An examination of environmental policy regarding the 2008 Koi Herpesvirus (CyHV-3) outbreak in Lake Simcoe, Ontario, Canada: the disposal of *Cyprinus carpio carpio* L. on First Nation and off-reserve land

by

Kira Jade Cooper

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Environmental Studies
in
Environment and Resource Studies

Waterloo, Ontario, Canada, 2013

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.
I understand that my thesis may be made electronically available to the public.

- Kira Jade Cooper

Abstract

Koi Herpesvirus (KHV), a species-specific DNA virus of the family Herpesviridae, is responsible for mass mortalities of common carp (*Cyprinus carpio carpio* L.) throughout the world. KHV's broad geographical distribution and relatively high mortality rate among infected fish, creates significant disposal issues when die-offs occur, especially taking into account the body burden of contaminants in the fish. In locales where adequate disposal facilities are unavailable, or are unable to accommodate additional loadings of contaminated fish carcasses, concerns regarding human and environmental health are raised. During the summer of 2008, residents of the Lake Simcoe Region of southern Ontario, Canada, were faced with a massive die-off of carp, infected with KHV. Carp within the Great Lakes and much of the world are known to bioaccumulate (and biomagnify) contaminants, such as, polychlorinated biphenyls (PCBs), pesticides (e.g., dichlorodiphenyltrichloroethane, DDT, and toxic metals (e.g., mercury). These contaminants have been associated with numerous adverse effects on both human and environmental health, and are thus of important considerations when planning for large-scale carcass disposal, following fish die-offs. Although suites of microbiological tests and water quality assessments are frequently conducted to identify causative factors during extensive fish-kills - assessments of relative contaminant burdens in the carcasses, which should dictate the most appropriate method of carcass disposal - are rarely performed. A case study on Snake Island, Lake Simcoe, Ontario was conducted to further examine the implications of this policy. Soil samples from two known disposal sites and three presumed control locations were sampled on Snake Island and sent to the Analytical Services Unit of Queen's University for chemical analysis. Although none of the soil samples exceeded any legal guidelines in the present study, there is still concern as future die-offs of other fish species or piscivorous birds and the disposal of large numbers of carcasses may be an issue.

Acknowledgements

I would graciously like to extend my most sincere thanks to Professor Leonard Tsuji, Professor Stephen Murphy and Professor Ken Oakes for their guidance throughout the duration of this research and to the Chippewas of Georgina Island for supporting this study. I would like to thank the Social Science Humanities Research Council (SSHRC) and the Ministry of Natural Resources (MNR) for kindly funding this project.

I would like to recognize the following individuals and their associated organizations:

Stephen Matusiak- Canadian Food Inspection Agency (CFIA)
Edward Malek- Canadian Food Inspection Agency (CFIA)
Kim Klotins- Canadian Food Inspection Agency (CFIA)

Shane deSolla- Environment Canada

Kevin Joynes- Ontario Ministry of Agriculture Forestry and Rural Affairs (OMAFRA)

Wil Wegman- Ontario Ministry of Natural Resources (OMNR)

Professor John Lumsden- Guelph University

Claire Malcomson- Environmental Defense

Jeff J. Andersen- Lake Simcoe Region Conservation Authority (LSRCA)

Professor Toshifumi Minamoto- Kobe University (Japan)

Chris Walster- The Island Veterinary Associates Ltd (United Kingdom)

Mark Newell- Sir Sandford Fleming College

Ontario Ministry of the Environment- Ontario Sport Fish Monitoring Program

Chippewas of Georgina Island Band Members: Sylvia McCue, Mike McCue

Snake Island cottagers: Peter and Åse Petersen, Tom and Mette Cooper, Peter Morrison

Fieldwork assistance provided by: Sean Cooper (York University)

Cartographic assistance provided by: Harold Morrison

Editing assistance provided by: Jeff Samuel

Laboratory assistance provided by: Anne Grant, University of Waterloo- Ecology Lab

Photo credits: Kira Cooper, Claire Malcomson, Wil Wegman

Dedication

Dedicated to the Lake Simcoe carp…

The most hideous and yet beautiful creature I have come to love, even in spite of your herpes…

Carpe diem!

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1.0 Introduction

The ecological integrity of aquatic ecosystems throughout much of the world is being degraded by human activities, such as, land development, habitat destruction and pollution associated with urbanization (Fraser et al., 2003). In Canada, such anthropogenic activities in the Great Lakes region threaten the ecological integrity of these freshwater ecosystems. In Ontario, smaller water bodies like Lake Simcoe have multiple stressors simultaneously imposed on them, such as, surface water runoff from intensive agriculture, urbanization and land development; all of which are known to transport contaminants (e.g., persistent organic pollutants, POPs) to the aquatic environment (Ministry of the Environment, 2009). Historically, great concern at the international, national and local levels about both the distribution of POPs in the environment and the associated health effects for exposed biota has been noted; as POPs are known to bioaccumulate and bioconcentrate in exposed biota. However, little work has been done with respect to how contaminants in fish tissue can be transported from the aquatic ecosystem to the terrestrial ecosystem following mass die-offs and subsequent carcass disposal.

In 2008, more than fifteen different water bodies between Peterborough, Lake Simcoe and Simcoe County (MNR, 2009) were faced with a die-off of common carp as a result of the species-specific Koi Herpesvirus (KHV) (also known as carp nephritis and gill necrosis virus, CNGV) (Perelberg et al., 2005) and cyprinid herpes virus-3 (CyHV-3)) (Minamoto et al., 2009). Although it remains unclear exactly how many carp died, conservative estimates of carcass volume range upwards of 13,000 fish (Garver et al., 2010: 1244), and this estimate does not include the carcasses which were disposed of on private property or were left to decompose *in situ*. The result was that approximately 110 tonnes of carp were disposed of in waste management facilities throughout the region (MNR, 2009), which raised numerous concerns regarding the removal and

disposal of large volumes of fish over a relatively short period of time. Especially taking into account that the 2008 die-off followed a 2007 fish die-off in the Kawartha Lakes region of Ontario resulting in the disposal of 12,000-24,000 carp (MNR, April 2008; 1).

Following the mass mortality of *C. carpio* in 2008, the Ontario Ministry of Natural Resources (MNR) advised residents to double-bag the carcasses for roadside pickup, so that carcasses could be disposed of as waste in municipal landfills (MNR, July 2008). The MNR advisories stated that as an alternative to waiting for municipal pickup to landfill the fish, the carcasses could be buried on a landowner's property at a minimum depth of two feet (and also requested that people do not flush the fish down the toilet!) (MNR, May 2008). The MNR advisory clearly stated that "the virus does not affect humans" and that "carp infected with KHV are safe to eat and handle" (MNR, May 2008).

It is well documented by various Ontario government agencies that Ontario carp contain elevated levels of contaminants. The Ontario Ministry of the Environment (MOE) in association with the MNR have routinely published fish consumption guidebooks for Ontario anglers to minimize human exposure to contaminants through consumption of fish caught in Ontario. These advisories have been established in accordance with Health Canada's tolerable daily intake (TDI) for contaminants (Gilbertson and Carpenter, 2004). The "Guide to Eating Ontario Sport Fish" has consistently published advisories for carp from the Lake Simcoe watershed based on contaminants, such as, mercury, PCBs, and pesticides (MOE, 2009; 103). Such restrictive consumption advisories for carp in Lake Simcoe are fairly representative of those for many other lakes across Ontario, and beyond (Haenen et al., 2004; Matsui et al., 2008; MOE, 2009).

Consumption advisories for carp become more stringent as fish length increases, reflecting the bioconcentration potential of a relatively long-lived, benthivorous omnivore. In some Ontario lakes, carp consumption recommendations are restricted to very small, relatively young individuals, if consumption of carp of any size are not completely advised against (MOE, 2009; 106). Considering these Ontario agencies were aware (or should have been aware) that carp bioconcentrate contaminants, as indicated in their own government publications, the suggestion that carp infected with KHV were safe for human consumption or disposal on public property appears, at least in some cases, to be exercising insufficient precautions to protect human health and the environment. Acknowledging that human consumption of contaminated fish tissue is of great importance – this topic is beyond the scope of my thesis - my study will focus primarily on the toxicological considerations with respect to safe disposal of carp carcasses after die-offs.

1.1 Background

KHV outbreaks, such as the 2008 episode in Lake Simcoe, are not confined to Canadian waterways; these types of outbreaks are becoming a global phenomenon with occurrences being reported throughout Europe, South Africa, the United States and much of Asia (Haenen et al., 2004; Matsui et al., 2008). Presently, KHV has spread throughout much of the world and as a result, it has been recognized as one of the most significant pathogens to affect common carp and koi on a global scale (Maya et al., 2010; Michel et al., 2010a; Dong et al., 2011). KHV has significantly reduced both wild and farmed carp populations in numerous countries (Matsui et al., 2008; Uchii et al., 2011). Given the nature of this fish as a significant source of food for the human population, particularly within Asian regions, and its widespread distribution in freshwater ecosystems around the globe, the epidemic potential of this virus is a direct concern for

the financial stability, food security and ecological integrity of freshwater ecosystems throughout much of the world.

In aquatic ecosystems, organisms are exposed to contaminants through water, food and sediment. The bioaccumulation of contaminants from water compounded with the bioconcentration of these substances through trophic amplification leads to an increase of contaminants in aquatic species. In many cases, age is considered an important factor for the accumulation of contaminants, especially for mercury and POPs (McIntyre and Beauchamp, 2007; 571). Bioaccumulation occurs when the total uptake of contaminants exceeds the rate that the contaminants are secreted or excreted from the body (572); over time, contaminant body burdens build up, resulting in higher concentrations in older individuals. Biomagnification, a term used to describe the increase in contaminant concentration in a species from the consumption of another organism (Gobas and Morrison, 2000; McIntyre and Beauchamp, 2007; 572), is an important consideration for the movement of contaminants in aquatic ecosystems, particularly with carp, which can scavenge decaying animal material already highly contaminated. Generally, the longer the food chain, and higher the fat content of the species, the rate of biomagnification increases (Rasmussen et al., 1990; Bentzen et al., 1996; Guildford et al., 2008).

It has been well documented that fish in Canadian waters harbor various legacy contaminants (e.g., POPs) associated with adverse human health outcomes. Fish from the Great Lakes and their tributaries frequently have relatively high body burdens of organic and inorganic contaminants (Gewurtz et al., 2009). These compounds generally enter waterways via industrial effluents, sewage runoff, inputs from agricultural practices (Giesy et al., 1997), combustion emissions, historical electric equipment, landfill sites, as well as numerous other point and non-point sources of pollution (Gewurtz et al., 2009). Carp are arguably one of the most contaminated (e.g., PCBs) freshwater fish species, due to a benthivorous and omnivorous diet (Moermond et al.,

2004). Thus, the handling of large numbers of this species *en mass* during die-offs raises practical concerns pertaining to human and environmental health.

The exact concentration of contaminants in fish from the Lake Simcoe region that have been disposed of in landfills, private and public property and on the First Nations Land of the Chippewas of Georgina Island remains unknown. However, using toxicological data provided by the MOE, it is possible to calculate relative contaminant loadings to estimate the relative risk associated with this disposal approach for a given site. A case study was conducted on Snake Island in Lake Simcoe, Ontario (land of the Chippewas of Georgina Island) to determine whether sites used to dispose carp during the 2008 die-off contained detectable levels of mercury (inorganic and organic), pesticides (e.g., DDT) and other hazardous substances known to accumulate in fish tissue (PCBs).

2.0 Literature Review

2.1 Global significance of carp fish

2.1.1 Carp ecology

The general vernacular "common carp" refers to a culmination of three separate kinds of wild carp, which originate from: Europe, East Asia (primarily Siberia) and Central Asia (Balon, 1995; 15). Carp husbandry is not a novel phenomenon, but rather it has been a cultural tradition in eastern nations, particularly China for the past 2000 years (Balon, 1995; 8).

Part of the reason why carp have been so successful at establishing themselves in new environments throughout the world is their highly prolific reproduction and rapid growth. Carp produce upwards of two million eggs per year, which hatch in 3 to 6 days (RBG, 1998; 3). Hatchlings can grow to a maximum size of 120 cm and live up to 40 years of age (RBG Fish Fact Sheet 2001; 2). In optimal growing conditions (water temperatures between 23°C- 30°C, this species can grow 2 to 4 percent of their body weight per day (FAO, 2012). A very hardy species (Johnson, 1954; Mark, 1966; Balon, 1995; 27), carp can endure, among other things, low-oxygen levels (0.3-0.5 mg/L), temperature fluctuations, and waters with very high concentrations of saline (5%) (FAO, 2012; 2). Carp can also be transported outside of water in damp moss and can survive long periods of starvation (Balon, 1974; Balon, 1995; 27).

Carp are benthivores and eat a variety of foods found primarily in sediment. As carp forage, they uproot plants searching for benthic prey, increasing water turbidity, while releasing dissolved nutrients such as nitrates and phosphates (Hoffman, 1995; 74). In the process, carp are exposed to hydrophobic contaminants, such as, PCBs, mercury

and pesticides, which adsorb to sediment and biomagnify in the fish as they are consumed. The preferred spawning grounds for the Lake Simcoe carp are located in the Holland Marsh, Cooks Bay, and in the weedy bays and slow rivers during May and June when the water starts to warm (MacCrimmon and Skobe, 1970; 72). The Holland Marsh for example acts as a reservoir of contaminants (e.g., DDT), as chemicals have been historically applied as pesticides or chemical fertilizers.

2.2 History of carp in the Laurentian Great Lakes

The ecological stability of aquatic ecosystems is greatly influenced and often threatened with the introduction of invasive species. The majority of exotic species in the Laurentian Great Lakes region were introduced during early European settlement in the 17th century (Mills et al., 1993; 2). A resident of Orange County, New York was the first to introduce the common carp to North America in 1831. This resident had imported the fish (which was local to Eurasia) from France with the intention of propagating this species in his private ponds (DeKay 1842; Mills et al., 1993). Later, it was noted that carp were being deliberately released into the Hudson River where they were being caught by anglers. By 1879, several years after it was first introduced in private ponds, the United States Fish Commission released carp fry imported from Europe into the Great Lakes (Mills et al., 1993).

During the 1880s, common carp were released in the Great Lakes as part of government stocking programs as well as by the deliberate release by local citizens (Emery, 1985; Mills et al., 1993; 3). It was the intention of the government and the local population to stock fish such as common carp in this water body, in order to establish a successful fishing industry which would compensate for the decline of the east coast fisheries (Emery, 1985; Mills et al., 1993; 42). Carp were officially introduced to Canada in 1881,

as part of a Lake Ontario hatchery project that aimed to produce inexpensive fish for consumption (McCrimmon, 1968; RBG, 1998; 3). By the 1890s, this plan backfired and carp quickly became considered a nuisance species because of their highly destructive habits, which threatened the success of more desired fish species (Krueger and May, 1991; Mills et al., 1993; 42).

2.3 History of carp in Lake Simcoe

By the 19th century, millponds in the Holland River (at the south end of Lake Simcoe) were stocking carp that had been imported from Europe. The first carp believed to have entered Lake Simcoe occurred in 1896, when a Dam broke at Dyke's Pond near Newmarket, Ontario (MacCrimmon and Skobe, 1970; 69). Lake Simcoe carp were noted for growing rapidly which developed a demand for carp as a food fish following their first introduction (MacCrimmon and Skobe, 1970; 69).

Shortly thereafter in the early 1900s, the destructive nature of the carp became rather well known and this once desired species was no longer revered (MacCrimmon and Skobe, 1970; 69). By 1902, approximately six years after carp entered Simcoe, the spawning beds of the Maskinonge River were destroyed forcing many native fish species to move further up the Holland River to spawn. The loss of the wild rice beds, which had supported an array of waterfowl were almost completely destroyed, as a result of the introduction of carp (MacCrimmon and Skobe, 1970; 69).

In 1909, the first commercial carp seine license was issued and by 1911, 462,406 lbs of fish was harvested (MacCrimmon and Skobe, 1970; 70). Around this time, the mirror carp, a genetically similar variation of the common carp was in demand for a short time. By 1912, the annual catch of carp decreased significantly to 123, 871 lbs (MacCrimmon

and Skobe, 1970; 70). This downward trend went as low as 84,452 pounds taken in 1918 - and peaked again in 1923 with a reported catch of 347,409 lbs - and then proceeded again to decline (MacCrimmon and Skobe, 1970; 71).

In 1928, a significant die-off of carp occurred requiring local authorities to remove dead fish from the shoreline (MacCrimmon and Skobe, 1970; 71). Although the cause of the die-off was never officially determined, it was reported that not all carp were killed and fishing reports in 1928 and 1938 accounted for a catch of approximately 50,000 lbs of carp (MacCrimmon and Skobe, 1970; 72). Between 1910 and 1969, the commercial carp fishery in Lake Simcoe documented a total catch of 4,000,000 lbs (MacCrimmon and Skobe, 1970; 71). The amount of fish harvested outside of the monitored fishery remains unknown. Presently, it remains unknown as to what triggered the die-off and how the 50,000 lbs of dead fish was disposed.

2.4 Global importance of carp

2.4.1 Carp in the aquaculture, food fish, trade industry

Cyprinus carpio (L.) otherwise known as common carp, have become dispersed into many freshwater ecosystems around the world (Rahel, 2007; Matsui et al., 2008). Native to Eastern Europe and Central Asia, these non-indigenous North American fish have entered many waterways in Ontario, including the Great Lakes, where they were deliberately released in 1879 (Mills et al., 1993) and later detected in Lake Simcoe in 1896 (Ministry of the Environment, 2009; 56). Known for their adaptability to new and often poor environments, Cyprinid fish (Cyprinidae family) such as carp, koi and goldfish exhibit a high tolerance level for changes in the water temperature as well as oxygen deprivation (Williams et al., 2008); therefore, they are able to successfully become established in foreign and often degraded environments.

Globally, aquaculture is the fastest growing source of animal protein producing upwards of 52.5 million tonnes per annum (FAO, 2010; Murray, 2012 in press) Carp are an important source of revenue in the world economy, representing approximately fourteen percent of the global freshwater fish production as of 2002 (FAO, 2012). In 2004, estimates suggest that upwards of 3.3 million tons of carp were produced globally, with an annual increase of upwards of 10 percent per year (http://fao.org; Matsui et al., 2008). Because carp are farmed in numerous countries in large quantities, it is of particular economic value to understand the diseases which target this species as well as other related toxicological issues (Williams et al., 2008) that could pose threats to this industry. Because of the virulence of this virus, outbreaks of KHV are extremely detrimental to not only fish husbandry and related industries, but also to human and environmental health if carcasses are not appropriately disposed of.

2.4.2 Current threats to carp species and

Due to the extensive carp production and domestication worldwide, wild populations of common carp are thought to be either extinct or critically endangered (Balon, 1995; 3). Feral progeny from cultured carp have spread throughout much of the world and though they are may not be genetically identical to their historical lineage, they have still managed to rapidly colonize expanses of freshwater ecosystems. Due to the extremely high reproductive capacity of carp, they are recognized as "one of the most prolific freshwater fishes" (MacCrimmon and Skobe, 1970; 72).

2.5 Contaminant uptake in aquatic ecosystems

2.5.1 Contaminant uptake in aquatic ecosystems

Toxins have become integrated in global aquatic ecosystems through various processes such as atmospheric deposition, point sources of pollution, agricultural and road runoff (Allan, 1986; Canadian Council of Ministers of the Environment, 1995). The implications of exposure to various sediment-bound toxins has been documented and analyzed at both acute and chronic exposure levels in both laboratory (Thomas et al. 1986; Kosalwat and Knight 1987; Dawson et al. 1988; Long and Morgan 1990; Burton 1991, 1992; Burton et al. 1992; Lamberson et al. 1992; Canadian Council of Ministers of the Environment, 1995) and field studies (Canadian Council of Ministers of the Environment, 1999) with focuses on various benthic species (plants and invertebrates) as well as benthivores.

The bioaccumulation and biomagnification of sediment-bound contaminants in aquatic taxa has been well researched (Foster et al. 1987; Knezovich et al. 1987; Canadian Council of Ministers of the Environment, 1995: 5). Results suggest that sediments, as eloquently described by the Canadian Council of Ministers of the Environment (1995) "represent potentially significant hazards to the health of aquatic organisms and to the overall health of aquatic ecosystems". Thus, the Canadian Council of Ministers of the Environment advocate the use of sediment quality guidelines (SQGs) to assess associated risk factors in various aquatic systems to protect organisms from exposure to such contaminants. Currently, there lacks adequate scientific literature to support SQG's for all of Canada (Canadian Council of Ministers of the Environment, 1995), but they are being used in areas such as the Great Lakes to compare the historical contaminant concentrations to current values (CCME, 1999; Marvin et al., 2003).

