RELATIONSHIP OF PLASMA LIPIDS, THYROID HORMONES, AND VITAMIN A WITH ENVIRONMENTAL CONTAMINANTS MEASURED IN BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*) IN BRITISH COLUMBIA AND SOUTHERN CALIFORNIA

by

Lillian Sylvia Cesh

B.S., Zoology, University of Calgary, 1999

PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ENVIRONMENTAL TOXICOLOGY

In the
Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

Summer 2005

APPROVAL

Name: Lillian Sylvia Cesh

Degree: Master of Environmental Toxicology

Title of Project:

Relationship of plasma lipids, thyroid hormones and vitamin A with environmental contaminants measured in bald eagles in British Columbia and Southern California

Examining Committee:

Chair: Dr. C. Lowenberger, Assistant Professor

Dr. T. Williams, Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. J. Elliott, Research Scientist
Canadian Wildlife Service, and Adjunct Professor
Department of Biological Sciences, S.F.U.

Dr. C. Kennedy, Associate Professor
Department of Biological Sciences, S.F.U.

Dr. M. Moore, Associate Professor
Department of Biological Sciences, S.F.U.
Public Examiner

August 10, 2005
Date Approved

SIMON FRASER UNIVERSITY

PARTIAL COPYRIGHT LICENCE

ABSTRACT

Bald eagles (*Haliaeetus leucocephalus*) are considered an appropriate indicator species to monitor ecosystem contamination. In this study, bald eagles nestlings were used to 1) investigate the importance of analyzing and controlling for plasma lipid levels when interpreting contaminant levels; 2) determine the relationship between contaminants and thyroid hormones as well as vitamin A; and 3) examine spatial and temporal trends of organochlorines and polybrominated diphenyl ethers in British Columbia and southern California. No significant relationships were found between contaminants and lipid levels. Nevertheless, significant differences between sites for both p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE) and polychlorinated biphenyls (PCBs) were detected. Only p,p'-DDE and PCB levels showed a negative correlation with thyroid hormones. No significant relationships were found between contaminants and vitamin A. In British Columbia, trans-Nonachlor and PCB levels significantly decreased between 1993 and 2003, but Hexachlorobenzene (HCB) and p,p'-DDE have not.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my two main supervisors, Dr. Tony Williams, and Dr. John Elliott for their excellent guidance, support, assistance, and for finding funding to support me during the completion of my degree. It was an honour and pleasure to work with both of you. I would also like to thank Dr. Chris Kennedy for his support, suggestions, and help in preparing for my defence.

I would like to thank Dave Garcelon and Peter Sharpe with the Institute for Wildlife Studies for allowing me to collaborate with the bald eagle project on Santa Catalina Island, California. Peter Sharpe collected the blood samples and eagle measurements on the island.

I would like to acknowledge everyone who helped out with the field work. Sandi Lee of the Canadian Wildlife Service organized the field equipment, travel accommodations, and assisted with blood sampling and data collection. Ian Jacobs and Darcy Haycock climbed the trees and retrieved the eagle nestlings. Ian Moul and Chris Gill were our guides on Vancouver Island. Shari Weech and Sandi Lee collected the blood samples and eagle measurements in the Fort St. James area.

I would like to acknowledge participants of the captive feeding experiment. Sandi Lee and Chris Gill of the Canadian Wildlife Service performed the experiment. Staff at the Orphaned Wildlife Rehabilitation Centre assisted with the study and allowed the captive bald eagles at the facility to be used for this experiment.

I would like to acknowledge the laboratory staff led by Suzanne Trudeau and Bryan Wakeford at the National Wildlife Research Centre in Ottawa, Ontario for completing the analysis of organochlorines, PCBs, total lipids determined both gravimetrically and colorimetrically, thyroid hormones, and vitamin A. Robert Letcher and his assistants completed the analysis of PBDEs. Thank you for your proficient work and helping me learn the methodology used at the lab.

I would like to thank all of my lab mates for their support, suggestions, and helping me improve my study and presentations; Jim Dale, Courtney Albert, Kristen Gorman, Kat Salvante, Oli Love, and Dana Seaman. I would like to thank Daniel Guertin for his patience, understanding, support, and for proofreading the many versions of my thesis. Karen Vey for her enthusiasm and support of my research, and my family for their continued love and support.

Funding for this research project was provided by the Georgia Basin Action Plan, Science Horizons, Canadian Wildlife Service, and Simon Fraser University.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
GENERAL INTRODUCTION

Environmental contaminants

The introduction and use of synthetic chemicals in the environment has had a severe impact on wildlife (Blus 1996; Clark et al. 1998; de Wit 2002; Elliott and Harris 2001/2002; Kannan et al. 2004; Kendall 2003; Murk et al. 1996). It has been estimated that worldwide, approximately 70,000 chemicals are in common use, and the chemical industry markets an additional 200-1000 new synthetic chemicals each year (Moeller 1997). As a result, wildlife are constantly exposed to these chemicals in the environment from their use in pesticides, herbicides, flame retardants, industry, as well as many other sources (de Wit 2002; Hoffman et al. 1990; Kannan et al. 2004; Kendall 2003). In areas of high exposure, adverse effects such as immunotoxicity, endocrine disruption, physical deformities, sex reversal, reduced reproduction, and mortalities have been noted in many species (Blus et al. 1997; Bowerman et al. 2000; Elliott and Harris 2001/2002; Garcelon 1994; Hayes et al. 2003; Somers et al. 1993) .

The chemicals most problematic with regard to wildlife are those that are persistent and bioaccumulate due to their structure and chemical nature (Elliott and Harris 2001/2002; Murk et al. 1996; Somers et al. 1993). Chemicals such as dichlorodiphenyltrichlorethane (DDT) and polychlorinated biphenyls (PCBs) are lipophilic and are stored in the fat tissue, and consequently accumulate up the food chain (Blus 1996; Clark et al. 1998; Le Boeuf et al. 2002). Species at the top of the food chain

are therefore more susceptible to environmental contaminants. Bald eagles (*Haliaeetus leucocephalus*) are considered to be highly vulnerable to contaminants not only because of their position on the food chain, but also because their scavenging behaviour may put them at greater risk of consuming poisoned prey (Elliott and Harris 2001/2002). Due to this high susceptibility, bald eagles have been used extensively as an indicator species for monitoring contaminants in the environment (Anthony et al. 1993; Bowerman et al. 2000; Donaldson et al. 1999; Elliott and Norstrom 1998; Garcelon 1994; Gill 1993).

Factors affecting toxicity due to environmental contaminants

There are a number of factors that influence the final toxic expression in wildlife due to environmental contaminants. The amount of xenobiotic that the animal is exposed to, duration of exposure, the route of exposure, and species differences all play a role (Hoffman et al. 1990; Klaassen 2001). Differences among species may exist due to differing feeding habits, physiology, and the ability to metabolize the toxins (Hoffman et al. 1990; Klaassen 2001). Also, natural occurring stressors, such as temperature extremes, nutritional deficits, and disease may influence toxicity of xenobiotics (Hoffman et al. 1990). Knowing these factors will aid in the assessment of dangers and risks posed by the toxins and help in the remediation.

The chemical and structural properties of environmental contaminants will also affect their toxicity to wildlife (Klaassen 2001). Environmentally relevant chemical properties include solubility, vapour pressure, Henry Law constant, octanol-water partitioning coefficient (K_{OW}), organic carbon-water partition coefficient, sediment-water partitioning coefficient, octanol-air partitioning coefficient, bioaccumulation factor, and

half-life (Klaassen 2001). Based on these properties, contaminants will vary in their toxic effects they pose on wildlife.

Effects of contaminants on bald eagle

Historically, bald eagles were abundant across North America, but populations declined drastically in the mid-late 1900's, due to a combination of habitat loss, human persecution, and the use of organochlorine pesticides (Elliott and Harris 2001/2002). The bald eagle population in the contiguous United States was listed for protection in 1978 under the Endangered Species Act of 1973 (Buehler 2000). In Canada, bald eagles were designated endangered in Ontario and New Brunswick (1973 and 1976 respectively). In the other Canadian provinces and in Alaska, population declines did not occur or were not as severe (Elliott and Harris 2001/2002). Since 1980, as contaminant levels dropped and human persecution decreased, bald eagle populations have increased dramatically (Buehler 2000). However, in some areas such as the Great Lakes region, Maine, along the lower Columbia River in Oregon, and the southern coast of California, eagle populations have not rebounded as quickly due to continued contaminant problems from exposure to elevated concentrations of chlorinated hydrocarbons (Elliott and Harris 2001/2002).

The levels of chlorinated hydrocarbons in North America have been monitored in bald eagles since the 1960's (Anthony et al. 1993; Bowerman et al. 1998; Dominguez et al. 2003; Donaldson et al. 1999; Elliott and Harris 2001/2002). Most commercial uses of DDT, dieldrin, and PCB's were banned in the US and Canada between 1969 and 1974, due to detrimental effects on the environment and wildlife (Elliott and Harris 2001/2002; Elliott and Norstrom 1998). In bald eagles, these contaminants have been shown to cause eggshell thinning, reduced breeding success, behavioural defects, physical deformations

and mortality (Elliott and Norstrom 1998; Gill and Elliott 2003). Although the contaminant levels have decreased since the 1970's, areas such as southern California and the Great Lakes region are still experiencing these harmful effects (Elliott and Harris 2001/2002).

The Channel Islands, off the coast of California are severely impacted from high contaminant levels (Garcelon 1994, 1997; Garcelon and Roemer 1990). From the 1940's to the 1970's, Los Angeles area industries discharged approximately 1,800 metric tons of DDT and PCBs into ocean waters off the southern California coast. Surveys completed in 1992 and 1993 by the United States Geological Survey estimated more than 100 metric tons of DDT and 10 metric tons of PCBs remain in the sediments on the ocean bottom of the Palos Verdes Shelf (Lee 1994). These levels are still environmentally significant affecting the survival and breeding of wildlife in the area. Bald eagles were a resident breeding species on the Channel Islands from before the turn of the century until at least the 1930's (Kiff 1980). The last confirmed nesting of an eagle on the Channel Islands was in 1947 and by the early 1960's bald eagles were extirpated from all of the Channel Islands (Kiff 1980). In the early 1980's bald eagles were reintroduced on Santa Catalina Island, but they are still not able to reproduce on their own due to continuing high contaminant levels (Garcelon 1997).

Over most of British Columbia there have been no reports of widespread bald eagle population declines (Elliott and Norstrom 1998; Gill 1993). However, several localized populations residing in the Strait of Georgia, the west coast of Vancouver Island, Johnstone Strait, and the Queen Charlotte Islands have continued to show low productivity (Gill 1993). It has been suggested that this decrease in bald eagle

productivity is a result of low food supply during the breeding season and in some locations due to the industry in the area (e.g. paper and pulp mills) (Elliott and Norstrom 1998; Gill 1993). The long term monitoring of these sites continues to provide important information on the status of the local environments, health of the bald eagle population, changes in contaminant levels, and the ability to detect new contaminants such as polybrominated diphenyl ethers (PBDEs) (Elliott and Norstrom 1998; Gill and Elliott 2003).

Polybrominated diphenyl ethers (PBDEs) are chemicals that recently have become a concern in relation to wildlife (de Wit 2002; Ikonomou et al. 2002; Manchester-Neesvig et al. 2001). PBDEs are widely used flame retardants and their concentrations appear to be increasing exponentially in the environment (Ikonomou et al. 2002). Because PBDEs are mixed and not chemically bound into the material they are used in, they are able to migrate from the material during its lifetime (Eriksson et al. 2001). One of the first reports of PBDEs in the environment appeared in 1981 (Anderson and Blomkist 1981). Since then, PBDEs have been shown to be persistent compounds that appear to have an environmental dispersion similar to that of PCBs and DDT (Eriksson et al. 2001). They also have a similar molecular structure to polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), and biphenyls (PCBs) (Manchester-Neesvig et al. 2001). Since these chemicals are known to be toxic, persistent, and bioaccumulative, there is concern that PBDEs will have the same detrimental effects on wildlife and humans as PCBs (Ikonomou et al. 2002).

Lipids in relation to environmental contaminants

Many environmental contaminants are lipophilic (such as organochlorines), therefore the partitioning of these contaminants within the body should depend on the concentration of plasma and stored lipids in the body (Hebert and Keenleyside 1995). Many factors can cause fluctuations in lipid levels, such as breeding, migration, age, mass, feeding, and fasting (Groscolas 1982; Guglielmo et al. 2002; Jenni-Eiermann and Jenni 1994, 1996; Phillips et al. 1989; Williams et al. 1992). Due to the association between lipids and lipid soluble contaminants, the contaminants themselves should also be influenced by these fluctuations in the body lipids. However, few studies have explicitly investigated this correlation between plasma lipids and contaminants to understand the impacts and importance (but see Bustnes et al. 2001; Elliott and Norstrom 1998; Gill 1993).

The movement of lipids within the body and variation in lipid levels between individuals, could potentially influence the contaminant results obtained by sampling from plasma (Fig 1.1). For example, two individuals with the same contaminant body burden, but differing in the amount of lipids in the plasma, could have a higher concentration of contaminants in the plasma due to more contaminants partitioning into plasma lipids (Fig 1.1-a). If the contaminant levels are not adjusted for the effect of higher plasma lipid levels, the individual with the higher percentage of lipids in the plasma would appear to have a higher contaminant level, which is not representative of the body burden. Thus, contaminant levels should be "normalized" to correct for the influence of plasma lipids to correctly assess the body burden via plasma analysis. This problem becomes more complicated if a blood sample was taken from an individual who

has recently eaten a food item high in lipids (but free of contaminants), because there will be a transient increase in plasma lipids due to digestion and assimilation (Fig 1.1-b). The extent to which this transient increase in plasma affects the movement of the contaminants in body lipid stores would depend on the partitioning rates and the time required for the lipids and contaminants to reach equilibrium between the plasma and the body. The partitioning rate will be determined by the percentage of body fat, the body fat to plasma lipid ratio, and the digestion time for the individual. Finally, a third level of complexity is attained if the individual blood sampled has recently eaten a food item high in lipids and high in contaminants. In this situation many more factors come into play, such as the contaminant concentration in the food item, the contaminant concentration already in the body, lipid status of the food item, in the plasma, and the lipid stores of the individual.

Thesis structure

In Chapter 2 a methodological question was addressed concerning the importance of analyzing and controlling for plasma lipid levels when interpreting contaminant results. The objectives for this chapter were firstly to determine the levels of chlorinated hydrocarbons and PCBs in British Columbia. Samples were collected from nests that have been previously monitored to compare the levels of contaminants to earlier studies. Samples were also collected from a new site in southern California known for its high levels of contamination for comparison and collaboration purposes. The second objective was to investigate the importance of analyzing and controlling for plasma lipid levels when interpreting contaminant results. The third objective was to determine which measure of plasma lipids is most appropriate (triglycerides, non-esterfied fatty acids, or

total lipids) when interpreting contaminant results. This has been an under-explored topic in toxicology, and we hope to aid in the understanding of the relationship between lipids and contaminants.

In Chapter 3, the relationships between p,p'-DDE, PCBs and PBDEs with thyroid hormones and vitamin A levels in bald eagles was investigated. The first objective was to compare levels of p,p'-DDE, PCBs, and PBDEs between sites and secondly, to determine the relationship between plasma levels of these chemicals and thyroid hormone and vitamin A levels. Thyroid hormones and vitamin A are essential for proper growth, development, cell differentiation, immunology, vision, and reproduction and there is some evidence that these hormonal pathways are disrupted by p,p'-DDE, PCBs, and PBDEs.

In Chapter 4, spatial and temporal trends of chlorinated hydrocarbons in nestling bald eagles in British Columbia and southern California were determined. Contaminant samples measured in B.C. in 2003 were compared to levels measured in 1993 to investigate the long term trends of these chemicals. Long term monitoring of these contaminants in bald eagles may provide important information on the health of eagle populations and the local environments, and show changes in contaminant levels.

In Chapter 5, conclusions and a general synthesis of the preceding chapters is presented. This chapter highlights and summarizes the key points illuminated in this thesis and makes suggestions for future research.

1.1 Figures

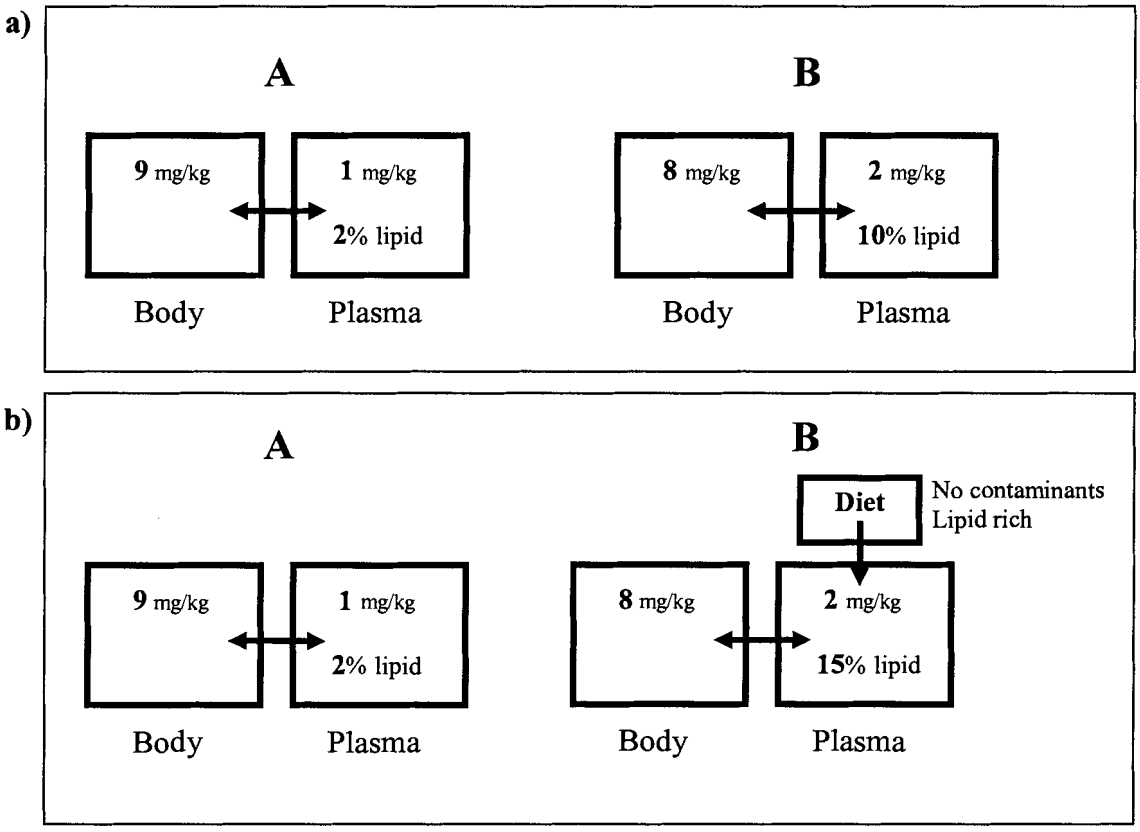

Figure 1.1 Diagram showing the interaction of lipids and lipid soluble contaminants and the influence of feeding. The top scenario shows two individuals with the same contaminant body burden (10 mg/kg), but individual B has more plasma lipids (10%), therefore more contaminants will partition into the plasma. In the bottom scenario, individual B has a transient increase in plasma lipids due to its diet, but no intake of contaminants. The effect on the movement of contaminants depends on the partitioning rate between the plasma and the body.

1.2 Reference List

Anderson, O., and Blomkist, G. (1981). Polybrominated aromatic pollutants found in fish in Sweden. Chemosphere 10, 1051-1060.

Anthony, R. G., Garrett, M. G., and Schuler, C. A. (1993). Environmental contaminants in bald eagles in the Columbia River Estuary. Journal of Wildlife Management 57, 10-19.

Blus, L. J. (1996). Environmental contaminants in wildlife. CRC Press Inc.

Blus, L. J., Wiemeyer, S. N., and Bunck, C. M. (1997). Clarification of effects of DDE on shell thickness, size, mass, and shape of avian eggs. Environmental Pollution 95, 67-74.

Bowerman, W. W., Best, D. A., Grubb, T. G., Zimmerman, G. M., and Giesy, J. P. (1998). Trends of contaminants and effects in bald eagles of the Great Lakes basin. Environmental Monitoring and Assessment 53, 197-212.

Bowerman, W. W., Best, D. A., Grubb, T. G., Sikarskie, J. G., and Giesy, J. P. (2000). Assessment of environmental endocrine disruptors in bald eagles of the Great Lakes. Chemosphere 41, 1569-1574.

Buehler, D. A. (2000). Bald Eagle. The Birds of North America 506, 1-40.

Bustnes, J. O., Skaare, J. U., Erikstad, K. E., Bakken, V., and Mehlum, F. (2001). Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gull (*Larus Hyperboreus*). Environmental Toxicology and Chemistry 20, 1046-1052.

Clark, K. E., Niles, L. J., and Stansley, W. (1998). Environmental contaminants associated with reproductive failure in bald eagles (*Haliaeetus leucocephalus*) eggs in New Jersey. Bulletin of Environmental Contamination and Toxicology 61, 247-254.

de Wit, C. A. (2002). An overview of brominated flame retardants in the environment. Chemosphere 46, 583-624.

Dominguez, L., Montevecchi, W. A., Burgess, N. M., Brazil, J., and Hobson, K. A. (2003). Reproductive success, environmental contaminants, and trophic status of nestling bald eagles in eastern Newfoundland, Canada. Journal of Raptor Research 37, 209-218.

Donaldson, G. M., Shutt, J. L., and Hunter, P. (1999). Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. Archives of Environmental Contamination and Toxicology 36, 70-80.

Elliott, J. E., and Harris, M. L. (2001/2002). An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Reviews in Toxicology, 1-60.

Elliott, J. E., and Norstrom, R. J. (1998). Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. Environmental Toxicology and Chemistry 17, 1142-1153.

Eriksson, P., Jakobsson, A., and Fredriksson, A. (2001). Brominated Flame Retardants: A novel class of developmental neurotoxicants in our environment? Environmental Health Perspectives 109, 903-908.

Garcelon, D. K. (1994). Effects of organochlorine contaminants on bald eagle reproduction at Santa Catalina Island, pp. 1-16. Institute for Wildlife Studies, Arcata.

Garcelon, D. K. (1997). Effects of organochlorine contaminants on bald eagle reproduction at Santa Catalina Island. Montrose Trustee Council.

Garcelon, D. K., and Roemer, G. W. (1990). The reintroduction of bald eagles on Santa Catalina Island, California. Memoirs of the Natural History Foundation of Orange County 3, 63-68.

Gill, C. E. (1993). Environmental contaminants, food availability, and reproduction of bald eagles (*Haliaeetus leucocephalus*) on Vancouver Island, British Columbia. In Biological Sciences, p. 149. Simon Fraser University, Burnaby.

Gill, C. E., and Elliott, J. E. (2003). Influence of food supply and chlorinated hydrocarbon contaminants on breeding success of bald eagles. Ecotoxicology 12, 95-111.

Groscolas, R. (1982). Changes in plasma lipids during breeding, molting, and starvation in male and female emperor penguins (*Aptenodytes forsteri*). Physiological Zoology 55, 45-55.

Guglielmo, C. G., O'Hara, P. D., and Williams, T. D. (2002). Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). The Auk 119, 437-445.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C., and Vonk, A. (2003). Atrizine-induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): laboratory and field evidence. Environmental Health Perspectives 111, 568-575.

Hebert, C. E., and Keenleyside, K. A. (1995). To normalize or not to normalize? Fat is the question. Environmental Toxicology and Chemistry 14, 801-807.

Hoffman, D. J., Rattner, B. A., and Hall, R. J. (1990). Wildlife Toxicology. Environmental Science and Technology 24, 276-283.

Ikonomou, M. G., Rayne, S., Fischer, M., Fernandez, M. P., and Cretney, W. (2002). Occurrence and congener profiles of polybrominanted diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. Chemosphere 46, 649-663.

Jenni-Eiermann, S., and Jenni, L. (1994). Plasma metabolite levels predict individual body-mass changes in a small long distance migrant, the garden warbler. The Auk 111, 888-899.

Jenni-Eiermann, S., and Jenni, L. (1996). Metabolic differences between the postbreeding, moulting and migratory periods in feeding and fasting passerine birds. Functional Ecology 10, 62-72.

