THE RISK OF NON-TARGET SPECIES POISONING FROM BRODIFACOUM USED TO ERADICATE RATS FROM LANGARA ISLAND, BRITISH COLUMBIA, CANADA

by

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Abstract

In 1995, the Canadian Wildlife Service attempted to eradicate introduced Norway rats (*Rattus norvegicus*) from Langara Island and adjacent Cox and Lucy Islands with the application of the second generation anticoagulant rodenticide brodifacoum. However, anticoagulant rodenticides are non-species specific pesticides and pose a poisoning risk to non-target species. This thesis addresses the short term poisoning impacts to non-target species from brodifacoum used to eradicate the rats from Langara and Lucy Island.

In 1994, during testing of the baiting protocol on Lucy Island, the native dusky shrew (*Sorex monticolus elassodon*) population size fell from an estimated 25 unique shrews/ha before the baiting to four unique shrews/ha after the baiting. This prompted a monitoring program in three regions on Langara Island in 1995. While shrews were attracted to bait in stations, the decline in their population was non-significant. Shrews in breeding condition were at greater risk of poisoning likely due to their ability to range widely. Shrews entered and chewed on bait blocks in up to 80% of bait stations.

The risk of secondary poisoning to avian scavengers from poisoned toxic rat carcasses was investigated. In 1994, common ravens (*Corvus corax*) were identified as the most significant scavenger of rat carcasses. In 1995, two of 15 radio-collared Norway rats poisoned with brodifacoum died above ground and one was scavenged. Whole body brodifacoum residues from other rats found dead above ground ranged from 2.40-16.51 mg/kg. Between 1995 and 1996, 20 raven

remains were found or reported. In 1995, 13 raven livers tested positive for brodifacoum. Ravens were secondarily poisoned from scavenging rat carcasses and primarily from raiding bait stations. Brodifacoum was detected in Northwestern crows (*Corvus caurinus*) 9 months after the cessation of baiting on Lucy Island in 1994, but before the baiting on Langara Island. Brodifacoum residues were detected in the plasma of 15% of bald eagles (*Haliaeetus leucocephalus*) sampled (0.037-1.74 ppm).

The invertebrates as a source of brodifacoum to non-target species was investigated. Snails (*Vespericola* sp. and *Haplotrema* sp.) and banana slugs (*Ariolimax* sp.) were common and abundant invertebrates found feeding on bait in stations. The blue coloured bait could be seen through the translucent bodies and the molluscs tested positive for brodifacoum. Carrion insects readily consumed rat carcasses containing brodifacoum. Blowfly larva (*Calliphora* sp.) tested positive for brodifacoum residues. The invertebrates found feeding on the bait and carrion insects were a secondary and tertiary poisoning risk to non-target species such as the song sparrow (*Melospiza melodia*).

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Abbreviations

ANOVA	analysis of variance	ppm	parts per million
CWS	Canadian Wildlife Service	PT	prothrombin time
dbh	diameter at breast height	PWRC	Pacific Wildlife Research Centre
GIT	gastro-intestinal tract	s	standard deviation
ha	hectare	SAS	trademark, SAS Institute Inc.
HAG	height above ground	s.e.	standard error
ND	none detected	ug	micro gram
NWRC	National Wildlife Research Centre		

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...to my parents for the many years of love and encouragement.

Chapter 1. General Introduction

The introduction of rats (*Rattus* spp.) to oceanic islands can have significant consequences for local wildlife populations, particularly for burrow-nesting seabirds which can decline in abundance or eventually be extirpated (Moors and Atkinson 1984). Introduced rats have been implicated as a significant factor in the decrease or extirpation of the Langara Island, British Columbia, Canada (see section 1.2 and Figure 1.2) breeding population of seabirds that was once described as "immense" and "astronomical" (Drent and Guiguet 1961). Seabirds that previously utilised Langara as a breeding site included: tufted puffins (*Lunda cirrhata)*, Leach's storm petrels (*Oceanodroma leucorha)*, fork-tailed storm petrels (*O. furcata),* Cassin's auklets (*Ptychoramphus aleutica),* rhinoceros auklets (*Cerorhinca monocerata)*, and ancient murrelets (*Synthliboramphus antiquus)* (Campbell et al. 1990). But, since the 1950's, the breeding populations on the Island have declined significantly or have been extirpated. Cassin's and rhinoceros auklets and Leach's and fork-tailed storm petrels no longer breed on Langara, while only a small breeding population of tufted puffins exists on nearby Cox Island (Taylor and Kaiser 1993). In 1993, the nesting population of ancient murrelets were estimated to be less than 10% of historical numbers, and have declined by 40% since the late 1980's (Harfenist 1993; Bertram 1989).

The ship rat (*Rattus rattus)*, likely introduced during the fur trade in the early 1800's, was present on Langara Island, but has been displaced by the Norway rat (*Rattus norvegicus*), likely introduced in the 1940's. The introduction of the Norway

rat coincided with the decline in the seabird colony (Taylor 1993), and there is evidence for significant rat predation on murrelet eggs, chicks, and adults (Bertram and Nagorsen 1995, Harfenist 1993, Bertram 1989).

Similar problems have been reported elsewhere, but since 1981, New Zealand biologists have successfully eradicated introduced rats from small oceanic islands with the use of anticoagulant rodenticides. Recent rat eradication programs in New Zealand used the anticoagulant brodifacoum, dispensed from fixed, evenly spaced bait stations on offshore islands (Taylor and Thomas 1993; 1989).

In 1995, the Canadian Wildlife Service attempted the complete eradication of Norway rats from Langara Island and adjacent Cox and Lucy Islands with the application of the second generation anticoagulant, brodifacoum (IUPAC 3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin) (Figure 1.1), using a technique developed in New Zealand (Taylor and Kaiser 1993). This was the first time this method (Section 1.3) was employed in North America and it could have potential for other islands in the Queen Charlotte archipelago (Bertram and Nagorsen 1995). However, the use of brodifacoum to eradicate rats on offshore islands poses a risk of primary and secondary poisoning to non-target species. Primary poisoning results when the bait and anticoagulant are consumed directly by a non-target animal. Secondary poisoning occurs when a primarily poisoned animal is consumed by a predator or scavenger (Colvin et al. 1988).

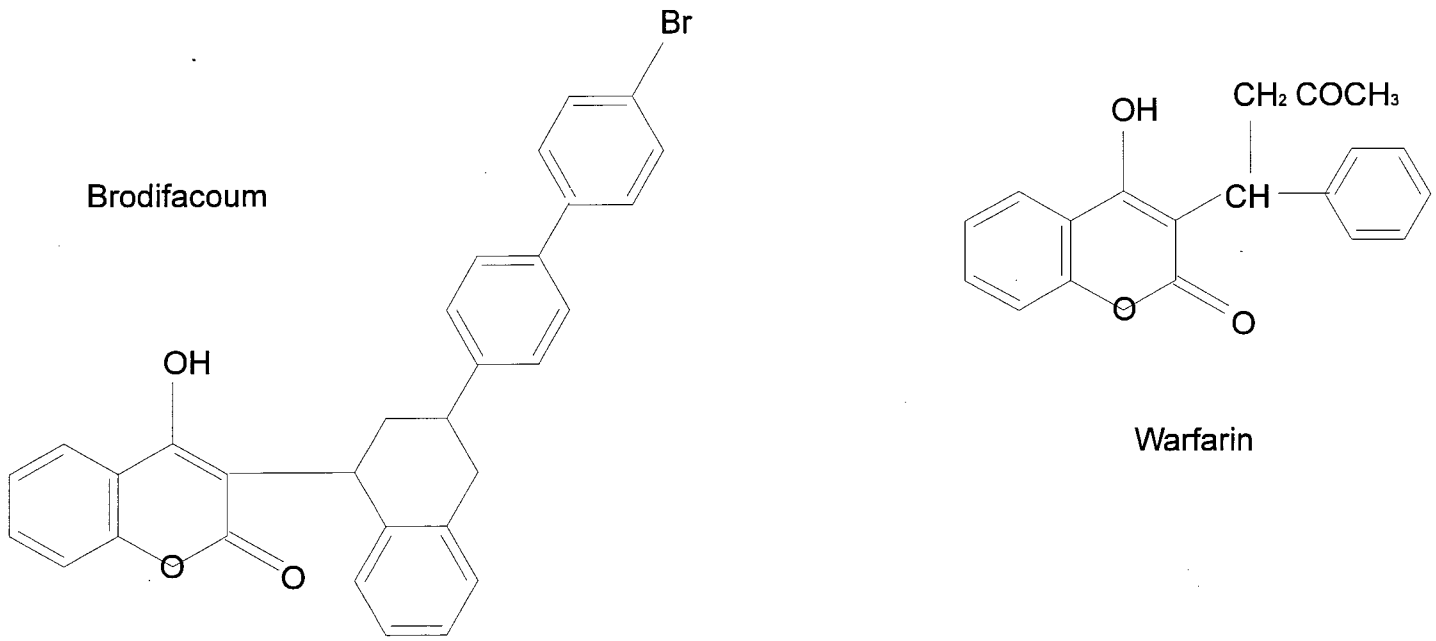


Figure 1.1. Chemical structure of brodifacoum. The more common and familiar anticoagulant warfarin is presented for comparison purposes. Note the 4-hydroxycoumarin ring system common in both brodifacoum and warfarin.

1. 1 Objectives of Research

The objective of the Canadian Wildlife Service was to examine the feasibility of eradicating introduced rodents from seabird colonies by balancing the long term benefits against the relative costs. The benefits are the removal of a significant, exotic predator species from islands which are important seabird breeding areas along the Pacific Coast. The costs, other than financial, include the effects on the native avian and mammalian species that live on and around these seabird colonies.

There were three main areas of poisoning concern for non-target species: 1) Langara Island has a native population of dusky shrews (*Sorex monticolus elassodon* Osgood). Deer mice (*Peromyscus maniculatus*) were also known to once inhabit the island. The New Zealand baiting model was designed to ensure eradication of the introduced rats and mice because there were no native small mammals to be concerned about. 2) Rats are known to die above ground after ingesting a lethal dose of an anticoagulant (Cox 1990) posing a possible secondary poisoning hazard for native predators. However, few researchers have investigated the actual proportion of rats dying above ground, and the importance of rats in the diet of the local predators and scavengers on Langara Island was unknown. 3) Invertebrates are important in eliminating excess bait and poisoned carcasses from the environment, however, they may introduce brodifacoum into the food chain, and pose a possible secondary or tertiary poisoning risk.

The overall goal of this thesis was to investigate the risk of non-target species poisoning with the use of brodifacoum to eradicate rats from Langara Island. There

were three main objectives of my study: 1) monitor the native small mammal population over the course of the baiting. 2) evaluate the risk of secondary poisoning to avian scavengers and predators from poisoned rats, and 3) assess the invertebrates as a source of introduction of brodifacoum into the ecosystem.

In Chapter 2, I summarise the mode of action of anticoagulants and the physiological basis for the non-target species poisoning concern. In Chapter 3, I examine the results of the endemic small mammal population monitoring before and after the intensive baiting period (Section 1.3). In Chapter 4, I examine the role played by rats in the secondary poisoning hazard to the local avian predators and scavengers. In Chapter 5, I determine the transfer of brodifacoum into the ecosystem, particularly by invertebrates consuming bait and poisoned rat carcasses. In Chapter 6, I summarise the results from the studies and I propose recommendations for minimising non-target primary and secondary poisoning hazards when eradicating rats from seabird colonies along the British Columbia coast. I also conducted two laboratory experiments to assist in the evaluation of data collected in Chapter 4 and these are described in Appendix A. Environmental aspects of brodifacoum are presented in the appendices.

1.2 Study Areas

1.2.1 Langara Island

Langara Island (54^0 14'N, 133^0 W), also known as Kiis Gwaii or North Island, is located at the northwestern tip of the Queen Charlotte archipelago (Haida Gwaii),

British Columbia, Canada (Figure 1.2). The Island is 3300 ha in size and is relatively flat rising to a maximum elevation of 160 m. The shoreline is highly variable ranging from rocky and sandy beaches to steep cliffs and bluffs. The Island is ringed by three bands of dominant vegetation that include: sitka spruce (*Picea sitchensis*) predominating along the shoreline, western hemlock (*Tsuga heterophylla)*, and western red cedar (*Thuja plicata*) dominating the interior. Thick growths of salal (*Gaultheria shallon*) are abundant in areas, while Nootka reed grass (*Calmagrostis nutkaensis*) is found along the shoreline. The forest floor is predominantly open, and covered with moss, and moss covered logs and stumps. Raised bogs are found inland amongst the lakes.

There were two areas at which people reside year-round on the Island. At Langara Point, on the north-west tip, is the Langara Lightstation with two families living permanently (Figure 1.3). At the southern most tip of Langara Island were the fishing lodges located in and around Henslung Cove. There were two permanent land-based lodges, West Coast Fishing Club and Langara Island Lodge on Iphigenia Point. Three other, seasonal, floating fishing lodges are barged in early spring and leave by early fall. Float planes regularly drop off and pick up tourists throughout the fishing season.

1.2.2 Lucy Island

Lucy Island located in close proximity to Langara Island (about 300m) is 40 ha in area, 1400 m long and 300 m across at its widest. The shoreline is highly variable consisting of coarse gravel beaches and boulders to rocky shelves and outcrops. The island is relatively flat with a maximum elevation of 69 m above

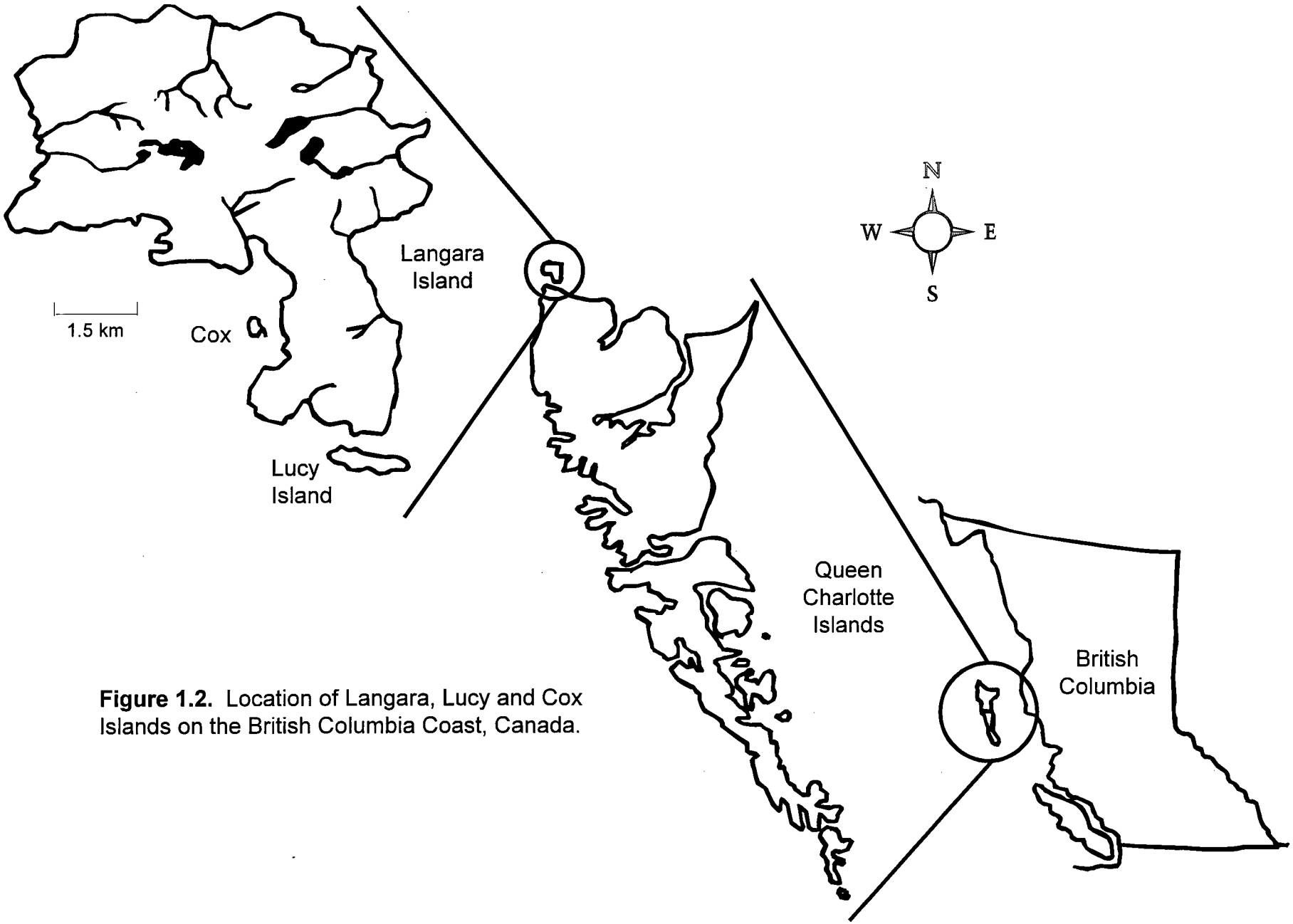


Figure 1.2. Location of Langara, Lucy and Cox Islands on the British Columbia Coast, Canada.

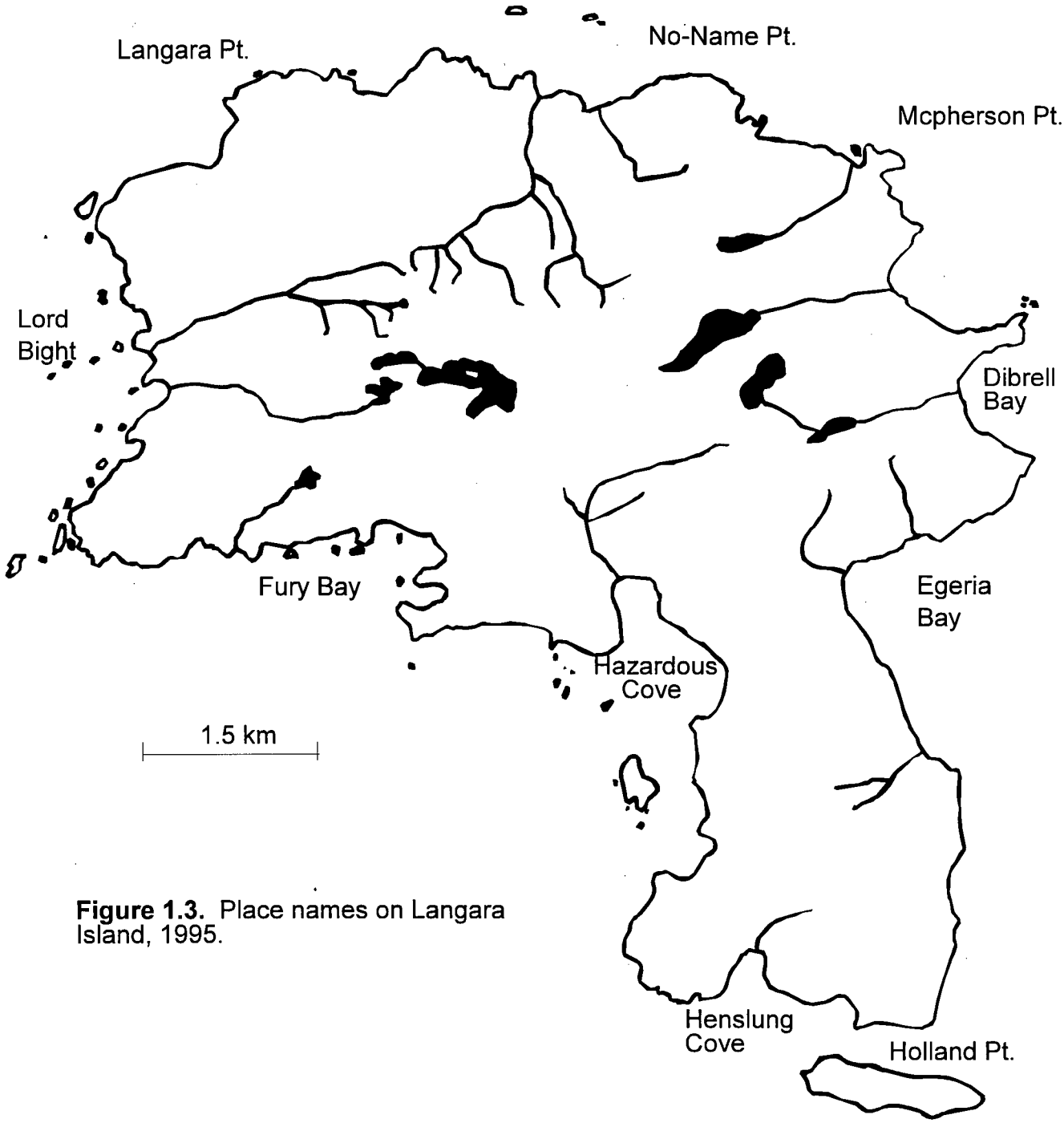


Figure 1.3. Place names on Langara Island, 1995.

sea level. Old growth forest predominates, but with heavy patches of windfall containing thick second growth running along the central portion of the Island. The dominant vegetation is similar to that found on Langara.

1.3 Baiting Protocol

The following is a summary of the baiting protocol of the Langara Island Seabird Habitat Restoration Project. The protocol is described in detail in Kaiser et al. (1997). The baiting was carried out with up to 70 people working out of five field camps dispersed around Langara Island.

The bait stations were deployed approximately every 100m and fastened down with two 60 cm long wires (Figure 1.4). The bait blocks measured approximately 3.5 cm x 3.5 cm x 2 cm , and weighed 20 g. The bait consisted of brodifacoum (Ratak+ ^{TM}) at a concentration of 0.005%, or 1 mg brodifacoum per bait block. The carriers and attractants were a mixture of blood, bone, wheat, tallow and castor sugar (Kaiser et al. 1997). Paraffin wax bound the bait together and "weatherproofed" the block.

The baiting protocol was tested on Lucy Island beginning July 12, 1994 (Buck 1995). Each station was armed with three bait blocks. The stations were checked each day and all activity was recorded. Activity included: bait disappearance, chew marks by rats, shrews or invertebrates. Baits chewed by shrews or slugs were destroyed and replaced. On day 19, baits were placed into plastic bags to minimise exposure to non-target species. On August 17, 1994, all baits were removed from the stations.

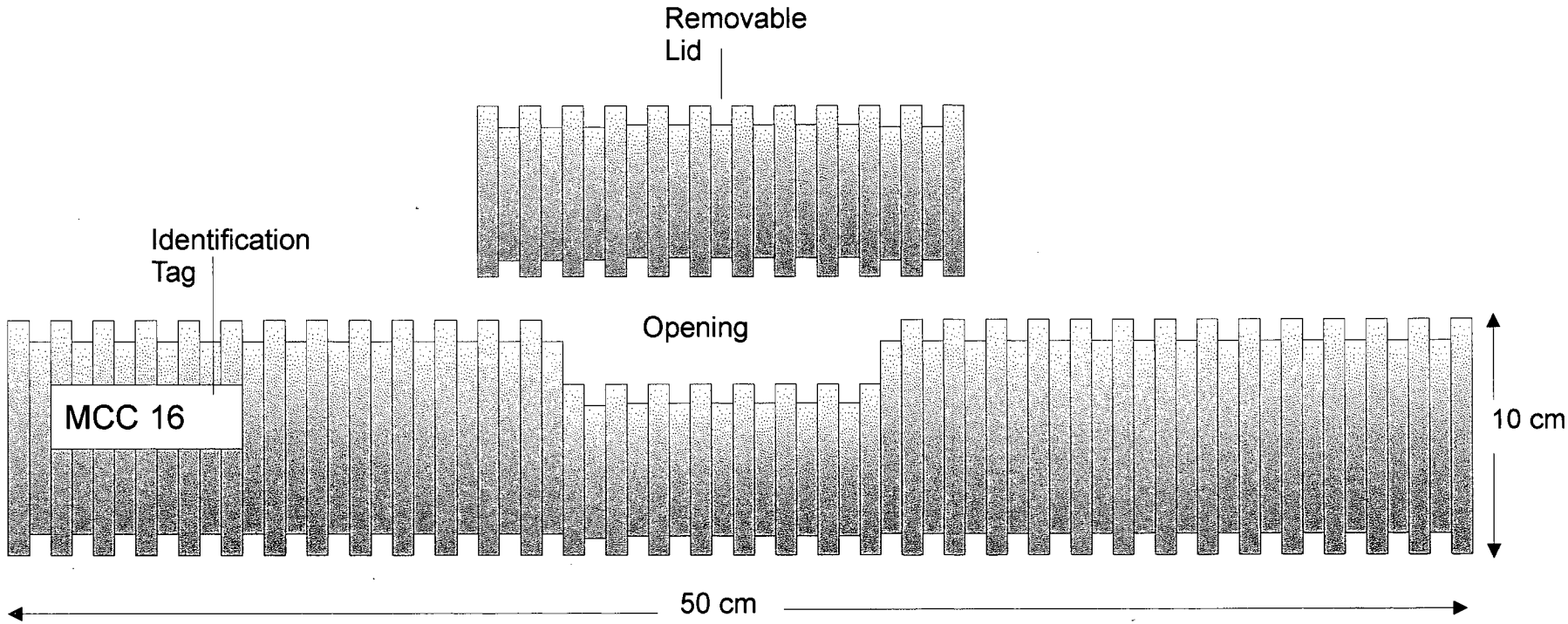


Figure 1.4. Bait Station Design used on Lucy, Langara and Cox Islands. The stations were made of orange coloured PVC corrugated drainage pipe. The lid was removable for placement of bait and checking of stations. The lid snapped into place to protect the bait from weather and most non-target species. Each station was labelled with a unique identification number. The stations were staked to the ground with 2- 60 cm long wires bent into a horseshoe shape and placed on either end (adapted from Taylor 1993).

Rats were discovered again on Lucy Island in late August 1994, and in 1995, the Island was re-baited in attempt to eradicate the remaining rats. On July 11 1995, intensive baiting began on Langara Island. Each of the 3848 bait stations (Figure 1.5) were armed with 6 to12 bait blocks , monitored every 2 to 3 days, and replenished as necessary until early August. For the post-intensive baiting period between August 1995 and August 1997, each station on Langara Lucy and Cox Islands was armed with three bait blocks wrapped in plastic produce bags and placed on an aluminium or plastic tray to keep the bait dry. The bait stations were checked for evidence of rat use in September 1995, and again in February, May, and August 1996. The stations and bait were removed from the Islands during the summer of 1997.