2.5.2 Contaminant uptake in carp

Carp are omnivorous fish that consume aquatic vegetation, decaying matter and benthic prey (Crivelli, 1981; Garcia-Berthou, 2001; Matsui et al., 2008). They use their jaws as shovels to dig into substrate and expose food detected by their sensory barbells, which they swallow whole and pass through the tooth-like structure in the back of their throat (Alderton, 2005). According to Moermond et al (2004) carp accumulate PCBs typically in the following three ways: "uptake through invertebrate food, uptake from fast-desorbing fractions in ingested sediments and uptake from water". When carp feed, they both displace sediment (Scheffer et al., 2003, Moermond et al., 2004) and ingest sediment (Michelsen et al., 1994; Tolonen et al., 2000; Moermond et al., 2004), which can be approximated using the following contaminant uptake model developed by Moermond et al. (2004):

$$\frac{dC_{fish}}{dt} = k_{abs,in}C_w + k_{ass1,in}C_{food} + k_{ass2,in}C_{sedfast} -$$

C_w - dissolved concentration of PCBs in water (µg/kg)
C_{fish} - concentration of PCBs in fish (µg/kg)
C_{food}- concentration of PCBs in invertebrate food (µg/kg)
$C_{sedfast}$ - concentration of fast-desorbing PCB concentration in sediment (µg/kg)

$k_{abs,in}$ (absorption rate constant from water (µg/kg^{-1} wet wt)/ (µg/kg x L^{-1} x d))
$k_{ass1,in}$ (assimilation rate from invertebrate food (µg X kg-1 wet wt / (µg x kg-1 x L^{-1} x d))
$k_{ass2,in}$ (assimilation rate from sediment (µg X kg^{-1} wet wt / (µg x kg^{-1} x L^{-1} x d) (which is dependent on food ingestion rate king (kg x kg -1 x d^{-1})
$k_{excr,out}$ (excretion rate into water (d^{-1}))
$k_{eges,out}$ (ingestion rate with food (d^{-1}))
$k_{grdil,out}$ (growth dilution rate constant (d^{-1}))

(Moermond et al., 2004).

Much of the current technology and methods used to monitor contaminants in fish do not consider the ingestion of PCB-adsorbed sediment as a way of accumulating this toxin (Thomann et al., 1992; Hendricks et al., 2001; Moermond et al., 2004) and therefore many models are not effective in determining contaminants in benthivorous fish (Moermond et al., 2004). Great Lakes tributaries outside of Canadian jurisdictions, such as the Saginaw River in Michigan, indicate a similar presence of synthetic halogenated hydrocarbons (including PCBs, polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs)), which pose threats to both humans and wildlife (Allan et al., 1991; Brandon et al., 1991; Giesy et al., 1997).

Currently PCBs and tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) cause the majority of the dietary restrictions of fish from the Great Lakes (Sonzogni and Swain, 1984; Maxim and Harrington, 1984; Sonzogni et al., 1991; Williams et al., 1992; Gilbertson, 1992; Giesy et al., 1997). Due to the high concentrations of POPs and heavy metals, the Saginaw River and Saginaw Bay have been categorized as areas of concern in the Great Lakes by the International Joint Commission (International Joint Commission, 1993; Giesy et al., 1997). Similar cases have occurred in places like the Buffalo River, New York, where PCB isomers and other contaminants were found in amounts which exceeded the United States Food and Drug Administration's guidelines for fish consumption (Loganathan et al., 1995). Advisories as such, indicate that PCB-contaminated carp and other fish species are not restricted to the Lake Simcoe watershed, but are also national and international concerns. Viruses such as KHV, that are responsible for mass mortalities of carp around the world, raise the issue of proper means of disposal for contaminated fish and other biota.

2.5.3 Biological vectoring of contaminants in salmon

Semelparous and anadromous species such as salmon are more recently gaining attention as biovectors of persistent bioaccumulative contaminants (Blais et al., 2007; Baker et al., 2009). Salmon carcasses are integral to the nutrient balance in many freshwater ecosystems (Brickell and Goerging, 1970; Bilby et al., 1996; Lyle and Elliott; 1998; O'Toole et al., 2006). Nonetheless, as seen in the case of sockeye salmon (*Oncorhynchus nerka)* on the west coast of Alaska for example, fish carcasses are contributing to contaminant loading in the environment (Krümmel et al., 2003). These salmon are bioaccumulating contaminants throughout their lifecycle - and releasing them into the environment as their bodies decompose following spawning activities – resulting in the transportation of contaminants from the Pacific Ocean to freshwater systems in Alaska, (Krümmel et al., 2003; O'Toole et al., 2006; 102).

In the ocean, where PCB concentration is less than 1 ng/L (Fisk et al., 2001; Iwata et al., 1993; Krümmel et al., 2003), sockeye salmon are able to biomagnify PCBs to levels reaching 2,500 ng/g of lipid content (Schultz-Bull et al., 1998; Krümmel et al., 2003). Krümmel et al (2003) estimate that a spawning run of approximately one million Chinook salmon would lead to an increased PCB loading of 0.16 kg to the receptive freshwater ecosystem in which they will die (Krümmel et al., 2003; 255).

Research conducted in the Credit River, a tributary of Lake Ontario, by O'Toole et al., (2006) raised similar concerns regarding the addition of salmon carcasses as ecosystem amendments because of the high contaminant levels Chinook Salmon are known to mobilize from Lake Ontario and transport to spawning grounds following their death (O'Toole et al., 2006; 111). O'Toole et al (2006) have estimated that an approximate 75g of total PCBs and 35g of total DDT compounds to enter the Credit River occur as a

result of Chinook Salmon tissue decay after spawning based on recent size and contaminant trends (O'Toole et al., 2006; 111).

The implications of the bulk transfer of contaminants from salmon through oceanic to freshwater ecosystems can be further assessed by examining predatory species such as grizzly bears (*Ursus arctos horribilis* O.) and orcas/killer whales (*Orcinus Orcas* L.). Killer whales from the west coast of Canada, which almost exclusively consume salmon, have been noted for containing PCB concentrations of 146,300 ng/g (Ross et al., 2000; Krümmel et al., 2005; 7020).

2.6 Contaminants in the Laurentian Great Lakes and Lake Simcoe fish

Toxic equivalent (TEQ) quantification methods have been used for risk assessments and for developing regulations that indicate the relative risk for exposure to these compounds in relation to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (WHO, 2000), the most toxic of these contaminants (Bhavsar et al., 2008). TEQ is limited in its risk assessment because it does not take into account the differences in the toxicokinetics of each of the congeners (WHO, 2000). The WHO has modified the TEQ assessment called WHO-TEF (van den Berg et al., 1998; van den Berg et al., 2006; Bhavsar et al., 2008), which takes into consideration the toxic equivalent factor using relative potencies (REP), which are more accurate for risk assessments (Finley et al., 2003; Bhavsar et al., 2008).

2.6.1 Fish consumption advisories

Fish advisories in Ontario have been established for many of the persistent organic pollutants listed under the Stockholm Convention including: PCBs, pesticides (DDT, Toxaphene, Mirex), PCDDs, PCDFs (Stockholm Convention on Persistent Organic

Pollutants, 2008). In order to protect the health of fish eating individuals, a "Guide to Eating Ontario Sport Fish" is published every two years as part of a partnership with the Ministry of the Environment and Ministry of Natural Resources as reference for guide for fish contaminant levels and suggested dietary intake.

Aside from the Laurentian Great Lakes, Lake Simcoe is the largest inland lake in Southern Ontario with an angling industry that generates upwards of $100 million dollars per year (Gewurtz et al., 2011). Carp consumption advisories in this area are issued for the following contaminants: Mercury, PCBs, mirex/photomirex and pesticides, dioxins, furans and dioxin-like PCBs (MOE, 2012; 4).

2.6.2 Mercury (Hg)

Mercury is a highly toxic metal naturally released from environmental sources, such as, geological features, forest fires, oceans and volcanoes (CCME, 2003). Anthropogenic activities associated with the release of mercury include the burning of coal, waste incineration and manufacturing (ATSDR, 2011). While there are many forms of mercury, methylmercury (MeHg) is the most bioaccumulative and toxic (Wang et al., 2004). Exposure to mercury via the consumption of contaminated fish has been associated with numerous adverse health effects including neurotoxicity (Gilbertson and Carpenter, 2004; 240). Fish accumulate mercury primarily from their diet (Trudel et al., 2000 and Madenjian, Keir, Whittle, 2011) and through their gills via uptake of water (Madenjian et al., 2011).

Methylmercury (MeHg) is created through the process of mercury methylation. As a highly bioaccumulative contaminant that readily biomagnifies through food chains, MeHg typically enters organisms via their diet (Hall et al., 1997; Fowlie, Hodson and Hickey, 2008) and can be found typically, in the highest concentrations in larger and

older piscivorous species (Simoneau et al., 2005; Fowlie, Hodson and Hickey, 2008). Although Hg accumulates in aquatic sediments, sediment concentrations are low enough that few socio-ecological risks are associated with direct exposure to this matrix (Fowlie et al., 2008; 84). Pollutant levels in fish, according to Fowlie et al., (2008), more accurately represent the bioavailability of Hg (84) and therefore are more reliable indicators of Hg contamination in aquatic environments. Seasonal fluctuations of Hg concentrations in aquatic environments are important to consider when monitoring fish for contaminant guidelines (Fowlie et al., 2008; 84). Minamata disease and other "environmentally induced diseases" (Gilbertson and Carpenter, 2004; 242) further stress the need to understand chronic low-dose exposure to toxicants such as mercury.

2.6.3 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls are a group of contaminants potentially comprised of 209 congeners that were originally produced in the United States, Germany, France, the United Kingdom, Japan, Spain and Italy (Berger et al., 2001). Presently, North Korea and Russia are still producing these compounds, which were banned in the United States in 1977 out of fear that these chemicals posed a threat to human health (Berger et al., 2001). According to the World Health Organization (WHO), PCBs have been designated as contaminants "in almost every component of the global ecosystem" (WHO, 1993: p.149; Berger et al., 2001). PCBs are lipophilic contaminants that are readily absorbed (EPA, 1999: EPA-823-F-99-019) through the skin, lungs and gastrointestinal system (Tsuji et al., 2005). Because of the chemical stability of these compounds, PCBs bioaccumulate and persist both in biota and in the environment (Berger et al., 2001).

PCBs enter waterways primarily by atmospheric deposition from up to 1609 km from the original source of pollution (Eisenreich et al., 1981; Suchan et al., 2004). Once

dispersed, PCBs accumulate in fat tissues of biota as they have low solubility in water (EPA, 1999; EPA-823-F-99-019) and many of these contaminants have been associated with detrimental human health outcomes (Suchan et al., 2004). The primary manner by which humans are exposed to PCBs is via diet, particularly the consumption of fish, fish products and locally harvested game (EPA, 1999; EPA-823-F-99-019).

It is thought that the introduction of PCBs into the Great Lakes watershed began in the 1930s (Tanabe, 1988; Bhavsar et al., 2007), but were not detected until 1968 (Veith, 1968; Li et al., 2009). Although the amount of PCBs entering the Great Lakes from point sources and atmospheric deposition has been reduced over the past decades, the amount of legacy PCBs persisting in the sediments is considered a significant source of contamination (Li et al., 2009). The accumulation, transportation, and reduction of previously deposited PCBs in sediments of the Great Lakes have been thoroughly underrepresented in academic literature (Li et al., 2009).

Although PCBs are no longer in production in most countries, there is still widespread legacy contamination in sediments and biota (WHO, 2000). The WHO (2000, p.1) states that "the ability of PCBs to co-distil, volatize from landfills into the atmosphere (adsorption to aerosols with a particle size of <0.05-20 μm), and resist degradation at low incinerating temperatures, makes atmospheric transport the primary mode of global distribution" (WHO, 2000, 1). PCBs have been widely employed as dielectrics and heat exchange fluids because they are very versatile in many electrical and industrial applications. Their versatility comes from the fact that they are relatively fire resistant, conduct little electricity, have high thermal conductivity, are resistant to degradation caused by heat stressors and have low water solubility (WHO, 2000).

Between 1970-1980, the input of PCBs in the Great Lakes declined rapidly (Baumann and Whittle, 1988; Jeremiason et al., 1994; Swackhammer, 2005; Bhavsar et al., 2007),

with very little detectable input in the 1990s (De Vault et al., 1996; Hickey et al., 2006; Bhavsar et al., 2007). Research conducted by Bhavsar et al. (2007) indicates that although the current PCB content of the Great Lakes is significantly less than it has been historically, there is still a potential health risk (Bhavsar et al., 2007). Research conducted by Li et al. (2009), indicates that the accumulation and input of PCBs annually in the sediments of the Great Lakes is approximately 30% less than it was in the 1980s, which is a decrease from 420 tons (Eisenreich, 1987, Li et al., 2009) to the current 300 ± 50 tons (Li et al., 2009).

Li et al. (2009) order the Great Lakes commencing with the lake with the highest inputs as follows: Lake Erie → Lake Ontario → Lake Michigan → Lake Huron → Lake Superior. Lake Ontario has shown the most obvious decrease since the 1980s. It is assumed that the decrease in Lake Ontario has occurred as a result of "gradient-induced upward and downward diffusions through the sediment column, as well as *in situ* PCB degradation" (Li et al., 2009; 142). The lateral movement of contaminants in lakes is assumed to be consistent with the movement and suspension of sediments (Li et al., 2009). It is believed that the congener distribution throughout the sediment is most likely a result of the changes in both sales and production of the chemical (Li et al., 2009). Other factors influencing the gradual breakdown and dispersal of the most frequently persistent PCB congeners is anaerobic reductive dechlorination which occurs when microbial dehalorespiration is initiated by the aggregation of large quantities of halogenated compounds (Li et al., 2009; 145).

In order to quantify the amount of PCBs present in sediment and approximate the PCB inventory or total amount per determined unit area, sediment coring as described by Li et al. (2009) is suggested. Also, a thorough inventory of wave movements is recommended since the movement of water along the basin of the lake can move and re-suspend particles (Li et al., 2009). PCBs become dispersed through lake ecosystems

by adhering to sediments, which are deposited onto the basin of the water body (MacDonald et al., 1993), where they can become a concern to aquatic health (Bhavsar et al., 2007). The lipophilicity of the contaminant increases with a higher level of chlorination (WHO, 2000) and congeners with lower chlorination are more volatile (WHO, 2000). In non-industrial areas believed to be free of contamination, ambient air levels of PCBs were detected at 0.02 ng/m^3 in contrast to heavily industrialized areas, where levels exceeding 650 µm/m^3 (WHO, 1993; WHO, 2000). Research conducted in the United States indicated that 92% of detectable PCBs were in vapour form (WHO, 1993; WHO, 2000). In order to test for PCBs in vapour phase, biomonitors, such as plant foliage, are recommended by the WHO (WHO, 2000) and should be considered as part of the risk assessment.

The general public is most commonly exposed to PCBs through food, particularly fish (WHO, 2000). The World Health Organization's (WHO) tolerable monthly intake of dioxins, furans and PCBs is 70 picograms per kg of body weight (based on the "cumulative exposure to dioxins and furans from all sources including food and water") (WHO, 2004). Due to the fact that many contaminants have been legislated and are no longer in production, sediments throughout the world, as described by Moermond et al. (2004), have in many cases become sinks for lipophilic contaminants (Moermond et al., 2004; 4503). Risk assessments in aquatic ecosystems therefore need to consider the remobilization of sediment-bound hydrophobic contaminants in order to determine the potential risks these compounds pose to the biotic community (Moermond et al., 2004; 4503).

2.6.4 Dichlorodiphenyltrichloroethane (DDT) and pesticides

Dichlorodiphenyltrichloroethane (DDT) is a synthetic persistent organochlorine pesticide once widely used in agricultural applications to limit the infestation of insects

(ATSDR, 2011), as well as a means to eradicate malaria and other vector-borne diseases in endemic countries (WHO, 2007; EPA, 2012). Though the production of the substance is highly controlled by the International Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention on Persistent Organic Pollutants, 2008), and banned in many countries, the World Health Organization (WHO) asserts that at present, there are no other alternatives that are as efficient and economically viable to combat these diseases by targeting the vector of the disease (WHO, 2007).

Dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) are DDT metabolites created via microbial action in soil (ATSDR, 2011). Lipophilic, these substances adhere to sediment, bioaccumulate, and can interact with plant and animal receptors (ATSDR, 2011). Health concerns associated with exposure to these chemicals include effects on embryonic development, mutagenic effects, and DDT is recognized as a probable carcinogen (EPA, 2012). The majority of the population is exposed to DDT and its metabolites via food, primarily the consumption of fish (Health Canada, 2007).

2.7 Koi herpesvirus (KHV)

2.7.1 Global significance and current status of Koi herpesvirus (KHV)

Cyprinid herpesvirus-3, CyHV-3 or Koi Herpesvirus (KHV), part of the *Herpesviridae* family (Aoki et al., 2007; Uchii et al., 2011) is a herpesvirus that induces a species-specific disease in *Cyprinus carpio carpio* L. (Honjo et al., 2011 in press) responsible for mass mortalities of carp throughout the world. Koi Herpesvirus (KHV), also known as carp interstitial nephritis and gill necrosis virus (CNGV) (Pikarsky et al., 2004; Ronen et al., 2003; Ilouze et al., 2006a) and Cyprinid herpes virus-3 (CyHV-3) (Waltzek et al., 2005 Ilouze et al., 2006) was first isolated in 1998 in the United States (Pokorova et al., 2005)

after confirmed reports of outbreaks both in Israel and the USA (Hedrick et al., 2000). This species-specific herpesvirus is an extremely contagious pathogen that has spread extensively throughout the world (Perelberg et al., 2005) targeting exclusively common carp (Sadler, Marecaux and Goodwin, 2008; 71). KHV is vectored through water and can therefore be spread in areas with low carp density (Matsui et al., 2008). Research conducted by Minamoto et al., (2009) in Lake Biwa, Japan, clearly indicates that once KHV has entered a body of water, the virus will remain in the aquatic system for a significant amount of time (Minamoto et al., 2009).

KHV has caused massive die-offs of farmed ornamental and common carp in fish farms (Perelberg et al., 2005) and die-offs throughout Europe, South Africa, the United States and Asia (Haenen et al., 2004; Matsui et al., 2008). Between 2000-2004, the aquaculture industry in Israel alone lost $3 million annually from fish deaths associated with KHV (Perelberg et al., 2005; 3401). Research conducted by Matsui et al., (2008) indicates that KHV may be the first viral disease targeting freshwater environments that has had a severe global impact resulting in the death of massive fish populations. Lack of KHV testing equipment and methods in the aquatic environment continues to hinder ecologists' progress in better understanding the disease (Matsui et al., 2008).

Although little is known about how the virus initially spreads, it has been widely accepted that the primary mode of KHV dissemination occurs as a result of the introduction of healthy looking infected carp or koi to naïve populations (Matsui et al., 2008). It is suspected that a significant factor in the rapid spread of the disease is associated with the widespread trade of carp both as ornamental and food fish (Perelberg et al., 2005). It is relatively unknown as to how the virus is spread (Minamoto et al., 2009), but it is assumed that transmission occurs through the feces of infected carp into the environment (Haramoto et al., 2007), where it is spread to other carp. In order for the virus to multiply, water temperatures need to be between 15-25°C (Gilad et al.

2003; Haramoto et al., 2007) and it is for this reason that there are no outbreaks in the winter months.

Although there are no outbreaks in the winter, the virus is able to remain active in the water long enough to cause an outbreak in the following spring (Haramoto et al., 2007). KHV and other viruses can attach to solid material in the aquatic ecosystem by adhering to debris and by becoming mixed in the growing material, and the virus is able to remain active and collect in shellfish (Landry et al., 1983, Matsui et al., 2008). Epifaunal shellfish are consumed by carp and koi in freshwater systems and therefore, it is assumed that there is a direct correlation between the accumulation of KHV in shellfish species and carp infections (Matsui et al., 2008), making detection of the virus extremely difficult in aquatic environments.

