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Aquatic Forensics
Determination of Time Since Submergence
Using Aquatic Invertebrates

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TECHNICAL REPORT
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EXECUTIVE   SUMMARY

Research examining the development, species, and sequence of invertebrates associated with submerged pig carrion commenced August 31, 1996 in Coastal Lower Mainland, B.C. The goal of this research is to create an invertebrate successional database for use in estimated time of submergence in aquatic human death investigations. Carrion insect databases exist for above ground and buried carcasses in the Coastal Lower Mainland, B.C. It is not known whether invertebrate succession is predictable in the aquatic environment or which invertebrates would be involved in the decompositional process. If present, this predictable sequence may be used to determine time since submergence and hence, time since death.

SOMMAIRE

Le 31 août 1996, dans le district continental sud, sur la côte de la Colombie-Britannique, on a entrepris des recherches sur le développement, les espèces et la sequence des invertebres dans la decomposition de carcasses de porcs immergées. Ces recherches permettront de créer une base de données sur l'ordre de succession des invertebres. Ces données serviront au cours des enquêtes sur les noyades à determiner le moment où s'est produite l'immersion des victimes. 11 existe des bases de données sur les insectes necrophages qui s'attaquent aux carcasses trouvées à l'air libre et enfouies dans le sol sur la côte de la Colombie-Britannique. On ignore si la succession des invertebres est prévisible dans l'eau et quels sont les invertebres qui interviennent dans le processus de decomposition. S'il existe une sequence previsible, elle pourrait servir à determiner à quel moment remonte l'immersion et, par consequent, le décès.

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ABSTRACT

Research commenced fall 1996 to determine time since submergence of carrion using aquatic invertebrate succession. This research indicated that aquatic invertebrates colonize carrion sequentially. Once the analysis has been completed, the data will be used to assist in determining time of submergence and hence time since death. The sequence of aquatic invertebrate colonization varies slightly with habitat. Data collected from this research will form the basis for a database for victims killed in aquatic habitats. This work, if already completed, could have assisted in the investigation of eight deaths in 1996 in the Vancouver area alone.

ABSTRACT (non-technical)

Forensic entomology is the study of insects associated with carrion in order to determine elapsed time since death in homicide investigations. Organisms (invertebrates) colonize the carrion in a predictable sequence in terrestrial and buried scenarios. This sequence is different if the body is submerged in a lake or stream habitat. This sequence of organisms colonizing the carrion, or corpse, can be used to determine time since submergence, which may aid in determining time since death. This research will be valuable in criminal investigations currently pending, as well as in the future.

INTRODUCTION

Forensic entomology is the study of insects and other arthropods related to criminal investigations (Goff, 1993). Insects are often the first witnesses to death, arriving in a predictable sequence. This sequence is governed by a wide range of rapid and complex chemical , biological and physical changes as carrion decomposes from a fresh state to a skeleton. Each stage of decomposition is attractive to different species of arthropods. When the sequence of colonizing insects is known; an analysis of the arthropod fauna on carrion can be used to determine the elapsed time since death in human death investigations. Time of death is very important in focusing an investigation into the correct time frame, determining timeline prior to death, and may be of value identifying the victim (Anderson and VanLaerhoven 1996).

Forensic entomology is accepted in courts worldwide and has been used in homicide investigations in North America for over 15 years (Anderson and VanLaerhoven 1996; Catts and Goff 1992; Greenberg 1985). Although within the first few hours after death, a pathologist can provide a reasonable estimate of the postmortem interval based on medical and scene parameters, these methods become less precise over time (Coe and Curran 1980; Van den Oever 1976). In fact, after 72 hours, entomological evidence usually is the most accurate, and frequently the only method of determining time of death (Kashyap and Pillai 1989). Consequently, there is frequently no scientific, defensible method of determining elapsed time since death in these cases, except for the correct understanding and analysis of the arthropod evidence.

However, no work has been done to determine time of death using fauna1 succession on bodies disposed of in natural water sources. Lord and Burger (1983) stated that one of the most important factors that will alter the normal course of insect succession in carrion is immersion in fresh water. Many homicide victims are disposed of in bodies of water, which makes determining time of death extremely difficult. The only carrion ecology studies conducted in water have concentrated on terrestrial insects alone, which colonized the exposed regions of the corpse (Payne and King 1972). Experience with aquatic homicide cases have shown that reliance on terrestrial insects is extremely limiting, because time of exposure of cadavers varies with water depth and amount of exposure as the body will rise and sink as it

decomposes (Anderson pers. comm.). If the body is colonized while floating, then sinks as gases are lost, any terrestrial insects will drown. The body may be recolonized as the body surfaces, but will not give an accurate time of death. There are a few examples of case studies using aquatic insects (Siver et al. 1994, Hawley et al. 1989) but these are rare. No experimental work has examined aquatic succession on carrion, despite the enormous potential.