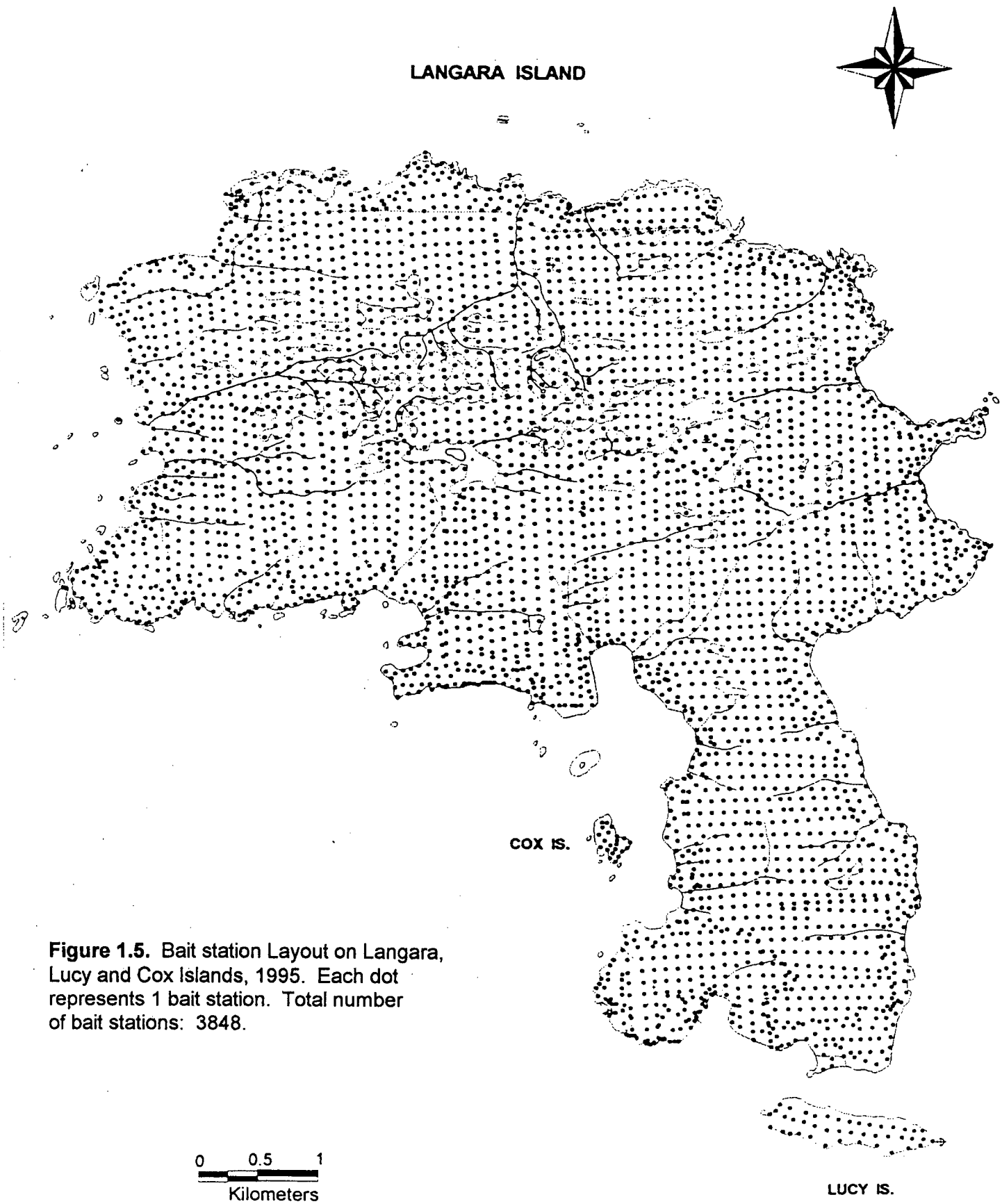


Figure 1.5. Bait station Layout on Langara, Lucy and Cox Islands, 1995. Each dot represents 1 bait station. Total number of bait stations: 3848.

Chapter 2. Hemostasis and Mode of Action of the Anticoagulant Rodenticides: the Physiological Basis for Non-Target Species Poisoning Concern.

2.1 Introduction

Hemostasis is one of the many physiological characteristics that is shared by birds and mammals. Hemostasis has two major functions: (1) to prevent blood loss from sites of vascular disruption and (2) prevent pathologic thrombosis (excess clotting) by limiting clot formation to sites of vascular disruption (Brandt 1991). Hemostasis can be viewed as two forces which continuously oppose and balance each other. Any shift in the balance of forces will result in excess thrombosis or bleeding.

2.2 Hemostasis

Under balanced conditions, hemorrhaging is controlled by clot formation which results from a series of proenzyme to enzyme transformations culminating in the conversion of fibrinogen to fibrin (Kase et al. 1980). In mammals and birds, this coagulation or enzyme cascade (Macfarlane 1964) can be activated by one of two ways: extrinsically or intrinsically.

Intrinsic activation involves contact activation such as the exposure of the blood to the subendothelium *in vivo* or, contact of blood to a negatively charged surface such as glass *in vitro* (Macfarlane 1964; Rapaport 1987). Recently, the well studied human coagulation system has revealed that contact activation is not required for normal hemostasis (Jesty and Nemerson 1995). Past studies have indicated that birds lack an effective intrinsic clotting mechanism (Didisheim et al. 1959). However, Doerr and Hamilton (1981) have provided evidence of a

functioning intrinsic mechanism in chickens, but this plays a minor role in the overall coagulation function in comparison to the extrinsic pathway. This is similar to the accepted human model in that the coagulation cascade is initiated by tissue factor (thromboplastin) and not necessarily the intrinsic pathway (Jesty and Nemerson 1995).

The extrinsic pathway involves a tissue factor (or thromboplastin) that normally resides in the endothelium and other tissues such as the brain (Griminger 1986). The thromboplastin is present on the surface of many cell types and is not normally in contact with the circulating blood (Jesty and Nemerson 1995). Upon tissue damage, the thromboplastin, now exposed to blood, binds with factor VII forming an enzymatically active complex which initiates the clotting cascade (Rapaport, 1987) (Figure 2.1).

The mammalian and avian extrinsic pathway coagulation cascades are functionally similar (Griminger 1986; Belleville et al. 1982; Kase 1978). Once the factor VII-thromboplastin complex forms, it initiates the activation of factor X which provides a positive feedback increasing the enzymatic activity of the factor VII-thromboplastin complex (Rapaport 1987). This complex along with activated factor XI then activates factor IX. The activated factor VII-thromboplastin and factor IX complexes are required to activate sufficient factor X to generate enough thrombin to maintain hemostasis (Rapaport 1987). Thrombin (activated factor II) transforms fibrinogen into fibrin followed by the stabilization of the fibrin clot. Interruption of this cascade at any step will prevent the formation of the fibrin clot and hemorrhaging may continue uncontrolled. The introduction of an anticoagulant, such as

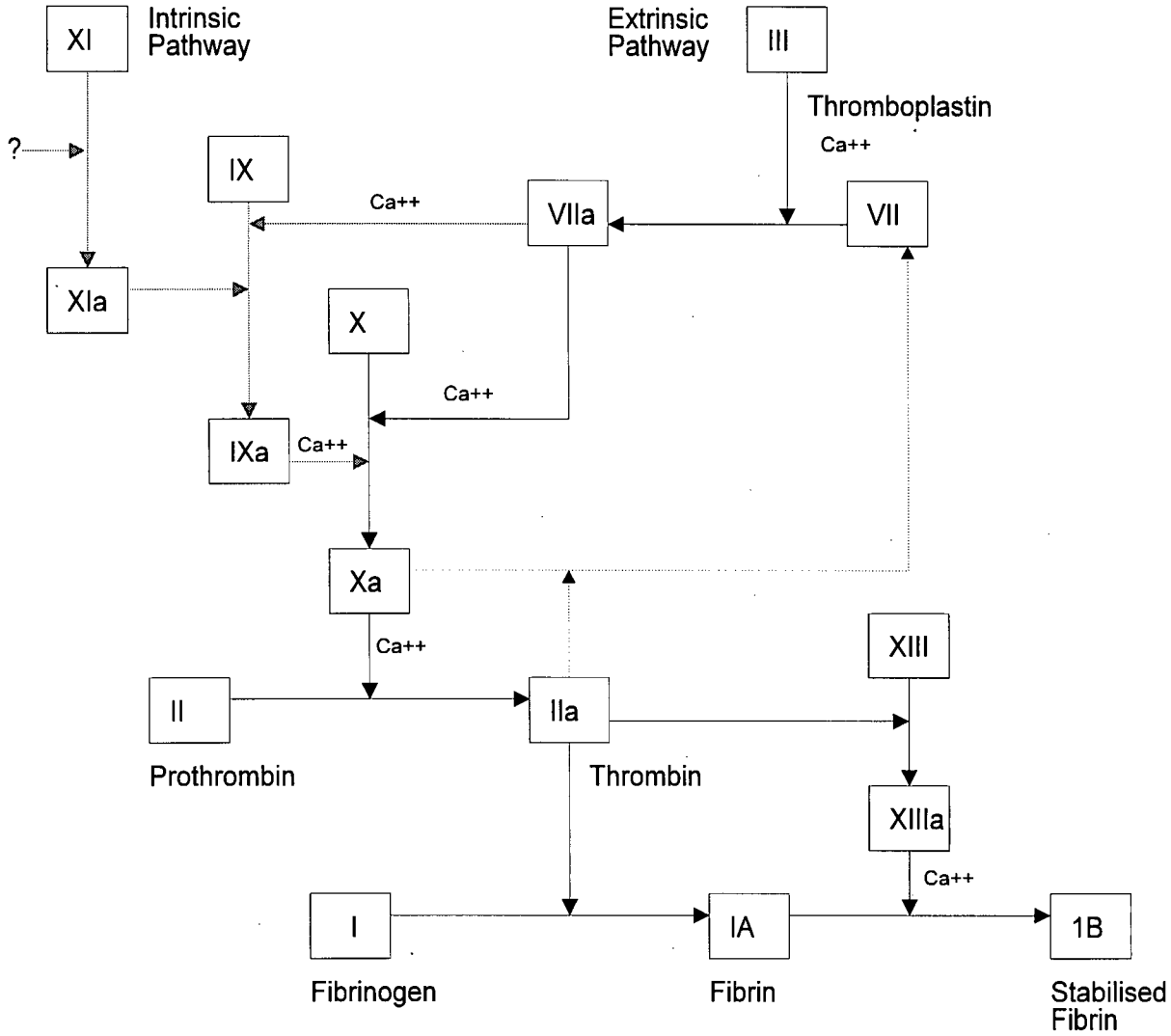


Figure 2.1. A diagrammatic representation of the coagulation cascade. The grey arrows refer to the intrinsic pathway. The roman numerals represent the clotting factors. The lower case a, represents the activated form of that clotting factor. Dashed lines represent positive feedback loop enhancing the coagulation cascade (adapted from Sturkie 1986 and Rapaport 1987).

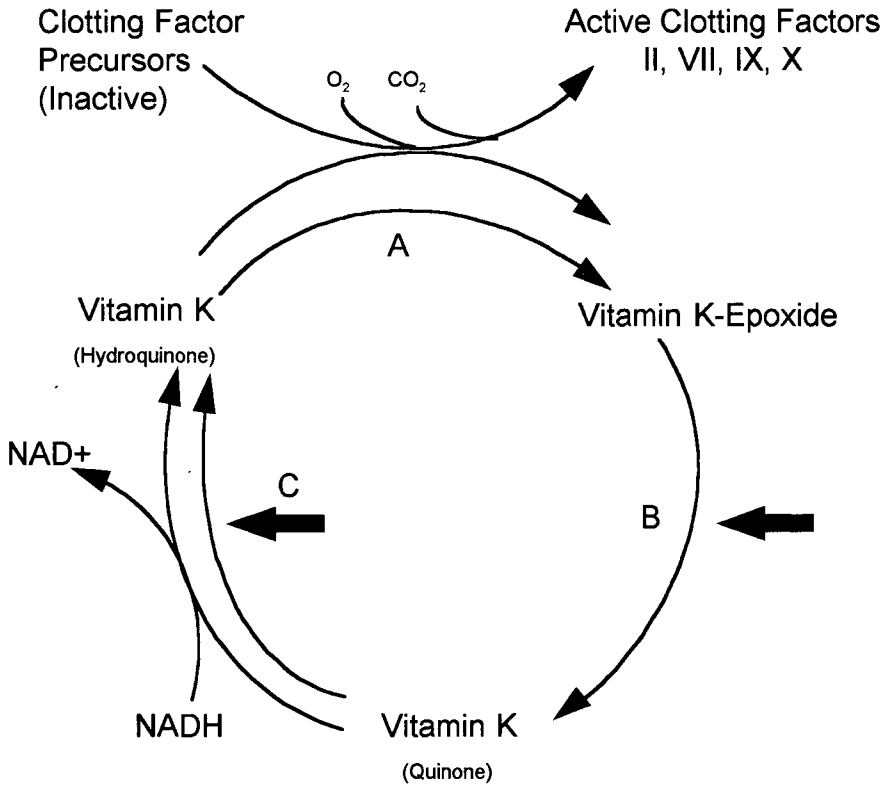
brodifacoum, in sufficient doses, will result in the inhibition of production of some clotting factors required in the extrinsic pathway.

2.3 Vitamin K_1 and the Mode of Action of the Anticoagulants

The metabolic role of vitamin K_1 is to activate the vitamin K dependent clotting factors- II (prothrombin), VII, IX, and X (Rapaport, 1987; Suttie 1980) by contributing a carboxy side chain by post-translational carboxylation of selected glutamic residues to form gamma-carboxyglutamic acid residues (Figure 2.2). These amino acid residues are necessary to chelate divalent calcium to interact with phospholipid containing membranes, their normal site of activation (Goodman et al., 1985). Vitamin K_1 now in its oxide form, can be reduced to its original form by the epoxide reductase enzyme. The recycled vitamin K_1 is now available for further activation of the vitamin K dependent clotting factors. The anticoagulants interfere with this process, resulting in the release of inactive clotting factors into the blood stream.

All coumarins, the group of anticoagulants to which brodifacoum belongs, have the same mode of action. Anticoagulants bind to the warfarin binding protein (Thijssen and Baars 1989) in the endoplasmic reticulum of the liver hepatocytes (Searcey et al. 1977) and inhibit the epoxide reductase enzyme preventing the reduction of vitamin K_1 -epoxide. The 4-hydroxycoumarins do not necessarily bind to the reductase enzyme, but it has been speculated that they interact with a subunit structure with which the enzyme has to interact for normal functioning (Thijssen and Baars 1989). The inihibition of the reductase enzyme leads to a build up of hepatic

Figure 2.2. The vitamin K metabolic activities in rat liver microsomes (black arrows indicate where the anticoagulants inhibit the cycling of vitamin K). Step A is a carboxylase-epoxidase enzyme not inhibited by coumarins. Step B, is the epoxide reductase enzyme which is inhibited by the anticoagulants. This leads to rapid depletion of Vitamin K stores and build up of Vitamin K epoxide. Step C is also a reductase enzyme inhibited by anticoagulants, however, an alternative pathway exists allowing this reaction to proceed (Adapted from Mount et al. 1982;Suttie 1980).



vitamin K_1 epoxide (Leck and Park 1981; Caldwell et al. 1974) and the level of activated clotting factor production declines or is inhibited (Choonara et al. 1988; Leck and Park 1981). The extent to which the anticoagulant inhibits the production of the clotting factors is dose dependent (Thijssen and Baars 1989; 1987). In liver, there are specific, high affinity, saturable binding sites for anticoagulants (Thijssen and Baars 1989; Huckle et al. 1989a; Huckle et al. 1989b; Parmar et al. 1987). The anticoagulants need to saturate the binding sites to initiate the anticoagulant effect (Parmar et al. 1987).

Without the carboxylation of the vitamin K dependent clotting factors, they are released into the blood with little or no enzymatic viability and the level of active factors declines. The anticoagulant effect develops gradually over time as the level of active clotting factors declines according to their different rates of biologic decay (Rapaport 1987). There exists a threshold of active clotting factors below which significant bleeding will result (Hoffman et al. 1988). Without active clotting factors, any trauma induced or spontaneous bleeding is uncontrollable and death results from hypoxia, and hypovolemic shock.

All birds and mammals share the coagulation characteristics that make them susceptible to anticoagulant rodenticides. The differences in sensitivity to anticoagulants results primarily from varying ability to metabolise or excrete these compounds (Huckle et al. 1989b). In general, birds are less susceptible to anticoagulants as they are more readily able to metabolise the compound while mammals are unable to metabolise the anticoagulants before the lethal

anticoagulant effect takes place (Huckle et al. 1989b). Further discussion into potential sub-lethal and long term effects of brodifacoum exposure is presented in Appendix C.

Chapter 3.0 The Short Term Impacts of Brodifacoum Baiting on the Native Small Mammals.

3.1 Introduction

In North America, native small mammals are abundant and ubiquitous in a variety of habitats including offshore islands. The use of anticoagulants to eradicate introduced rats from these islands may alter the abundance and composition of the native small mammal populations. There is little information on how controlling or eradicating rats affect resident native small mammal populations, particularly on offshore islands.

Historical records indicate that the dusky shrew and the deer mouse were the only native small mammals known to inhabit Langara Island (Foster 1965, I. Mctaggart Cowan, pers. comm.). Based on morphological measurements and pelage colour, the shrews on Langara Island have been included with the north Moresby Island race (*elassodon*) (Foster 1965). Deer mice were known to be present in the 1940's but had apparently been extirpated by the 1960's when Foster (1965) recorded shrews but no deer mice. No evidence of deer mice was found during recent trapping campaigns (Harfenist 1993; Bertram 1989). The disappearance of the deer mouse coincides with the introduction of the Norway rat (Taylor 1993). However, in September 1994, one deer mouse was trapped in Lord Bight on the west coast of Langara Island (C. French, pers. comm.) which may have represented a previously undetected population.

The native small mammals are at risk of poisoning because they share many characteristics with the target rodent species. The risk of primary poisoning is

related to the palatability and availability of the bait in space and time. The bait consisting of both animal and grain products may be attractive to both dusky shrews and deer mice. Dusky shrews are normally insectivorous, but are known to consume carrion (Cox 1990; V. Craig, UBC, pers. comm.). They are inquisitive and readily eat animal carcasses, showing a preference for organs, particularly the liver, in which anticoagulants accumulate (Cox 1990).

The objective of this study was to identify the native small mammal species at risk of poisoning, and monitor the short term population changes, abundance and composition, over the intensive baiting period.

3.2 Materials and Methods

Non-target Small Mammal Identification and Population Monitoring

In 1994, a 1ha grid of 49 trap stations (7x7), 15.2 m apart, with one Longworth-style live trap located within 2m of each station was established on Lucy Island (Figure 3.1). Only one grid was used due to the unavailability of traps, and no control grid was used. A three evening arming and check period was employed. The traps were baited with a mixture of peanut butter and oats, and coarse brown cotton was supplied for bedding. The traps were armed in the early evening and checked 5-6 hours later, at dusk or just after. This was repeated for the following two evenings.

Live trapping was conducted once before and once after the baiting, in July 1994, 3 days before the start of the baiting and in August after the bait was removed from the stations. In May 1995 and August 1995, a single trap session on the established grid was performed.

In 1995, Langara Island was divided into three treatment regions: the existing Ancient Murrelet colony at Mcpherson Point (a high population of rats), the east coast in Egeria Bay (lower rat population), and the west coast region in Lord Bight (possible remnant population of deer mice) (Figure 3.1). In each of the regions, two 1-ha grids, each with 49 trap stations (7x7), 15.2 m apart, were established. Longworth style live traps were placed on all grids except for two which had small Shermann traps. Each grid was trapped twice before the baiting began and twice after the intensive baiting period.

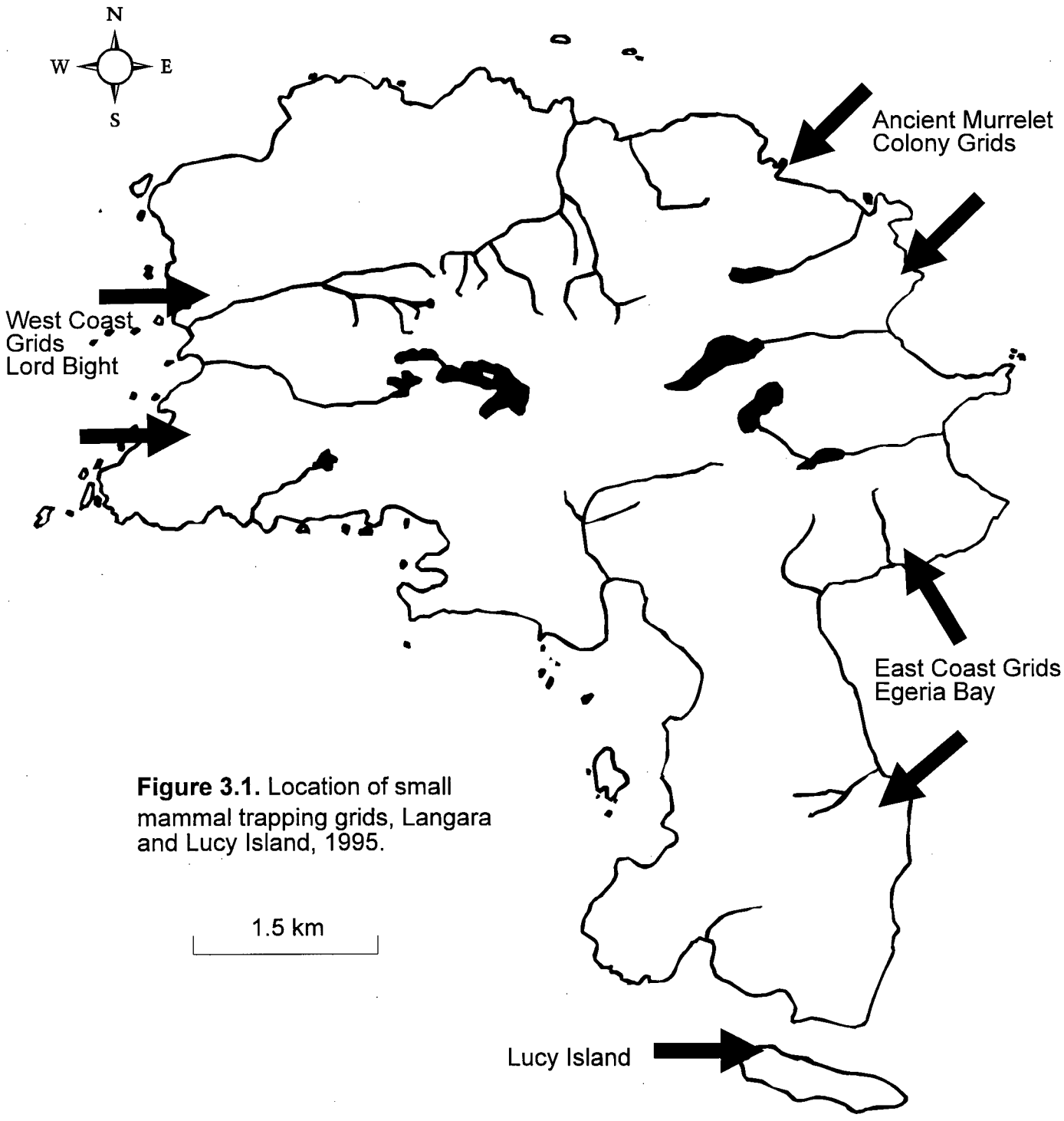


Figure 3.1. Location of small mammal trapping grids, Langara and Lucy Island, 1995.

Graham Island, 1 km across Parry Passage from Langara Island, was used as a control site. Two control grids were established, however, one was removed after continuous disruption by a female black bear and her two cubs during the second trap session in June 1995.

A three evening arming and check period was employed on Langara and Graham Islands following the same procedures used on Lucy Island. This short arming time was utilised because shrews die if left in traps over night (Sullivan 1990). Trapping was performed in the evening to increase the chances of trapping any deer mice present on Langara or Lucy Islands.

Each shrew captured was weighed with an Avinet spring balance, and marked for future identification with a blonde hair dye in a unique combination of dots on its fur and/or toe-nail clipped. I chose not to toe-clip because if any bait was consumed, lethal hemorrhaging may have been induced. Due to the difficulty in sexing juvenile or non-sexually active shrews, only obvious reproductive condition was noted (Craig 1995; Hawes 1977). Individuals were released immediately after processing. The traps were locked open between trap sessions, and also between checks and arming times.

Shrews caught five or more times (Craig 1995) were used for estimation of range size and distance traveled calculated with the computer program CALHOME (Kie et al. 1994). Range size was calculated using 90% minimum convex polygons (MCP) for comparison to other studies (Craig 1995; Hawes 1977).

Shrew use of Bait Stations

Bait station operators were asked to record shrew activity in bait stations. Training in the identification of shrew chewed bait blocks was provided prior to the baiting. Shrew chews were identified from the size and pattern of the incisor marks. Any shrew chewed bait block was replaced to prevent it being recounted during future visits. However, only data collected by identified, reliable bait station operators were used.

Calculations and Statistical Analyses

Population Size Changes

The data are reported as the number of individuals/ha and unique shrews /100 trap nights (TN). Success per 100 TN was calculated following Nelson and Clark (1973) as:

$$CE = A \times 100 / (TU - IS/2)$$

Where CE= catch effort; A= number of animals; TU= trap units (TU= PxIxN where P= number of trapping intervals (3 nights); I= length of trapping intervals (1 night); and N=number of traps (49 traps); S= number of traps closed and empty or with a recaptured shrew. Where possible, the Schnabel/Schumacher-Eschmeyer method was used to estimate population size (Krebs 1989). Confidence intervals were calculated using the Poisson distribution.

The trap success data were square root transformed in an attempt to normalize the data. I used a two way ANOVA for repeated measures (Kuehl 1994).

Analysis was carried out with the *JMP* statistical package (SAS 1995) with the following statistical model:

$$Y_{ijk} = \mu + R_i + T_j + (RT)_{ij} + E_{ijk}$$

where Y_{ijk} = unique shrews/100TN, R_i = effect of region, and T_j = effect of the jth time, and $(RT)_{ij}$ = the two way interaction between time and region, and E_{ijk} = random error. A significance level of $P<0.05$ was chosen a priori.

3.3 Results

Only dusky shrews (63 unique individuals) were trapped on Lucy Island in 1994 and 1995, and on Langara Island (182 unique individuals) in 1995. On Graham Island, deer mice (32 unique individuals) were abundant on control grids (Table 3.1), and dusky shrews (18 unique individuals) were also captured.

Table 3.1. Abundance of deer mice (*Peromyscus maniculatus*) /100 Trap Nights on Graham Island, before and after the intensive baiting period on Langara Island, 1995.

	Before Baiting		Post Baiting	
Location	May	July	August	Mid-August
East Grid	7.9	-	-	-
West Grid	8.8	21.0	17.4	4.3

Lucy Island

In 1994, there was a decline in the number of unique shrews captured after the baiting (Figure 3.2, Table 3.2). By May 1995, trap success rebounded to half the pre-baiting 1994 estimate. Unique number of shrews captured again declined over the baiting period in 1995 (Table 3.2). The proportion of shrews in breeding condition declined in both years over the course of the baiting period (Table 3.3).

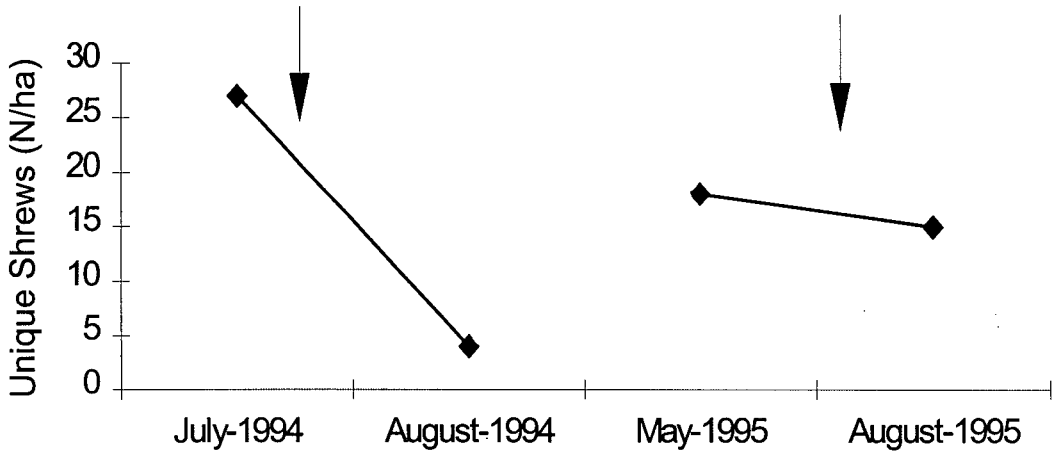


Figure 3.2. The number of unique dusky shrews (*Sorex monticolus*) captured on Lucy Island, 1994 and 1995. The vertical arrows indicate when the brodifacoum baiting for introduced rats began. The bait was removed in mid August, 1994, but was reapplied in July 1995.