There are two standard methods to detect KHV. The first method is by clinical observation which looks for histopathological indications of infection, such as, lesions on the skin and on internal organs (Gray et al., 2002), gill necrosis and proliferation (Pokorova, 2005; Hedrick et al., 2000; Gray et al., 2002; Matsui et al., 2008). Other physical symptoms (e.g., discoloration, hyper-production of mucus or behavioural changes (e.g., loss of appetite, erratic swimming, an increase in respiration frequency; Hedrick et al., 2000; Gray et al., 2002; Matsui et al., 2008) are also used in initial assessment. It is assumed that the virus' primary entry route into the host is via dermal contact (Costes et al., 2009), which is consistent with changes in the mucus layer of the skin; thus increasing susceptibility to secondary infections (Adamek et al., 2013). As indicated by Matsui et al. (2008), these symptoms are not unique to KHV and therefore detection of the virus or viral DNA (using forms of DNA amplification and enzyme-linked immunosorbent assays) is necessary to definitely identify KHV infection (Matsui et al., 2008; 1264).

Methods to determine the presence of KHV in the natural environment have not been extensively developed (Matsui et al., 2008). Microporous filters can be used to test samples in water and sediment, and the available molecular biological tests are able to detect minute quantities of viral DNA (Matsui et al., 2008). Where testing experiences the most significant obstacle is the extraction of concentrated amounts of KHV from large bulk samples (Matsui et al., 2008) and in this case, especially where the virus is assumed to be in low concentrations. TaqMan PCR system (operating in real-time) was developed to quickly test for KHV (Gilad et al., 2004; Haramoto et al., 2007).

Koi which survive outbreaks of KHV often become carriers of the virus and transmit KHV to naïve koi which ultimately leads to another outbreak of the disease once conditions are adequate (St-Hilaire et al., 2005; Sadler, et al., 2008; 71). Although KHV is a species-specific virus, research conducted by Sadler, Marecaux and Goodwin (2008) reveals that even long after an outbreak of KHV, exposed *Carassius auratus* (L.) (common goldfish) tested positive for the CyHV-3 genome. Though this does not explicitly prove that goldfish are definite carriers of the virus, it suggests that there is a need to test other species that are in contact with *C. Carpio*, which could ultimately vector the disease.

CyHV-3 is no longer detectable in water after three days without a host (Shimizu et al., 2006; Uchii et al., 2011) suggesting that in water, free floating CyHV-3 has a relatively short lifespan. For fish species such as carp, which aggregate in order to spawn, the incidence of disease transmission is believed to increase (Uchii et al., 2011). It was noted by Uchii et al (2011; 245) that breeding season coincides with warmer water temperatures of 15-25°C (Barus et al., 2002; Uchii et al., 2011) that are ideal for the propagation of the virus (Gilad et al., 2003; Uchii et al., 2011). In areas with high turbidity (which is often where spawning activities occur) and elevated concentrations of chlorophyll (as common to eutrophic sites), the virus adheres to both inorganic and

organic particles where it avoids predation and degradation (Minamoto et al., 2009). The relationship between chlorophyll and turbidity indicate that in nutrient-rich areas, KHV is more prevalent.

The largest recorded KHV outbreak to date was in 2004 in Lake Biwa, Japan where between 60-80% of the carp population (approximately 100,000 carp) was decimated (Matsui et al., 2008; Uchii et al., 2011). As a means of disposal, carp bodies were incinerated with other garbage and then buried in a pit (Professor Toshifumi Minamoto, Kobe University, *pers. Comm*.). Uchii et al (2011) have noted that there have been confirmed cases of carp die-offs in Japan as a result of the virus, though none as severe as the case of Lake Biwa (Uchii et al., 2011; 224). KHV has been detected in the winter months when water temperature is below 10°C which suggests that even outside of the known virus reproductive temperature, carp are actively excreting (via feces) or re-suspending KHV that had been attached to sediment through their benthivorous foraging activities (Minamoto et al., 2009). Host cell temperature outside of the optimum range of 15-25°C exhibit minimal virus replication (Gilad et al., 2003; Uchii et al., 2011).

The fact that the majority of the fish killed in the Lake Biwa outbreak were adults greatly supports this theory as it is the sexually mature carp that would be exposed to the infection while breeding in large groups (Uchii et al., 2009). Uchii et al (2011) detected CyHV-3 in significantly higher concentrations in mating versus non-mating sites suggesting that spawning areas become "hot-spots" for infection transmission by accumulating the virus during the breeding process (Uchii et al., 2011; 249). Other factors considered to contribute to disease susceptibility include temporary immunity suppression induced by the increase in sex hormones in the spring (Watanuki et al., 2002; Uchii et al., 2011).

2.7.2 Koi herpesvirus outbreaks in southern Ontario (2007-2008)

Peterborough, Lake Simcoe, Simcoe County and fifteen additional water bodies experienced a mass carp die-off during the summers of 2007 and 2008 (MNR, 2009). It was determined that the primary etiological agent responsible for the widespread mortalities was KHV (Perelberg et al., 2005) and CyHV-3 (Minamoto et al., 2009). The removal and disposal of large volumes of fish over a relatively short period of time raises various practical, financial and health concerns.

Conservative estimates of carcass volume during the outbreak affecting Lake Simcoe suggest that upwards of 13,000 fish were disposed of in 2008 (Garver et al., 2010: 1244), not considering the thousands, which were disposed of on private property or were left to decompose *in situ*. Estimates suggest 110 tonnes of carp were disposed of in waste management facilities throughout the region (MNR, 2009). The 2008 die-off was a continuation of the 2007 incident that affected the Kawartha Lakes region resulting in the disposal of 12,000-24,000 carp (MNR, April 2008; 1).

In order to help facilitate the removal of fish waste, the Ontario Ministry of Natural Resources (MNR) issued a series of recommendations and fact sheets which instructed residents to double-bag the carcasses for roadside pickup, where they would be disposed of as waste in municipal landfills (MNR, July 2008). The MNR recommendation advisories stated that as an alternative to municipal waste collection, fish carcasses could be buried on a landowner's property at a minimum depth of two feet. The MNR also requested that people do not flush the fish down the toilet!) (MNR, May 2008). The MNR advisory clearly stated that "the virus does not affect humans" and that "carp infected with KHV are safe to eat and handle" (MNR, May 2008).

Although the zoonotic potential for KHV is assumed to be quite low, (*Walster, pers comm*.), and this virus may not be a direct concern for humans as stated by the MNR (May 2008), other toxicological and bacteriological concerns are raised.

2.8 Aquatic animal health

2.8.1 Fish epizootics and mass die-offs

Although relatively little is known about the transmission of infectious animal diseases in the wild, this phenomenon is now considered as one of the most significant threats to biological diversity worldwide (Daszak et al., 2000; Smith et al., 2009; Uchii et al., 2011). In recognition of this, countries such as Canada have developed initiatives such as The National Aquatic Animal Health Program to address concerns pertaining to diseases which threaten the health of Canadian aquatic environments and the revenue it generates in domestic and foreign markets.

The National Aquatic Animal Health Program (NAAHP) is a partnered initiative between Fisheries and Oceans Canada and the Canadian Food Inspection Agency. The mandate of the NAAHP is to "protect Canada's aquatic resources by preventing the introduction or spread of infectious diseases in wild or farmed aquatic animals" (DFO, 2011). In addition, the NAAHP acts in accordance with the World Organization for Animal Health (OIE), whose aim is to protect animal health at a global level by monitoring, mitigating and limiting the spread of infectious diseases (OIE, 2013).

The Centre for Aquatic Animal Health Research and Diagnostics (CAAHRD), as part of Fisheries and Oceans Canada, acts as a scientific research base providing diagnostics and expertise in support of programs such as the NAAHP and other regional aquatic health initiatives (DFO, 2011). The goal of this program is to mitigate the movement of

fish diseases and ensure they are not spread via fish or fish-based products within Canada or our trading partners (DFO, 2011).

Though Canada has programs to monitor the spread of exotic and endemic fish diseases, and in some cases has bilateral monitoring programs established (as in the case of Viral hemorrhagic septicemia virus, DFO, 2011), what fails to be recognized is the need for a standardized disposal procedure that includes both pathogenic and toxicological tests to ensure that the handling and end processing of the dead fish do not induce long-term concerns for human and environmental health.

2.8.2 Animal disposal

In Canada, it is the responsibility of the individual province or territory to dispose of wild animal carcasses. Although support is offered at the Federal level through organizations such as the Canadian Food Inspection Agency (CFIA) who advises provincial or territorial staff how to effectively implement disposal legislation and practices and Environment Canada, Provincial Natural Resources and Ministries of the Environment are chiefly responsible. Regulations for the disposal of farmed animal carcasses, products and by-products are under provincial and territorial jurisdictions. CFIA ultimately acts as a body of knowledge and expertise to assist and ultimately approve the disposal plan in accord with its Foreign Animal Disease Emergency Response Program (FADERP) (CFIA, 2012).

The CFIA has developed infrastructure to aid in the detection, prevention, containment and eradication of foreign animal diseases in Canada (CFIA, 2012). In the event of an outbreak requiring rapid and immediate intervention, this authority can call both provincial and local governments and invested stakeholders to assist in the

implementation of procedures which work to avoid potentially devastating losses to the agri-food industry at large (OMAFRA, April 7, 2011). In the case of an Emergency, provincial ministerial roles are outlined in the Emergency Management Ontario (EMO) and Civil Protection Act (Emergency Management Ontario, 2006-2009).

The Foreign Animal Disease Emergency Response Plan (FADERP) was developed out of a partnership between the CFIA, EMO and OMAFRA (Ontario Ministry of Agriculture Food and Rural Affairs) as a means to:

i. *clearly align terminology with the Provincial Emergencies Response Plan (PERP)*
ii. *to augment CFIA's disease control capability with the provincial emergency management system*

(Adapted directly from OMAFRA, April 7, 2011).

In essence, the FADERP outlines the involvement of various organizations that follow the lead of the CFIA in the event of a highly contagious foreign animal disease in Ontario. EMO and OMAFRA's roles as "provincial emergency coordination agencies" and other involved agencies are also described (OMAFRA, April 7, 2011). Within this act, a *foreign animal disease* refers to "a range of biological threats (that are not normally found in Canada) to livestock, poultry and wildlife" (OMAFRA, April 7, 2011).

In Ontario for example, a wild fish die-off is initially investigated by the Ontario Ministry of Natural Resources (OMNR) and Environment Canada. If the cause of death is determined to be related to a disease regulated by the CFIA, these authorities are required under the Health of Animals Act and Health of Animals Regulations to report back to the CFIA who in turn conducts their own follow up investigations. The CFIA has the authority to investigate any disease outbreak that threatens Canadian aquatic resources (CFIA, 2012). If the die-off is believed to have occurred as a result of pollution then it is Environment Canada who continues with the investigation and all other

causes of death are followed up by the OMNR. If KHV proves to be enzootic in wild carp populations, then disease control activities may not be necessary (Klotins, CFIA *pers. Comm*.).

The following diseases are listed by the CFIA as *reportable diseases.* There is a legal responsibility to anyone who suspects an outbreak of these diseases to contact the CFIA immediately due to the inherent ecological and economic risk factor (CFIA, 2013):

- Koi herpesvirus disease
- Spring viraemia of carp
- Viral haemorrhagic septicaemia
  (CFIA, 2013).

2.8.3 Fish disposal

Though suites of histopathological tests and water quality assessments are frequently conducted during large fish-kills, what needs to be further developed is a suite of standardized contaminant tests to quantify the relative chemical loading contained in the fish carcasses to safely and effectively determine the most precautious method of disposal. The United States Environmental Protection Agency (EPA) for example has clearly indicated that fish are significant sources of toxicants, specifically mercury. The EPA has also set forth stringent regulations on the handling and disposal of mercury products, none of which has any mention of fish (EPA, 2012).

During mass die-offs of fish in Canada and particularly Ontario, the associated cleanup and disposal responsibilities are often points of contention between authorities, local municipalities, homeowners and cottagers. Issues pertaining to the logistics of carp removal (cost, resources, labour, disposal facility allocation) can delay the removal of carcasses and cause unrest with local residents. In addition, the invasion of scavenger species, such as, fish eating birds adds further complexity, especially in cases when they

too die as a result of consuming the contaminated fish and require subsequent disposal as well. In 2011, Nottawasaga Bay experienced a die-off of fish and waterfowl as a result of a Botulism E. epidemic, which required the removal of over 1,000 birds, many of which were transported to the local waste facility (John Cooper, Fish and Wildlife Services , *pers. Comm*.). Lake Erie also experienced a widespread fish kill the following year when tens of thousands of carcasses washed up on shore. A rapid change in temperature and steep decline in oxygen levels are thought to be the primary factors responsible for the mass mortalities, with dead fish and piscivorous birds littering approximately 40 km of Lake Erie shoreline (Brennan, 2012).

Ontario Regulation 105/09 under the Food and Safety and Quality Act (2001) outlines the appropriate disposal method for deadstock off of farming operations. Fish are neither listed in Reg. 105/09 nor Reg. 106/09 (Nutrient Management Act- Disposal of Dead Farm Animals) as a species of dead animal to be treated and disposed of as deadstock (Reg. 105/09 Section 2:1, Reg. 106/09 Section 2:1-7). In these Acts, the handling, disposal and inspection of deadstock is limited to a list of specific individuals (Reg. 105/09 Section 3:5). Those who satisfy the list of criteria, who are therefore regarded as "certain persons" have a series of disposal options outlined under Section 7 which include the following options for non-farmed animals as indicated in the Act 105/09 (none of which outline the proper creation of disposal pits, or the handling of dead fish):

Disposal Options:

7. (1) A custodian shall, in accordance with the relevant requirements set out in this Regulation, dispose of deadstock by,

A. using the services of a licensed collector;
B. delivering it to a disposal facility;
C. delivering it to an approved waste disposal site; or
D. delivering it for the purposes of a post mortem activity to a veterinarian who agrees to accept the responsibilities of a custodian with respect to the deadstock.

(Ontario Regulation 105/09 made under the Food Safety and Quality Act, 2001).

Though landfills are often selected due to their relatively low-cost and simplistic design, they have the potential to pollute the environment (Butt et al., 2008; 962). Producing solid, liquid and gaseous waste, landfills as a means of waste disposal raise significant concerns pertaining to management of waste and its potential adverse effects on the landscape. Leachates and emissions from landfills are capable of polluting the atmosphere, lithosphere and hydrosphere, and therefore poses a hazard to human and environmental health (Butt et al., 2008; 952).

In order to sufficiently protect human and animal health when dealing with dead animals, proper disposal practices are necessary (Kalbasi et al., 2005; Xu et al., 2008; Akdeniz et al., 2010). In North America, the primary disposal method used for mortalities was rendering (converting animal by-products into useable materials) prior to the bovine spongiform encephalopathy (BSE) outbreak which raised concerns pertaining to the long-range transportation of "pathogen-contaminated carcasses" (Stanford et al., 2007; Wilkinson, 2007; Benson et al., 2008; Guan et al., 2008; Glanville et al., 2009; Guan et al., 2009; Stanford et al., 2009; Xu et al., 2009; Akdeniz et al., 2010; 1981).

Research conducted by Akdeniz et al (2010) indicated that biologically secure barriers are needed during emergency composting scenarios to limit the risk of disease transmission and reduce the survival time of pathogens. Though the focus of their research was primarily epidemiological, the same concerns apply with regards to handling fish with elevated contaminants. The risk of contracting a communicable disease poses a greater threat at the immediate or shortly thereafter contact with the carcass than the long-term implications pertaining to chronic exposure to persistent contaminants. Though authorities may more commonly overlook concerns pertaining to

chemical exposure, it is no less valid than the initial epidemiological risks identified by health agencies.

Active disposal piles used for carcasses should be left undisturbed in order to reduce aerosol transmission of pathogens (Ahn et al., 2008a., Ahn et al., 2008b; Akdeniz et al., 2010; 1981). This knowledge should also be applied to cases where communicable diseases are not detected so to limit the movement of volatile contaminants such as some PCBs congeners and mercury. The tools needed to effectively handle, monitor and limit disease transmission in emergency disposal situations have not been sufficiently developed (Akdeniz et al., 2010), nor have they been to mitigate the movement of contaminants out of disposal sites into the environment.

It is unlikely that biologically and chemically secure barriers were employed in any of the disposal sites on private or public property during the 2008 carp die-off, and therefore it can be assumed that there is no medium in place to retain leachate. Also, if the decaying carcasses were not immediately disposed of, secondary infections could have set in as well. In order to adequately sanitize the composted material, a consistent temperature of 70°C or above must be reached (Butt et al., 2008; 386) and again, it is unlikely that the carcasses disposed as compost were sufficiently sterilized.

2.8.4 Botulism and communicable diseases

Seasonal die-offs of fish and other species in the Great Lakes region are not uncommon. Outbreaks of botulism in this locale have been responsible for large die-offs of fish and bird species, but so far have not been associated with any outbreaks of botulism in humans (MNR October 24, 2011). Botulism is induced by the ingestion of the bacterium *Clostridium botulinum*, which is naturally occurring in aquatic sediments (MNR October 24, 2011). Of the seven recognized types of Botulism, only four are noted to induce

human botulism: A, B, E and F. The other types, primarily C and D are responsible for mammalian, avian and fish botulisms (WHO Fact Sheet N° 270; August 2002), and G is found in soils. Botulism E., unlike some species-specific viruses, is a public health concern as it is transferable from fish to humans via direct contact as well as consumption (WHO Fact Sheet N° 270; August 2002).

During the fall of 2011, there was a large fish die-off as a result of a Botulism E. outbreak in Ontario. According to John Cooper from Fish and Wildlife Services (*pers. comm*., 2012), reports began emerging from MNR sources regarding the death of 8 sturgeon and one carp in south-eastern Georgian Bay. Throughout October, these reports continued as the number of reported deaths increased to approximately 120 fish of five species. Waterfowl such as the Canada Goose (*Branta canadensis*) and six other species were also being reported dead in the general area of Parry Sound North to Collingwood. By October 22nd, thousands of dead birds were washing up along the South East shorelines of Nottawasaga Bay. Two days later, the Provincial Park Staff removed approximately 1000 dead birds from Wasaga Beach Provincial Park. According to the Fish and Wildlife Services (John Cooper, Fish and Wildlife Services *pers. comm*, 2012). , 70% of the birds were long-tailed ducks, 20% grebes (both pied-billed and red-necked species), and the remainder was a mix of common loons, white-winged scoter, ring-billed bull and herring gulls. Throughout the remainder of October, 20 dead lake sturgeon, carp, common carp and a Canada Goose were also reported by the Wasaga Beach Provincial Park. The carcasses from this epizootic were taken to the Wasaga Beach Municipal waste site for disposal (John Cooper, Fish and Wildlife Services *pers. comm*, 2012).

The disposal of birds and fish on private land is the responsibility of the individual property owner (MNR October 24, 2011). To assist residents with the removal of carcasses, the MNR (via Fish and Wildlife Services) developed a fact sheet for the

general public that included the following:

To dispose of dead fish or birds along your shoreline
• You are responsible for disposing of dead birds and fish on your property.
• Bury them or dispose of them in the garbage.
• Wear rubber gloves or cover your hands with plastic bags while handling the carcasses, and dispose of the gloves or bags in the garbage.
• Wash your hands thoroughly with soap and water after handling the carcasses.
• Wash any tool that came into contact with the animal with a disinfectant.
(MNR October 24, 2011).

Diseases such as KHV, VHS or spring Viraemia of carp which are suspected to be a threat to aquatic ecosystems are sent to federally (Government of Canada) regulated laboratories which test for a variety of reportable and notifiable diseases (CFIA, 2012). In Canada, the federal government is responsible for monitoring the movement of fish domestically and abroad (CFIA). VHS has been confirmed in Lake Simcoe as a freshwater virus capable of infecting 28 species of fish (MNR, December 2011).

The issue of aquatic and semi-aquatic animal disposal is not limited solely to cases involving disease outbreaks, nor are cases limited to natural landscapes. Recently, concerns were raised about the discovery of mass graves at the famous Marineland attraction in Niagara Falls, Ontario. Currently, the Ontario Ministry of the Environment is investigating the burial sites used for various porpoises, pinnipeds, ruminants, and other large terrestrial mammals. Their inquiry is assessing whether there has been, or will likely be, adverse impacts on soil, groundwater and nearby watercourses from what is assumed to be large, unlined, untreated graves (Diebel and Casey, 2012).

3.0 Objectives and Rationale:

3.1 Objectives

The purpose of this study is to determine whether or not sites used for the disposal of carp carcasses in the Lake Simcoe KHV die-off were a human or environmental health concern as a result of contaminant loading. In order to effectively assess the implications of these disposal sites in the environment, the following objectives were established.