Aquatic ecology studies have shown that colonization on a substrate depends on many factors, such as size of the object, texture, position, flow of water, water temperature, current speed, water depth, presence of aquatic flora and fauna *etc.* (Peckarshy 1986; Sheldon 1983). There are several factors that may be used in determining the duration of submergence including succession of arthropod fauna on the remains, presence of specific life stages of aquatic insects which may be seasonal in specific aquatic habitats, the presence of specific structures built by aquatic insects, and the presence of indicator species, specific to certain microhabitats. Once an organism has located a substrate, substrate characteristics will determine whether that organism stays. The substrate may act as an anchoring site, a food source, or protection *etc.* (Haskell *et* al. 1989). Colonization of a substrate in water is predictable and has been documented over time on various inert substances (Tevesz 1985, Sheldon 1983), and has been used in a forensic case (Moran 1983). Research is needed to determine if a progression on corpses (or carrion) exists and whether it can be used to determine time of submergence,

OBJECTIVES

The main objective of this research was to study aquatic invertebrate development and succession on carrion, in the hope of developing a methodology to determine time of death/submergence for homicide victims found in aquatic environments. In order to achieve this objective, the invertebrate succession and rate of decomposition on submerged pig *(Sus scrofa* L.) carcasses was examined in both lake and stream habitats in the Coastal Lower Mainland, B.C. beginning August 31, 1996. As well, changes in water chemistry due to carcass decomposition, seasonal differences, and clothed carcasses *versus* unclothed carcasses will be examined. This research provided a unique opportunity for interaction and cooperation

between police officers, the B.C. coroners service and university researchers in order to develop the technology and knowledge to determine time of submergence.

RESEARCH MODELS

Pig carcasses were used in experiments because they have been widely accepted as human models for decompositional studies (Goff 1993). Pigs are similar to humans since they are omnivorous and possess a similar digestive system including their gut fauna which is important in the evaluation of the decomposition process of carcasses. 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). Mucus is secreted by glands of the large intestine which prepares chyme for elimination by action of bacteria. These bacteria ferment any remaining carbohydrates and release H_2, CH_4, and CO_2 gas. Differences exist between the types of bacteria found in the intestinal tract of the pig and humans, but the end result is the production of the same gases in the gastrointestinal tract which cause refloat (Tortora and Anagnostakos 1984).

Pigs are relatively hairless and have skin tissue similar to that of humans, in fact it has been used in human skin grafts. Catts and Goff (1992) claim that a 23 kg (50 lb.) pig is approximately equivalent to an average adult male torso, which is the main site of decomposition and insect colonization. However, smaller pigs (20 lb.) were used to prevent the bloated pigs from getting caught between the bars of the cages and preventing the carcass from decomposing naturally.

RESEARCH LOCATION

The research was conducted at the University of British Columbia's Malcolm Knapp Research Forest in Maple Ridge, B.C (Figure 1). The forest, 5153 hectares in size, was chosen because it is characteristic of the Western Coastal Hemlock biogeoclimatic zone, which was the zone including the Coastal and Lower Mainland British Columbia, the majority of Vancouver Island, the Gulf Islands and extends up the Pacific Coast to the Alaska border
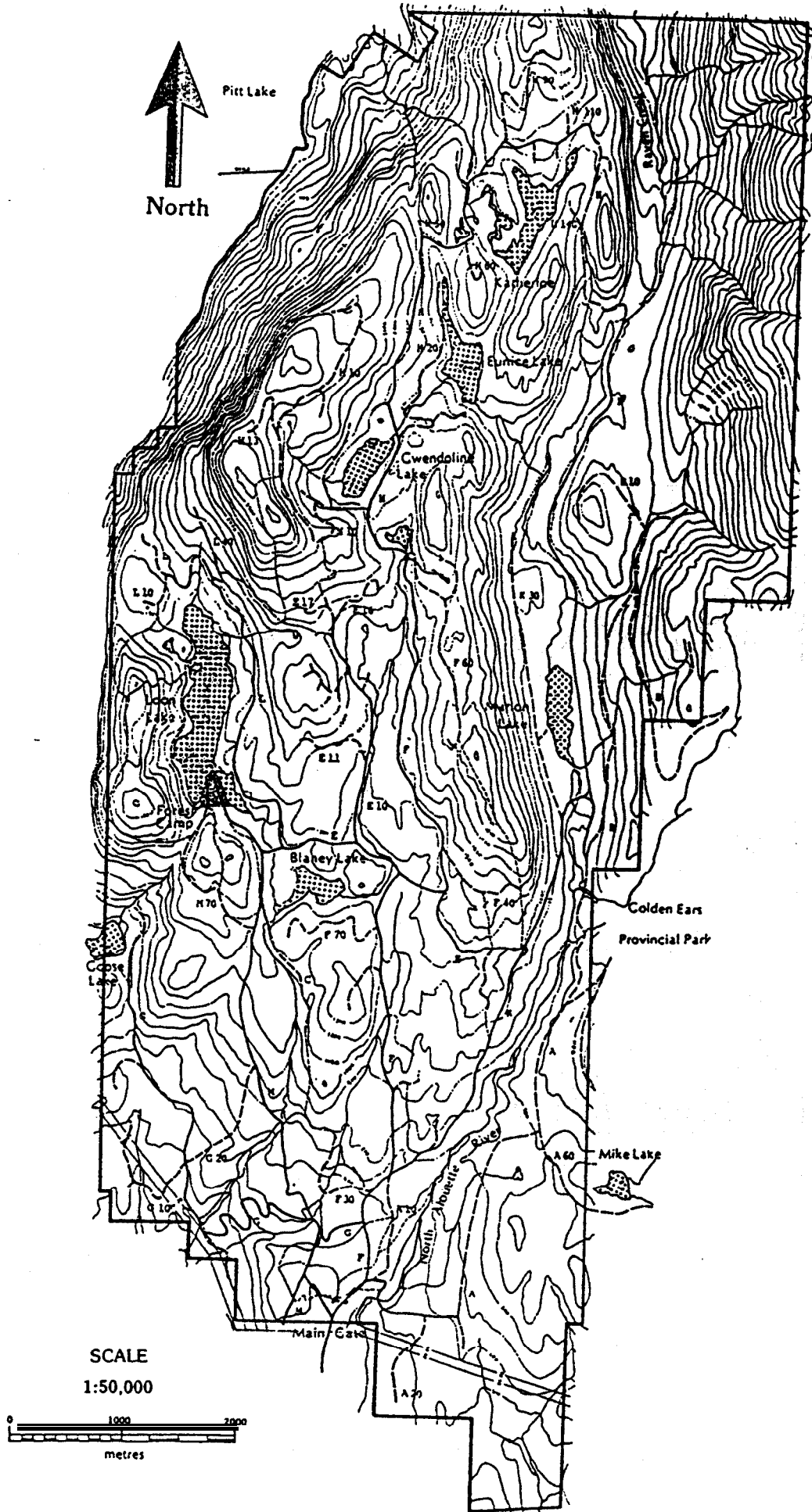
Figure 1. U. B.C. Malcolm Knapp Research Forest