Kannan, K., Kajiwara, N., Le Boeuf, B. J., and Tanabe, S. (2004). Organochlorine pesticides and polychlorinated biphenyls in California sea lions. Environmental Pollution 131, 425-434.

Kendall, R. J. (2003). Wildlife Toxicology Revisited. Environmental Science and Technology, 178A-183A.

Kiff, L. F. (1980). Historical changes in resident populations of California Islands raptors. In The California Islands: Proceedings of a Multidisciplinary Symposium (D. M. Power, ed., pp. 651-673. Santa Barbara Museum of Natural History, Santa Barbara.

Klaassen, C. D., ed. (2001). Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill Companies Inc.

Le Boeuf, B. J., Giesy, J. P., Kannan, K., Kajiwara, N., Tanabe, S., and Debier, C. (2002). Organochloride pesticides in California sea lions revisited. BMC Ecology 2, 1-8.

Lee, H. (1994). The distribution and character of contaminated effluent-affected sediment, Palos Verdes Margin, Southern California, p. 237. U.S. Geological Survey, Menlo Park.

Manchester-Neesvig, J. B., Valters, K., and Sonzogni, W. C. (2001). Comparison of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PCBs) in Lake Michigan salmonids. Environmental Science and Technology 35, 1071-1077.

Moeller, D. W. (1997). Toxicology. In Environmental Health, pp. 15-22. Harvard University Press, Cambridge.

Murk, A. J., Boudewijn, T. J., Meininger, P. L., Bosveld, T. C., Rossaert, G., Ysebaert, T., Meire, P., and Dirksen, S. (1996). Effects of polyhalogenated aromatic hydrocarbons and related contaminants on common tern reproduction: integration of biological, biochemical, and chemical data. Archives of Environmental Contamination and Toxicology 31, 128-140.

Phillips, D. L., Pirkle, J. L., Burse, V. W., Bernert, J. T., Hernderson, L. O., and Needham, L. L. (1989). Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Archives of Environmental Contamination and Toxicology 18, 495-500.

Somers, J. D., Goski, B. C., and Barbeau, J. M. (1993). Accumulation of organochlorine contaminants in double-crested cormorants. Environmental Pollution 80, 17-23.

Williams, T. D., Ghebremeskel, K., Williams, G., and Crawford, M. A. (1992). Breeding and moulting fasts in macaroni penguins: do birds exhaust their fat reserves. Comparative Biochemistry and Physiology 103A, 783-785.

CHAPTER 2
EFFECT OF PLASMA LIPID CONTENT ON INTERPRETING VARIATION IN CHLORINATED HYDROCARBON CONCENTRATION IN BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*)

2.1 Abstract

Many studies investigating contaminant levels, such as organochlorines have either ignored variation in lipid levels among study species or have just assumed that the contaminants must be normalized without investigating the relationship in detail. This study investigated the importance of analyzing and controlling for circulating lipid levels when interpreting contaminant concentrations measured in blood. The association of lipid variation with body mass and age of the eagle chicks, as well as feeding effects was examined. Blood samples were collected from nestling bald eagles at 5 sites in British Columbia and 1 site in southern California. All samples were analyzed for a suite of organochlorine pesticides and polychlorinated biphenyls. Samples were also assayed for plasma triglycerides, non-esterfied fatty acids, and for total lipids determined gravimetrically and colorimetrically. Plasma lipid concentrations showed considerable variability among individual eagle nestlings, but mass and age were not confounding factors. Sequential blood sampling of captive bald eagles showed a consistent rise and return to baseline of triglycerides during a 24 hour period. Due to variation in time of feeding of nestling eagles, sampling of wild eagles could occur at any point within the normal period of lipid flux. Therefore, no significant relationships were found between the plasma concentrations of contaminants and any measure of lipids (triglycerides, non-esterfied fatty acids, or total lipids). Nevertheless, we were able to detect significant differences among sites for both p,p'-DDE and PCBs demonstrating that blood sampling is still a valid technique for measuring exposure of eagles to lipid soluble contaminants.

2.2 Introduction

The relationship between contaminant concentrations and lipids is complex and many factors affect the movement of contaminants and their concentrations in the plasma. Lipid stores are constantly changing due to seasonal cycles and changes in an individual's physiological condition. Life stages, such as breeding and migration cause extreme variation in plasma lipid concentrations (Groscolas 1982; Jenni-Eiermann and Jenni 1996). Age, mass, feeding, and fasting are also factors that have been shown to influence lipid stores (Guglielmo et al. 2002; Jenni-Eiermann and Jenni 1994; Phillips et al. 1989; Williams et al. 1992). Since many contaminants are lipophilic (e.g., chlorinated hydrocarbons, PDBEs) these contaminants are often found associated with the lipids and are influenced by their movement. Contaminants associated with stored tissue lipids do not cause toxicity, however when lipids are brought into the blood stream to be used, contaminants passively follow due to their lipophilic nature and are then able to exert their toxic effects (provided they reach a site of action). Therefore, when interpreting contaminant levels in birds, it is potentially important to consider ecological and physiological factors, e.g., feeding and breeding ecology and physiological condition (Van Den Brink et al. 1998).

Many contaminant studies have either ignored variation in plasma lipid concentrations among study species or have just assumed that the contaminants must be normalized without investigating the relationship in detail (Jenni-Eiermann et al. 2002; Kern et al. 2001; Sasaki et al. 2001; Totzke and Bairlein 1998). In this chapter, the relationship between plasma lipids and contaminant plasma levels in nestling bald eagles was investigated, accounting for lipid variation in association with body mass and age of

chicks, as well as by examining feeding effects. The gravimetric and colorimetric methods, as well as measurement of specific lipids such as triglycerides and non-esterfied fatty acids were compared to determine the best measure of lipids when interpreting contaminant levels. The specific objectives of this study were: 1) to investigate the importance of analyzing and controlling for plasma lipid levels when interpreting contaminant levels; 2) to determine which measure of plasma lipids is most appropriate when investigating variation in contaminant levels (triglycerides, non-esterfied fatty acids, or total lipids); and 3) to examine spatial variation in the levels of chlorinated hydrocarbons in British Columbia and in southern California, taking lipid variation into account.

2.3 Methods

Study Areas

Blood samples were collected from bald eagles at 5 different sites in British Columbia and 1 site in California. The B.C. sites include the Delta-Richmond area (lower Fraser Valley), Abbotsford-Chilliwack area (central Fraser Valley), Nanaimo-Crofton area (southeast Vancouver Island), Barkley Sound (southwest Vancouver Island), and Fort St. James area (northern B.C.). Samples were also collected from Santa Catalina Island, California. (Fig. 2.1). The sites were chosen to maximize detection of variation in contaminant levels caused by industrial practices in the area and lipid variation due to differences in feeding and physiological factors.

The Fort St. James area in northern B.C. was the most remote site and the only non-marine site. The Barkley Sound area has had relatively low levels of human

disturbance and historically has been shown to have lower contaminant levels compared to other coastal sites in B.C. (Elliott and Harris 2001/2002; Gill 1993). The Delta-Richmond/ Fraser Valley area is surrounded by a productive estuary, and has been modified by clearing of forests and wetlands for agriculture, urban, and industrial use (Gill 1993). The Nanaimo-Crofton area has been altered extensively by urban developments, logging, and industry (such as paper and pulp mills), causing higher contaminant levels, but suitable bald eagle habitat still exists (Elliott and Norstrom 1998). Santa Catalina Island, located 22 miles west of Los Angeles, is known to be a highly contaminated site due to dumping of chemicals from a local chemical company from the 1940's to 1970's. Due to the high level of contaminants in the water, the bald eagles on the island are not able to reproduce without assistance.

Field work and sample collection

In British Columbia, surveys were conducted either by helicopter or by boat to locate bald eagle nests, to determine which nests had chicks (preferably 4-8 weeks old), and to observe accessibility by land. A professional tree climber was hired to climb the nest trees and lower the chicks in a soft canvas bag. The eagles were weighed and a CWS band was attached to the left tarsus. Measurements such as length and depth of the bill, length of the left hallax, and wing chord (if available) were recorded. Up to 24 mL of blood was withdrawn from the brachial vein using a 12 mL sterile syringe and a 21 gauge needle. Blood was transferred to heparinized vacutainers and stored upright on ice. Samples were centrifuged within 6 hours of collection, the plasma drawn off, transferred to chemically cleaned (acetone/hexane) glass vials or cryotubes, and stored at -20°C.

On Santa Catalina Island, CA, blood samples were collected by the Institute for Wildlife Studies in conjunction with an ongoing bald eagle reintroduction program. When bald eagle nestling reached approximately 8 weeks old, biologists hiked to the nests. All of the nests on the island are on rocky cliffs, therefore a tree climber was not necessary. A 10 mL blood sample was collected following the same procedure as mentioned above. The samples were frozen at -20°C and shipped to Simon Fraser University.

Metabolite assays

Free glycerol and triglyceride were assayed via sequential color endpoint assay (Trinder reagent A and B, respectively, Sigma-Aldrich Canada, Oakville, Ontario), using 5 μL of sample with 240 μL and 60 μL of reagents A and B respectively. A reading was taken at 540 nm after 10 minutes of incubation at 37°C after the addition of each reagent. Triglyceride concentration (mmol L^{-1}) was calculated by subtracting free glycerol from total glycerol. Non-esterfied fatty acids (NEFA) were assayed via an endpoint assay (WAKO USA, Richmond, Virginia), using 3 μL of sample, 6 μL of standard, 120 μL of reagent A, and 240 μL of reagent B. The plate was incubated at 37°C for 10 minutes after the addition of each reagent and the reading taken at the end at 550nm. Uric acid was also assayed; methods and results are in the appendix.

All assays were run in 400 μL flat-bottom 96-well microplates (NUNC, Denmark) and read with a microplate spectrophotometer (Biotec 340EL). Each plate was run with a standard curve based on a serial dilution of 2.54 mmol glycerol (Sigma-Aldrich) for the triglyceride-glycerol assay. Each plate also included a 19-day old hen plasma pool used to calculate inter-assay coefficient of variation. Inter-assay coefficients

of variation were 8.0% (n=3), 12.9% (n=3), 3.9% (n=3) 8.3% (n=3) for glycerol, triglyceride, NEFA, and uric acid, respectively.

Total lipids were determined using the colorimetric and gravimetric method by staff at the Canadian Wildlife Service National Wildlife Research Center (NWRC). For the colorimetric method, samples (20 μL) were combined with 0.20 mL of concentrated sulfuric acid in a cuvet and mixed thoroughly on a vortex mixer. Cuvets were then placed in boiling water for 10 minutes and then cooled in cold water for 5 minutes. Approximately 10 mL of phosphor-vanillin reagent, made by combining 350 mL of vanillin reagent, 50 mL of water, and 600 mL of concentrated phosphoric acid, was added to each cuvet. Samples were mixed well on a vortex mixer and placed in a 37°C water bath for 15 minutes. Cuvets were then cooled for 5 minutes and absorbances measured within 30 minutes at 540 nm on a Spectronic 70 (Bausch & Lomb).

Total lipids were determined using the gravimetric method by combining 1 to 2 mL of sample with 4 mL of hexane in a centrifuge tube, which was then mixed using an Ultra-Turrax homogenizer for 2 min. The contents of the tube were then centrifuged to separate the hexane and plasma layers. The hexane was then passed through sodium sulphate to remove any moisture. This process was repeated twice more and the sodium sulphate washed with hexane after the final extract. The three hexane extracts were combined on a preweighed aluminium dish, the hexane was then evaporated, and the dish reweighed to determine the amount of lipid. Lipid was then calculated on the basis of grams per mL plasma and expressed as a percentage.

Contaminant analysis

All chemical analyses were performed by staff at the Canadian Wildlife Service National Wildlife Research Center (NWRC) which included determination of organochlorines (OCs) and polychlorinated biphenyls (PCBs). The suite of organochlorines analyzed included: chlorobenzenes (tetra, penta, and hexa.) hexachlorocyclohexanes, chlordane related compounds (oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor and heptachlor epoxide), p,p'-DDT and metabolites (p,p'-DDE and p,p'-DDD), mirex, photomirex, and dieldrin. Sixty-seven major PCB congeners were analyzed and summed to present the level of total PCBs.

The analysis of contaminants followed standard procedures and is described in full in the CWS Laboratory Service Methods Manual (MET-CHEM-OC-04D). Briefly, the plasma samples were denatured with formic acid (1:1 v/v). Internal standards were added to plasma samples before denaturation with formic acid. The extraction of OC/PCBs was done with activated C18 cartridges and elution with DCM/Hexane (1:1). The DCM/hexane extract was cleaned up by Florisil column chromatography. Samples were quantitatively analyzed by capillary gas chromatography coupled with a mass selective detector operated in selected ion monitoring mode. The instruments used were the HP 5890 GC with HP Mass Selective Detector HP 5971A for PCB analysis, and Agilent 6890 GC with HP Mass Selective Detector Agilent 5973 for OC analysis. As part of the quality control, blanks and CWS reference material (2003 Herring Gull QA) were run concurrently with the samples. The nominal detection limit for all compounds was 0.1 ng/g wet weight. Residues were not corrected for internal standard recoveries which were typically between 70 % and 90 %.

Captive Study Feeding Experiment

This experiment was performed in the fall of 1998 by Chris Gill and Sandi Lee in conjunction with the Canadian Wildlife Service and the Orphaned Wildlife Rehabilitation Centre (Delta, B.C.). Four non-releasable bald eagles (1 sub-adult, 3 adult) at the Orphaned Wildlife Rehabilitation Centre were used for this experiment. The eagles were housed in a flight cage measuring 102' x 48' x 18'. The experiment involved the eagles fasting for 2 days, then each bird was fed a known amount of herring (between 265-300g). Blood samples (2cc) were taken at time intervals 0, 30, 60, 120, 360, 600 mins., and at 24 hours after feeding. The triglyceride and NEFA levels were measured in the blood plasma for each time interval using the same methods described above and at the same time.

Statistics

All statistical analyses were performed using SAS v.9.1 software package (2002-2003). All of the contaminants (DDE, HCB, t-Nonachlor, PCBs) were non-normally distributed, therefore were log transformed ($\log_{10} + 1$). Triglyceride and non-esterfied fatty acid levels were also non-normally distributed and were therefore log transformed. Total lipids were normally distributed. Possible confounding factors of mass and age on the lipid status of the chicks was investigated by running a linear regression between these variables and each metabolite (triglycerides, non-esterfied fatty acids, and total lipids). Site differences were determined using analysis of variance (ANOVA) with a Student Newman Keuls test for pair-wise comparisons among sites. To ensure mass and age of the chick would not change our results, the ANOVA analyses were rerun with mass or age as a covariate. The correlation between mass and age was also tested using a

linear regression model. The correlation between different lipid measures and the relationship between contaminants and lipids was determined using linear regression models. Since no correlation was found between the contaminants and lipids, the contaminants were not normalized for lipids. Site differences were again detected using ANOVA, and to determine which sites were different, a Student Newman Keuls test was performed. To ensure that lipid normalizing the contaminants would not change our results, we ran an ANOVA with each lipid metabolite as a covariate. Unless stated otherwise, results were considered significant if $p < 0.05$.

2.4 Results

Variation and covariation in lipid measures

Lipid measures used included triglycerides and non-esterfied fatty acid assays, and total lipids determined gravimetrically and colorimetrically (Table 2.1). There was considerable variability among individuals for all lipid measures (Table 2.1); however, only the total lipids determined colorimetrically varied significantly among sites ($F=2.97$, $p=0.0291$, $df=5$). Correlations between all lipid measures were investigated. Significant correlations were found between total lipids determined gravimetrically and triglycerides ($r^2=0.3308$, $p=0.0021$), as well as NEFA and triglycerides ($r^2=0.2698$, $p=0.0019$; Fig. 2.2), Correlations were also seen between triglycerides and total lipids determined colorimetrically ($r^2=0.1812$, $p=0.0152$) and total lipids determined gravimetrically with total lipids determined colorimetrically ($r^2=0.5187$, $p<0.0001$; Fig. 2.3). All other correlations were non-significant ($p>0.05$).

Potential factors affecting variation in plasma lipid levels

Effect of mass and age

Since samples were collected from several sites with chicks of varying mass and age, we investigated the relationship between plasma lipids and these factors. There was no significant relationship between body mass of the chicks and any of the plasma lipid measures (triglycerides, non-esterfied fatty acids, or total lipids; $p>0.15$ in all cases; Fig. 2.4). There was also no significant relationship between age of the chicks and any of the plasma lipids (triglycerides, non-esterfied fatty acids, or total lipids) ($p>0.2$ in all cases; Fig. 2.5). Predictably, there was a strong correlation between mass and age ($F=84.12$, $p<0.0001$, $df=1$; Fig. 2.6), which suggests that mass is dependent on age but not plasma lipid status.

Site-specific variation in plasma lipid levels controlling for mass and age

Site differences in plasma lipid measures with mass and age as covariates were investigated at B.C. sites. On Santa Catalina Island, mass of the chicks was not available; therefore these data could not be included in the analysis. Only plasma triglycerides showed a significant differences among sites when mass was a covariate ($F=4.07$, $p=0.0160$, $df=4$). When age was used as a covariate, again only triglycerides showed significant differences among sites ($F=3.50$, $p=0.0187$, $df=5$; Table 2.1).

Effects of feeding on lipid variability

The captive experiment investigating feeding effects in bald eagles showed that levels of triglycerides increase approximately 2 fold after eagles were fed, and the average increase in triglycerides was 0.249 mg/mL (Fig. 2.7). The range in triglycerides

in fasted bald eagles was 0.241 to 0.701 mg/mL. The wild bald eagle chicks varied in triglycerides from 0.501 to 3.605 mg/mL. In general, levels were much higher in free-living chicks compared with captive birds. Non-esterfied fatty acids also showed a consistent trend, decreasing by 0.3 mg/mL in the first 2 hours after feeding and then slowly rising back to pre-feeding levels (Fig. 2.7). The range in non-esterfied fatty acids in fasted bald eagles was 0.293 to 0.791 mg/mL. The NEFA values in wild bald eagle chicks varied from 0.205 to 2.046 mg/mL. In the captive experiment, both triglycerides and NEFA had returned to their starting levels within 24 hours. This study identified the pattern of plasma lipids before and after feeding and shows that feeding status influences plasma lipid levels, however direct investigation of this factor was not possible in free-living chicks where feeding status was unknown.

Relationship between lipids and contaminants

The relationship between each measure of lipid (total lipids determined gravimetrically and colorimetrically, triglycerides, and NEFA) and the contaminant levels (p,p'-DDE, total PCBs, PCB 153, P99, P118, P138, and P180) was determined. There was no significant relationship between any measure of lipid and p,p'-DDE or PCB contaminant levels ($p>0.05$ in all cases, Figs. 2.8 and 2.9). The only significant relationship was between total lipids (determined colorimetrically) with trans-nonachlor and HCB ($F=8.80$, $p=0.0057$, $df=1$ in both cases; Fig. 2.10).

Site-specific variation in contaminant levels

We analyzed variation in chlorinated hydrocarbons in relation to sampling location using raw data, not correcting for variation in plasma lipids since no correlation

between these variables was found. Plasma samples from 34 bald eagle chicks were analyzed and wet weight concentrations are presented in Table 2.2. Due to low levels of many of the contaminants and individual congeners, we decided to focus on p,p'-DDE and total PCBs. For p,p'-DDE, samples from Santa Catalina Island, CA had significantly higher levels than all of the British Columbia sites ($F=3.15$, $p=0.0222$, $df=5$; Fig. 2.11). In British Columbia, Barkley Sound had the highest level of p,p'-DDE, followed by the Nanaimo/Crofton area, lower and central Fraser Valleys, and then the Fort St. James area (Fig 2.11), however, overall these site differences within BC were not significant ($F=2.54$, $p=0.639$, $df=4$).

There were significant differences among sites for total PCBs, not correcting for variation in plasma lipids ($F=5.11$, $p=0.0019$, $df=5$). The highest concentration of total PCBs were in samples from the Nanaimo/Crofton area and Barkley Sound (35.6 and 22.3 μg/kg, wet weight respectively; Fig.2.11). Santa Catalina Island, CA showed the next highest concentration (12.3 μg/kg, wet weight). Mean concentrations of individual PCB congeners generally followed the geographical pattern of the total PCBs; for example, highest concentration of PCBs 99 and 153 were also at Nanaimo/Crofton area and Barkley Sound (Table 2.2).

Site-specific variation in contaminant levels controlling for plasma lipids, age, and mass

Site specific variation in contaminant levels was investigated controlling for plasma lipids to see if our results would change. p,p'-DDE and PCBs were significantly different between sites ($p<0.05$ in all cases) regardless of which plasma lipids were used to normalize the data, which supports our earlier findings. To ensure that site differences in the relationship between contaminants and plasma lipid levels were not affecting our

pooled results, a regression of the data by site was conducted (Fig. 2.12). There was a random pattern between sites, therefore pooling the data will correctly portray the relationships of interest. There were no significant differences among sites when mass was used as a covariate for any of the measured contaminants ($p>0.05$ in all cases). Only p,p'-DDE was significant among sites ($F=3.19$, $p=0.0258$, $df=5$) when age was used as a covariate.

2.5 Discussion

The most important findings in our study are: 1) plasma lipid levels showed considerable variability among individual eagle nestlings; 2) mass and age were not confounding factors in lipid variability, but the feeding status of the birds was the most important factor; 3) plasma lipids (triglycerides, non-esterfied fatty acid, and total lipids determined gravimetrically and colorimetrically) were not significantly correlated with contaminant levels in bald eagle chicks; and 4) site differences in contaminant levels were detected.

Use of blood samples to monitor chlorinated hydrocarbon levels

The use of blood sampling as a non-destructive technique for assessing both exposure and effects of environmental toxicants in avian wildlife has been validated by many studies (Bustnes et al. 2001; Elliott and Norstrom 1998; Henriksen et al. 1998). These studies have shown a high correlation between contaminant levels in the blood and fat storage tissues such as fat depots, liver, and muscles. This is because blood and tissues rapidly attain equilibrium with each other resulting from high tissue perfusion rates, and the fact that time to achieve intertissue equilibrium of lipophilic and persistent

contaminants is generally much faster than whole-body contaminant clearance rates (Bustnes et al. 2001). Since many contaminants are highly lipid soluble, there is a strong association with plasma and tissue lipids, therefore the contaminants are affected by their movements. When interpreting contaminant levels in birds, it is therefore important to consider causes of lipid variation and investigate the relationship between contaminants and plasma lipids.

Variation in plasma lipid levels

Lipid levels in bald eagle nestling plasma, which was measured using various techniques (triglyceride and non-esterfied fatty acid assays, and total lipids determined gravimetrically and colorimetrically) showed considerable variability among individuals. The eagle nestlings varied in total percent lipids (determined colorimetrically) from 0.470 to 1.34% and triglyceride levels varied from 0.501 to 3.619 mg/mL. Ferrer et al.(1998) found that in Spanish Imperial eagle chicks (*Aquila adalberti*; 34-75 day old) the range of triglyceride values was between 0.46-2.68 mg/mL. In juvenile Swainson's hawks (*Buteo swainsoni*), triglyceride values were found to be between 0.79 and 2.58 mg/mL (Sarasola et al. 2004) Both studies show very similar triglyceride values to our findings and demonstrate the normal range of values for lipids in raptors.

Even though all measures of plasma lipids produced the same results, total lipids determined colorimetrically incorporate the greatest amount of lipids, therefore we suggest that this be considered the best measure of plasma lipid concentration. The gravimetric method of lipid determination measures primarily triglyceride levels in blood plasma which comprises approximately 60% of the total lipid volume (Christie and Moore, 1972). The colorimetric method measures both triglycerides and phospholipids

(which comprise 32% of the total lipid volume) circulating in the plasma (Frings and Dunn 1970). The colorimetric method, therefore accounts for 92% of the total lipid volume in the blood.

Factors contributing to plasma lipid variability

Many studies have shown a correlation between lipid levels with mass and age of animal species (Jenni-Eiermann et al. 2002; Kern et al. 2001; Sasaki et al. 2001; Totzke and Bairlein 1998). Since the nestling bald eagles we sampled were of different masses and age, these are potential confounding factors that needed to be considered before site comparisons can be made. We did not find a significant correlation between either age or mass and any of our plasma lipid measures, therefore, they were not considered further in the analysis. It appears that age and mass do not play a significant role in plasma lipid variation, at least in bald eagle chicks over the range of ages and body masses we sampled, and does not need to be considered in future studies.