1. To quantify the concentration of contaminants left in the pits after carp carcasses had decomposed.
2. To assess, based on the level of contamination, whether or not Ontario's current fish disposal practices are adequate in protecting human health and the ecological integrity of affected and neighbouring communities.
3. To develop a case study to evaluate the effectiveness of various response strategies to future aquatic pandemics involving large die-offs of potentially contaminated biota.

3.2 Rationale

Carp are a sentinel species known to bioaccumulate and bioconcentrate contaminants. As such, a mass die-off like the one involving KHV raises concerns regarding the movement of aquatic toxicants to terrestrial ecosystems via the transportation and disposal of contaminated carcasses.

4.0 Methodology

4.1 Field Methods

4.1.1 Soil sample collection and extraction

Georgina Island Reserve N^0 33A, located in the south end of Lake Simcoe, Ontario is comprised of three islands; Snake, Fox and Georgina (see Figure 1). Owned and managed by the Chippewas community, these lands are inhabited by the Chippewas First Nations community and seasonal cottagers. Permission to conduct research was granted by the Band and conducted in a manner as to respect the cultural, aesthetic and ecological value of the landscape.

During the summer of 2008, the shorelines of these islands were inundated with carp carcasses as a result of the Koi Herpesvirus outbreak. Although each of the three islands were faced with disposal issues pertaining to the abundance of dead fish, Snake Island was chosen as a case study due to its accessibility to the mainland, seasonal as opposed to year round inhabitation, minimal infrastructure, and because it was assumed that the limited development on the island would be reflected in negligible background contaminant levels. There are 227 leased cottages on Snake Island (Chippewas of Georgina Island, n.d.), which are seasonably occupied and left vacant over the winter months.

In order to dispose of the fish carcasses on Snake Island, four pits were mechanically created using a Bobcat™ skid-steer at the time of the die-off by members of the band and island curators. The disposal sites were neither capped nor lined and therefore the contents were susceptible to predation and dispersal throughout the environment. At the time of the die-off, the pits were left open for a minimum of five days before the soil was backfilled into the holes and the contents were left untreated.

Due to financial and temporal constraints, only two of the four sites were sampled. The two sites chosen for sampling were the most easily identifiable in the landscape, characterized by deep depressions outlining the peripheral circumference of the pit. A standardized deterministic random design technique was employed in order to capture representative samples of the site. Though other methods such as the geostatistical approach as per described by the Environmental Sciences Group (1999) could have been used, given the relatively small size of the pits and obvious landscape markers, it was decided that this method was the most applicable. In addition, a total of three randomly selected control sites were randomly selected along the south half of the island in order to assess background contaminant levels.

In order to determine if these sites were actual disposal sites, the center of pits were exhumed with a manual coring auger 50 cm below the surface to remove what was assumed to be backfill. It was generally at this depth that bone matter was discovered and collected for further analysis. Once evidence of bones and fish matter was located, digging ceased so not to disturb the site and reduce the amount of disruption of the soil and to restrict the volatilization of contaminants. Bone material was collected and the coring commenced in what was believed to be the center of the pit. Control sites were selected in relatively undisturbed areas and were sampled in the same manner as the disposal pits.

The first series of samples were taken at the following intervals; 30 cm, 60 cm, 90 cm and 120 cm using a manual coring auger. This instruments' ability to penetrate coarse substrate without disturbing the land for safety, ecological and aesthetic reasons made this piece of equipment the most effective non-mechanized form of sampling for the island. When sites were revisited approximately one year after the initial sampling period, a mechanical auger was used to sample the same sites at the same depths

because the ground was relatively impenetrable from the onset of frost and commencement of winter.

Sampling of the first batch of soil was conducted two years after the die-off and was analyzed for PCBs using a semi-quantitative immunoassay method. This method was determined to be inaccurate giving false positives, due to matrix interference; thus, a quantitative method was employed that measured for a suite of POPs in the soil. Subsequently, the sites were re-sampled in the same manner in the following year, and three new control sites were selected, as the initial control sites were no longer locatable.

During the soil collection process, it was discovered in site one that there was rotting carp remains. These particular carcasses were disposed of in double lined garbage bags within the pits and as a result, the contents had not fully decomposed. Two tissue samples were extracted from the site using a trowel at a depth of 90 cm. The carp tissue samples were treated with the same quality control as soils.

4.1.2 Quality control

Between each sample, the augers were washed in an acetone (Comet Chemical Company Ltd., 100% concentration) bath (waste collected and disposed of according to handling procedure for organic solvents) to reduce cross-contamination between sites. Samples were double wrapped and sealed in whirlpack bags, labeled and packaged in a cooler with icepacks to maintain a constant temperature of approximately 4°C to reduce biological activity until they were placed in a freezer prior to laboratory analysis. Samples were taken commencing with the first extraction in the center and then outwardly at a 90° angle to the right from the first sample, 1 meter from the center. This was repeated in a clockwise formation repeating the same 90° angle for a total of four

samples originating from the center. Protective eyewear, boots, gloves and facemasks were worn for the sampling to reduce exposure to volatile and lipophilic compounds that could potentially be in the soil.

4.1.3 Worm sample collection and extraction

Earthworms are often used as sentinel species for quantifying the bioavailability of organic chemicals in soil because ***i)*** they live in close contact with the soil***, ii)*** they have a thin permeable membrane through which contaminants can pass, ***iii)*** they also consume a significant amount of soil as part of their diet (Jager et al., 2005). Because each species of earthworm interacts differently with soil, the contaminant uptake varies between organisms (Jager et al, 2005). Earthworms (likely *Lumbricus* sp., but not definitively identified), were collected at each of the sites where soils were obtained to estimate contaminant bioavailability. This species of invertebrate was chosen for examination because it was common to all sites, simple to collect and most importantly because it is known to bioaccumulate organic contaminants in soil.

Within the drilosphere (the portion of the soil which is most influenced by earthworms) (Brown et al., 2000; Hickman and Reid, 2008), contaminants, once sorbed to organic material and soil particles, can become mobilized through contact with earthworms (Verma and Pillai, 1991; Gevao et al., 2001; Hickman and Reid, 2008) and subsequent bioturbation. The effects of earthworms in contaminated soils differ depending on the species (Jager et al, 2005), the particular compound in the site as well as the physiochemical properties of the soil (Hickman and Reid, 2008). *Pheretima posthuma* exposed to DDT have been reported to release sorbed fractions (Verma and Pillai, 1991), in contrast to to *E. fetida* exposed to PCB congener Aroclor 1248, which proved to be highly bioaccumulative due to digestive related processing (Tharakan et al., 2006).

Earthworm samples were taken from the two disposal pits that were sampled in order to determine whether or not these species were acting as biological vectors mobilizing the contaminants from the soil into the environment. Worms were collected using a trowel and stored in sealed and labeled whirlpack bags. (Worms were collected to a maximum depth of 120 cm). The second series of collection was conducted late in late December at which point worms were difficult to locate and were assumed to have migrated down into the soil horizon for overwintering.

4.1.4 Shipping and handling of samples

Soil and worm samples were handled and shipped to the Analytical Services Unit Laboratory of Queen's University, Kingston, ON. Soil samples were frozen until shipment. Earthworms were rinsed in distilled water, and following a 24-hour depuration period on moist paper towel to ensure full excretion of gut contents. Worms were then frozen in tinfoil and sealed in whirlpack bags and frozen until shipment at -20^{o}C. Worms were depurated so that contaminant levels reflected their tissues rather than the soil in their guts. Worm and soil samples were sent to the laboratory in an insulated container with icepacks to ensure that temperature remained constant and did not thaw during the shipping process. Once received, samples were homogenized for analyses. (Other tissue samples that were obtained in the disposal sites were inseparable from soil and therefore homogenized in the lab for analysis).

4.2 Laboratory Methods

The samples were contracted to the Analytical Services Unit at Queen's University in Kingston, Ontario. (See Appendix for detailed methods).

5.0 Results

Contaminant levels in the disposal pits were lower than the estimated loading value calculated from data obtained from the Ontario Ministry of the Environment- Sport Fish Contaminant Monitoring Program. There was no clear pattern associated with the dispersal of the various chemicals through the soil horizons. In the case of PCBs, all soil samples were below the detection limit and detectable in the carp tissue and composite worm samples (0.2 μg/g). Both total mercury and total DDT levels were significantly more elevated than PCB concentrations, however no legal thresholds were exceeded in any of the three tested mediums.

Table 1: Laboratory results for soils

	∑DDT (ng/g)[a]	PCB (μg/g)[b]	∑Hg (ng/g)[c]
Control 1			
0-30 cm	92.8	<0.1	67.5
30-60 cm	110.6	<0.1	57.3
60-120 cm	78.00	<0.1	36.8
Control 2			
0-30 cm	<0.1	<0.1	59.2
30-60 cm	<0.1	<0.1	31.8
60-120 cm	<0.1	<0.1	10.4
		<0.1	
Control 3			
0-30 cm	43.40	<0.1	163
30-60 cm	6575.40	<0.1	449
60-120 cm	3692.30	<0.1	398
Site 1			
0-30 cm	48.80	<0.1	81.2
30-60 cm	15.20	<0.1	41.5
60-120 cm	6.40	<0.1	27
Site 2			
0-30 cm	3.00	<0.1	56
30-60 cm	46.90	<0.1	47
60-120 cm	111.60	<0.1	42.8

(Sample size of 250 g)
[a] ∑DDT: 2,4- DDE, 4,4-DDE, 2,4- DDD, 4,4-DDD, 2,4-DDT, 4,4-DDT
[b] PCB: Aroclors 1221, 1232, 1242, 1248, 1254, 1260

[c] ∑Hg: Combined organic and inorganic forms of mercury
All samples were analyzed at the Analytical Services Unit at Queens University, Ontario, Canada.

Table 2: Laboratory results for carp tissue

	% Lipid[a]	∑DDT (ng/g)[b]	PCB (µg/g)[c]	∑Hg (ng/g)[d]
Tissue 1	5	401.40	<0.1	53.1
Tissue 2	2.6	75.90	<0.1	51.8

(Sample size of 50 g)
[a] % lipid determined using gravimetric determination
[b] ∑DDT: 2,4- DDE, 4,4-DDE, 2,4- DDD, 4,4-DDD, 2,4-DDT, 4,4-DDT
[c] PCB: Aroclors 1221, 1232, 1242, 1248, 1254, 1260
[d] ∑Hg: Combined organic and inorganic forms of mercury
All samples were analyzed at the Analytical Services Unit at Queens University, Ontario, Canada.

Table 3: Laboratory results for worms

	∑DDT (ng/g)[a]	PCB (µg/g)[b]
Control Worms 1	134.30	<0.2
Control Worms 2	<5.0	<0.2
Worms Site 1	16.00	<0.2
Worms Site 2	112.00	<0.2

(Composite sample size of 150 g)
[a] ∑DDT: 2,4- DDE, 4,4-DDE, 2,4- DDD, 4,4-DDD, 2,4-DDT, 4,4-DDT
[b] PCB: Aroclors 1221, 1232, 1242, 1248, 1254, 1260
All samples were analyzed at the Analytical Services Unit at Queens University, Ontario, Canada.

6.0 Discussion

6.1 Contaminant loading from carp carcasses to terrestrial ecosystems

The contaminants measured in the present study were not detectable at elevated concentrations *in situ* four years after their burial. It is assumed that following fish decomposition, microbial action and predation by detrivores such as worms act as vehicles for the remobilization of these substances. Assumed receptors for the contaminants are groundwater and biota though it remains inconclusive as for how much each are receiving and where these substances migrate thereafter. Based on MOE data, a loading of 200 carp to each site would not exceed legal contamination thresholds. Mass disposal pits at the municipal scale if unlined and uncapped could potentially exceed these guidelines.

Table 4: Soil Quality Guidelines for the Protection of Env. and Human Health

Chemical	Agriculture (mg/kg dry weight)	Residential/ Parkland (mg/kg dry weight)	Estimated loading for 200 carp (mg/kg)
Mercury	6.6	6.6	0.4
PCBs	0.5	1.3	0.002
DDT (total)	0.7	0.7	0.015
CCME Soil Quality Guidelines for the protection of Environmental and Human Health * Calculated based on 2001 pre-die-off contaminant concentrations for Lake Simcoe carp (MOE, 2012) on an assumption of 200 fish carcasses per site			

6.2 PCB Degradation

Given that PCBs are extremely persistent and they have only been *in situ* within burial pits since the summer of 2008, it is highly unlikely that lower soil concentrations than loading estimates would predict is primarily due to degradation, but it may well be a factor worthy of future study as factors modifying the attenuation of PCBs in organically enriched soils and sediments is largely unknown (Li et al., 2009).

In the Great Lakes for example, anaerobic dechlorination and dissolution into water are two significant factors affecting PCB persistence (Li et al., 2009). In Lake Michigan for example, other factors such as the deep burial of contaminated sediment are also considerations for the decrease in PCB concentration (Li et al., 2009). Li et al. (2009) concluded that "even if PCB degradation was widespread and substantial, the ultimate elimination of PCBs from the Great Lakes by nature may take decades or centuries to complete" (p. 146).

6.3 Disposal policy ramification suggestions

Ramifications to disposal policies are suggested in order to ensure that areas used for fish disposal following die-offs do not become dumping grounds for hazardous waste and that human and environmental health are sufficiently protected from contaminant exposure. Future research is needed in order to accurately map the movement of these substances in disposal pits immediately following die-offs because the bioavailability of certain toxins decreases as time progresses from when the contaminant first entered the soil (Alexander, 2000; Jager et al. 2005; 293).

7.0 Future Recommendations

7.1 The Future of KHV and similar outbreaks

With technological developments and rampant globalization, the rapid transportation of alien aquatic species is becoming a growing concern (Minchin, 2007). Considering the development of these new technologies and the growth in the fish trade industry, it is probable that the risk of KHV infection is increasing (Pokorova et al., 2005). Although not all carp will contract the virus, *C. carpio* that survive an outbreak of KHV develop CyHV-3 antibodies and later become carriers (Uchii et al., 2009); thus, retaining the virus in carp populations to produce disease once conditions are adequate. It is probable that there are carp in Lake Simcoe and its tributaries with acquired immunity, enabling the spread of the virus to infect other fish and cause another mass die-off. In order to ensure that humans and environmental health is not compromised, a thorough risk assessment is in order to ensure a precautionary disposal procedure so as not to contaminate land and exposed biota with various pollutants.

7.2 Areas of future research

The case study of Snake Island can serve as a preliminary study from which further investigations could be conducted. Future sample collection should be conducted immediately following die-offs to map the dispersal regime of the contaminants through the ecosystem. Also, worm samples should be taken immediately following decomposition in the warmer season so to ensure an adequate collection of biota for analysis. The movement of lipophilic contaminants, PCBs for example, is highly influenced by soil's physical properties such as pH, moisture content and organic matter in addition to the substances' (congener's) chemical properties (CCME, 3). In

order to have better accounted for this, these parameters should also be considered in future research.

Temporal and financial restraints greatly limited the number of samples that were analyzed.

8.0 Concluding Remarks

The absence of detectable contaminants in the disposal pits suggests that the carcasses did not exhibit relatively high body burdens of the contaminants measured in the present study and/or contaminants have moved from the initial site into the environment. Potential receptors for the contaminants are assumed to be groundwater, the atmosphere, biota and humans. These factors have been overlooked by the demand for the timely and effective removal of carp carcasses. Future cases of fish die-offs are opportune events that need to be effectively monitored, with the movement of contaminants in terrestrial environments via bulk transport from disposed carcasses examined. Additional research is suggested to further analyze the implementation of fish disposal policies that take into consideration the bulk transport of legacy contaminants from improperly disposed fish carcasses.

It has also come to our attention that local residents should be informed of the potential health risks associated with the handling of carcasses (considering both disease and toxicological threats) as this may influence where and whether or not they dispose of carcasses on their private properties or consult other disposal options. Ramifications to disposal policies are suggested in order to ensure that areas used for fish disposal following die-offs do not become dumping grounds for hazardous waste and that human and environmental health are sufficiently protected from chronic low-dose or acute contaminant exposure.

It has become apparent that fish have been overlooked as vectors of contaminants by regulatory bodies in Ontario and in cases where large quantities of fish mass requires disposal, concerns pertaining to larger health related issues are raised. This study is an important pilot project in demonstrating the necessity for future aquatic pandemic response plans to account for the potential transfer of contaminants from aquatic to

terrestrial environments via improperly devised disposal sites on both private and First Nations land.

Appendix 1.0: Sample site descriptions

a- Site One: (Lat: 44.310685 Lon: -79.477484)

The first sampled site was located approximately two meters from the center of the foot path *Nahwuhyee Meekun*. The site was estimated to be 120-200 cm deep and was mechanically created (estimations based on the depth that bones were discovered at). Though the initial exhuming of the site could have led to the volatilization of some chemicals, it was necessary to ensure that the pit was in fact located. The reputability of the locations is subject to human error and therefore as a precautionary measure, it was determined that it was necessary to locate fish matter before sampling. Also, taking into consideration leaching and the inversion of soil atop the mound, the corer would not be able to penetrate deep enough to enter the pit if it was deeper than 120 cm. This site had an abundance of fish matter, which was collected into whirlpack bags using a sterile stainless steel trowel. The bags were taken into the laboratory to be photographed and retained for future testing. The soil was extremely compacted and had a clay texture with large rocks that impeded digging.

b- Site Two: (Lat: 44.309786 Lon: -79.486384)

The second site used for sampling was manually created by a long-time resident of Snake Island on their property as following instructions of the MNR. The pit was dug approximately 120-140 cm deep as the MNR had instructed. In order to sample, it was necessary to dig down 30 cm to locate bone matter. Once this depth was reached and it became evident that the pit had been located, samples were taken in the same pattern as they were in the previous sites. Fish remains were also collected and brought into the laboratory.

c- Site Three: (Lat: 44.309786 Lon: -79.486384)

The third site was also mechanically created approximately 1 m from another footpath on the island. This site remained exposed as bone fragments and fish skeletons were visible atop the rocky terrain. Bones and fish remains were gathered, however little soil or organic matter was present. Unlike the other sites, this disposal area remained an open dumping ground for fish and therefore the carcasses were left exposed to scavengers and subsequently predation. This site had minimal soil to sample and therefore was not during the second series of sampling, as it was not considered to be representative of disposal pits.

d- Site Four: (Lat: 44.316336 Lon: -79.483407)

The fourth site was not as visually apparent as the other locations. There was a large depression in the earth, however the location was only an estimation based on anecdotal evidence. There was a definite stench to the area and the soil was a lot moister than the other locations. This site is located approximately six meters from a well-traveled footpath. Bones were not located at a depth of 80 cm anywhere in the area within a 4-meter radius. The site was still sampled for the initial examination however; it was not opened for further investigation after the first samples were retrieved because the assumed size was larger than what could be investigated without significant landscape alteration and significant excavation.

Appendix 2.0: Analysis

While detectable concentrations of pesticides, mercury and PCBs were confirmed in soil and worms adjacent to burial pits, legacy contamination at sites presumed to be controls precluded meaningful comparisons. Using data obtained by the MOE, the

following trends associated with contaminant loading and percent decrease, bulk loading and body burden can be concluded.

Appendix 2.1: Mercury (Hg) Analysis

Following the 2008 die-off, 13 carp collected live from Lake Simcoe in August, were sampled in order to determine approximate body burden contaminant levels. These fish contained an average mercury concentration of 0.071666667 µg/g, approximately 73% lower than the 0.2645 µg/g average of the 2001 samples (MOE data). This 73% decrease in mercury concentration is consistent with the fact that a large percentage of the Lake Simcoe carp population was annihilated as a result of the KHV outbreak. Surviving carp sampled in 2008 after the die-off were substantially smaller on average than those sampled in 2001(1878.5 g as opposed to 7712.5) which is consistent with the reduced mercury body burden as would be expected in smaller carp.

Using the reference conditions prior to the die-off, it can be assumed that given the mean 2001 mercury body burden of 0.2645 µg/g, and mean sampled carp size of 7712.5 g, the average carp has approximately 2039.9 µg of mercury or 2 mg of mercury per fish. Soil quality guidelines as developed by the CCME state an inorganic mercury concentration limit of 6.6 mg/kg dry weight for agricultural, residential and parkland areas (CCME Soil Quality Guideline Summary Table, 6). With such factors considered, mercury contained within 4 fish with an average body burden of 2 mg, if concentrated by decay/leachate into soil above an aquitard of about 1 kg, would exceed these guidelines that have been developed as a means to protect environmental and human health.