As we studied faunal succession in relation to the decomposing carcasses, it was important to determine the natural invertebrate fauna of the area. Therefore, control water analysis and invertebrate collecting was performed one week prior to carcass placement and each week during sampling.

EXPERIMENTAL   DESIGN

Fresh sacrificed pigs (Canadian Council on Animal Care Approval Certificate) were immediately transported to research sites. Once located at the field site, carcasses were weighed and then half clothed (Figure 2), as clothing has been found to have an effect on terrestrial insect succession (Dillon and Anderson 1996), and may, therefore, have an effect on aquatic succession. Pig carcasses were placed in the middle of the cages so that no parts of the carcass were close to the edge of the cage (Figure 3). These cages protected the carcasses from large predators but it did not impede access to small fish and arthropods, or affect the natural rise and fall of the carcass during decomposition.

The carcasses were examined for arthropod succession initially twice a week following death and then every l-2 weeks. Photographs were taken with a Nikon® F-401X before sampling occurred. High speed film, International Standards Organization (ISO) 400, was used to ensure proper exposure in the dim light, and allowed a shutter speed of 1/500 sec. which was fast enough to prevent camera motion from blurring the overall picture. Invertebrate species present on the carcass were collected directly with forceps and placed in ethanol.

Temperature was monitored using a temperature-recording datalogger (SmartReader® 1, Young Env. Systems, B.C.), which recorded ambient, internal, water temperatures. Two loggers with multiple channels were used in carcasses in each habitat. The loggers were attached to the top of cage to record ambient temperatures, and a temperature probe were inserted into the carcasses (Figure 4 and 5).

In order to determine the natural biota of each aquatic environment, 250 mL water samples were taken prior to the experiment and at each sampling time. Natural decomposition changed the water chemistry which may have affect composition of organisms collected; therefore, carbon dioxide and pH were recorded near the carcass and for a control. In the lake

Figure 2. Volunteer dressing pig carcass.

Figure 3. Final adjustments to cage set-up,

Figure 4. Pig carcass in the lake habitat, just after death.

Figure 5. Pig carass in th stream habitat, just after death.

habitat, the control was located a minimum of 5 m from the cage but was always across the water inflow/outflow pathway to prevent cross contamination. In the stream habitat, the control was located 4 m upstream from the caged carcass.

In the lake habitat, a 250 mL sample of sediment was taken from within the cage and at the control site. In the stream habitat, sediment could not be removed during sampling; therefore, faunal collection was sampled by using a surber instead of an aquatic net, as used in lake environment. The surber was randomly placed on the stream bed, larger rocks within the frame were turned over and the flow of the stream washed the organisms into the net. Once sediment and surber collections were brought to the lab, they were sorted into a dissecting tray and examined with a 10 X hand lens for macro invertebrates. The invertebrates were transferred with forceps to a labelled vial with 95 % ethanol, for later identification.