Feeding has also been shown to cause variation in plasma lipid levels (Jenni-Eiermann and Jenni 1996; Leclercq et al. 1974). In a study by Jenni-Eiermann and Jenni (1996), it was shown that feeding passerine birds have higher levels of plasma triglycerides and free fatty acids. Phillips et al. (1989) also demonstrated that total blood lipid levels increased following a fatty meal in humans. In chickens, serum lipid concentrations increased rapidly during meal periods, but returned to steady state concentrations within about 2 hours of feeding (Leclercq et al. 1974). This short-term fluctuation in lipid status after feeding may be a confounding factor for site comparisons when individuals of different (often unknown) feeding status are sampled. A blood

sample taken during this time of lipid fluctuation could provide skewed results, therefore this may be an important consideration when interpreting contaminant results.

The captive experiment performed in this study shows the same trend and variability of plasma lipids after feeding as reported in previous studies. Plasma triglyceride levels increased approximately two fold after feeding while the non-esterfied fatty acids decreased two fold. This is consistent with other feeding studies (Jenni-Eiermann and Jenni 1994) showing metabolites that are known to characterize reabsorption (ex. triglycerides) increase after feeding and metabolites which are characteristic of fasting decrease (e.g. NEFA). The birds in the captive experiment showed individual lipid variation. In the fasted state, the triglyceride levels in the birds ranged from 0.16 to 0.55 mg/mL, and after feeding they ranged from 0.51 to 0.88 mg/mL. On average, the increase in triglycerides after feeding was 0.249 mg/mL. The individual variation seen in the 34 wild birds is greater, most likely due to a larger sample size, demonstrating differences in energetic status. Variability in the wild can be attributed to bald eagle nestlings constantly being fed in order to support their rapid growth, differences in feeding rates between nests, as well as dissimilarity in food type (Gill and Elliott 2003).

Based on the feeding experiment, it takes 2-10 hours for the triglyceride levels to peak even though they start at different baselines, and takes up to 24 hours to return to the starting level. Given that nestling bald eagles may feed at different times of the day (Elliott et al. 2005) birds will be at varying stages of assimilation and digestion. Since we do not know the feeding status of the wild birds, the relationship between organochlorines and the feeding status is unknown.

Effect of plasma lipid content on interpreting chlorinated hydrocarbon concentrations

Many contaminant studies have either ignored variation in lipid levels among study species or have just assumed that the contaminants must be normalized without investigating the relationship in detail (Anthony et al. 1993; Clark et al. 1998; Hebert and Keenleyside 1995; Henny et al. 1981; Tansy et al. 2003). It is well known that lipophilic contaminants accumulate in proportion to tissue and plasma lipid content; therefore, the contaminants should be normalized for lipids (Hebert and Keenleyside 1995). If there is not a significant correlation between the contaminants and the lipid levels, Hebert and Keenleyside (1995) argue that normalizing the data will introduce more unexplained variability to the results. Therefore, investigating the relationship between the concentration of contaminants and the level of plasma lipids is key to interpreting contaminant results.

In this study, we did not find significant relationships between any measure of plasma lipid (triglycerides, non-esterfied fatty acids, and total lipids determined gravimetrically or colorimetrically) and the major chlorinated hydrocarbons measured in plasma (DDE and PCBs), suggesting that there is no need to lipid normalize contaminant data. These results are consistent with those of Bustnes et al. (2001), who concluded that body condition and blood lipids are not a major factor when interpreting chlorinated hydrocarbon levels. They also showed that blood sampling is an acceptable measure of body burden. Bustnes et al (2001) investigated the long and short-term variability in blood concentration of organochlorines in glaucous gulls, however, it should be noted that both their study and ours were not able to account for feeding effects, which could have changed the results. Other studies investigating contaminant levels in nestling bald

eagles also did not find correlations between contaminants and plasma lipids (measured using the colorimetric method; (Dominguez et al. 2003; Donaldson et al. 1999)

Elliott and Norstrom (1998) did find many strong correlations between contaminants and total plasma lipids determined gravimetrically in eagle nestlings, therefore corrected their contaminant data using analysis of covariance. Gill (1993), also reported significant correlations between contaminant and plasma lipids in adult eagles, therefore normalized the corresponding contaminant data, but this relationship was not found in eagle nestlings. Since these correlations vary between studies, it is important to examine the relationship to avoid misinterpreting chlorinated hydrocarbon levels.

Site-specific differences in plasma contaminant levels

Overall, the site specific patterns of contaminant levels matched patterns of land-use development, the degree of urbanization, and the level of industrial development at each of the sites we sampled. Bald eagles sampled at Santa Catalina Island, CA have the highest mean DDE level, due to extensive chemical discharge from industry in the area (Garcelon 1997, Lee 1994). The Fort St. James area in northern B.C. had the lowest level of contamination due to its remote location and limited industry in the area. Barkley Sound showed high levels of contaminants even though it is a fairly remote site. This is most likely due to effluent from industry in the surround area and the pattern of ocean currents. The Nanaimo/Crofton area showed high levels of PCBs. This area has been altered extensively by urban developments, logging, and industry (such as paper and pulp mills), resulting in higher contaminant levels (Elliott and Norstrom 1998). These site differences were consistent regardless of whether contaminant values were normalized using blood lipid levels.

2.6 Figures

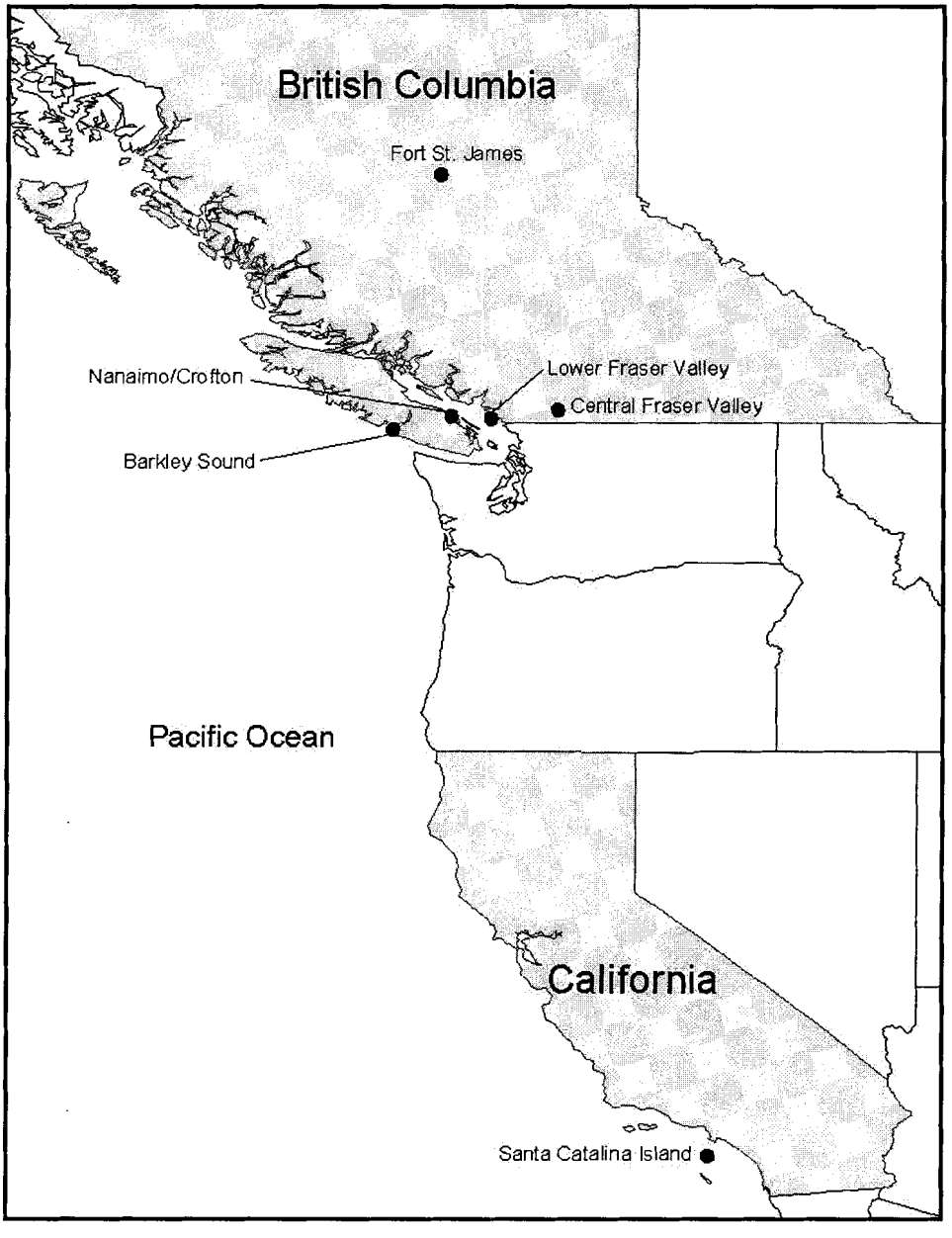

Figure 2.1 Location of study sites

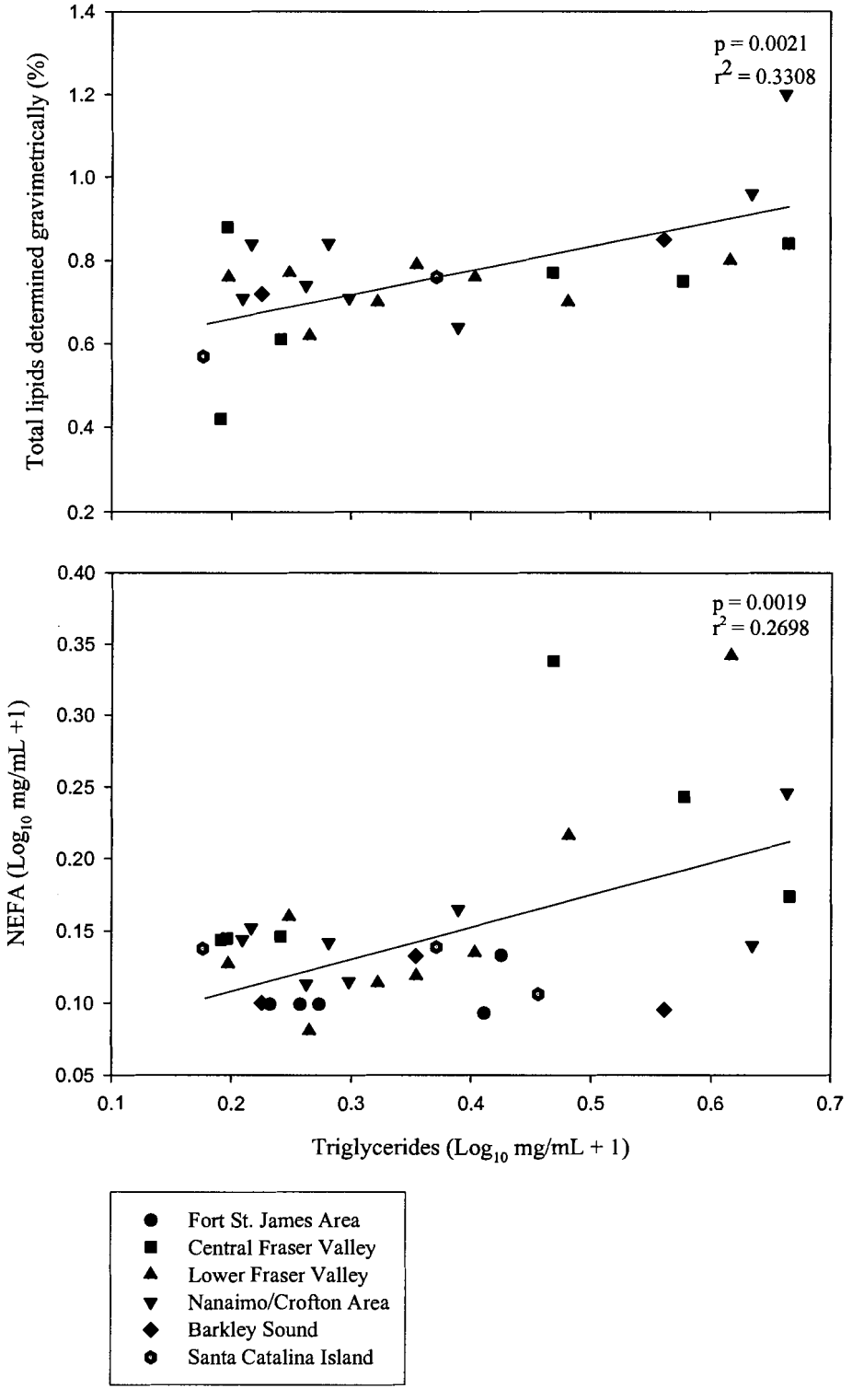

Figure 2.2 Correlation between total lipids determined gravimetrically and non-esterfied fatty acids (NEFA) with triglycerides

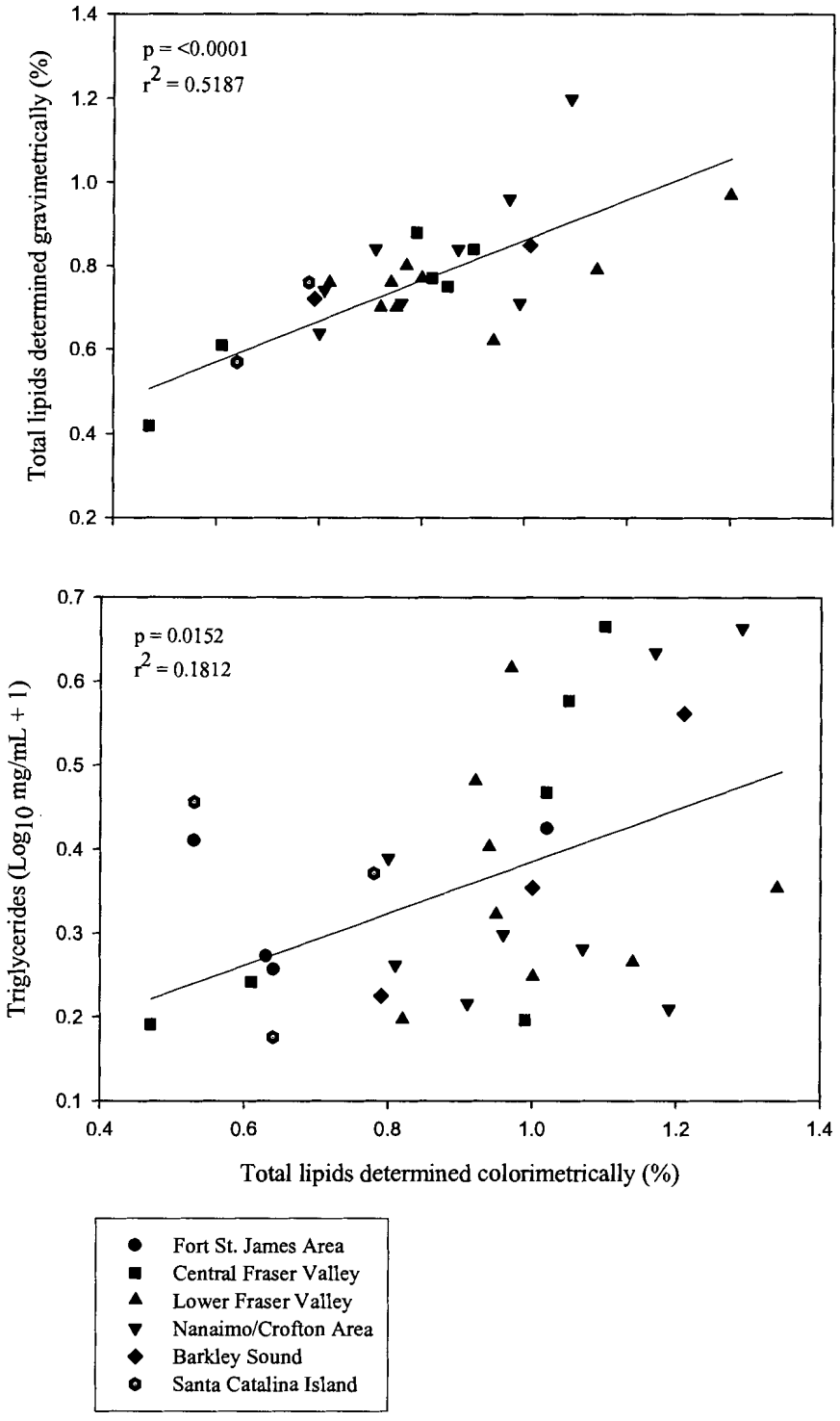

Figure 2.3 Correlation between total lipids determined gravimetrically and triglycerides with total lipids determined colorimetrically

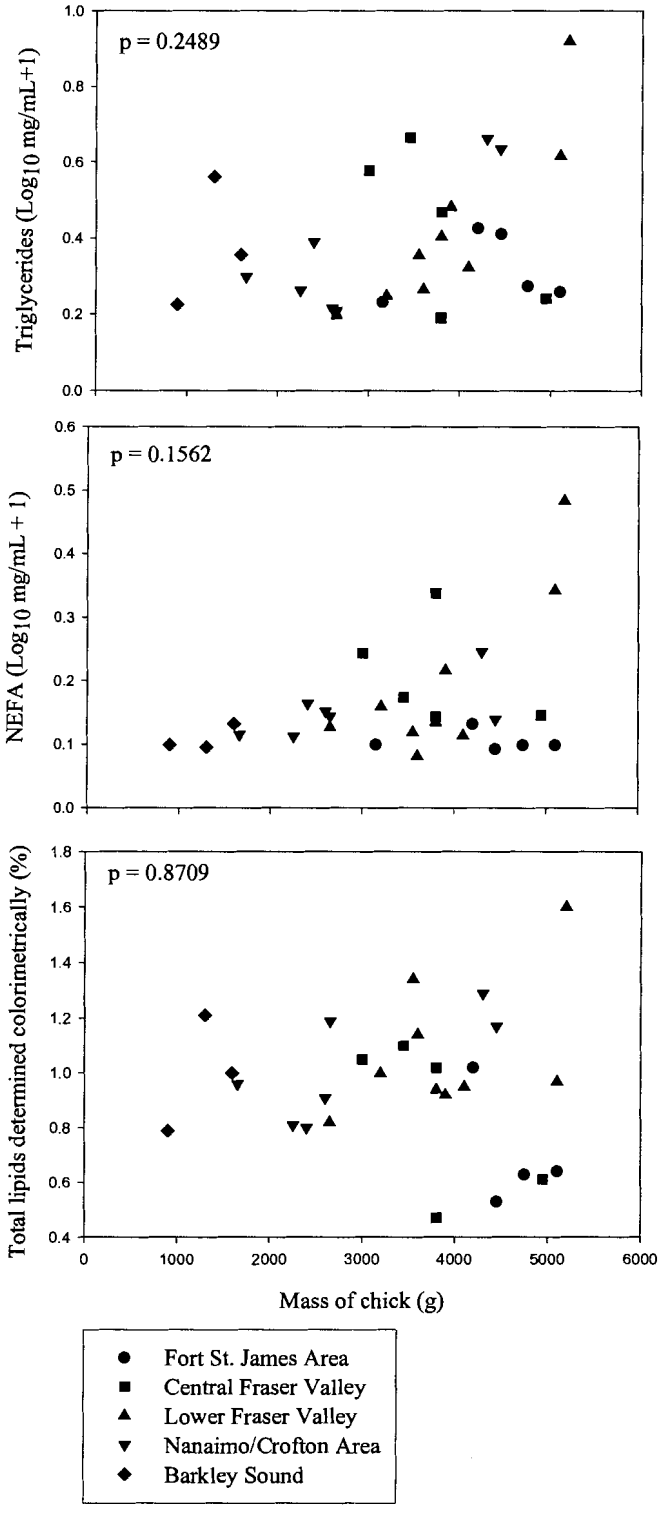

Figure 2.4 Relationship between plasma lipids (triglycerides, non-esterfied fatty acids (NEFA), and total lipids determined colorimetrically) and mass of the bald eagle chicks at all sites.

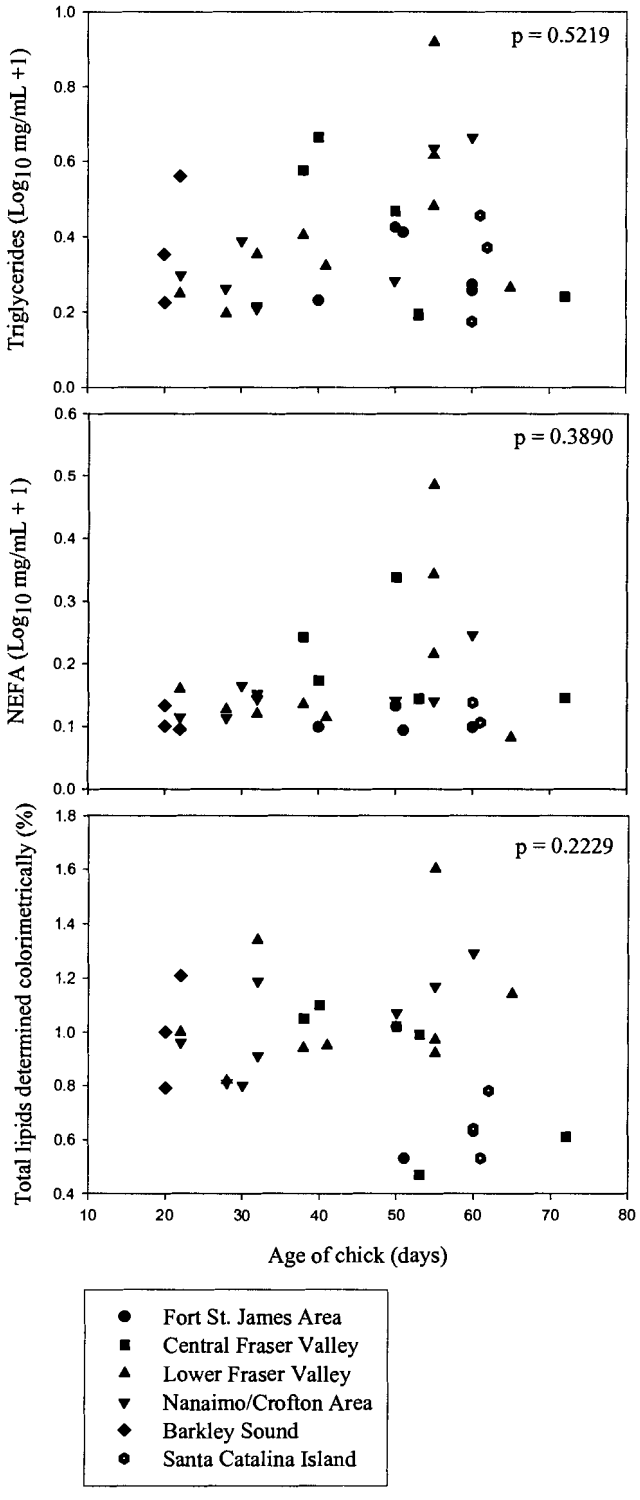

Figure 2.5 Relationship between plasma lipids (triglycerides, non-esterfied fatty acids (NEFA), and total lipids determined colorimetrically) and age of the bald eagle chicks at all sites.

