ASSESSING THE TOXICITY OF PRINCE EDWARD ISLAND STREAM SEDIMENTS USING ASIAN MEDAKA EMBRYOLARVAL BIOASSAYS

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Biology

Faculty of Science

University of Prince Edward Island

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Charlottetown, P.E.I

November, 2005

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ISBN: 0-494-10349-3
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ABSTRACT

Soil erosion and the sedimentation of streams have become major environmental concerns on Prince Edward Island (PEI). I conducted two experiments examining the potential toxicity of aquatic sediments. In the first experiment, I developed a methodology of exposing fish to sediment and assessed the relative toxicity of refrigerated and frozen sediments, both of which are commonly used storage conditions. Indian medaka (*Oryzias dancena*) embryos were used to conduct experiments on sediments collected from three sites on the Wilmot River (a watershed that contains mostly agriculture) and one site on the West River (a watershed that contains more forest than agriculture). Survivorship, hatching times, hatching lengths, and developmental abnormalities at hatching were used as endpoints. Medaka exposed to frozen sediments were found to be significantly smaller, exhibit more developmental abnormalities and, in some cases, hatch earlier than those exposed to refrigerated sediments. These results indicate that freezing sediments may result in increased toxicity to fish. Methods developed in this experiment should prove valuable in assessing whole sediment toxicity with other vertebrate models.

In the second experiment, Japanese medaka (*Oryzias latipes*) embryos were used to assess the relative toxicity of sediment from the Wilmot River sites, one control site on Priest Pond Creek (within a watershed containing little agriculture), and one control group containing only embryo-rearing solution. This experiment was conducted in order to determine if Wilmot River sediments are accumulating toxic concentrations of agricultural related contaminants. Possible changes in sediment toxicity during the

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summer and fall were also examined for each of the Wilmot River sites with the prediction that summer sediments would be more toxic than fall sediments due to the high amounts of pesticides applied on fields during the summer months. Refrigerated sediments were used to conduct experiments using the procedures developed in the first experiment. In general, medaka exposed to the Wilmot River sediments hatched earlier, were smaller at hatching, and exhibited more developmental abnormalities than those of one or both of the control groups. Furthermore, contrary to my prediction, December sediments were generally more toxic to medaka than those collected in early July and sediments collected on July 24 were generally more toxic than July 2 sediments. These results suggest that Wilmot River sediments are more toxic to medaka and that this toxicity increases between early July and December. The substances and mechanisms responsible for this increase in toxicity have not yet been identified. Since medaka appear to be less sensitive to contaminants than native salmonid species, these results may have important implications for environmental management issues on PEI.

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ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Kevin Teather for all his help and for being patient while reading and editing my novel :) Kevin - I really appreciated how quickly you went through the drafts. Thanks also to my committee members Dr. Christian Lacroix and Dr. Marva Sweeny-Nixon and my external committee member Collins Kamunde. A big thanks to Koji Inue who volunteered to extract and sequence the DNA from my unknown medaka species. This was very much appreciated.

I would also like to extend my thanks to the various professors, associates, and staff members who have helped out with this project; Daryl and Todd - thanks for your advice and helping me select my sites. Marina and Donna - thank you for your input on the statistics of my project. Gilbert and Pat - thanks for all your help with autoclaving, helping me find materials to use in this project, and always making sure I had a place to do my research during all the Duffy renovations. I would also like to thank Kaye for all her help, especially for when I needed a pair of scissors to cut my tin foil, and helping me figure out how to work the photocopier and laminator. Furthermore, I would like to thank Yogi for all her help with shipping and handling.

Thank-you to Don Jardine, Jonathon Bentley, and Blair Ramsey who voluntarily helped with the collection of my sediment. When I collected sediments in March, all roads to my sites were completely snow covered. To make sediment collection easier, Blair Ramsey and his son drove my father and I around on skidoos to all the sites on the Wilmot River, which was greatly appreciated. Thanks also to Jonathon, who snowshoed with me to my site on the West River in March and who helped collect my sediments in

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July and December. He also took all of the wonderful photos of me and my sites that are in my presentation!

Further, I would like to say thank you to the various awards and organizations that have helped fund this project and pay my tuition. Thank you to the Canadian Water Network who provided funding for this project. Thanks also to the Henry Havelock McFarlane Ecology Award, the Biology Club Award, and the Harvey Moore Wildlife Fund who all helped with tuition costs. Also, thanks to the Biology Department, specifically, Kevin, Donna, Pedro, Liz, and Terrie who all let me help out in their teaching labs as a TA or an Instructor.

Thanks to all of my friends in the DuffyVale Trailer Park for making my thesis writing more entertaining. I lived in Trailer #2 with Mike, Melissa, Karen, Tracy, Billy, Josh (Youshy), Rob (Eor), Todd (Pepper), sometimes Shawn, and a mixture of Larry/Kevin/Pedro. We had a great time this year and made many memorable memories :) Some of my favorites moments this year were; creating the DuffyVale sign with Billy and Horace, hanging out at the Wave on Friday evenings, reading up on the Phd comics with Youshy, Todd's answering machine, talks with Eor and the gang, and trying to trap a Sasquatch (a.k.a. Samquanch) near Tignish.

On a more serious note, I would like to thank all my friends, family, and my boyfriend for all their support throughout my Masters, especially with their patience and help with my new gluten free diet. During my masters, I was diagnosed with celiac disease, and had to drastically change my diet. I was very thankful that it wasn't more serious and that I could be put into remission by following a strict gluten free diet. I

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would just like to thank all the people who helped me through it and continue to support me. Mom and Nani - thank you for taking the time to cook for me. I really appreciated those meals when I didn't always feel like cooking and those desserts were delicious. Mom and Dad - thank you for buying all those gluten free cookbooks and new cookware, and thanks for taking the time to find and buy some of my very expensive food! Jonathon - thanks for being there and supporting me. Marc - thanks for understanding and for doing your best to help. Tara - thanks for introducing me to Thai Kitchen! Yum!

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TABLE OF CONTENTS

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LIST OF FIGURES

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CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW

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1.1 INTRODUCTION

An excess of sediments in rivers can directly affect fish by interfering with feeding (Waters 1995), spawning, and survival (MacNeil and Curry 2002, Salo and Cundy 1987). Sediments can also accumulate lethal or sublethal concentrations of contaminants which may negatively impact fish. For example, lethal concentrations of pesticides were detected in river sediments after a fish kill that occurred on Prince Edward Island (PEI) in 1999 (Mutch 2000c), while sublethal concentrations of a wide variety of pesticides have been detected in PEI river sediments (Mutch *et al.* 2002, Savard *et al.* 1999). Sublethal concentrations of contaminants may cause reduced growth (Van den Belt et al 2003), decreased mobility (Zhou and Weis 1998), changes in hatching times (González-Doncel *et al.* 2003), reduced fecundity rates (Walker *et al.* 1989), and increased number of developmental abnormalities (Lefebvre *et al.* 2003).

The first objective of this study was to compare the toxicity of refrigerated sediments and frozen sediments using a vertebrate model, Indian medaka (*Oryzias dancena*) embryolarval bioassays. This experiment was undertaken to develop proper methodology for further investigations and to provide needed information on the effects of refrigerating and freezing sediment on aquatic vertebrates. Results from this experiment were used in evaluating whether freezing is an appropriate sediment storage method for sediment toxicity testing using fish embryolarval bioassays. The second objective of this study was to assess the relative toxicity of potentially contaminated whole sediments using Japanese medaka (*Oryzias latipes*) embryolarval bioassays.

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Japanese medaka newly fertilized eggs were exposed to whole sediments from a stream heavily impacted by agriculture and compared with those exposed to sediments from a stream with minimal agricultural impact. While this study was designed primarily to further methodological development of a sediment toxicity bioassay using a vertebrate model, it also provides information concerning the potential impact of agriculture on sediment toxicity.

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1.2 LITERATURE REVIEW

Sedimentation is a major environmental stressor of streams and rivers. The main source of sediment in aquatic habitats is soil erosion (Beasley 1972), caused by a number of activities which include forestry, agriculture, construction, urban runoff (USEPA 2000), mining, and natural erosion (Waters 1995). Thus, most of the excess sediment in rivers and streams is derived directly from anthropogenic sources. Of all these activities, poor agricultural practices are regarded as the leading source of increased sedimentation in streams and rivers (USEPA 2000). Poor agricultural practices include ploughing on slopes, farming on flood plains, ploughing in the fall, exposing ploughed soil for long periods of time, and inadequate buffer zones (Waters 1995).

Sediments are highly mobile and can travel long distances through stream currents before being deposited into a specific region. Lyman *et al.* (1987) indicated that the source of sediment contamination may be extremely localized; however, the contaminated sediment may affect an extensive area. Sediment settling areas include lakes, impoundments, reservoirs, harbours, bays, deltas, and estuaries (Lyman *et al.* 1987). Sediments are also deposited in streambeds where they may linger for up to one year or be abraded within a few days (Waters 1995). Slow moving areas within a river are major targets for the accumulation of sediment (Lyman *et al.* 1987).

1.2.1 Geological History and Prince Edward Island Soils

Prince Edward Island (PEI) is located in the Appalachian mountain distribution (MacDougall *et al.* 1988). Appalachian rocks, which are approximately 350 to 450

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million years in age, form the foundation layer of rock on PEI with sedimentary rocks situated above this foundation consisting of siltstone, sandstone, and conglomerate (DeGrace 1999). Sandstone is located underneath approximately 60% of the land surface area and is the major constituent of rocks on PEI (von de Poll 1981, MacDougall *et al.* 1988). Prince Edward Island red beds originated from small intertwined streams that deposited sediment at the bottom of mountains which were formed at the same time as the Atlantic Ocean (DeGrace 1999). Prince Edward Island bedrock, which is comprised mostly of sandstone, originated from the Triassic and the Permo-Carboniferous periods (Prest 1973). Deposits found on or near the surface of Prince Edward Island consist of glacial deposits, glaciolacustrine and glaciofluvial deposits, postglacial deposits, and early postglacial marine and glaciomarine deposits (Prest 1973).

Prince Edward Island was shaped by various glaciers that formed thousands of years ago (DeGrace 1989). During the last glacial period, it is probable that a great deal of ice was situated directly on Prince Edward Island (Prest and Grant 1969). The last glaciers on PEI began melting approximately 7,000 to 15,000 years ago (DeGrace 1989). During deglaciation, the sea level rose to levels as high as 25 m on PEI. In response, PEI was separated into three islands. However, sea levels eventually subsided and have not significantly changed in the last 4,000 years (DeGrace 1989).

In general, Prince Edward Island soils are acidic, shallow, and have a low organic matter content consisting mostly of fine sand and silt and low amounts of clay (Jacobs and Associates Ltd 1997). The soil types found on Prince Edward Island cannot aggregate as well as other soil types. The term soil aggregation can be defined as the

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clumping of organic particles and minerals that are bound to decaying organic matter and clay (Jacobs and Associates Ltd 1997). Soils with increased numbers of clay particles and coarser sand particles will not erode as quickly as soils with more sand and silt particles. Because Prince Edward Island soils do not aggregate, they are highly vulnerable to erosion. This is particularly true during the winter months as a result of freezing and thawing cycles (Jacobs and Associates Ltd 1997). Air temperatures during PEI winters can drastically fluctuate, resulting in 40°C temperature differences during short time periods (MacDougall *et al.* 1988). Winter soil erosion occurs when the snow melts and washes the top few centimeters of thawed soil into nearby rivers. Jacobs and Associates Ltd (1997) reported that soil erosion caused by winter soil thawing can result in up to 80% of total soil loss per year on Prince Edward Island.

1.2.2 Agriculture on Prince Edward Island

The total land area of Prince Edward Island is 566,560 ha and just over half (260,000 ha) of this land is devoted to farming (Agriculture, Fisheries, Aquaculture, and Forestry 2003). Land in potato production or potato rotation makes up the highest proportion of total crop acreage. The amount of land planted with potatoes has dramatically increased since the late 1980's from approximately 28,000 ha in 1989 (Jacobs and Associates Ltd 1997) to an estimated 1/6 of farmland (43,000 ha) in 2003 (Statistics Canada Agricultural Division 2003).

Extensive potato production on Prince Edward Island contributes to substantial soil erosion and sedimentation in island rivers. On PEI, most potatoes are planted in soils

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that are highly susceptible to erosion (DeHaan 2002). The amount of soil erosion on moderate and steep slopes typically varies between 20 and 40 tonnes per hectare per year but can be as great as 90 tonnes per hectare per year (Jacobs and Associates Ltd 1997). Generally, increased potato production results in increased soil erosion, which results in increased sediment accumulation in the streams.

Conventional farming practices also use extensive amounts of pesticides to protect potatoes and other crops. On Prince Edward Island, 785,978 kg of active ingredient of pesticides were sold in 2001 (Reeves 2002). The most commonly used pesticides that were sold in 2001 have been categorized into three groups based on the total weight sold (Reeves 2002). Two pesticides, Mancozeb and Chlorothalonil, exceeded 50,000 kg active ingredient in amounts sold, while eight others (Dimethoate, Diquant, Glyphosate, MCPA (amine salts), Metalaxyl-m, Metiram, Metribuzin, and Metobromuron) sold between 10,000 and 50,000 kg of active ingredient. Twenty five others were sold in amounts less than 10,000 kg. Many of these pesticides have been detected in local island stream sediments and stream water. For instance, a variety of pesticides were detected in sediments and/or river water during a monitoring program from 1996 to 1998. These included, chlorothalonil, azinphos-methyl, linuron, metalaxly, metribuzin, dimethoate, alpha and beta endosulfan and atrazine (Mutch *et al.* 2002).