On each visit, an aquatic net lined with muslin, was used to sweep the area within the cage as well as at the control site for each carcass location. The muslin was placed in a ziplock bag and transferred to the laboratory. The muslin was rinsed with distilled water into a dissecting tray and examined with a 10 X hand lens (for macro invertebrates). The invertebrates were transferred with forceps to a labelled vial with 95 % ethanol, for later identification. A thorough investigation of each carcass was done each sample date. Representatives of each invertebrate species found were photographed, recorded, and placed into vials of 95 % ethanol, for later identification. All invertebrates will be identified using a Meiji® EMZ5 Dissecting Microscope and the appropriate keys. All samples will be checked and unknowns identified by Linde Looy, R.P.Bio., Aquatic Biologist of Fraser Environmental Services, Surrey, British Columbia.

RESULTS

Temperature

Graph 1 depict maximum and minimum ambient, water, and internal carcass temperatures recorded for the first 18 days since death for the stream habitat and lake habitat. Unlike ambient temperatures, no diurnal temperature changes in water or internal carcass temperatures occurred in either habitat. Water temperatures strongly correlated to internal

Graph 1. Maximum and minimum mean ambient and internal carcass temperatures for both stream and lake sites, located in the Coastal Western Hemlock biogeoclimatic zone of British Columbia.

Stream 1, Fall 1996

Lake 1, Fall 1996

Days Since Death
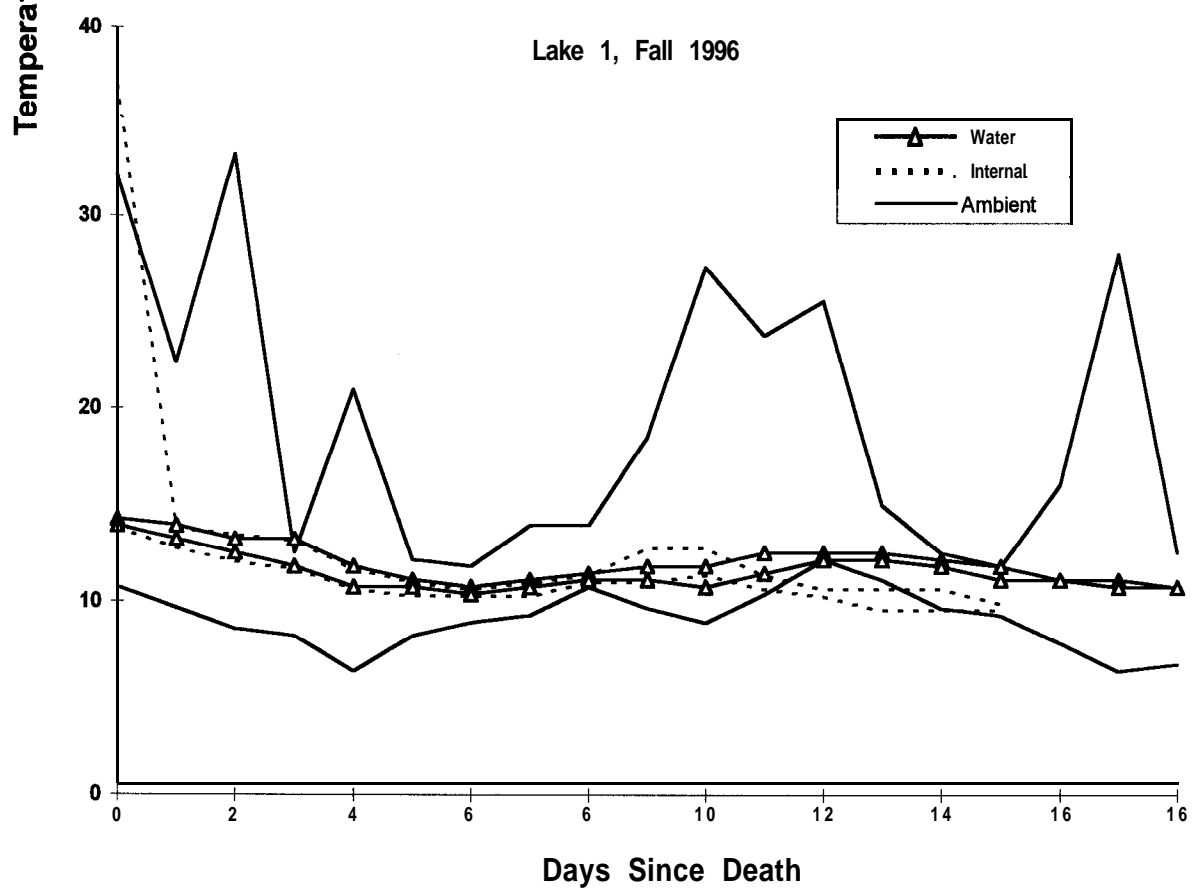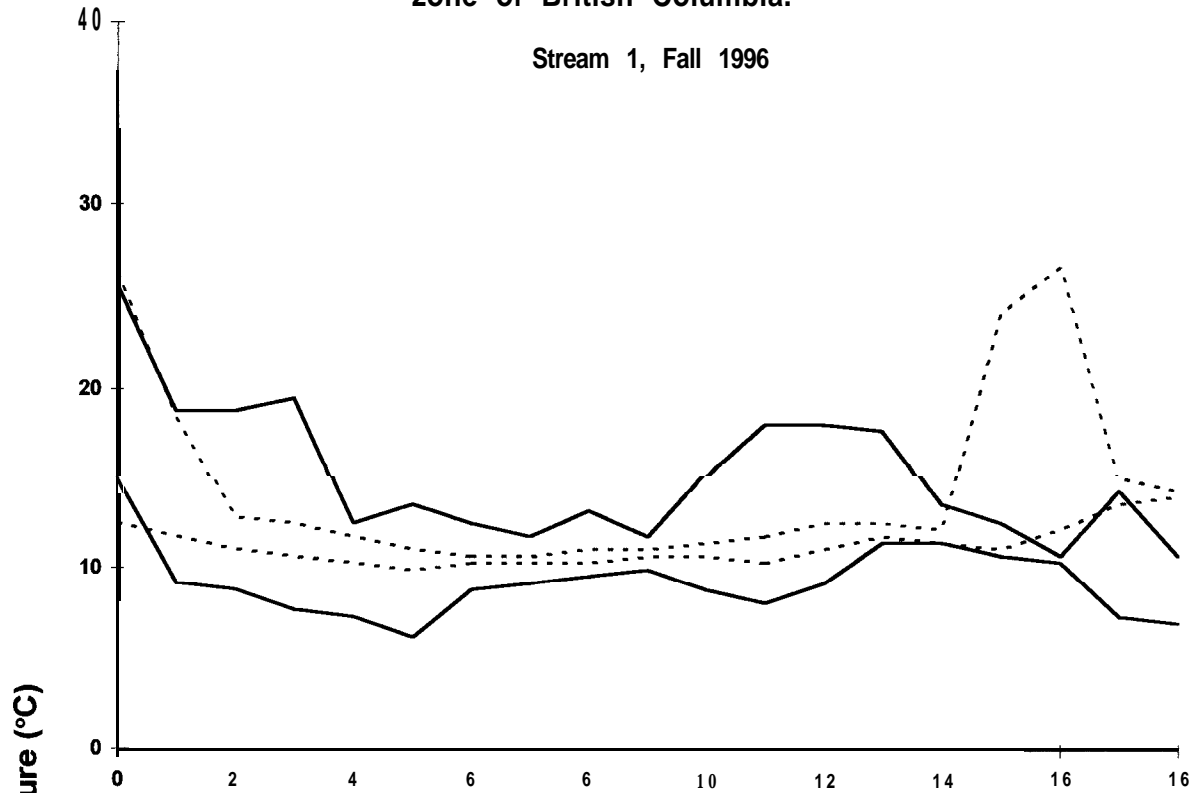
carcass temperatures, during the 18 days following death. Both aquatic habitats averaged approximately 12°C (water temperatures).