Table 3.2. Dusky shrew (*Sorex monticolus*) trap success (# shrews caught/100 Trap Nights) and population estimate before and after brodifacoum bait application Lucy Island, 1994/1995.

		Before Baiting	Post Baiting
1994	Trap Success	19.9	2.8
	Population Estimate	56.4 (32-155)[b]	ND[a]
1995	Trap Success	11.8	10.5
	Population Estimate	28.1 (19-185)[b]	13.2 (11-47)[b]

a Not Determined. All shrews were trapped on the third evening.
b 95% Confidence Interval

Table 3.3. Proportion of dusky shrews (*Sorex monticolus*) in breeding condition (testes scrotal, nipples large) before and after baiting on Lucy Island, 1994/1995 (n in brackets).

	Pre-Baiting	Post-Baiting
1994	0.40 (10)	0.25 (1)
1995	0.56 (10)	0.13 (2)

Langara Island

There was no significant interaction between time and regions (Table 3.4). Overall, there was no statistical difference in trap success before and after the intensive baiting period on Langara Island (Figure 3.3). Trap success was very low on the small Shermann trap grids (Table 3.4), but removing those grids from the analysis had no effect.

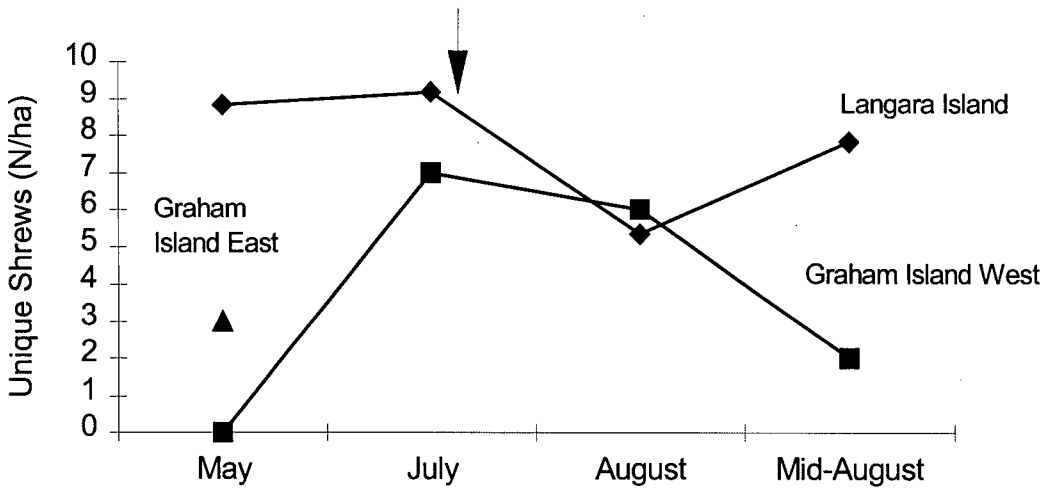


Figure 3.3 Mean unique number of dusky shrews (*Sorex monticolus*) captured on Langara Island and on the control grids on Graham Island, 1995. Vertical arrow indicates when brodifacoum baiting for introduced rats began on Langara Island.

Table 3.4. Dusky shrew (*Sorex monticolus*) trap success (# caught/ 100 Trap Nights) by trap session and region on Langara Island and Graham Island, 1995.

		Before Baiting		Post Baiting	
Region	Grid Location	May	July	August	Mid-August
Langara Island					
Murrelet Colony	No-Name Pt	14.3	1.4[a]	6.9	17.1
	Explorer Bay	3.4	9.1	6.8	3.6
East Coast	Egeria North	3.0	11.1	2.8	7.0
	Egeria South	3.5	3.5	1.4	0.7
West Coast	Lord Bight North [a]	0.7	1.4	0.0	1.4
	Lord Bight South	12.5	10.3	3.5	3.5
Graham Island	East	2.4	-	-	-
(Control)	West	0.0	5.7	5.7	1.1

a. Small Shermann traps used on these grids.

While statistically not significant, the regional number of unique shrews captured per hectare declined between the start of the baiting and the third trap session in all regions on Langara Island. The greatest decline occurred on the east coast, dropping by 75% (Figure 3.4).

On the Graham Island control grid, there was an accumulated trap mortality of three shrews during the second and third trap session. During the last trap session, 31, 35 and 36 of the 49 traps over the three trap nights, on the grid were reported closed and empty.

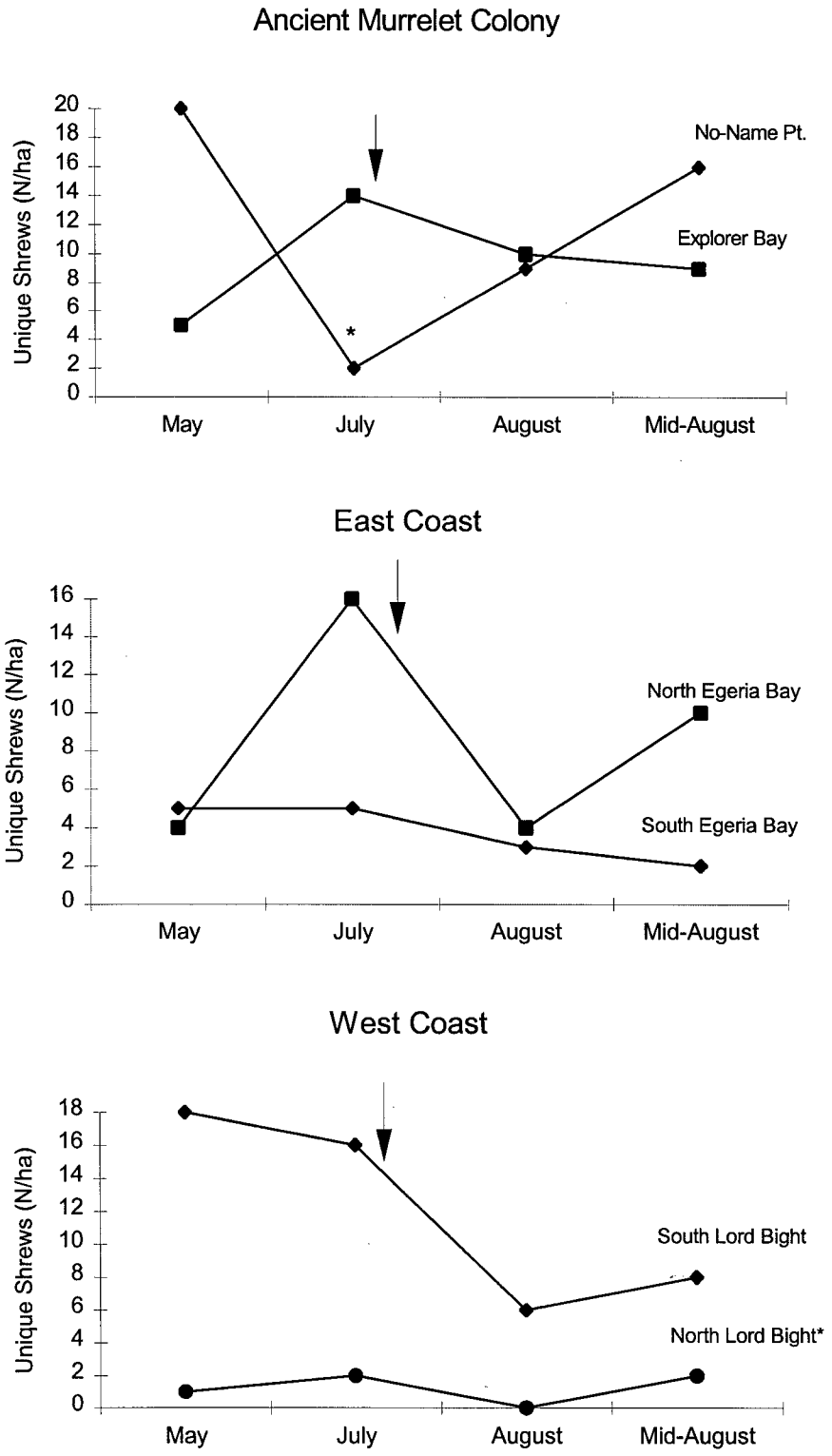


Figure 3.4. Unique number of dusky shrews (*Sorex monticolus*) captured on each study grid in the three regions on Langara Island, 1995. Vertical arrows indicate when brodifacoum baiting began. Longworth traps were used on all grids except where denoted by a * when small Shermann traps were used.

The proportion of shrews captured in breeding condition did not change on the control grid and remained at 50% (Table 3.5). However, the proportion of shrews in breeding condition declined at all three baited regions, with the most drastic decline (%) occurring on the West Coast (Table 3.5).

Table 3.5. Proportion of dusky shrews (*Sorex monticolus*) in breeding condition before and after intensive baiting, Langara Island, 1995 (mean of two grids per region; sample size in brackets).

	Murrelet Colony	East Coast	West Coast	Graham Island (Control)
Pre-Baiting	0.44 (18)	0.43 (7)	0.49 (18)	0.50 (5)
Post-Baiting	0.39 (17)	0.21 (4)	0.06 (1)	0.50 (4)

Shrew Use of Bait Stations

Shrew incisor marks on bait blocks were small, and readily identifiable. The shrews chewed the edges of the blocks, working their way around all edges, never consuming the whole block. Conversely, rat incisor marks were larger and they would begin and continue to chew on one side of the bait block.

On Lucy Island shrews chewed 38 bait blocks in 28% of the bait stations in 1994 (Buck 1995). Shrews were also photographed with automatic cameras exiting bait stations. By day 20 of the baiting program on Lucy Island in 1995, shrews had visited 80% of 42 bait stations and chewed one or more bait blocks in each station. In Henslung Cove, shrews had chewed 157 bait blocks in 50% of the 28 stations in that region over the course of the intensive baiting period. In the interior of the

Island, shrews had chewed 151 bait blocks in 80% of 53 stations visited. On Langara Island, bait station operators reported shrews "stumbling" out of stations, indicating possible anticoagulant intoxication (Cox 1990). As well, during checks of the live traps on the North Egeria grid in early August, small, blue colored shrew feces were noted in one trap indicating that at least one shrew had been feeding on the bait.

Shrew Ranging Distances

Only four individual shrews were captured five or more times, with the number of recaptures ranging from five to eight. The shrew 90% MCP range size varied between 112 m^2 to 2700 m^2. The maximum distances traveled between traps ranged from 61.8 m to 91.2 m (Table 3.6).

Table 3.6. Number of captures, weight, maximum distance traveled and 90% MCP range size for dusky shrews (*Sorex monticolus*) caught five or more times, Langara Island, 1995.

Shrew No.	Location	Number of Captures	Weight g	Mean Distance m	Maximum Distance m	90% Range Area m^2
2	Murrelet Colony	8	8.3	40.3	90.2	2700.0
5	Murrelet Colony	6	6.8	54.9	91.2	1238.0
6	Lord Bight	5	7.8	75.7	76.5	2475.0
32	Murrelet Colony	5	5.8	54.6	61.8	112.5

3.4 Discussion

Shrews were abundant in the coastal region of Langara Island prior to the baiting, but there was no evidence of deer mice. The results suggest that the dusky shrew was able to co-exist with introduced rats, perhaps because of niche differences resulting in low interspecific competition. Rats may have avoided preying on shrews because they secrete a distinctive pungent, acrid odor making them unpalatable (Churchfield 1990; Hawes 1976). Deer mice were apparently extirpated from the coastal region of Langara Island either through predation, competition or both. Other islands in the Queen Charlotte archipelago contain populations of deer mice except where there are rats; the exception are the larger islands, Graham, Moresby, Kunghit and Lyell Island that have populations of both deer mice and rats (Foster 1965). The individual deer mouse trapped in Lord Bight in 1994 may have represented a remnant population, or may have been accidentally transported from Graham Island by the survey crew.

Despite use of bait stations by shrews, and declines over the course of the baiting, there were no significant short-term impacts on shrew abundance on Langara Island. Rat control programs have been shown to significantly impact other non-target small mammal species. During anticoagulant poisoning for rat control in the spring and summer on farms in England, non-target woodmice (*Apodemus sylvaticus*) entered stations and fed on bait (Cox and Smith 1990). There was a 75% reduction in the population size of woodmice after treatment while the control populations increased (Cox and Smith 1990). The individual survivorship of marked

individuals was between 0-19% on baited farms and 50% on control farms (Cox and Smith 1990). No estimates of survivorship could be made for the shrews on Langara Island.

According to the trapping record, the Graham Island (control grid) dusky shrew abundance decreased while the Langara abundance increased between the third and fourth trap session. It was expected that the reverse would be measured, i.e., shrews on Langara Island would decrease in abundance as compared to the control site. However, on the control grid, the trapping of non-target deer mice, high number of closed but empty traps and trap mortality of shrews could account for the low number of shrews trapped on this grid during the third and fourth trap session.

The Lucy Island population of shrews declined sharply over the course of the baiting in 1994 and was the reason a monitoring program was established on Langara Island in 1995. In 1995, shrews on Lucy Island did not decline in abundance as in 1994, but the proportion of shrews in breeding condition followed a similar pattern of decline. On Langara Island, the greater the regional decline in number of shrews captured after the intensive baiting, the greater the decline in the proportion of shrews in breeding condition (Figure 3.5). There was no change in the proportion of breeders on the control grid over the same time period. This decline in the proportion of shrews in breeding condition may be due to seasonal changes such as recruitment of juveniles or it may represent a specific poisoning impact on shrews in breeding condition. This seems to suggest that the individual risk of primary poisoning was greater for individuals in breeding condition. As these

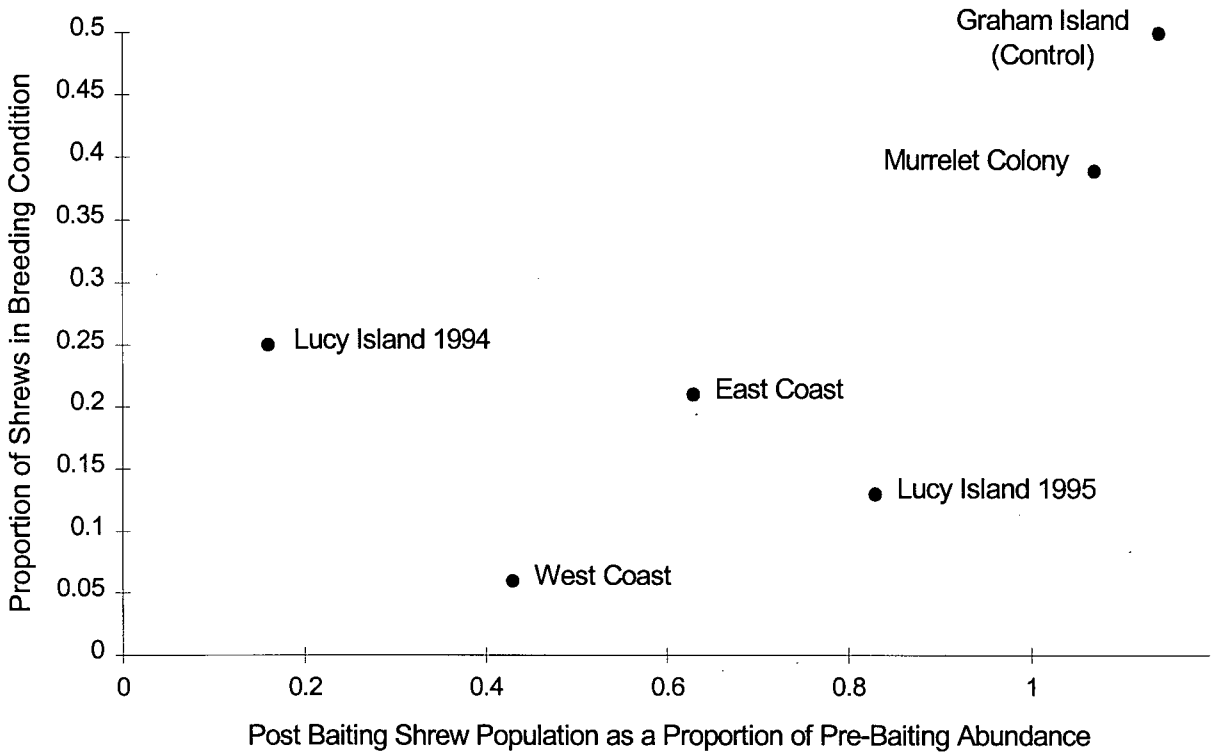


Figure 3.5. The proportion of dusky shrews (*Sorex monticolus*) in breeding condition correlated to the post baiting shrew population estimate as a proportion of the pre-baiting population estimate (r = 0.64).

territories were vacated, dispersing juveniles and other non-breeding shrews may have competed to fill those vacant territories (Churchfield 1990). This would lead to an increasing density of shrews which may explain the increased population during the fourth trap session in mid-August on four of the six grids on Langara Island.

The risk of primary poisoning to shrews from gaining access to the bait in stations is greater for shrews in breeding condition because they range more than non-breeders (Hawes 1977). The greater ranging distances of breeding shrews increased the chance of encountering brodifacoum in stations. Breeding males may

be at greater primary poisoning risk than the breeding females, non-breeders and juveniles, as they range significantly further afield (Hawes 1977). An extreme movement of one scrotal adult male was noted in Lord Bight. This individual was live-trapped in a field camp, marked and released, and was subsequently recaptured 7 hours later on the North Lord Bight grid, 181.5 m away- almost twice the average distance between bait stations. The range sizes calculated for dusky shrews on Langara Island were smaller than the ranges calculated by Hawes (1977) but similar to those found by Craig (1995). The distance between stations was adequate to spatially exclude some shrews.

This study addressed the short term impacts of brodifacoum baiting on the shrew population. What remains unknown is the impact of having bait left in bait stations wrapped in plastic bags for the 2 year period after this study ended. It can be concluded that shrews were attracted to bait in the stations and that there were short term impacts on shrews including breeding individuals. However, they were not extirpated from Langara or Lucy Island. Shrew trap success rebounded 9 months after bait was removed from Lucy Island. Thus, cessation of the baiting could allow for the shrew population to increase to pre-baiting levels within 1 year. The long term impacts of brodifacoum baiting on the non-target dusky shrew would require further investigation; however, there are unlikely to be any significant long term impacts.

Chapter 4. An Evaluation of the Secondary Poisoning Hazard to Avian Wildlife.

4.1 Introduction

The secondary poisoning risk to birds from feeding on anticoagulant killed rodents is well known and has been demonstrated in the laboratory (Newton et al. 1990; Radvanyi et al. 1988; Townsend et al. 1981; Mendenhall and Pank 1980). Assessing the actual hazard under field conditions is difficult because pharmacological susceptibility is not necessarily an indicator of ecological susceptibility (Moore 1966) and predators and scavengers are not expected to consume only contaminated animals (Townsend et al. 1984). The risk of secondary poisoning to avian predators and scavengers from brodifacoum poisoned Norway rats is related to exposure factors such as the behaviour of the target species during the latent period, the location of carcasses (above or below ground), the anticoagulant residue loading in the target species, and the behaviour and diet of the non-target species (Record and Marsh 1988; Taylor 1993; Kaukeinen 1982).

Anticoagulant poisoned Norway rats demonstrate altered behaviour which potentially makes them more susceptible to predation and scavenging. Norway rats exposed to a lethal dose of an unidentified anticoagulant showed reduced thigmotactic behaviour (moving in contact with a vertical surface such as a wall) and spent significantly more time in open areas than under cover as compared to the controls (Cox and Smith 1992). While in the open, rats were observed sitting motionless or staggering about shortly before death. On death, 50% died in open

areas, apparently deliberately moving out of nest boxes into open areas just before dying.

During the field testing of the baiting protocol on Lucy Island in 1994, one of three brodifacoum poisoned radio-collared Norway rats died above ground away from a burrow (Howald 1995). Carcasses of poisoned rats also were found on the beach and other open areas under the forest canopy.

Secondary poisoning of non-target species from the use of brodifacoum to control rats and voles has been demonstrated in the field. Eastern screech-owls (*Otus asio*) died after exposure to brodifacoum after broadcast application to control voles in orchards (Colvin and Hegdal 1988; Merson et al. 1984). In New Zealand, Western weka (*Gallirallus australis australis*) and Stewart Island weka (*Gallirallus australis scotti*) died after consuming rats that had fed on bait containing brodifacoum (Taylor 1984).

Common ravens (*Corvus corax*), Northwestern crows (*Corvus caurinus*) and bald eagles (*Haliaeetus leucocephalus*) were considered to be at risk of secondary poisoning from feeding on dead or dying Norway rats on Langara Island (Taylor and Kaiser 1993). The importance of rats in their diets is unknown, but, they are opportunistic scavengers and predators that may take advantage of a new prey source.

To evaluate the secondary poisoning risk of the three avian species, an integrated study was undertaken to determine the extent to which rats will die above ground, and to evaluate the poisoning of non-target species. The main objective

was to evaluate the secondary poisoning risk to avian predators and scavengers from the use of brodifacoum to control Norway rats on Langara Island. The specific hypothesis tested was that dying or dead rats would be available to predators and scavengers, thus putting them at risk of secondary poisoning.

4.2 Materials and Methods

4.2.1 Carcass Locations of Brodifacoum Poisoned Norway Rats

Fifty Tomahawk live traps were armed for 400 trap nights & 400 trap d in early July 1995, between Mcpherson Point and No-Name Point, in an attempt to capture 5 rats of each age (juvenile/adult) and sex class. Trapped rats were anaesthetised with halothane, sexed, weighed with a spring balance, ear tagged with a fingerling tag, and fitted with a PD-2C radio collar (Holohil Systems, Ontario, Canada). Four adult males, five adult females, five juvenile males and five juvenile females were radio-collared between three d before the start of the program to the day the intensive baiting began. One adult male and one adult female were trapped on day 2 of the program.

Each rat was located at least once per day by tracking the signal to its location and taking a bearing and measurement to the nearest bait station. At any one time, a rat was determined to be alive if, when holding the antenna steady, the signal strength became weaker or stronger indicating that the animal was moving, or if the rat was found to be alive at a future time point. The time to death was calculated as the number of d elapsed between the start of the baiting program and the date of last known activity +24 h.

4.2.2 Brodifacoum Residues in Norway Rats Found Dead Above Ground

Rats found dead by bait station operators and research staff were sexed, weighed and frozen in pre-labelled whirl-pak bags. Five adult male, four adult

female, and three juvenile male rats were sent to the National Wildlife Research Centre, Hull, PQ for tissue preparation for brodifacoum residue analysis. The liver, GIT (gastrointestinal tract, including its contents), and the carcass were individually homogenised and analysed for brodifacoum as described below. The data were log transformed ($X_i = \log_{10}(x+1)$). Analysis of the data was carried out using the *JMP* statistical package (SAS, 1995) with a two way ANOVA. The statistical model was:

$$Y_{ijk} = \mu + A_i + T_j + (AT)_{ij} + E_{ijk}$$

where Y_{ijk} = log brodifacoum residue measured, A_i = the effect of the ith age/sex class, and T_j = effect of the jth tissue, $(AT)_{ij}$ = the two-way interaction between dose effect and time of blood collection, and E_{ijk} = random error.

4.2.3 Norway Rat Scavenger Identification

In 1994, a year before the baiting operation began, non-poisoned, snap-trapped rats were laid out in open, exposed areas such as on beaches, open areas under the forest canopy, and around bald eagle nesting trees. Automatic, infra-red motion sensing cameras were used to identify scavengers. A total of 29 rats were put out, 18 with cameras, and 11 on Egeria Bay beach where scavenging species were identified from tracks left behind on moist sand, or by direct observation. Time to scavenging was only roughly estimated between visits to carcass sites or from direct observation.

4.2.4 Effects on Predators and Scavengers

4.2.4.1 Common Ravens

All bait station operators, research and Langara Lightstation staff were briefed before the baiting program began and were encouraged to report observations involving ravens, or to turn in any carcasses, pellets, or other remains they found. Such carcasses were labelled and frozen for necropsy at the University of British Columbia, Department of Animal Science by Dr. Malcolm Mcadie, a veterinarian experienced in examining pesticide poisoned birds. Livers were removed and frozen for brodifacoum residue analysis.

Source of Brodifacoum Poisoning to Ravens

The source of brodifacoum was determined from protein electrophoresis of the gizzard contents and by evaluation of the gizzard and intestinal contents for Norway rat hairs and bait fragments. Each gizzard was opened along its length and the contents, if any, were removed and the cutica gastrica rinsed with water from a squeeze bottle. If there was an adequate amount, a sample was frozen and sent with control samples of Norway rat, shrew, raven, snails, and bait to the Alberta Natural Resources Service, Enforcement-Field Services, Forensic Laboratory, Edmonton, Alberta for identification by polyacrylamide gel electrophoresis (McClymont et al. 1982). The remaining gizzard contents were rinsed through two layers of cheesecloth and a 60 mesh sieve lined with filter paper before using a dissecting microscope (7-30x) to identify remains and estimated percent per volume.

The intestines were cut into three sections and the contents squeezed into a pre-washed glass dish. The contents were examined for hair or bait crumbs under a dissecting microscope. All hair was identified to species under a microscope by comparison against control samples of Norway rat, dusky shrew, black-tailed deer (*Odocoileus hemionus columbianus*), human and published micrographs of deer, shrew, and Norway rat (Moore et al. 1974; Adorjan 1969).

Raven Activity-1996

In April 1996, the potential ongoing raven exposure to brodifacoum on Langara Island was investigated. Formerly active nest sites were visited at Mcpherson Point and Hazardous Cove. New nests were located by walking up to 200 m inland parallel to the shoreline; one observer between the beach and cliff bottom, the other along the ridge that rings the island. Regurgitated pellets and prey remains were collected from under and around the nest sites.

A total of 107 bait stations reported active (baits removed and/or stations disrupted) were visited. The plastic bags, aluminum trays used to keep the bait off the floor of the station, and bait remains were collected and examined for beak marks. Plastic bags were examined under a dissecting microscope and compared against reference examples of raven-torn plastic bags. Bags were examined for stress bars and/or beak marks.

4.2.4.2 Northwestern Crows

Crows (25) were collected by shotgun between May and August 1995. Each crow was weighed and sexed, and selected morphological measurements were taken before the livers were removed and placed into pre-labelled whirl-pak bags and frozen. The livers were pooled for brodifacoum analysis based on collection dates and/or location. Control livers from pre-baiting snap-trapped rats were also sent for brodifacoum analysis.

4.2.4.3 Bald Eagles

Using a fish snare (Jackman et al. 1993; Cain and Hodges 1989), bald eagles were trapped and a blood sample drawn between days 7 and 47 of the intensive baiting campaign (Table 4.1 and Table 4.2). Three eagles were trapped prior to the start of the eradication in order to test the trapping methods and to obtain control blood. Trapping was concentrated where eagles commonly roosted on the coast, and ranged from the Langara Island Lightstation east to Mcpherson Point, south along the east coast to Holland Point, and west to Cox Island. Trapping was attempted, but unsuccessful, on the west coast due to the incessant northwest/westerly ocean swell which hindered trapping. We commonly observed eagles flying from all regions of the island and most individuals likely were present in our trapping region during the sampling period.

Table 4.1. Bald eagle (*Haliaeetus leucocephalus*) trapping success using the floating fish set; Langara Island, 1995; (n=148 sets).

Description	Rate	No. Eagles
Induced off Roost	49%	73
Attempted to Pick up Bait	84%	61
Trapped	36%	22

Table 4.2. Bald eagle (*Haliaeetus leucocephalus*) trapping results, Langara Island, 1995.

Age Class	Number Trapped
Adult	13
Sub Adult	3
Old Immature	2
Young Immature	4

Each eagle was assigned an age class based on plumage patterns (Bortolotti 1984), weighed with a 10 kg Pesola spring scale, and the wing chord, tail length, culmen, and tarsus diameter measured. Sex was determined from the bill depth and hallux length measurements (Bortolotti 1984).