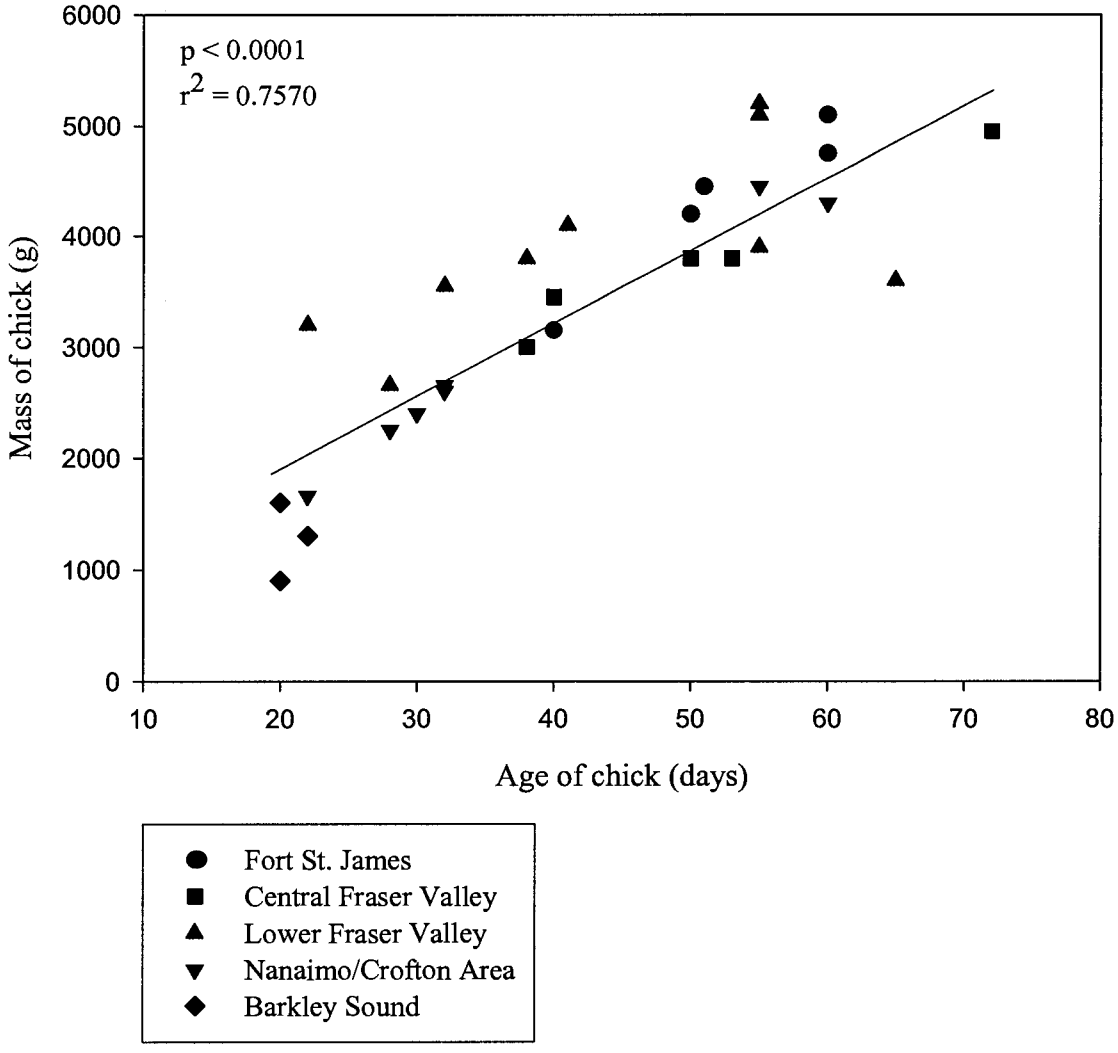

Figure 2.6 Relationship between mass of the bald eagle chicks and their age

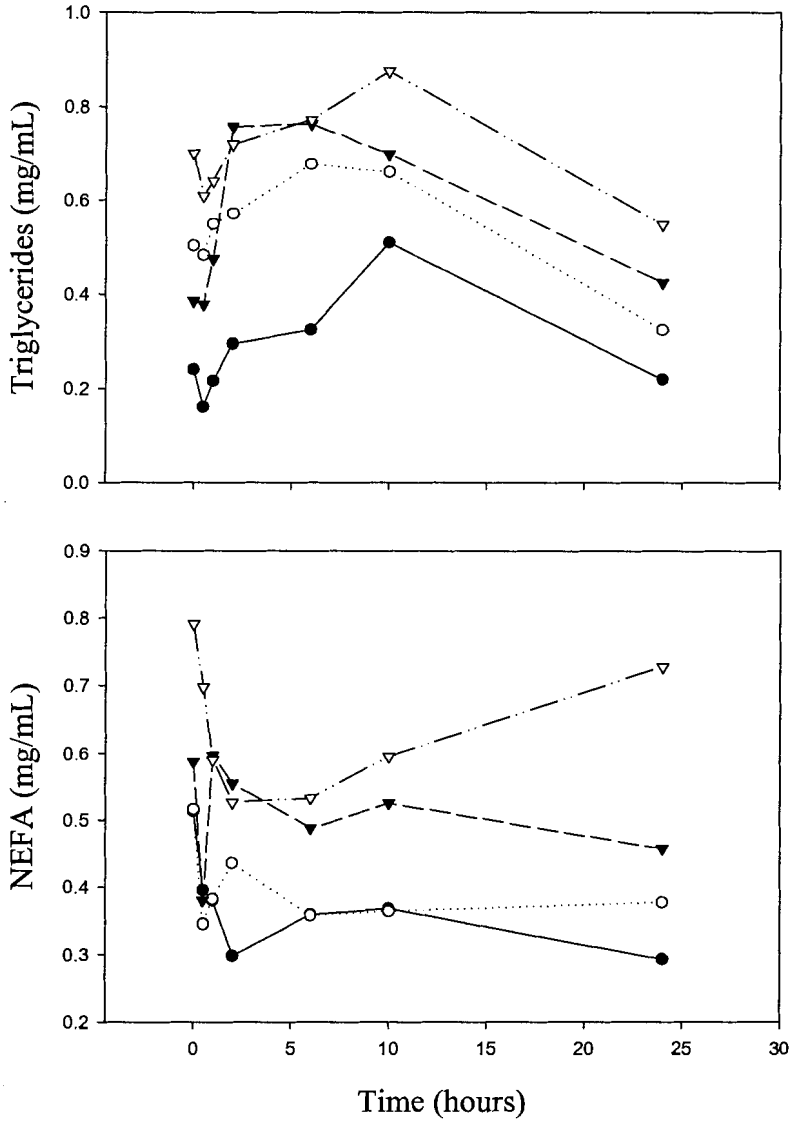

Figure 2.7 The levels of triglycerides and non-esterfied fatty acids in the plasma of four captive bald eagles (1 sub-adult, 3-adult) taken at regular intervals after being fed a known amount

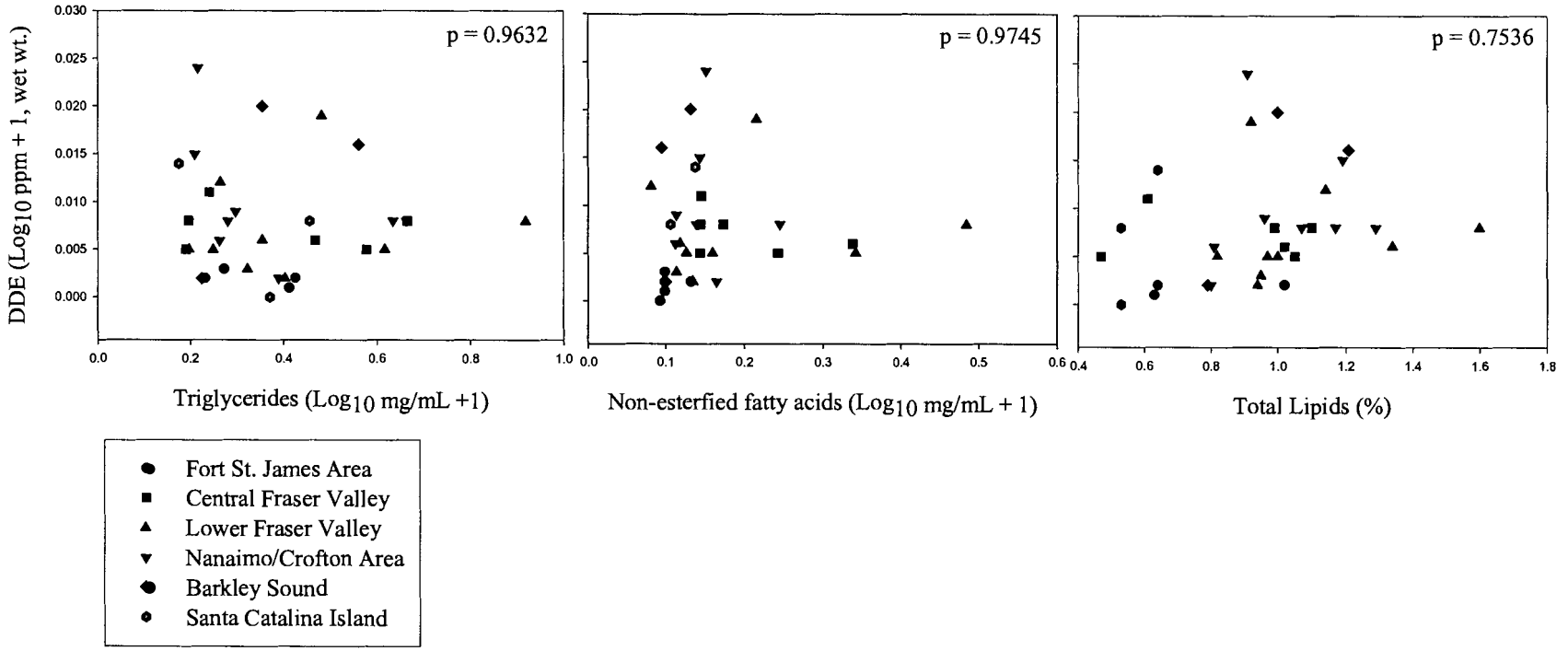

Figure 2.8 Relationship between plasma levels of p,p'-DDE and plasma lipids (triglycerides, non-esterfied fatty acids, and total lipids determined colorimetrically) in bald eagle nestlings

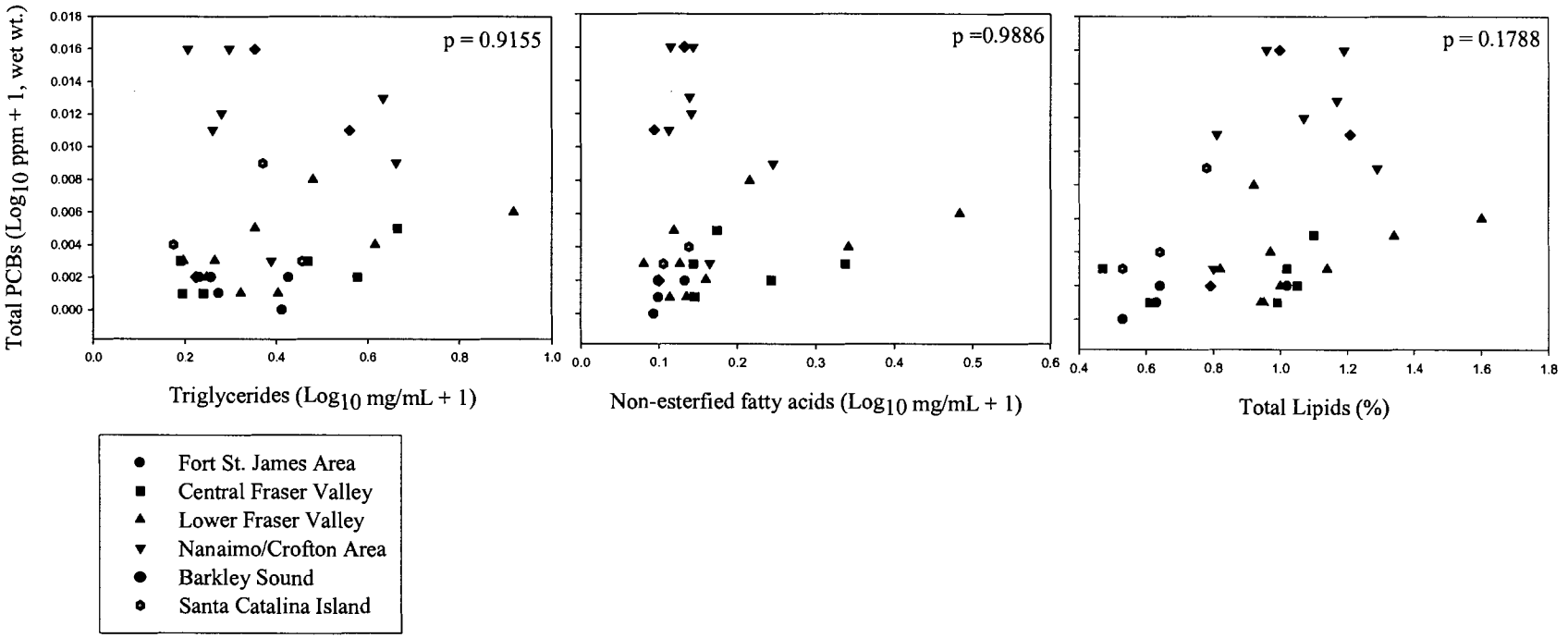

Figure 2.9 Relationship between plasma levels of total PCBs and plasma lipids (triglycerides, non-esterfied fatty acids, and total lipids determined colorimetrically) in bald eagle nestling

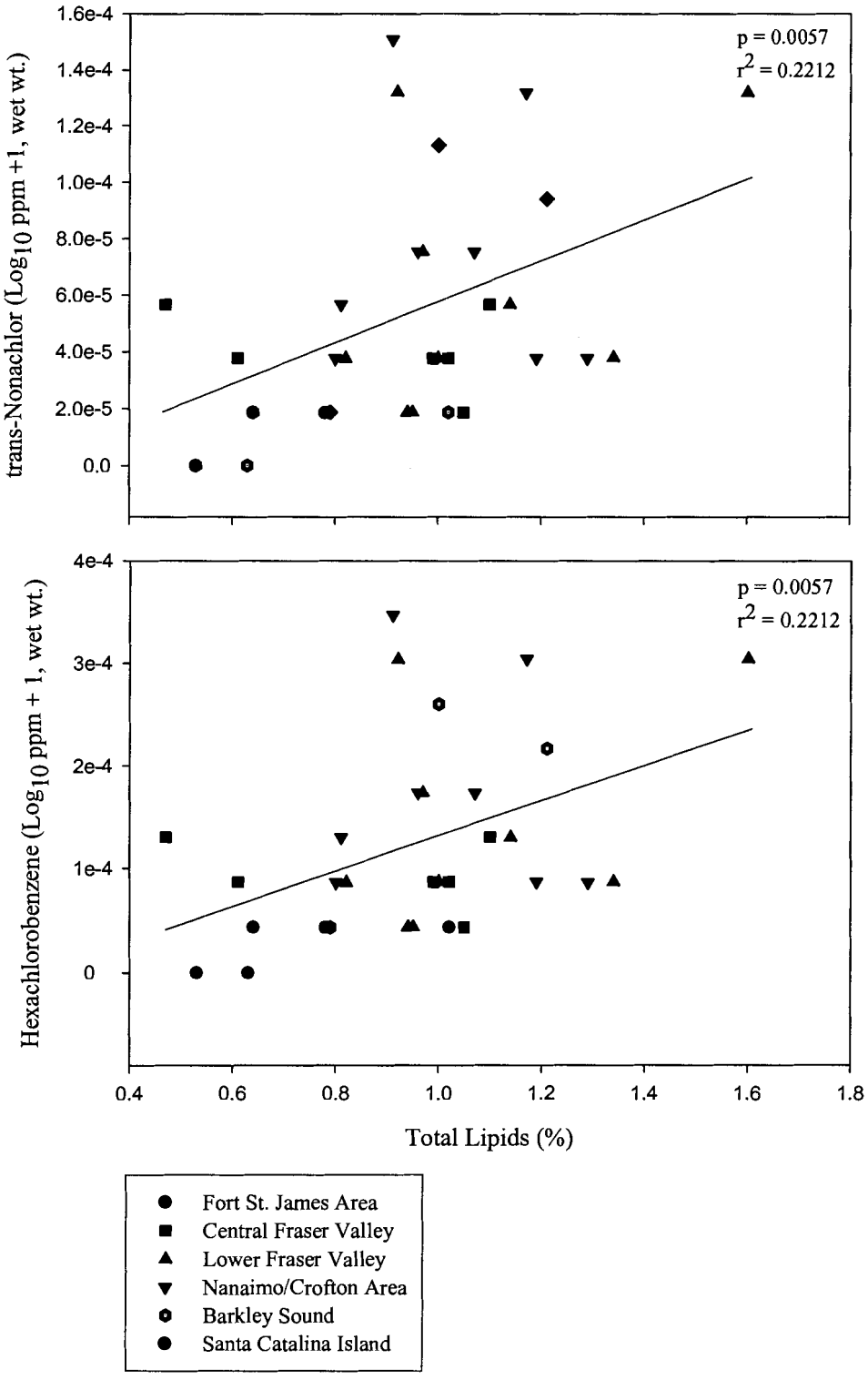

Figure 2.10 Relationship between plasma trans-Nonachlor and hexachlorobenzene levels with total plasma lipids determined colorimetrically in bald eagle nestling

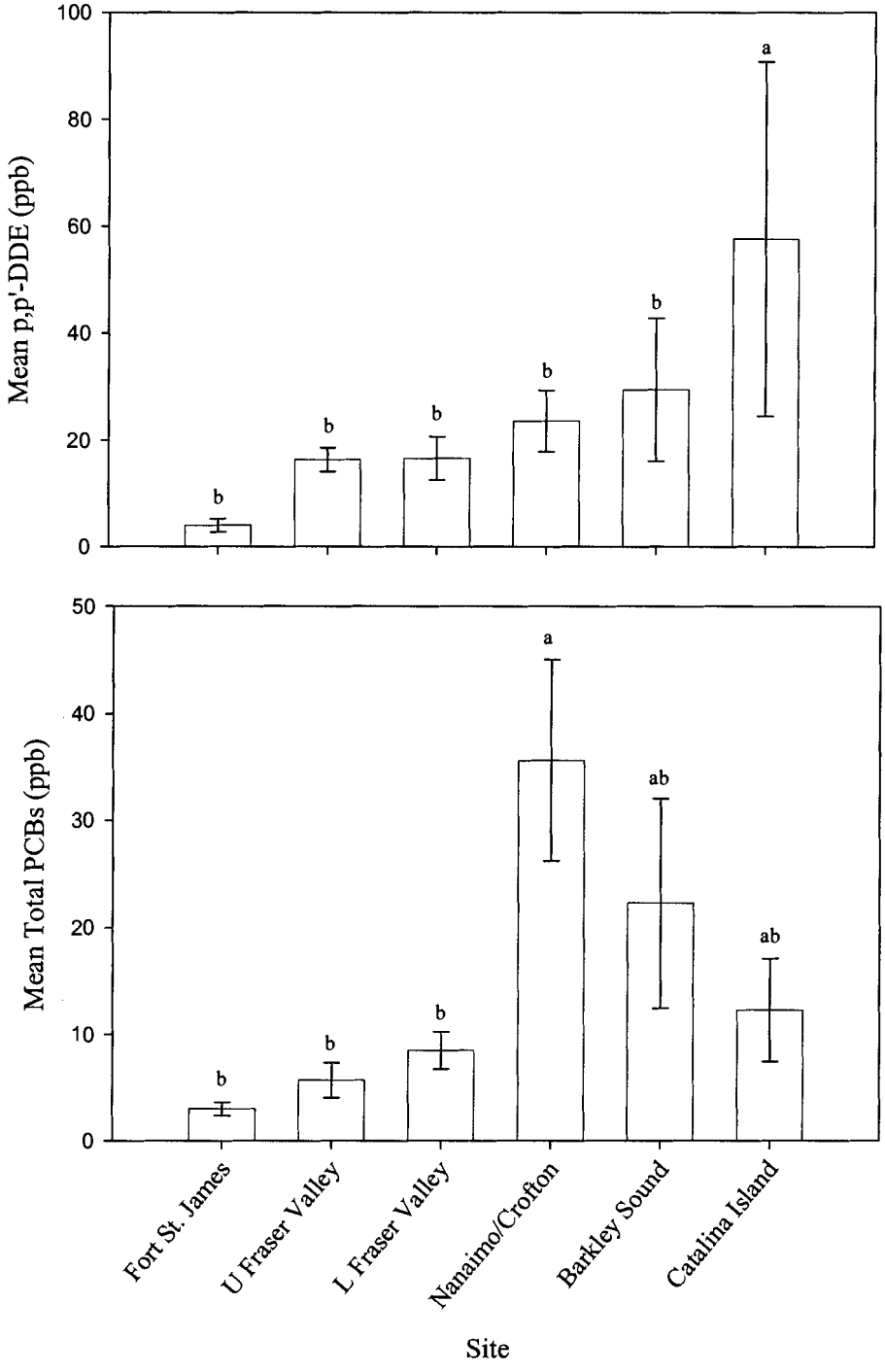

Figure 2.11 Residue levels of p,p'-DDE and total PCBs in plasma samples of bald eagles nestlings collected in British Columbia and in southern California. Means that do not share the same lowercase letter were significantly different ($p<0.05$).

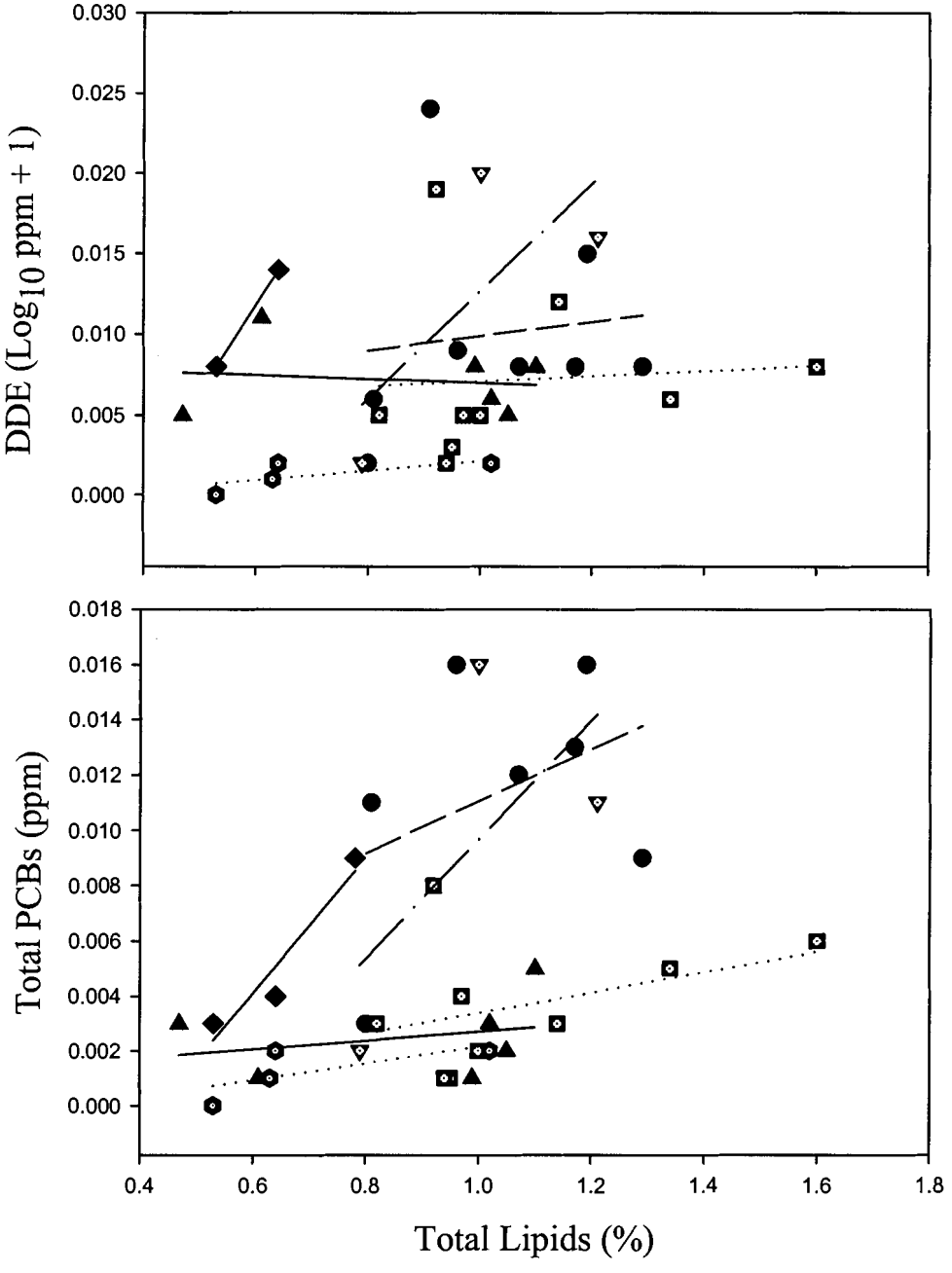

Figure 2.12 Linear regressions of the relationship between DDE and total PCBs with total plasma lipids (determined colorimetrically) for each site in bald eagle nestlings. (Nanaimo/Crofton: circle, dash line; Lower Fraser Valley: open square, dotted line; Central Fraser Valley: up triangle, solid line; Barkley Sound: down-open triangle, dash-dot line; Santa Catalina Island: diamond, solid line; Fort St. James: open hexagon, dotted line)