Extensive amounts of fertilizers are also applied on agricultural farmland in order to replenish soil nutrient loss due to crop farming. According to Statistics Canada (2001), at least 1,368 applications of manure and 1,232 applications of fertilizer were applied on PEI farmland in 2000; however, Statistics Canada indicated that this number is under-

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reported. The application methods of manure included solid spreaders, liquid surface spreaders, and an irrigation system. Fertilizers can contain additives such as, nitrogen, phosphorous, and metals (Haygarth 2002), which can negatively affect non target terrestrial and aquatic organisms. The sources of nitrogen are decayed animal and plant matter, fertilizers, nitrogen in soil fixation, and animal wastes. Fertilizers are distributed on soils in a variety of forms including urea, ammonium, and nitrate.

1.2.3 Fates of Agricultural Additives

A surplus of nitrogen on agricultural land, usually due to fertilizer application, can lead to nitrogen leaching; however, some nitrogen leaching can occur without the addition of fertilizers (Haygarth 2002). Nitrogen leaching from soil could ultimately enter groundwater and then river water through springs. In combination with water, nitrogen can also move in and above soils and contaminate surface waters (National Research Council Staff 1993). According to Carpenter *et al.* (1998), high quantities of fertilizers on agricultural crops can increase the quantity of nutrients in soils, which may potentially enter aquatic habitats through soil erosion and negatively affect aquatic organisms.

The fate of pesticides in the environment depends on many factors such as hydrolysis, adsorption, volatilization, photolysis, absorption, desorption, degradation, mobility, persistence, and pesticide physiochemical properties (Shaw and Chadwick 1998). Physiochemical properties of pesticides such as, molecular weight, melting point, boiling point, solubility in water, and their partition coefficient between water and

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sediment can ultimately affect the transfer of pesticides in the environment. For example, the partition coefficient can be used to determine how tightly pesticides bind to sediments and their accumulation potential in organic tissue (Shaw and Chadwick 1998). In addition, the volatility of a pesticide will help determine if the pesticide will remain on the surface of soil (Shaw and Chadwick 1998).

In general, pesticides tend to adsorb to the organic matter of soils (Huang *et al.* 1984, Leenheer and Ahlrichs 1972, Saltzman *et al.* 1972, Hance 1965). Nevertheless, pesticides can also adsorb into the mineral and organic matter colloids (Saltzman *et al.* 1972) and the minerals aluminum and iron (Huang *et al.* 1984) of soils. For example, Hance (1965) discovered that the organic matter in the soil was responsible for the adsorption of urea, and its derivatives found in the pesticides diuron, linuron, and monuron. In addition, Leenheer and Ahlrichs (1971) found that carbaryl and parathion adsorbed to the organic matter content of soils. Saltzman *et al.* (1972) indicated that the pesticide parathion adsorbed more to organic matter than minerals and found that the elimination of organic matter content from soil lowered the adsorption of the pesticide parathion in soils containing mostly minerals. Once pesticides are attached to soils they can be transferred out of their original location by water (Saltzman and Yaron 1986, Hall *et al.* 1972). For example, 0.16% of atrazine was lost annually from agricultural fields through eroded sediment (Hall *et al.* 1972).

Decreased levels of pesticides in soils can be due to microbial degradation, chemical hydrolysis, photolysis, volatility, leaching, and runoff (Haygarth 2002). Certain pesticides will also continually adsorb and desorb within the sediment and water, but this

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will ultimately depend on the pesticides binding capacity to the sediment and the concentration of the pesticide in the sediment (Widenfalk 2005). The degradation rates of pesticides in sediments depends on pesticide characteristics such as solubility, and structure as well as environmental conditions such as temperature, soil structure, and microbial activity (Haygarth 2002). For example, the half-life of chlorothalonil under aerobic soil can vary from 10 to 40 days; however, the half life under anaerobic aquatic soil (similar to sediments) is 5 to 15 days (United States Environmental Protection Agency 1999a). Saltzman *et al.* (1972) reported that microbes will degrade pesticides within the sediment and produce metabolites. Those degraded metabolites can be less toxic or more toxic than the pesticide (Widenfalk 2005).

Agriculturally related metals and atmospheric deposition of metals from industrial sources can potentially result in metal contaminated river sediments. The sources of metals in the agricultural industry include fertilizers, medicine, animal manure, feed, atmospheric deposition of metals, and roughage (Haygarth 2002). Mercury can be deposited on soil through atmospheric deposition by industrial sources involved in chlorine production, hazardous and medical waste incineration and by industrial boilers, municipal waste combustors, and utility coal boilers (Evers 2005). Airborne mercury can eventually deposit in watersheds, potentially harming aquatic organisms (Evers 2005).

Metals can bind to soil components such as, organic matter, carbonates, magnesium oxides, iron oxides (Filgueiras *et al.* 2004, Yu *et al.* 2001a, Yu *et al.* 2001b), residual metals (Filgueiras *et al.* 2004), silicates, sulfides, and clay minerals (Calmano 1996). The attachment of metals to these substances is determined by metal

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chemical bioavailability and mobility. Soils may be exposed to pesticides and metals on a continuous basis. Metals that are poorly bound to sediments can be readily accessible to biota. However, metals which are strongly attached to sediments may only be available to aquatic organisms after geochemical weathering (Calmano 1996).

1.2.4 Agriculture and Fish

Fish kills usually occur during crop growing season and after heavy rain. Thirty known or suspected pesticide related fish kills have occurred in PEI streams from 1965 to 2000 (Mutch *et al.* 2002). Furthermore, two pesticide-related fish kills occurred on the Wilmot River during the summer of 2002 (Gormley *et al.* 2005). Lethal concentrations of pesticides are usually discovered after fish kills or obvious runoff events. For example, after a fish kill on the Westmoreland River in 1999, lethal concentrations of endosulfan and chlorothalonil were detected in the standing water and sediment, respectively (Mutch 2000c). Lethal concentrations of azinphos-methyl and endosulfan were also detected in water after fish kills on the Huntley River in 1998 and the Indian River in 2000 (Mutch 1999, Mutch 2001b). In addition, the fungicide chlorothalonil was detected at lethal concentrations in field, buffer, and stream sediments after a fish kill in Fullerton's Creek in 2000 (Mutch 2001a). After a fish kill on the Valleyfield River in 1999, lethal concentrations of azinphos-methyl were detected in sediment and standing water (Mutch 2000b).

Lethal concentrations of pesticides do not linger in flowing river systems; sublethal concentrations of pesticides, however, are often detected on a regular basis in

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river surface water and river sediments on Prince Edward Island. For example, sublethal concentrations of 12 different pesticides were frequently detected in the Big Pierre Jacques and Long Creek river water and/or sediments during the summer months of 1996 and 1997 (Mutch *et al.* 2002). Sublethal concentrations of pesticides in stream sediments and river water have also been detected in the Mill River, Wilmot River, Found's River, and Souris River during a monitoring program in 2003 and 2004 (Murphy and Mutch 2005). In 2003, the pesticides chlorothalonil and metalaxyl were detected in Wilmot River surface water and the fungicide Mancozeb was detected in Wilmot River sediments in 2004 (Murphy and Mutch 2005). In general, lethal concentrations of pesticides are observed only following runoff events associated with heavy rain; however, sublethal concentrations may occur throughout the year.

The toxicity of aquatic sediments are typically evaluated using invertebrates as test organisms. Fewer studies have tested the toxicity of contaminated sediments to fish and little research has been conducted on the sub-lethal effects of whole sediments on fish. For the purpose of this review, I examine studies that have investigated the sub-lethal effects of sediments contaminated by pesticides, metals, and various other substances on fish.

Sublethal effects of contaminants on fish are less apparent in the natural environment than are lethal effects. Exposure to sublethal concentrations of contaminants may result in altered hatching timing (Strmac *et al.* 2002), reduced length and growth (Teather *et al.* 2001, Rombough and Garside 1982), reduced mobility (Heath *et al.* 1993), abnormal reproductive abilities (Rowe 2003), and developmental deformities

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(Gray and Metcalfe 1999). Sublethal effects may have critical repercussions on long term survival and reproductive success of exposed individuals, thus ultimately resulting in changes in the population structure.

Early life stages of fish, such as embryolarval and juvenile stages, are usually more sensitive to contaminants (McKim 1977) and, for this reason, most studies have focused on the effects of contaminants on early development (e.g., Gormley and Teather 2003, Strmac *et al.* 2002, Villalobos *et al.* 2000, Nimrod and Benson 1998, Cooper *et al.* 1993, Wisk and Cooper 1990). Typical endpoints have included hatching time, growth, mobility, developmental abnormalities, and subsequent reproduction.

Developmental abnormalities are common among fish that have been exposed to contaminants. Spine alterations, pericardial edema, heart abnormalities, deformed tails, and tail lesions were noticed in Japanese medaka exposed to sublethal concentrations of the fungicide Acrobat MZ (Teather *et al.* 2001). Mondon *et al.* (2001) also found that developmental abnormalities occurred in greenback flounders (*Rhombosolea tapirina*) after exposure to contaminated sediments, while Cooper *et al.* (1993) found developmental abnormalities in Japanese medaka exposed to sediments contaminated with furans and dioxins. Strmac *et al.* (2002) found that zebrafish (*Danio rerio*) had spinal deformities, yolk sac resorption, heart edema, and yolk sac edema after exposure to sediment acetone extracts from a river suspected to be polluted with pesticides, heavy metals, and polyaromatic hydrocarbons. In addition, fathead minnows (*Pimephales promelas*) exposed to sediment extracts contaminated with zinc exhibited a wide range of developmental abnormalities such as pericardial edema, gut edema, and fin abnormalities

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(Dawson *et al.* 1988).

Although many studies have reported alterations in hatching time in fish that were exposed to contaminants (e.g., González-Doncel *et al.* 2003, Strmac *et al.* 2002, Todd and Van Leeuwen 2002, Strmac and Braunbeck 1999), others have found no such differences (e.g., Teather *et al.*2005, Teather *et al.* 2001, Gray and Metcalfe 1999, Mauck *et al.* 1978). Hatching time, when affected, may be delayed (González-Doncel *et al.* 2003, Todd and Van Leeuwen 2002, Rach *et al.* 1998, Fent and Meier 1994) or advanced (Strmac *et al.* 2002, Cooper *et al.* 1993). In general, hatching time in fish exposed to contaminants can be quite variable; therefore, predicting the effect of contaminants on hatching times of fish may prove to be difficult.

Exposure to contaminants may suppress growth in fish (Teather *et al.* 2005, Rowe 2003, Van den Belt *et al.* 2003, Brauner and Wood 2002, Knörr and Braunbeck 2002, Mondon *et al.* 2001, Teather *et al.* 2001), increase growth (Gray and Metcalfe 1999), or have no effects on growth (Gormley and Teather 2003, Teather *et al.* 2001, Owens and Baer 2000, Fjeld *et al.* 1998, Weis and Weis 1995). Teather *et al.* (2005) found that Japanese medaka that were exposed to azinphos-methyl were significantly smaller at one week post hatching when compared to the control group. In a previous study, Teather *et al.* (2001) found a significant reduction in medaka length at hatching after embryos were exposed to certain concentrations of Acrobat TM® but did not find a subsequent difference in the growth rates of fish exposed to either this pesticide or Tattoo C®. In addition, Rowe (2003) found that the growth of hatchling sheepshead minnow (*Cyprinodon variegatus*) exposed to contaminated sediment was significantly reduced

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compared to controls. Similarly, the growth of greenback flounder *Rhombosolea tapirina* was significantly reduced when they were exposed to contaminated sediments and a metal contaminated diet (Mondon *et al.* 2001). Therefore, the most likely outcome of contaminant exposure to fish is reduced growth.

1.2.5 Sediment Collection, Storage, and Exposure Techniques

Proper collection, storage and exposure methodology is important in obtaining accurate experimental results when testing the toxicity of aquatic sediments. Therefore, techniques for each will be reviewed. In addition, sediment ratios for whole sediment testing and methods of exposing fish to whole sediments are also examined and compared. Core samplers and surface grab samplers are typically used to collect sediment (Palmer 1984). Core samplers are recommended for the collection of contaminated sediment and for collecting different types of sediments. Palmer (1984) suggested using a two inch diameter stainless steel corer and a CAB (cellulose acetate butyrate) plastic liner for such samples. According to Palmer (1984), surface grab samplers are generally used for sediment collection when the core sampler cannot be used due to sand or gravel, and to collect large amounts of material on the surface. Collected sediment can be placed in new glass jars rinsed with test site water and covered with aluminum foil or lids lined with Teflon (Palmer 1984). Anderson *et al.* (1984) indicated that the top 2 cm of surface sediment is reasonable for sediment toxicity testing. Sediment samples can be placed directly into an ice chest filled with wet ice in order to maintain a temperature of about 4°C (Palmer 1984).

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Sediment grain size is an important factor to consider when analyzing contaminated sediment. Lyman *et al.* (1987) reported that finer sediments will accumulate contaminated substances more than coarser sediments due to the higher surface area available for bonding. Förstner and Salomons (1980) found that metal content in sediment decreases with an increase in sediment particle size and recommended a grain size fraction of less than 63 μm for sediment toxicity experimentation. Sediments can be classified according to grain size diameter as follows: boulders > 256 mm, rubble 64 to 256 mm, gravel 2 to 64 mm, sand 0.06 to 2.0 mm, silt 0.004 to 0.06 mm, and clay 0.004 mm (Palmer 1984). Based on this classification, silt, clay, and some sand particles would be acceptable for sediment toxicity testing.