Up to 10 ml of blood was drawn from the brachial vein. The blood was first collected into heparinized Vacutainer tubes for brodifacoum residue analysis, and if there was enough, 4.5 ml was placed into a Vacutainer tube containing buffered sodium citrate and immediately placed on wet ice for PT evaluation (Brown 1988). A single drop of blood was placed on to a slide for a smear. The bird was banded and released. The banding data has been submitted to the province of British Columbia, Ministry of Environment, and the National Wildlife Research Centre, Hull, PQ.

The blood was centrifuged at 2000 x g for 15 min and the plasma pipetted into pre-labelled 5 ml cryovials, and immediately frozen (-20^0 C). PTs were measured within 6-8 h of collection using the Coulter P/T Fibrinogen following the procedures for manual evaluation (Brown 1988). For control PTs, blood was drawn from bald eagles at the Orphaned Wildlife (OWL) rehabilitation centre in Delta, B.C. and in rehabilitation centres on Vancouver Island. The PT for each sample was measured three times. Normal human plasma was used before and after each sample to ensure that the test was effective. The reported PT is an average of the two closest times.

4.2.5 Brodifacoum Residue Analysis

Frozen tissues were shipped to the National Wildlife Research Centre, Hull, PQ for preparation for analysis. Tissue homogenisation was carried out using chemically cleaned instruments to avoid contamination. Extracts of tissue were analysed at NovaMann International, Mississauga, Ontario using high pressure liquid chromatography (HPLC) with two detection systems: a) post column reaction and measurement of fluorescence of brodifacoum and b) ultraviolet spectrum scanning (for details of procedure see Hunter 1983). The compound identified as brodifacoum using florescence matched the UV spectrum of the brodifacoum standard. The limit of detection was 0.005 mg/kg.

As part of the quality control, NovaMann confirmed quantitative recovery of brodifacoum from liver and reported mean recoveries of 76% for fortifications at the

0.5 and 1.0 mg/kg levels. Fortified rat liver samples were also prepared in the field, wrapped in foil packets, placed into pre-labelled polyethylene whirl pack bags and shipped on ice. The tissues and foil were rinsed with known volumes of methanol and tissues homogenised. The homogenised tissues were divided into two thirds/one third by weight. The larger portion was extracted at NovaMann, the smaller stored at NWRC. The mean quantitative recovery of brodifacoum was 54% for fortifications between 6.4 to 23 mg/kg (Table 4.3). All original chromatography graphical tracings from the instrumental analyses were carefully examined at NWRC by Dr. Bryan Wakeford to ensure both quantitative and qualitative assessment of the brodifacoum residues were correct.

Table 4.3. Quantitative recovery of brodifacoum from Norway rat (*Rattus norvegicus*) liver fortified on Langara Island in August, 1995.

Tissue Type	Tissue + Methanol Sent (g)	Fortified Level (ug)	Brodifacoum Reported (ug)	Percent Recovery
Liver	10.3	0.0	0.0	Control
Liver	6.8	14.22	6.94	48.8
Liver	5.3	16.25	6.97	42.9
Liver	13.7	6.97	6.22	89.2
Liver	4.7	7.83	2.79	35.6

Bald eagle plasma was analysed for brodifacoum residue (Murphy et al. 1989) at the Department of Agriculture, State of Illinois Veterinary Diagnostic Laboratory, USA. The limit of detection was 0.005 ppm. An aliquot of the spike solution was sent to the lab with the samples for analysis. The quantitative recovery was 78.1% for a concentration of 115 ug/ml spike solution.

4.3 Results

4.3.1 Carcass Locations of Brodifacoum Poisoned Norway Rats

The majority (86.7%) of the radio-collared rats died underground in their burrows and therefore were unavailable to avian scavengers (Table 4.4). At 8 d following initial baiting, an adult female was found dead on the beach above the high-tide line in a puddle of water, 24 h after her last known activity.

Another radio collared adult female was tracked into the forest canopy 10 d after the start of the baiting. The remains, including the head (with the radio collar around the neck), forelegs and thoracic cage (organs removed) were found on the branch of a coastal Western Hemlock (*Tsuga heterophylla*) (dbh = 51 cm) about 10 m above the ground. The tree was located 36 m south of bait station MCB19, and >225 m from the north shoreline of Langara Island. It is unclear whether it was scavenged or preyed on. There was no evidence of ejected pellets or other remains under the tree. This rat was known to have been active 4 d before, with no evidence of further movement until the signal was tracked to the tree.

The interval between the start of the intensive baiting and detected death for 15 rats ranged from 3 to 9 d (Table 4.5). There was no significant difference ($P>0.05$) in time to death between sex and/or age class. The signals for 2 adult males could not be detected possibly because of transmitter malfunction, or them having left the region. The collar and ear-tag of a juvenile female were found under a log, while the radio of another adult female was found because either it had slipped off, or the rat had been preyed upon.

Table 4.4. Carcass locations of brodifacoum poisoned radio-tagged Norway rats (*Rattus norvegicus*), Langara Island, 1995.

Age Class	Above Ground	Below Ground	Predated or Scavenged	Unknown
Juvenile Female	0	4	0	1
Juvenile Male	0	5	0	0
Adult Female	1	2	1[a]	1
Adult Male	0	2	0	2
Total	1	13	1	4[b]
	6.7%	86.7%	6.7%	-

a Found in the forest canopy. It is unclear if it was scavenged or preyed on.
b Not included in percentage calculations.

Table 4.5. Interval between start of poisoning and detected death of radio-tagged Norway rats (*Rattus norvegicus*), Langara Island, 1995.

Age and Sex Class	Mass (g) mean[a] (range)	mean ± s.e. Days to Death Post Start of Baiting[b]	n
Juvenile Female	90 (52-145)	6 ± 1.15	4
Juvenile Male	74.4 (50-105)	8 ± 0.32	5
Adult Female	278.6 (198-353)	6 ± 1.47	4
Adult Male	237.5 (163-298)	6 ± 0.0	2
Mean		7 ± 2	

a n=5 for all age classes except Adult Male with n=4.
b Days to Death= Days to last known activity + 1 day.

Other, non-radio tagged rats, of both age and sex classes were found dead above ground and collected opportunistically by the baiting and research crews (Appendix A). The locations of all rats found above ground is in Figure 4.1.

4.3.2 Residues in Norway Rats Found Dead Above Ground

The brodifacoum residue concentrations in selected rat tissue can be found in Table 4.6. There was a significant interaction between age/sex class and tissue types ($P<0.05$) (Table 4.6). Carcass brodifacoum residue concentrations were similar across all age/sex classes. Liver concentrations were similar across all age/sex classes. The adult female liver brodifacoum residue concentration was significantly less than the juvenile male but similar to the adult male (Table 4.6).

The absolute (mg) residue in the tissues of the rats found dead above ground are presented in Table 4.7. The whole carcass residue load (mg) of the rats ranged from 0.097-1.809 mg or 0.097-1.809 bait block equivalents.

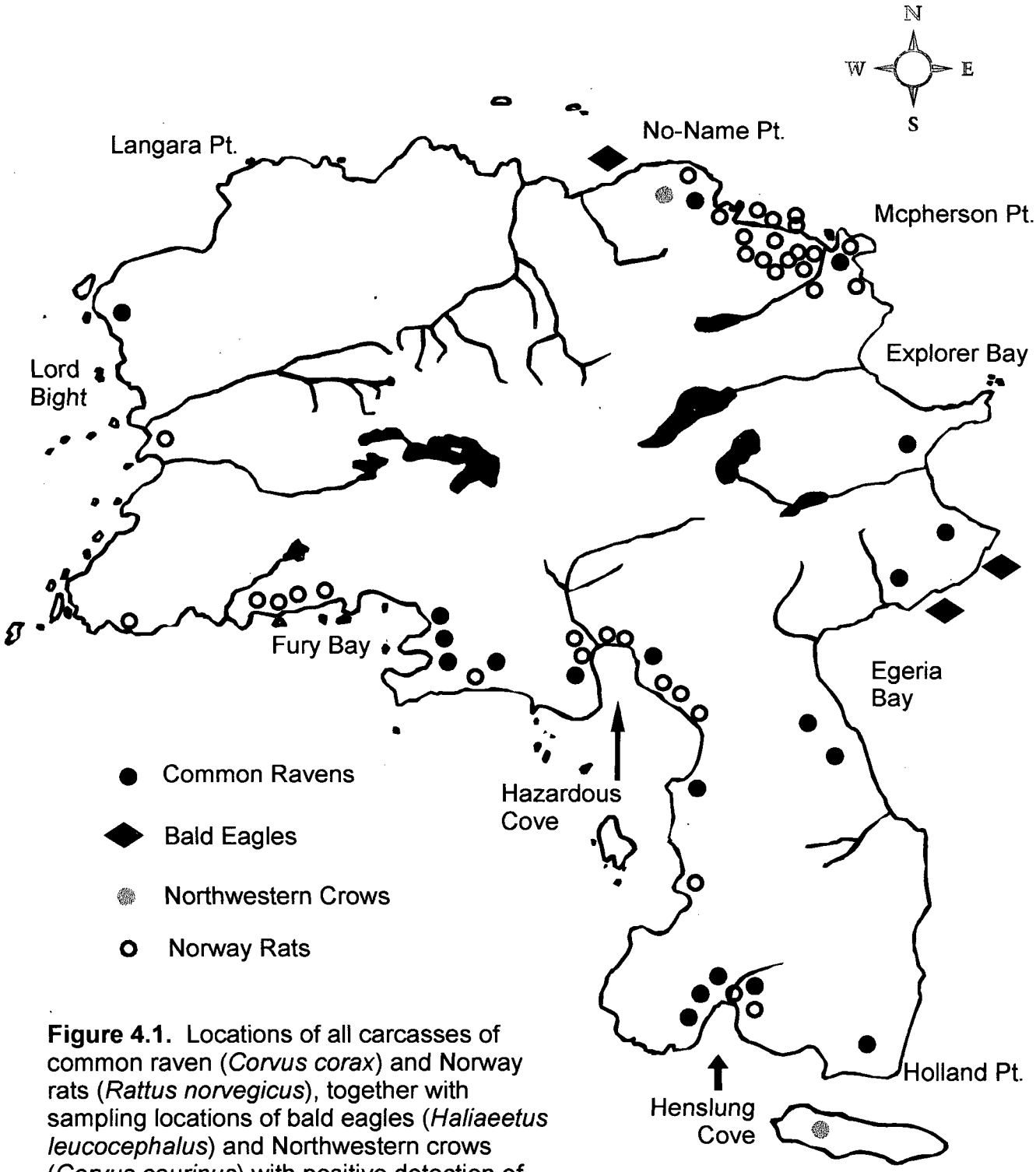


Figure 4.1. Locations of all carcasses of common raven (*Corvus corax*) and Norway rats (*Rattus norvegicus*), together with sampling locations of bald eagles (*Haliaeetus leucocephalus*) and Northwestern crows (*Corvus caurinus*) with positive detection of brodifacoum residue, 1995.

Table 4.6. Brodifacoum residue concentrations (mg/kg) in Norway rats (*Rattus norvegicus*) found dead above ground, Langara Island, 1995; (means, 95% confidence limits in brackets; means that do not share the same superscript were significantly different at $P<0.05$).

	Carcass	n	Liver	n	GIT	n	Whole Body	n
Adult Male	3.60^{ab} (2.53-4.99)	5	24.78^{def} (14.33-42.38)	5	20.03^{cdef} (13.82-28.83)	4	7.08^{g} (3.92-9.17)	4
Adult Female	1.57^{a} (0.26-4.23)	4	35.05^{def} (8.21-146.15)	3	9.94^{bc} (2.20-36.43)	4	5.61^{g} (1.39-12.19)	3
Juvenile Male	2.53^{ab} (0.21-9.31)	3	35.30^{ef} (7.12-161.21)	3	55.34^{f} (6.49-422)	3	8.63^{g} (1.77-25.97)	3

Table 4.7. Brodifacoum residues (mg) in Norway rats (*Rattus norvegicus*) found dead above ground, Langara Island, 1995; (mean± s.e., range in brackets).

Age and Sex	Carcass	Liver	GIT	Whole Body
Adult Male	0.702±0.101 (0.495-1.001)	0.235±0.033 (0.188-0.327)	0.474±0.141 (0.181-0.791)	1.472±0.260 (0.897-1.809)
Adult Female	0.439±0.138 (0.101-0.757)	0.646±0.130 (0.388-0.802)	0.270±0.078 (0.042-0.383)	1.327±0.242 (0.872-1.697)
Juvenile Male	0.058±0.021 (0.037-0.100)	0.039±0.013 (0.015-0.061)	0.108±0.053 (0.045-0.214)	0.205±0.086 (0.097-0.375)

3.3 Scavenger Identification

Common ravens were identified to be the most significant avian scavenger (80%) of Norway rats (Table 4.8). Northwestern crows also were photographed at six carcass sites and suspected of scavenging three other rats.

Table 4.8. Frequency of avian scavengers of unpoisoned Norway rat (*Rattus norvegicus*) carcasses, Langara Island, 1994.

Species	Number	Percent	Time to Scavenging
Raven	12	80	1-24 h
Crow	3	20	1-14 d
Total	15	100	

Crows, however, were also attracted to three other Norway rat carcasses after they were scavenged or buried by the necrophagous beetle *Nicrophorus* sp., or attacked by other insects (Table 4.9). Photographs of an adult bald eagle were

taken at a carcass that was not subsequently scavenged, but buried by the beetle *Nicrophorus* sp. Song sparrows (*Melospiza melodia*) were photographed at three rat carcasses that had been attacked by carrion insects.

Table 4.9. Identified scavengers of unpoisoned Norway rat (*Rattus norvegicus*) carcasses, Langara Island, 1994.

Scavenger Class	Number	Percent
Avian	15	52
Insect	5	17
Unknown	4	14
Not Scavenged	3	10
No Data	2	7
Total	29	100

Ravens scavenged carcasses within 1 h of placement for six carcasses and between 4-24 h for the remainder. The carcasses were suspected to have been scavenged by crows anywhere from 1 h to 14 d after placement. The cameras failed to take pictures of the scavenging species or took pictures for no apparent reason at 11 carcasses.

4.3.4 Effects on Predators and Scavengers

4.3.4.1 Common Ravens

A total of 13 ravens were found dead during the intensive baiting campaign between days 12 and 47 after the start of the baiting (Table 4.10 and Figure 4.1). The coastline between Fury Bay eastward to Iphigenia Point, yielded six poisoned ravens between days 13 and 20, or about 1.14 ravens/km of coastline. The east

coast from Holland Point north to Mcpherson point yielded four dead ravens or 0.38 ravens/km coastline. Three were also found in Henslung Cove late in August, 1995. All were within 300 m of the shoreline.

Liver residue analyses confirmed all 13 ravens were exposed to brodifacoum. The level of brodifacoum residue ranged from 0.985 mg/kg to 2.522 mg/kg with a mean of 1.353 mg/kg. There were no significant differences in residue levels between mature and immature birds (two tailed t-test $P>0.05$) or between male and females (two tailed t-test; $P>0.05$) (Table 4.11).

Table 4.10. Brodifacoum residues, gut contents, and sites of hemorrhage in common ravens (*Corvus corax*) found dead, Langara Island, 1995.

Raven #	Date Found	Location Found	Sex	Age[a]	Weight (kg)	Condition Score /5[b]	Brodifacoum Liver Residue mg/kg	Gut Contents Rat Hair (+/-)[c]	Gut Contents Bait (+/-)	Primary Sites of Hemorrhage
1	July 22	Mcpherson Point	F	IM	0.95	3	1.061	-	-	Pulmonary hemorrhage
2	July 24	Hazardous Cove	M	IM	1.08	3	1.490	-	-	Pulmonary hemorrhage
3	July 27	Hazardous Cove	F	M	1.20	4	1.001	-	-	Intracoelemic hemorrhage
4	July 29	Fury Bay	ND[d]	M	1.23	4	2.522	+	+	Breast muscle
5	July 28	Dadens	M	IM	1.00	3	0.985	+	-	Pulmonary hemorrhage
6	July 29	Fury Bay	ND	M	1.09	ND	1.354	-	-	ND
7	July 29	Fury Bay	M	M	1.41	3	1.223	+	-	Pulmonary hemorrhage
8	July 31	Hazardous Cove	F	M	1.22	3	1.229	+	-	Pulmonary hemorrhage
9	August 3	Dibrell Bay	F	M	1.20	4	1.729	-	-	Breast, abdomen, neck muscles
10	August 8	S. Egeria Bay	M	M	1.39	4	1.380	-	-	Free blood in caudal air sacs
11	August 23	Henslung Cove	ND	IM	1.23	3	1.187	-	+	Pulmonary hemorrhage
12	August 26	Henslung Cove	M	IM	1.42	4	1.022	-	+	Pulmonary hemorrhage
13	August 26	Henslung Cove	M	IM	1.02	4	1.410	+	+	Pulmonary hemorrhage

a- IM= Immature/ M= Mature
b- Condition Score is out of 5: 1= Emaciated; 2= Poor, 3= Fair, 4= Good, 5= Very Good
c- (+/-) denotes presence/absence of either rat hair or bait in the analysed intestines and/or gizzard contents.
d- Not determined, carcass too autolytic.

Table 4.11. Common raven (*Corvus corax*) liver brodifacoum residue levels (mg/kg), Langara Island, 1995 (geometric mean; 95% confidence interval in brackets).

Age		Sex	
Immature (n=6)	Mature (n=7)	Male (n=6)	Female (n=4)
1.18 (0.98-1.41)	1.45 (1.07-1.91)	1.24 (1.03-1.48)	1.24 (-0.82-28.05)

Necropsy revealed that 69% of the ravens had died from severe pulmonary hemorrhaging and the remainder of intramuscular or intracoelemic hemorrhaging (Table 4.12). One death could not be determined due to autolysis, however, the liver brodifacoum concentration was similar to the others.

Table 4.12. Primary sites of hemorrhage in common ravens (*Corvus corax*) found dead, Langara Island, 1995.

Primary Site of Hemorrhage	Percent	No.
Pulmonary	69%	9
Breast Muscle	15%	2
Intracoelemic	8%	1
Undetermined	8%	1

Source of Brodifacoum Poisoning to Ravens

Only seven raven gizzards contained sufficient material for protein electrophoresis. A protein band matching rat or muskrat haemoglobin was seen in one. No rat serum albumin was observed. No protein bands matching shrew controls were observed and no bands from bait or snails. Four samples showed protein matching raven albumin.

Table 4.13. Food remains in the gizzards and intestines of 13 common ravens (*Corvus corax*) found dead, Langara Island, 1995.

Contents	Frequency (%)	No.
Rat Hair	38	5
Bait	31	4[a]
Invertebrate		
Marine	46	6
Other	15	2
Vegetation		
Marine	46	6
Other	46	6
Vertebrate Bone	8	1
Unidentified	46	5

a- Hair characteristic of Norway rat also found in two.

Five (38%) of the gizzard and intestinal contents contained hair characteristic of Norway rats, including the contents from the raven which showed positive for rat or muskrat haemoglobin in protein electrophoresis (Table 4.13). The gizzard contents of one raven contained bait block fragments mixed with 11 unidentified avian bone fragments (1.09 ± 0.12 cm long x 0.46 ± 0.05 cm wide (mean±s.e.)). No rat hair was found in the gizzard or intestinal contents.

Three regurgitated raven pellets were found in Egeria Bay on day 35 post start of baiting (16.8 ± 1.9 g, 4.45 ± 0.31 cm long, 2.66 ± 0.08 cm wide (mean± s.e.)). All were situated on logs, two on the beach above the high tide line, and one under the forest canopy. The blue brodifacoum bait predominated in the pellets, although hairs characteristic of Norway rat were also found in each.

Raven Activity - 1996

Five nests were visited, however, none were active (Table 4.14). A nest in Dibrell Bay was empty and the bark and tops of branches under the nest were coated with blue tinted faecal matter. The remains of a scavenged raven were found nearby. Prey remains under the nest included chitons and 12 regurgitated pellets consisting primarily of bait and seven distinct piles (1- >100 pieces) of bait crumbs. Stretched pieces of plastic bag that had wrapped the bait were found in 58% of the pellets. Three more regurgitated pellets consisting of the blue bait were found between No-Name Point and Dibrell Bay, while searching for raven nests.

Table 4.14. Inactive common raven (*Corvus corax*) nests, Langara Island, 1996.

Nest #	Geographic Location	Year of Last Known Activity	Tree Species	Tree dbh[a]	Nest HAG[b]
1	Mcpherson Point	1995	Sitka Spruce	-	18 m
2	Mcpherson Point	-	Western Hemlock	-	10 m
3	Mcpherson Point	1994	Western Hemlock	-	7 m
4	Dibrell Bay	-	Sitka Spruce	1.16 m	15 m
5	Hazardous Cove	1995	Western Hemlock	0.73 m	7.30 m

a dbh: Diameter at breast height.
b HAG: Height above ground.

The prey remains under the nest in Hazardous Cove consisted of snails (5 *Haplotrema* sp. and 1 *Vespericola* sp.), two limpet shells, black-tailed deer hair, eight chitons and the skull of an adult Norway rat.

Plastic bags were found outside 54 of 107 bait stations investigated. Tear patterns characteristic of the raven positive controls were found on 98% of the bags. The imprint of a beak and/or bill tip of ravens were found on the aluminum trays from 12 stations. The imprints were within 1.7±0.26 (mean ± s.e.) cm of the tray edge which faced either opening of the stations. Bait crumbs were found outside stations or on top of nearby logs at nine bait stations.

The remains of seven more ravens were found or reported including a fresh, dead raven at the top of the beach in Hazardous Cove (Table 4.15). Necropsy results confirmed its death was due to severe, bilateral pulmonary hemorrhaging.

Four pairs of ravens were observed flying along the north end of the island between Langara Lightstation and Mcpherson Point, in Egeria Bay, Hazardous Cove, and at Lord Bight on the west coast. This indicates that some ravens were still alive on Langara Island in 1996.

Table 4.15. Dates, location and condition of common ravens (*Corvus corax*) found or reported dead, Langara Island, 1996.

Raven #	Date	Location	Condition	Source
1	April	Radar Point	Skeletal remains	J. Schweers, pers. comm.
2	May	Dibrell Bay	Scavenged/Feathers/Bones	Author
3	May	No-Name Point	Feathers	J. Elliott, pers. comm.
4	May	Henslung Cove	Skeletal remains	Author
5	May	Egeria Bay	Skeletal remains	Author
6	May	Hazardous Cove	Fresh	Author
7	August	Egeria Bay	Skeletal remains	G. Kaiser, pers. comm.

4.3.4.2 Northwestern Crows

A summary of information on the crows collected is in Appendix Table 4-3. Brodifacoum was detected in one pooled sample of crow livers collected from Lucy Island, 12 d after the start of the baiting campaign in 1995 (Table 4.16). A crow was found dead in Henslung Cove on August 7, 1995 but no brodifacoum was detected. Trace amounts of brodifacoum (0.048 mg/kg) were detected in one crow collected in May 1995 before the intensive baiting began on Langara Island.

Table 4.16. Brodifacoum residue levels in livers of Northwestern crows (*Corvus caurinus*), Langara Island, 1995.

Pool No.	Crow #'s	Brodifacoum (mg/kg)
1[a]	1	0.048
2[a]	15, 16	ND[c]
3	2, 3, 4, 5	ND
4	6, 7, 8	0.019
5	9, 10, 11	ND
6	12, 13, 14	ND
7	15, 16	ND
8[b]	17	ND
6	18	ND
9	19, 20, 21	ND
10	22, 23, 25	ND
11	24	LA[d]

a Collected in May 1995.
b Found dead in Henslung Cove.
c None detected. Detection limit <0.01 mg/kg.
d Lost in analysis.

4.3.4.3 Bald Eagles

A total of 22 bald eagles were trapped; two before the baiting began, and 20 over the course of the baiting for a 36% trapping success rate. Another eagle was

rescued from a surge channel at No-Name Point 3 d before the eradication program. Mainly adult birds were targeted although four young of the year were also trapped. The overall sex ratio was 13 males:10 females. A detailed summary of eagles trapped is in Appendix Table 4-2.

Brodifacoum was detected in the plasma of three individuals (15%). Each was from a different age class and two were female. The greatest plasma residue was detected in the subadult trapped at Cohoe Point (Table 4.17 and Figure 4.1).

Table 4.17. Bald eagle (*Haliaeetus leucocephalus*) plasma brodifacoum residues and prothrombin times, Langara Island, 1995.

No.	Date	Location	Sex	Age Class[a]	Brodifacoum Residue (mg/kg)	PT (secs)
1	July 5	Margaret Point	M	A	ND	-
2	July 6	Dibrell Bay	F	A	ND	-
3	July 7	No-Name Point	M	SA	ND	-
4	July 18	Explorer Bay	M	A	ND	-
5	July 19	Mcpherson Point	M	A	ND	-
6	July 23	Iphigenia Point	M	OI	ND	-
7	July 31	Iphigenia Point	F	A	ND	-
8	August 1	No-Name Point	M	YI	0.041	-
9	August 1	No-Name Point	F	SA	ND	-
10	August 3	No-Name Point	M	A	ND	147
11	August 4	No-Name Point	F	A	ND	261
12	August 10	Egeria Bay	F	A	0.037	-
13	August 10	Cohoe Point	M	A	ND	177
14	August 10	Cohoe Point	M	A	ND	201
15	August 10	Dibrell Bay	F	A	ND	234
16	August 11	No-Name Point	M	SA	ND	224
17	August 12	Andrews Point	M	A	ND	151
18	August 13	Cohoe Point	F	SA	1.74	221
19	August 14	Andrews Point	M	YI	ND	284
20	August 15	Margaret Point	F	YI	ND	185
21	August 25	Iphigenia Point	F	OI	ND	122
22	August 26	Dadens	F	A	ND	226
23	August 26	Henslung Cove	M	YI	ND	122
					Mean ± s.e.	197 ± 14

a A= Adult; SA= Sub-Adult; OI= Old Immature; YI= Young Immature (Bortolotti 1984)

PTs were not performed for the adult and young immature bird exposed to brodifacoum either due to lack of blood collected, or because lipids in the plasma made them unsuitable for testing. The subadult eagle PT was 221 s (Table 4.17). The mean PT for all eagles was 197 s which was significantly longer (one tailed t-test $P<0.05$) than the mean control PT of 125 s (Table 4.18).

Table 4.18. Bald eagle (*Haliaeetus leucocephalus*) control prothrombin times.

Number	Sex	Age[a]	PT (secs)
1	M	YI	70
2	F	YI	177
3	M	OI	151
4	M	YI	125
5	M	YI	59
6	F	A	149
7	F	OI	108
8	F	YI	146
9	F	YI	145
10	M	YI	145
11	M	YI	97
		mean ± s.e.	125 ± 11

a YI= Young Immature; OI= Old Immature; A= Adult

4.4 Discussion

4.4.1 Carcass Locations of Brodifacoum Poisoned Norway Rats

A total of 13.4% of radio collared Norway rats died above ground and all were adult females. This is likely an underestimate of the actual frequency of death above ground, because other non-radio collared rats found dead above ground included all age and sex classes. The location of death does not appear to be age or sex related. In 1994, one of three radio-collared rats died above ground after the poisoning operation on Lucy Island (Howald 1995). These results are in apparent contrast to New Zealand operations where all 16 radio-collared Norway rats on Ulva Island died in their burrows (Taylor 1993) and no poisoned rats were found on the surface of Hawea Island (Taylor and Thomas 1989). Further, only four Norway rats were found above ground on Breaksea Island in New Zealand after similar brodifacoum baiting for rats (Taylor and Thomas 1993). However, there was no indication of a quantified search method in these studies.