2.7 Tables

Table 2.1 Means, standard errors, and range of the mass, age, and the different plasma lipid measures used in bald eagle nestlings by site

Site	n	Mass (g)	Age (days)	Means and Standard Errors of Lipid Measures			
				Triglycerides (mg/mL)	Non-esterfied fatty acids (mg/mL)	Total Lipids Gravimetric Method (%)	Total Lipids Colorimetric Method (%)
Fort St. James Area	5	4330.0 ± 331.13 (3150.0 - 5100.0)	52.5 ± 3.72 (40.0 - 60.0)	1.126 ± 0.204 (0.708 - 1.661)	0.273 ± 0.021 (0.238 - 0.357)	- -	0.705 ± 0.108 (0.530 - 1.020)
Central Fraser Valley	6	3800.0 ± 322.88 (3000.0 - 4950.0)	51.0 ± 4.97 (38.0 - 72.0)	1.700 ± 0.529 (0.554 - 3.619)	0.601 ± 0.128 (0.393 - 1.175)	0.712 ± 0.070 (0.420 - 0.880)	0.873 ± 0.108 (0.470 - 1.100)
Lower Fraser Valley	9	3900.0 ± 275.00 (2650.0 - 5200.0)	43.4 ± 4.90 (22.0 - 65.0)	1.404 ± 0.296 (0.573 - 3.128)	0.476 ± 0.112 (0.205 - 1.196)	0.763 ± 0.032 (0.620 - 0.970)_	1.076 ± 0.082 (0.820 - 1.600)
Nanaimo/Crofton Area	8	2901.4 ± 400.15 (1660.0 - 4450.0)	38.6 ± 5.01 (22.0 - 60.0)	1.544 ± 0.428 (0.618 - 3.605)	0.425 ± 0.052 (0.296 - 0.761)	0.830 ± 0.064 (0.640 - 1.200)	1.025 ± 0.065 (0.800 - 1.290)
Barkley Sound	3	1266.7 ± 202.76 (900.0 - 1600.0)	20.7 ± 0.67 (20.0- 22.0)	1.526 ± 0.581 (0.680- 2.640)	0.287 ± 0.035 (0.244 - 0.357)	0.785 ± 0.065 (0.720 - 0.850)	1.000 ± 0.121 (0.790 - 1.210)
Santa Catalina Island	3	- -	61.0 ± 0.58 (60.0 - 62.0)	1.235 ± 0.395 (0.501 - 1.857)	0.343 ± 0.033 (0.277 - 0.377)	0.665 ± 0.095 (0.570 - 0.760)	0.650 ± 0.072 (0.530 - 0.780)

Table 2.2 Geometric means and the range of contaminants measured in bald eagle nestling blood plasma for each site

Location	n	Residue Levels (ug/kg, wet weight)								
		p,p'-DDE	HCB	t-Nonachlor	Total PCBs	PCB 99	PCB 118	PCB 153	PCB 138	PCB 180
Fort St. James Area	5	3.00 0.7 - 8.0	0.07 0.05 - 0.1	0.05 0.05 - 0.05	2.64 1.0 - 4.0	0.10 0.05 - 0.2	0.05 0.05 - 0.05	0.36 0.1 - 0.7	0.05 0.05 - 0.05	0.18 0.1 - 0.3
Central Fraser Valley	6	15.66 11.4 - 25.7	0.20 0.1 - 0.3	0.46 0.3 - 0.8	4.51 1.5 - 12.7	0.09 0.05 - 0.2	0.36 0.05 - 1.5	0.84 0.4 - 2.1	0.66 0.5 - 2.0	0.17 0.05 - 0.6
Lower Fraser Valley	9	13.56 5.4 - 44.5	0.26 0.1 - 0.7	0.40 0.2 - 0.9	7.11 2.8 - 18.6	0.27 0.1 - 0.8	1.53 0.7 - 3.4	1.35 0.5 - 3.0	0.92 0.4 - 2.3	0.64 0.4 - 1.3
Nanaimo/Crofton Area	8	19.12 4.5 - 57.7	0.35 0.2 - 0.8	1.33 0.3 - 5.2	28.87 7.6 - 97.0	0.78 0.2 - 2.4	5.04 2.1 - 12.2	5.27 1.2 - 18.0	3.55 0.7 - 12.6	2.40 0.7 - 9.0
Barkley Sound	3	18.38 3.5 - 48.2	0.31 0.1 - 0.6	0.78 0.3 - 1.3	15.19 3.6 - 36.6	0.48 0.1 - 1.1	1.99 0.5 - 4.4	3.18 0.8 - 7.6	2.43 0.7 - 5.4	1.50 0.4 - 3.5
Santa Catalina Island	3	41.25 18.0 - 123.4	0.08 0.05 - 0.1	0.52 0.3 - 0.8	10.66 6.5 - 21.9	0.39 0.2 - 1.0	2.24 1.4 - 4.2	2.02 1.4 - 4.2	1.49 1.0 - 3.0	0.62 0.4 - 1.2

2.8 Appendix

Glycerol and Uric Acid Methods and Results

Free glycerol was assayed via a sequential color endpoint assay (Trinder reagent A and B, respectively, Sigma-Aldrich Canada, Oakville, Ontario), using 5 µL of sample with 240 µL and 60 µL of reagents A and B respectively. A reading was taken at 540 nm after 10 minutes of incubation at 37°C after the addition of each reagent. Uric acid was also assayed via color endpoint assay (WAKO USA, Richmond, Virginia), using 5 µL of sample with 300 µL of reagent, with a reading taken at 550 nm after 10 minutes of incubation at 37°C.

Table 2.3 Means and standard error of mass and age of bald eagle nestlings. Means and standard error of glycerol, and uric acid levels measured in plasma of bald eagle nestlings for each of the sites

Site	n	Mass (g)	Age (days)	Glycerol (mg/mL)	Uric Acid (mg/mL)
Fort St. James Area	5	4330.0 (331.13)	52.5 (3.720)	0.048 (0.007)	1.240 (0.159)
Central Fraser Valley	6	3800.0 (322.88)	51.0 (4.967)	0.180 (0.031)	0.995 (0.264)
Lower Fraser Valley	9	3900.0 (275)	43.4 (4.902)	0.116 (0.023)	0.871 (0.131)
Nanaimo/Crofton Area	8	2901.4 (400.15)	38.6 (5.010)	0.093 (0.013)	0.910 (0.169)
Barkley Sound	3	1266.7 (202.76)	20.7 (0.667)	0.064 (0.012)	0.987 (0.365)
Santa Catalina Island	3	- -	61.0 (0.577)	0.087 (0.604)	0.604 (0.096)

2.9 Reference List

Anthony, R. G., Garrett, M. G., and Schuler, C. A. (1993). Environmental contaminants in bald eagles in the Columbia River Estuary. Journal of Wildlife Management 57, 10-19.

Bustnes, J. O., Skaare, J. U., Erikstad, K. E., Bakken, V., and Mehlum, F. (2001). Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gull (*Larus Hyperboreus*). Environmental Toxicology and Chemistry 20, 1046-1052.

Clark, K. E., Niles, L. J., and Stansley, W. (1998). Environmental contaminants associated with reproductive failure in bald eagles (*Haliaeetus leucocephalus*) eggs in New Jersey. Bulletin of Environmental Contamination and Toxicology 61, 247-254.

Dominguez, L., Montevecchi, W. A., Burgess, N. M., Brazil, J., and Hobson, K. A. (2003). Reproductive success, environmental contaminants, and trophic status of nestling bald eagles in eastern Newfoundland, Canada. Journal of Raptor Research 37, 209-218.

Donaldson, G. M., Shutt, J. L., and Hunter, P. (1999). Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. Archives of Environmental Contamination and Toxicology 36, 70-80.

Elliott, J. E., and Norstrom, R. J. (1998). Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. Environmental Toxicology and Chemistry 17, 1142-1153.

Elliott, J. E., and Harris, M. L. (2001/2002). An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Reviews in Toxicology, 1-60.

Elliott, K. H., Gill, C. E., and Elliott, J. E. (2005). The influence of tide and weather on provisioning rates of chick-rearing bald eagles in Vancouver Island, British Columbia. Journal of Raptor Research 39, 1-10.

Frings, C. S., and Dunn, R. T. (1970). A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. In 21st National meeting of the American Association of Clinical Chemists, Vol. 53, pp. 89-91, Denver, Colorado.

Ferrer, M., and Dobado-Berrios, P. (1998). Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. Comparative Biochemistry and Physiology Part A 120, 209-217.

Garcelon, D. K. (1997). Effects of organochlorine contaminants on bald eagle reproduction at Santa Catalina Island. Montrose Trustee Council.

Gill, C. E. (1993). Environmental contaminants, food availability, and reproduction of bald eagles (*Haliaeetus leucocephalus*) on Vancouver Island, British Columbia. in Biological Sciences, p. 149. Simon Fraser University, Burnaby.

Gill, C. E., and Elliott, J. E. (2003). Influence of food supply and chlorinated hydrocarbon contaminants on breeding success of bald eagles. Ecotoxicology 12, 95-111.

Groscolas, R. (1982). Changes in plasma lipids during breeding, molting, and starvation in male and female emperor penguins (*Aptenodytes forsteri*). Physiological Zoology 55, 45-55.

Guglielmo, C. G., O'Hara, P. D., and Williams, T. D. (2002). Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). The Auk 119, 437-445.

Henny, C. J., Griffin, C. R., Stahlecker, D. W., Harmata, A. R., and Cromartie, E. (1981). Low DDT residues in plasma of bald eagles (*Haliaeetus leucocephalus*) wintering in Colorado and Missouri. The Canadian Field Naturalist 95, 249-252.

Henriksen, E. O., Gabrielsen, G. W., and Skaare, J. U. (1998). Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls, *Larus hyperboreus*. Chemosphere 37, 2627-2643.

Hebert, C. E., and Keenleyside, K. A. (1995). To normalize or not to normalize? Fat is the question. Environmental Toxicology and Chemistry 14, 801-807.

Jenni-Eiermann, S., and Jenni, L. (1994). Plasma metabolite levels predict individual body-mass changes in a small long distance migrant, the garden warbler. The Auk 111, 888-899.

Jenni-Eiermann, S., and Jenni, L. (1996). Metabolic differences between the postbreeding, moulting and migratory periods in feeding and fasting passerine birds. Functional Ecology 10, 62-72.

Jenni-Eiermann, S., Jenni, L., and Piersma, T. (2002). Plasma metabolites reflect seasonally changing metabolic processes in a long-distance migrant shorebird (*Calidris canutus*). Zoology 105, 239-246.

Kern, M., Bacon, W., Long, D., and Cowie, R. J. (2001). Possible roles for corticosterone and critical size in the fledging of nestling pies flycatchers. Physiological and Biochemical Zoology 74, 651-659.

Leclercq, B., Hassan, I., and Blum, J. C. (1974). The influence of force feeding on the transport of plasma lipids in the chicken (*Gallus gallus l.*). Comparative Biochemistry and Physiology 47B, 289-296.

Lee, H. (1994). The distribution and character of contaminated effluent-affected sediment, Palos Verdes Margin, Southern California, p. 237. U.S. Geological Survey, Menlo Park.

Phillips, D. L., Pirkle, J. L., Burse, V. W., Bernert, J. T., Hernderson, L. O., and Needham, L. L. (1989). Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Archives of Environmental Contamination and Toxicology 18, 495-500.

Sarasola, J. H., Negro, J. J., and Travaini, A. (2004). Nutritional condition and serum biochemistry for free living Swanson's Hawks wintering in central Argentina. Comparative Biochemistry and Physiology Part A 137, 697-701.

Sasaki, O., Yamamoto, N., Togashi, K., and Minezawa, M. (2001). Effects of age, environments, and sex on plasma metabolites in young Holstein calves. Asian-Australasian Journal of Animal Sciences 15, 637-642.

Tansy, C. L., Senthilkumar, K., Pastva, S. D., Kannan, K., Bowerman, W. W., Masunaga, S., and Giesy, J. P. (2003). Concentrations and profiles of polychlorinated biphenyls, -dibenzo-p-dioxins, and -dibenzofurans in livers of mink from Southern Carolina and Louisiana, U.S.A. Environmental Monitoring and Assessment 83, 17-33.

Totzke, U., and Bairlein, F. (1998). The body mass cycle of the migratory garden warbler (*Sylvia borin*) is associated with changes of basal plasma metabolite levels. Comparative Biochemistry and Physiology Part A 121, 127-133.

Van Den Brink, N. W., Van Franeker, J. A., and De Ruiter-Dijkman, E. M. (1998). Fluctuating concentrations of organochlorine pollutants during a breeding season in two Antarctic seabirds: adelie penguin and southern fulmar. Environmental Toxicology and Chemistry 17, 702-709.

Williams, T. D., Ghebremeskel, K., Williams, G., and Crawford, M. A. (1992). Breeding and moulting fasts in macaroni penguins: do birds exhaust their fat reserves? Comparative Biochemistry and Physiology 103A, 783-785.

CHAPTER 3
EFFECT OF p,p'-DDE, PCBs, AND PBDEs ON THYROID HORMONE AND VITAMIN A LEVELS IN BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*)

3.1 Abstract

Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants that have a similar molecular structure to other halogenated aromatics such as, 1,1-dichloroethylene *bis*[*p*-chlorophenyl] (p,p'-DDE), polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), and biphenyls (PCBs) and their levels are increasing exponentially in the environment. Since halogenated aromatics are known to be toxic, persistent, and bioaccumulative, there is concern that PBDEs will have similar detrimental effects on wildlife and humans. Studies have shown that p,p'-DDE, PCBs and PBDEs can impair the metabolism of vitamin A and decrease circulating thyroid hormones, which are required for proper growth, development, cell differentiation, immunology, vision, and reproduction. The objectives of this study are to investigate the levels of p,p'-DDE, PCBs, and PBDEs in bald eagles resident in British Columbia and southern California, and to measure the levels of plasma vitamin A and thyroid hormones (T3 and T4) in bald eagles. Blood samples were collected from nestling bald eagles at 6 different sites; 5 in British Columbia and 1 in southern California. The results show a significant negative correlation between p,p'-DDE and both thyroid hormones (T3 and T4). A significant negative relationship between PCBs and T4 levels in the plasma was also observed, but not with total T3. There were no significant relationships between PBDEs and T4 or T3. None of the contaminants showed a correlation with vitamin A. This data suggests that p,p'DDE and PCBs may be negatively affecting the endocrine system in nestling bald eagles.

3.2 Introduction

Many environmental contaminants have been shown to be endocrine disruptors, especially the polyhalogenated aromatic hydrocarbons (PHAHs) (Brouwer et al. 1998; Builee and Hatherill 2004; Hallgren and Darnerud 2002; Hallgren et al. 2001; Hose and Guillette 1995; Murk et al. 1996). Common chemicals in this class include dichlorodiphenyltrichlorethane (p,p'-DDT), its metabolite 1,1-dichloroethylene *bis*[*p*-chlorophenyl] (p,p'-DDE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (Brouwer et al. 1998; Builee and Hatherill 2004). This group of chemicals has been shown to be capable of compromising thyroid function, specifically altering normal synthesis, secretion, transport, metabolism, binding, and excretion of thyroid hormones (Bassett and Williams 2003; Brouwer et al. 1998; Builee and Hatherill 2004; Dawson 2000; Verreault et al. 2004). Thyroid hormones are essential for normal growth and development (especially of sex organs and the brain), therefore any disruption in their levels could cause drastic effects, especially in the offspring of exposed individuals (Bassett and Williams 2003; Brouwer et al. 1998; Builee and Hatherill 2004; Dawson 2000; Hallgren and Darnerud 2002; Zoeller 2002).

Since the introduction of PHAHs into the environment in the 1940s and 50s, PHAHs have become widespread global contaminants known to be persistent, toxic and to bioaccumulate up the food web (Brouwer et al. 1998; Hallgren and Darnerud 2002; Hewitt et al. 2002). They have been found in high concentrations in both wildlife and humans around the world (Dawson 2000; Hewitt et al. 2002; Hose and Guillette 1995; Newson et al. 2004; Verreault et al. 2004). PHAHs have broad ranging effects on lipid

peroxidation, oxidative phosphorylation, sex steroids, thyroid hormones, and can accelerate cellular apoptosis (Brouwer et al. 1998; Choksi et al. 2003; Dawson 2000; Hallgren et al. 2001; Murk et al. 1996; Smits et al. 2002; Verreault et al. 2004).

A recent group of chemicals of concern in relation to the effect of PHAHs on humans and wildlife are polybrominated diphenyl ethers (PBDEs) (de Wit 2002; Ikonomou et al. 2002; Manchester-Neesvig et al. 2001). PBDEs are widely used flame retardants and their concentrations appear to be increasing exponentially in the environment (Ikonomou et al. 2002). Because PBDEs are mixed and not chemically bound to the material in which they are used, they are able to migrate from the material during its lifetime (Eriksson et al. 2001). One of the first reports of PBDE in the environment appeared in 1981 (Anderson and Blomkist 1981). Since then, PBDEs have been shown to be persistent compounds that appear to have an environmental dispersion similar to that of PCBs and p,p'-DDT (Eriksson et al. 2001). They also have a similar molecular structure to polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), and biphenyls (PCBs) (Manchester-Neesvig et al. 2001). Since these chemicals are known to be toxic, persistent, and bioaccumulative, there is concern that PBDEs will have the same detrimental effects on wildlife and humans as PCBs (Ikonomou et al. 2002). However, few studies have investigated the effects of these chemicals in the natural environment or in a species considered to be at risk due to its high trophic status and feeding strategy, such as the bald eagle. Therefore, the specific objectives of this study are: 1) to determine the levels of p,p'-DDE, PCBs, and PBDEs in bald eagle chicks in British Columbia and in southern California and 2) to examine the correlation between p,p'-DDE, PCBs and

PBDEs with thyroid hormones (triiodothyronine (T3) and thyroxine (T4)) and vitamin A in bald eagle chicks during their growth phase.

3.3 Methods

Study Areas, Sample Collection, and Contaminant Analysis

Blood samples were obtained from 6 different sites as described in Chapter 2 (see Fig. 2.1 for map). Sample analysis for p,p'-DDE and PCBs are also described in Chapter 2. The analysis of PBDEs was carried out in the laboratory of R.J. Letcher with the National Wildlife Research Centre (Ottawa, ON). All of the bald eagle plasma samples collected in British Columbia were analyzed for PBDEs (total PBDEs, BDE 47, 99,100, 138, 153, 183, and 209) BB101, and total hexbromocyclododecane (total HBCD).

Procedures used for the extraction and determination of brominated flame retardants (BFRs) in plasma were based on methods previously described (Sandala et al. 2004; Verreault et al. 2005a; Verreault et al. 2005b). Briefly, plasma samples (2.0 – 3.0 g) were spiked with the internal standards/recovery surrogates, BDE30 (25 uL of 428 pg/uL; for total-HBCD, and PBDEs). After spiking, samples were acidified with 1 mL HCl (6 M) and 3 mL of 2-propanol was added. The denatured plasma was extracted three times with 6 mL methyl-*tert*-butyl-ether (MtBE)/*n*-hexane (50:50 volume ratio (VR)). The solution was partitioned with 6 mL KOH (1 M in 50% ethanol), and two phases were obtained: an aqueous phase containing the deprotonated HPCs and an organic phase containing the neutral BFRs (i.e., PBDEs, and total-HBCD). The aqueous phase was acidified with H_2SO_4 and the HPCs were back-extracted with 6 mL MtBE/*n*-hexane (50:50 VR), then dried over Na_2SO_4. The extracts were concentrated to 1 mL, the solvent

2,2′,4-trimethylpentane (iso-octane) was added, and finally reduced to 100 μL under a gentle flow of nitrogen for analysis by gas chromatography-mass spectrometry in the negative chemical ionization mode (GC-MS(ECNI)).

The organic phase from the KOH partitioning step was concentrated, and fractionated with a Florisil® column (8.0 g, 1.2% H2O deactivated) (Magnesium silicate, F100 – 500, 60 – 100 mesh) (Fisher Scientific, Ottawa, ON, Canada). Three eluted fractions were collected and combined, 38 mL *n*-hexane, 34 mL DCM/*n*-hexane (15:85 VR), and 54 mL DCM/*n*-hexane (50:50 VR), which contained all the neutral brominated compounds (PBDEs and total-HBCD). This combined fraction was concentrated to 1 mL by rotary evaporation, and reduced to 100 μL in iso-octane under a gentle flow of nitrogen for GC-MS(ECNI) analysis.

For the brominated compounds, the determination was performed with GC-MS(ECNI). An Agilent gas chromatograph (GC) 6890 equipped with a 5973 quadrupole mass spectrometer (MS) detector was used. Mean recovery (±1 standard error) of the internal standards was on average 90 ± 7% for BDE30. Concentrations were recovery-corrected as an internal standard method of quantification was used to reduce heterogeneity within and between analyte classes. Blank samples with each batch of 5 samples were used to monitor interferences and co-eluting contamination. No substantial background contamination was encountered for any BFR or HPC analytes. The analytical precision of quantitative determinations was tested by repeated injections of standard compounds, and where sample amount permitted, duplicate analyses of selected bald eagle samples was performed. Duplicate samples demonstrated on average 10% variation of analyte concentrations. The method limit of quantification (MLOQ) for individual

analytes was based on a minimum of 10 times the noise level (S/N). MLOQs in bald eagles ranged between 0.001 and 0.01 ng/g wet weight (ww). Plasma lipid levels were determined colorimetrically, using a sulfo-phospho-vanillin reaction (Frings and Dunn 1970) and color endpoint assays were used to determine triglyceride and non-esterfied fatty acids levels as described in Ch.2.

Thyroid hormone and vitamin A analysis

Thyroid hormones thyroxin (T4) and triiodothyronine (T3) were analyzed by staff at NWRC using Coat-A-Count Canine T4 and T3 kits (Diagnostic Products Corp). These solid phase radioimmunoassays use tubes coated with either monoclonal T4 or T3 antibodies. After an adequate incubation period (2 hours at 37°C), the bound and free fractions are separated and the radioactivity quantified. Sample results are interpolated from a standard curve generated by counting samples containing known quantities of unlabeled T4 or T3.

Vitamin A was also analyzed by the staff at NWRC. For the analysis, the internal standard retinyl acetate was added to 100 ul of plasma. The retinol-protein complex was then dissociated by the addition of acetonitrile. The retinol and 3,4-didehydroretinol were extracted with successive volumes of hexane. The separation of the organic and aqueous phases was achieved by centrifugation and the organic phases were combined and evaporated to dryness with nitrogen. The residues were re-dissolved in methanol, filtered and were analysed using a Varian HPLC system (9010-2332, 91000-2778). The separation was achieved in less then 6 min by a gradient method using methanol: dichlorometane with a 15 cm ODS ZORBAX column. The retinoid compounds were then detected with a UV/VIS detector (9050-0664) set at 325 nm. The calibration standard

curve ranged from 0.8 to 17 ng per injection. A verification standard was included and analyzed with each set of samples for quality assurance.

Statistics

All statistical analyses were performed using SAS v.9.1 software package (2002-2003). All of the contaminants (DDE, HCB, t-nonachlor, PCBs, PBDEs) were non-normally distributed, therefore were log transformed ($\log_{10} + 1$) to approximate normal. T3 levels were also non-normally distributed and were therefore log transformed; T4 and vitamin A were normally distributed.

The relationship between contaminants and lipid metabolites was determined using linear regression models. Since, no correlation was found; the contaminants were not normalized for lipids. Site differences were determined using analysis of variance (ANOVA) and to determine which sites were different a Student Newman Keuls test was performed.

The relationship between contaminants and thyroid hormones/vitamin A was also investigated using linear regression models. Site differences were determined using ANOVA and to determine which sites were different a Student Newman Keuls test was performed. Unless stated otherwise, results were considered significant if $p < 0.05$.

3.4 Results

Contaminant levels

Plasma samples from 34 bald eagle chicks were analyzed. Wet weight concentrations for p,p'-DDE and PCBs are presented in Table 2.1 and PBDE concentrations are presented in Table 3.1. Due to low levels of many of the contaminants

and individual congeners, it was decided to focus mainly on p,p'-DDE, total PCBs, and total PBDEs. Site differences were found for all contaminants (Fig. 2.11 and 3.1).