Sediments used in toxicity testing are frequently sieved or mixed. However, according to Northcott and Jones (2000), sieving and mixing can alter chemical and physical properties in the sediment. A disruption in chemical and physical properties can ultimately affect the concentrations of pollutants in sediment (Northcott and Jones 2000). For example, mixing sediments altered the concentration of PCB's in sediment in a study by Burgess and McKinney (1997) . Certain protocols, however, require sediment sieving and mixing. The American Society for Testing and Materials (1998 *in* Northcott and Jones 2000) recommends using a sieve with a pore size of 1 to 2 mm or larger and reducing the amount of water used during sieving. Therefore, sediments should be separated with a sieve having a relatively large pore size and with a limited amount of water in order to minimize alterations in sediment contaminant concentrations.

The appropriate sediment storage temperature for toxicity testing has not yet been

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agreed upon (Nebeker *et al.* 1984). Research has demonstrated that freezing sediment can either increase (Geffard *et al.* 2004, Jung and Bailey 2004, Beiras *et al.* 1998), decrease (Stemmer *et al.* 1990, Schuytema *et al.* 1989, Malueg *et al.* 1986, Nebeker *et al.* 1984, Thomson *et al.* 1980), have little effect (Stenberg *et al.* 1998), or have no effect on the toxicity of sediment (Carr *et al.* 1989). Studies have recommended refrigerating (Schuytema *et al.* 1989, Malueg *et al.* 1986) or freezing (Stenberg *et al.* 1998) sediments before testing their toxicity. Malueg *et al.* (1986) reported that sediment spiked with copper stored at 5°C was more toxic to *Daphnia magna* than sediment stored at -20°C. Schuytema *et al.* (1989) reported that the LC_{50} (the concentration of a contaminant which kills 50% of exposed individuals) value of *Hyalella azteca* exposed to sediment spiked with DDT stored at 4°C was significantly higher than the LC_{50} value of sediment stored at -20°C. On the other hand, Jung and Batley (2004) found that selenium concentrations in frozen sediments were noticeably higher than in refrigerated sediments. Stenberg *et al.* (1998) found that microorganisms had a greater effect on refrigerated sediments (+2°C) than on frozen sediments and thus recommended freezing sediments at -20°C. Carr *et al.* (1989) found that the toxicity of sediment porewater to polychaetes *Dinophilus gyrociliatus* stored at 4°C was not significantly different from that of the sediment stored at -80°C.

Based on the results listed above, it is not surprising to learn, short sediment storage times are generally recommended (Northcott and Jones 2000, Becker and Ginn 1995, Stemmer *et al.* 1990). For example, Stemmer *et al.* (1990) found that *Daphnia magna* exposed to sediment spiked with selenite and stored for 72 h, 1 week and 2 weeks

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were significantly less toxic than sediments stored for 24 h and 48 h; however, sediments stored for 3 weeks were significantly more toxic. They speculated that microorganism or chemical pathways may have been altered causing the observed toxicity fluctuations and suggested that chemical and microorganism pathways *in vitro* may be different than those *in vivo* conditions. Based on these results, Stemmer *et al.* (1990) suggested beginning experiments within 24 h in order to obtain consistent results. Becker and Ginn (1995) found that the percent survival of *Rhepoxynius abronius* exposed to control sediment increased significantly over time, but the percent survival among *Rhepoxynius abronius* exposed to toxic sediment decreased overtime. They also proposed that sediments used in toxicity studies should be tested as soon as possible after initial field collection. Although short sediment storage times are recommended for sediment toxicity testing, longer storage times may be necessary and acceptable (DeFoe and Ankley 1998, Stenberg *et al.* 1998). DeFoe and Ankley (1998) found that storage times periods of 18 to 70 weeks were acceptable for sediment toxicity. In addition, Stenberg *et al.* (1998) indicated that storage times of up to 13 months were acceptable. Presumably, the appropriate storage time and temperature will be dependent on the type of suspected contaminants in the sediment.

1.2.6 Sediment Exposure

Sediment test phases are methods of exposing organisms to potentially toxic sediments. There are five sediment test phases commonly used in sediment toxicity testing (Burton 1991). These include; the (i) elutriate phase (water extractable), (ii)

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extractable phase (non-water extractable), (iii) *in situ* phase, (iv) interstitial water phase (porewater), and (v) whole sediment assays (see Table 1-1). Each test phase has advantages and disadvantages and selecting one phase that will satisfy experimental objectives can be difficult (Burton 1991). Various studies have used more than one test phase to evaluate an organism's response to the sediment (Geffard *et al.* 2003, Strmac *et al.* 2002, Sasson-Brickson and Burton 1991) while others have used only one (Dawson and Stebler 1988, Ankley *et al.* 1990, Day *et al.* 1994, Mondon *et al.* 2001).

When compared in experiments, test phases were shown to differ in toxicity. For example, Sasson-Brickson and Burton (1991) found that the elutriate phase was significantly less toxic than either the interstitial water phase or the solid phase (whole sediment test phase). In contrast, Hoke *et al.* (1995) found that the elutriate phase was usually more toxic than the porewater phase (interstitial water phase). Liß and Ahlf (1997) compared the toxicity of sediment with the elutriate phase, whole sediment phase, and the porewater phase. They indicated that the interstitial water phase is not sufficient for toxicity testing and they suggested that all three tests should be used for assessing the toxicity of sediments. In comparison, Sasson-Brickson and Burton (1991) did not find a significant difference between the toxicity of the interstitial water phase and the solid phase. Generally, the sediment test phase used in an experiment will largely depend on the objectives, the available resources, and the test organism.

The *in situ* test phase is an appropriate test phase for the assessment of sediment toxicity since this test is more realistic than laboratory tests (Baudo *et al.* 1999). I general, the *in situ* test phase involves exposing organisms directly in the natural

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Table 1-1 Brief descriptions of sediment test phases

Sediment Test Phase	Brief Description
In situ	Involves exposing organisms directly to sediment in the natural environment (Baudo *et al.* 1999).
Whole sediment	Organisms are exposed to whole sediment in the laboratory (Viganò *et al.* 1995).
Extractable	Involves exposing organisms to freeze-dried sediment extracts which are placed in a solvent (acetone, methanol) (Strmac *et al.* 2002).
Elutriate	Organisms are exposed to the water phase (overlying waters) of a sediment:water ratio (Sasson-Brickson and Burton 1991).
Interstitial water	Organisms are exposed to the water within the sediment (porewater) (Sasson-Brickson and Burton 1991).

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environment (Baudo *et al.* 1999). Although the in situ test phase seems best for sediment toxicity testing, there are disadvantages that make this test difficult to carry out and interpret. Being a fairly new measure of sediment toxicity (Baudo *et al.* 1999), there is little information regarding the methodology of these tests. Visits to test sites need to be frequent, especially for experiments requiring daily observation of test organisms. Confounding variables such as temperature fluctuations, predation, and variable food resources could confound results. Due to these potential problems, the *in situ* test phase was eliminated as a possible test phase for this type of study.

The whole sediment test phase is the next most realistic approach to exposing organisms to natural sediment conditions (Burton 1991). Briefly, this test involves collecting sediment from the test site and exposing organisms to the sediment in a laboratory environment (Day *et al.* 1994, Viganò *et al.* 1995). The whole sediment test phase has been used in a variety of studies as a method of exposing aquatic organisms to contaminated sediments (Day *et al.* 1994, Viganò *et al.* 1995, Mondon *et al.* 2001, Shin *et al.* 2002, Duft *et al.* 2003). This test is useful since it measures the toxicity of the actual sediment, instead of sediment extractions, elutriates, and pore waters (Burton 1991). One main disadvantage of the whole sediment test phase is the potential changes in the chemical, microbiological, and physical properties of sediment after collection (see above). However, this problem cannot be circumvented since a certain lag time between sediment collection in the field and sediment testing in the lab is unavoidable. In addition, disrupting the stable state between sediment and water during sediment collections is inevitable (Dave and Nilsson 1996). The whole sediment test phase is an

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appropriate test for this study since it closely mimics natural environmental exposure conditions, while reducing many of the confounding variables noted previously. Therefore, the whole sediment test phase was selected to be used for this study.

1.2.7 Fish exposure to sediment

Tanks (Hutchinson *et al.* 2003, Rowe 2003, Mondon 2001, Viganò *et al.* 2001, Hopkins *et al.* 2000), chambers (Ankley *et al.* 1990), glass vessels (Viganò *et al.* 1995), individual wells (Strmac *et al.* 2002), petri dishes (Dawson and Stebler 1988) and vials (Munkittrick *et al.* 2003, Cooper *et al.* 1993) have all been used to expose fish to contaminated sediments. Some of these studies expose groups of fish to contaminated sediments in non replicated or poorly replicated vessels (Mondon 2001, Viganò *et al.* 1995, Dawson and Stebler 1988) and consequently may be difficult to statistically analyze due to pseudo-replication (Hurlbert 1984). Other studies have exposed fish to contaminated sediments in replicated vessels (Munkittrick *et al.* 2003, Strmac *et al.* 2002, Cooper *et al.* 1993). For example, Strmac *et al.* (2002) exposed zebrafish embryos individually to contaminated sediment extracts and Cooper *et al.* (1993) exposed medaka embryos individually to contaminated whole sediments. In addition, Munkittrick *et al.* (2003) exposed Japanese medaka embryos individually to sediment extracts. In order to avoid complications resulting from pseudoreplication in this project, medaka embryos were exposed individually or in groups of two to five and all treatments were replicated.

While many studies have used a 1:4 (sediment : water) ratio for whole sediment

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testing (Malueg *et al.* 1986, Stemmer *et al.* 1990, Viganò *et al.* 1995, Nebeker *et al.* 1984), other sediment to water ratios have also been used (Day *et al.* 1994, Stemmer *et al.* 1990). According to Stemmer *et al.* (1990), sediment to water ratios of 3:1 and 1:8 (with greater surface area) can result in complete and partial lethality among *Daphnia magna* respectively. They found that a sediment to water ratio of 1:4 did not affect the survival of *Daphnia magna*. Thus, Stemmer *et al.* (1990) conclude that a 1:4 (sediment:water volume) ratio (approximately) is appropriate for most tests.

1.2.8 Study Objectives

The main objectives of this study were to:

- develop methodology for exposing fish to sediments.
- compare the toxicity of refrigerated and frozen stream sediments using a vertebrate model, Indian medaka (*Oryzias dancena*) embryolarval bioassays.
- assess the effects of potentially contaminated stream sediments on fish using Japanese medaka (*Oryzias latipes*) embryolarval bioassays.
- compare the seasonal toxicity of Wilmot River sediments using Japanese medaka embryolarval bioassays.

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CHAPTER 2:

GENERAL MATERIALS AND METHODS

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2.1 MATERIALS AND METHODS

2.1.1 Wilmot River Site Selection

Three test sites were selected on the Wilmot River. This river was selected since the watershed is highly impacted by agriculture (Fig 2.1) and there have been a number of major fish kills due to pesticide runoff (Gormley *et al.* 2005). Thus, the sediments are expected to contain agricultural chemicals or chemical metabolites. Two sites were selected on the main branch of the Wilmot River. The first of these sites was located upstream above an impoundment (Arsenaults Pond) and is referred to as the upstream site (WU) (Fig 2-1). The second site on the main branch was located downstream, also above an impoundment (Marchbanks Pond) and is referred to as the downstream site (WD). The third site was selected on a tributary of the main branch and was located between the two sites on the main branch and is designated as the tributary site (WT).

2.1.2 Sediment Collection, Sieving, and Storage

At each site, the top few centimeters of sediment were collected from three or more locations from the bottom of the river bed and placed into autoclaved 1000 ml glassware (canning jars). Approximately 2 L of sediment were collected from each site with an autoclaved stainless steal spoon. Immediately after collection, the jars containing sediment were placed inside an insulated container with ice. Upon returning to the lab, the jars were placed in the refrigerator at a temperature of approximately 5 ± 2 °C until experiments began. Within two days of collection, sediments were washed through an autoclaved sieve having a mesh size of 2 mm using 1 L or less of distilled water. The