During anticoagulant poisoning operations on farms in England, a small proportion (%) of the Norway rat population was found to have died above ground, the majority died below ground (Harrison et al. 1988; Fenn et al. 1987). In laboratory enclosure trials with wild Norway rats, 67% were reported to have died in open areas after receiving a lethal dose of brodifacoum (Cox and Smith 1992). Similarly, Gemmeke (1990) showed that captive anticoagulant poisoned rodents died above ground as often as below. More work is required in this area to accurately determine

the proportion of rats that die above ground, thus making themselves available to scavengers.

The remains of the adult female found in the tree confirms that some rats were either scavenged or preyed on. The scavenger or predator could not be identified but the location of the tree 225 m inland suggests that a raven was likely responsible. Bald eagles were rarely observed under the forest canopy after the ancient murrelets had left the colony in mid-June. Ravens, however, were regularly observed in the forest and were identified as the most significant scavenger of rats.

The mean time to death for the radio-collared rats was 7 d after the start of the baiting program. Although the precise length of the latent period was not known, it does indicate that the majority of rats were dead and available to scavengers a week after the baiting program began. For predators, the greatest opportunity to catch a live but toxic rat, was within the first week of the baiting campaign.

4.4.2 Residues in Norway Rats Found Dead Above Ground

The presence of brodifacoum in the carcasses of poisoned rats found above ground poses a secondary poisoning hazard to the identified scavengers. The liver contained the greatest concentration of brodifacoum as compared to the carcass, but was not significantly greater than the GIT. This preferential storage in the liver is consistent with other anticoagulants such as Warfarin, bromadiolone and flocoumafen (Huckle et al. 1989a; Huckle et al. 1989b; Lechevin and Vigie 1992; Newton et al. 1990). The liver is the target organ for anticoagulant rodenticides where they act to

prevent the reduction of the vitamin K epoxide by inhibiting the epoxide reductase enzyme (Mount 1988). However, the liver constitutes only 4.1% by weight of the rat, while brodifacoum is present throughout the carcass. Thus, adult rats present more of a hazard than smaller rats because of their weight which allows for greater brodifacoum accumulation.

The GIT was the tissue that resulted in the significant interaction effect between age/sex class and tissue type. This was likely a result of the presence of unassimilated bait found in the alimentary tracts of some individuals. On dissection, some stomachs were found to be packed with bait and the intestines were tinted with the colour of the bait. The juvenile males contained the greatest concentration of brodifacoum in the GIT suggesting that they preferred and continued to feed on the bait even after hemorrhaging had begun. Adult females appeared to contain the least amount of brodifacoum concentration and this may reflect the demands placed on her, such as providing for the pups by bringing back bait to the burrows. This could account for the high concentration in the juvenile GITs.

The mean whole body brodifacoum residue concentrations found in the rats on Langara seemed to be the highest yet reported and appears to reflect the saturation baiting strategy used. Dubock (1984) reported a mean carcass residue level of 3.2 mg/kg in rats after saturation baiting with brodifacoum, although 8% contained a concentration above 10 mg/kg. Merson et al. (1984) live trapped, euthanised and analysed the whole body brodifacoum burdens of meadow voles (*Microtus pennsylvanicus*) and found them to range from 2.07± 0.17 mg/kg (mean±s.e) to

4.07± 0.20 mg/kg after aerial application of bait. In laboratory trials, brodifacoum concentrations of voles fed 10 mg/kg brodifacoum ranged from a mean of 0.40 to 0.53 mg/kg, while voles fed 50 mg/kg brodifacoum bait ranged from a mean of 2.17 to 5.21 mg/kg (Kaukeinen 1982). In general, carcass residue concentrations are positively related to the rate of anticoagulant application (Merson et al. 1984; Dubock 1984).

The distribution of brodifacoum across different tissues may provide a degree of secondary poisoning protection, if there is rejection of any of those tissues. For example, golden eagles were observed to reject the stomach and entrails of strychnine poisoned Richardson ground squirrels (*Spermophilus richardsoni*) resulting in no detectable strychnine poisoning impact to any eagles (Graham 1977). If a brodifacoum poisoned rat is eviscerated and the GIT not consumed, between approximately 30-50% of the total brodifacoum residue is thereby avoided. However, there was wide variability between each age and sex class in tissue retention of brodifacoum. This variation may be due to the small sample size and further work is required.

4.4.3 Common Ravens

Common ravens were the most significantly impacted species in the study. Although no population estimates were made, the 20 ravens found dead between July 1995 and August 1996 were likely a minimum number affected. Carcass searching studies have had low success rates, due to the efficiency with which scavengers and

predators removed the carcasses, as well as the low efficiency of human searchers (Linz et al. 1991; Brown et al. 1988; Mineau and Collins 1988; Balcomb 1986; Stutzenbaker 1984). Therefore, the impact on the raven population can only be estimated.

On Saltspring Island, Brenchley (1985) estimated the minimum raven nesting density to be 26/100 km^2 . A high estimate relative to Europe where 17 nests/100 km^2 was the highest estimated density (Newton et al. 1982). Assuming Langara could sustain similar densities, there may have been five to eight territorial, nesting pairs with between three to seven fledglings each (Ehrlich et al. 1988) before the baiting began. Therefore, the pre-baiting common raven population may have been from 20 to 72 individuals, and the impact of the poisoning operation on the local raven population appears to have been heavy. However, the presence of four pairs in 1996 indicates that some individuals survived, or that empty territories were taken over by immigrants from nearby Graham Island.

Necropsy Results and Liver Residue Analyses

The necropsies confirmed that all ravens died of acute, multifocal hemorrhage, symptomatic of anticoagulant poisoning (M. Mcadie, pers. comm.). These results are in accordance with other studies investigating anticoagulant poisoning in avian predators (Mendenhall and Pank 1980; Newton et al. 1990). The body condition scores indicate that none of the birds were emaciated or in poor condition, and there

was no evidence of disease that may have predisposed the ravens to the effects of brodifacoum.

The role of brodifacoum poisoning could not be determined when only feathers and/or skeletal remains were found. Some mortality may have occurred over the winter, independent of brodifacoum use. However, the locations and evidence where individuals were found suggests that brodifacoum poisoning was the ultimate cause of death, even though predation may have been the proximate cause or they were scavenged after death.

As brodifacoum binding in the liver is a saturable process resulting in a steep dose response (Thijssen and Baars 1989; Godfrey 1985), and death from anticoagulants is delayed allowing for some metabolism and excretion of the ingested dose (Godfrey 1985), the relationship between the concentration measured and the initial exposed dose is complex and difficult to interpret. Godfrey et al. (1985) found that liver levels are unsuitable for quantifying exposure to brodifacoum because of the lack of correlation between liver residue concentration and dosing levels. However, it can be concluded that the positive analysis of brodifacoum in the liver together with the necropsy results are symptomatic of brodifacoum poisoning.

Source of Brodifacoum Poisoning to Ravens

Some raven mortality from scavenging dead rats was expected, however, primary poisoning from raiding bait stations was not. The first indication that primary poisoning contributed to raven mortality came after the bait spill at the Langara Fishing

Lodge in Henslung Cove. Two to three 20 l buckets containing old bait blocks awaiting incineration, were left open and unattended overnight. The following morning, the fishing lodge manager reported a "sea of black" ravens picking up bait blocks in their bills and flying off with them. Over the next 4 d, three ravens were found dead and two were observed falling off their roost and picked up dead. This may have been an isolated primary poisoning event because it was not uncommon to have piles of garbage accumulate before incineration, and to observe ravens work open holes in bags and boxes. The presence of the bait buckets with open lids presented an easy opportunity for ravens to investigate a new food source. The attraction to the bait indicates the palatability of this toxic food source to ravens, however, it is not known if ravens were responsible for raiding bait stations during the intensive baiting in 1995. There were no reports of ravens raiding bait stations during the initial campaign and stations were not investigated as a source of poisoning. If ravens were removing bait from stations, it would have been assumed rats were responsible.

The beak marks on the aluminum trays, regurgitated pellets of bait containing bits of plastic bag, and torn bags found outside bait stations, confirmed that ravens raided bait stations over the winter of 1995/1996. The systematic checking of bait stations by operators might have been observed by ravens and led to their investigating the stations, finding the bait blocks, and being primarily poisoned. Ravens preyed on pinyon jay (*Gymnorhinus cyanocephalus*) eggs and young within 24 h after researchers had climbed trees with nests (Marzluff and Balda 1992).

Similarly, in the Okanagan Valley of British Columbia, ravens have been observed systematically checking orchards for songbird nests, perhaps imitating researchers studying them (A. Preston pers. comm.).

In May 1996, eight bait stations, including four all in order and along a line, that were checked and re-armed 2 d previously, had the bait removed. The design of the stations and how they were staked down, allowed ravens to employ one of three tactics to gain access to the bait in them: reaching into the station and pulling the aluminum tray and bagged bait blocks out, lifting one end of the station so that the bait rolled closer to either opening, or uprooting the stations from the stakes so the bait rolled out. In New Zealand, western wekas and keas (*Nestor notabilis*) have been observed reaching into the stations and pulling bait out (Eason and Spurr 1995; Taylor and Thomas 1993). However, the action of the ravens on Langara is apparently the first reported case of non-target species completely disrupting bait stations to gain access to the bait. Clearly, the bait stations were inadequate to exclude common ravens and need to be modified for future rat eradication programs.

The primary and secondary poisoning appears to have had a large impact on the local common raven population on Langara Island. In New Zealand, the entire population of western weka was exterminated from Tawhitinui Island after feeding on brodifacoum bait intended for ship rats, and by eating dead or dying rats (Taylor 1984 in Eason and Spurr 1995). Similarly, 80-90% of Stewart Island weka were primarily and secondarily poisoned (Eason and Spurr 1995). Both wekas and common ravens

share an aggressive and inquisitive behaviour and opportunistic, omnivorous diet (Falla et al. 1983; Ehrlich et al. 1988) which contributed to their decline in population.

The gizzard analysis confirmed that ravens were not consuming exclusively either rats or bait during the initial campaign but the content analysis is not a sensitive enough indicator to determine the source of poisoning. No rat hair, rat protein or bait were detected in six dead ravens in 1995 probably because of the delayed time to death after ingestion of brodifacoum allowing for evacuation of the GIT contents (Godfrey 1985). Captive barn owls feeding on brodifacoum poisoned rats died between 5-6 d after the last poisoned rat was consumed (Mendenhall and Pank 1980). It is possible, however, that brodifacoum exposure may have originated from another source such as invertebrates found feeding on the bait (See Chapter 5).

The presence of bait crumbs along with Norway rat hairs may indicate that ravens were both primarily and secondarily exposed to brodifacoum, or that the bait crumbs originated from the rats. The latter seems more likely because on dissection, rat stomachs and intestines were laden with bait crumbs. However, the presence of bait crumbs without the detection of Norway rat hair, does not preclude the possibility of secondary poisoning. For ravens 11 and 12, where only bait crumbs were detected in their gut contents, it seems probable that primary poisoning was the cause because they were found within 4 d after the bait spill in Henslung Cove.

Secondary Poisoning and Toxicological Significance

Rats were confirmed as a source of brodifacoum to ravens, however, it is unclear if one or more rats were required to impair hemostasis and cause death. The

LD_{50}, the single acute, oral dose required to cause death to 50% of a population, is used as an index of toxicity and demonstrates a pharmacological risk of brodifacoum. The LD_{50} of brodifacoum for the raven is unknown, however, I calculated a value that offers 95% species protection, with 95% and 50% confidence, from 10 published brodifacoum LD_{50} values representing nine major families of birds (Aldenberg and Slob 1993). In other words, the LD_{50} with 95% and 50% confidence estimate for 95% species protection based on published values (Table 4.19). For any unknown species the LD_{50} would be above 0.56 mg/kg (50% confidence) or 0.105 (95% confidence). The closest related species to the raven with published LD_{50} values are from the Order Passeriformes, specifically the house sparrow (*Passer domesticus*) with an LD_{50} >6 mg/kg, blackbird (*Turdus merula*) and hedge sparrow (*Prunella modularis*) >3 mg/kg (Godfrey 1985). These values represent the highest dose administered.

Caution must be used when estimating hazard because the close taxonomic relation cannot be consistently used to predict the sensitivity of birds to pesticides (Hill 1994). Therefore, the LD_{50} range for ravens may be between 0.56 and >6 mg/kg. With a mean weight of 1.19 kg for all ravens found dead over the summer of 1995, as little as 0.69 mg to > 7.14 mg brodifacoum could result in a 50% chance of lethal hemorrhaging. A single adult rat, with a mean residue level of 1.4 mg, would result in the equivalent dose of 1.17 mg/kg if consumed whole. Captive common ravens fed diphacinone poisoned adult rats consumed everything except the skin and bones (J. Marzluff, pers. comm.). However, small rats were eaten whole and a pellet was produced. The regurgitation of a pellet would decrease the amount of brodifacoum

ingested and possibly, decrease the risk of hemorrhaging. Barn owls feeding on brodifacoum poisoned mice and producing pellets, reduced the amount of consumed rodenticide by an average of 25%, thus lowering their risk of hemorrhage (Gray et al. 1994). Assuming ravens regurgitated a pellet containing 25% brodifacoum, this would decrease the single brodifacoum dose to 0.88 mg/kg. This would still be within the estimated LD_{50} range, thus a single adult rat may pose a secondary poisoning hazard to ravens.

Primary Poisoning and Toxicological Significance

The toxicological significance of bait blocks to ravens is difficult to accurately interpret because ravens regurgitate a pellet (due to the high, indigestible, paraffin wax content) that likely contains brodifacoum. Each block contains 1mg brodifacoum, enough for a raven to have a 50% chance of haemorrhaging, if all the brodifacoum was ingested. Obviously, the chance of lethal poisoning increases with the number of blocks that are consumed, and as each station was armed with three bait blocks over the winter months, each raven was exposed to as much as 3 mg brodifacoum at each bait station it raided. It seems probable that any one raven upon discovery of the easy food source found in a bait station, continued to raid more than one station.

Table. 4.19. Species and LD_{50} values used for calculating the value that offers 95% bird species protection with 95% and 50% confidence limits.

Common Name	Latin Name	LD_{50} (mg/kg)	Reference
Black-Backed Gull	*Larus dominicanus*	< 0.75	Godfrey 1985
Pukeko	*P. porphyrio melanotus*	0.95	Godfrey 1985
California Quail	*Callipepla californica*	3.3	Godfrey 1985
Mallard Duck [a]	*Anas platyrhynchos*	2.3	Godfrey 1985; Ross et al. 1978
Harrier Hawk	*Circus approximans*	10.0	Godfrey 1985
Ring Necked Pheasant	*Phasianus colchicus*	10.0	Godfrey 1985
Paradise Shelduck	*Tadorna variegata*	> 20.0	Godfrey 1985
Chicken [a]	*Gallus gallus*	3.15	Ross et al. 1977; Taylor 1993
Japanese Quail	*Coturnix japonica*	11.6	Ross et al. 1976

a Mean of LD_{50} values in literature.

Confidence limits for hazardous concentrations based on logistically distributed brodifacoum LD_{50} value calculated following Aldenberg and Slob (1993). Two rules were followed as employed at the National Wildlife Research Centre (P. Mineau, pers. comm.):

1. Include known absolute LD_{50} values. Do not use < or > LD_{50} values if they are within the range of the absolute values.
2. If < or > LD50 values fall outside of absolute LD_{50} range, include and new range has been established.

The 95% and 50% confidence limit for brodifacoum LD_{50} data is 0.11 mg/kg and 0.56 mg/kg.

4.4.4 Northwestern Crows

Brodifacoum was detected in low levels in the sampled crows. The crow collected in May 1995 was exposed to brodifacoum from Lucy Island because brodifacoum was not available on Langara Island until July 1995. It is unknown if exposure occurred during the baiting on Lucy Island in 1994, or if exposure occurred after the bait was removed from the stations in August 1994. The biological half-life of brodifacoum has been estimated to range between 120-220 d (Godfrey 1985; Parmar et al. 1987). With such a range, exposure may have occurred during the baiting on Lucy Island. Brodifacoum was still present and available on Lucy Island 9 months after the removal of the bait from the stations. In May 1995, four old bait blocks (9.6 g) were found under a log on the west side of Lucy Island and contained 10.99 mg/kg brodifacoum. Snails found near the bait were collected and contained a concentration of 0.910 mg/kg brodifacoum. Therefore, it is possible that crows could have been exposed to brodifacoum from preying on invertebrates, that had fed on old bait, during the winter of 1994/1995.

Inspection of the adult rat carcasses visited by crows, but not scavenged, revealed patches of missing hair. The rats had also been moved from their original placement. The photographs revealed that crows were grabbing the carcasses by the tails, legs and flanks. The crows were apparently unable to fly off with the larger rats and were unable to break the skin of fresh, dead rats. Thus, brodifacoum exposure from scavenging rats was minimised by the crow's inability to carry whole rats to their

perches for consumption, and their inability to break the skin to gain access to the contaminated flesh.

4.4.5 Bald Eagles

Bald Eagles were confirmed to have been exposed to brodifacoum but no mortality was detected during the intensive baiting in 1995. Similarly, no evidence of lethal poisoning to New Zealand falcons (*Falco novaeseelandiae*) or moreporks (*Ninox novaeseelandiae*), the main avian predators at risk, was noted on Breaksea Island in New Zealand, though they did prey on rats (Taylor and Thomas 1993). Further, comparable numbers of southern great skuas (*Catharacta lonnbergi*) and New Zealand Falcons were seen before and after a similar baiting operation on Hawea Island in New Zealand (Taylor and Thomas 1989), but, these numbers could have been maintained by immigration.

Eagles were not attracted to rat carcasses put out for them, but may have preyed on rats during the latent period. Eagles are known to take rodents and other mammals associated with a body of water, such as muskrats (*Ondatra zibethicus*) (Knight et al. 1991; Watson et al. 1990). On the San Juan Islands, rabbits were identified as the eagle's main prey item (Retfalvi 1970). Eagles spend much of their time perching on trees overlooking the water and shoreline (Gerrard and Bortolotti 1988) and may have opportunistically preyed on a rat if it displayed symptoms of anticoagulant poisoning near or in the intertidal zone. Rats were likely available for diurnal eagles because 35% of rats were live-trapped during daylight h and were

known to utilise the beaches, because the contents of a rat burrow on Lucy Island contained a carapace and legs of a shorecrab (Howald 1995). One of the radio-collared rats (see above) and other rats were found dead on or near the beach, and two rats were found to have died sometime in the afternoon, including one on the beach edge above the winter high tide line.

The apparent low brodifacoum exposure of the bald eagle population was likely due to the highly productive marine environment around Langara Island which is the major, if not exclusive, foraging area. Bald eagles were commonly observed fishing for Pacific sandlance (*Ammodytes hexapterus*), Pacific herring (*Clupea harengus*) and salmon (*Oncorhynchus* spp.). In 1994, over 100 eagles were observed fishing off the west coast of Langara Island. Knight et al. (1990) reported that, for coastal nesting bald eagles, seabirds are the predominant prey item. The spatial separation of the foraging and baiting areas reduced the risk of poisoning to bald eagles. Likewise, the potential for barn owl mortality with brodifacoum baiting around farm buildings appeared to be low because they preyed on rodents in grasslands away from the buildings (Hegdal and Blaskiewicz 1984).

The remains of five eagles (4 juveniles and 1 adult) were found in 1996. However, the carcasses were too autolytic to determine cause of death. The talons exhibited multiple focal areas of reddish discoloration, possibly representing excoriation of scales or subcutaneous hemorrhage (M. Mcadie, pers. comm.) an indication of possible brodifacoum exposure. However, the talons were too desiccated to distinguish between lesions and artefacts of post mortem change. The

lesions were similar to those seen in thermal burns from electrocution (M. Mcadie, pers. comm.) but there are no electrical power lines on or near Langara Island.

Although eagle mortality was detected 10 months after the initiation of the baiting program, the role of brodifacoum poisoning could not be ascertained. Brodifacoum may have been responsible for some mortality because of the widespread primary poisoning of ravens which, in turn, could have been preyed on or scavenged by eagles. A dead bald eagle was reported next to the remains of a scavenged raven near the lightstation (J. Schweers, pers. comm.). At least three other remains of ravens were scavenged, however, the scavengers could not be identified. Food items collected under bald eagle nests in the San Juan Islands included common ravens and Northwestern crows (Knight et al. 1990).

It is important to note that winter mortality of eagles would likely have occurred independent of brodifacoum use. Overwinter mortality rates for eagles in Alaska ranged from 5% to 29% (Bowman et al. 1995). It was estimated that 46% of first-year eagles, and 9% of adults died over the winter in Maine (McCollough 1982; 1986 in Gerrard and Bortolotti 1988). On Langara Island, it was not uncommon to find bones and/or carcasses of bald eagles before baiting began.

The effect of brodifacoum poisoning on the breeding population appears to have been negligible. Nesting success declined from 57% in 1995 to 35% in 1996; but there was a decline from 39% to 10% nesting success in South Moresby National Park in the Queen Charlotte archipelago for the corresponding period (J. Elliott, pers. comm.).

Toxicological Significance of Residue Levels and Prothrombin Times

No brodifacoum LD_{50} data are available for bald eagles. The above estimate of 0.56 mg/kg is likely low for bald eagles because the mean LD_{50} for two more closely related species, the harrier hawk (*Circus approximans*) -10.0 mg/kg) and the American kestrel (*Falco sparverius*) -8.20 mg/kg (Taylor 1993) is much higher. Thus, using a mean weight of 4.5 kg (Range: 3.6 - 5.6 kg) for all bald eagles sampled, the dose required for a 50% chance of lethal hemorrhaging may be as low as 2.52 mg (LD_{50}: 0.56 mg/kg) but is more likely around 39 mg brodifacoum. This is equivalent to 1.4 - 21.6 adult male rats each with a brodifacoum residue loading of 1.809 mg. Thus, the risk of an eagle hemorrhaging after ingesting a single rat appears to be low.

The 4.3 kg sub-adult eagle trapped on day 33 contained the highest concentration of brodifacoum (1.74 mg/l plasma). Assuming the plasma volume (PV) in bald eagles is similar to that in red-tail hawks (mean of 3.5 ml/100g body weight, Bond and Gilbert 1958), the sub-adult eagle would have a PV of 0.151 litres. This yields a total plasma brodifacoum loading of 0.262 mg at the time when the blood sample was drawn. The immature and female bald eagles had total plasma brodifacoum loadings of 0.0052 mg and 0.0073 mg respectively.

The plasma brodifacoum levels, however, are a poor indicator of the initial dose (Appendix 1; Huckle et al. 1989b). The plasma brodifacoum loading of Japanese quail (*Coturnix japonica*) 24 h post dosing with brodifacoum at 1.4, 0.7 and 0.35 mg/kg, represented only 1.16%, 0.32% and 0.19% of the original dose respectively (Appendix 1). Assuming that quail are a representative model and that the eagles

were trapped and a blood sample drawn around 24 h after exposure, a rough estimate of the original dose can be made. The plasma concentration of the sub-adult eagle (1.74 ppm) was greater than the plasma concentration of the Japanese quail at 24 h post dosing, therefore, it was likely exposed to more than 1.4 mg/kg brodifacoum or more than 6 mg brodifacoum, which is equivalent to more than 3.3 rats (1.809 mg brodifacoum/rat). Again, a single rat was not likely the source of brodifacoum, but more likely having come from consuming a raven or several rats.

The PT is used to screen for deficiencies in the vitamin-K dependent clotting factors. The use of a mammalian thromboplastin for avian PT determinations results in wide variation within and among samples (Appendix A; Griminger 1986). As well, a sub-lethal exposure to brodifacoum would likely not have been detected because a 30% concentration of these factors may provide a normal PT (Brown 1988; Hoffman et al. 1988). As well, a markedly increased PT would likely have corresponded to a positive detection of brodifacoum residue in the plasma. Therefore, it cannot be concluded that the bald eagles were at risk of hemorrhaging because a significantly longer PT was detected. Savarie et al. (1979) found golden eagles (*Aquila chrysaetos*) experienced increases in the PT from the control time of 23 s to 900 s (39 times longer than the control time) without mortality after feeding on diphacinone contaminated meat. Some of those golden eagles, however, did experience hemorrhaging.

The effectiveness of the mammalian thromboplastin to detect a significant increase in PT was investigated (Appendix A). The thromboplastin kit was able to

detect a significant increase in the PT of Japanese quail dosed with brodifacoum. The quail had a significant longer PT at 2.5 times the mean control time at 72 h post dose. Assuming quail are an effective model, no eagles were clinically at risk of hemorrhaging, i.e., all were less than 2.5 times the control time, as detected by the Coulter PT fibrinogen kit. The risk of hemorrhaging to the exposed individuals appeared to be low.

4.5 Conclusions

Secondary and primary poisoning studies can be divided into three levels as defined by Colvin and Hegdal (1987): hazard to individuals, short term population effects and long-term population effects. This study was designed to address the hazard to scavengers and predators at an individual level. A secondary poisoning hazard to avian predators and scavengers does exist from the use of brodifacoum to eradicate rats from seabird colonies along the British Columbia coast. Norway rats were shown to die above ground and be available for avian scavengers and predators. Common ravens were the species most significantly impacted as a result of both primary and secondary poisoning. The population effect remains unquantified, however, breeding pairs were observed alive one year after the baiting program was initiated. There was a low risk of brodifacoum poisoning to bald eagles from dead toxic rats, although plasma brodifacoum levels established that brodifacoum was present in the food chain.

Appendix Table 4-1. Dates, locations, weight, age and sex of Norway rats (*Rattus norvegicus*) found dead above ground, Langara Island, 1995.

Rat #	Date Reported/ Turned In	Location Found	Weight (g)	Age Class	Sex
1	-	Mcpherson Pt.	-	A	F
2	-	Mcpherson Pt.	-	J	M
3	-	Mcpherson Pt.	-	A	F
4	-	Mcpherson Pt.	-	J	M
5	-	Lord Bight	222	A	M
6	July 13	Hazardous Cove	150	J	M
7	July 14	No-Name Pt.	-	A	M
8	July 14	Hazardous Cove	58	J	M
9	July 15	Mcpherson Pt.	102	J	M
10	July 15	Mcpherson Pt	52	J	M
11	July 15	Mcpherson Pt.	-	J	F
12	July 15	No-Name Pt.	390	A	F
13	July 16	Mcpherson Pt	120	J	F
14	July 16	No-Name Pt.	43	J	M
15	July 18	No-Name Pt	351	A	F
16	July 18	Hazardous Cove	278	A	M
17	July 18	Mcpherson Pt.	373	A	F
18	July 18	Mcpherson Pt.	62	J	F
19	July 19	Mcpherson Pt.	21	J	M
20	July 19	Mcpherson Pt.	20	J	M
21	July 20	No-Name Pt.	35	J	M
22	July 20	No-Name Pt.	25	J	-
23	July 20	Henslung Cove	285	A	M
24	July 20	Hazardous Cove	220	A	M
25	July 20	Hazardous Cove	35	J	M
26	July 20	Fury Bay	326	A	M
27	July 20	Fury Bay	225	A	F
28	July 20	Fury Bay	26	J	-
29	July 20	Fury Bay	22	J	M
30	July 20	Fury Bay	25	J	M
31	July 21	Hazardous Cove	-	-	-
32	July 22	Henslung Cove	-	-	-
33	July 24	Hazardous Cove	-	-	-
34	July 25	Hazardous Cove	-	-	-
35	July 25	Hazardous Cove	-	-	-

Appendix Table 4-2. Dates, locations, and selected morphological measurements of bald eagles (*Haliaeetus leucocephalus*) trapped on Langara Island, 1995.