Relationship between contaminants and thyroid hormones

For the organochlorines, only p,p'-DDE showed a significant negative correlation both with total T4 (TT4)($F=5.15$, $p=0.0304$, $df=1$), and total T3 (TT3) ($F=4.39$, $p=0.0445$, $df=1$; Fig. 3.2). Total PCBs were also significantly negatively correlated with T4 ($F=5.73$, $p=0.0231$, $df=1$), but not T3 ($F=0.84$, $p=0.3670$, $df=1$; Fig. 3.3). All of the individual PCB congeners (PCB153, 99, 118, 138, and 183) showed the same trend as the sum of PCBs (Table 3.1). No relationship was found between any of the PBDEs and the thyroid hormones (T4 or T3) ($p>0.05$ in all cases; Fig. 3.4). Levels of T3, T4, and vitamin A are presented by site in Table 3.2.

Relationship between contaminants and vitamin A levels

No significant relationships were found between any of the contaminants and vitamin A levels ($p>0.05$ in all cases; Figs. 3.2, 3.3, and 3.4), although some contaminants showed negative trends. Total PCBs, BDE 99, and BDE 138 all approached significance with vitamin A ($F=3.40$, $p=0.0746$, $df=1$; $F=3.40$, $p=0.0516$, $df=1$; $F=4.22$, $p=0.0516$, $df=1$ respectively).

3.5 Discussion

The most important findings in this study are 1) there was a significant negative correlation between both p,p'-DDE and PCBs and circulating levels of thyroid hormones in nestling bald eagles, 2) PBDEs at the plasma concentrations found in this study did not

appear to be negatively influencing thyroid hormone levels in bald eagles, and 3) contaminants measured in this study did not have a negative affect on vitamin A levels.

Relationship between contaminants, thyroid hormones and vitamin A

In the present study, significant negative correlations were found between plasma levels of p,p'-DDE and PCBs with circulating thyroid hormones in nestling bald eagles. Both T3 and T4 were negatively correlated with p,p'-DDE, but only T4 was reduced in relation to PCBs. Similar results have been reported in other studies for other species. For example, Verreault et al. (2004) found decreased levels of T4 and T4:T3 ratio in glaucous gulls (*Larus hyperboreus*) in relation to p,p'-DDE and PCBs, as well as other organochlorines. Smits et al. (2002) found levels of T3 to be significantly depressed in PCB exposed American kestrels. In another study of nestling bald eagles, Newson et al. (2004) found PCBs were negatively correlated with thyroid free T3 index.

In contrast to p,p'-DDE and PCBs, the present study showed no significant correlations between PBDEs and circulating thyroid hormones in nestling bald eagles. To date, few studies have examined the effects of PBDEs in wildlife species; most studies have been performed in the laboratory. Hallgren et al. (2001) found that PBDEs decreased thyroxine levels in rats and mice exposed to PBDEs, and showed a greater reduction in T4 when a mixture of PCBs and PBDEs was administered (Hallgren and Darnerud 2002). This latter experiment is probably more environmentally relevant, because both compounds are ubiquitous in many ecosystems. Zhou et al. (2001) did find a dose dependant depletion of T4 (up to 80% reduction) and T3 (up to 30%) in weaning rats exposed to PBDEs, but the doses used were much higher then we found in the present study in nestling bald eagles.

No significant correlations were found between any of the contaminants (p,p'-DDE, PCBs and PBDEs) with vitamin A. However, laboratory studies have shown a decrease in vitamin A levels after exposing rats and mice to PCBs and PBDEs (Hallgren et al. 2001). In birds, these correlations have also been reported; Champoux et al.(2002), found significant negative correlations between retinol and PCBs as well as p,p'DDE in two species of herons (*Ardea herodias* and *Nycticorax nycticorax*). In nestling black guillemots (*Cepphus grille*), liver retinol concentrations significantly decreased with ΣPCB exposure (Kuzyk et al. 2003). Vitamin A and the thyroid hormones are both transported via the same transport protein transthyretin (Brouwer 1991; Brouwer et al. 1986), therefore one might expect similar effects given that contaminants affect the same transport protein. However, retinol is also bound to retinol-binding proteins, and this configuration may prevent it from being excreted as quickly as the thyroid hormones (Brouwer et al. 1998).

Possible mechanisms of thyroid hormone disruption

Many studies have suggested possible mechanism(s) for organoaromatic contaminant induced alterations in thyroid hormone and vitamin A levels. Specifically, three levels of contaminant interaction of the thyroid hormone system have been experimentally demonstrated: 1) effects on thyroid gland function and regulation, 2) effects on thyroid hormone metabolism, and 3) effects on thyroid hormone transport binding proteins (Brouwer et al. 1998; Builee and Hatherill 2004; Dawson 2000). With some compounds, such as PCBs, the contaminants are thought to interfere at all three levels of the thyroid hormone system, which increases the risk of a prolonged disruption

of this system and associated physiological functions, including developmental disturbances (Brouwer et al. 1998; Builee and Hatherill 2004; Dawson 2000).

Our study of bald eagles supports the idea that contaminants interfere with the thyroid hormone system through interactions with plasma transport processes for thyroid hormones, but not vitamin A. The transport system consists of a complex of two proteins: transthyretin (TTR), which contains two binding pockets for thyroid hormones, and a retinol binding protein (RBP), which contains a binding site for the vitamin A analog, retinol (Brouwer et al. 1998; Dawson 2000). The contaminants used in this study have a similar molecular structure to TTR, which allows these chemicals to mimic T4 and bind to TTR (Figure 3.5) (Brouwer 1991; Brouwer et al. 1986; Newson et al. 2004). These contaminants also have a higher binding affinity, binding with three to ten times more affinity (especially their hydroxylated metabolites) than T4 , therefore T4 is displaced from its binding sites (Brouwer et al. 1998). This results in reduced amounts of T4 reaching the target tissues, and the unbound fraction of T4 thus being more readily excreted. Also, there may be a temporary increase in the level of free T4 in the plasma, which may increase enzymatic degradation of T4 into T3 or reverse T3, the inactive form of thyroid hormone through selenoproteins ID-I and ID-II (Builee and Hatherill 2004). As a result of increased degradation of T4 , its levels are decreased with increased or normal T3 (and reverse T3 levels) and TSH levels become unregulated by pituitary and thyroid gland interactions (Fig. 3.6 and 3.7) (Builee and Hatherill 2004).

Many studies have investigated the effects of contaminants binding to TTR. Using radiolabelled tetrachlorobiphenyl (H-TCB), Brouwer et al. (1986) was able to examine its association with TTR and levels of T4 and retinol in the plasma of rodents. They found a

significant decrease in both T4 and retinol due to a direct interaction of the metabolite of TCH with TTR. Hydroxy metabolites of several PCBs, PCDDs, and PCDFs were also shown to compete competitively for thyroxine on the transthyretin binding sites in rodents (Dawson 2000).

Studies on birds have shown mixed results in regards to competitive binding of PHAHs with TTR. This may be due to most of the T4 being associated with albumin rather than TTR (Dawson 2000). Only about 10-30% of T4 is bound to TTR. The TTR found in birds has a lower affinity for T4 than in mammals, but is higher for T3, which may also play a role in the lack of negative correlations between PHAHs and T4 levels (Dawson 2000).

Vitamin A is also affected by contaminants binding to TTR (Brouwer et al. 1986; Champoux et al. 2002; Kakela et al. 2003). Normally, vitamin A binds to RBP and then as a complex binds to TTR, which is transported to the target tissues (Builee and Hatherill 2004). Contaminants may interfere with this process by binding to TTR, causing a conformational change that prevents the RBP from binding to TTR, therefore not effectively transporting retinol to its target tissues. Consequently, retinol is left unbound, which increases its chances of being excreted (Builee and Hatherill 2004). Although a significant negative correlation between the contaminants and retinol was not seen in this study, numerous contaminant studies have shown a decrease in retinol. Kakela et al. (2003) found a decrease in retinol in relation to PCBs in mink and Newson et al. (2004) showed a decrease in retinol in relation to OH-PCBs in bald eagles.

In conclusion, the present study showed a weak negative association between T4 and T3 and plasma p,p'-DDE levels and a decrease in T4 in relation to plasma PCBs.

This suggests that these contaminants may be having a negative effect on the endocrine system of nestling bald eagles. PBDEs did not show a correlation with either thyroid hormone (T3 or T4) or vitamin A, but the hydroxy metabolites may be of greater concern and should be investigated. The hydroxy metabolites of PCBs and PBDEs has been found to be a higher affinity for TTR, and therefore may be more detrimental to the endocrine system (Brouwer 1991; Dawson 2000; Lans et al. 1994; Newson et al. 2004).

3.6 Figures

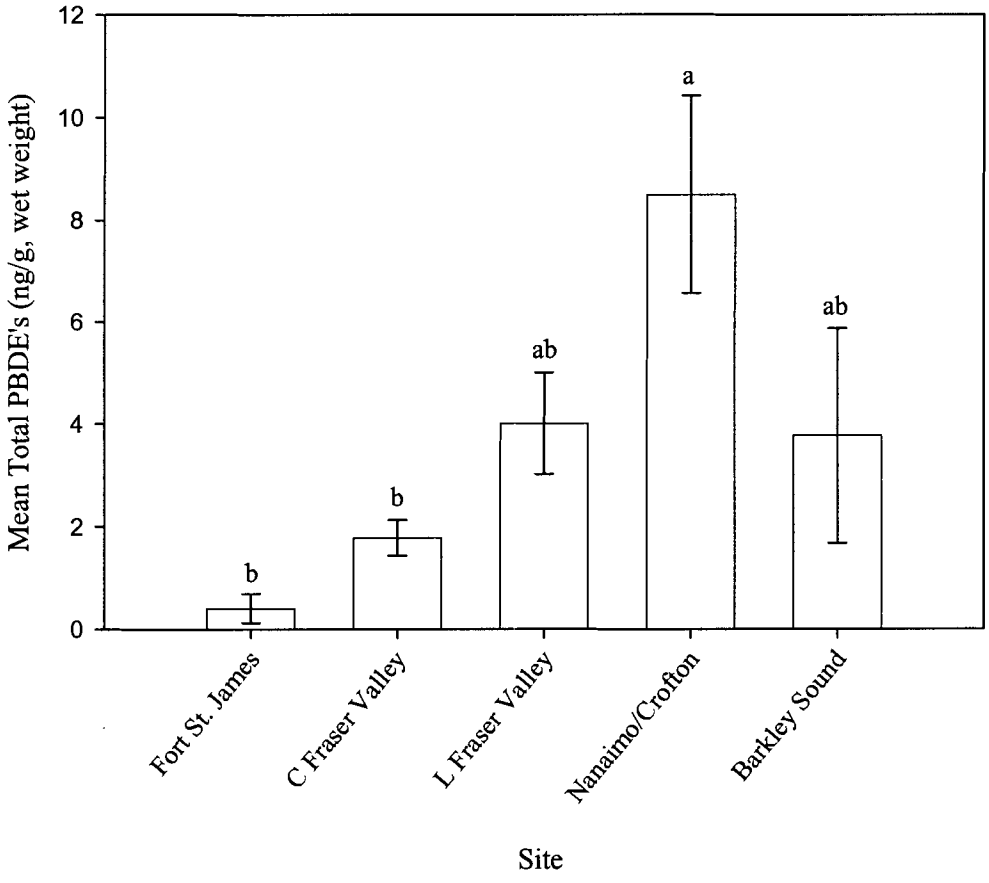

Figure 3.1 Residue levels of total PBDEs in plasma samples of bald eagles nestlings collected in British Columbia. Means that do not share the same lowercase letter were significantly different ($p<0.05$).

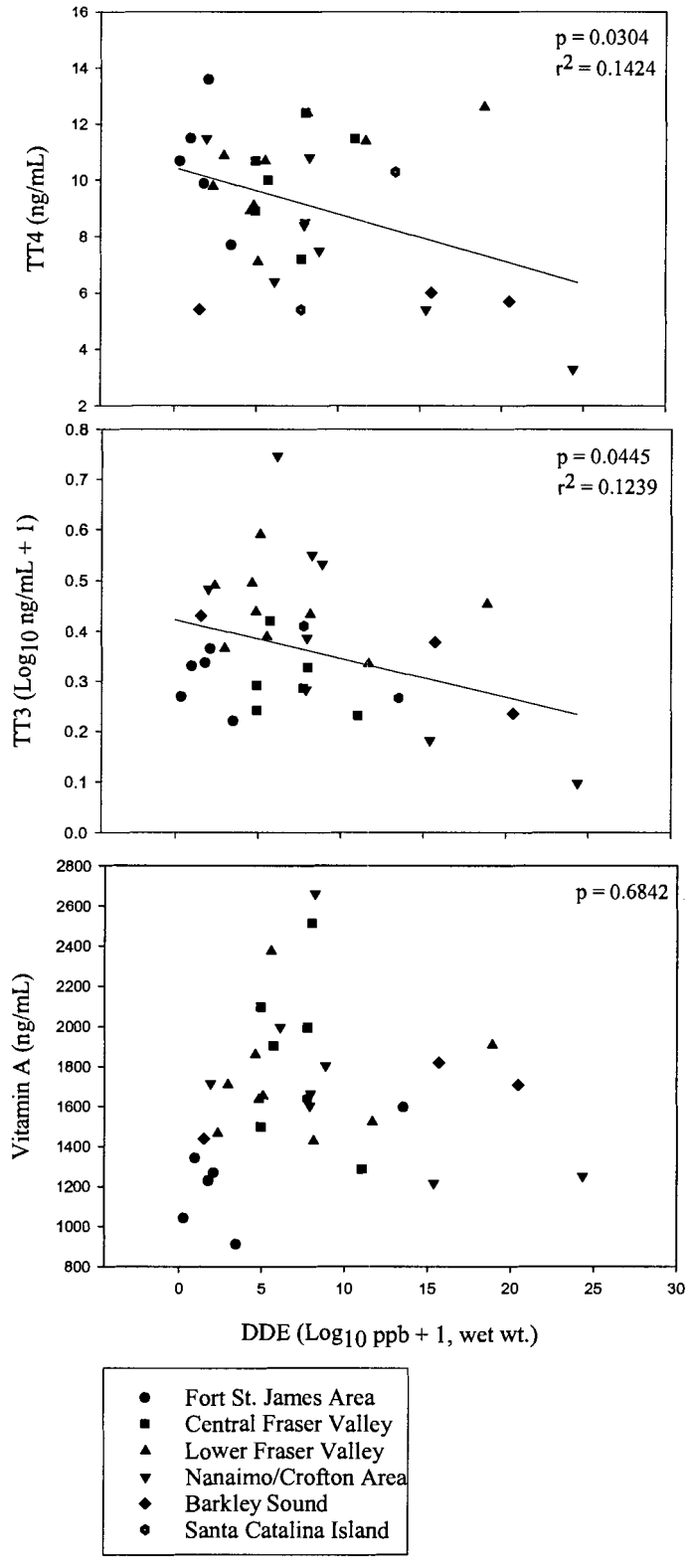

Figure 3.2 Relationship between total T4, total T3, and vitamin A with p,p'-DDE in the plasma of bald eagle nestlings

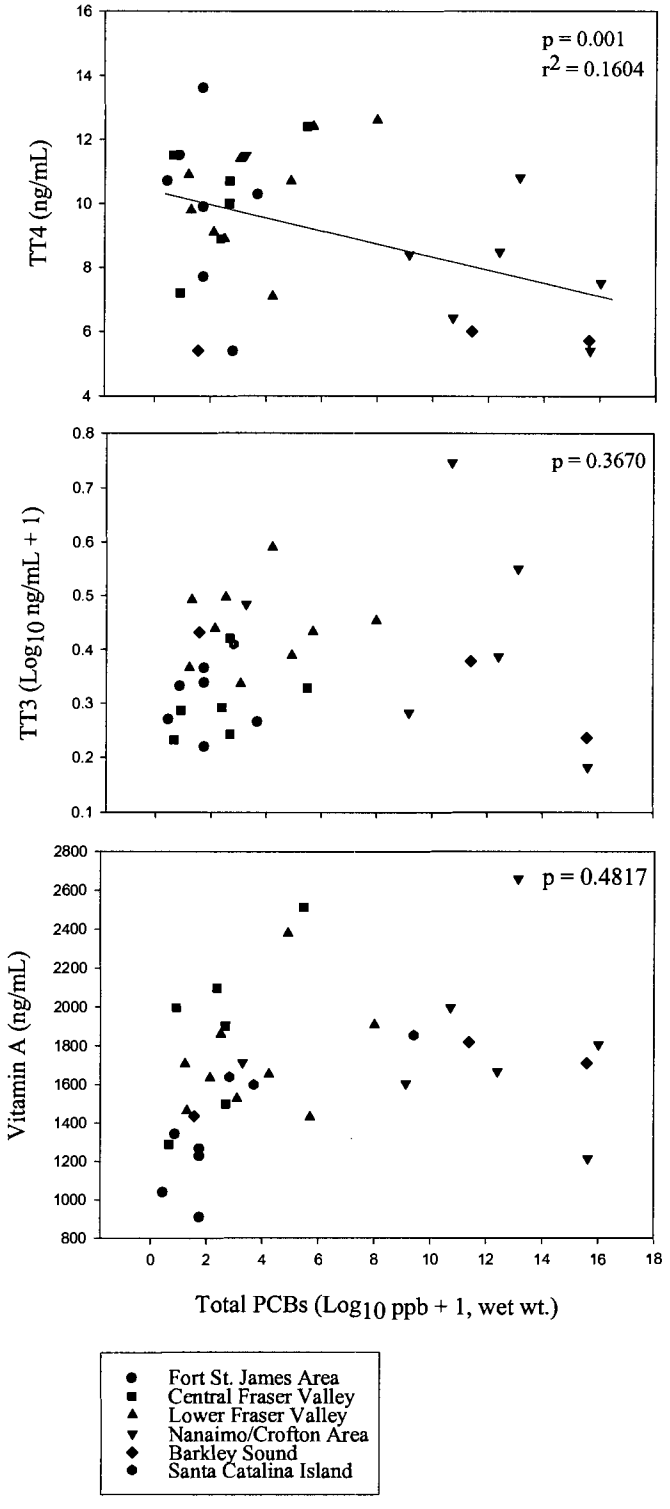

Figure 3.3 Relationship between total T4 , total T3, and vitamin A with total PCBs in the plasma of bald eagle nestlings

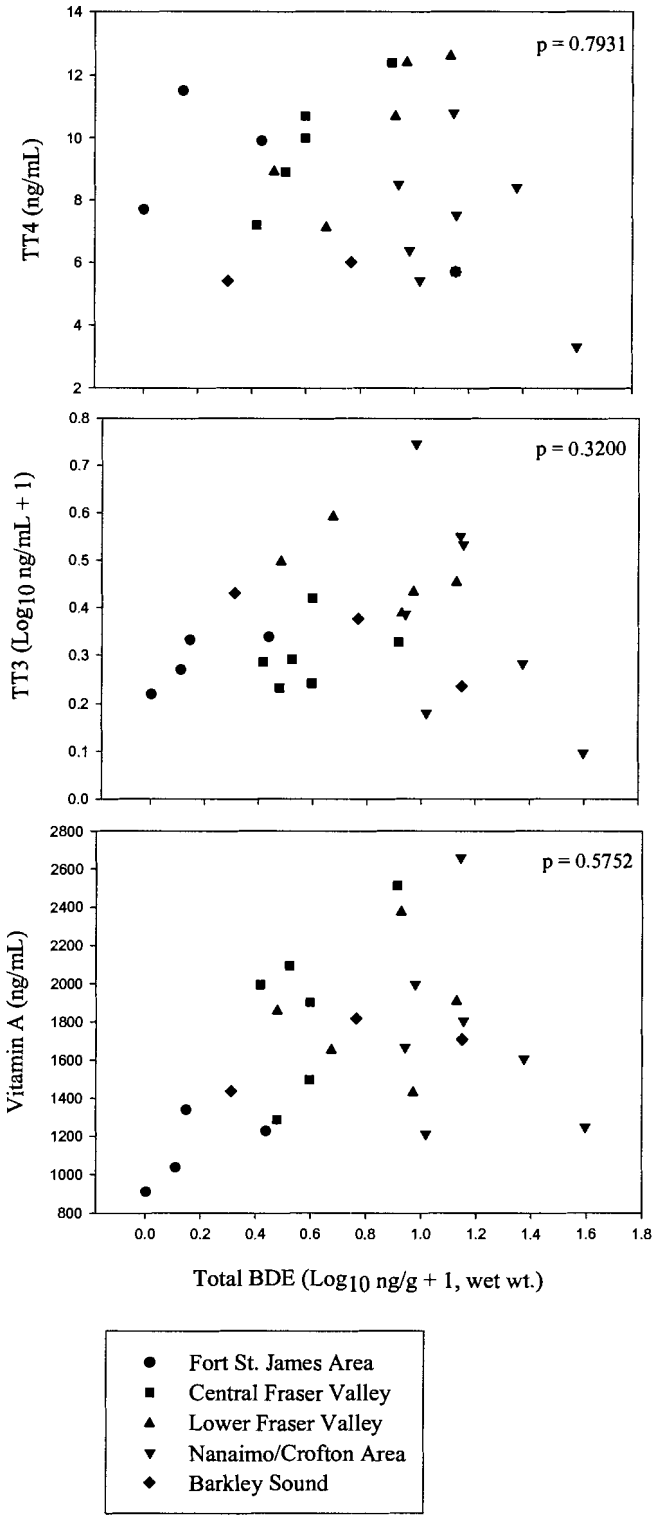

Figure 3.4 Relationship between total T4 , total T3, and vitamin A with total PBDEs in the plasma of bald eagle nestlings

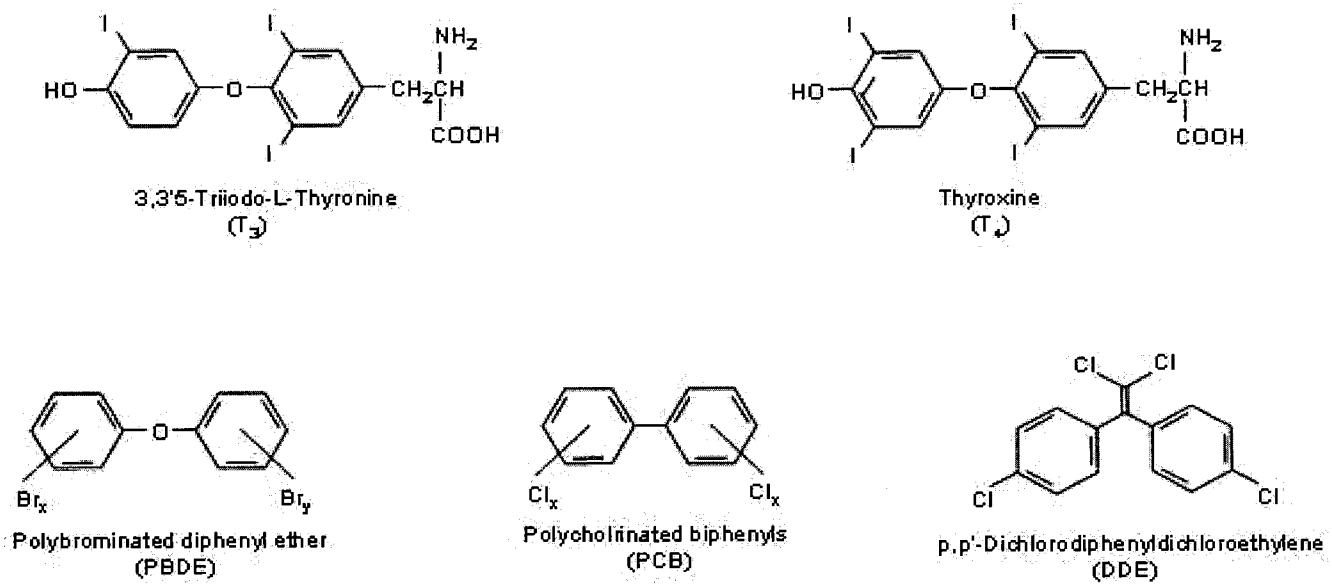

Figure 3.5 Diagram showing the similar structures of the thyroid hormones to contaminants such as PBDEs, PCBs, and DDE