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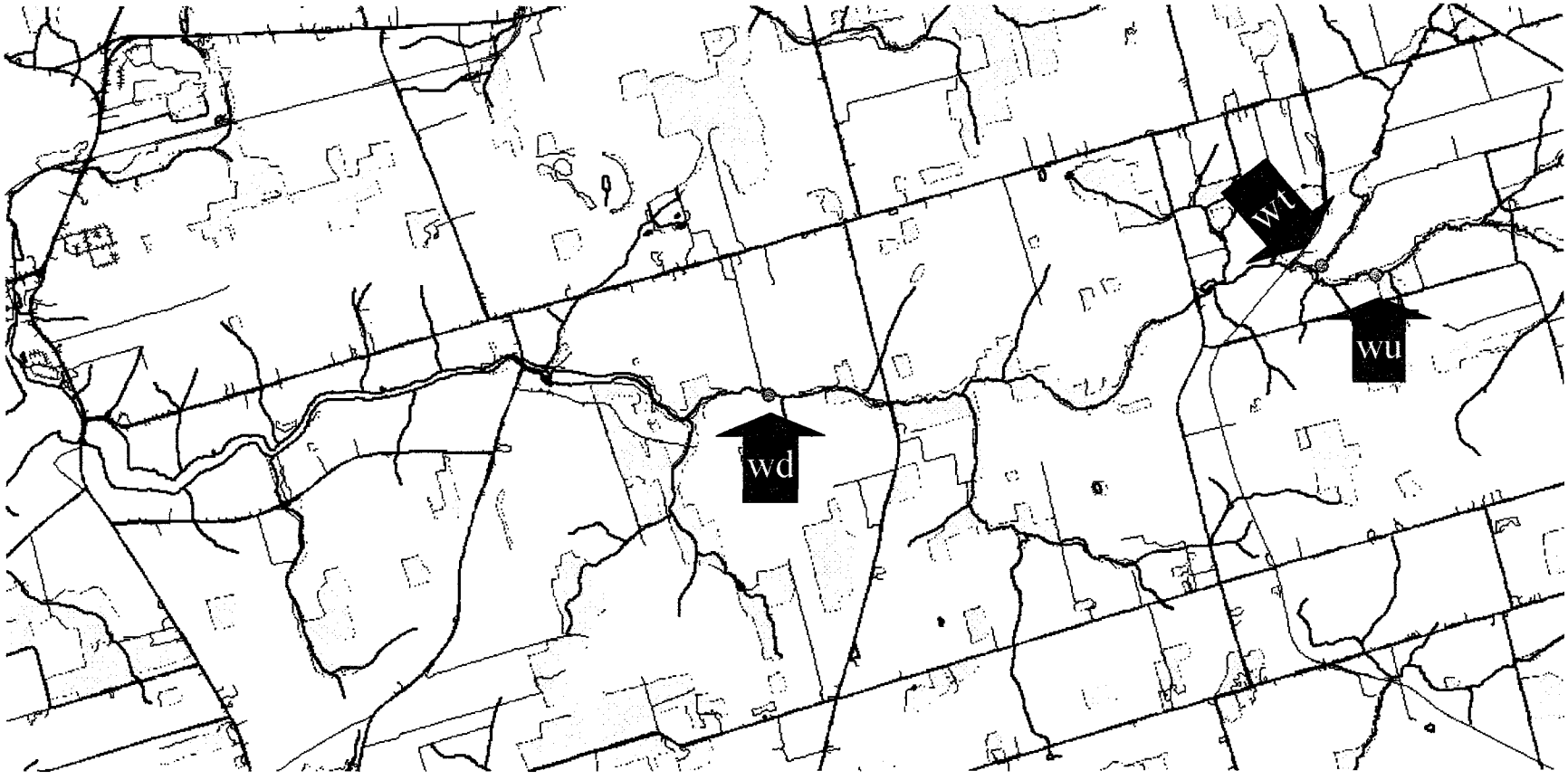

Fig 2-1 Landuse map of the Wilmot River watershed indicating the selected upstream site (WU), the selected downstream site (WD), and the selected site on the tributary (WT). Land use in this watershed is dominated by agriculture (78%), with a smaller amount in forest (11%), and the remaining amount (11%) is made up of industrial areas, sports fields, school, abandoned farmland, and wetlands (Fig 2-1) (Department of Agriculture and Forestry 2000). Beige indicates agriculture and green indicates forest.

sieved sediment was thoroughly mixed with a stainless steel spoon, placed into autoclaved1000 ml jars, and allowed to settle for 24 h. The sediment was then divided into portions to be used in the refrigerated and frozen trials. All sediments were stored in autoclaved stainless steal beakers or glass jars. Sediment assigned for refrigeration was placed in the refrigerator at approximately 5 ± 2 °C. All sediments were stored in the refrigerator for approximately the same time period. Sediment assigned for frozen trials was placed in the freezer approximately 2 weeks after collection. Frozen sediments were stored at -20°C and removed from the freezer approximately two days before testing began. All utensils used for sieving sediment were throughly washed with a laboratory dishwasher using distilled water between each use.

2.1.3 Study organisms

2.1.3.1 Japanese medaka

Japanese medaka (*Oryzias latipes*) are native to Taiwan, southeastern Asia, and Japan (Kirchen and West 1976). Medaka are a freshwater killifish that feed mainly on mosquito larvae and are typically found in rice paddies (Metcalfe *et al.* 1999). Japanese medaka can survive well in air temperatures varying from 5°C to 35°C (Kirchen and West 1976). The meristic characters of Japanese medaka include 30-31 vertebrae, 6-7 dorsal fin rays, 18-20 anal fin rays, 9-10 pectoral fin rays, 6 pelvic fin rays, and 5/6 principal caudal fin rays (Roberts 1998).

In captivity, Japanese medaka should be maintained in aquaria containing spring water or safe dechlorinated tap water (Kirchen and West 1999). Medaka do best in

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temperatures between 20 °C and 25 °C with a temperature drop of 5 °C at night (Kirchen and West 1976). Medaka can be fed brine shrimp and tropical fish food (Metcalfe *et al.* 1999) and they should be fed sparingly three times a day (Kirchen and West 1976).

Although the natural breeding season of Japanese medaka occurs from April to September, medaka can be induced to breed during other months by adjusting the photoperiod, increasing the water temperature, and adjusting their feeding regime (Kirchen and West 1976). To induce breeding, the photoperiod should be increased to a 16:8 (light:dark) cycle (Kirchen and West 1976). Water temperature during the day should vary from 25 °C to 28 °C and the temperature should decrease during the night (Kirchen and West 1976). According to Kirchen and West (1976) increased egg production will occur when dry food is provided one hour after the lights turn on. Medaka are oviparous and one female can produce up to 70 eggs in one clutch (Kirchen and West 1976) and up to 3,000 eggs in a single breeding season (Metcalfe *et al.* 1999). Medaka eggs develop optimally at temperatures between 15 and 25 °C. Hatching times of Japanese medaka are quite variable. For example, medaka embryos can hatch as early as seven days post fertilization at 25°C (Naruse 1994). Teather *et al.* (2000) found that the median time for medaka embryos to hatch was 11 days at 27 ± 2 °C; however, some embryos did not hatch until three to five weeks post fertilization. Fry are approximately 4.5 mm in length at hatching and they will grow to approximately 2 to 4 cm in length (Kirchen and West 1976). Medaka mature within two to six months and can live more than four years (Kirchen and West 1976).

Medaka are appropriate for research studies since they can be easily bred and

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maintained in the laboratory. Females can be induced to produce eggs daily (Kirchen and West 1976) and the egg chorion is transparent, facilitating the examination of developmental stages (Metcalfe *et al.* 1999). In addition, early life stages of medaka seem to be especially sensitive to contaminants (Wisk and Cooper 1990, Owens and Baer 2000, Teather *et al.* 2001, Gormley and Teather 2003). One potential disadvantage is the fact that Japanese medaka are not native to Prince Edward Island (PEI). Native salmonid species on PEI include Atlantic salmon *(Salmo salar)* and brook trout (*Salvelinus fontinalis*) (Guignion *et al.* 2002). The response of Japanese medaka and native salmonids to several different contaminants has been compared in Table 2-1. Based on the results of these studies, Japanese medaka appear to be less sensitive to contaminants than Atlantic salmon and brook trout.

2.1.3.2 Indian medaka

The species of medaka used to conduct experiments in Chapter 3 is different than the species used in Chapter 4. Japanese medaka are normally used for experimentation in the Biology Department at the University of Prince Edward Island. However, due to the unavailability of Japanese medaka in North America at the time of purchase, *Oryzias dancena* were substituted for *Oryzias latipes*. Unfortunately, the North American supplier (Ward's Natural Science) did not indicate this change at the time of purchase and due to the similarity of the two species, experiments were completed before a positive identification was made. Ward's was unable to confirm the identification of the species; therefore, frozen medaka specimens were shipped to Koji Inoue at the Ocean Research

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Table 2-1 Comparing the LC50 ug/L of Japanese medaka *(Oryzias latipes)*, Atlantic salmon *(Salmo salar)*, and brook trout *(Salvelinus fontinalis)* exposed to DDT, Cadmium chloride, and nitric acid.

	Oryzias latipes	***Salmo salar***	***Salvelinus fontinalis***
DDT			
LC50 µg/L	380	6.2, 4.6, 8.4	30
Duration (days)	1	1	1
Source	Tsuji et al. (1986)	Mayer and Ellersieck (1986)	Gardner (1973)
Cadmium chloride			
LC50 µg/L	30,000	1500 - 2700	5080, 4320-5970
Duration (days)	2	24	4
Source	Tsuji et al. (1986)	Rombough and Garside (1982)	Holcombe et al. (1983)
Nitric acid			
LC50 µg/L	340,000	700	4100
Duration (days)	2	4	4
Source	Tsuji et al. (1986)	Grande and Anderson (1983)	Holcombe et al. (1976)

Institute at the University of Tokyo, Japan. DNA sequencing was done on two individuals using a known DNA sequencing method for medaka species (see Takehana *et al.* 2005). The DNA of both specimens identified the species as *Oryzias dancena*.

Indian medaka are widely spread throughout India, Myanmar, and Bangladesh (Roberts 1998). They are usually located along the coast and are tolerant to both brackish and fresh water aquatic habitats (Roberts 1998). Indian medaka are slightly different in appearance than Japanese medaka. The meristic characters of Indian medaka include 28-29 vertebrae, 6-7 dorsal fin rays, 6 pelvic fin rays, 20-24 anal fin rays, 10-11 pectoral fin rays, and 6/6 principal caudal fin rays (Roberts 1998). Unlike Japanese medaka, Indian medaka are not commonly used in research experiments; thus, little information is available on the species. Due to the lack of information on Indian medaka, they were maintained under the same environmental conditions and provided with the same diet as Japanese medaka.

2.1.4 Egg collection

Between breeding cycles, medaka were maintained under a 16:8 light/dark cycle at approximately 20 °C. Medaka were fed Nutrafin® freeze dried red grubs (guaranteed 56% minimum crude protein, 8% minimum crude fat, 4% maximum crude fiber, 5% maximum moisture, and 10% maximum ash), and/or Nutrafin$_{®}$ staple food (guaranteed 46% minimum crude protein, 5% minimum crude fat, 2% maximum crude fiber, and 8% maximum moisture) twice daily. Medaka were induced to breed by increasing the temperature to 25 °C ± 2 °C, and by providing live brine shrimp (*Artemia salina*) once or

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twice daily in addition to a single feeding of freeze dried red grubs and/or staple food. Breeding tanks were checked 3-4 times per day and eggs were manually removed from the abdomen of each female shortly after they had copulated. Eggs were immediately placed into an autoclaved petri-dish containing embryo-rearing solution (1% NaCl, 0.03% KCL, 0.04% CaCl, 0.163% $MgSO_4$ (Kirchen and West 1976) and a few drops of methylene blue to reduce fungal infections. Eggs were then examined under a dissecting microscope; unfertilized or dead eggs were discarded and fertilized eggs were set aside for sediment exposures. Fertilized eggs were easily distinguishable from unfertilized eggs since fertilized eggs have oil drops that migrate towards the vegetable pole of the embryo.

2.1.5 Experimental Design

In the first experiment (Chapter 3), both refrigerated and frozen sediments were used, while only refrigerated sediments were used in the second experiment (Chapter 4). The initial set up for the first part of each experiment took place over 5-16 days (see Chapters 3 and 4 for details) since 12-14 jars were prepared for each group. Experimental procedures were identical for both frozen and refrigerated sediments. Autoclaved Bernardin® 250 ml wide mouth mason jars and autoclaved snap lids were used in all trials. Each jar consisted of 20 ml of sediment and 150 ml of embryo rearing solution and were prepared daily for trials. The content of the jars were thoroughly mixed with a magnetic stir bar for 2 min and allowed to settle for 24 h at 5 °C ± 2 °C. Approximately 5 h before exposures, jars were placed in an environmental chamber at a

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temperature of 25 °C ± 2 °C. Immediately before exposures, autoclaved glass mini-petri dish bottoms were gently placed in the jars on top of the sediment. These dishes were used to reduce predation from invertebrates living in the sediment and to facilitate observation of the eggs under the dissecting microscope. One to five fertilized medaka eggs were then placed directly on top of the dishes. The total number of eggs varied daily as a result of unpredictable egg production by females. At this point, most of the sediment particles had settled on the bottom of the mason jars; however, a small amount remained suspended in the embryo-rearing solution. This was left to settle out directly on top of the eggs and thus there was some direct contact between the eggs and the sediment. Jars were immediately placed into an environmental chamber at 25 °C ± 2 °C and a 16:8 hour light:dark photoperiod.

2.1.6 Endpoints

2.1.6.1 Survivorship

Medaka eggs were checked once daily for mortality. Eggs in which embryos had died are easily distinguished as methylene blue passes through the chorion and dyes the embryos blue.

2.1.6.2 Hatching Time

Eggs were checked once daily for hatching. Hatching was characterized as a complete hatch from the embryo, a partial hatch from the embryo, or any part of the larvae that broke through the chorion.

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2.1.6.3 Lengths

Magnified photographs of each medaka were obtained using a dissecting microscope with an attached video camera and transferred to a computer using the software program Snappy® software (Play Inc., Rancho Cordova, CA, USA). Lengths were determined by measuring from the tip of the head to the end of the notochord directly on the computer screen to the nearest 0.5 mm. These figures were subsequently converted to actual lengths using a known conversion factor (see Teather *et al.* 2000).

2.1.6.4 Developmental Abnormalities

Newly hatched medaka fry were throughly examined for a variety of different developmental abnormalities (Table 2-2).

2.1.7 Sediment Analysis

Frozen sediment from each site and collection date was shipped on January 19, 2005 to The Aquatic Ecosystem Protection Research Branch at the National Water Research Institute in Burlington, Ontario for analysis.

2.1.8 Data analysis

All data were checked for normality using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests in SPSS (Inc., 1989-2002). and the Anderson-Darling normality test in Minitab ®. Data were considered normal when all three tests indicated that the data were normal. All data were analyzed with the statistical programs, SPSS Inc and/or the

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Table 2-2 Description of developmental abnormalities found in fish and examples of studies that have observed those abnormalities.