For arguments sake, if approximately 200 average size fish (2001 reference size) were placed in a pit for disposal then 200 fish X an approximate mercury burden of 2 mg/fish

would be equivalent to a 400 mg (0.4 g) loading of mercury to the site. If we extrapolate this to cover an approximate 20,000 carp (to consider both the carp accounted for in the landfills and that which was either disposed of on private property or left to decompose *in situ*) that died as a result of the outbreak then it can be assumed that approximately 40, 000 mg or 40 g of mercury was loaded into the Lake Simcoe region via the contaminated carp carcasses.

Though it remains hypothetical, if the assumption is that 200 fish buried at each site then approximately 40.8 grams of mercury were added. If we multiply the average carp size (7712.5g) X the estimated number of carp at each site (200) then we have a total mass (prior to backfill) of 1542500 g (or 1542.5 kg) fish carcasses deposited in each location. If we assume the pits are backfilled with a quarter of the volume of the carcasses to cover the large mass of bodies and fill the spaces in between, then we can assume 1542500 g (1542.5 kg) / 4 = 385625 g (385.625 kg) of soil was added to the pits. As the organic fish matter decomposes and becomes integrated into the soil, the initial soil mercury loading concentration can be estimated using the following equation: 407991.250 µg (carp matter)/ 1542500 g (soil) = 0.2645 mg/kg. From the samples analyzed in the lab, the highest detected Hg soil concentration was 81.2 ng/g (equivalent to 0.0000812 mg/kg) suggesting there was no barrier to Hg moving off site, either by leaching or biota movements.

Appendix 2.2: Polychlorinated Biphenyl (PCB) and DDT Analysis

Hg percent values indicated the largest decline from all compounds from the 2001 fish data to that recorded in 2008 after the die-off. PCB levels also indicated a relatively high decrease whereas DDT levels did not result in a significant change. Where Hg levels really stood out from the other contaminants was the estimated body burden levels where it recorded 40.8 gram burden compared to a 0.014824967 g DDT burden.

Appendix 3.0: Mercury percent decrease calculations:

Percent decrease was calculated as follows:

% decrease= [(2001 AVG ∑Hg concentration- 2008 AVG ∑Hg concentration)/
2001 AVG ∑Hg concentration)] x 100
% decrease= [(0.2645-0.071666)/ 0.2645] x 100
% decrease = 72.9

AVG ∑Hg= average total Hg concentration for sampled fish in the given year

Appendix 3.1: Mercury body burden calculations:

Mean Hg body burden = 0.2645 µg/g
Average carp mass (using reference size 2001 samples) = 7712.5 g
Estimated number of carp= 200

∑Hg loading= mean body burden X average carp mass X estimated number of carp
∑Hg loading = 0.2645 µg/g X 7712.5 g X 200
∑Hg loading = 407991.250 µg or 40.8 g

Appendix 4.0: PCB percent decrease:

% decrease= [(2008 AVG ∑PCB concentration- 2001 AVG ∑PCB concentration)/
2001 AVG ∑PCB concentration)] x 100
% decrease= [(242-77.69)/242] x 100
% decrease = 68%

AVG ∑PCB= average total PCB concentration for sampled fish in the given year

*AVG ∑PCB= average total PCB concentration for sampled fish in the given year (Average was calculated based on the total of number sampled in that season as the number of fish sampled for each contaminant varies each year. For example, in 2001, 10 fish were sampled for PCB while 13 were sampled in 2008. PCB content was analyzed comparing ∑PCB values (ng/g) as opposed to individual congeners (pg/g) as the sampled congeners were not consistent between samples, however a total PCB level

was and to maintain consistency between samples, this figure was used for comparison purposes).

Appendix 4.1: PCB body burden calculations:

Body Burden= AVG 2001 carp mass X 2001 AVG ∑PCB
= 7712.5 g X 242 ng/g
= 1866425 ng
= 0.0018 g per fish

Appendix 5.0: DDT percent decrease calculations

∑DDT= op-DDT+ pp-DDD + pp-DDT + (DDT+ Metabolites)

AVG ∑DDT= average total DDT concentration for sampled fish in the given year

% decrease= [(2008 AVG ∑DDT concentration- 2001 AVG ∑DDT concentration)/
2001 AVG DDT concentration)] x 100
% decrease= [(192.22-150.15)/ 192.22] x 100
% decrease = 22%

*AVG ∑DDT= average total DDT concentration for sampled fish in the given year (Average was calculated based on the total of number sampled in that season as the number of fish sampled for each contaminant various each year. For example, in 2001, 9 fish were sampled for DDT while 13 were sampled in 2008).

Appendix 5.1: DDT body burden calculations

Body Burden= AVG 2001 carp mass X 2001 AVG ∑DDT
= 7712.5 g X 192.22 ng/g
= 1482496.7 ng
= 0.014824967 g

Appendix 6.0: Percent Decrease and Estimated Body Burden for Lake Simcoe Carp (2001, 2008)

Chemical	Percent Decrease (%)	Estimated Body Burden (g)
Hg*	73	40.8
PCB*	68	0.0018
DDT**	22	0.014824967

* AVG ∑Hg= average total Hg concentration for sampled fish in the given year
**AVG ∑PCB= average total PCB concentration for sampled fish in the given year

***AVG ∑DDT= average total DDT concentration for sampled fish in the given year
-For example, in 2001, 9 fish were sampled for DDT while 13 were sampled in 2008).
(Source: Ministry of the Environment, 2012)

To better contrast the correlation between size and body burden, the largest sampled carp was 13300 grams and the smallest 120 grams. Below contrasts their contaminant concentrations:

Appendix 7.0: Lake Simcoe Common Carp Samples

	Size (g)	∑Hg (µg/g)	∑DDT (ng/g)	∑PCB (ng/g)
Largest sampled Lake Simcoe carp	13300	0.5	435	440
Smallest sampled Lake Simcoe carp	120	0.03	16	20

(Source: Ministry of the Environment, 2012)

References

Adamek, M., Syakuri, H., Harris, S., Rakus, K.L., Brogden, G., Matras, M., Irnazarow, I., Steinhagen, D. 2013. Cyprinid herpesvirus 3 infection disrupts the skin barrier of common carp (*Cyprinus carpio* L.). *Veterinary Microbiology* **162**: 456–470.

Ahn, H.K., Richard, T.L., Glanville, T.D., 2008a. Optimum moisture levels for biodegradation of mortality composting envelope materials. *Waste Manag*. **28**: 1411–1416.

Ahn, H.K., Richard, T.L., Glanville, T.D., 2008b. Laboratory determination of compost physical parameters for modeling of airflow characteristics. *Waste Manag*. **28**: 660–670.

Alderton, D. 2005. Aquarium and pond fish. DK Publishing Inc., New York.

Allan, R.J. 1986. The role of particulate matter in the fate of contaminants in aquatic ecosystems. Sci. Ser. 142. Inland Waters Directorate, National Water Research Institute, Burlington, ON. 128 pp.

Allan, R.J. et al., 1991. Toxic Chemicals in the Great Lakes and Associated Effects, Vols. 1,2. Environment Canada, Ottawa ON, Canada.

Akdeniz, N., Koziel, J.A., Ahn, H.K., Glanville, T.D., Crawford, B.P. 2010. Field scale evaluation of volatile organic compound production inside biosecure swine mortality composts. *Waste Management.* 30: 1981-1988.

Ardó, L., Jeney, Z., Adams, A., Jeney, G. 2010. Immune responses of resistant and sensitive common carp families following experimental challenge with Aeromonas hydrophila. *Fish & Shell Fish Immunology* **29**: 111-116.

Ariav, R., Tinman, S., Paperna, I. and Bejerano, I. 1998. Presented at the EAFP 9th International Conference, Rhodes, Greece.

Arora, M., Kiran, B., Shweta, R., Rani, A., Kaur, B., Mittal, N. 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry* **111**: 811–815.

Aroson, K.J., Miller, A.B., Wolcott, C.G., Stems, E.E., McCready, D.R., Lickley, L.A., Fish, EB; Hiraki, GY; Holloway, C; Ross, T; Hanna, WM; Ser Gupta, SK; Weber, JP. (2000). Breast adipose tissue concentrations and polychlorinated biphenyls and other organochlorines and breast cancer risk. Cancer Epidemiology Biomarkers and Prevention **9** (1): 55-63.

ATSDR. 2011. DDT, DDE, AND DDD CAS # 50-29-3, 72-55-9, 72-54-8. Accessed from: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=81&tid=20

ATSDR. 2001. Mercury. Accessed from: http://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=24.

Baker, M.R., Schindler, D.E., Holtgrieve, G.W., St. Louis, V.L. 2009. Bioaccumulation and Transport of Contaminants: Migrating Sockeye Salmon As Vectors of Mercury. *Environ. Sci. Technol.* **43:** 8840-8846.

Balon, E.K. 1995. The common carp, *Cyprinus carpio*: its wild origin, domestication in aquaculture, and selection as colored nishikigoi. *Guelph Ichthyology Reviews* ISSN: 1181-8549. Number 3: 1-56. Institute of Ichythyology, University of Guelph, Guelph Ontario.

Barus, V., Penaz., M., Kohlmann, K. (2002) *Cyprinus carpio* (Linnaeus 1758). In: *The Freshwater Fishes of Europe, Vol. 5, Part 3, Cyprinidae 2, Part III: Carassius* to *Cyprinus*, Gasterosteidae (Eds P.M. Banarescu & H.-J. Paepke), pp. 85-17 9. AULA-Verlag, Wibelsheim Germany.

Barrena, R., Artola, A., Vázquez, F., Sánchez, A. 2009. The use of composting for the treatment of animal by-products: Experiments at lab scale. *Journal of Hazardous Materials* **161**: 380-386.

Baumann, P.C., Whittle, D.M. 1988. The status of selected organics in the Laurentian Great Lakes-an overview of DDT, PCBs, dioxins, furans and aromatic hydrocarbons. *Aquatic Toxicology* **11**: 241-257.

Bell, D., Clode, S., Fan, M., Fernandes, A., Foster, P., Jiang, T., Loizou, G., MacNicoll, A., Miller, B., Rose, M., Tran, L. 2007. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar (Han) rat II: chronic dosing causes developmental delay. *Toxicol. Sci.* **99**: 214–223.

Benson, E.R., Malone, G.W., Alphin, R.L., Johnson, K., Staicu, E., 2008. Application of in-house mortality composting on viral inactivation of Newcastle disease virus. *Poult. Sci*. **87**: 627–635.

Bentzen, E., Mackay, D., Hickie, B.E., and Lean, D.R.S. 1999. Temporal trends of polychlorinated biphenyls (PCBs) in Lake Ontario fish and invertebrates. *Environ. Rev.* **7**: 203–223.

Bentzen, E., Lean, D.R.S., Taylor, W.D. Mackay, D. 1996. Role of food web structure on lipid and bioaccumulation of organic contaminants by lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* **53**: 2397-2407.

Berger, DF; Lombardo, JP; Jeffers, RM; Hunt, AE; Bush, B; Casey, A; Quimby, F. 2001. Hyperactivity and impulsiveness in rats fed diets supplemented with either Aroclor 1248 or PCB-contaminated St. Lawrence river fish. *Behavioral Brain Research* **126** (1-2): 1-11.

Berti, P.R., Receveur, O., Chan, H.M., Kuhnlein, H.V. 1998. Dietary Exposure to Chemical Contaminants from Traditional Food among Adult Dene/ Métis in the Western Northwest Territories, Canada. Environmental Research Section A. 76: 131-142. ARTICLE NO. ER973797.

Beveridge, M.C.M., Sikdar, P.K., Frerichs, G.N., Millar, S. 1991. The ingestion in suspension by the common carp *Cyprinus carpio* L. *Journal of Fish Biology* **39** (6): 825-831.

Bhavsar, S.A., Hayton, A; Jackson, D.A. 2008. Uncertainty analysis of dioxin-like polychlorinated biphenyls-related toxic equivalents in fish. *Environmental Toxicology and Chemistry* **27** (4): 997-1005.

Bhavsar, S.P., Jackson, D.A., Hayton, A., Reiner, E.J., Chen, T., Bodnar, J. 2007. Are PCB levels in fish from the Great Lakes still declining? *Journal of Great Lakes Research* **33** (3): 592-605.

Blais, J.M., MacDonald, R.W., Mackay, D., Webster, E., Harvey, C., Smol, J.P. 2007. Biologically mediated transport of contaminants in aquatic systems. *Environ. Sci. Technol.* **41**: 1075-1084.

Brandon, D.L., Lee, C.B., Simmers, J.W., Tatum, S.H. and Skogoerboe. 1991. Information summary, area of concern: Saginaw River and Saginaw Bay. Miscellaneous paper E1-91-7. U.S. Environmental Protection Agency, Chicago, IL.

Braune, B.M. and Norstrom, R.J. 1989. Dynamics of organochlorine compounds in herring gulls. III. Tissue distribution and bioaccumulation in Lake Ontario gulls. Environmental Toxicology and Chemistry **8**: 957-968.

Breivik, K., Wania, F., Muir, D.C., Alaee, M.,Backus, S., Pacepavicus, G. 2006. Empirical and modeling evidence of the long-range atmospheric transport of decabromodiphenyl ether. Environmetal Science and Technology **40** (15): 4612-4618.

Brennan, R.J. September 7th, 2012. Lack of oxygen killed Lake Erie fish, test shows. The Star. Accessed from: http://www.thestar.com/news/canada/article/1253189--lack-of-oxygen-killed-lake-erie-fish-tests-show

Bretzinger, A., Fischer-Scherl, T., Oumouma, R., Hoffmann, R. and Truyen, U. 1999. Mass mortalities in koi, Cyprinus carpio, associated with gill and skin disease. Bull. *Eur. Assoc. Fish Pathol.* 1**9:** 182–185.

Bright, D.A., Dushenko, W.T., Grundy, S.L., Reimer, K.J., 1995. Evidence for short-range transport of polychlorinated biphenyls in the Canadian Arctic using congener signatures of PCBs in soils. Sci. Total Environ. **160** (161): 251–263.

Brouwer, A; Longnecker, M.P., Birnbaum, L.S., Cogliano, J. Kostynaik, P., Morre, J., Schantz, S., Winneke, G. 1999. Characterization of potential endocrine-related health effects at low-dose levels of expose to PCBs. *Environmental Health Persepectives* **107** (Suppl 4): 639-649.

Burton, G.A., and B.L. Stemmer. 1988. Evaluation of surrogate tests in toxicant impact assessments. *Toxic. Assess*. **3**: 255–269.

Burton, G.A., M.K. Nelson, and C.G. Ingersoll. 1992. Freshwater benthic toxicity tests. In: Sediment toxicity assessment, G.A. Burton, ed., pp. 213–240. Lewis Publishers, Inc., Boca Raton, FL.

Butt, E.E., Lockley, E., Oduyemi, K.O.K. 2008. Risk Assessment of landfill disposal sites- State of the art. *Waste Management* **28**: 952-964.

Cambra, K., Martınez, T., Urzelai, A., Alonso, E., 1999. Risk analysis of a farm area near a lead- and cadmium-contaminated industrial site. *Journal of Soil. Contamination*. **8**: 527–540.

Canadian Gazette. 2008. Canadian Environmental Protection Act 1999 PCB Regulations. **142** (19): 2078-2140.

Canadian Council of Ministers of the Environment. 1995. Protocol for the derivation of Canadian sediment quality guidelines for the protection of aquatic life. CCME EPC-98E. Prepared by Environment Canada, Guidelines Division, Technical Secretariat of the CCME Task Group on Water Quality Guidelines, Ottawa. [Reprinted in Canadian environmental quality guidelines, Chapter 6, Canadian Council of Ministers of the Environment, 1999, Winnipeg.]

Canadian Council of Ministers of the Environment CCME , 1989. Guidelines for management of wastes containing poly- chlorinated biphenyls PCBs. Report CCME-TSrWM- REE008 E. Manual EPS 9 / HA/ 1 revised. September 1989.

Castrillon, J., Gomez-Campos, E., Aguilar, A., Berdié, L., Borrell, A. 2010. PCB and DDT levels do not appear to have enhanced the mortality of striped dolphins (Stenella coeruleoalba) in the 2007 Mediterranean epizootic *Chemosphere* **81**: 459–463.

CFIA, 2012. Aquatic Animal Health. Accessed from: http://www.inspection.gc.ca/animals/aquatic-animals/eng/1299155892122/1320536294234

CFIA, 2013. Federally Reportable Aquatic Animal Diseases in Canada 2013. Accessed from: http://www.inspection.gc.ca/animals/aquatic-animals/diseases/reportable/2013/eng/1339174937153/1339175227861

Charya, N.S., Kamalaa, C.T. and Suman Raj, D.S.S. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety* **69**: 513–524.

Chippewas of Georgina Island. N.d. Lands/Membership and Estates. www.georginaisland.com/landsAndMemberships.html.

Clarke, B.O. and Smith, S.R. 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environment International* **37**: 226–247.

Colborn, T; vom Saal, FS; and Soto, AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* **101**(5): 378-384.

Couch, J.A., and J.C. Harshbarger. 1985. Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcinogenesis Rev*. **3:** 63–105.

Crivelli, A.J. 1981. Biology of the common carp *Cyprinus carpio* in the Camargue southern France. *Journal of Fish Biology* **18**: 271-290.

Dawson, D.A., E.F. Stebler, S.L. Burks, and J.A. Bantle. 1988. Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo–larval assays. *Environ. Toxicol. Chem*. **7**: 27–34.

DeKay, J. E. 1842. *Natural History of New York.* 1. *Zoology. Reptiles and Fishes. Part IV-Fishes.* Albany, NY: W.A. White and 1. Visscher.

De Vault, DS; Hesselberg, R; Rogers, PW; Feist, TJ. 1996. Contaminant trends in lake trout and walleye from the Laurentian Great Lakes. *Journal of Great Lakes Research* **22** (4): 884-895.

DFO (Department of Fisheries and Oceans. 2011. Tracking Aquatic Animal Diseases: the Fisheries and Oceans Canada (DFO) Centre for Aquatic Animal Health Research and Diagnostics (CAAHRD)Accessed from: http://www.dfo-mpo.gc.ca/science/publications/article/2011/02-28- 11-eng.html.

DFO (Department of Fisheries and Oceans). 2012. National Aquatic Health Program - FAQ's. Accessed from: http://www.dfo-mpo.gc.ca/science/enviro/aah-saa/nfaq-eng.htm

Diebel, L., Casey, L. 2012. Marineland agrees to external water-quality inspections. The Toronto Star. Accessed from: http://www.thestar.com/news/canada/2012/10/04/marineland_agrees_to_external_waterquality_inspections.html

Diliberto, J., Jackson, J., Birnbaum, L., 1996. Comparison of 2,3,7,8- tetrachlorodibenzo-p-dioxin disposition following pulmonary, oral, dermal and parental exposures to rats. *Toxicol. Appl. Pharmacol*. **138**: 158–168.

Dodder N.G., Strandberg B, Hites RA. 2002. Concentrations and spatial variations of polybrominated diphenyl ethers and several organochlorine compounds in fishes from the northeastern United States. *Environ Sci Technol* **36**: 146–151.

Domingo, J.L. and Bocio, A, 2007. Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: A literature review. *Environment International* **33**: 397–405.

Dong, C. Weng, S., Li, W., Li, X., Liang, Q., He, J. 2011. Characterization of a new cell line from caudal fin of koi, Cyprinus carpio koi, and first isolation of cyprinid herpesvirus 3 in China. *Virus Research*. **161:** 140-149.

Dudka, S., Miller, W.P., 1999. Permissible concentrations of arsenic and lead in soils based on risk assessment. *Water Air Soil Pollution* **113**:127–132.

EC, European Commission. 2006a. Soil protection — the story behind the strategy. Luxembourg: Office for Official Publications of the European Communities; ISBN 92-79-02066-8.

Eimers, M.C., and Winter, J.G. 2005. *Lake Simcoe Water Quality Update 2000–2003*. Lake Simcoe Environmental Management Strategy Implementation Technical Report Imp. B.20. Lake Simcoe Region Conservation Authority, Newmarket, ON.

Eisenreich, SJ; Loonez, B; Thornton, JD. 1981. Airborne organic contaminants in the Great Lakes Ecosystem. *Environmental Science and Technology* **15**: 30.

Eisenreich, S.J. 1987. The chemical limnology of non-polar organic contaminants: polychlorinated biphenyls in Lake Superior. In Hites, R.A. Eisenreich, S.J. (Eds.), Source and Fate of Aquatic Pollutants. *American Chemical Society:* Chapter 13.