No.	Date	Location	Weight kg	Sex	Age Class	Bill Depth mm	Hallux mm	Culmen cm	Tarsus Diameter cm	Tail Length cm	Wing Chord cm
1	July 5	Margaret Point	-	M	A	35.2	37.4	4.7	1.60	-	-
2	July 6	Dibrell Bay	-	F	A	39.5	42.0	-	1.35	-	-
3	July 7	No-Name Point	4.4	M	SA	35.2	36.4	5.06	1.36	-	-
4	July 18	Explorer Bay	4.2	M	A	33.2	40.2	5.06	1.59	26.1	56.5
5	July 19	Mcpherson Point	3.9	M	A	33.5	39.0	-	1.57	-	59.5
6	July 23	Iphigenia Point	-	M	OI	32.2	40.2	4.94	1.30	29.4	60.5
7	July 31	Iphigenia Point	5.1	F	A	38.1	46.0	5.96	1.56	30.1	62.7
8	August 1	No-Name Point	3.6	M	YI	32.0	39.0	4.32	1.82	31.6	60.1
9	August 1	No-Name Point	5.4	F	SA	38.9	41.9	6.06	1.64	33.0	63.5
10	August 3	No-Name Point	4.5	M	A	34.0	40.8	5.02	1.47	28.3	60.5
11	August 4	No-Name Point	5.2	F	A	38.7	46.2	5.66	1.49	28.8	62.3
12	August 10	Egeria Bay	5.6	F	A	37.9	44.4	5.78	1.63	28.4	63.5
13	August 10	Cohoe Point	3.8	M	A	35.0	39.3	4.89	1.31	28.1	59.4
14	August 10	Cohoe Point	5.2	M	A	32.6	43.4	5.55	1.75	29.5	61.1
15	August 10	Dibrell Bay	5.3	F	A	37.9	43.2	5.65	1.55	27.8	60.2
16	August 11	No-Name Point	4.1	M	SA	32.9	38.7	4.91	1.34	28.6	61.5
17	August 12	Andrews Point	4.2	M	A	32.9	40.8	5.22	1.42	29.2	60.4
18	August 13	Cohoe Point	4.3	F	SA	34.7	41.6	5.35	1.50	29.9	59.5
19	August 14	Andrews Point	3.8	M	YI	32.8	38.1	4.96	1.24	29.1	56.1
20	August 15	Margaret Point	4.4	F	YI	35.3	41.5	5.13	1.53	28.3	59.1
21	August 25	Iphigenia Point	4.2	F	OI	36.0	41.2	5.38	1.47	28.0	58.5
22	August 26	Dadens	5.0	F	A	36.8	44.3	5.17	1.61	29.5	59.8
23	August 26	Henslung Cove	4.1	M	YI	36.5	37.0	4.60	1.49	28.6	60.5

Appendix Table 4-3. Dates, locations and selected morphological measurements of Northwestern crows (*Corvus caurinus*) collected on Langara and Lucy Island, 1995.

Crow #	Date	Location	Weight (g)	Sex	Wing Chord cm	Tail Length cm	Bill Depth cm	Hallux cm	Tarsus L cm	Tarsus D. cm
1	May	No-Name Point	375	M	-	-	1.90	1.70	-	0.60
2	July 10	Lucy Island	298	F	27	-	1.58	1.7	4	0.44
3	July 10	Lucy Island	319	M	26	-	1.57	1.41	3.82	0.42
4	July 10	Lucy Island	388	M	28	-	1.53	1.5	3.74	0.55
5	July 10	Lucy Island	350	F	27.3	-	1.62	1.39	3.4	0.45
6	July 22	Lucy Island	382	M	26.5	15.1	1.74	1.69	3.06	0.41
7	July 22	Lucy Island	405	M	27.5	16.6	1.9	1.6	3.59	0.46
8	July 22	Lucy Island	390	M	23.8	14.8	1.82	1.58	3.07	0.38
9	July 22	No-Name Point	340	F	24.8	13.9	1.8	1.49	3.16	0.36
10	July 22	No-Name Point	388	M	28.3	15.7	1.76	1.48	4.5	0.59
11	July 23	Mcpherson Point	400	M	25.5	14	1.77	1.46	3.32	0.46
12	August 10	Mcpherson Point	333	F	24.8	15.2	1.6	1.42	4	0.42
13	August 5	Mcpherson Point	362	M	27.3	15.2	1.67	1.46	3.98	0.39
14	August 5	Mcpherson Point	349	F	25.3	15.8	1.56	1.46	4.21	0.36
15	May	Mcpherson Point	339	F	25.9	15.8	1.55	1.56	4.38	0.36
16	May	Mcpherson Point	324	F	25.6	14.9	1.59	1.58	3.94	0.38
17	August 7	Henslung Cove	334	F	24.7	16.9	1.43	1.44	4.16	0.42
18	August 16	Mcpherson Point	414	M	26.6	16.5	1.65	1.63	3.54	0.38
19	August 17	Lucy Island	440	M	26.2	16.4	1.74	1.72	3.48	0.41
20	August 17	Lucy Island	451	M	25.3	14.9	1.59	1.58	4.50	0.42
21	August 17	Dadens	327	M	27.9	15.2	1.63	1.42	4.90	0.41
22	August 17	Cohoe Point	382	M	27.8	15.9	1.66	1.42	4.57	0.41

Appendix Table 4-3. cont.

Crow #	Date	Location	Weight (g)	Sex	Wing Chord cm	Tail Length cm	Bill Depth cm	Hallux cm	Tarsus L cm	Tarsus D. cm
23	August 17	Cohoe Point	446	M	29.1	16.3	1.84	1.51	4.50	0.46
24	August 21	Lucy Island	370	F	27.3	15.2	1.46	1.41	4.7	0.37
25	August 19	Egeria Bay	410	M	27.7	15.3	1.56	1.57	4.72	0.42

Chapter 5. The Uptake of Brodifacoum by Invertebrates Feeding on Bait Containing the Rodenticide Brodifacoum or Norway Rat carcasses Poisoned with Brodifacoum.

5.1 Introduction

Invertebrates play an important role in the nutrient cycling process by consuming animal carcasses and organic matter, releasing nutrients for primary consumers. The bait used as the rodenticide carrier and attractant provides a rich and readily available food source for invertebrates (Stejskal et al. 1994). Similarly, animal carcasses resulting from brodifacoum poisoning are likely attractive to carrion insects. Invertebrates that feed on the bait and/or carcasses, therefore, may be a secondary or tertiary poisoning risk to non-target species that may otherwise be at low risk of poisoning. To-date, the uptake of brodifacoum residues by invertebrates from anticoagulant poisoned carcasses has not been studied.

The objective of this study was to identify the invertebrates that are attracted to and consume brodifacoum poisoned rats and the bait blocks within bait stations. The specific hypothesis tested was that invertebrates consuming brodifacoum poisoned rat carcasses and/or bait would carry detectable levels of brodifacoum.

In 1994, different methods of collecting and preserving invertebrates for brodifacoum assay were tested as there was no established method available in the literature. The results allowed me to establish a protocol for tissue collection. In 1995, invertebrates were again collected using the protocol established and analysed for brodifacoum residue.

5.2. Materials and Methods

5.2.1 Invertebrate Collections

5.2.1.1 Collection of Invertebrates at Bait Stations

In 1994, invertebrates found in bait stations on Lucy Island were collected and frozen (- 5 Celsius) individually in plastic bags. In 1995, on Langara Island, bait station operators were requested to document the presence of any invertebrates present in stations. Samples of invertebrates found in the bait stations were collected during the baiting campaign. The samples were pooled and frozen for analysis (See below).

5.2.1.2 Collection of Carrion Insects

In 1994, different methods of collection and preservation of invertebrates exposed to brodifacoum were tried:

1. Preservation in 95% Ethanol

1994 pilot study: Ethanol is an effective preservative for tissue samples when they cannot be quick frozen in the field. However, it is also a solvent and may leach brodifacoum from tissue samples. Rat carcasses were laid out in three habitats: along the shoreline in Nootka reed grass, in the ancient murrelet colony, and in the interior. Within each location, two carcasses, one snap trapped rat (unpoisoned) and one brodifacoum poisoned rat were spaced approximately 50 m apart, and covered with a cage made with

hardware cloth (1 cm x 1 cm mesh) to exclude avian scavengers. Each carcass was checked daily or every other day until sufficient numbers of insects were present feeding on the carcasses. The carcass and a shovel full of soil around the carcass were carefully placed into a (30 cm X 20 cm) wooden box and wrapped with a cotton shirt to allow for gas and moisture exchange. Insects were allowed to exit the box from a 5 cm diameter hole through a down-pipe elbow into a glass jar filled with 95% ethanol. Insects were allowed to consume the carcass for approximately 12 wk before the lid was removed, and remaining insects collected and placed into the ethanol filled jars.

1995: carrion insects were again collected using the previous boxes. The design of the box was modified to minimise handling of carcass and insects to prevent contamination. Three brodifacoum poisoned rats were placed individually into boxes with a shovel full of soil and covered with noseum netting. A funnel was placed on one end for an entrance. The exit opening was a 5 cm diameter downpipe elbow into a jar filled with 95% ethanol. Only insects that voluntarily left the carcass into the ethanol were analysed. Due to the low number of insects collected, insects from all three samples were pooled for analysis.

2. **Preservation by Freezing**

1994 pilot study: The Langara Lightstation staff offered the use of a -5 Celsius freezer and I decided to run the insect collection trials again and freezing the samples. The fresh collected carrion insect experiment was conducted between July and August 1994. Three brodifacoum poisoned and six unpoisoned rat carcasses were each placed in a 10 cm diameter, 30 cm long PVC drainage pipe tubes (Figure 5.1). The tube had a ventilation hole cut in the top and covered with noseum netting, while small holes were cut in the bottom to prevent accumulation of rainwater. A funnel with the same diameter as the tube was placed over one end for an entrance. On the opposite end, a 2.5 cm diameter clear PVC tubing (10 cm long) was connected to the tube and led into a glass jar. Insects that were in the glass jar were collected every 7 d for 4 wk, sorted by species, and frozen for future analysis. Due to the low numbers of individuals collected each day, individuals of each species were pooled across time for brodifacoum residue analysis.

3. **Preservation by Hexane Cleaned Aluminum Foil and Freezing**

The results of the carrion insect collection trials in 1994 revealed that there was contamination of control samples from both ethanol and frozen preservation. In 1995, carrion insects were opportunistically collected from

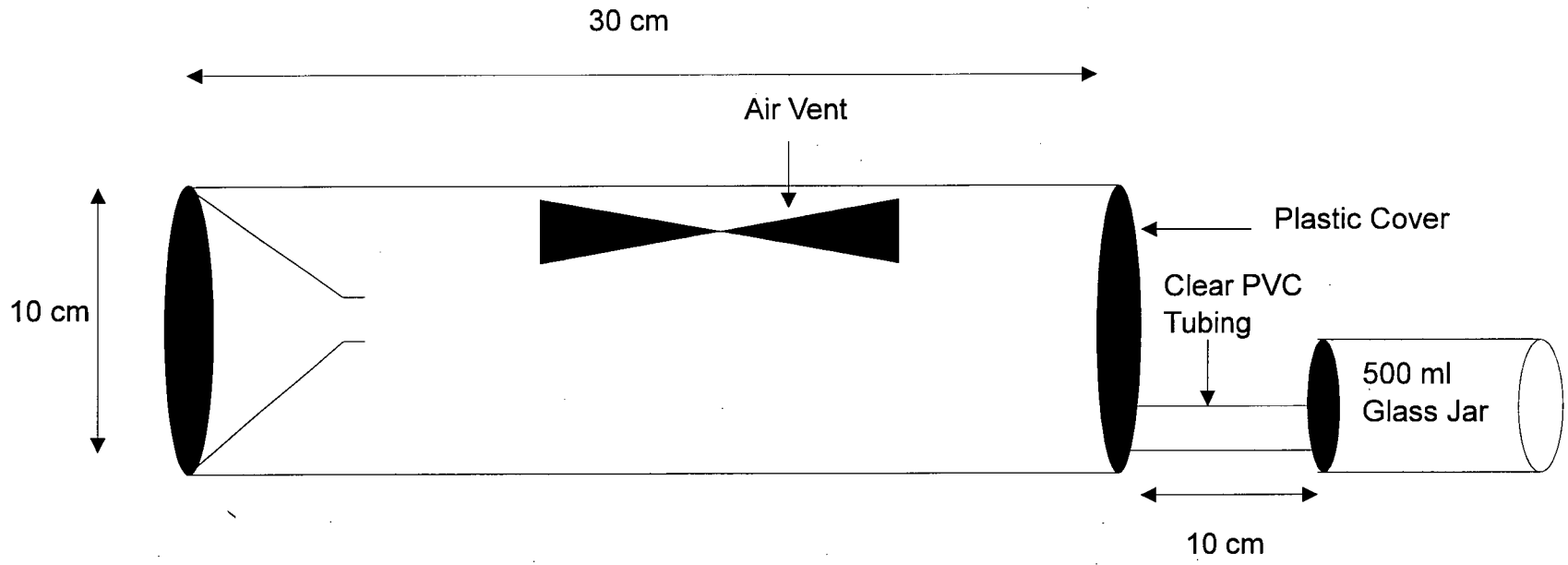


Figure 5.1. Carrion Insect Trap used to capture and hold insects attracted to rat carcasses on Langara Island, 1994. Note the funnel over the opening of the tube on the left hand side, and the 2.5 cm diameter PVC tubing exiting the tube into the 500 ml glass jar for holding insects. The glass jar has small air holes in the removable lid. Insects were collected at regular intervals from the glass jar and frozen. The black triangles were the ventilation holes and were covered with noseum netting.

brodifacoum poisoned rat carcasses. Surface feeding carrion insects were collected with chemically cleaned (hexane) instruments and wrapped in chemically cleaned aluminum foil to prevent contamination. The packets were slipped into pre-labelled whirl-pack bags and frozen.

5.2.2 Sample Preparation

Samples were shipped frozen to NWRC, Hull, PQ. After the samples were prepared, one-third (by weight) of all tissues were archived at NWRC.

5.2.2.1 Bait Station Invertebrates

The snails and slugs had their shells removed and the soft tissue weighed. The shells were rinsed with methanol in a 1 mm diameter kitchen strainer into a cup holding the soft tissue.

5.2.2.2 Carrion Insects

1. **Samples Preserved in 95% Ethanol**

   The insects were separated and the ethanol was allowed to evaporate from the insects for approximately 1 h and the contents weighed. The insects were homogenised and an aliquot of both insects and the ethanol was analysed for brodifacoum residue (See below).

2. Frozen Samples

The insects were removed from containers, counted and weighed. Methanol was used to rinse any brodifacoum residue from original container. The insects were chopped into fine pieces with chemically cleaned scissors to facilitate homogenisation.

3. Samples Frozen in Hexane Cleaned Aluminum Foil

The insects were separated from the foil packages and weighed. The foil was rinsed with methanol into a cup holding the insects. Insects were chopped with chemically cleaned scissors into fine pieces to facilitate homogenisation. Homogenisation was done in chemically cleaned glass jars. A chemically cleaned shaft homogeniser was used for all samples.

5.2.3 Brodifacoum Assay

Extraction of brodifacoum residues from prepared samples was carried out at NovaMann International, Mississauga, Ontario, following the procedure established by Hunter (1983). The limit of detection was 0.01 ppm.

As part of the quality control, clean invertebrate and Canada goose (*Branta canadensis*) liver samples were fortified with brodifacoum and shipped along with treatment invertebrates in 1994. In 1995, clean snails and slugs were collected from Graham Island, 1 km across from Langara Island, and fortified with brodifacoum in the field. Samples were individually wrapped in chemically cleaned foil packets and

slipped into pre-labelled polyethylene whirl pack bags. These control samples were handled the same way as those invertebrates collected from rat carcasses and bait stations.

All original chromatography graphical tracings from the instrumental analyses were verified by Bryan Wakeford at the NWRC laboratory.

5.3. Results

5.3.1 Brodifacoum Assay

Quality Control: In 1994, the brodifacoum residue recovery from fortified samples was 14% from insect samples and 0.0% from the liver sample (Table 5.1). In 1995, recovery of brodifacoum ranged between 0.0% and 127% (Table 5.2). All control samples did not test positive for brodifacoum.

Table 5.1. Brodifacoum residue recovery from fortified samples, Langara Island, 1994.

Tissue Type	Fortified Level ug Brodifacoum	Tissue Weight g	Brodifacoum Reported ug	% Recovery
Liver	5.4	2.2	None Detected	0.0
Insect	9.9	4	1.4	14%

Table 5.2. Brodifacoum residue recovery rate from fortified samples prepared on Langara Island, 1995.

Tissue Type	Spike Solution Added (ul) 115 ug/ml	ug Brodifacoum Added	Fortified Level (ug/g)	Brodifacoum Reported (ug)	Percent Recovery
Vespericola sp. Snail	56	6.4	0.30	7.16	111.2%
Vespericola sp. Snail	54	4.14	0.34	5.26	127.1%
Vespericola sp. Snail	100	11.5	0.40	11.91	103.5%
Vespericola sp. Snail	100	11.5	1.46	5.42	47.11%
Ariolimax sp. Slug	100	11.5	0.85	0.00	0.00%
Ariolimax sp. Slug	74	8.51	-[a]	-	-
Ariolimax sp. Slug	90	10.35	0.34	3.02	29.2%
Ariolimax sp. Slug	52	5.98	0.26	4.00	66.9%
Spike Solution		115 ug/ml		93.14	81.0%
Haplotrema sp. Control Snail	0.00	0.00	0.00	0.00[b]	-
Ariolimax sp. Control Slug	0.00	0.00	0.00	0.00[b]	-
Ariolimax sp. Control Slug	0.00	0.00	0.00	0.00[b]	-

a Not Processed because of leakage of contents in transport.
b Control Sample.

5.3.2 Bait Station Invertebrates

The terrestrial molluscs, banana slugs (*Ariolimax* sp.) and snails (*Vespericola* sp. and *Haplotrema* sp.) were the most common and abundant invertebrates found in bait stations and feeding on the bait. The blue coloured bait could be readily seen through the translucent body on those individuals found feeding on bait. Blue casts from both snails and slugs were evident around stations on leaves and moss, as well as inside stations.

The mean daily reported proportion of bait stations with slugs and snails were similar at 8.9% and 8.2% respectively (two-tailed t-test; $P>0.05$) (Figure 5.2). The proportion of bait stations with one or more snails ranged from 1.9% to a high of 17.4%. The brown shelled snail, *Vespericola* sp. was reported in a mean of 5.8% bait stations daily, significantly more than the yellow/green coloured snail, *Haplotrema* sp. (3.0%) (one-tailed t-test; $P<0.05$) (Figure 5.3). The brodifacoum residue levels for the terrestrial molluscs ranged from a low of 0.54 ug/g in banana slugs to a high of 4.13 ug/g in *Haplotrema* sp. snails (Table 5.3).

Other invertebrates found in bait stations included crickets (Order Orthoptera) two millipedes (Class Diplopoda, *Harpaphe* sp. and one unidentified) and harvestmen (Order Phalagida) although they were uncommon. The blue coloured bait could be seen through the translucent body of the crickets found in bait stations. The cylindrical millipedes burrowed into the bait blocks. Generally the millipedes were found burrowed into the bait blocks alone, however they were also

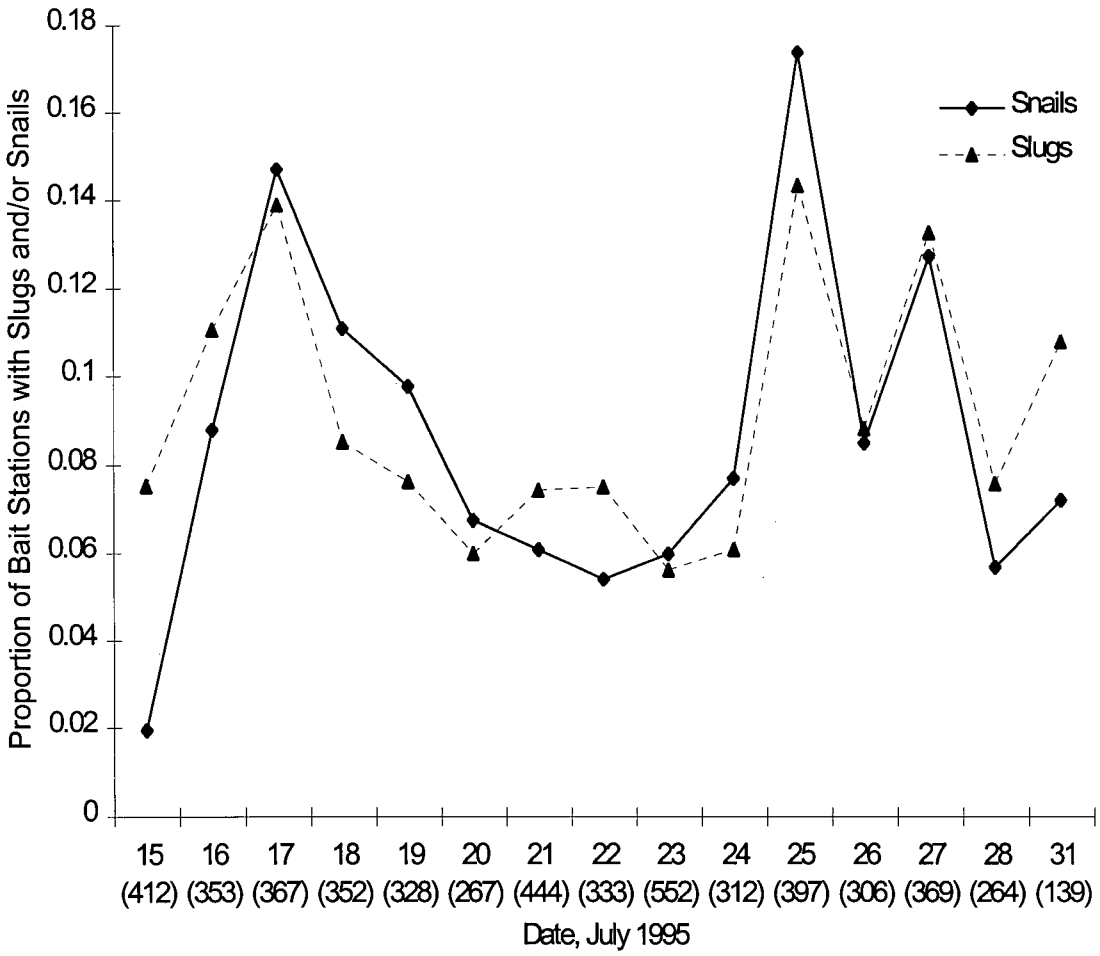


Figure 5.2. Proportion of bait stations with snails and banana slugs over the course of the intensive baiting period, Langara Island, 1995 (number of bait stations in brackets).

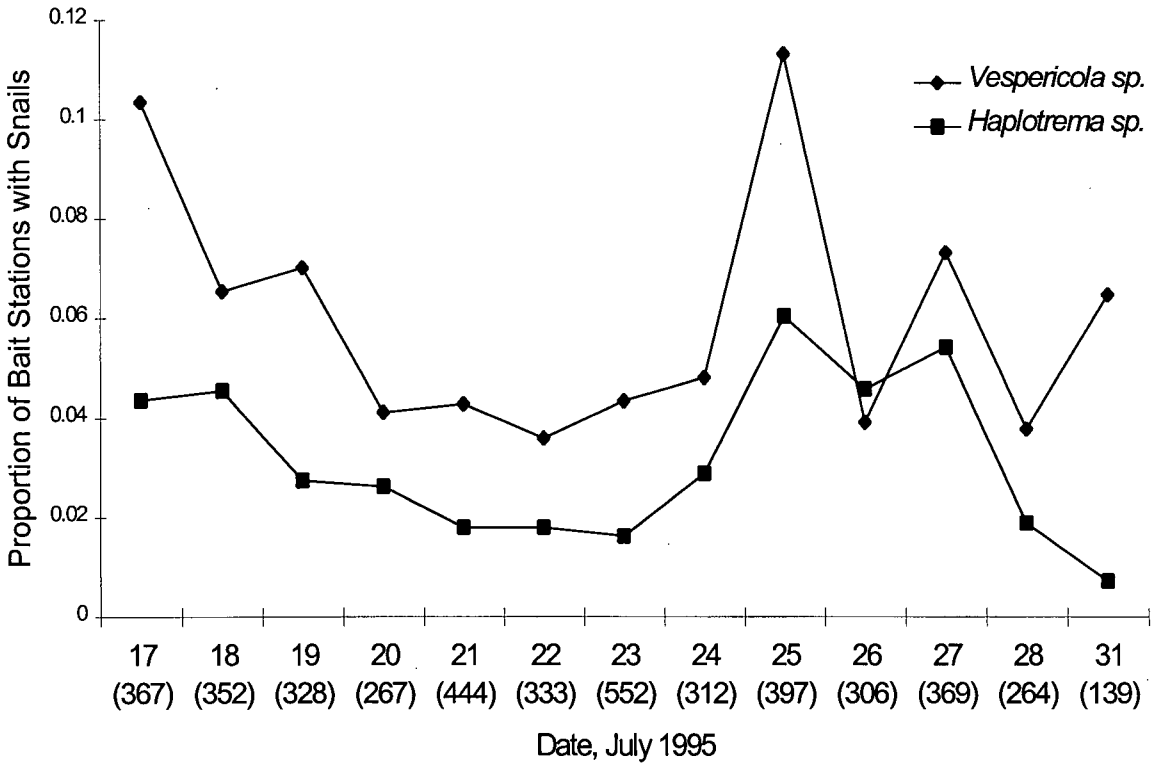


Figure 5.3. Proportion of bait stations with the terrestrial snails, *Vespericola* sp. and *Haplotrema* sp., over the course of the intensive baiting period (number of bait stations in brackets).

Table 5.3. Brodifacoum residues in invertebrates found in bait stations, Langara Island, 1995.

Common Name	Taxonomy	No. Individuals	Mean Weight g	Brodifacoum ug/g	ug Brodifacoum per individual
Banana Slug	*Ariolimax* sp.	13	10.2	2.90	29.55
		13	17.1	0.54	9.21
Snail	*Vespericola* sp.	13	0.85	1.53	1.29
		40	0.62	1.42	0.88
Snail	*Haplotrema* sp.	9	1.16	3.57	4.12
		30	0.87	4.13	3.58
		15	0.91	4.04	3.66
Cricket	Order Orthoptera	10	0.04	8.17	0.33
Cylindrical Millipede	Class Diplopoda	30	0.08	2.74	0.22
Millipede	*Harpaphe* sp.	4	0.2	3.70	0.74
Predatory Centipede	Class Chilopoda	1	0.2	0.0	0.0
Ground Beetle	Family Carabidae	3	0.1	0.0	0.0
Daddy Long Legs	Order Phalangida	7	0.014	266.4	3.81

reported in groups of three or more. Harvestmen or daddy long legs were found to contain the highest concentration of brodifacoum (Table 5.3.)

Only terrestrial molluscs and one millipede were found to feed on bait in stations on Lucy Island in 1994. Brodifacoum residues could not be detected in *Haplotrema* sp. snails but were detected in *Vespericola* sp. (Table 5.4). Brodifacoum residues in banana slugs were at detection limits.

Table 5.4. Brodifacoum residues in invertebrates found in bait stations, Lucy Island, 1994.

Common Name	Genus	No. Individuals	Mean Weight (g)	Brodifacoum (ug/g)	ug Brodifacoum per individual
Snail	*Vespericola* sp.	46	0.40	2.462	0.984
Snail	*Haplotrema* sp.	6	0.27	0.000	0.000
Banana Slug	*Ariolimax* sp.	8	3.94	0.002	0.002
Millipede	*Harpaphe* sp.	1	0.56	1.607	0.900

5.3.2. Carrion Insects

5.3.2.1 Preservation in 95% Ethanol

There were four genus that were the most abundant at rat carcasses; these include three genus from the Order Coleoptera (Beetles): *Nicrophorus* sp., *Necrophilus* sp., and *Catops* sp. The fourth group were the blowflies or blue-bottle flies from the Order Diptera, Family Calliphoridae, Genus *Calliphora.*

In 1994, brodifacoum residues were detected in the pooled insects collected from brodifacoum poisoned and control rat carcasses (Table 5.5). Brodifacoum residues were also detected in the ethanol used for the preservation of insects.