Water Chemistry

In the lake habitat, peaks in the carbon dioxide levels occurred twice for both within cage sample and control (Graph 2). However, carbon dioxide levels within cages seemed delayed and lagged behind control carbon dioxide levels approximately 7 days. The peaks may be due to an accumulation of detritus within the cages and from the control sites. Increased detritus was observed with increases in water levels. A comparison of water levels and carbon dioxide levels is currently in progress.

In the stream habitat, the control displayed similar peaks in the carbon dioxide levels as seen in the lake habitat. However, within the cage, carbon dioxide levels appeared to accumulate and not fluctuate with the increasing amounts of detritus.

All pH measurements obtained during sampling for both habitats are illustrated in Graph 3. Ph levels for both habitats were consistent at 5-5.5. Minor fluctuations were observed in stream and lake sites. A comparison of pH readings and carbon dioxide levels over time is currently being conducted.

Control and Experimental Carcasses

No differences in decomposition or invertebrate activity was observed between control (undisturbed) and experimental (disturbed) carcasses found within similar stream or lake habitats. Rates of decomposition were similar; carcasses entered each stage similarily, and inflation and deflation of carcasses with respect to the water surface also occurred within the same time frame. Times of arrival and diversity of invertebrate fauna were also comparable. Based on these preliminary results, observational data from experimental and control carcasses were combined.

Decomposition

For summary of visual observations refer to Table 1 and Figures 6-13. In general, carcasses were completely submerged following placement into the aquatic environments. Aquatic invertebrates such as Caddisflies (Trichoptera) immediately colonized submerged facial regions of carcasses and remained for approximately two weeks. Resurfacing of carcasses in

Graph 2. Water carbon dioxide levels over time in both stream and lake habitats, Coastal Western Hemlock biogeoclimatic zone, British Columbia.