Developmental Abnormality	Description	Examples
Developmental arrest	medaka in embryo is extremely underdeveloped	Helmstetter and Alden (1995), Gray et al. (1999)
Swollen Abdomen/yolk sac edema/deformed yolk sac	yolk sac/abdomen is abnormally large	Helmstetter and Alden (1995), Zha and Wang (2005), Gray and Metcalfe
small eyes and head	eyes and head are smaller than normal	Villalobos et al. 2000
pericardial edema	a space between the heart and the body wall	Villalobos et al. 2000, Marty et al. 1990, Zha and Whang 2005, Teather et al. 2001, Gray et al. 1999
tubular heart "rudimentary heart"	heart is tube shaped	Villalobos et al. 2000
eye protrusion	-	Barahona-Fernandes 1982
Distortion of eyes	eyes peel	Zha and Wand 2005
degeneration of eye cup	-	Solomon and Faustman 1987 Smithberg 1962
incomplete development of bilateral symmetry of eyes	-	example not found
Ophthalmic edema	bubble on eye	example not found
anophthamia	complete absence of an eye.	example not found

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Abnormality	Description	Examples
Asymetric eyes (anisophthalmia or microphthalmia)	an eye that has an abnormal smallness	Solomon and Faustman 1987 Gray et al. 1999
Cranial abnormality	cranium is abnormal	Andrades et al. 1996
lordosis	spine is curved forwards	Andrades et al. 1996 Barahona-Fernandes 1982
kyphosis (severe, slight)	spine is curved backwards	Solomon and Faustman 1987
scoliosis (severe, slight)	spine is curved laterally	Couch et al. 1977 Barahona-Fernandes 1982
Lordotic/scoliotic/kyphotic syndrome (LSK syndrome)	lordosis, kyphosis, and scoliosis	Fraser et al. 2004
Lack of pectoral fin(s)	-	Fischer et al. 2003
Fin deformity	-	Suzuki et al. 2000
premature	hatched prematurely	
stasis of circulation	The normal flow of body fluids has stopped.	Gray et al. 1999 Smithberg 1962
hemorrhages	severe bleeding	Zha and Wang 2005 Gray and Metcalfe 1999 Gray et al. 1999
bubbles/growths	clear	example not found
Inconsistent blood circulation	-	Gray and Metcalfe

statistical program Minitab ® (Inc., 2000). Parametric data were compared using one-way ANOVA's, or two sample t-tests. Non-parametric data were compared using the Kruskall-Wallis or the Mann Whitney U tests. *Post hoc* testing of significant ANOVA's was carried out using Tukey's *post hoc* multiple comparison test. *Post hoc* testing of significant Kruskall-Wallis tests was carried out using Mann-Whitney U-tests analyzed with the application of a Bonferroni correction, in which the p-value was adjusted based on the number of comparisons.

The number of eggs produced daily by adult medaka females was unpredictable; therefore, each day the number of eggs available for exposures varied. To avoid pseudoreplication, the mean hatching times and lengths for eggs in each jar were calculated. Using these values, the mean hatching times and lengths were calculated for the entire group. In addition, the proportion of surviving individuals and the proportion of developmental abnormalities were calculated for each jar and these values were subsequently used for the entire group.

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CHAPTER 3

COMPARING THE TOXICITY OF REFRIGERATED AND FROZEN STREAM SEDIMENTS USING *ORYZIAS DANCENA* EMBRYOLARVAL BIOASSAYS

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3.1 INTRODUCTION

Aquatic sediments can be a major environmental concern since they may act as a reservoir for a variety of anthropogenic contaminants (Yang *et al.* 2005, Galanopoulou *et al.* 2004, Glasby *et al.* 2004, Ruiz-Fernández *et al.* 2003). As a result, many researchers have investigated the toxicity of potentially contaminated sediments to aquatic organisms. The majority of these studies have been conducted in the laboratory, requiring temporary or long term storage of collected sediments. Many investigators freeze sediments for use in toxicology studies (Strmac *et al.* 2002, Liß and Ahlf 1997, Knaebel *et al.* 1996). Refrigerating sediments at approximately 4°C is also a common storage method (Bettinetti *et al.* 2002, DeFoe and Ankley 1998, Moore *et al.* 1995, Landrum *et al.* 1994, Ho and Quinn 1993, Hoke *et al.* 1990). In many studies, the storage temperature of sediments is not indicated (Rowe 2003, Mondon *et al.* 2001, Day *et al.* 1994, Hoke and Prater 1980, Prater and Anderson 1977). There has been some debate concerning the appropriate sediment storage temperature for sediment toxicity testing (Northcott and Jones 2000, Burton 1991, Carr *et al.* 1989). Approximately 20 years ago, Nebeker *et al.* (1984) noted that the issue of freezing or refrigerating sediments before toxicity testing had not been resolved. This controversy was due to the lack of studies on the effects of temperature on contaminated sediments (Malueg *et al.* 1986). While there has been some progress in resolving this issue since that time, more comparative data, particularly with vertebrates, is needed to fully address the problem.

Although some studies have found that freezing does not alter sediment toxicity (Carr *et al.* 1989) or has a minimal impact on the toxicity of sediments (Stenberg *et al.*

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1998), the majority of studies have discovered that freezing sediments disrupts the contaminants in some way (Geffard *et al.* 2004, Jung and Batley 2004, Stemmer *et al.* 1990). A variety of tests have demonstrated that freezing sediments can increase (Geffard *et al.* 2004, Jung and Batley 2004, Beiras *et al.* 1998) or decrease (Geffard *et al.* 2004, Stemmer *et al.* 1990, Schuytema *et al.* 1989, Malueg *et al.* 1986, Nebeker *et al.* 1984, Thomson *et al.* 1980) the toxicity of these sediments. In addition, Rutledge and Fleeger (1988) found that the vertical profile or structure of sediments is altered after freezing. After comparing the toxicity of refrigerated and frozen sediment using biological assays, most researchers have concluded that sediments used in toxicity testing should be stored at 4°C and used as soon as possible (Northcott and Jones 2000, Beiras *et al.* 1998, Maleug *et al.* 1986). However, frozen storage at -20°C has also been recommended (Sternberg *et al.* 1998). Freezing sediments for toxicity testing is more practical and thus still commonly used as a storage method in experiments. Unlike fresh or refrigerated samples, frozen sediments provide researchers with more time for experimental preparation; this is important since sampling days can be limited due to experiment restrictions (Stenberg *et al.* 1998).

In addition to the uncertainty regarding storage temperature, the majority of studies that have assessed the toxicity of sediments have used aquatic invertebrates (e.g., Norton *et al.* 1999, Stemmer *et al.* 1990, Dillon *et al.* 1994, Schuytema *et al.* 1989) or microorganisms (e.g., Stenberg *et al.* 1998). Little or no research has been conducted to compare the toxicity of refrigerated and frozen sediments using aquatic vertebrates. Thus, the objective of this study is to compare the toxicity of refrigerated and frozen

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sediments using Indian medaka as a vertebrate model. Based on studies using aquatic invertebrates, I predict that frozen sediments will be more toxic than refrigerated sediments to this species.

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3.2 MATERIALS AND METHODS

General details regarding the study organisms, site selection, methodology, and data analysis from this study are provided in the General Methods section. Information specific to this investigation is provided below.

3.2.1 Study Organism

In this study, Indian medaka (*Oryzias dancena*) were used to compare the toxicity of refrigerated and frozen whole sediments. Medaka were purchased from Ward's Natural Science in Rochester, NY.

3.2.2 Site Selection

Three sites (WU, WT, WD) were selected on the Wilmot River (refer to Fig 2-1). One control site was selected on the West River (WR), Prince Edward Island, since this watershed has the least amount of agricultural activity of any rivers neighbouring the Wilmot River and is expected to have sediments that are less toxic than those in the Wilmot. Landuse in the West River watershed consists of 41% agriculture, 51% forest, and 8% other which includes industrial areas, sports fields, schools, abandoned farmland, and wetlands (Department of Agriculture and Forestry 2000).

3.2.3 Sediment Collection, Sieving, and Storage

Due to snow covered roads, sites could only be accessed by snowshoeing or by snowmobile; thus, sediments could not all be collected on the same day. Sediments were

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collected from the Wilmot River and the West River on March 14, 2004 and March 15, 2004, respectively. Sediment to be used in frozen trials was stored in the refrigerator for approximately 16 days at 5 °C ± 2°C and then stored in the freezer at -20 °C for approximately 43 days. Remaining sediment was placed in the refrigerator for use in experiments at 5 °C ± 2°C for no longer than 16 days after collection. Therefore, sediments assigned for both refrigerated and frozen trials remained in the refrigerator for approximately the same time period.

3.2.4 Egg collection and Experimental Design

Fourteen and twelve jars were used to set up refrigerated and frozen trials over a period of 11 and 8 days respectively. The total sample size and the total number of eggs for refrigerated and frozen trials are provided in Table 3-1.

3.2.5 Endpoints

Medaka eggs were checked daily for survivorship and hatching. The notocord of all hatchlings were measured to the nearest 0.01mm and each hatchling was checked for developmental abnormalities.

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Table 3-1 The total sample size (jars) and the total number of medaka eggs exposed to refrigerated and frozen Wilmot River (WU, WD, WT) and West River (C) sediments.

	WU	**WD**	**WT**	**C**
Refrigerated				
Jars	14	14	14	14
Embryos	51	50	50	50
Mean eggs per jar	3.6	3.6	3.6	3.6
Frozen				
Jars	12	12	12	12
Embryos	41	42	42	44
Mean eggs per jar	3.4	3.5	3.5	3.7

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3.3 RESULTS

Freezing sediments did not affect the survivorship of medaka (p's > 0.05, Mann Whitney U tests, Fig 3-1). There were no significant differences in the hatching times of medaka exposed to refrigerated and frozen West River, WD, and WT sediments (Fig 3-2). However, medaka exposed to frozen WU sediment hatched significantly earlier than medaka exposed to the same refrigerated sediment ($p < 0.05$, two sample t-test). Medaka exposed to frozen Wilmot River and West River sediments were significantly smaller in length than those exposed to refrigerated sediments (p's < 0.05, two sample t-tests, Mann Whitney U test, Fig 3-3). In addition, medaka exposed to frozen Wilmot River and West River sediment exhibited significantly more developmental abnormalities than medaka exposed to refrigerated sediments (p's < 0.05, Mann Whitney U tests, Fig 3-4).

Sediments from the West River were expected to be less toxic than those in the Wilmot River since the West River watershed had noticeably less agriculture (78% vs. 41%, respectively). However, there were no significant differences in the survivorship, hatching, lengths, or developmental abnormalities between medaka exposed to frozen or refrigerated West River and Wilmot River sediments (p's >0.05, one way ANOVA's, Kruskall Wallis's, Table 3-2).

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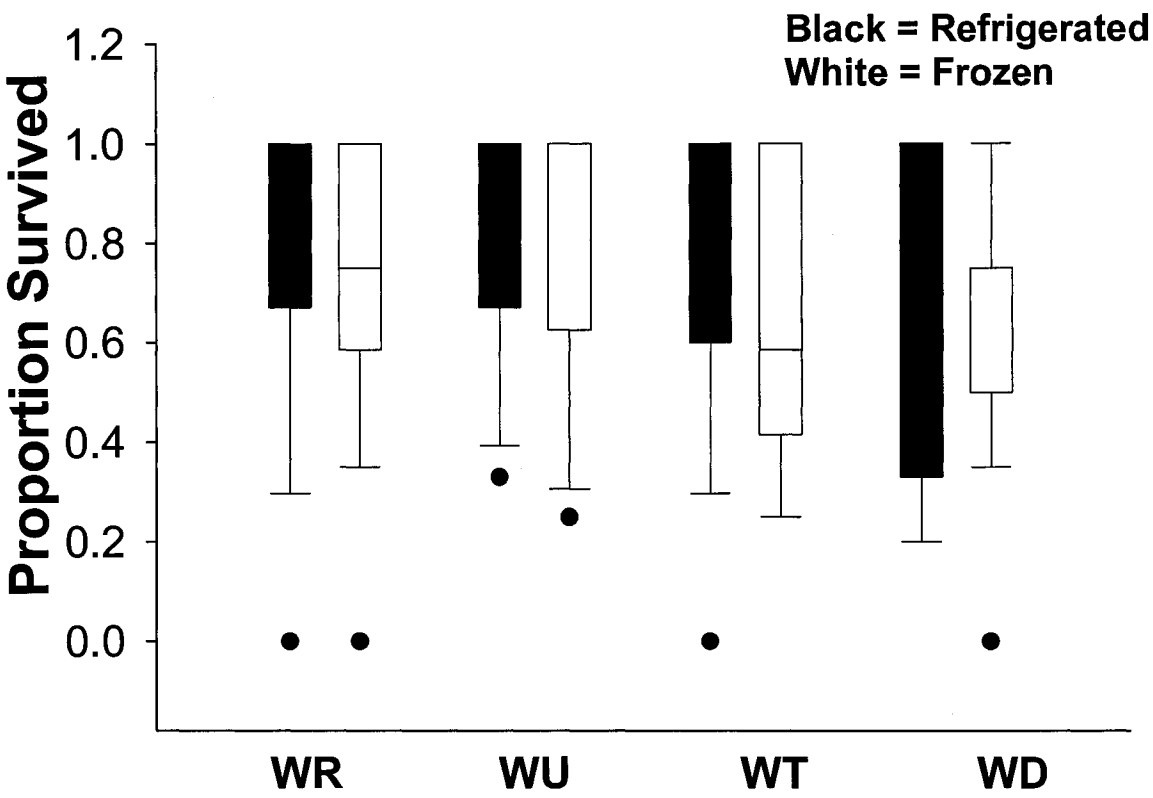

Figure 3-1 Survivorship of Indian medaka exposed to refrigerated (black) and frozen (white) West River (WR) and Wilmot River (WU, WT, WD) sediments. The line in the center represents the median. The box is delineated by the 25th percentile and the 75th percentile; while top and bottom whiskers represent the 90th and 10th percentiles respectively. Individual points indicate data that fall outside the listed percentiles.

