made in the future if the occurrence and abundance of species could be related to the decompositional stages of a carcass.

Colonization by aquatic invertebrates appeared to be influenced by season (Tables 4, 5) especially for mayfly and stonefly nymphs, and dytiscids. Experiments initiated in the spring would indicate whether these trends were due to season or decompositional stage of the carcass.

4.5 Comparison with Water Death Investigations

The striking absence of detailed descriptions of decomposition in water death investigations limited the comparison of forensic and research observations to just 23 % of the 65 possible freshwater cases (Table 8). Of that small percentage, only one case report mentioned the existence of aquatic organisms on the bodies. Four case reports mentioned postmortem animal activity, presumably by mammals. The highest consistency between forensic and research observations was seen in case descriptions with long PMIs. However, the criteria for comparison were very general for long PMIs, and therefore more cases seemed to be consistent with the research. If detailed descriptions were made in future forensic investigations, a checklist might be developed of characteristics that could define the decompositional stage, and hence aid in determining time of submergence.

5. RECOMMENDATIONS

Although my data are not definitive in themselves, a number of recommendations can be made on the basis of my research.

1. If invertebrate succession is ever to be used in determining the time of submergence, a database of the invertebrates for a given habitat and region, must be developed.

2. An understanding of the ecology of the species found on the carrion is needed in order to clarify their relationship with time of submergence.

3. Because of the long duration when certain species are present, decompositional stages should also be used in determining time of submergence.

4. More research should be done on decompositional stages of carrion in water.

5. Pig carcasses (or models) should all be approximately the same size when conducting research. This ensures that differences observed would be due to the factors being tested and not variation in carcass size.

6. To determine if colonization of a particular species on carrion was due to season or decompositional stage, future research should be done in all seasons.

7.   More research needs to be done on calliphorids in moist environments, which retard larval development and cause high mortality during pupal emergence, skewing any estimations of time of death or submergence.

8.   More awareness and education are needed of the potential for using invertebrate succession in conjunction with decompositional stages as an aid in determining time of submergence. Proper collection of invertebrates from crime scenes and autopsies will be vital in developing this potential.

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