Ellyson, T. September 17th, 2012. Disease hits deer hard; season not in danger. Columbus Telegram. Accessed from: http://columbustelegram.com/news/local/disease-hits-deer-hard-season-not-in-danger/article_a9595a44-00c0-11e2-a3d0-001a4bcf887a.html

Emery, L. 1985. *Review of fish species introduced into the Great Lakes,* 1819-1974. Great Lakes Fishery Commission Technical Report No. 45. Great Lakes Fishery Commission, Ann Arbor, Michigan.

Environment Canada, and U.S.EPA. 2003. *State of the Great Lakes 2003*. Environment Canada and United States Environmental Protection Agency, Ottawa, ON and Washington, DC. EPA 905-R-03-004.

Environment Canada, 2006. Guidelines for the Management of Wastes Containing Polychlorinated Biphenyls (PCBs) http://www.ec.gc.ca/drgd-wrmd/default.asp?lang=En&n=95EC9FA8-1&offset=3&toc=show#anchor13

Environment Canada. (April 8, 2011). *PCB Regulations*: An Overview. Accessed from: http://www.ec.gc.ca/bpc-pcb/default.asp?lang=en&n=E794BDF1-1&printerversion=true. Date of Access: June 8th 2011.

Environmental Protection Agency (EPA), 2012. DDT-A Brief History and Status. Accessed from: http://www.epa.gov/pesticides/factsheets/chemicals/ddt-brief-history- status.htm.

Environmental Protection Agency (EPA). 2012. Mercury Basic Information. Accessed from: http://www.epa.gov/hg/about.htm

Environmental Protection Agency (EPA). 2002. Great Lakes Strategy 2002 (available at http://www.epa.gov/glnpo/gls/glstoc.html). ———. 2003. *Update: National listing of fish and wildlife advisories*. Office of Water, U.S.EPA., Washington, DC. EPA-823-F-03-003.

ESG (Environmental Sciences Group.) 1999a. Mid-Canada Line 1998 site assessment/delineation. Kingston, Ontario: Royal Military College. Environmental Sciences Group.

Evans, D.O., Nicholls, K.H., Allen, Y .C., and McMurtry, M.J. 1996. Historical land use, phosphorus loading, and loss of fish habitat in Lake Simcoe, Canada. *Can. J. Fish. Aquat. Sci.* **53**: 194–218.

Evans, D.O. 2006. *Effects of hypoxia on scope-for-activity of lake trout: defining a new dissolved oxygen criterion for protection of lake trout habitat.* Habitat and Fisheries Unit, Aquatic Research and Development Section, Ministry of Natural Resources, Peterborough, ON. Technical Report 2005-01.

Ewald, G., Larsson, P., Linge, H., Okla, L., Szarzi, N. 1998. Biotransport of organic pollutants to an inland Alaska lake by migrating Sockeye salmon (Oncorhyncus nerka). *Arctic* **51**: 40–47.

FAO. World Aquaculture 2010. Food and Agricultural Organisation of the United Nations 2010. http://www.fao.org/docrep/014/ba0132e/ba0132e.pdf.

FAO and WHO. 2010. Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption Executive Summary. 25-29 January 2010, Rome, Italy. Accessed from: *ftp://ftp.fao.org/FI/.../risk_consumption/executive_summary.pdf* . Date of Access: June 8, 2011.

Fein, G.G., Jacobson, J.L., Jacobson, S.W., Schwartz, P.M., Dowler, J.K. 1984. Prenatal exposure to polychlorinated biphenyls. *American Journal of Epidemiology* **105** (2): 315-320.

Fernandes, A., Mortimer, D., Rose, M., Gem, M. 2010. Dioxins (PCDD/Fs) and PCBs in offal: Occurrence and dietary exposure. *Chemosphere* **81**: 536–540.

Ficko, S.A., Rutter, A., Zeeb, B.A. 2010. Potential for phytoextraction of PCBs from contaminated soils using weeds. *Science of the Total Environment* **408:** 3469–3476.

Finley, BL; Connor, KT; Scott, PK. 2003. The use of toxic equivalency factor distributions in probabilistic risk assessments for dioxins, furans and *Journal of Toxicology and Environmental Health* **66** (6): 533-550.

Fisk, A. T.; Hobson, K. A.; Norstrom, R. J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the north water polynya marine food web. *Environ. Sci. Technol*. **35**: 732- 738.

Fitchko, J.1986. Literature review of the effects of persistent toxic substances on Great Lakes biota. International Joint Commission, Windsor, ON, Canada.

Foster, G.D., S.M. Baksi, and J.C. Means. 1987. Bioaccumulation of trace organic contaminants from sediments by Baltic clams *(Macoma balthica)* and soft-shell clams *(Mya arenaria*). *Environ. Toxicol. Chem*. **6**: 969–976.

Fraser, D.G., Mabee, W., Slaymaker, O. 2003. Mutual vulnerability, mutual dependence: The reflexive relation between human society and the environment. *Global Environmental Change* **13**: 137-144.

Fuller, G.B. and Hobson, W.C. In *PCBs and the Environment*; Waid, JS., Ed.; CRC Press: Boca Raton, 1986; pp. 101-125.

Garcia-Berthou, E. 2001. Size- and depth-dependent variation in habitat and diet of the common carp (*Cyprinus carpio). Aquatic Sciences* **63**: 466-476.

Gewurtz, S.B., Bhavsar, S.P., Jackson, D.A., Fletcher, R., Awad, E., Moody, R., Reiner, E.J. 2010. Temporal and spatial trends of organochlorines and mercury in Fishes from the St. Clair River/Lake St. Clair corridor, Canada. *Journal of Great Lakes Research* **36**: 100–112.

Gewurtz, S.B., Lega, R., Crozier, P.W., Whittle, D.M., Fayex, L., Reiner, E.J., Helm, P., Marvin, C.H., Tomy, G.T. 2009. Factors influencing trends of polychlorinated naphthaneles and other dioxin-like compounds in Lake Trout (*Salvelinus namaycush)* from Lake Ontario, North America (1970- 2004). *Environmental Toxicology and Chemistry*. 28, **5**: 921–930, 2009.

Ghosh M, Singh SP. 2005. A review of phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research* **3**:1-18.

Giesy et al., 1997. Polychloronated Dibenzo-p-Dioxins, Dibenzo-furans, Biphenyls and 2,3,7,8-Tetrachlorodibenzo-p-Dioxin equivalents in fishes from Saginaw Bay, Michigan. 1997. *Environmental Toxicology and Chemistry* **16**: 713-724.

Giesy, J.P., Bowerman, W.W., Mora, M.A., Verbrugge, D.A., Othoudt, R.A., Newsted, J.L., Summer, C.L., Aulerich, R.J., Bursian, S.J., Ludwig, J.P., Dawson, G.A., Kubiak, T.J. Best, D.A., and Tillitt, D.E. 1995. Contaminants in fishes from Great Lakes- influenced sections and above dams of three Michigan Rivers: III. Implications for health of bald eagles. *Archives of Environmental Contamination and Toxicology* **29** (3): 309-321.

Giesy, J.P., Jude, D.J., Tillitt, D.E., Gale, R.W., Meadows, J.C., Zajieck, J.L., Peterman, P.H., Verbrugge, D.A., Sanderson, J.T., Schwartz, T.R., Tuchman, ML.1997. Polychloronated Dibenzo-*p*-Dioxins, Dibenxonfurans, Biphenyls and 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin equivalents in fishes from Saginaw Bay, Michigan. 1997. *Environmental Toxicology and Chemistry* **16** (4): 713-724.

Giesy, J.P., Verbrugge, D.A., Othoudt, R.A., Bowerman, W.W., Mora, M.A., Jones, P. D., Newsted, J.L., Vandervoort, C., Heaton, S.N., Aulerich, R.J., Bursian, S.J. Ludwig, J.P., Dawson, G.A., Kubiak, T.J., Best, D.A., and Tillitt, D.E. 1994. Contaminants in fishes from the Great Lakes-influenced sections above dams of three Michigan rivers. II Implications for health of mink. *Archives of Environmental Contamination and Toxicology* **27** (2):213-223.

Gilad, O., Yun, S., Zagmutt-Vergara, F.J., Leutenegger, C.M., Bercovier, H., Hedrick, R.P. 2004. Concentrations of a Koi herpesvirus (KHV) in tissues of experimentally-infected *Cyprinus carpio koi* as assessed by real-time TaqMan PCR. *Diseases of Aquatic Organisms* **60**: 179-187.

Gilad, O., Yun, S., Adkison, M.A., Way, K., Willits, N.H., Bercovier, H. and Hedrick, R.P. 2003. Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* **84**: 2661-2668.

Gilbertson, M. 1992. PCB and dioxin research and implications for fisheries research and resource management. *Canadian Journal of Fisheries and Aquatic Sciences* **17**: 26-27.

Gilbertson M. and Carpenter D.O. 2004. An ecosystem approach to the health effects of mercury in the Great Lakes basin ecosystem. *Environ Res*. **9** (3): 240-6.

Gobas FAPC, Morrison HA. Bioconcentration and biomagnification in the aquatic environment. In: Boethling RS, Mackay D, editors. Handbook of property estimation methods for chemicals: Environmental health and sciences. Boca Raton, FL, U.S.A: Lewis; 2000. p. 189–231.

Goodwin, A.E. 2002. First Report of Spring Viraemia of Carp Virus (SVCV) in North America. *Journal of Aquatic Animal Health* **14:** 161–164.

Goodwin, A.E., Khoo, L., Lapatra, S.E., Bonar, C., Key, D.W., Garner, M., Lee, M.V., Hanson, L. 2006. Goldfish Hematopoietic Necrosis Herpesvirus (Cyprinid Herpesvirus 2) in the USA: Molecular Confirmation of Isolates from Diseased Fish. *Journal of Aquatic Animal Health* **18**: 1 1–18.

Goyette, D., D. Brand, and M. Thomas. 1988. Prevalence of idiopathic liver lesions in English sole and epidermal abnormalities in flatfish from Vancouver Harbour, British Columbia, 1986. Regional Program Report 87-09. Environment Canada, Vancouver. 48 pp.

Gray, W.L., Mullis, L., LaPatra, S.E., Groff, J.M., Goodwin, A. 2002. Detection of koi herpesvirus DNA in tissues of infected fish. *Journal of Fish Diseases* **25**: 171-178.

Gregory-Eaves, I., Demers, M. J., Kimpe, L., Krummel, E. M., Macdonald, R. W., Finney, B. P., Blais, J. M. 2007. Tracing salmon- derived nutrients and contaminants in freshwater food webs across a pronounced spawner density gradient. *Environ. Toxicol. Chem*. **26** (6): 1100–1008.

Guan, J., Chan, M., Ma, B., Grenier, C., Wilkie, D.C., Pasick, J., Brooks, B.W., Spencer, J.L., 2008. Development of methods for detection and quantification of avian influenza and Newcastle disease viruses in compost by real-time reverse transcription polymerase chain reaction and virus isolation. *Poult. Sci.* **87** (5): 838–843.

Guildford, S.J., Muir, D.C.G., Houde., Evans, M.S., Kidd, K.A., Whittle, D.M., Drouillard, O.K., Wang, X., Anderson, M.R., Bronte, C.R., Devault, D.S., Haffner, D., Payne, J., Kling, H.J. 2008. PCB Concentrations in Lake Trout (Salvelinus namaycush) Are Correlated to Habitat Use and Lake Characteristics. *Environ. Sci. Technol. 42:* 8239–8244.

Guimaladevi, I., Knon, T., Venugoapl, M.N., Sakai, M. 2004. Detection of koi herpesvirus in common carp, *Cyrpinus carpio* L., by loop-mediated isothermal amplification. *Journal of Fish Diseases* **27** (10): 583-589.

Haenen, O.L.M., Way, K; Bergmann, S.M. and Ariel, E. 2004. The emergence of koi herpesvirus and its significance to European aquaculture. Bulletin of the European Association of Fish Pathologists **26**: 293-307.

Hale R.C, La Guardia M.J., Harvey E.P., Matteson Mainor T., Duff ,W.H, Gaylor M.O. 2001. Polybrominated diphenyl ether flame-retardants in Virginia freshwater fishes (USA). *Environ Sci Techno.l* **35**: 4585– 4591.

Haramoto, E., Kitajima, M., Katayama, H., Ohgaki, S. 2007. Detection of koi herpesvirus DNA in river water in Japan. *Journal of Fish Diseases* **30** (1): 59- 61.

Hashimm M.A., Mukhopadhyay, S. Sahu, J.N., Sengupta, B. 2011. Remediation technologies for heavy metal contaminated groundwater. *Journal of Environmental Management* **92**: 2355-2388.

Health Canada. (2007a). *Toxicological reference values of organic contaminants - internal reference document.* Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Health Products & Food Branch, Health Canada, Ottawa.

Health Canada. (2007b). *Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption.* Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch.

Heaton, S.N. Bursian, S.J., Giesy, J.P., Tillit, D.E., Render, J.A., Jones, P.D., Verbrugge, D.A., Kubiak, T.J., and Aulerich, R.J. 1995. Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. *Archives of Environmental Contamination and Toxicology* **28** (3): 334- 343.

Hedrick R.P., Gilad O., Yun, S., Spangenberg, J.V., Marty, G.D., Nordhausen, R.W., Kebus, M.J., Bercovier, H., & Eldar, A., (2000) A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *Journal of Aquatic Animal Health* **12**: 44-57.

Hedrick RP. and Sano, T. 1989. Herpesviruses of fishes. In: *Viruses of Lower Vertebrates* (ed. By W. Ahne & E. Kurstak), pp. 161-170. Springer-Verlag, Berlin.

Helm, P.A., et al., (2011). Lake-wide distribution and depositional history of current- and past-use persistent organic pollutants in Lake Simcoe, Ontario, Canada. *J. Great Lakes Res* **37:** 132-141.

Hendricks, AJ; Van der Linde, A; Cornelissen, G; Sijm, DTHM. 2001. *Environmental Toxicology and Chemistry* **20**: 1399-1420.

Hickey, J.P., Batterman, S.A., Chernyak, S.M. 2006. Trends of chlorinated organic contaminants in Great Lakes trout and walleye from 1970 to 1998. *Archives of Environmental Contamination and Toxicology* **50** (1): 97-110.

Hickman, Z.A. and Reid, B.J. 2008. Earthworm assisted bioremediation of organic contaminants. *Environment International* **34**: 1072–1081.

Hites R.A., Foran J.A., Carpenter D.O., Hamilton M.C., Knuth B.A., Schwager S.J. 2004. Global assessment of organic contaminants in farmed salmon. *Science* **303**: 226–9.

Hoffman, R.C. Environmental change and the culture of common carp in medieval Europe. *Guelph Ichthyology Reviews* ISSN 1181-8549. Number 3: 56-85. Institute of Ichythyology, University of Guelph, Guelph Ontario

Honjo, M.N; Minamoto, T., Kawabata, Z. 2011 (in press). Reservoirs of Cyprinid herpesvirus 3 (CyHV-3) DNA in sediments of natural lakes and ponds. *Veterinary Microbiology*. doi:10.1016/j.vetmic.2011.09.005.

Horwitz, KB., and McGuire, WL. 1978. Estrogen control of progesterone receptor in human breast cancer: Correlation with nuclear processing of estrogen receptor. *Journal of Biological Chemistry* **253** (7): 2223-2228.

Ikonomou M.G., Rayne S., Addison R.F. 2002. Exponential increases of the brominated flame retardants, polybrominated diphenyl ethers, in the Canadian Arctic from 1981 to 2000. *Environ Sci Technol* **36**:1886–1892.

Ilouze, M., Dishon, A. and Kotler, M. 2006b. Characterization of a novel virus causing a lethal disease in carp and koi. *Microbiol. Mol. Biol. Rev*. **70**: 147–156.

International Association for Great Lakes Research (IAGLR). 2002. Linking science and policy for urban nonpoint source pollution in the Great Lakes Region. http://www.iaglr.org/scipolicy/nps/nps_iaglr02.pdf. November 2002.

International Joint Commission. 1993. IJC recommends United States, Canada phase out 11 toxics persistent in lakes. Environmental Report-BNA 24: 1063. Windsor, ON, Canada.

Iwata, H., Tanabe, S., Sakal, N., Tatsukawa, R. 1993. Distribution of persistent organochlorines in the ocean air and surface seawater and the role of ocean on their global transport and fate. *Environ. Sci. Technol*. **27**: 1080-1098.

Jacobs, M., Ferrario, J. and Byrne, C. 2002. Investigation of polychlorinated dibenzo-p-dioxins, dibenzo-p-furans and selected coplanar biphenyls in Scottish farmed Atlantic salmon (Salmo salar). *Chemosphere* **47**: 183–191.

Jager, T., Van Der Wal, L., Fleuren, R.H.L.J., Barendregt, A., Hermens, J.L.M. 2005. Bioaccumulation of organic chemicals in contaminated soils: evaluation of bioassays with earthworms. *Environmental Science and Technology* **39**: 293-298.

Jeremiason, J.D., Hornbucle, K.C., Eisenreich, S.J. 1994. PCBs in Lake Superior, 1978-1992-Decreases in water concentrations reflect loss by volatilization. *Environmental Science and Technology* **28** (5): 903-914.

Kalbasi, A., Mukhtar, S., Hawkins, S.E., Auvermann, B.W., 2005. Carcass composting for management of farm mortalities: a review. *Compost Sci. Util*. **13**: 543–559.

Kelley, G.O., Waltzek, T.B., McDowell, T.S., Yun, S.C., Lapatra, S.E., Hedrick, R.P. 2005. Genetic relationships among Herpes-like viruses Isolated from sturgeon. *Journal of Aquatic Animal Health* **17**: 297-303.

Khan, A. 2012. Extreme Summer Heat Causing Massive Fish Die Off. International Science Times. Accessed from: http://www.isciencetimes.com/articles/3600/20120806/extreme- summer-heat-causing-massive-fish-die.htm

Kimbrough, R.D., 1995. Polychlorinated biphenyls (PCB) and human health: an update. *Crit. Rev. Toxicol*. **25**: 133–163.

Knezovich, J.P., F.L. Harrison, and R.G. Wilhelm. 1987. The bioavailability of sediment-sorbed organic chemicals: A review. *Water Air Soil Pollut*. **32**: 233–245.

Kosalwat, P., and A.W. Knight. 1987. Chronic toxicity of copper to a partial life cycle of the midge, *Chironomus decorus*. *Arch. Environ. Contam. Toxicol*. **16**: 283–290.

Kristie A; Vanpatten, KA; Nunan and Lightner.2004. Seabirds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. *Aquaculture* **241** (1-4): 31-46.

Krueger, C. C., and May, B. 1991. Ecological and genetic effects of salmonid introductions in North America. *Can. J. Fish. Aquat. Sci.* **48** (Suppl. 1): 66-77.

Krümmel, E.M; Macdonald, R.W; Kimpe, L.E; Gregory-Eaves, I; Demers M.J; Smol, J.P; Finney, B; Blais. J.M; 2003. Delivery of pollutants by spawning salmon. *Nature* **425**: 255-256.

Kuhnlein, H.V. and Chan, H.M. 2000. Environment and Contaminants in Traditional Food Systems of Northern Indigenous Peoples. *Annual Rev. Nutrition*. **20**: 595-626.

Lamberson, J.O., and R.C. Swartz. 1992. Spiked-sediment toxicity test approach. In: Sediment classification compendium. EPA 823-R-92- 006. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Lamberson, J.O., T.H. DeWitt, and R.C. Swartz. 1992. In: Sediment toxicity assessment, G.A. Burton, ed., pp. 183–211. Lewis Publishers, Inc., Boca Raton, FL.

Landry, EF; Vaughn, JM; Vicale, TJ; Mann, R. 1983. Accumulation of sediment associated viruses in shellfish. *Applied and Microbiology* **45:** 238-247.

Larson, P., Olka, S., Rydlink, S., Westoo, B. 1990. Contaminated Sediment as a Source of PCBs in a River System. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 746-754.

Larsson, P. 1985. Contaminated sediments of lakes and oceans act as sources of chlorinated hydrocarbons for release to water and atmosphere. *Nature* **317**: 347–349.

Lavado, R., Thibaut, R., Raldu´, D, Mart´ın, R., Porte, C. 2004. First evidence of endocrine disruption in feral carp from the Ebro River. *Toxicology and Applied Pharmacology* **196**: 247–257.