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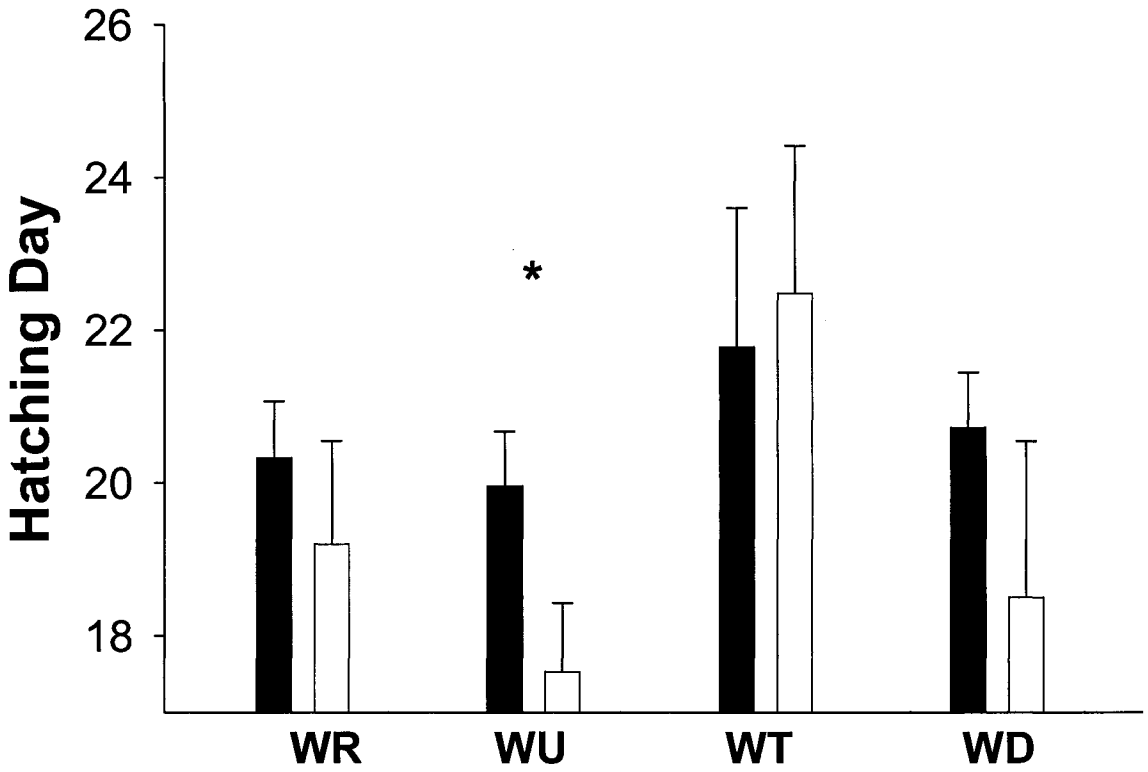

Figure 3-2 Hatching days of Indian medaka exposed to refrigerated (black) and frozen (white) West River (WR) and Wilmot River (WU, WT, WD) sediments. Asterisks indicate significant differences ($p < 0.05$) between frozen and refrigerated sediments for each site. Bars represent the mean and whiskers represent the standard error. All data were normally distributed except WT refrigerated data.

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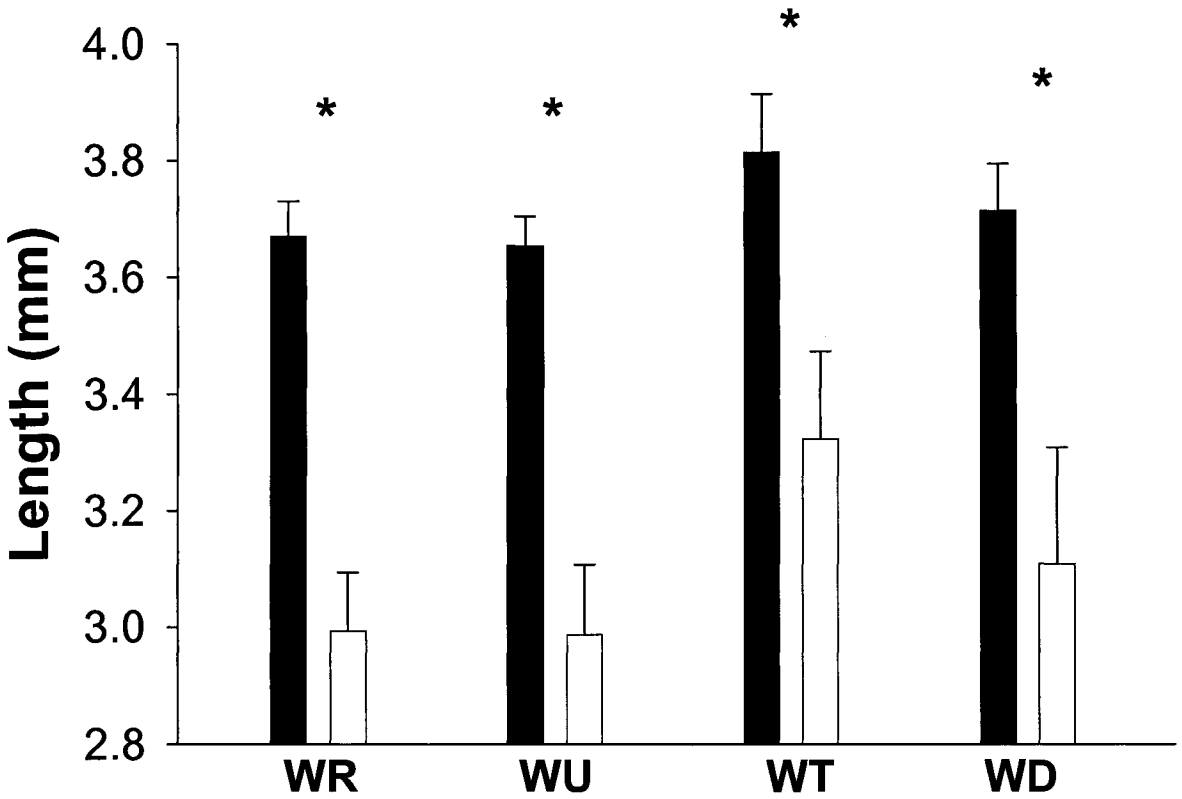

Figure 3-3 Lengths of Indian medaka exposed to refrigerated (black) and frozen (white) West River (WR) and Wilmot River (WU, WT, WD) sediments. Asterisks indicate significant differences ($p<0.05$) between frozen and refrigerated sediments for each site. Bars represent the mean and whiskers represent the standard error. All data were normally distributed except WT refrigerated data.

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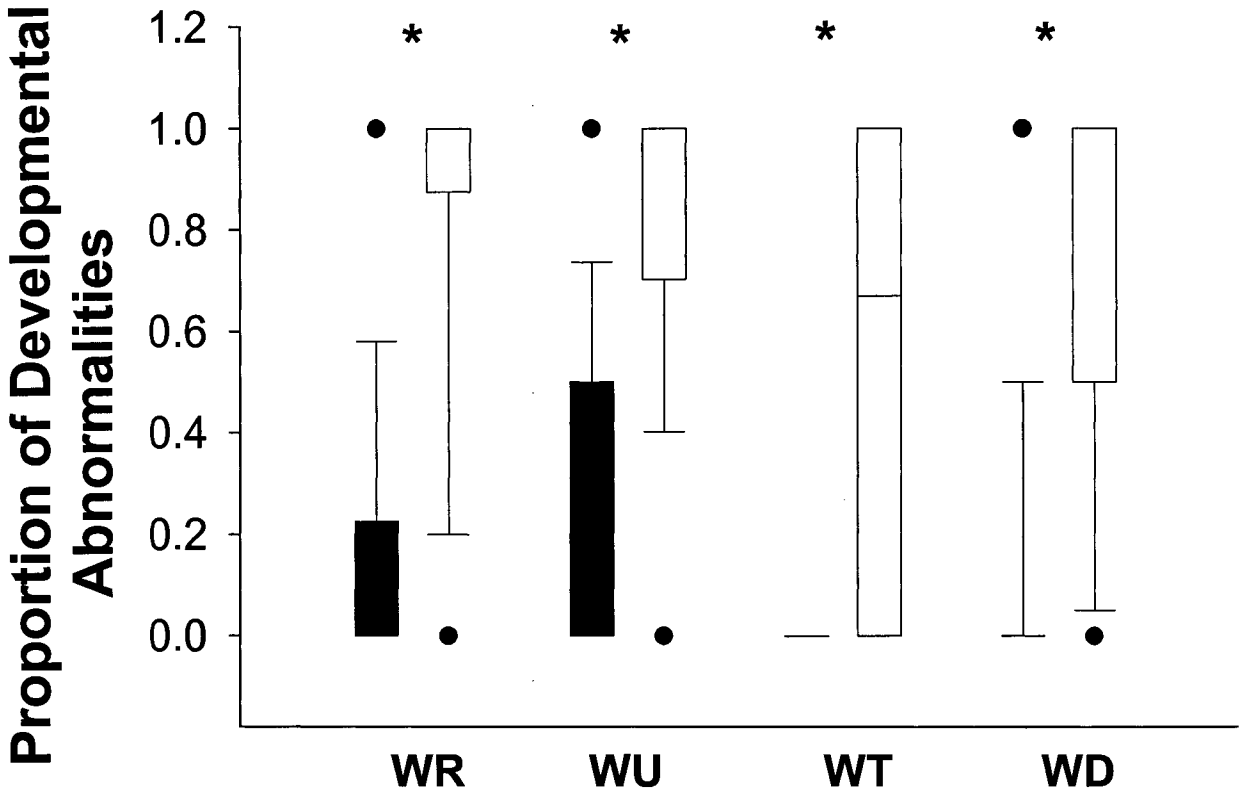

Figure 3-4 Proportion of developmental abnormalities of Indian medaka exposed to refrigerated (black) and frozen (white) West River (WR) and Wilmot River (WU, WT, WD) sediments. Asterisks indicate significant differences ($p<0.05$) between frozen and refrigerated sediments for each site. The line in the center represents the median. The box is delineated by the 25th percentile and the 75th percentile; while top and bottom whiskers represent the 90th and 10th percentiles respectively. Individual points indicate data that fall outside the listed percentiles.

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Table 3-2 P-values for the survivorship, hatching, lengths, and abnormalities of Indian medaka exposed to refrigerated and frozen West and Wilmot River sediments.

	refrigerated sediments p-values	frozen sediments p-values
Survivorship	0.636	0.417
Hatching	0.199	0.128
Lengths	0.305	0.344
Abnormalities	0.205	0.238

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3.4 DISCUSSION

The objective of this study was to test potential differences in toxicity of refrigerated and frozen sediments using a vertebrate model, the Indian medaka (*Oryzias dancena*). Survivorship of embryos that were exposed to refrigerated or frozen sediments did not differ. However, medaka that were exposed to frozen sediments were smaller and had more developmental abnormalities than those exposed to refrigerated sediments. In addition, hatching times of some treatment groups were decreased when medaka eggs were exposed to frozen sediments. Since refrigerated and frozen sediments were stored in the fridge for approximately the same time period, freezing sediments would have most likely increased in toxicity as opposed to refrigerating sediments decreasing in toxicity.

Many studies have shown an increase in the toxicity of contaminated sediments upon freezing. For example, Geffard *et al.* (2004) found that frozen sediment elutraites, which are the overlying waters of sediment (Sasson-Brickson and Burton 1991), increased the number of abnormalities in *Crassostrea gigas* oysters. Jung and Batley (2004) reported that the selenium concentrations in frozen sediments were noticeably higher than in refrigerated sediments. Beiras *et al.* (1998) found that freezing sediments prior to experiments negatively affected the percentage of normal *Crassostrea gigas* larvae.

The mechanism or changes resulting in increased toxicity of frozen sediments is often difficult to determine (Malueg *et al* 1986). Freezing sediments could alter the equilibrium of the toxicant within the sediments (Beiras *et al.* 1998). For example, certain contaminants that were bound to the refrigerated sediments could be released after

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freezing (Beiras *et al.* 1998). In addition, chemical reactions caused by freezing could create new toxicants that were not present in fresh or refrigerated sediments (Beiras *et al.* 1998). Both of these scenarios could explain the increase in toxicity of frozen sediments, in this study.

Jung and Batley (2004) offered another explanation for the observed increase in toxicity in frozen sediments. They suggested that microorganisms and invertebrates may be destroyed during freezing and release contaminants through cell rupturing. They found that selenium concentrations were noticeably higher in the frozen sediments than in those stored at 4 °C. They suggested that consumed selenium was released upon the rupture of the cells of microorganisms during freezing and ultimately increased the concentration of selenium in the frozen sample. In addition, Schuytema *et al.* (1989) found that the concentration of soluble organic carbon (SOC) was significantly higher in frozen sediments than in sediments stored at 4 °C. They speculated that the microorganisms and invertebrates that were destroyed during freezing may have released SOC. In the present study, the microorganism or macroinvertebrate scenario may be plausible, especially since invertebrates were observed in the sediments.