Brodifacoum residues were not detected in insects collected into ethanol in 1995 (Table 5.6).

5.3.2.2 Preservation by Freezing

In 1994, brodifacoum residues were detected only in *Nicrophorus* sp. larvae (Table 5.7). Brodifacoum residues were not detected in the more common and abundant blowfly larva or adult forms. Brodifacoum residues were detected in adult blowflies collected from a control carcass (Table 5.7).

5.3.2.3 Preservation in Chemically Cleaned Aluminum Foil and Freezing

Brodifacoum residues were detected in the blowfly larvae collected from the radio-collared Norway rat carcasses in 1995 (Table 5.8). The concentration was variable and ranged from 0.27-11.39 ug/g larva. Small ants (Family Formicidae) were collected from two rat carcasses, but no brodifacoum residues were detected.

Table 5.5. Number of and brodifacoum residues in carrion insects collected from brodifacoum poisoned Norway rats and control Norway rats, into 95% ethanol, Langara Island, 1994.

Taxonomy			Brodifacoum			Control		
Order	Genus	Life Stage	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3
			Number of Insects			Number of Insects		
Coleoptera	*Necrophilus* sp.	Adult	2	1	3	17	2	3
		Larva	32	1	68	0	22	77
	Catops sp.	Adult	0	0	18	14	2	3
	Nicrophorus sp.	Adult	0	0	0	0	0	0
		Larva	3	0	0	0	0	0
Diptera	*Calliphora* sp.	Adult	16	42	11	49	2	14
		Larva	43	93	72	22	13	55
	Unidentified	Adult	0	3	121	10	8	2
Total			96	140	293	112	49	154
Weight (g)			7.7	5.9	10.46	7.6	1.4	4.1
Brodifacoum[a]	Insects		+	+	+	+	ND	+
Brodifacoum	Ethanol		+	+	ND[b]	ND	ND	ND

a Positive detection of brodifacoum residues indicated by a + sign.
b ND= None Detected

Table 5.6. Insects collected and preserved in 95% ethanol with no brodifacoum residues detected, Langara Island, 1995.

Order	Family/Genus	Common Name	Association	Number
Coleoptera (Beetles)	*Catops* sp.	Carrion beetle	Carrion	184
	Necrophilus sp.	Carrion beetle	Carrion	2
	Nicrophorus sp.	Carrion beetle	Carrion	1
	Family Rhizophagidae	Root-Eating beetles	Decaying wood	3
	Family Dermistidae	Skin beetles	Carrion & Vegetation	18
	Unidentified	-	-	54
Diptera (Flies)	*Calliphora* sp.	Blowflies	Carrion	4
	Family Ptychopteridae	Phantom Crane flies	Decaying Vegetation	5
	Family Trichoceridae	Winter Crane flies	Decaying Vegetation	1
	Family Phoridae	Humpbacked flies	Decaying Vegetation	7
	Family Platypezidae	Flat-Footed flies	Vegetation	3
	Family Asilidae	Robber flies	Insect Larva	1
	Family Anthomyiidae	Anthomyiid flies		2
	Unidentified	-	-	2
Collembola		Springtails	Soil	>63
Diplura		Diplurans	Soil	4
Lepidoptera		Moths	Vegetation	1
Araneae		Spiders	Insects	5
Acari		Spiders	Insects	2
Total				>362
Brodifacoum (mg/kg)				None Detected

Table 5.7. Brodifacoum residues in carrion insects collected from brodifacoum poisoned Norway rat carcasses, collected fresh, Langara Island, 1994.

Genus	Life Stage	No.	Mean Weight (g)	Brodifacoum (ug/g)	ug Brodifacoum per Individual
Nicrophorus sp.	Larva	13	1.02	0.860	0.877
Necrophilus sp.	Adult	18	0.18	ND[a]	< Detection Limits
Catops sp.	Adult	193	0.002	ND	< Detection Limits
Calliphora sp.	Larva	204	0.02	ND	< Detection Limits
Calliphora sp.	Adult	25	0.14	ND	< Detection Limits

a None Detected

Table 5.8. Brodifacoum residues in blowfly larva collected from brodifacoum poisoned Norway rat carcasses, Langara Island, 1995.

No. Larva in Pool	Mean Larva Weight (g)	ug Brodifacoum per Individual Larva	Brodifacoum Concentration ug/g Larva
100	0.05	0.24	4.81
100	0.06	0.15	2.65
15	0.05	0.12	2.54
56	0.06	0.02	0.27
40	0.02	0.26	11.39
25	0.09	0.18	2.09
33	0.08	0.06	0.75
60	0.02	0.05	2.28

5.4. Discussion

In 1995, collection and preservation protocols were established to rectify the contamination problem encountered in 1994. However, the level of brodifacoum residue detected from pooled samples was highly variable mostly because of the variable recovery rates from sample to sample by the assay procedure used. The procedure for assaying the 4-hydroxycoumarin anticoagulants has been published with mean recovery rates between 89-91% (Hunter 1983). However, the results provided by NovaMann Laboratories showed recovery rates ranging from 0 to 127% from the spiked samples. No explanation was offered by NovaMann.

Another factor which may have contributed to the variability is that the brodifacoum residues detected may represent unassimilated brodifacoum in the gut of some invertebrate samples and assimilated brodifacoum into the tissues of other samples.

The control samples did not test positive for brodifacoum, thus, I am confident that the qualitative brodifacoum residue analysis was sound and brodifacoum was detected from samples in 1995. The data presented indicate that invertebrates feeding on bait and brodifacoum poisoned rats did contain detectable levels of brodifacoum residue. Carrion insects were a potential tertiary poisoning risk and the invertebrates feeding on the bait were a secondary poisoning risk to non-target species. In New Zealand, invertebrates were observed to feed on baits, and brodifacoum residues were found in beetles

collected from bait stations containing bait intended for rats on Stewart Island, however, no data were presented (Eason and Spurr 1995). The only reported secondary poisoning of insectivorous birds was in a zoo where birds in an aviary died after feeding on ants and cockroaches that had eaten bait containing brodifacoum (Godfrey 1985).

The blue coloured bait could be readily seen through the translucent bodies of the snails and slugs found feeding on the bait. The brodifacoum residues in the gut of Norway rats represented between 30-50% of the whole body residue level (See Chapter 4). The blowfly larva collected likely had a gut full of carrion containing brodifacoum residues. The implication is that the secondary/tertiary poisoning hazard to non-target species is greater from an invertebrate that has been recently feeding on bait/carrion containing brodifacoum versus one that has had time to excrete the contents containing brodifacoum. In other words, the poisoning risk can be defined as short and long term. The short term risk may be greater than the long term risk because of the presence of brodifacoum in the gut of the invertebrate. In order to elucidate the above possibility, known amounts of brodifacoum could be fed to invertebrates and the residue levels from pooled or individual samples can be analysed at various time points after feeding including when the bait has passed through the gut. This would allow for measurement of the brodifacoum residues in the gut versus levels of brodifacoum retained in the tissues.

The attractiveness of the bait to snails and slugs indicate that any excess bait dispersed by rats over the course of the intensive baiting period would be consumed. The amount of bait dispersed by rats and not consumed is unknown. However, bait crumbs were found in and around burrows, under logs, and along runs used by rats. On Lucy Island in 1994, a *Vespericola* sp. snail was found to be feeding on the bait crumbs outside a burrow and had excreted a blue cast. In May 1995, 9 months after the removal of the bait from the stations, four old bait blocks (9.6 g) were found under a log on the west side of Lucy Island and contained 10.986 ug/g brodifacoum. Snails found near the bait were collected and contained a concentration of 0.910 mg/kg brodifacoum. Thus, the snails and slugs could be an ongoing secondary poisoning risk until the bait has degraded or is consumed.

No brodifacoum residues were detected in the ethanol preserved insects in 1995. This may be due to the low consumption of the carcasses by carrion insects. The carcasses were not being consumed by carrion insects as found the previous year using a similar design trap. This may be due to the high number of other wild rat carcasses that were available to insects or the altered design of the trap attracted fewer carrion insects. Most of the other insects were associated with vegetation and soil, and were a result of placing a shovel full of soil in with the carcass.

The brodifacoum residue data from blowfly larva collected in 1995 were more reliable due to the different collection technique and increased attention to

minimising or eliminating contamination due to collection. It can be concluded that blowfly larva consuming rat carcasses containing brodifacoum pose a tertiary poisoning risk to non-target species. In 1994, song sparrows and Northwestern crows were photographed at rat carcasses that had been attacked by carrion beetles (Chapter 4). They were at risk of tertiary poisoning in 1995, if the carrion insects consuming carcasses did carry a load of brodifacoum residue. Pooled samples of song sparrow livers tested positive for brodifacoum (Appendix 2). Unfortunately, the highly variable quantitative brodifacoum residue data preclude estimating actual risk of poisoning from the invertebrates.

The confirmation of brodifacoum residues in invertebrates indicates that insectivorous non-target species may be exposed to brodifacoum and need to be considered in future eradications. The saturation baiting strategy used on Langara Island provided a constant food supply for snails, slugs and other invertebrates. The carrion insects rapidly and readily consumed brodifacoum poisoned rat carcasses and subsequently attracted song sparrows and Northwestern crows (Chapter 4). The above data indicate that to minimise potential non-target species poisoning, less brodifacoum in the form of bait and/or poisoned carcasses should be available to invertebrates.

In conclusion, brodifacoum residues were detected in all invertebrates attracted to bait in the stations. The positive detection of brodifacoum residues in carrion insects and bait station invertebrates indicates that they were a tertiary and secondary poisoning risk to insectivorous species.

Chapter 6. Conclusions and Recommendations

The overall objective of this thesis was to investigate the short term poisoning hazard to non-target species from brodifacoum bait used to eradicate rats from Langara and surrounding Lucy and Cox Islands. Brodifacoum residues were detected in every level investigated: carrion insects, terrestrial molluscs, songbirds, ravens, Northwestern crows, and bald eagles - the top of the food chain. The results presented in this thesis provide the basis for future work by identifying pathways and species at risk of poisoning during similar operations along the British Columbia coast.

6.1 Conclusions

6.1.1 Native Small Mammal Study

1. While Dusky shrews were attracted to the bait in the stations, the decline in their population was non-significant in all regions on Langara Island, indicating they were at low risk of extirpation.
2. Adult breeding Dusky shrews appeared to be at greater risk of poisoning than non-breeders and juveniles.

6.1.2 Secondary Poisoning Risk to Avian Scavengers and Predators

1. Rats dying above ground are a consequence of anticoagulant poisoning and is not age or sex related.

2. The mean whole body brodifacoum residue concentrations in Norway rats found dead above ground were the highest yet reported in the literature.
3. Common ravens were at an extreme risk of primary and secondary poisoning:
    i. Ravens were able to gain access to the bait in the stations.
    ii. Ravens were identified as the most significant scavenger of Norway rat carcasses.
4. Bald eagles were exposed to brodifacoum, however, no mortality was detected.
5. Brodifacoum was detected in Northwestern crows 9 months after the Lucy Island baiting in 1994.

6.1.3 Study of Brodifacoum Transport into the Ecosystem

1. The bait blocks were highly attractive to terrestrial snails and slugs.
2. Brodifacoum residues were detected in snails, slugs, blowfly larva, and other species, however, it is unclear if it was unassimilated brodifacoum in the gut and/or brodifacoum retained in the tissues.

6.1.4 Detection of Brodifacoum Exposure through Plasma Residue Analysis and Prothrombin Time Evaluation.

1. Plasma brodifacoum residue analysis is an effective indicator of exposure in birds.
2. The use of a mammalian derived thromboplastin is only an effective tool to detect a severely affected bird.

6.2 Recommendations

The following eight recommendations are presented to minimise the non-target species poisoning during similar rat eradication operations in the future. They are presented from a non-target species perspective and some recommendations may not be economically or operationally feasible under all conditions.

1. Switch from saturation baiting to pulsed baiting. Utilising a pulsed baiting strategy, where the bait is available in limited quantities and is only replenished at pre-specified intervals, has been shown to reduce the residue load of target rodents (Merson et al. 1984; Kaukeinen 1982). Alternatively, reduction of the concentration of the active ingredient has also been shown to reduce the target species residue level (Kaukeinen 1982). The pulsed baiting strategy would also reduce the total amount of brodifacoum released into the environment, and available for potential transport into the ecosystem by invertebrates. The cost is an increased time to eradication which could translates into an increased economic cost.

2. Shift the intensive baiting period to the time of the year when the rat population is at its lowest. This would reduce the absolute number of rats dying above ground and therefore, decreasing availability to scavengers and predators. The late winter months are likely the time rat populations are lowest. However, it may not

be feasible to have people working on and around offshore islands during winter months off the coast of British Columbia when severe storms are common.

3. The use of anticoagulants that are less toxic to non-target species should be investigated and considered. Brodifacoum is very effective for controlling rodents due to its high toxicity to the target species. However, it is also highly toxic to non-target bird species at dose levels that were readily available during the Langara Island Seabird Habitat Restoration Project. The use of a less toxic anticoagulant would reduce the primary and secondary poisoning risk to birds. But it is generally avoided because of the risk of rats developing bait shyness or resistance. On the other hand, there are other anticoagulants that are as toxic as brodifacoum to rats, but less toxic to birds. For example, flocoumafen has a LD_{50} of 0.25-0.56 mg/kg for Norway rats (similar to brodifacoum) (Huckle et al. 1989b) but is approximately 26 times less toxic to Japanese quail (flocoumafen LD_{50}= >300 mg/kg (Huckle et al. 1989b); brodifacoum LD_{50}= 11.6 mg/kg (Ross et al. 1976)).

4. Re-design bait stations to exclude Common Ravens. Studies with different bait station designs such as an S-shape or simply, a longer station should be undertaken to determine the shape that does not compromise acceptance by rats but excludes ravens.

5. Use bait formulations that are less attractive to birds. The use of a bait formulation that would make the baits unpalatable to birds but maintains high attractiveness to the target species would be ideal. In the United States, methyl anthranilate is used as an effective non-lethal bird repellent. It has been used effectively as a non-lethal bird repellent in the field (Askham 1994; Avery 1992; Cummings et al. 1991; Glahn et al. 1989). It is not yet registered for use in Canada (P. Mineau, pers. comm.), however, further study into the effectiveness of this product, or others, to prevent primary poisoning to birds otherwise attracted to the bait is warranted.

6. Use non-removable bait blocks in stations. Even if recommendation 4 is followed, rats may disperse large quantities of bait and potentially make them accessible to non-target species. The bait blocks should be fastened down so that they cannot be removed but must be consumed within the stations thus minimising dispersal. Alternatively, the bait could be reformulated into a brick (15 cm x 15 cm x 2.5 cm) that cannot be physically removed from the station but requires rats to feed directly on the bait in station. Either option would reduce the number of visits to a station by an operator, thus, reducing the number of crew required to check stations. On the down side, fixing the bait may lead to dominant rats defending stations and preventing conspecifics from gaining access to bait. As a consequence, the time to eradication may be lengthened.

7. Monitor non-target species before, during, and after eradication campaigns.

i. *Avian scavengers.* Common ravens are the non-target species at greatest risk of poisoning. Ravens are common scavengers in seabird colonies in the Queen Charlotte archipelago (Rodway et al. 1990;1988). Their aggressive and inquisitive behaviour put them in the highest risk of poisoning of all species studied and thus, could be used as an indicator of non-target species poisoning. They could indicate the availability of brodifacoum to non-target species. Population estimates before, during and post eradication should be made and compared against one or more control site(s).

If an effective trapping method can be developed, blood sampling of adult and juvenile ravens during and post eradication can be used to monitor the exposure of individuals to brodifacoum. If future eradications are pursued in late winter/early spring when ravens are nesting, pre-fledging raven chicks may be a readily accessible source of plasma. The plasma brodifacoum residue analysis is an effective, inexpensive indicator of exposure. A laboratory that is already set up for the analysis should be used to ensure quality control.

ii. *Native small mammals.* Many seabird colonies in the Queen Charlotte archipelago harbor populations of native deer mice and/or dusky shrews. Shrews are unlikely to be extirpated from islands following the regime of the Langara Island Seabird Habitat Restoration Project. However, the operation has the potential to alter population dynamics. Small mammal populations should be monitored for changes in the short term and long term. Variables such as population size,

demographics including age and sex structure changes should be measured and quantified. A control site, with similar small mammal composition and island size, should be monitored concurrently.

8. Training in the safe handling and disposal of bait blocks is essential. At least three of the 13 ravens found dead were primarily poisoned as a direct result of a bait spill. This incident indicates that a strict protocol in the handling and disposal of bait blocks is required. A training program specifically designed to provide training for island restoration crews dispensing and disposing of bait should be developed.

Adherence to all or some of these recommendations would reduce the non-target species poisoning risk substantially. In particular, if only the first four recommendations are followed, the non-target species poisoning risk should already be lessened significantly.

6.3 Future Directions

This thesis only investigated the short term impacts of the poisoning operation and does not address the long term impacts. Over 14, 000 bait blocks were dispensed from bait stations across the island over the intensive baiting period. However, the 1,100 active bait stations were concentrated primarily within a few hundred meters of the shoreline, leaving the possibility of ongoing primary and secondary poisoning risks to non-target species. The long-term effects of the

baiting on both the shrew and raven population should be investigated. Endpoints such as mortality should be continued to be monitored but also sub-lethal effects such as reproduction.

The use of radio-telemetry would be useful to monitor the hazard to individual common ravens. This technology allows for the determination of the location, time, and potentially, cause of death. If brodifacoum poisoned ravens were preyed on or scavenged by eagles, radio telemetry could possibly answer this question.

Laboratory investigation into the LD_{50} of brodifacoum to local species, such as the common raven, would be useful for determining risk of poisoning from brodifacoum residues found within species investigated in this thesis. Similarly, laboratory investigation into the biological significance of brodifacoum plasma residues on prothrombin time (a measure of risk of hemorrhaging) in birds would be useful to interpret residue data.

Further investigations into modelling accumulation and transfer of residues, such as that developed by Smith et al. (1990), as it applies to island restoration should be undertaken so as to increase the availability of the tools in assessing the non-target species poisoning cost.

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Appendix A. The Detection of Exposure to Brodifacoum in Japanese Quail through Plasma Brodifacoum Residue Analysis and Prothrombin Time Evaluation

Introduction

In 1995, the Canadian Wildlife Service attempted to eradicate introduced Norway rats from 3,300 hectare Langara Island at the north-western tip of British Columbia's Queen Charlotte archipelago by use of the anticoagulant brodifacoum. The island, however, is also home to breeding Bald Eagles, Common Ravens and other wildlife which prompted concerns of non-target species poisoning. Bald Eagles, the top predator on Langara Island, were identified to be at risk from secondary poisoning from potentially scavenging/preying on rats and non-target species exposed to brodifacoum. Bald Eagles were trapped and blood samples drawn for prothrombin time (PT) determinations and plasma brodifacoum residue analysis to determine if exposure occurred. The PTs were evaluated using the commercially available Coulter PT/Fibrinogen kit, a mammalian (rabbit brain) thromboplastin. The validity of using a mammalian source thromboplastin for avian PT determinations for brodifacoum exposed birds needs to be evaluated.

One of the many tests to assess the hemostatic system, the PT is used to indicate the relative risk of hemorrhaging of individuals suspected of anticoagulant exposure. The PT measures the extrinsic portion of the coagulation cascade (Brown 1988), and is an indicator of the vitamin K dependent clotting factor activity (Rapaport 1987). After whole blood is collected and the calcium is bound by sodium

citrate to prevent coagulation, tissue thromboplastin is mixed with plasma, and time to clotting is noted (Brown 1988). The PT is compared against a known normal and indicates the degree of clinical anticoagulation.

A PT can be determined for avian species using homologous or heterologous thromboplastins. The PT which most accurately reflects the *in-vivo* system, however, is best estimated with a homologous thromboplastin. Didisheim et. al. (1959) found that when homologous thromboplastin is used for PT determinations in avian plasma, the prothrombin time is no longer than many mammalian species. However, the PT is prolonged when a mammalian source thromboplastin is used for avian PT determinations (Kase 1978; Tahira et al. 1977; Didisheim et al. 1959). The literature is unclear, but there is a suggestion that factor VII is in low concentration or absent in avian blood (Walz et al. 1975; Stopforth 1970), which may account for the increased PT if avian thromboplastin has no physiological need to bind with factor VII. Kase (1978) found evidence of factor VII in avian plasma while Belleville et al. (1982) demonstrated the mammalian equivalent of factors V, VII, IX, and X in Japanese Quail plasma. The resulting prolonged prothrombin times, when using a mammalian source thromboplastin in avian plasma (and vice versa), may reflect a difference between avian and mammalian coagulation systems, or that they are less specific manifestations of the class specificity of these protein interactions (Kase et al. 1980). However, further studies by Belleville et al. (1982) and Doerr and Hamilton (1981), have demonstrated a fundamentally similar coagulation pathway as in the well studied human system, thus the differences are

likely a result of the class specificity of the protein reactions. Therefore, the use of a mammalian thromboplastin for evaluating avian PT may be adequate for comparison purposes if the PT are reproducible between individuals. The objective of this experiment was to evaluate if brodifacoum residue could be detected in the plasma of birds, and if the prothrombin test kit detect an increase in prothrombin time. The secondary objective was to assess whether brodifacoum plasma residue levels or prothrombin time change is a more sensitive indicator of exposure to low levels of brodifacoum.

Materials and Methods

1.0 Plasma Brodifacoum Residues

Eighteen male Japanese quail (*Coturnix japonica*) were obtained from the UBC Quail Genetic Resource Centre. The birds were put on a 12L/12D light regime with water and food provided *ad libitum*. After a 6 day acclimatisation period the birds were randomly divided into six treatment groups of two with six as reserves. The experimental design was a 2 x 3 factorial, with two dose levels of brodifacoum and three time points for blood collection. The two dose levels were 0.7 mg/kg and 1.4 mg/kg (6% and 12% of the LD_{50} respectively (Ross et al. 1976). The three time points for blood collection were 24 h, 5 d and 10 d post dosing. After processing the 24 hour groups, 2 of the 1.4 mg/kg group had died between 24-36 h post dosing, 1 by 3 d, and 1 by 4 d post dosing. Necropsy results revealed massive localised hemorrhaging on the top of the head and side of the neck. Japanese Quail tend to jump in reaction to noise and possibly causing them to hit their heads on the ceiling of the battery. Thus, the remaining 6 reserve quail were dosed at a lower level of 0.35 mg/kg and blood collected from 2 birds each at 24 h, 6 d and 10 d post dosing.

Trunk blood was collected into heparin coated test tubes and transferred into 1 ml cryovials for centrifuging. The plasma was pipetted into 1 ml prelabelled cryovials and frozen at -20 C until shipment with the livers to the Department of Agriculture, State of Illinois, Veterinary Laboratory Service for HPLC analysis (Murphy et al. 1989; Hunter 1983). The detection limit was 0.002 ppm.

All birds were examined for signs of internal bleeding and livers extracted and

frozen for residue analysis.

The 0.7 and 0.35 mg/kg (instead of 1.4 mg/kg) dose groups data were treated as a 2 (dose levels) x 3 (time points) experiment for analysis. Analysis of the data was carried out using the *JMP* statistical package (SAS, 1995) with the following statistical model:

$$Y_{ijk} = \mu + D_i + T_j + (DT)_{ij} + E_{ijk}$$

where Y_{ijk} = Residue level, D_i = the effect of the ith dose level, and T_j = effect of the jth time point of blood collection, $(DT)_{ij}$ = the two-way interaction between dose effect and time of blood collection, and E_{ijk} = random error. The data was common log transformed in attempt to normalise the data and the analysis rerun using the above model.

An ANOVA was used to analyse the 1.4, 0.7 and 0.35 mg/kg dose groups at 24 h post dosing.

2.0 Prothrombin Time Validation

Twenty seven, 4 month old male Japanese quail (*Coturnix japonica*) were obtained from the UBC Quail Genetic Resource Centre. The birds were put on a 12L/12D light regime with water and food provided *ad libitum*. After a 6 day acclimatisation period the birds were randomly divided into nine treatment groups of three. The experimental design was a 3 x 3 factorial, with three dose levels of brodifacoum and three time points post-exposure for prothrombin time evaluation. The three dose levels were 0.0 mg/kg (control), 0.7 mg/kg (a low and sub-lethal

dose), and 1.4 mg/kg (a high and potentially lethal dose as determined from a pilot study conducted earlier). The three time points for prothrombin time evaluation were 24 h, 72 h and 120 h post dosing (Stopforth, 1970).

After processing the 24h and 72 h treatment groups, it was obvious that the highest dose group were not showing signs of internal bleeding or morbidity after exposure. The 120h groups were therefore not processed, and the birds were given a 7 week rest period (to minimise any effects of prior exposure) and redosed at 13.5 mg/kg (the upper 95% confidence interval of the LD_{50} (Ross et al. 1976)). Blood was collected from the jugular vein but trunk blood was collected from those with which difficulties were encountered in bleeding. Blood collected was immediately transferred into a test tube containing buffered sodium citrate. Prothrombin times were measured within 6 h of collection using the Coulter P/T Fibrinogen kit (Lot # N1222295) for manual evaluation (tilt-tube method) (Brown, 1988).

All birds were sacrificed after blood collection and examined for signs of internal bleeding.

Since blood collected from decapitation showed a significantly shorter PT time than blood collected via the jugular vein, the PT measurements were converted to prothrombin time ratio (PTR) to eliminate the bias due to collection method (Miletitch 1995). The PTR is the ratio of the dose group PT to the mean control PT (PTR= Sample PT/ Mean Control PT).

The data was square root (SQRT) transformed for analysis. Initial analysis showed that there was no difference in the PTR in the control groups of the first and

the added treatment, the data were therefore treated as a 4 (dose levels) x 2 (time points) experiment for analysis. Analysis of the data was carried out using the *JMP* statistical package (SAS 1995) with the following statistical model:

$$Y_{ijk} = \mu + D_i + T_j + (DT)_{ij} + E_{ijk}$$

where Y_{ijk} = (SQRT) PTR measured, D_i = the effect of the ith dose level, and T_j = effect of the jth time point of blood collection, $(DT)_{ij}$ = the two-way interaction between dose effect and time of blood collection, and E_{ijk} = random error.

Results

1.0 Plasma Brodifacoum Residues

Log transformation had no effect on analysis. The data is presented using the arithmetic values.

1.1 *Plasma Residue Levels*

There was no significant interaction between time and dose.

1.1.1 *Time post Exposure*

There was no significant difference in brodifacoum plasma residues at 24 h between the 1.4, 0.7, and 0.35 mg/kg dose groups. The plasma residues declined significantly ($P<0.05$) between d 1 (0.028 ± 0.005 ppm) and 5 (0.005 ± 0.005 ppm) d post dosing, but not between d 5 and 10 (0.002 ± 0.005 ppm) (Figure A-1).

1.1.2 *Effect of dose level*

There was a significant dose effect. The brodifacoum plasma residue was significantly less ($P<0.05$) in the 0.35 mg/kg dose group (0.005 ± 0.004) than the 0.7 mg/kg group (0.018 ± 0.004) (Figure A-2).

1.2 Liver Residue Levels

There was no significant interaction effect, or significant effect of time or dose on the level of residue between groups (Table A-1).