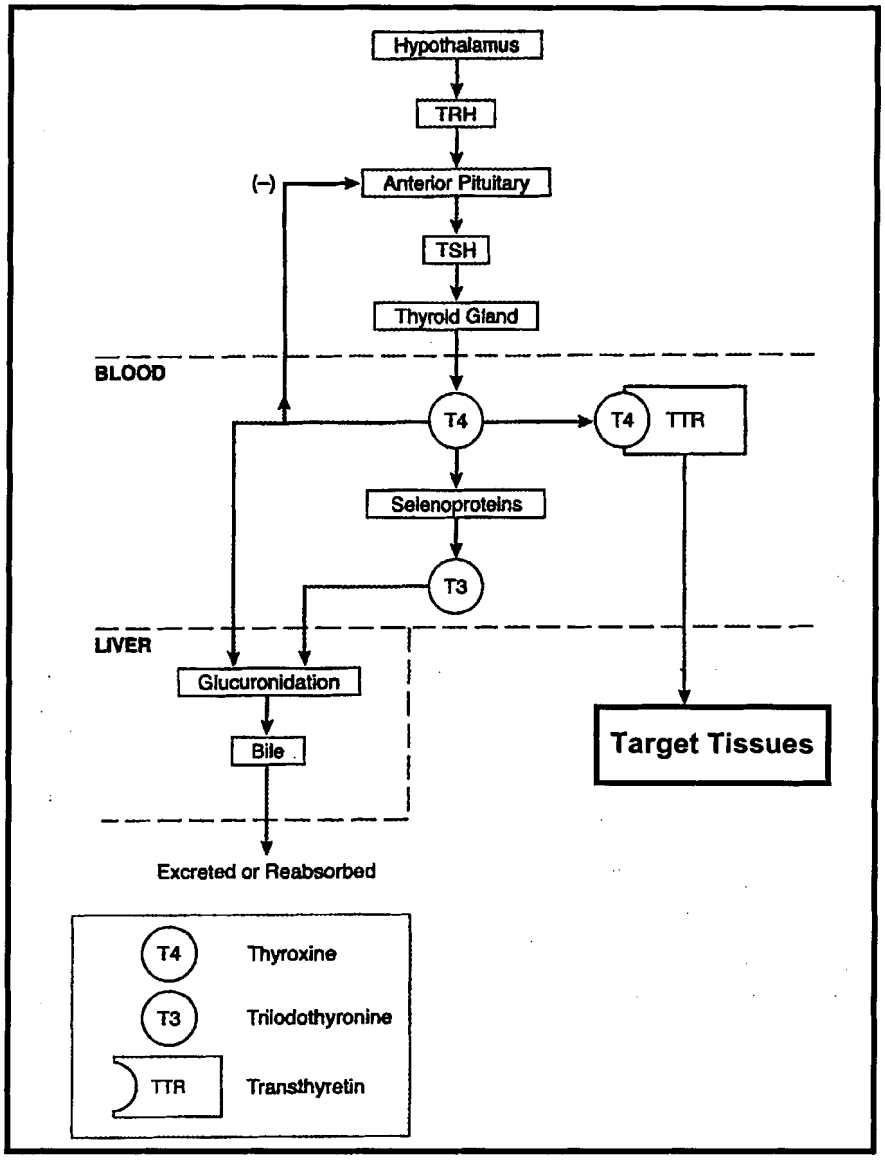

Figure 3.6 **Removal and secretion of thyroxin from the hypothalamus-anterior pituitary-thyroid axis (HPT-axis). Regulation of thyroxin secretion is anticipated in the anterior pituitary as indicated by the negative arrow. Continual regulation of thyroxin occurs in the blood, liver, and neuronal system of the body, initiating cascade-response functions. Thyroxin in the blood is synthesized by the enzymatic activity of a selenoprotein into triidothyronine or transported via transthyretin receptor to target tissues such as the brain. In the liver, glucuronidation of thyroid hormone occurs and are further processed into bile where excretion and reabsorption may occur. Compare the normal function of the HPT-Axis to PHAH exposure in Figure 3.7 (modified from Builee and Hatherill, 2004).**

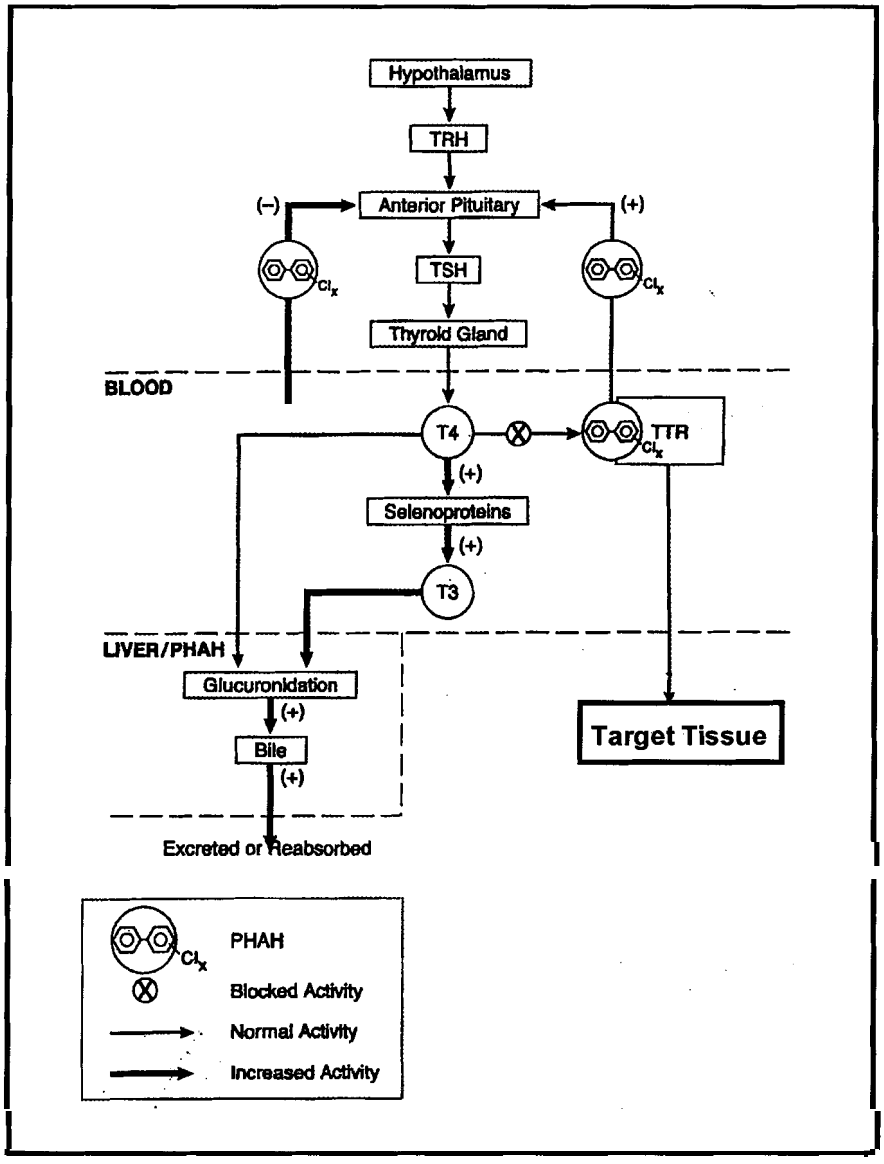

Figure 3.7 The HPT-Axis when exposed to PHAHs. PHAHs may interfere with the binding of T4 to TTR, therefore T4 will no longer be effectively transported to the target tissues and more will be excreted (modified from Builee and Hatherill, 2004).

3.7 Tables

Table 3.1 Geometric means and the range of polybrominated diphenyl ethers (PBDEs) measured in bald eagle nestling blood plasma for each site

Location	n	Residue Levels (ng/g, wet weight)							
		Total BDEs	BDE-47	BDE-100	BDE-99	BDE-153	BDE-138	BDE-183	BDE-209
Fort St. James Area	4	0.118 0.005 - 1.25	0.015 0.005 - 0.42	0.014 0.005 - 0.30	0.096 0.005 - 0.53	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005
Central Fraser Valley	6	1.648 1.15 - 3.40	0.749 0.26 - 1.00	0.407 0.25 - 0.83	0.284 0.10 - 0.85	0.119 0.07 - 0.21	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005
Lower Fraser Valley	6	3.428 1.23 - 7.08	1.249 0.47 - 3.54	0.680 0.34 - 1.48	0.727 0.23 - 1.02	0.165 0.005 - 2.28	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005
Nanaimo/Crofton Area	7	7.478 4.27 - 18.87	3.933 2.15 - 10.73	1.713 1.02 - 4.78	1.297 0.35 - 3.43	0.215 0.02 - 0.53	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005
Barkley Sound	3	2.416 0.62 - 7.74	1.073 0.27 - 3.2	0.487 0.13 - 1.33	0.645 0.14 - 2.78	0.173 0.08 - 0.43	0.005 0.005 - 0.005	0.005 0.005 - 0.005	0.005 0.005 - 0.005

Table 3.2 Means, standard errors, and range of triiodothyronine (T3), thyroxin (T4), and vitamin A in nestling bald eagle plasma for each site

Site	n	T3 (ng/mL)	T4 (ng/mL)	Vitamin A (ng/mL)
Fort St. James Area	5	0.31 ± 0.03 (0.22 - 0.37)	10.68 ± 0.97 (7.70 - 13.60)	1156.8 ± 79.08 (911.00 - 1341.00)
Central Fraser Valley	6	0.30 ± 0.03 (0.23 - 0.42)	10.12 ± 0.76 (7.20 - 12.40)	1882.00 ± 178.61 (1288.00 - 2513.00)
Lower Fraser Valley	9	0.44 ± 0.03 (0.34 - 0.59)	10.32 ± 0.59 (7.10 - 12.60)	1727.90 ± 97.52 (1428.00 - 2376.00)
Nanaimo/Crofton Area	8	0.41 ± 0.08 (0.10 - 0.75)	7.76 ± 0.96 (3.30 - 11.50)	1737.90 ± 161.44 (1213.00 - 2660.00)
Barkley Sound	3	0.35 ± 0.06 (0.24 - 0.43)	5.70 ± 0.17 (5.40 - 6.00)	1654.00 ± 113.53 (1436.00 - 1818.00)
Santa Catalina Island	3	0.34 ± 0.07 (0.27 - 0.41)	7.85 ± 2.45 (5.40 - 10.3)	1696.00 ± 79.39 (1597.00 - 1853.00)

3.8 Reference List

Anderson, O., and Blomkist, G. (1981). Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere* 10, 1051-1060.

Bassett, J. H. D., and Williams, G. R. (2003). The molecular actions of thyroid hormone in bone. *TRENDS in Endocrinology and Metabolism* 14, 356-364.

Brouwer, A. (1991). Role of biotransformation in PCB-induced alterations in vitamin A and thyroid hormone metabolism in laboratory and wildlife species. *Biochemical Society Transactions* 19, 731-736.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, A., and Visser, T. J. (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicology and Industrial Health* 14, 59-84.

Brouwer, A., Van den Berg, K. J., Blaner, W. S., and Goodman, D. S. (1986). Transthyretin (prealbumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid metabolism. *Chemosphere* 15, 1699-1706.

Builee, T. L., and Hatherill, J. R. (2004). The role of polyhalogenated aromatic hydrocarbons on thyroid hormone disruption and cognitive function: a review. *Drug and Chemical Toxicology* 27, 405-424.

Champoux, L., Rodrigue, J., Desgranges, J.-L., Trudeau, S., Hontela, A., Boily, M. H., and Spear, P. A. (2002). Assessment of contamination and biomarker responses in two species of herons on the St. Lawrence River. *Environmental Monitoring and Assessment* 79, 193-215.

Choksi, N. Y., Jahnke, G. D., St. Hilaire, C., and Shelby, M. (2003). Role of thyroid hormones in human and laboratory animal reproductive health. *Birth Defects Research* 68, 479-491.

Dawson, A. (2000). Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review. *Ecotoxicology* 9, 59-69.

de Wit, C. A. (2002). An overview of brominated flame retardants in the environment. *Chemosphere* 46, 583-624.

Eriksson, P., Jakobsson, A., and Fredriksson, A. (2001). Brominated Flame Retardants: A novel class of developmental neurotoxicants in our environment? *Environmental Health Perspectives* 109, 903-908.

Frings, C. S., and Dunn, R. T. (1970). A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. In 21st National meeting of the American Association of Clinical Chemists, Vol. 53, pp. 89-91, Denver, Colorado.

Hallgren, S., and Darnerud, P. O. (2002). Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated parafins (CPs) in rats - testing interactions and mechanisms for thyroid hormone effects. *Toxicology* 177, 227-243.

Hallgren, S., Sinjari, T., Hakansson, H., and Darnerud, P. O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid and vitamin A levels in rats and mice. *Archives of Toxicology* 75, 200-208.

Hewitt, E. A., Crain, D. A., Gunderson, M. P., and Guillette, L. J. J. (2002). Thyroid status in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference sites on Lake Okkeechobee, Florida, USA. *Chemosphere* 47, 1129-1135.

Hose, J. E., and Guillette, L. J. J. (1995). Defining the role of pollutants in the disruption of reproduction in wildlife. *Environmental Health Perspectives* 103, 87-91.

Ikonomou, M. G., Rayne, S., Fischer, M., Fernandez, M. P., and Cretney, W. (2002). Occurrence and congener profiles of polybrominanted diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. *Chemosphere* 46, 649-663.

Kakela, A., Kakela, R., Hyvarinen, H., and Nieminen, P. (2003). Effects of Aroclor 1242 and different fish-based diets on vitamins A_1 (retinol) and A_2 (3,4-didehydroretinol), and their fatty acyl esters in mink plasma. *Environmental Research* 91, 104-112.

Kuzyk, Z. Z. A., Burgess, N. M., Stow, J. P., and Fox, G. A. (2003). Biological effects of marine PCB contaminants on black guillemot nestlings at Saglek, Labrador: liver biomarkers. *Ecotoxicology* 12, 183-197.

Lans, M. C., Spiertz, C., Brouwer, A., and Koeman, J. H. (1994). Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *European Journal of Pharmacology* 270, 129-136.

Manchester-Neesvig, J. B., Valters, K., and Sonzogni, W. C. (2001). Comparison of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PCBs) in Lake Michigan salmonids. *Environmental Science and Technology* 35, 1071-1077.

Murk, A. J., Boudewijn, T. J., Meininger, P. L., Bosveld, T. C., Rossaert, G., Ysebaert, T., Meire, P., and Dirksen, S. (1996). Effects of polyhalogenated aromatic hydrocarbons and related contaminants on common tern reproduction: integration of biological, biochemical, and chemical data. Archives of Environmental Contamination and Toxicology 31, 128-140.

Newson, S. C., Sandau, C. D., Brown, S. B., Elliott, J. E., Gill, C. E., and Norstrom, R. J. (2004). PCBs and their hydroxylated metabolites in bald eagle plasma - comparison to thyroid hormone and retinol levels, p. 20, Ottawa.

Sandala, G. M., Sonne-Hansen, C., Dietz, R., Muir, D. C. G., Valters, K., Bennett, E. R., Born, E. W., and Letcher, R. J. (2004). Methyl sulfone and hydroxylated PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from east Greenland. Science of the Total Environment 331, 125-141.

Smits, J. E., Fernie, K. J., Bortolotti, G. R., and Marchant, T. A. (2002). Thyroid hormone suppression and cell-mediated immunomodulation in American kestrels (*Falco sparverius*) exposed to PCBs. *Archives of Environmental Contamination and Toxicology* 43, 338-344.

Verreault, J., Skaare, J. U., Jenssen, B. M., and Gabrielsen, G. W. (2004). Effects of organochlorine contaminants on thyroid hormone levels in Artic breeding glaucous gulls, *Larus hyperboreus*. *Environmental Health Perspectives* 112, 532-537.

Verreault, J., Gabrielsen, G. W., Chu, S., Derek, C., Muir, G., Andersen, M., Hamaed, A., and Letcher, R. J. (2005a). Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian artic top predators: glaucous gulls and polar bears. Environmental Science and Technology (in press).

Verreault, J., Gabrielsen, G. W., Muir, D. C. G., Chu, S., Gebbink, W., and Letcher, R. J. (2005b). Novel organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Artic. Environmental Toxicology and Chemistry (in press).

Zhou, T., Ross, D. G., DeVito, M., and Crofton, K. M. (2001). Effects of short term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weaning rats. *Toxicological Sciences* 61, 76-82.

Zoeller, R. T. (2002). Thyroid hormone, brain development, and the environment. *Environmental Health Perspectives* 110, 355-361.

CHAPTER 4
SPATIAL AND TEMPORAL TRENDS OF CHLORINATED HYDROCARBONS IN NESTLING BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*) IN BRITISH COLUMBIA AND SOUTHERN CALIFORNIA

4.1 Abstract

Spatial and temporal trends of chlorinated hydrocarbons measured in nestling bald eagles in British Columbia and southern California were investigated. Contaminant levels were compared using concentrations measured in British Columbia in 1993 and 2003. In the ten year span, spatial patterns have changed very little. Only levels of total PCBs in central Fraser Valley have significantly decreased between 1993 and 2003. Overall, there was no significant difference in levels of p,p'-DDE or hexachlorobenzene, but significant decreases were found for trans-nonachlor and PCBs in British Columbia. Long term monitoring of contaminants continues to provide important information on the health of bald eagles, status of the local environments, changes in contaminant levels, and provides the ability to detect new contaminants.

4.2 Introduction

Bald eagles (*Haliaeetus leucocephalus*) have been shown to be an appropriate indicator species to monitor ecosystem contaminant concentrations (Bowerman et al. 2003; Bowerman et al. 1998; Donaldson et al. 1999; Elliott and Norstrom 1998). Due to their position at the top of the food chain and their scavenging behavior, bald eagles bioaccumulate a wide array of lipophilic contaminants (Elliott and Harris 2001/2002; Roe 2004). This includes chemicals such as dichlorodiphenyltrichlorethane (DDT), its metabolite 1,1-dichloroethylene *bis*[*p*-chlorophenyl] (DDE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and recently polybrominanted diphenyl ethers (PBDEs).

Since the 1960's, the levels of chlorinated hydrocarbons in North America have been monitored in bald eagles (Bowerman et al. 1998; Dominguez et al. 2003; Donaldson et al. 1999; Elliott and Harris 2001/2002). Most commercial uses of dichlorodiphenyltrichlorethane (DDT) and polychlorinated biphenyls (PCBs) were banned in the US and Canada between 1969 and 1974, due to their detrimental effects on the environment and wildlife (Elliott and Harris 2001/2002; Elliott and Norstrom 1998). In bald eagles, these contaminants have been shown to cause eggshell thinning, reduced breeding success, behavioral defects, physical deformations and mortality (Bowerman et al. 2003; Bowerman et al. 1998; Elliott and Norstrom 1998; Gill and Elliott 2003). Although the overall contaminant levels have decreased since the 1970's, areas of high exposure such as southern California and areas of the Great Lakes region are still

experiencing these harmful effects (Bowerman et al. 2003; Elliott and Harris 2001/2002; Garcelon 1994).

In British Columbia, bald eagles have been increasing since the legislated ban of DDT and PCBs, although bald eagle numbers in some areas of B.C. have been slow to recover from high levels of contaminants, low food supply during the breeding season, and habitat loss as discussed previously (e.g., Nanaimo/Crofton area; (Elliott and Harris 2001/2002; Elliott et al. 1998). Elliott and Harris (2001/2002) showed that in B.C., the regional rate of increase in the population size of bald eagles from 1966 to 1996 was greater than 1.5% each year. By the early 1990s organochlorine levels in the Strait of Georgia were reported to be below levels associated with reproductive impairment in bald eagle eggs (Elliott and Norstrom 1998). The long term monitoring of these contaminants in bald eagles continues to provide important information on the health of bald eagles, status of the local environments, changes in contaminant levels, and provides the ability to detect new contaminants.

The primary objective of this study was to evaluate spatial and temporal trends of chlorinated hydrocarbons in bald eagle plasma in British Columbia and southern California measured in 1993 and 2003.

4.3 Methods

Study Areas, Sample Collection, and Contaminant Analysis

Blood samples were obtained from bald eagle nestlings at 6 different sites as described in Chapter 2 (see Fig. 2.1 for map). Sample analysis and results for p,p'-DDE,

PCBs, trans-nonachlor, and hexachlorobenzene are also described in Chapter 2 (Fig. 2.11, Table 2.2).

Statistics

All statistical analyses were performed using SAS v.9.1 software package (2002-2003). All of the contaminants (DDE, HCB, t-nonachlor, ΣPCBs) were non-normally distributed, therefore were log transformed ($\log_{10} + 1$) to approximate normal.

Site differences were determined using analysis of variance (ANOVA) and to determine which sites were different a Student Newman Keuls test was performed.

The relationship between contaminants and lipid metabolites was determined using linear regression models. Since no correlation was found between plasma lipids and contaminant concentration levels, the contaminants were not normalized to plasma lipid content. To compare contaminant levels in 1993 to those found in 2003, a Wilcoxon rank test was used. Unless stated otherwise, results were considered significant if $p < 0.05$.

4.4 Results

Spatial trends in contaminant levels

The spatial trends in contaminant levels collected in 2003 are fully discussed in chapter 2, therefore to avoid repetition they will not be discussed in this chapter (Fig. 2.11; Table 2.2).

The site specific changes in contaminant levels was investigated comparing levels measured in 1993 and 2003 at four of the same sites (central and lower Fraser Valley, Nanaimo/Crofton area, and Barkley Sound; Fig 4.1- 4.4). In the ten year time span, the

spatial patterns have changed very little. Only the levels of total PCBs in central Fraser Valley have significantly decreased between 1993 and 2003 (Z=2.2874, p=0.0452, df=1).

Temporal trends in contaminant levels

Overall, there was no significant difference in levels of p,p'-DDE in British Columbia (pooling all sites) comparing levels measured in 1993 vs. 2003 (Z=1.8178, p=0.0747, df=1) From Figure 4.1, the levels in 2003 appear higher then in 1993 at Barkley Sound, the Nanaimo/Crofton area, and central Fraser Valley, but lower in the lower Fraser Valley, although these differences are not significant. Levels of trans-nonachlor significantly decreased over the ten years pooling all data (Fig.4.2; Z=-2.1953, p=0.0325, df=1), but levels of HCB did not (Fig.4.3; Z=-0.3299, p=0.7428, df=1).

Concentrations of total PCBs in plasma of nestling bald eagles significantly declined between 1993 to 2003 (pooling all sites measured) in British Columbia (Z=2.1727,p=0.0343, df=1; Fig. 4.4). The largest decrease in total PCBs was noted in the central Fraser Valley (Z=2.2874, p=0.0452, df=1).

4.5 Discussion

Site specific contaminant levels

Overall, the site specific patterns of contaminant levels matched patterns of land-use development, the degree of urbanization, and the level of industrial development at each of the sites sampled. Santa Catalina Island, CA has the highest mean p,p'-DDE level, which is consistent with a known source of contamination in that area (Garcelon 1997; Lee 1994). From the 1940's to the 1970's, Los Angeles area industries discharged approximately 1,800 metric tons of p,p'-DDT and PCBs into ocean waters off the

Southern California coast (Lee 1994). Surveys completed in 1992 and 1993 by the United States Geological Survey found more than 100 metric tons of p,p'-DDT and 10 metric tons of PCBs remaining in the sediment on the ocean bottom of the Palos Verdes Shelf (Lee 1994). These levels are still environmentally significant and may affect the survival and breeding of wildlife in the area, especially bald eagles.

The Fort St. James area in northern B.C. had the lowest level of contamination due to its remote inland location and limited industry in the area. Barkley Sound had high levels of contaminants, despite being fairly remote. The high level of contamination may be due to industry in the surrounding area and/or due to oceanic currents transporting toxins from contaminated sites to this area. The Nanaimo/Crofton area showed high levels of PCBs, which is consistent with previous studies (Elliott and Harris 2001/2002; Elliott et al. 1998). The Nanaimo-Crofton area has been altered extensively by urban development, logging, and industry (such as paper and pulp mills), causing higher contaminant levels (Elliott et al. 1998). Over the last ten years, the level of contaminants measured at the B.C. sites has changed very little. The lack of significance is mostly likely due to having a small sample size. Nevertheless, all sites (including remote locations) have detectable amounts of the measured contaminants demonstrating the persistence of these chemicals and their ability to be transported long distances in the environment.