Lake, Fall 1996

Stream, Fall 1996

· · · · · Within Cage--Mean

- Control--Mean

Carbon Dioxide Levels
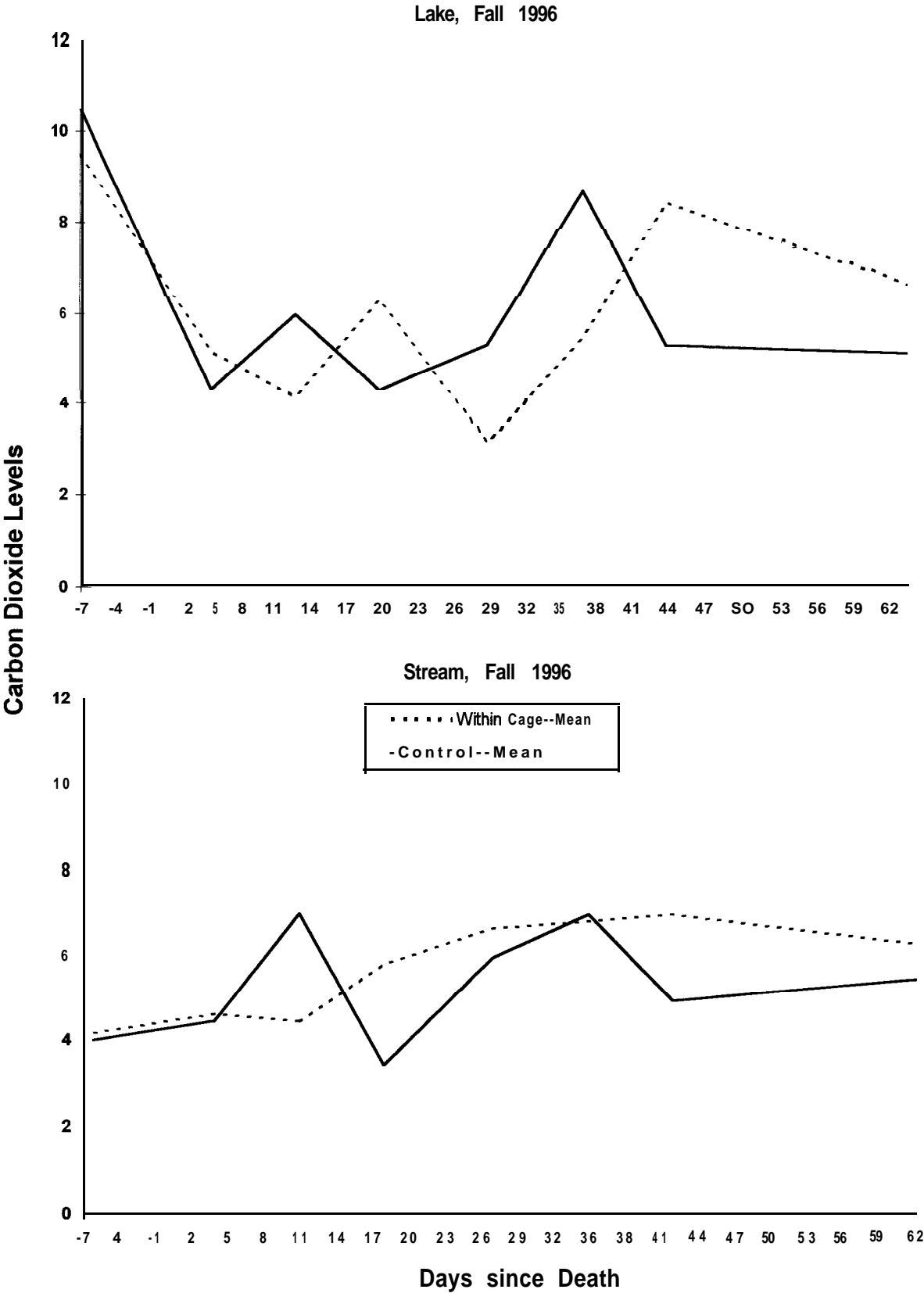
Days since Death

Graph 3. PH levels in water over time in both stream and lake habitats, Coastal Western Hemlock biogeoclimatic zone, British Columbia.
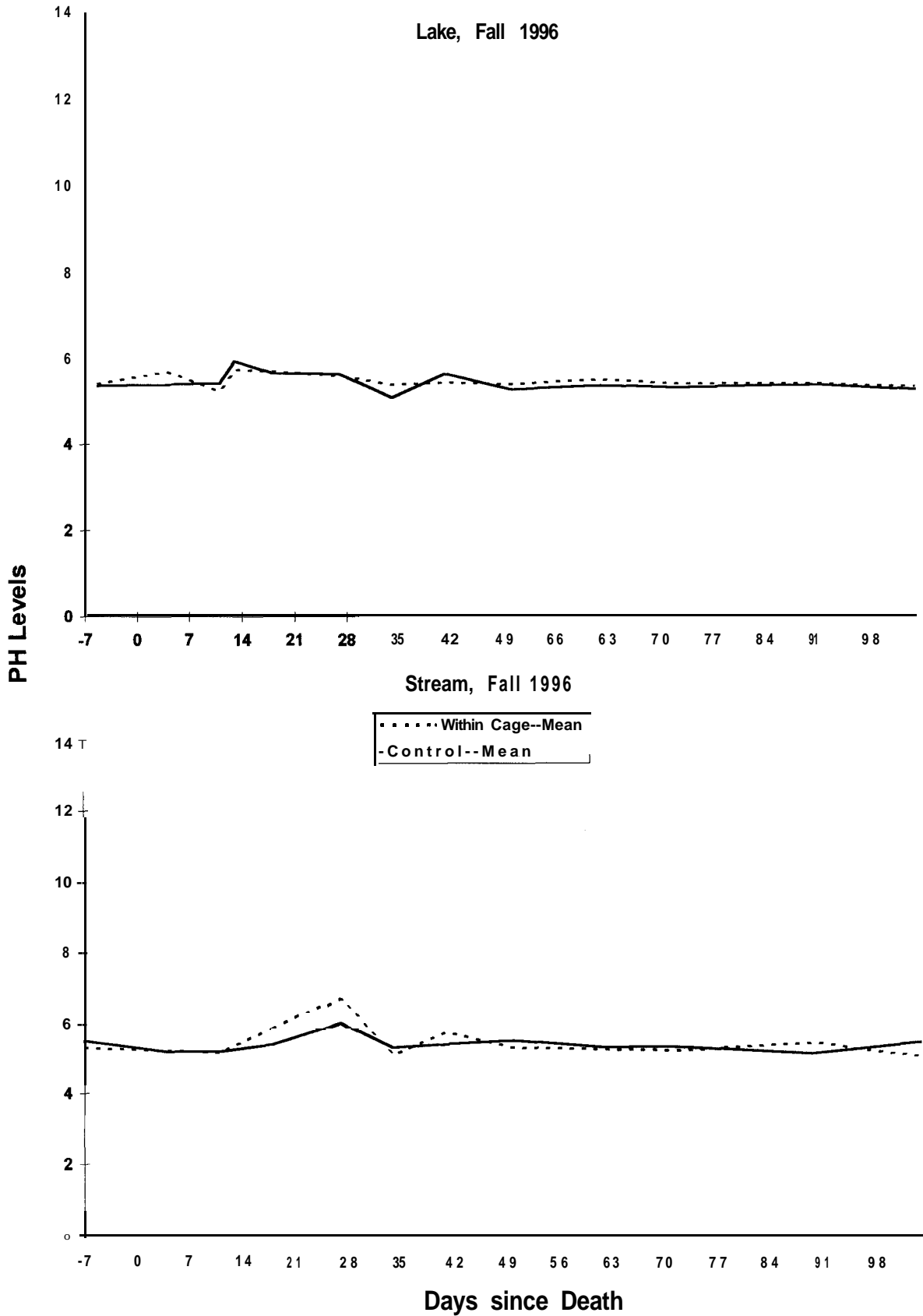

Lake, Fall 1996

Stream, Fall 1996

······Within Cage--Mean

-Control--Mean

PH Levels

Days since Death

Table 1. Preliminary results of observations

Days Since Death	Stream	Lake
0	Experiment started Rigs partially exposed	Experiment started Rigs 7/8 submerged
2-4	Eggs laid along edge of clothing	3/4 to 7/8 submerged Trichoptera (caddisflies) < 50
11-13	Exposed skin paler in colour Submerged skin brownish	Eggs of *Calliphoridae vomitoria* present Blood worms, Trichoptera, Silphidae present
18	Bloat Clothing starting to restrict bloat Lard-like substance on head No organism visible near natural orifices Staphylinidae present Mink scavenging on pig #4	Bloat Clothing starting to restrict bloat Very few Trichoptera present
27	No flies near scavenged areas Flies landing and laying eggs on exposed skin Staphylinidae and other Coleoptera present	Staphylinidae present under clothing Silphidae (∼5) Calliphoridae and Muscidae present Maximum bloat
34	Exposed skin hardened Really floating - Maximum Bloat Scavenged carcass completely submerged	
41	Hair falling out (sign of decay in terrestrial habitat) Dipteran larvae present under clothing, leaves, bars Decreasing bloat	Hair and skin falling off Decreasing bloat
50	Very few larvae present No evidence of pupae Hair falling out Substantial flaking of the skin Hoof fell off	White powdery substance deposited on skin Lard-like substance on abdomen starting to harden Waxy substance soaked through clothing and starting to harden
82	Exposed skin hardened and hairless	
91		Most exposed skin hairless No living Dipteran larvae
109	Other scavenged carcass completely submerged Orangy colour to hardened, exposed skin	1/4 exposed
124	No Dipteran larvae found Exposed skin still orangy colour	Coleoptera larvae in the submerged "mucus-like" substance coating the the carcass 1/4 to 1/3 floating Orangy colour to the exposed, hardened **skin**
193	Exposed skin still orangy Stomach still somewhat bloated New scavenging	1/8 exposed Blubbery appearance to submerged non-clothed areas Stoneflies present within blubbery All tissue on feet removed, no bone visible Head skeletonized (head was submerged)

Figure 6.   Calliphoridae eggs laid along the edge of clothing.