Letcher, R.J., Lemmen, J.G., van der Burg, B., Brouwer, A.,Bergman, A., Giesy, J.P., van den Berg, M. 2002. *In Vitro* effects of Aryl Methyl Sulfone Metabolites of Polychlorinated Biphenyls and 2,2-Bis(4-chlorophenyl)-1,1-dicholoethene on 17B-Estradiol-induced gene expression in several bioassay systems. *Toxicological Sciences* **69**: 362-372.

Li, A., Rockne, K.J., Sturchio, N., Song, W., Ford, J.C., Wei, H. 2009. PCBs in sediments of the Great Lakes- Distribution and trends, homolog and chlorine patterns, and in situ degradation. *Environmental Pollution* **157** (1): 141-147.

Li, M.H. and Hansen, LG. (1997). Consideration of enzyme and endocrine interactions in the risk assessment of PCBs. *Reviews in Toxicology* **1**: 71-156.

Loganathan, B.G., Kannan, K., Watanabe, I., Kawano, M., Irvine, K., Kumar, S., Sikka, HC. 1995. Isomer-specific determination and toxic evaluation of Polychlorinated Biphenyls, Polychlorinated/brominated Dibenzo-*p*-dioxins and Dibenzofurans, Polybrominated Biphenyl Ethers, and Extractable Organic Halogen in Carp from the Buffalo River, New York. *Environmental Science and Technology* **29**: 1832-1838.

Long, E.R., and L.G. Morgan. 1990. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. National Oceanic and Atmospheric Administration Tech. Memo. NOS OMA 52. Seattle, WA. 175 pp. + app.

Loring, D.H., and R.T.T. Rantala. 1992. Manual for the geochemical analysis of marine sediments and suspended particulate matter. *Earth- Sci. Rev*. **32**: 235.

MacDonald, C.R., Metcalfe, C.D., Balch, G.C., Metcalfe, T.L. 1993. Distribution of PCB congeners in seven lake systems: Interactions between sediment and food- web transport. *Environmental Toxicology and Chemistry* **12**: 1991-2003.

Macek, T., Mackova, M., Kas, J. (2000) Exploitation of plants for the removal of organics in environmental remediation. *Biotechnology* **18** (1): 23-34.

Madenjian, C.P., Keir, M.J., Whittle, D.M. 2011. Sexual difference in mercury concentrations of lake trout (Salvelinus namaycush) from Lake Ontario. *Chemosphere* **83**: 903–908.

Malins, D.C., M.M. Krahn, M.S. Myers, L.D. Rhodes, D.W. Brown, C.A. Krone, B.B. McCain, and S.L. Chan. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbour: Relationships with hepatic neoplasms and other hepatic lesions in English sole *(Parophrys vetulus)*. *Carcinogenesis* **6**(10): 1463–1469.

Matsui, K., Honjo, M., Kohmatsu, Y., Uchii, K., Yonekura, R, Kawabata, Z. 2008. Detection and significance of koi herpesvirus (KHV) in freshwater environments. *Freshwater Biology* **53** (6): 1262-1272.

Maxim, L.D. and Harrington, L. 1984. A review of the food and drug administration risk analysis for polychlorinated biphenyl congeners in blood of Wisconsin sport fish consumers. *Archives of Environmental Contamination and Toxicology* **20**: 56-61.35-45.

Maya, I., Maya, D., Ariel, D., Moshe, K., Arnon, D., 2010. The outbreak of carp disease caused by CyHV-3 as a model for new emerging viral diseases in aquaculture: a review. *Ecol. Res.* **26** : 885-592.

McIntyre, J.K., Beauchamp, D.A. 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. *Science of the Total Environment* **372**: 571-584.

McLeod, P.B., Van den Heuvel-Greve, M.J., Luoma, S.N., Luthy, R.G., 2007. Biological uptake of polychlorinated biphenyls by Macoma balthica from sediment amended with activated carbon. *Environ. Toxicol. Chem*. **26**: 980– 987.

Michel, B., Fournier, G., Lieffrig, F., Costes, B., Vanderplasschen, A., 2010a. Cyprinid herpesvirus 3. *Emerg. Infect. Dis*. **16** (12): 1835–1843.

Mills, E.L., Leach, J.J., Carlton, J.T., Secor, C.L. 1993. Exotic Species in the Great Lakes: A History of Biotic Crises and Anthropogenic Introductions. *Internal. Assoc. Great Lakes Res. J. Great Lakes Res*. **19** (1): 1-54.

Miller, M.A. 1994. Organichlorine concentration dynamics in Lake Michigan Chinook salmon (Oncohynchus tshawytscha). *Archives of Environmental Contamination and Toxicology* **27** (3): 367-374.

Mills, E.L., Leach, J.H., Carlton, J.T., Secor. 1993. Exotic species in the Great Lakes: A History of Biotic Crises and Anthropogenic Introductions. *Journal of Great Lakes Research* **19** (1): 1-54.

Minamoto, T., Honjo, M.N., Uchii, K., Yamanaka, H., Suzuki, A.A., Kohmatsu, Y., Iida, T., Kawabata, Z. 2009. Detection of cyprinid herpesvirus 3 DNA in river water during and after an outbreak. *Veterinary Microbiology* **135** (3- 4): 261-266.

Minchin, D. 2007. Aquaculture and transport in a changing environment: Overlap and links in the spread of alien biota. *Marine Pollution Bulletin* **55** (7-9): 302-313.

Ministry of Natural Resources (MNR) December 2011. Ontario's VHS Management Zone and the Lake Simcoe Management Zone. Accessed from:www.mnr.gov.on.ca/en/Business/LetsFish/2ColumnSubPage/23 471.html. Accessed March 2012).

Ministry of Natural Resources (MNR) 2009. Carp Die-off 2009: Lessons Learned.

Ministry of Natural Resources. May 2008. Fact Sheet Koi Herpes Virus. http://www.mnr.gov.on.ca/241236.pdf

Ministry of Natural Resources. (July, 2008). Carp die-off in Central Ontario. http://www.mnr.gov.on.ca/248890.pdf

Ministry of the Environment. 2009. "Guide to Eating Ontario Sport Fish 2009-2010" Queen's Printer for Ontario.

Ministry of the Environment. 2009. Lake Simcoe Protection Plan. http://www.ene.gov.on.ca/en/water/lakesimcoe/index.php

Moermond, C.T.A., Roozen, F.C.J.M., Zwolsman, J.J.G. and Koelmans, A.A. 2004. Uptake of Sediment-Bound Bioavailable Polychlorobiphenyls by Benthivorous Carp (Cyprinus carpio). *Environmental Science and Technology* **38**: 4503-4509.

Murray, A.G. 2012 (in press). Epidemiology of the spread of viral diseases under aquaculture. *Current opinion in Virology* **3**: 1-5.

Nielsen, H.M., Ødegård, J., Gjerde, B., Ardo, L., Jeney, G., Jeney, Z. 2010. Genetic analysis of common carp (Cyprinus carpio) strains I: Genetic parameters and heterosis for growth traits and survival. *Aquaculture* **304**: 14–21.

Norén K., Meironyté D. 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere* **40**: 1111–1123.

Norstrom R.J., Simon M., Moisey J., Wakeford B., Weseloh D.V. 2002. Geographical distribution (2000) and temporal trends (1981–2000) of brominated diphenyl ethers in Great Lakes her- ring gull eggs. *Environ Sci Technol* **36:** 4783–4789.

Ohtsubo, Y., Kudo, T., Tsuda, M., Nagata, Y., 2004. Strategies for bioremediation of polychlorinated biphenyls. *Appl. Microbiol. Biotechnol*. **65**: 250–258.

Olling, M., Derks, H., Berender, P., Liem, A., de Jong, A., 1991. Toxicokinetics of eight labelled PCDDs and PCDFs in lactating cows. *Chemosphere*. **23**: 1377–1385.

Olson, J., 2003. In: Schecter, A., Gasiewicz, T. (Eds.), Dioxins and Health. Wiley Interscience, Hoboken, NJ, USA. ISBN:0-471-43355-1.

Oropesa, A.L., Garcia-Cambero, J.P., Gomez, L., Roncero, V., Soler, F. 2008. Effect of long-term exposure to simazine on histopathology, hematological, and biochemical parameters in *Cyprinus carpio. Environmental Toxicology.* **24** (2): 187-199.

O'Toole, S., Metcalfe, C.,Craine, I., Gross, M. 2006. Release of persistent organic contaminants from carcasses of Lake Ontario Chinook salmon (*Oncorhynchus tshawytscha*). *Environmental Pollution* **140** (1): 102-113.

Patil M., Kulkarni R.S. 1993. Ovarian and hepatic biochemical response to sumaach (a crude form of HCG) in fish, *Notopterus notopterus* Pallas, under pesticide treatment. *Geobios* **20**: 255-259.

Perelberg, A., Smirnov, M., Hutoran, M., Diamant, A., Bejerano, I. and Kotler, M. (2003) Epidemiological description of a new viral disease afflicting cultured Cyprinus carpio in Israel. Isr. *J. Aquaculture-Bamidgeh* **55**: 5–12.

Perelberg, A., Ronen, A., Hutoran, M., Smith, Y., Kotler, M. 2005. Protection of culture *Cyprinus carpio* against a lethal viral disease by an attenuated virus v accine. *Vaccine* **23** (26): 3396-3403.

Pikarsky, E., Ronen, A., Abramowitz, J., Levavi-Sivan, B., Hutoran, M., Shapira, Y., Steinitz, M., Perelberg, A., Soffer, D. and Kotler, M. 2004. Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. *J. Virol*. **78**: 9544–9551.

Pizzol, L., Agostini, P., Marcomini, A. 2011.Regional risk assessment for contaminated sites Part 2: Ranking of potentially contaminated sites. *Environment International* **37**: 1307–1320.

Pokorova, D., Vesely, T., Piackova, V., Reschova, S., Hulova, J. 2005. Current knowledge on koi herpesvirus (KHV): a review. *Veterinary Medicine-Czech* **50** (4): 139-147.

Poland, J.S., Mitchell, S., Rutter, A. 2001. Remediation of former military bases in the Canadian Arctic. *Cold Regions Science and Technology* **32**: 93–105.

Rahel F.J. (2007) Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. *Freshwater Biology* **52** (4): 696-710.

Rasmussen, J.B., Rowan, D.J., Lean, D.R.S., Carey, J.H. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. *Can. J. Fish. Aquat. Sci.* **4**: 2030-2038.

Restum, J.C., Bursian, S.J., Giesy, J.P.,Render, J.A., Helferich, W.G., Shipp, E.B., Verbrugge, D.A. 1998. Multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron, on Mink. 1. Effects on mink reproduction, kit growth and survival, and selected biological parameters. 1998. *Journal of Toxicology and Environmental Health. Journal Part A*. **54**: 343-375.

Rice, D.C. Neurotoxicity of lead, methylmercury and PCBs in relation to the Great Lakes. *Environmental Health Perspectives* **103** (Supppl. 9): 1-8.

Rijkenberg, M.J.A. and Depree, C.V. 2010. Heavy metal stabilization in contaminated road-derived sediments. *Science of the Total Environment* **408**: 1212–1220.

Ronen, A., Perelberg, A., Abramowitz, J., Hutoran, M., Tinman, S., Bejerano, I., Steinitz, M., Kotler, M. 2003. Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. *Vaccine* **21** (32): 4677-4684.

Safe, S.H. 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Critical Reviews in Toxicology* **24**: 87-149.

Salomons, W., N.M. de Rooij, H. Kerdijk, and J. Bril. 1987. Sediments as a source for contaminants. *Hydrobiologia* **149**: 13–30.

Sarica, J., Amyot, M., Hare, L., Blanchfield, P., Bodaly, R. A., Hintelmann, H., Lucotte, M. 2005. Mercury transfer from fish carcasses to scavengers in boreal lakes. *Environ. Pollut*. **134**: 13–22.

Scheffer, M; Portielje, R; Zambrano, L. 2003. Fish facilitate wave resuspension of sediment. *Limnology and Oceanography* **48**: 1920-1926.

Scheider, W.A., Cox, C., Hayton, A., and Hitchin, G. 1998. Current status and temporal trends in concentrations of persistent toxic substances in sport fish and juvenile forage fish in the Canadian waters of the Great Lakes. *Environ. Monit. Assess***. 53**: 57–76.

Schultz-Bull, E. E.; Petrick, G.; Bruhn, R.; Duinker, J. C. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. 1998. *Chem*. **61**: 101-114.

Seemamahannop, R., Berthod, A., Maples, M., Kapila, S., Armstrong, D.W. 2005. Uptake and enantioselective elimination of chlordane compounds by common carp (Cyprinus carpio, L.). *Chemosphere* **59**: 493–500.

Shakkebaek, N.E., Rajpert-De Meyts, Jorgansen, E., Carlsen, E., Peterson, P.M., Giwercman, A., Andersen, A.G., Jensen, T.K., Anderson, A.M. and Muller, J. (1998). Germ cell cancer and disorders of spermatogenesis: An environmental connection? *Act A Pathologica Microbiological Et Immunologia Scandinavic*a **106** (1): 3-11.

Sharpe, R.M. and Skakkebaek, N.E. (1993). Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? (May 29) *The Lancet* **341**:1392-1395.

Shaun, T., Metcalfe, C., Craine, I., Gross, M. 2005. Release of persistent organic contaminants from carcasses of Lake Ontario Chinook salmon (*Oncorhynchus tshawytscha*). *Environmental Pollution* **140** (1):102-113.

Shephard, KL.1994. Functions for fish mucus. *Reviews for Fish Biology and Fisheries* **4** (4):401-429.

Sonzogni, W., Maack, L., Gibson, T., Degenhardt, D., Anderson, H., Fiore, B. 1991. Polychlorinated biphenyl congeners in blood of Wisconsin sport fish consumers. *Archives of Environmental Contamination and Toxicology* **20:** 56-61.

Sonzogni, W.C. and Swain, W.R. 1984. *Perspectives on human health concerns from Great Lakes contaminants in the Great Lakes*. John Wiley and Sons, New York, NY, USA, p.1-29.

Stanford, K., Nelson, V., Sexton, B., McAllister, T.A., Hao, X., Larney, F.J., 2007. Open-air windrows for winter disposal of frozen cattle mortalities: effects of ambient temperature and mortality layering. *Compost Sci. Util*. **15**: 257–266.

Stanford, K., McAllister, T.A., Reuter, T., Xu, W., Moyer, J.R., Larney, F.J., 2009. Biocontained mortality compost using liquid manure. *Compost Sci. Util*. **17** (3): 158–165.

Stapleton, H.M., Letcher, R.J., Li, J., Baker, J.E. 2004. Dietary accumulation and metabolism of Polybrominated Diphenyl Ethers by juvenile carp (*Cyrprinus carpio*). Environmental Toxicology and Chemistry **23**: 1939- 1946.

St-Hilaire S., Beevers N., Way K., Le Deuff R.M., Martin P. & Joiner C. (2005) Reactivation of koi herpesvirus infections in common carp Cyprinus carpio. *Diseases of Aquatic Organisms* **67**: 15–23.

Stockholm Convention on Persistent Organic Pollutants. 2008. The 12 Initial POPs under the Stockholm Convention. Accessed from: http://chm.pops.int/Convention/ThePOPs/tabid/673/Default.aspx. Date of Access: April 20th, 2012.

Suchan, P., Pulkrabová, J., Hajšlová, J., Kocourek, V. 2004. Pressurized liquid extraction in determination of polychlorinated biphenyls and organochlorine pesticides in fish samples. *Analytica Chimica Act A*. **520:** 193-200.

Suns, K.R., Hitchin, G.G., and Toner, D. 1993. Spatial and temporal trends of organochlorine contaminants in spottail shiners from selected sites in the Great-Lakes (1975–1990). *J. Great Lakes Res.* **19**:703–714.

Swackhamer, D.L. 2005. Aquatic processes and systems in perspective-The past, present and future of the North American Great Lakes: what lessons do they offer? *Journal of Environmental Monitoring* **7:** 540-544.

Tanabe, S. 1988. PCB problems in the future-foresight from current knowledge. *Environmental Pollution*. **50** (1-2): 5-28

Tharakan J., Tomlinson D., Addagada A., Shafagati A. 2006. Biotransformation of PCBs incontaminated sludge: potential for novel biological technologies. *Eng Life Sci.* **6** (1): 43–50.

The Barrie Examiner, October 24, 2011. Type E botulism to blame for bird deaths, MNR says. Accessed from: http://www.thebarrieexaminer.com/2011/10/24/type-e-botulism-to-blame-for-bird-deaths-mnr-says.

Tobin, ME. 2002. Developing methods to manage conflicts between humans and birds-three decades of change at the USDA National Wildlife Research Center. University of California, Davis. Pp. 91-96.

Tolonen, K.T., Karjalainen, J., Staff, S., Leppä, M. 2000. *Ecology of Freshwater Fish* **9**: 153-162.

TriState Neighbor, September 28th, 2012. Iowa cattle herds affected by virus common in deer. Accessed from: http://www.tristateneighbor.com/news/regional/article_4fbfdc04-0982- 11e2-8d66-001a4bcf887a.html

Trudel, M., Tremblay, A., Schetagne, R., Rasmussen, J.B., 2000. Estimating food consumption rates of fish using a mercury mass balance model. *Can. J. Fish. Aquat. Sci*. **57**: 414–428.

Tsuji, L.J.S., Wainman, B.C., Weber, J.P., Sutherland, C. et al. 2005. Protecting the health of First Nation Personnel at contaminated sites: A case study of Mid-Canada Radar Line Site 050 in Northern Canada. *Arctic* **58** (3): 233-240.

Tucker J.K., Cronin F.A. & Soergel D.W. (1996) Predation on zebra mussels (Dreissena polymorpha) by common carp (Cyprinus carpio). *Journal of Freshwater Ecology*, **11**: 363–375.

Uchii, K., Telschow, A., Minamoto, T., Yamanaka, H., Honjo, M.N., Matsui, K., Kawabata, Z. 2011. Transmission dynamics of an emerging infectious disease in wildlife through host reproductive cycles. *International Society for Microbial Ecology* **5**: 244-251.

UNEP (United Nations Environment Program). 1999. Guidelines for the Identification of PCBs and Materials containing PCBs. First Issue August. UNEP Chemicals, Switzerland.

UNEP. 2002. United Nations Environment Programme Chemicals. Mediterranean regional report. Regionally based assessment of persistent toxic substances. Global Environment Facility.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vio, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, M., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tykslind, M., Walker, N., Peterson, R.E. 2006. The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Science* **93** (2): 223-241.

Van den Berg, M., Birnbaum, L., Bosveld, A., Brunstroem, B., Cook, P., Feeley, M., Giesy, J., Hanberg, A., Hasegawa, R., Kennedy, S., Kubiak, T., Larsen, J., van Leeuwen, F., Liem, AKD., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D.,Tillit, D., Tykslind, M., Younes, M., Waern, F., Zacharewsk, T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs, for humans and wildlife. *Environmental Health Perspectives* **106** (12):775-792.

van Leeuwen, Feeley, M., Schrenk, D., Larsen, J.C., Farland, W., Younes, M. 2000. Dioxins: WHO's tolerable daily intake (TDI) revisited. *Chemosphere* **40**: 1095-1101.

Vasilyeva, G.K., Strijakova, E.R., 2007. Bioremediation of soil and sediments contaminated with polychlorinated biphenyls. *Microbiol*. **74**: 725–741.

Vasilyeva, G., Strijakova, E.R., Nikolaeva, S.N., Lebedev, A.T., Shea, P.J., 2010. Dynamics of PCB removal and detoxification in historically contaminated soils amended with activated carbon. *Environmental Pollution* **158**: 770–777.

Veith, G. 1968. Environmental Chemistry of the Chlorobiphenyls in the Milwaukee River. PhD dissertation, Water Chemistry Program, University of Wisconsin, Madison, Wisconsin.

Veldhoen, N., Ikonomou, M.G., Dubetz, C., MacPherson, N., Sampson, T., Kelly, B.C., Helbing, C.C. 2010. Gene expression profiling and environmental contaminant assessment of migrating Pacific salmon in the Fraser River watershed of British Columbia. *Aquatic Toxicology* **97**: 212–225.

Verbrugge, D.A., Giesy, J.P., Mora, M.A., Williams, L.L., Crawford, R.M., Rossman, R;., Moll, RA and Tuchman, ML. 1995. Concentrations of dissolved and particulate PCBs in water from the Saginaw River, Michigan. *Journal of Great Lakes Research* **21:** 219-233.

Verma A, Pillai M.K.K. 1991. Bioavailability of soil-bound residues of DDT and HCH to earthworms. *Curr Sci*. **61**(12): 840–3.