Contaminant levels in plasma of bald eagle nestlings have been monitored in other parts of North America including the Great Lakes region, and Newfoundland. The levels of p,p'-DDE found in Michigan in 1999-2002 (geometric means: 4-37 ppb; (Roe 2004) are comparable to levels found both in B.C. and southern California, but the levels

of total PCBs are higher (geometric means: 88-199 ppb; (Roe 2004). In Ohio, levels were measured between 1994-97 and for p,p'-DDE concentrations are lower (geometric means: 3-6 ppb), but for PCBs higher values are reported in Ohio (geometric means: 7-69 ppb; (Roe 2004). In Newfoundland, Dominguez et al. (2003) found the geometric means of p,p'-DDE to be between 2-9 ppb and for total PCBs to be between 10-30 ppb in the plasma of bald eagle nestlings. The levels of PCBs are comparable with this study, but the concentrations of p,p'-DDE is higher in B.C. and especially in southern California.

Temporal trends in contaminants in British Columbia

The level of total PCBs and trans-Nonachlor decreased between 1993 and 2003, but the levels of p,p'-DDE and hexachlorobenzene did not. Elliott and Harris (2001/2002) derived a critical level of p,p'-DDE and total PCBs in bald eagle plasma that was based on declines in productivity below the minimal sustainable rate. For p,p'-DDE the critical level was found to be 27.8 μg/kg and for total PCBs it was found to be 189 μg/kg. In British Columbia, some eagles in the Nanaimo/Crofton area and in Barkley Sound have exceeded this threshold, therefore may be experiencing serious negative effects due to elevated levels of p,p'-DDE. This is not the case with total PCBs. Based on the critical level derived by Elliott and Harris (2001/2002), bald eagles in B.C. have been well below the critical level for PCBs since 1993, therefore this chemical does not appear to be causing harm to the population. Critical levels for trans-Nonochlor and HCB have not yet been derived.

In conclusion, this study demonstrated the value and effectiveness of using bald eagles as indicator species to monitor ecosystem contaminant concentrations. Long term

monitoring of contaminants continues to provide important information on the health of bald eagles, status of the local environments, and changes in contaminant levels.

4.6 Figures

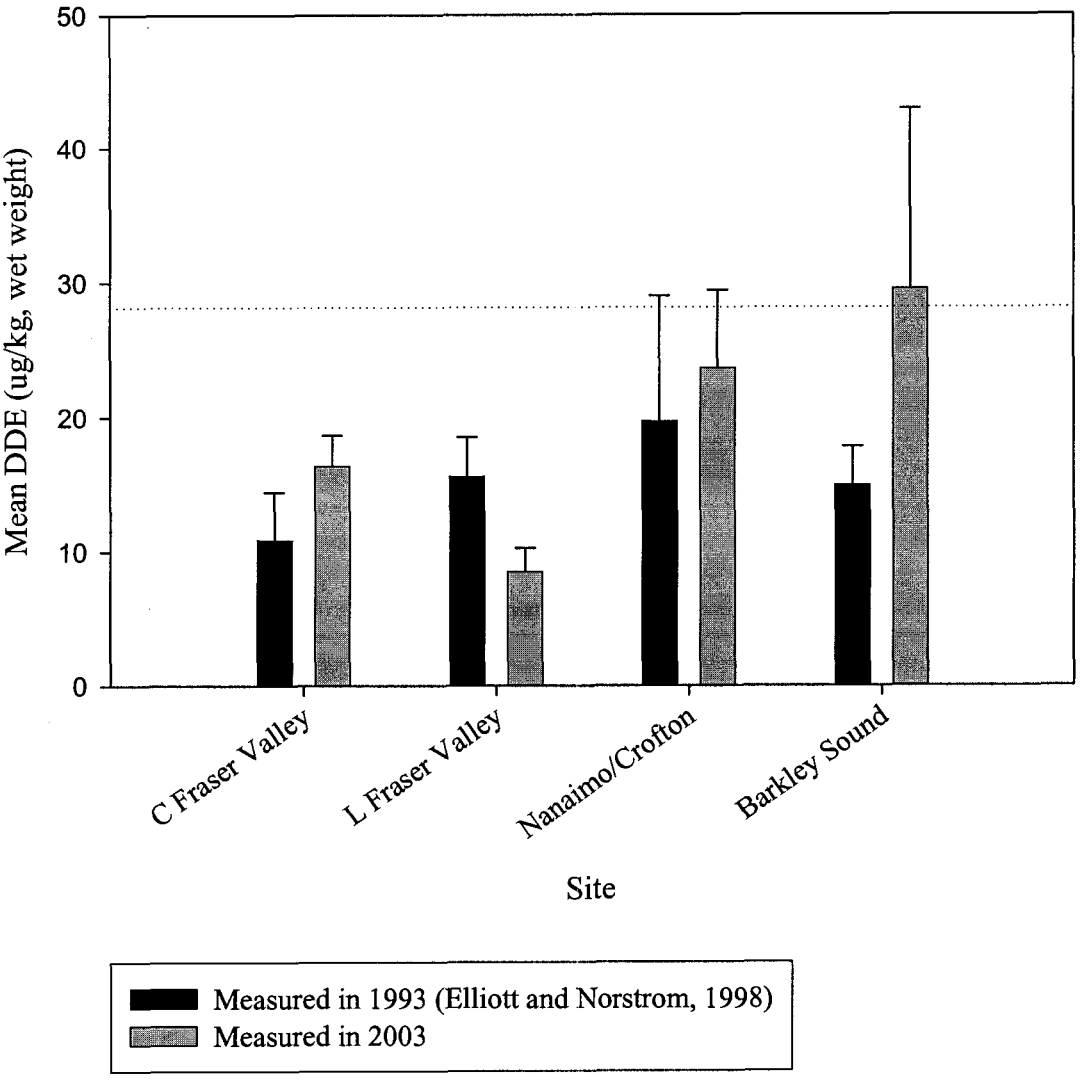

Figure 4.1 Residue levels of mean p,p'-DDE in plasma samples of bald eagle nestlings collected at sites in British Columbia in 1993 and 2003. Dotted line indicates the critical level (27.8 ug/kg) based on declines in productivity below the minimum sustainable rate (derived in Elliott and Harris 2001/2002).

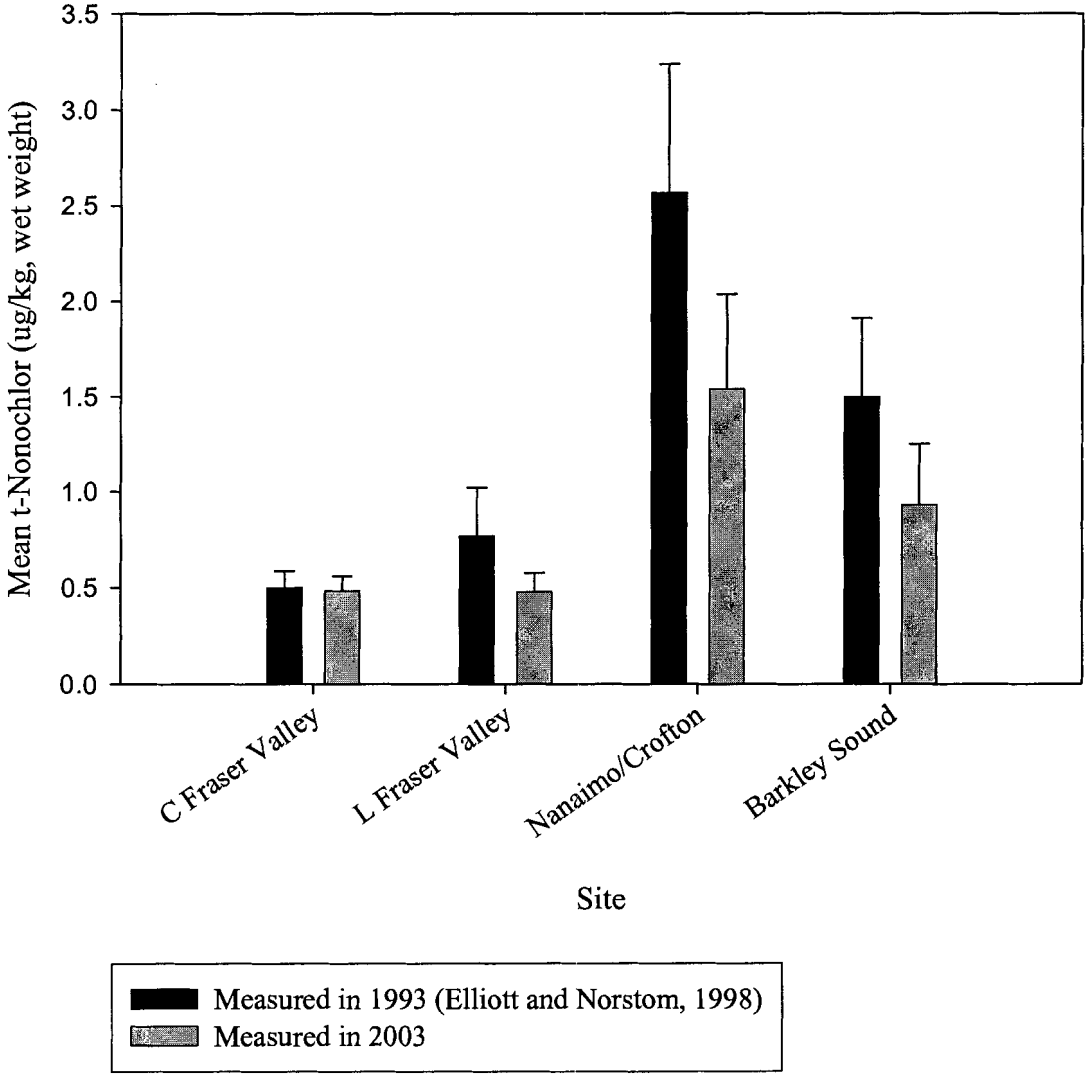

Figure 4.2 Residue levels of mean trans-Nonachlor in plasma samples of bald eagle nestlings collected at sites in British Columbia in 1993 and 2003

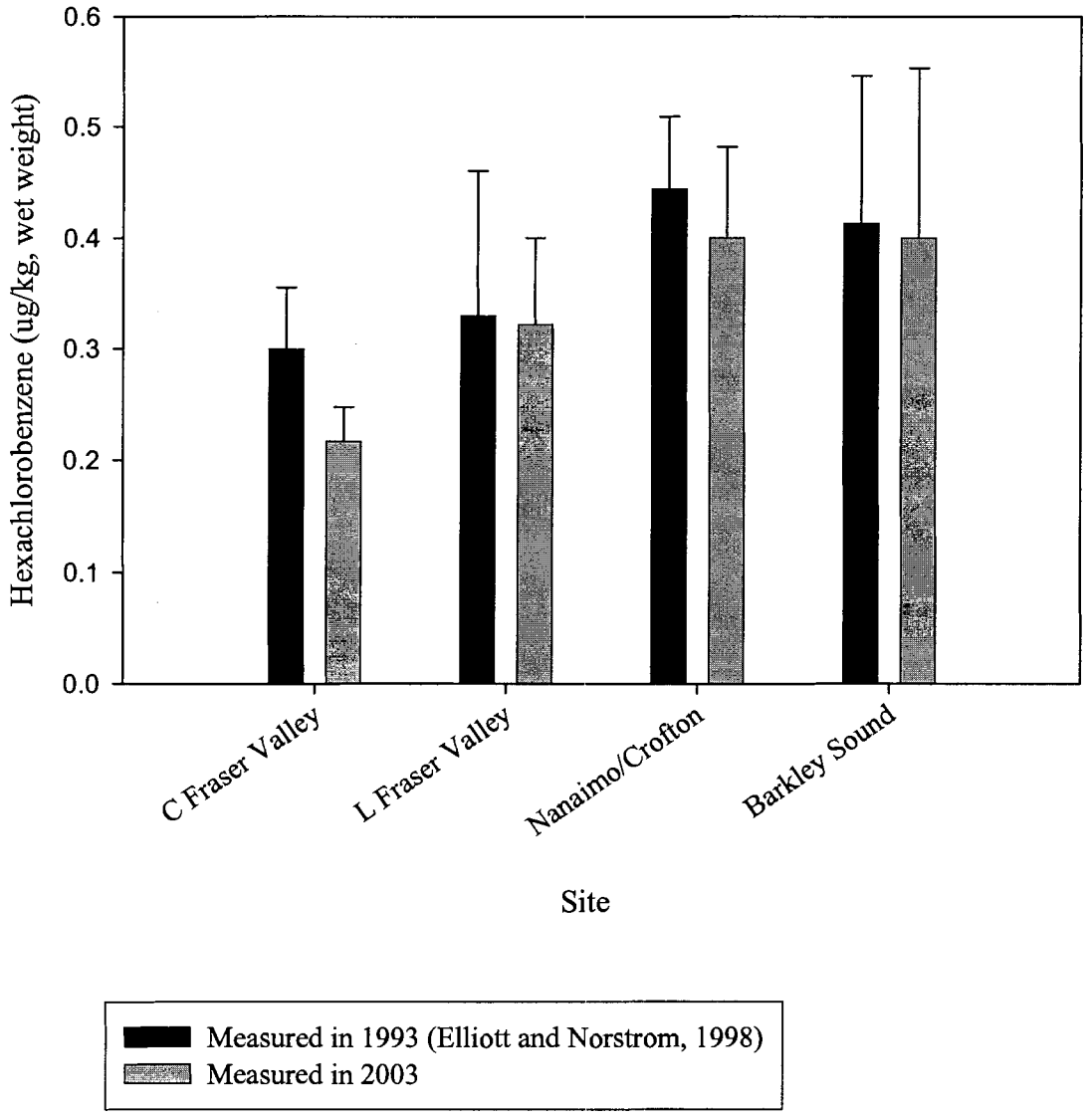

Figure 4.3 Residue levels of mean hexachlorobenzene (HCB) in plasma samples of bald eagle nestlings collected at sites in British Columbia in 1993 and 2003

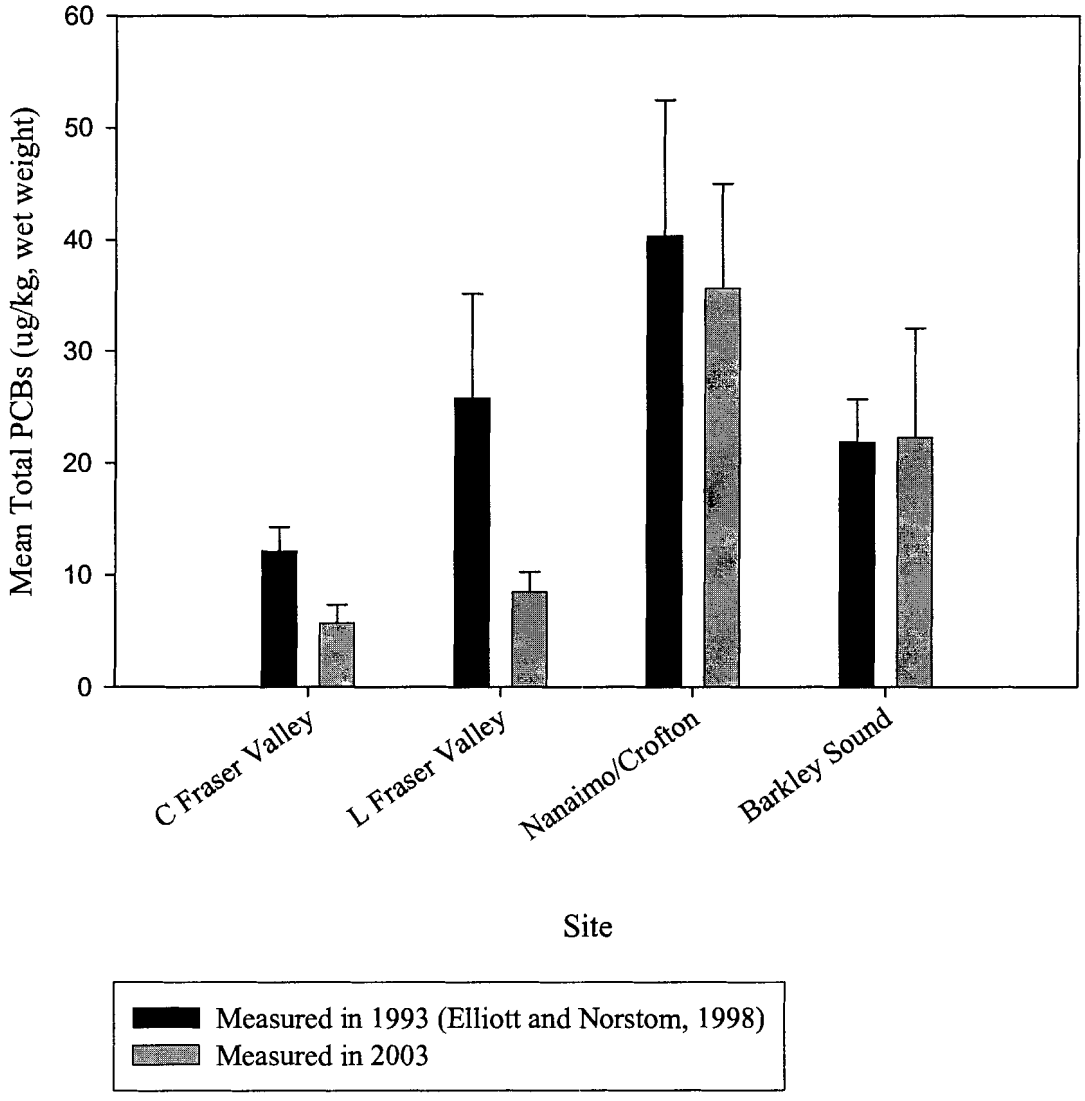

Figure 4.4 Residue levels of mean total PCBs in plasma samples of bald eagle nestlings collected at sites in British Columbia in 1993 and 2003

4.7 References

Bowerman, W. W., Best, D. A., Giesy, J. P., Shieldcastle, M. C., Meyer, M. W., Postupalsky, S., and Sikarskie, J. G. (2003). Associations between regional differences in polychlorinated biphenyls and dichlorodiphenyldichloroethylene in blood of nestling bald eagles and reproductive productivity. Environmental Toxicology and Chemistry 22, 371-376.

Bowerman, W. W., Best, D. A., Grubb, T. G., Zimmerman, G. M., and Giesy, J. P. (1998). Trends of contaminants and effects in bald eagles of the Great Lakes basin. Environmental Monitoring and Assessment 53, 197-212.

Dominguez, L., Montevecchi, W. A., Burgess, N. M., Brazil, J., and Hobson, K. A. (2003). Reproductive success, environmental contaminants, and trophic status of nestling bald eagles in eastern Newfoundland, Canada. Journal or Raptor Research 37, 209-218.

Donaldson, G. M., Shutt, J. L., and Hunter, P. (1999). Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. Archives of Environmental Contamination and Toxicology 36, 70-80.

Elliott, J. E., and Harris, M. L. (2001/2002). An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Reviews in Toxicology, 1-60.

Elliott, J. E., Moul, I. E., and Cheng, K. M. (1998). Variable reproductive success of bald eagles on the British Columbia coast. Journal of Wildlife Management 62, 518-529.

Elliott, J. E., and Norstrom, R. J. (1998). Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. Environmental Toxicology and Chemistry 17, 1142-1153.

Garcelon, D. K. (1994). Effects of organochlorine contaminants on bald eagle reproduction at Santa Catalina Island, pp. 1-16. Institute for Wildlife Studies, Arcata.

Garcelon, D. K. (1997). Effects of organochlorine contaminants on bald eagle reproduction at Santa Catalina Island. Montrose Trustee Council.

Gill, C. E., and Elliott, J. E. (2003). Influence of food supply and chlorinated hydrocarbon contaminants on breeding success of bald eagles. Ecotoxicology 12, 95-111.

Lee, H. (1994). The distribution and character of contaminated effluent-affected sediment, Palos Verdes Margin, Southern California, p. 237. U.S. Geological Survey, Menlo Park.

Roe, A. S. (2004). Spatial and temporal analyzes of environmental contaminants and trophic status of bald eagles in the Great Lakes region. In Environmental Toxicology, p. 83. Clemson University, Clemson.

CHAPTER 5
GENERAL SYNTHESIS AND CONCLUSIONS

Environmental contaminants continue to impact wildlife populations (Blus 1996; Clark et al. 1998; de Wit 2002; Elliott and Harris 2001/2002; Murk et al. 1996). Many of these contaminant are lipophilic and bioaccumulate up the food web, making top predators such as bald eagles at risk (Elliott and Harris 2001/2002). Due to their high trophic status, they have been used in some areas as indicator species for monitoring contaminants in the environment and the health of ecosystems (Anthony et al. 1993; Donaldson et al. 1999; Elliott and Norstrom 1998; Roe 2004).

In this study, we investigated using bald eagles nestlings 1) the importance of analyzing and controlling for plasma lipid levels when interpreting variation in chlorinated hydrocarbon levels; 2) the relationship between plasma contaminant levels and plasma thyroid hormone and vitamin A concentrations; and 3) spatial and temporal trends of organochlorines and polybrominated diphenyl ethers (PBDEs) in bald eagle plasma in British Columbia and southern California.

In Chapter 2, the following objectives of the study were achieved:

1. detected extensive variability of plasma lipids in bald eagle nestlings;
2. showed mass and age are not confounding factors in lipid variability, but feeding status of the birds may be an important factor;

3. showed that plasma lipids (triglycerides, non-esterfied fatty acids, and total lipids determined gravimetrically and colorimetrically) were not significantly correlated with contaminant levels in nestling bald eagles;
4. detected site differences for both p,p'-DDE and PCBs.

Future research is needed to account for feeding effects when interpreting contaminant results. This study should be done in a captive environment, controlling the food intake and performing sequential blood sampling of dosed birds.

In Chapter 3, the following objectives were achieved:

1. determined the levels of p,p'-DDE, PCBs, and PBDEs in bald eagles chicks in British Columbia and southern California;
2. detected relationships between contaminants and thyroid hormones; significant negative correlation between both p,p'-DDE and PCBs and circulating levels of thyroid hormone in nestling bald eagles was found;
3. detect relationships between contaminants and vitamin A; no negative effect on vitamin A was found.

Further research is needed on the effect of the metabolites of these chemicals, since it has been shown that the metabolites have a higher binding affinity for T4, therefore could have more of an effect.

In Chapter 4, the following objectives were achieved:

1. determine the levels of chlorinated hydrocarbons at sites in British Columbia which have been previously sampled;

2. determined spatial trends of contaminants in B.C. and southern California; Santa Catalina Island showed the highest level of p,p'DDE and Nanaimo/Crofton area had the highest level of total PCBs;
3. determine temporal trends of contaminants in B.C. between 1993 and 2003; significant decrease in total PCBs and trans-Nonachlor.

Although levels of some contaminants are decreasing, new contaminants are constantly replacing the old and increasing in the environment. It is important to continue monitoring the levels of contaminants in the environment and their effects to ensure the health of bald eagles, health of the ecosystem, and to assist in management decisions.

5.1 Reference List

Anthony, R. G., Garrett, M. G., and Schuler, C. A. (1993). Environmental contaminants in bald eagles in the Columbia River Estuary. *Journal of Wildlife Management* 57, 10-19.

Blus, L. J. (1996). *Environmental contaminants in wildlife*. CRC Press Inc.

Clark, K. E., Niles, L. J., and Stansley, W. (1998). Environmental contaminants associated with reproductive failure in bald eagles (*Haliaeetus leucocephalus*) eggs in New Jersey. *Bulletin of Environmental Contamination and Toxicology* 61, 247-254.

de Wit, C. A. (2002). An overview of brominated flame retardants in the environment. *Chemosphere* 46, 583-624.

Donaldson, G. M., Shutt, J. L., and Hunter, P. (1999). Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. *Archives of Environmental Contamination and Toxicology* 36, 70-80.

Elliott, J. E., and Harris, M. L. (2001/2002). An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. *Reviews in Toxicology*, 1-60.

Elliott, J. E., and Norstrom, R. J. (1998). Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. *Environmental Toxicology and Chemistry* 17, 1142-1153.

Murk, A. J., Boudewijn, T. J., Meininger, P. L., Bosveld, T. C., Rossaert, G., Ysebaert, T., Meire, P., and Dirksen, S. (1996). Effects of polyhalogenated aromatic hydrocarbons and related contaminants on common tern reproduction: integration of biological, biochemical, and chemical data. *Archives of Environmental Contamination and Toxicology* 31, 128-140.

Roe, A. S. (2004). Spatial and temporal analyzes of environmental contaminants and trophic status of bald eagles in the Great Lakes region. In Environmental Toxicology, p. 83. Clemson University, Clemson.