Alternatively, some microorganisms can survive under frozen conditions (Lund 2000, Sternberg *et al.* 1998, Hennessy *et al.* 1996, DiGirolamo *et al.* 1970), and subsequently contribute to microbial degradation of contaminants in the sediment. For example, Sternberg *et al.* (1998) indicated that some microorganisms can adapt to freezing and thawing cycles. Conversely, freezing can damage or destroy microbial activity (Lund 2000). The survival of microorganisms under frozen conditions will

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ultimately depend on factors such as the species, microbial strain, available nutrients, growth rate, surrounding environment, freezing rate, and the final temperature (Lund 2000). Microorganisms can degrade pesticides in soil (Kaufman and Blake 1973, Saltzman *et al.* 1972, Audus 1949) and may produce metabolites which are less toxic or more toxic than the original pesticide (Widenfalk 2005). In this study, refrigerated sediments were used for experimentation within 16 days of collection and frozen sediments were stored in the refrigerator for approximately the same time before being frozen. Thus, the frozen sediments used in this study had a longer sediment storage time than the refrigerated sediments, potentially altering more time for microbial degradation of potential contaminants within the frozen sediments, assuming active microorganisms were present. Degraded contaminants may have become more toxic than the original contaminants.

Longer sediment storage times may also result in increased ammonia levels within the sediment or in the overlying water of the sediment (Geffard *et al.* 2004, Moore *et al.* 1995) and contribute to increased in toxicity. Moore *et al.* (1995) discovered that the ammonia levels in the overlying water of certain test sediments increased and decreased in a cyclic pattern over time. Although they were uncertain of the cause of the cyclic pattern of ammonia levels, they speculated that ammonia levels may have been altered by the assimilation of ammonia by microorganisms. They suggested that if ammonia assimilating microorganims were present in their test sediments, ammonia concentrations would increase when microbial communities were low and decrease when these communities were high. As soon as these sediments are taken from storage and used in

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experiments, the increased levels of interstitial ammonia within the sediment would diffuse and stabilize into the water above the sediment. In general, sediments stored for long periods of time may accumulate increased ammonia concentrations due to decreased microbial activity. In this study, ammonia cycling due to microorganisms may have resulted in an increase in the toxicity of frozen sediments or a decrease in the toxicity of refrigerated sediments. For example, ammonia cycling among surviving microorganisms may have caused an increase in the toxicity of thawed frozen sediments during the medaka exposure period. Additionally, ammonia cycling may have caused a decrease in toxicity of refrigerated sediments during the medaka exposure period. Frozen sediments would most likely exhibit less microbial activity than refrigerated sediments since freezing would destroy the majority of the cells.

Most studies have compared the toxicity of refrigerated and frozen sediments using aquatic invertebrates (e.g., Norton *et al.* 1999, Stemmer *et al.* 1990, Dillon *et al.* 1994, Schuytema *et al.* 1989), and almost no studies have used aquatic vertebrates. Schuytema *et al.* (1989) found that the LC_{50} values for amphipods *(Hyalella azteca)* exposed to frozen sediments was significantly greater than those exposed to a cold stored sample. In addition, Dillon *et al.* (1994) found that the survival of mysid shrimp *Mysidopsis bahia* was more variable when exposed to sediments that were frozen. I found no studies that used vertebrate models to compare the toxicity of refrigerated and frozen sediments. The only related study was conducted by Hardy *et al.* (1987) who compared the toxicity of fresh and frozen sea-surface microlayers using sand sole eggs and found that freezing had no impact on the eggs.

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3.4.1 Conclusion

The results of this study indicate that freezing sediments increases their toxicity to the vertebrate model, Indian medaka. While the mechanism of increased toxicity is unclear, previous studies suggest a number of possibilities. Freezing sediments may potentially create or release toxicants that were not present in refrigerated sediments. Freezing sediments can also cause the cells of microorganisms and invertebrates to rupture and release contaminants, which would lead to an increase in the concentration of contaminants in the frozen sediments. Frozen sediments are usually stored for longer time periods than refrigerated sediments which allows more time for microbial degradation of contaminants attached to sediments; these derivatives can be more toxic than the original contaminant. Finally, decreased microbial activity within the sediment may increase levels of ammonia resulting in an increase of ammonia in the overlying waters of the sediment used in experiments. Therefore, according to the findings in this study, sediments used in toxicity tests involving vertebrate test subjects should be used as soon as possible and without freezing. Freezing sediments should only be used as a storage method when the fates of known toxicants within the experimental sediments are known.

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CHAPTER 4

ASSESSING THE TOXICITY OF WILMOT RIVER SEDIMENTS USING JAPANESE MEDAKA (*ORYZIAS LATIPES*) EMBRYOLARVAL BIOASSAYS

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4.1 INTRODUCTION

Aquatic sediment contamination is a major global issue (Munawar and Dave 1996). Both pesticides (Guzzella *et al.* 2005, Rajendran et al 2005, Wurl and Obbard 2005, Mutch *et al.* 2002) and metals (Qari *et al.* 2005, Santos *et al.* 2005, Muohi *et al.* 2003) are commonly found in aquatic sediments. Furthermore, high levels of nutrients such as nitrogen (Geffard *et al.* 2004, Hu *et al.* 2001, Mortimer *et al.* 1998, Van Sprang 1996, Ankley *et al.* 1990) and phosphorous (Cheung *et al.* 2003) have also been detected. Indeed, a certain degree of contamination exists in most aquatic sediments. In the present study, the toxicity of Wilmot River sediments, a river highly impacted by agriculture, was investigated using Japanese medaka (*Oryzias latipes*) embryolarval bioassays in order to see if agricultural contaminants from the fields surrounding the watershed are contaminating river sediments and thus have the potential to negatively impact fish populations. Agricultural contaminants that may be entering the Wilmot River through agricultural runoff or spray drift include pesticides, agricultural related metals, and high levels of nutrients.

Pesticides vary in their toxicity to fish. Some such as, azinphos-methyl can result in extensive fish mortality even at very low concentrations. The effects of lethal concentrations of pesticides are apparent following fish kill events (Gormley et al. 2005). Sublethal effects of pesticides to aquatic organisms are less obvious. For example, sublethal concentrations of certain pesticides can result in decreased growth (e.g., Teather *et al.* 2005), altered hatching times (e.g., González-Doncel *et al.* 2003), increased number of developmental abnormalities (e.g., Teather *et al.* 2001), reduced mobility

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(e.g., Beauvais *et al.* 2001), and decreased reproductive success (e.g., Nimrod and Benson 1998). A number of studies have demonstrated that pesticide bound sediments can also affect fish (DiPinto 1996), aquatic invertebrates (Schuytema *et al.* 1989, Wilcock *et al.* 1994, Muir *et al.* 1985), and a variety of micro-organisms (Widenfalk 2005). Sublethal effects of toxic pesticides may have critical repercussions on the long term survival of exposed fish, resulting in changes in the population and community structure.

Metals such as lead, copper, zinc, and cadmium are also common in the agricultural industry (Haygarth 2002). The sources of metals in agriculture include fertilizers, roughage, animal feed, animal manure, animal medicine, and the atmospheric deposition of metals unto agricultural land (Haygarth 2002). Like pesticides, sublethal concentrations of metals can result in abnormal fish development (e.g., Weis and Weis 1977), variation in hatching time (e.g., Dave and Xiu 1991), size reduction (e.g., Brauner and Wood 2002), respiratory impairment (e.g., Akiyama 1970), and decreased locomotion (e.g., Samson *et al.* 2001). Metals bound to sediments can also negatively affect fish, by decreasing growth (Rowe 2003, DelValls *et al.* 1998).

While nitrogen, ammonia, and phosphorous occur naturally in soils, a surplus of fertilizer and manure on agricultural fields can greatly increase the quantity of these nutrients (Carpenter *et al.* 1998). Subsequently, soil erosion from agricultural land may result in large amounts of excess nutrients entering aquatic systems (Carpenter *et al.* 1998). This surplus can adversely impact aquatic organisms. For instance, excess nutrients permit algal blooms and extensive plant growth. Upon decomposition of this

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excess organic matter, dissolved oxygen is depleted and many organisms, including fish, can suffer extensive mortality (Carpenter *et al.* 1998). Furthermore, high concentrations of ammonia , nitrates and nitrites can be directly toxic to fish (Tomasso and Carmichael 1986, Kincheloe 1979, Rubin and Elmaraghy 1977).

In sediment toxicity studies, various organisms have been exposed to whole sediments (Viganò *et al.* 1995), sediment extracts (Strmac *et al.* 2002), sediment *in situ* (Baudo *et al.* 1999), sediment elutriates, and porewater (Sasson-Brickson and Burton 1991). The *in situ* phase is the most realistic approach (Burton 1991). However, due to environmental complications of this method, a more practical method of exposing organisms to natural sediment conditions can be achieved by using whole sediments in a laboratory. This method has been used in a variety of studies as a method of exposing aquatic organisms to contaminated sediments (Day *et al.* 1994, Viganò *et al.* 1995, Mondon *et al.* 2001, Shin *et al.* 2002, Duft *et al.* 2003). The majority of whole sediment toxicity studies have used invertebrates or micro-organisms as test organisms and relatively few studies have used fish.

The first objective of this study was to compare the toxicity of sediments from a river highly impacted by agriculture with those from a river with little agricultural activity using Japanese medaka embryolarval bioassays. Japanese medaka exposed to Wilmot River sediments were expected to be shorter in length, have altered hatching times, exhibit more developmental abnormalities, and have lower survivorship than those in the control groups. The second objective of this study was to compare the toxicity of impacted sediments collected during the crop growing and non-growing seasons using the

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same medaka model to determine if there is seasonal variation in sediment toxicity. Sediment collected during the crop growing season were expected to be more toxic than those collected during the non-growing season since higher amounts of agricultural contaminants would have been utilized in the watershed during the summer. Therefore, medaka exposed to sediments collected during the growing season were predicted to be shorter in length, have altered hatching times, have increased developmental abnormalities, and have lower survivorship than those exposed to sediments collected in the non-growing season.

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4.2 MATERIALS AND METHODS

General details regarding the study organisms, site selection, methodology, and data analysis from this study are provided in the General Methods section. Information specific to this investigation is provided below.

4.2.1 Study Organism

Japanese medaka (*Oryzias latipes*) were purchased from the Carolina Biological Supply Company in North Carolina. Medaka were chosen for this study since they are commonly used in toxicity testing. They are easy to breed and maintain in the laboratory, and the egg chorion is transparent, facilitating the examination of developing embryos (see Metcalfe *et al.* 1999). Eggs typically hatch within 7 to 21 days (Kirchen and West 1976) and early life stages are particularly sensitive to contaminants (Cooper *et al.* 1993,Wisk and Cooper 1990, Owens and Baer 2000).

4.2.2 Site Selection

Test sites were selected on the Wilmot River (WU, WT, WD), a watershed known to be heavily impacted by agricultural runoff. In addition, there were two control groups. One control contained sediment collected from a site on the main branch of Priest Pond Creek (Fig 4-1). Priest Pond Creek was selected as a reference site since there is very little agricultural activity within its watershed (Fig 4-1). This control group will be referred to as Priest Pond control (PP). The control group without sediment contained only embryo rearing solution. This group will be referred to as the no sediment control

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Fig 4-1 Landuse map of Priest Pond Creek watershed indicating the selected control site (PP). Light brown indicates agriculture and green indicates forest habitat. This watershed consists mostly of forest (83%), and a small amount of agriculture (11%). The remaining amount (6%) is made up of areas such as industrial areas, sports fields, schools, abandoned farmland, and wetlands (Department of Agriculture and Forestry 2000).

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(CN).

4.2.3 Sediment Collection, Sieving and Storage

Sediments used in this study were collected during the growing season (July) and during the post-harvest (December), in order to see if there was a difference in sediment toxicity at different times of the year. Sediments were collected on July 2, July 24, and December 17 during the afternoon and early evening. On July 2 and December 17, sediment was collected from the Wilmot River sites and the control site on Priest Pond Creek. On July 24, sediment was only collected from the Wilmot River sites. Since sediments that have been frozen for storage appear to increase in toxicity (see Chapter 3), only refrigerated sediments, stored for no longer than two weeks, were used to conduct experiments in this study.

4.2.4 Experimental Design

Over a period of 5-6 days, 12 or14 jars containing sediments from each of the Wilmot River sites and/or the Priest Pond Creek site were used to set up experiments. Therefore, there were five groups in total, three from the Wilmot River, one from Priest Pond Creek, and one control with no sediment. Jars in the control group without sediment contained only 150 ml of embryo-rearing solution. The control group without sediment did not contain mini-petri dishes and the eggs were placed directly on the bottom of the jar. The total sample size for the control group with no sediment was 12 and the total number of jars in this group was 53 (n=53). The total sample sizes and total

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number of eggs for refrigerated experiments are provided in Table 4-1.

4.2.5 Endpoints

Medaka eggs were checked daily for survivorship and hatching. The notocord of all hatchlings was measured to the nearest 0.01mm and each hatchling was checked for developmental abnormalities.

4.2.6 Data Analysis

Statistical differences in seasonal toxicity were measured using a one-way ANOVA (parametric data) or Kruskal Wallis (nonparametric data) when three groups were compared. When two groups were compared, a two sample t-test or a Mann-Whitney U test was used.

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Table 4-1 Total number of jars and medaka embryos exposed to Wilmot River (WU, WD, WT) and/or Priest Pond Creek control sediments (PP) on July 2, July 24, and December 17.