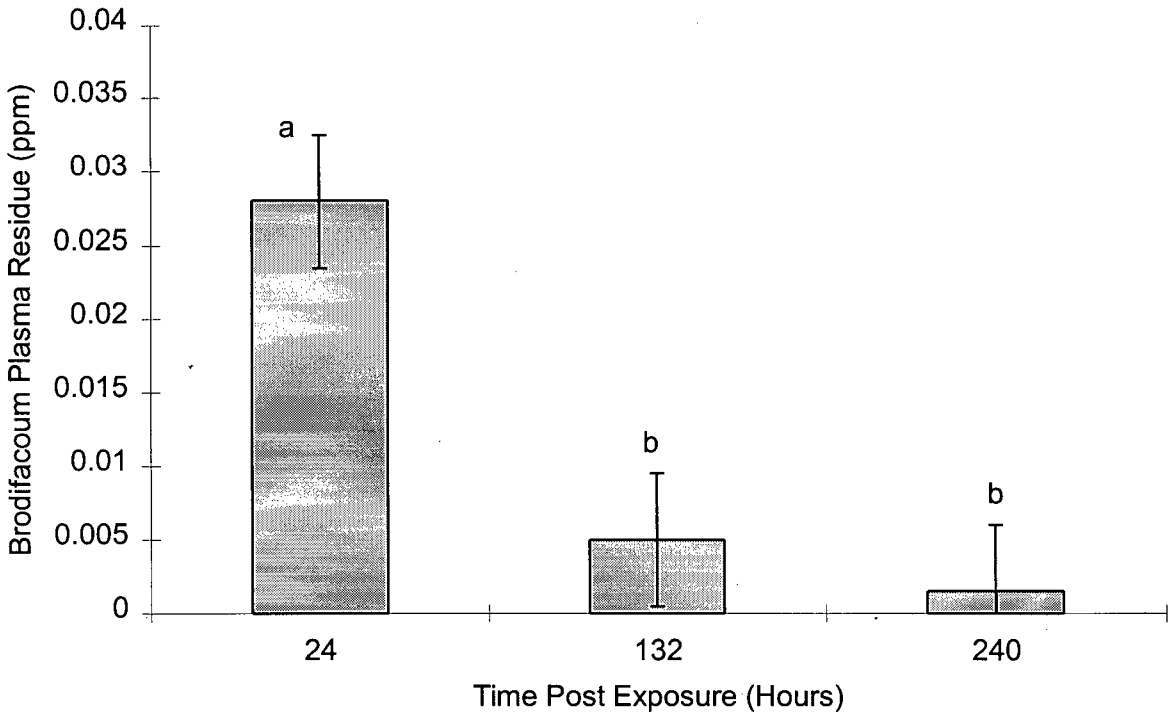

Figure A-1. Effect of time on brodifacoum plasma residue concentration (ppm) after a single oral dose of brodifacoum at 0.35 and 0.7 mg/kg (means that do not share the same letter were significant at $P<0.05$) (n=4 at each time point).

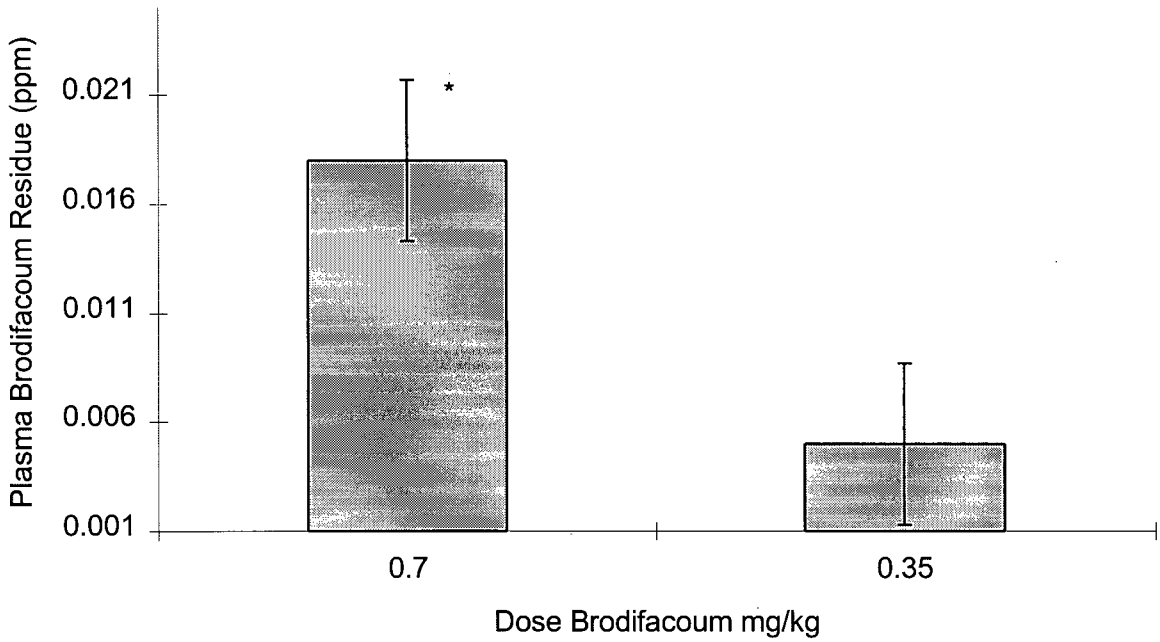

Figure A-2. Effect of dose on brodifacoum residue concentration (ppm) over 10 d (* significant at $P<0.05$) (n=2 at each dose level).

Table A-1. Liver brodifacoum residue in Japanese Quail after a single oral dose of brodifacoum (mean ±s.e., n=2 for each time/dose group).

Days Post Dose	Dose (mg/kg) 1.4	0.7	0.35
1	0.700 ± 0.100	0.52 ± 0.110	0.487 ± 0.12
5	-	0.443 ± 0.122	0.402 ± 0.003
10	-	0.354 ± 0.043	0.373 ± 0.037

1.3 Necropsy Results

Quail in the 1.4 mg/kg group showed hemorrhaging around the cranium and neck, and into the abdominal cavity. The 0.7 and 0.35 mg/kg groups showed no signs of hemorrhaging.

2.0 Prothrombin Time Validation

There was no significant interaction between time post exposure and dose level.

2.1 *Time post exposure*

One of the 4 birds dosed with 13.5 mg/kg brodifacoum showed signs of morbidity by 48 h and the remaining were bled for PT evaluation before 72 h post exposure. These were included in the 72 h group for analysis. The PTR of birds bled at 24 h (1.11± 0.068 sec) was significantly ($P<0.02$) less than those bled at 48 or 72 h post exposure (1.37±0.062 sec) (Figure A-3).

2.2 *Effect of dose level*

There was a significant ($P<0.02$) dose effect. The PTR for the control

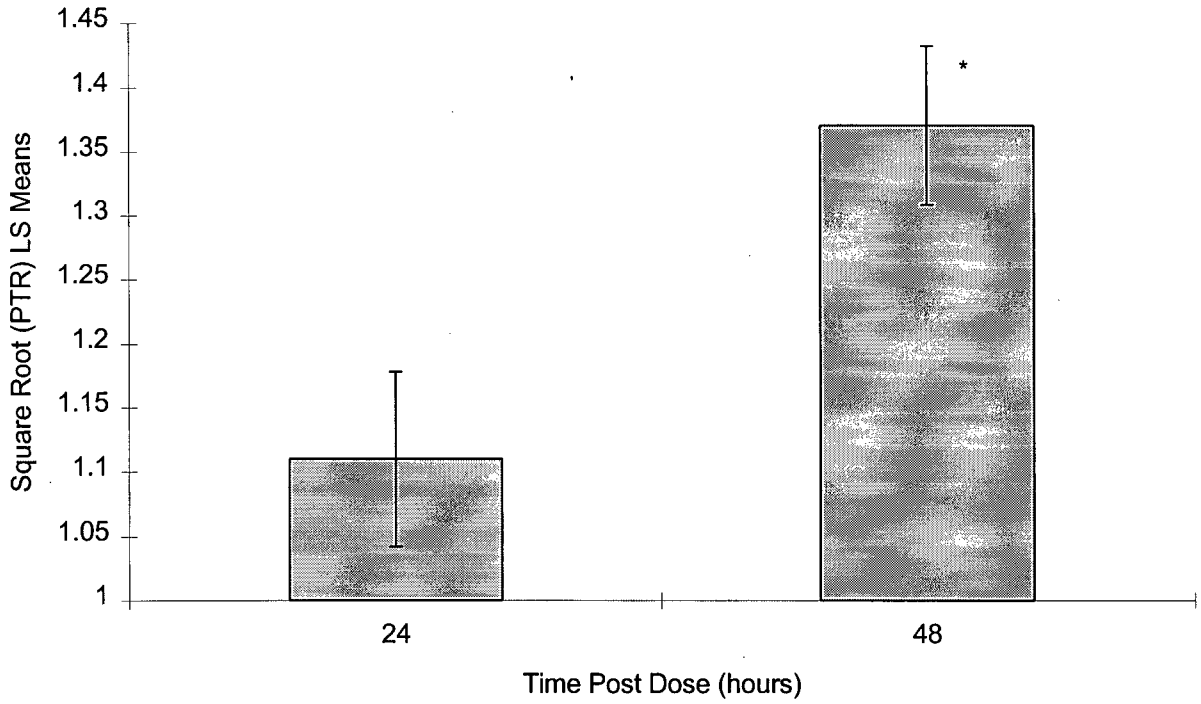

Figure A-3. Effect of time on the prothrombin time ratio (PTR) of Japanese Quail after a single oral dose of brodifacoum (* significant at $P<0.05$) (n=9 at each time point).

(1.00±0.082 sec) was not significantly different from those of the 0.7 mg/kg group (1.17±0.088 sec). The PTR of the 13.5 mg/kg dose group (1.47±0.099 sec) was significantly longer than the control and 0.7 mg/kg groups but not the 1.4 mg/kg group (1.31±0.099 sec). The 1.4 mg/kg and 0.7 mg/kg were not significantly different from each other (Figure A-4).

2.3 *Necropsy Results*

The control, 0.7 mg/kg and the 1.4 mg/kg groups showed no signs of hemorrhaging. The 13.5 mg/kg group showed signs of hemorrhaging. One individual was found dead at 48 h post dosing with extensive hemorrhaging in the breast muscle as well as frank blood in the abdominal and thoracic cavity. Another individual which had not died at 48 h, showed signs of hemorrhaging on the left body wall of the abdomen.

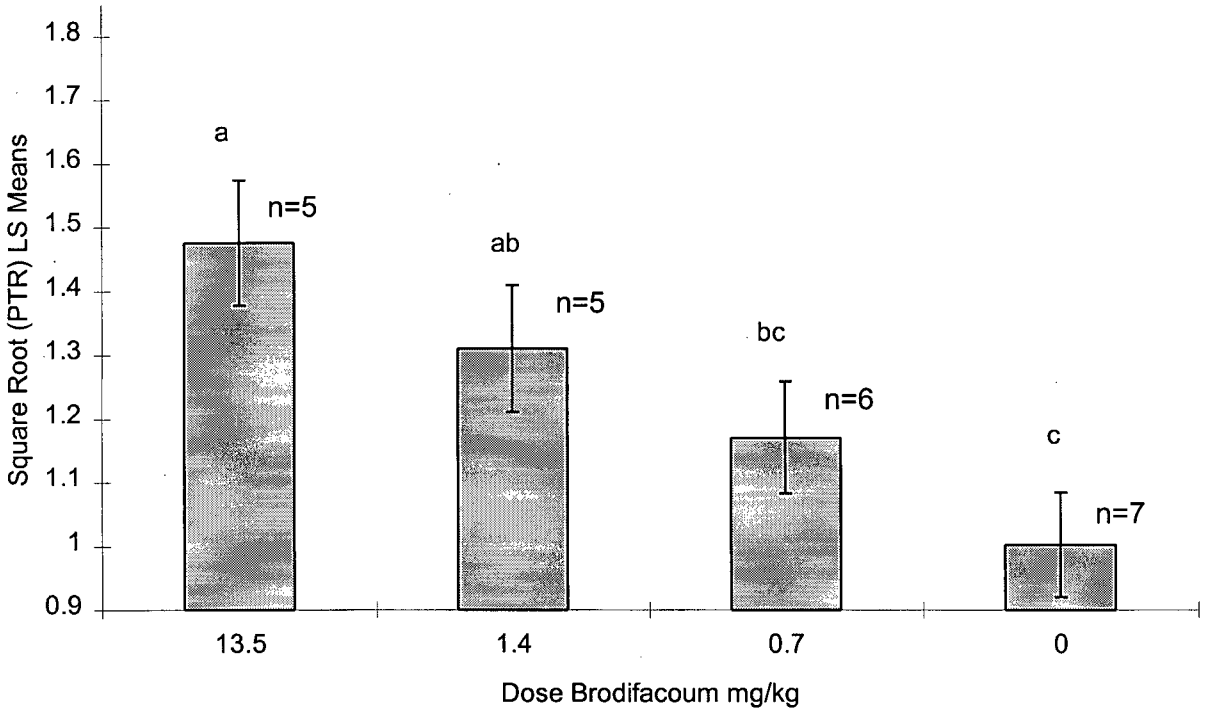

Figure A-4. Effect of dose of the prothrombin time ratio (PTR) of Japanes quail after a single oral dose of brodifacoum (means that do not share the same letter were significantly different at $P<0.05$).

Discussion

The results of the experiment indicate that the Coulter PT/Fibrinogen kit is able to detect an increase in the PT of avian plasma after exposure to brodifacoum. However, the kit was unable to detect a low, sublethal exposure to brodifacoum. Brodifacoum residue was detected in the plasma at all dose levels for up to 10 d.

In the PT experiment both time and dose were significant, likely from the 1.4 and 13.5 mg/kg doses. The lack of significant interaction between time and dose may be a result of the low sample size in each group and/or variation between samples. The variability is likely due to the use of mammalian thromboplastin for avian PT determinations and not the technique (Griminger 1986). Dorn and Muller (1965), using a rabbit brain thromboplastin, did not detect a significant increase in the PT s of chicks until 6 d of feeding vitamin K deficient and 0.1% sulfonamide feed. Conversely, the use of homologous thromboplastin detected a significant rise in the PT at 3 d. Thus, a mammalian thromboplastin would not be as sensitive as an avian thromboplastin for avian PT determinations. It would have taken a bigger decrease in prothrombin levels before it can be detected by mammalian thromboplastin. In other words, the PT/Fibrinogen kit may be useful to detect a high dose of brodifacoum but would not be sensitive enough to detect a low dose exposure.

The PT/Fibrinogen kit was unable to detect a low dose of brodifacoum that did not result in an increased PT or a high dose until the PT rose, 48-72 h after exposure. These results coincide with the accepted model that after an

anticoagulant binds in the liver and inhibits the recycling of vitamin K, enough time is required for the plasma concentration of the vitamin K dependent clotting factors to decline to a point below which the PT increases (Rapaport 1987; Hoffman et al. 1988).

The increased PTR indicates a risk of bleeding but not that hemorrhaging will occur. The 1.4 mg/kg and 13.5 mg/kg dose groups had similar PTRs at 48/72 h although only the latter group showed any signs of bleeding. In the brodifacoum residue experiment, individuals in the 1.4 mg/kg group, lethally haemorrhaged apparently brought on by trauma. This indicates that trauma may be required to induce hemorrhaging in individuals that have suppressed prothrombin levels. The prothrombin levels may have been enough to stop minor bleeding such as from spontaneous haemorrhages, but not a "major" wound (Doerr and Hamilton 1981). The 13.5 mg/kg group prothrombin levels may have been suppressed to the point beyond which minor, spontaneous hemorrhaging could not be controlled. The PTR of the 1.4 mg/kg group at 72 h suggests that these individuals were at risk of hemorrhaging although the conditions in the laboratory setting did not induce any detectable bleeding.

Brodifacoum was rapidly removed from the blood stream between days 1 and 5 post dosing followed by a slower insignificant elimination to the end of the study at day 10. In mammal serum, brodifacoum follows an exponential decay, persisting for 2-3 w (Muphy et al. 1985). Eason et al. (1996) reported that sub-lethal levels of brodifacoum (0.1 mg/kg) were detectable in plasma of possums (*Trichosurus*

vulpecula) for 35 d after oral administration of a sub-lethal dose.

The residues in quail liver indicate that brodifacoum is slowly eliminated. For a closely related compound, flocoumafen, the elimination half-life for quail liver is >100 d (Huckle et al. 1989b). In mammals, brodifacoum is extremely slowly eliminated (Mosterd and Thijssen 1991). Godfrey (1985) estimated the half-life of brodifacoum to be in excess of 150-200 d. Multiple long-term exposure to sub-lethal doses of brodifacoum have the potential to accumulate and suppress factor levels from a partially depressed state and may result in significant bleeding (Hoffman et al. 1988).

Although the test results are from a small sample size, they indicate that the use of a mammalian derived thromboplastin used for the PT determination of bald eagles on Langara Island in 1995 was valid and would have detected a severely anticoagulated bird. However, a high PT would likely correspond to a positive detection of plasma brodifacoum residue. When used in combination, the plasma brodifacoum residue and PT data can confirm exposure and associated risk of hemorrhaging. However, plasma brodifacoum residue analysis is a more sensitive indicator of exposure in birds.

Appendix B. Brodifacoum Exposure in the Song Sparrow.

Introduction

Song sparrows were photographed at three rat carcasses placed to identify scavengers of rats (Chapter 4). They were apparently attracted to insects that began to consume the rat carcasses. In chapter 5, carrion insects were found to test positive for brodifacoum after consuming brodifacoum poisoned rats and pose a tertiary poisoning risk to non-target species. The objective of this section was to determine if song sparrows were exposed to brodifacoum.

Methods

Song sparrows were collected by shotgun after the intensive baiting period beginning mid August 1995. After selected morphological measurements were taken, the livers were removed and frozen for analysis. The livers were pooled across time or location for analysis by HPLC as described in Chapter 4.

Results

Brodifacoum residues were detected in sparrows collected (Table A2.1). Selected morphological measurements and collection locations can be found in table A2.2.

Table B-1. Brodifacoum residues detected in Song Sparrows, Langara Island, 1995.

Pool	Sparrow	Location	Date	Tissue Analysed	Brodifacoum (ppm)
1	1, 4	North Egeria Bay	August 14	Liver	ND
2	2, 9	North Egeria Bay	August 16	Liver	0.643
3	6, 7	South Egeria Bay	August 14/16	Liver	ND
4	10, 11, 12	Lord Bight	August 21	Liver	0.567
5	3, 5, 8, 13	Egeria Bay/Lord Bight	August 14/16/21	Body	0.058

Table B-2. Morphological measurements from Song Sparrows collected on Langara Island, 1995.

No	Date Collected	Sex	Wing Chord (cm)	Tail Length (cm)	Hallux (cm)	Bill Depth (cm)	Tarsus Length (cm)	Tarsus Diameter (cm)
1	August 14	M	6.8	7.1	-	0.61	-	-
2	August 16	M	6.3	5.9	0.8	0.55	2.20	0.12
3	August 16	-	-	-	-	0.59	-	-
4	August 14	M	6.6	7.6	0.78	0.60	2.34	0.18
5	August 14	-	6.2	7.0	-	0.70	-	-
6	August 14	F	7.0	6.9	0.86	0.65	2.72	0.20
7	August 16	M	6.4	5.8	-	0.62	2.48	0.17
8	August 16	-	6.3	7.2	0.81	0.54	2.36	0.16
9	August 16	M	6.9	7.6	0.86	0.72	2.31	0.18
10	August 21	M	7.2	5.7	0.82	0.62	2.64	0.16
11	August 21	M	7.0	6.5	0.80	0.70	2.50	0.16
12	August 21	M	6.6	5.6	0.79	0.61	2.44	0.16
13	August 21	-	7.0	6.0	0.74	-	2.47	0.16

Discussion

Song sparrows were exposed to brodifacoum during the baiting campaign and were at risk of poisoning. It is unclear if the risk was from consuming carrion insects consuming brodifacoum poisoned rat carcasses or primarily from eating crumbs of bait that was found scattered around bait stations and along rat runs. In New Zealand, both granivorous and insectivorous birds were primarily poisoned after feeding on aerial distributed bait pellets intended for introduced rodents (Eason and Spurr 1995). Song sparrows are primarily insectivores, however, they are known to take seeds (Ehrlich et al. 1988). As well, they may have preyed on snails or other invertebrates feeding on the bait in the stations. Therefore, song sparrows were at risk of primary, secondary and tertiary poisoning. Primary poisoning from the bait crumbs, secondary from the invertebrates feeding on the bait, and tertiary from carrion insects.

The pooling of samples precludes quantifying the brodifacoum residue in individual carcasses as the brodifacoum may have originated from a single sparrow or all, likely diluting the concentration. However, it can be concluded that sparrows were at risk of brodifacoum poisoning and the primary, secondary and tertiary poisoning risk should be further investigated in future eradication operations.

Appendix C. Potential Sub-Lethal and Long Term Effects of Brodifacoum Exposure

There is no data from the literature on the long term effects of brodifacoum or the other anticoagulants on target or non-target species. This appendix identifies possible sub-lethal effects and potential long term effects of brodifacoum exposure to non-target species. The anticoagulant rodenticides are known to suppress clotting factor levels, affect bone mass, reproductively toxic, and are teratogenic with possible development effects.

The suppression of the clotting factor levels was the mechanism focused on in this thesis, and the mode of action is discussed in Chapter 2. However, there are long term implications of sub-lethal or repeated exposure to brodifacoum in that an increasingly smaller dose would potentiate the anticoagulant effect because of the already suppressed level of clotting factors. A normal PT may be obtained from human plasma with between 30-100% of normal vitamin K dependent clotting factor concentrations (Hoffman et al. 1988). Suppression of the clotting factor concentration below the threshold would result in an increased PT and possibly uncontrollable hemorrhaging. A significant decrease in the levels of the vitamin K dependent clotting factors lasted 43 days after ingestion of brodifacoum (Hoffman et al. 1988).

Bone mass may be compromised by sub-lethal or long term exposure to oral anticoagulants. Bone contains the vitamin K dependent protein osteocalcin which is part of the bone matrix, and levels in the blood may be used as an indicator of active bone deposition. The oral anticoagulants (phenprocoumon and acenocoumarol) reduce the activity of osteocalcin, which is activated by a vitamin K dependent reaction

(Van Haarlem et al. 1988). Patients on long term oral anticoagulant therapy showed significantly lower bone mass than controls (Fiore et al. 1990; Resch et al. 1991). Furthermore, it was found that a poor vitamin K status was associated with a high urinary calcium loss (Knapen et al. 1993). Therefore, young individuals with growing bones and female birds and mammals with high calcium demands in the breeding season are at greatest risk of sub-lethal effects of brodifacoum exposure.

The coumarin anticoagulants have been shown to be embryotoxic and teratogenic to rats. In rats, warfarin induced increased rates of embryolethality, hemorrhage and gross structural malformations (internal hydrocephalus and anomalies of skeletal ossification) (Mirkova and Antov 1983). Similarly, indiscriminate use of brodifacoum in fields was related to increased incidences of abortions and hemorrhage in sheep and goats in Egypt (Feinsod et al. 1986). In contrast, other laboratory studies have not demonstrated reproductive toxicity or teratogenicity of brodifacoum to rats or rabbits at various dose levels (Hodge et al. 1980a, 1980b, 1980c).

Anticoagulants have developmental impacts on the human fetus. In humans, there are two types of anticoagulant induced defects, depending on the time of administration of the anticoagulant: fetal warfarin syndrome and fetal wastage (Anonymous 1976). The most consistent feature of fetal warfarin syndrome is nasal hypoplasia leading to respiratory difficulty. Fetal wastage results also in central nervous system anomalies. Other common features includes bone abnormalities of the axial and appendicular skeleton (IPCS 1995), opthalmological malformations

leading to blindness, developmental delay, low birth weight, premature birth, mental retardation, and ear anomalies (Schardein 1985).

Although the above summary indicates that brodifacoum may have potential long term effects from sub-lethal exposure to non-target species, more work in these areas are required to confirm potential impacts. However, this summary identifies potential areas of investigation that may be conducted both in laboratory and field experiments. Clearly, the mortality endpoint as a result of the exposure to brodifacoum, the main focus of this thesis, is not the only non-target species impact that could be measured during future island restoration projects.

Appendix D. Environmental Aspects of Brodifacoum - Transport, Distribution and Transformation.

Air, Water and Soil

Air

Anticoagulant rodenticides have low volatility and increased levels in the air are unlikely (IPCS 1995).

Water

Brodifacoum is slightly soluble in water (<10 mg/l at 20 °C, pH 7). The vapor pressure is <0.13 mPa at 25 °C (IPCS 1995). It is a weak acid and does not readily form water-soluble salts (Worthing and Walker 1987). However, it is known to be toxic to fish. The brodifacoum 96-h LC_{50} to rainbow trout is 0.051 mg/l (Hill et al. 1976). This is equivalent to 1 bait block (20 g bait with brodifacoum at 0.005%) per 20 l of water that could be considered a toxic hazard to fish.

Soil

The adsorption and desorption of ^{14}C-brodifacoum in laboratory conditions has been investigated. Brodifacoum binds very strongly to soil particles and equilibria is established fairly rapidly with larger water:soil ratios despite very low brodifacoum water solubility (Newby and White 1978). Binding increases with greater organic matter in the soil (ICI 1984). Once bound to the soil particles, ^{14}C-Brodifacoum is effectively immobile and desorption is very slow (Jackson and Hall 1992; Newby and White 1978). Less than 2% of brodifacoum, added to

soil (0.6 and 6.0 kg/ha) with a pH from 4.3-7.1, organic matter from 6.8-72.1% and clay content from 5-19%, leached more than 2 cm (ICI 1984). This suggests that mobility of brodifacoum would be restricted to erosion processes, traveling with soil particles.

Once bound to soil particles, slow microbial degradation reduces brodifacoum to CO_2 and water (Taylor 1993; Shirer 1992). The half life of brodifacoum in soil ranges between 12-25 weeks, depending on soil conditions (ICI 1984). Hall and Priestley (1992) monitored the metabolism of ^{14}C-Brodifacoum in soil under aerobic conditions for 52 weeks. A mean of 35.8% of radioactivity recovered was $^{14}CO_2$. Radiolabelled brodifacoum was the major radiolabelled component in the soil extracts. A half-life of brodifacoum in soil was calculated to be 157 days.

No information on the abiotic degradation of brodifacoum is available. However, abiotic degradation of related second-generation anticoagulants have been described. Bromadiolone degrades rapidly on exposure to sunlight, with a half-life of 2.1 h (IPCS 1995). The photolytic half life of difenacoum at pH 5, 7, and 9 over 24 hours was calculated to be 3.3, 8.1 and 7.3 h (Hall et al. 1992). The hydrolytic half-life of brodifacoum was found to be in excess of 30 days, but no precise estimation was made (Jackson et al. 1991).

These data indicate that brodifacoum is a lipophilic compound relatively resistant to breakdown in soil. Any brodifacoum that is not consumed by target or non-target organisms and that is left in the environment will be present and

potentially available for transport into the ecosystem long after the bait has been removed from the stations.

Biological Retention of Brodifacoum

Biologically, brodifacoum is a lipophilic anticoagulant rodenticide relatively resistant to metabolism. Most (75%) of a 25 mg/kg dose of brodifacoum given to rats was retained principally in the liver, pancreas and the salivary glands, at ten days post dosing when the study ended (Godfrey 1985). The biological half-life was found to be 150-200 days (Godfrey 1985). Parmar et al. (1987) estimated the liver brodifacoum half-life to be 130 days in rats. Brodifacoum was detected in sheep liver 128 days post dosing at 0.2 and 2 mg/kg (Laas et al. 1985). Similarly, sheep consuming a single sub-lethal dose of 2 mg brodifacoum/kg showed 2 mg/kg in the liver 4 months after dosing (Rammell et al. 1984).

These data indicate that sub-lethally exposed mammals and likely birds, pose a long term poisoning risk to species preying on them. Long term retention of brodifacoum may also lead to accumulation of brodifacoum initiating the anticoagulant effect leading to hemorrhaging and death.