Walton, I. 1676. The complete angler or the contemplative man's recreation being a discourse of rivers, fishponds, fish and fishing not unworthy the perusal of most anglers, 5th edition (after the 1988 edition with modernized spelling by Bloomsbury Books, London. 224 pp).

Waltzek, T.B., Kelley, G.O., Stone, D.M., Way, K., Hanson, L., Fukuda, H., Hirono, I., Aoki, T., Davison, A.J. and Hedrick, R.P. (2005) Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family Herpesviridae. *J. Gen. Virol*. **86**, 1659– 1667.

Wang, Q., Kim, D., Dionysiou, D.D., Sorial, G.A., Timberlake, D. 2004. Sources and remediation for mercury contamination in aquatic systems- a literature review. *Environmental Pollution* **131**: 323-336.

Watanuki, H; Yamaguchi, T; Sakai, M. 2002. Suppression in function of phagocytic cells in common carp *Cyprinus carpio* L. injected with estradiol, progesterone or 11-ketotesterone. *Comp Biochem Physiol Part C Toxicol Pharmacol* **132**: 407-413.

Werner, D., Higgins, C.P., Luthy, R.G., 2005. The sequestration of PCB in Lake Hartwell sediments with activated carbon. *Water Res*. **39**: 2105–2113.

Werner, D., Ghosh, U., Luthy, R.G., 2006. Modeling polychlorinated biphenyl mass transfer after amendment of contaminated sediment with activated carbon. *Environ. Sci. Technol*. **40**: 4211–4218.

Weseloh, D.V., Ewins, P.J., Struger, J., Bishop, C.A., Postupalsky, S., Ludwig, J.P. 1995. Double-crested Cormorants of the Great Lakes: Changes in Population Size, Breeding distribution and Reproductive Output between 1913 and 1991. *Colonial Waterbirds* **18** (Special Publication): 48-59.

Wiegel, J., Wu, Q.Z., 2000. Microbial reductive dehalogenation of polychlorinated biphenyls. FEMS *Microbiol. Lett*. **32**: 1–15.

Wilkinson, K.G., 2007. The biosecurity of on-farm mortality composting. *J. Appl. Microbiol*. **102**: 609–618.

Williams, DR., Li, W., Hughes, M.A., Gonzalez, S.F., Vernon, C., Vidal, M.C., Jeney, Z., Jeney, G., Dixon, P., McAndrew, B., Bartfai, R., Orban, L., Trudeau, V., Rogers, J., Matthews, Fraser, LEJ., Gracey, AY., Cossins, AR., (2008). Genomic resources and microarrays for the common carp *Cyprinus carpio* L. *Journal of Fish Biology* **72** (9): 2095-2117.

Williams, L.L., Giesy, J.P., DeGalan, N., Verbrugge, D.A., Tillitt, D.E. Ankley, G.T., Welch, R.A. 1992. Prediction of concentrations of 2,3,7,8-TCDD equivalents (TCDD-EQ) from total concentrations of PCBs in fish fillets. *Environmental Science and Technology* **26**:1151-1159.

Winter, J.G., Eimers, M.C., Dillon, P.J., Scott, L.D., Scheider, WA; Willox, CC. 2007. Phosphorus Inputs to Lake Simcoe from 1990 to 2003: Declines in Tributary Loads and Observations on Lake Water Quality. *International Association of Great Lakes Research* **33**: 381-196.

World Health Organization (WHO), 1948. Preamble to the Constitution of the World Health Organization as adopted by the International Health Conference, New York, 19-22 June, 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, no. 2, p. 100) and entered into force on 7 April 1948.

World Health Organization (WHO). 1993. Polychlorinated biphenyls and terphenyls, 2nd ed. Geneva.

World Health Organization (WHO). 1993. Environmental Health Criteria 140: Polychlorinated biphenyls and terphenyls. 2ed. Geneva.

World Health Organization (WHO) 2000. Chapter 5.10 Polychlorinated Biphenyls. WHO Regional Office for Europe. Copenhagen, Denmark.

World Health Organization (WHO) Fact Sheet N˚ 270. August 2002. Botulism. Accessed from: www.who.int/mediacentre/factsheets/fs270/en/ February 2012.

World Health Organization (WHO) 2004. WHO Health-care waste management Fact Sheet N˚281. October 2004. www.who.int/mediacentre/factsheets/fs281/en/print.html.

World Health Organization (WHO). 2007. DDT The use of DDT in Malaria Control WHO Position Statement. Accessed from: http://www.who.int/ipcs/capacity_building/who_statement.pdf Date of Access: April 20th, 2012.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F. 2007. Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. *Environ. Sci. Technol.* **41**: 1584-1589.

Xie, P., Chen, Y., 1999. Threats to biodiversity in Chinese inland waters. *Ambio* **28** (8), 674–681.

Xu, W., Xu, Y., Jin, L., Reuter, T., Niu, D., You, J., Li, X., Stanford, K., McAllister, T.A., 2008. Development and evaluation of a composting system for emergency disposal of livestock mortalities and potential infectious manure. *J. Biotech*. 136: 709–710.

Ye, X., Schoenfuss, H.L., Jahnsm N.D., Delinsky, A.D., Strynar, M.J., Varns, J., Nakayama, S.F., Helfant, L., Lindstrom, A.B. 2008. Perfluorinated compounds in common carp (Cyprinus carpio) fillets from the Upper Mississippi River. *Environment International* **34**: 932-938.

Zabik, M.E., Zabik, M.J., Booren, Al. M., Nettles, M., Song, J.H; Welch, R; Humpfrey, H. 1995. Pesticides and total Polychlorinated Biphenyls in Chinook Salmon and Carp Harvested from the Great Lakes: Effects of Skin-on and Skin-off Processing and selected cooking methods. *Journal of Agriculture and Food Chemistry* **43** (4): 993-1001.

Zimmerman, J.R., Werner, D., Ghosh, U., Millward, R.N., Bridges, T.S., Luthy, R.G., 2005. Effects of dose and particle size on activated carbon treatment to sequester polychlorinated biphenyls and polycyclic aromatic hydrocarbons in marine sediments. *Environ. Toxicol. Chem*. **24**: 1594–1601.

Figure 1: Context Map

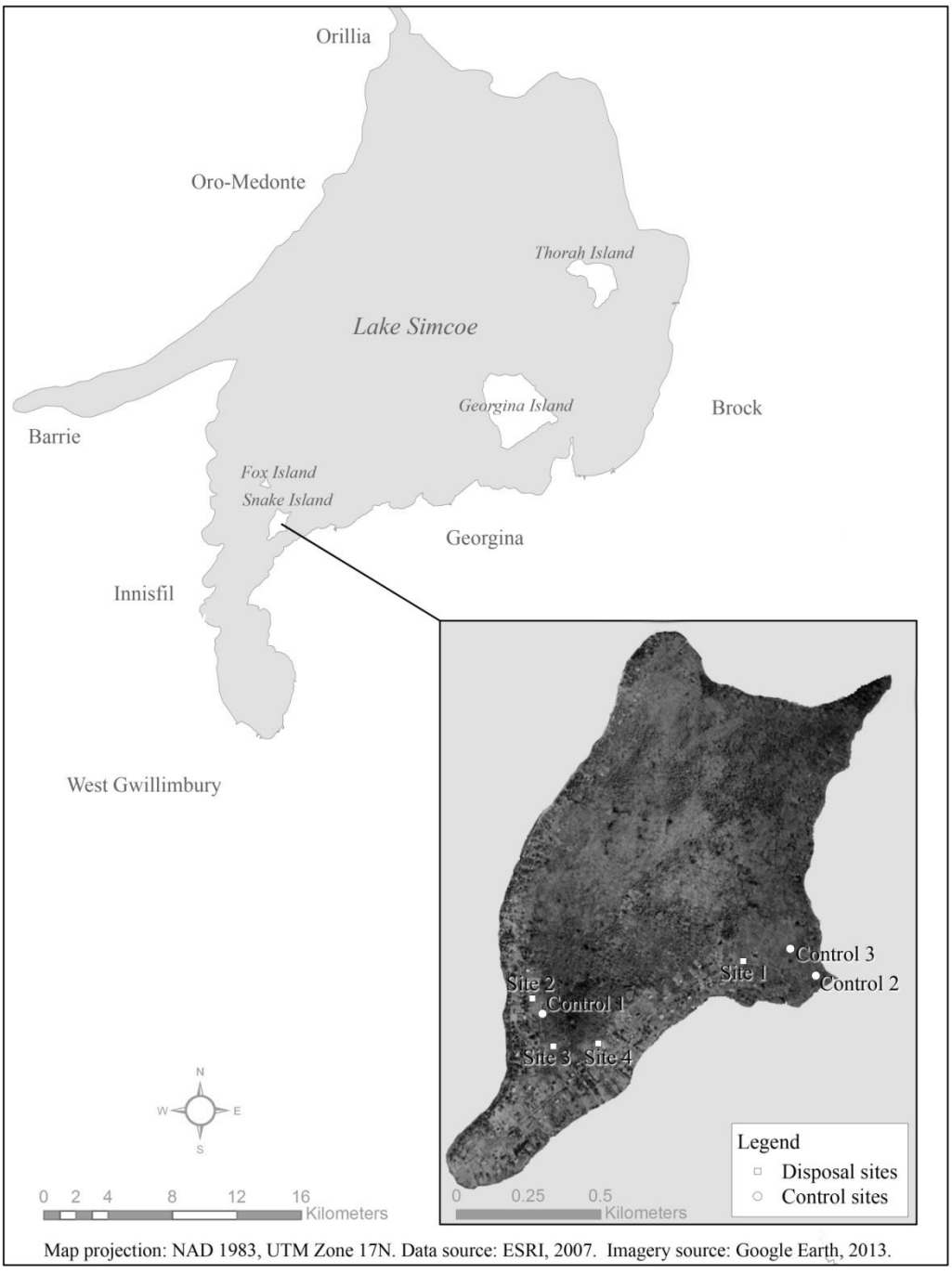

Figure 2: *Cyprinus carpio* found on the shore of Snake Island during the koi herpesvirus die-off.

(Photo credit: Kira Cooper, 2008)

Figure 3: *Cyprinus carpio* found floating during the koi herpesvirus die-off.

(Photo credit: Kira Cooper, 2008)

Figure 4: *Cyprinus carpio* left outside of garbage can by Island Grove parking lot (mainland directly across from Snake Island).

(Photo credit: Kira Cooper, 2008)

Figure 5: Cyprinus carpio carcass left on Lake Simcoe shoreline

(Photo credit: Claire Malcomson, 2008)

Figure 6: Cyprinus carpio spine collected from disposal site 1 during soil collection

(Photo credit: Kira Cooper, 2008)

Figure 7: Cyprinus carpio in Lake Simcoe summer 2012

(Photo credit: Kira Cooper, 2008)

Immunoassay methods

A Hach Pocket Colorimeter™ II Test Kit was used for the initial round of semi-quantitative PCB analysis. The detectable ranges for this instrument are between 1 ppm, 5 ppm, 10 ppm, 50 ppm. This instrument was chosen as it is a cost-effective and rapid detection method to determine whether or not the pits used to dispose carp carcasses had exceeded the legal contamination limit of 50 ppm PCB. Soil samples were stored at -20°C prior to analysis to ensure a constant temperature and to reduce any microbial interaction that could interfere with chemical readings. When sampling commenced, soils were homogenized using a sterile mortar and pestle, and placed in sterilized glass jars until analyzed.

Soil extraction: The following steps were conducted in accordance with the protocols provided with the Hach Pocket ColorimeterTM II Test Kit (see Hach Pocket ColorimeterTM II Test Kit Immunoassay Instruction Manual 59574-88) commencing at section 3 (Procedure for PCB in soil). Testing commenced with a 5 g aliquot of each soil sample added into an extraction vial where one scoop (HACH provided) of sodium sulfate was added. 10 mL of soil extractant (from HACH) was then added to the mixture, which was capped and shaken for one minute. 1.0-1.5 mL was removed from the mixture using a disposable pipette and deposited into a filtration barrel to screen out sediment through the microporous screen.

Immunoassay testing: The following steps were conducted according to the protocol provided for the Hach Pocket ColorimeterTM II Test Kit (see Hach Pocket ColorimeterTM II Test Kit Immunoassay Instruction Manual 59574-88) commencing at section 3.4 (procedure for soil extracts). Antibody cuvettes were labeled and placed in the provided Hach rack for storage. 0.5 mL of Diluent solution was pipetted into each cuvette using a precision pipet. Calibrator was then added to the cuvettes using a wiretrol™ pipet (new pipet tip was used for each sample to ensure there was no cross-contamination). A quantity of 50 µg of both calibrator and sample was used for samples testing for PCBs between the range of 1ppm and 5 ppm and 10 µg for tests in the 10 ppm and 50 ppm range.

Immediately following the addition of calibrator and sample, 0.6 mL of PCB enzyme conjugate were added to each cuvette and a 10 minute reaction was started. After ten minutes, the rack was mixed for 30 seconds (Hach, p. 2-3) and then another 5-minute reaction time was started. After the second reaction time, the rack was mixed one final time. Contents were then discarded and disposed of in accordance with proper laboratory methods and cuvettes were forcibly rinsed with deionized water. 0.5 mL of Color Developing Solution was added to the empty cuvettes for a 5-minute reaction time. After the reaction time had lapsed, 0.5 mL of Stop Solution was added in the

same order that the Color Developing Solution was added. One cuvette was labeled and filled with deionized (DI) water to serve as a zeroing cuvette to calibrate the machine. Upon calibration, cuvettes were added one at a time into the colorimeter, covered with the protective shield and readings were then recorded accordingly.

(Direct source: Analytical Services Unit, Queen's University)

Methods

Analysis of Polychlorinated Biphenyls (PCBs) in Soil, Paint and Concrete Samples

Analyses were conducted by the Analytical Services Unit, Queen's University, Kingston, Ontario. Each sample was clearly labelled and stored at low temperatures in a secured area before and after analysis.

All samples were spiked with an aliquot of decachlorobiphenyl (DCBP), a surrogate standard, prior to analysis by gas chromatography (GC) with electron capture detection (ECD). Samples were extracted with dichloromethane, using an orbital shaker or soxhlet apparatus. Extracts were concentrated using a rotoevaporator and the solvent was exchanged to hexane before cleanup of the sample, by passing the hexane containing the PCBs through a Florisil column.

Extraction Method
All samples were thoroughly homogenized before sampling for the analysis. Soil samples were subsampled for determination of wet/dry weight ratio.

Accurately weighed samples of wet soil (10 g) to which DCBP, sodium sulphate (40 g) and Ottawa sand (20 g) were added, were extracted 3 times for 20 minutes with 50 mL of dichloromethane on an orbital shaker. Alternatively accurately weighed samples of soil (10 g), paint (1 g) and concrete (1 g) to which DCBP, sodium sulphate (40 g) and Ottawa sand (20 g) were added, were extracted by soxhlet for 4 to 6 hours at 4 - 6 cycles per hour using 250 mL of dichloromethane.

The extract was then concentrated by rotoevaporation to approximately 1 mL, and 5 mL of hexane was added and again evaporated to 1 mL. This was repeated twice more, resulting in 1 mL of hexane solvent, which was then applied to a Florisil column for cleanup. The column was thoroughly rinsed with hexane and the eluent containing the

PCBs diluted to 10.0 mL. A GC vial (2 mL) was then filled and the sample analyzed by GC/ECD.

GC/ECD Analysis
Each sample was analyzed using an Agilent 6890 or 7890 gas chromatograph equipped with a ^{63}Ni electron capture detector (GC/ECD), a SPB™-1 fused silica capillary column (30 m, 0.25 mm ID x 0.25 μm film thickness). The chromatographic conditions were as follows: Sample volume - 1 μL, splitless injection, initial temperature – 100 °C for 2 min; ramp – 10 °C/min to 150 °C, 5°C/min to 300 °C; final time 5 minutes. Carrier gas used was helium with a flow rate of 2 mL/min. Nitrogen was used as a makeup gas for the ECD.
All values were reported as ppm (μg/g) on a dry weight basis.

Analysis of Chlorinated Pesticides in Soil Samples

Overview
Soil samples were analyzed for chlorinated pesticides by gas chromatograph with an electron capture detector (GC/ECD) and in some cases by gas chromatography with a mass spectrometer as a detector (GC/MS) operated in SIM mode after Soxhlet extraction and sample cleanup using SPE chromatography.

Sample Preparation and Extraction
Samples contained in Whirlpaks were thoroughly homogenized before weighing. Wet soil (10 - 15 grams) was transferred to a clean glass thimble, mixed thoroughly with sodium sulphate and Ottawa sand, and spiked with the surrogate, decachlorobiphenyl (DCBP) prior to extraction. A subsample was taken to determine the wet/dry weight fraction of the soil. The samples were Soxhlet extracted with 250 mL of dichloromethane for 4 hours (2-3 cycles per hour). Blank extracts received only DCBP, Ottawa sand and sodium sulphate. Control samples were run by spiking either pesticide-free soil or a blank with 1 of 2 chlorinated pesticide mixtures, each prepared independently from the other.

Sample Cleanup
The extract was concentrated by rotoevaporation to approximately 1 mL, and 5 mL of hexane was added and again evaporated to 1 mL. This was repeated twice more. The extract was quantitatively transferred and applied to a Florisil extraction column. The column was rinsed with hexane into a volumetric flask to a volume of 10.0 mL to create fraction 1. Dichloromethane was then applied to the same Florisil tube and a second 10.0 mL collected to create fraction 2. This latter fraction was rotoevaporated to approximately 1 mL and the solvent exchanged to hexane as described above and made up to a final volume of 10 mL. A GC vial (2 mL) of each fraction was filled and the

samples analyzed by GC/ECD. Either fraction may be analyzed by GC/MS if interfering compounds such as PCBs were present.

Analysis by GC/ECD
Each sample was analyzed using an HP 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector (GC/ECD), an SPB™-1 fused silica capillary column (30 m, 0.25 mm ID x 0.25 µm film thickness) and the HPChem station software. Carrier gas was helium at a flow rate of 2 mL/min. Nitrogen was used as a makeup gas for the ECD. GC/MS analysis used the following: an HP 5890 Series II Plus gas chromatograph equipped with an HP 5972 Mass selective detector, a PTE™-5 fused silica capillary column (30 m, 0.25 mm ID x 0.25 µm film thickness) and the HPChem station software. One characteristic ion plus the retention times for each target compound and surrogate were used for identification. All results are expressed as nanograms of pesticide per gram dry weight of soil (ppb).

Hg Analysis Summary
The method uses cold vapour atomic absorption spectrophotometry. The particular instrumentation (DMA-80) allows for direct measurement and has been designed to meet the criteria outlined in US EPA Method 7473. Very small quantities of liquid or solid sample are required with little or no preparation. Measurements are made of the total mass of mercury in a test portion and the range of the measurement capability is from 0.05 ng to 600 ng.

Solid samples are weighed and liquid samples are measured volumetrically into boats (quartz or nickel). Viable sample volumes range from 100 µl to a maximum boat capacity of 500 µl. The boats enter a chamber in the instrument where the sample is first dried and then thermally decomposed in a continuous flow of oxygen. The combustion products are carried off in the oxygen flow and are then further decomposed in a hot catalyst bed. Mercury vapors are trapped on a gold amalgamator tube and are subsequently desorbed for spectrophotometric quantitation at 254 nm. Long and short path length cells enable the measurement of low range (0.05 – 35 ng) and high range (35 – 600 ng) mercury contents respectively.

An ICP-AES, ICP MS (PlasmaCAL) stock standard of 1000 ppm Hg is used to make the working stocks and calibration standards. For soil and sediment analyses, a reference material such as MESS 3, Cabbage, or SS2 is included with each batch of samples (minimum 1 in 20 samples). An aqueous QC (or calibration check sample) is also included in all runs and is prepared from a source of Hg different from that used for the calibration standards. The aqueous QC is diluted to a concentration mid-range with respect to the assay standards.

Earthworm Analysis

All earthworm samples were prepared for analysis within 24 hrs of removal from soil. Live earthworms were washed under running water and patted dry. They were then placed onto moist paper towels inside whirlpacks and left for 48 hours, to allow for full depuration of gut contents. After depuration, post-depuration wet weight (g) was recorded and the earthworms were placed in a vented drying oven set at 25^{o}C for 12 h. Following dehydration, earthworm dry weight (g) was recorded. The dehydrated earthworms were then placed into clean whirlpacks and stored at – 15^{o}C until analysis.