Figure 7. Caddis fliess appeared on non-clothed areas of the submerged carcasses, 2 days after death.

Figure 8.   Lard-like substance (saponification) appeared on the head.

Figure 9. Mink scavenging on all pig carcasses in the stream environment.

Figure 10.   Hair falling out (sign of decay).

Figure 11. Dipteran larvae sheltered by clothing.

Figure 12. Substantial flaking of the skin, occurred in both stream and lake habitats.

Figure 13. Accumulation of algae and silt on the carcass.

both habitats occurred during the bloat stage, despite restricted bloat due to tight clothing. During this stage, exposed portions of carrion were predominately colonized by blow flies (Calliphoridae), and carrion beetles (Silphidae); whereas, submerged portions of carrion remained uncolonized. As this work is ongoing, complete identification of invertebrates is planned for summer of 1997. Clothing was found to alter invertebrate feeding activity on the carcasses. On exposed carcasses, invertebrates tend to feed under the clothing where they are protected. The opposite is true for submerged carcasses: invertebrates tend to colonize and feed on the non-clothed portions. Once carcasses began to deflate, marking the end of the bloat stage, exposed regions of carrion decreased. High blow fly larval mortality was observed. Adipocere formation began.

Considerable scavenging was observed on carcasses located in the stream habitat. Scavenging was concentrated on the lower and unclothed portions of the carcass. A mink was visually observed to be the cause of scavenging activity. Scavenging accelerated decomposition. Invertebrate activity was reduced on scavenged carcasses.

DISCUSSION

Temperature

Once carcasses cooled following death, there was little variation between water and internal carcass temperatures (Graph 1). There was no increase in internal temperatures observed. Goff (1993) states the increases in internal temperatures are partly due to the presence of maggot masses. However, in the aquatic environment, no maggot masses ever formed. Thus the water temperatures may reflect internal carcass temperatures and may be used in larval development. In addition, the absence of diurnal temperature changes in water or internal temperatures are very different from the terrestrial situation (Anderson and VanLaerhoven 1996) and may more accurately represent the temperatures at which maggots exist. This will be further analyzed.

Decomposition

        Historically, the rate of decomposition in both terrestrial and burial carrion experiments has been determined by measuring the loss of carcass weight over time (VanLaerhoven and Anderson 1996, Hewadikaram and Goff 1991). Loss of carrion weight has been attributed to losses in body fluids, maggot migration and decomposition (Hewadikaram and Goff 1991). However, in an aquatic environment, loss of body fluids can not be measured in this manner. Despite these reservations, changes in decomposition were still observed.

        Decomposition was delayed in aquatic enviornment in comparison to terrestrial. Decomposition occurred at a decreased rate in comparison to decomposition in a terrestrial environment. Naturally, this was expected as internal carcass temperatures were considerably lower and no maggot masses formed. In a terrestrial environment, decomposition of carrion during the fall in a shade environment was primarily propelled by scavenging activity (Dillon, 1997). Cold temperatures restricted insect activity in both aquatic and terrestrial environments.

        Decomposition changes happened earlier than previously reported for aquatic environments. European and North American literature suggests that decomposition in aquatic environments are delayed substantially due to the cooler temperatures and decompositon ceases at 8 'C (Simpson and Knight 1985). This does not seem to be the case, however research is still ongoing and thus results are only preliminary.

        There were differences in invertebrate succession between the stream and lake habitat. Because habitats influence invertebrate fauna, species colonizing carrion are habitat specific.

Scavenging

        Scavenging by mink was observed on all carcasses in the stream habitat. It appears that scavenging increases decomposition and limits species composition which corresponds with other environments. Feeding in terrestrial environments includes scavenging from the outside and "arthropod" feeding from the inside; in aquatic environments scavenging is only from the outside. Scavenging activity was allowed to continue and not prevented, because it may accurately reflects conditions imposed on a cadaver found in an aquatic environment.

<u>Clothing</u>

Invertebrate feeding activity was influenced by the presence or absence of clothing and depended on subermerged or emerged carrion. On the submerged portions of carcasses, clothing prevented aquatic feeding from invertebrates such as crayfish. Unclothed portions of submerged carrion were subject to feeding activity. However, on emerged portions of carrion, feeding activity reflected a terrestrial environment. Clothing provided protective shelter for insects such as Dipteran larvae, increasing their survival.

ACKNOWLEDGEMENTS

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