	WU	WD	WT	PP
July 2				
Jars	12	12	12	12
Embryos	55	54	55	54
Mean eggs per jar	4.6	4.5	4.6	4.5
July 24				
Jars	12	12	12	N/A
Embryos	42	43	44	N/A
Mean eggs per jar	3.5	3.6	3.7	N/A
December 17				
Jars	12	12	12	12
Embryos	38	38	38	39
Mean eggs per jar	3.2	3.2	3.2	3.3

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4.3 RESULTS

4.3.1 Comparing the toxicity of Wilmot River sediments to controls

4.3.1.1 Survivorship

The survivorship of medaka exposed to July 2 sediments from two of the Wilmot River sites was significantly lower than that of medaka in both control groups (*p*'s <0.005, Mann Whitney U test; Fig 4-2A). Sediment from only one of the Wilmot River sites collection on July 24 was associated with higher mortality in medaka (p's < 0.005, Mann Whitney U test; Fig 4-2B). Medaka that were exposed to both Wilmot River and Priest Pond Creek sediments collected on December 17 exhibited higher mortality than those in the control group with no sediment (p's ≤ 0.01, Mann Whitney U test; Fig 4-2C). Fewer than 50% of medaka survived exposure to WD and WU December 17 sediments. Wilmot River sediments collected from the downstream site (WD) resulted in particularly high mortality among medaka embryos, leaving only a few larvae to examine for developmental abnormalities and length. Due to the small sample size, medaka exposed to these sediments were omitted from hatching, length, and developmental abnormality statistical tests.

4.3.1.2 Hatching

Medaka exposed to Wilmot River sediments collected on July 2 and July 24 hatched significantly earlier than control medaka (p < 0.01, Tukey's *post hoc* multiple comparison; Fig 4-3A and B). There were no significant differences in the hatching times of embryos exposed to sediments collected on December 17 (p < 0.001, Tukey's *post hoc*

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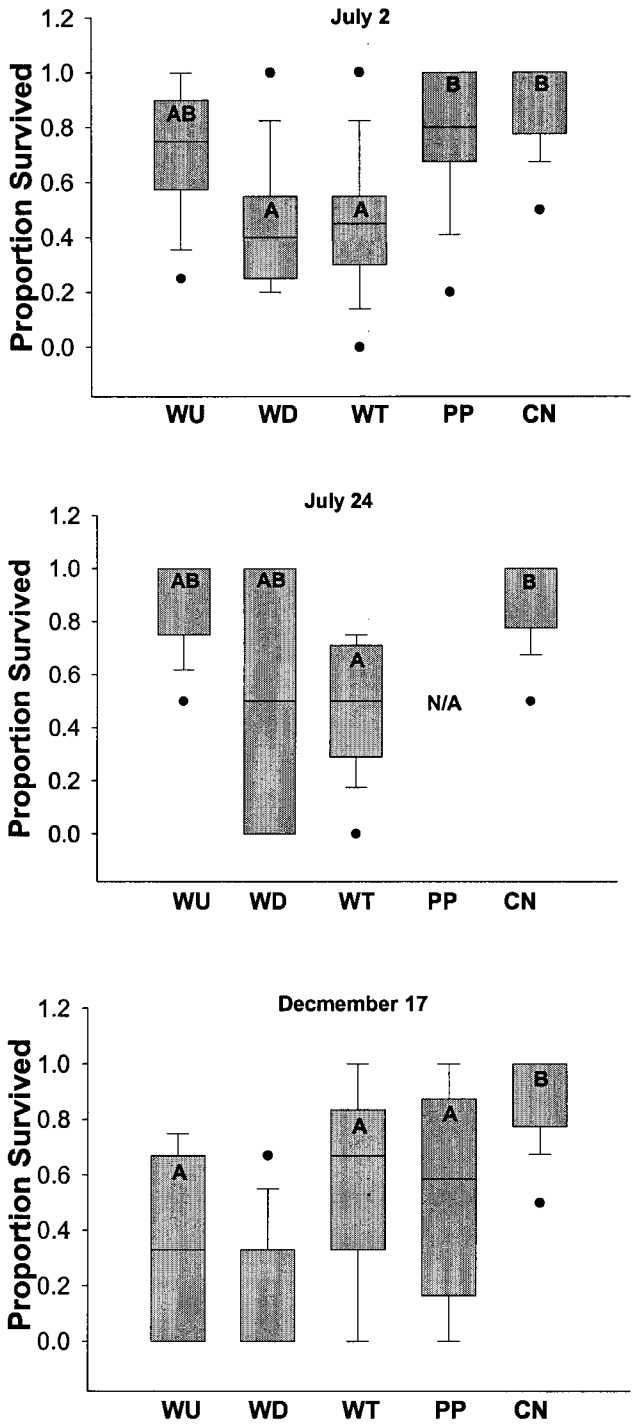

Figure 4-2 Survivorship of Japanese medaka exposed to Wilmot River sediments (WU, WD, WT) collected on July 2 (A), July 24 (B), and December 17 (C) and Priest Pond Creek sediments (PP) collected on July 2 and December 17. The control group with no sediment is indicated by CN. Different letters indicate significant differences.

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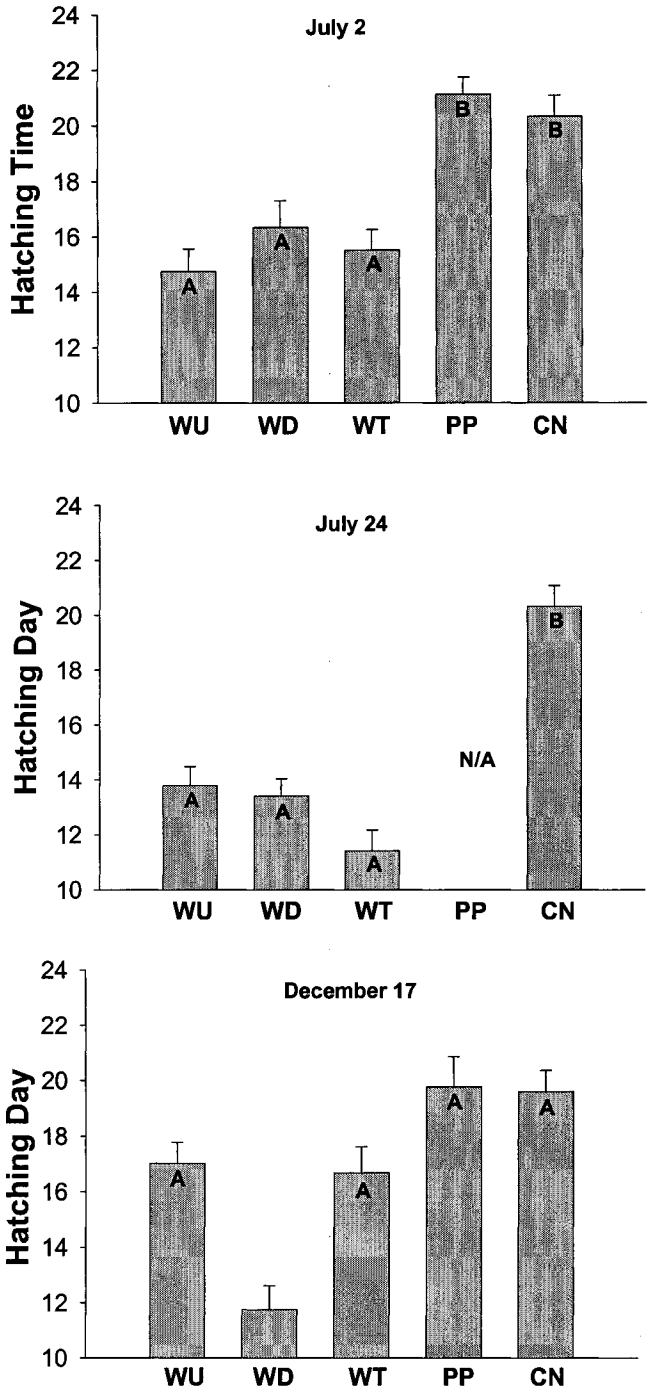

Figure 4-3 Hatching day of Japanese medaka exposed to Wilmot River sediments (WU, WD, WT) collected on July 2 (A), July 24 (B), and December 17 (C) and Priest Pond Creek sediments (PP) collected on July 2 and December 17. The control group with no sediment is indicated by CN. Different letters indicate significant differences. Whiskers indicate standard errors.

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multiple comparison; Fig 4-3C).

4.3.1.3 Hatching Length

Japanese medaka exposed to Wilmot River and Priest Pond Creek sediments collected on July 2 were significantly smaller at hatching than medaka in the control group with no sediment (p's < 0.001, Mann Whitney U test; Fig 4-4A). Medaka fry that had been exposed to Wilmot River sediments from this period were noticeably smaller than those exposed to sediment collected from Priest Pond Creek although the difference was significant for only one of these sites ($p < 0.01$, Mann Whitney U test). Fish that were exposed to all of the Wilmot River sediments collected on July 24 were significantly smaller than those in the control group with no sediment (p's < 0.001, Tukey's post hoc multiple comparison; Fig 4-4B). Medaka that were exposed to sediments from two Wilmot River sites and Priest Pond Creek sediments collected on December 17 were shorter than medaka in the control group with no sediment (p's < 0.001, Mann Whitney; Fig 4-4C).

4.3.1.4 Developmental Abnormalities

Fish that were exposed to Wilmot River and Priest Pond Creek sediments collected in July and December consistently exhibited more developmental abnormalities than the control group with no sediment (p's < 0.01, Mann Whitney U test; Fig 4-5). Medaka exposed to Wilmot River and Priest Pond sediments collected on July 2 had significantly more yolk sac protrusions than those in the control group without sediment

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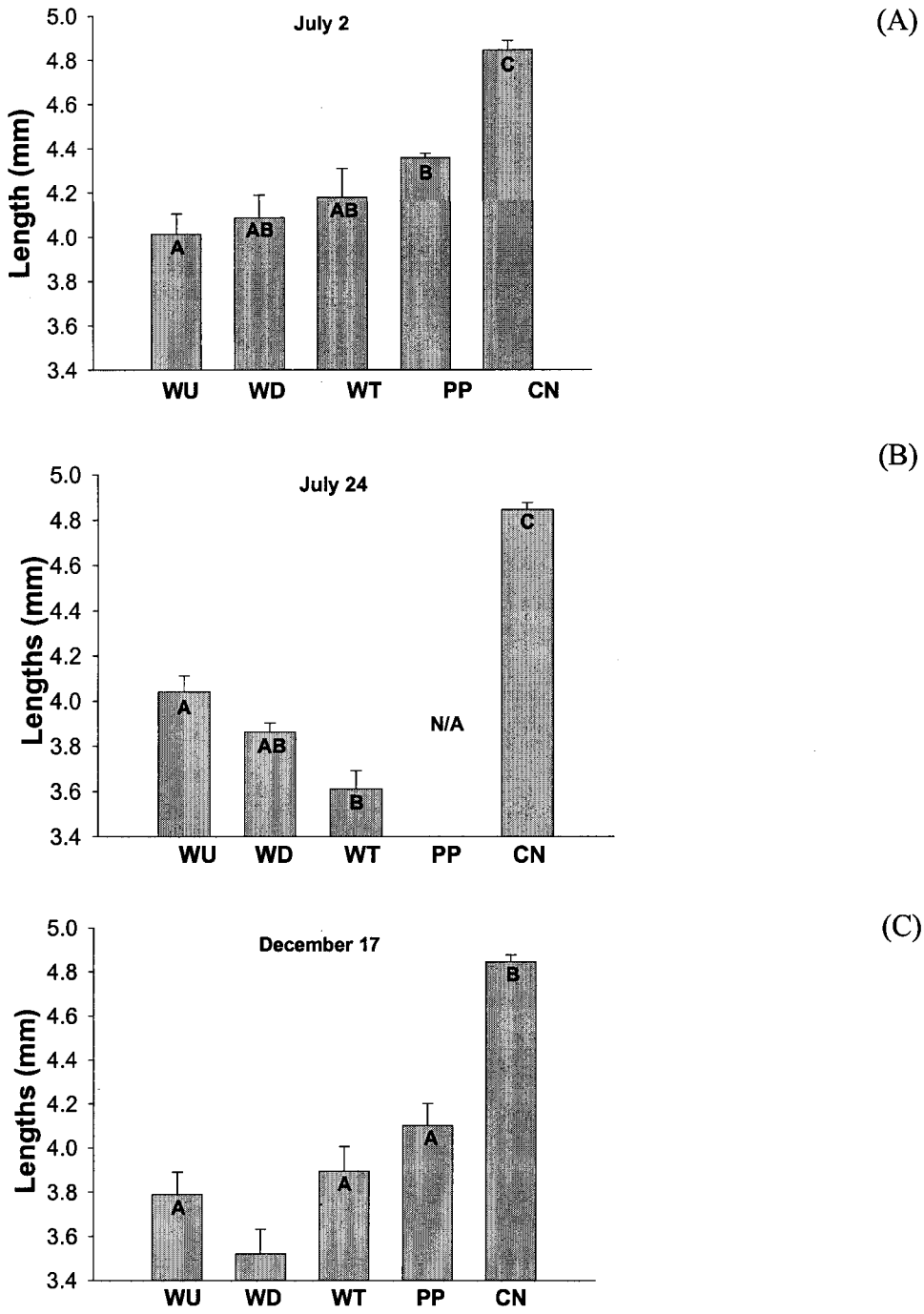

Figure 4-4 Lengths (mm) of Japanese medaka exposed to Wilmot River sediments (WU, WD, WT) collected on July 2 (A), July 24 (B), and December 17 (C) and Priest Pond Creek sediments (PP) collected on July 2 and December 17. The control group with no sediment is indicated by CN. Different letters indicate significant differences. Whiskers indicate standard errors.

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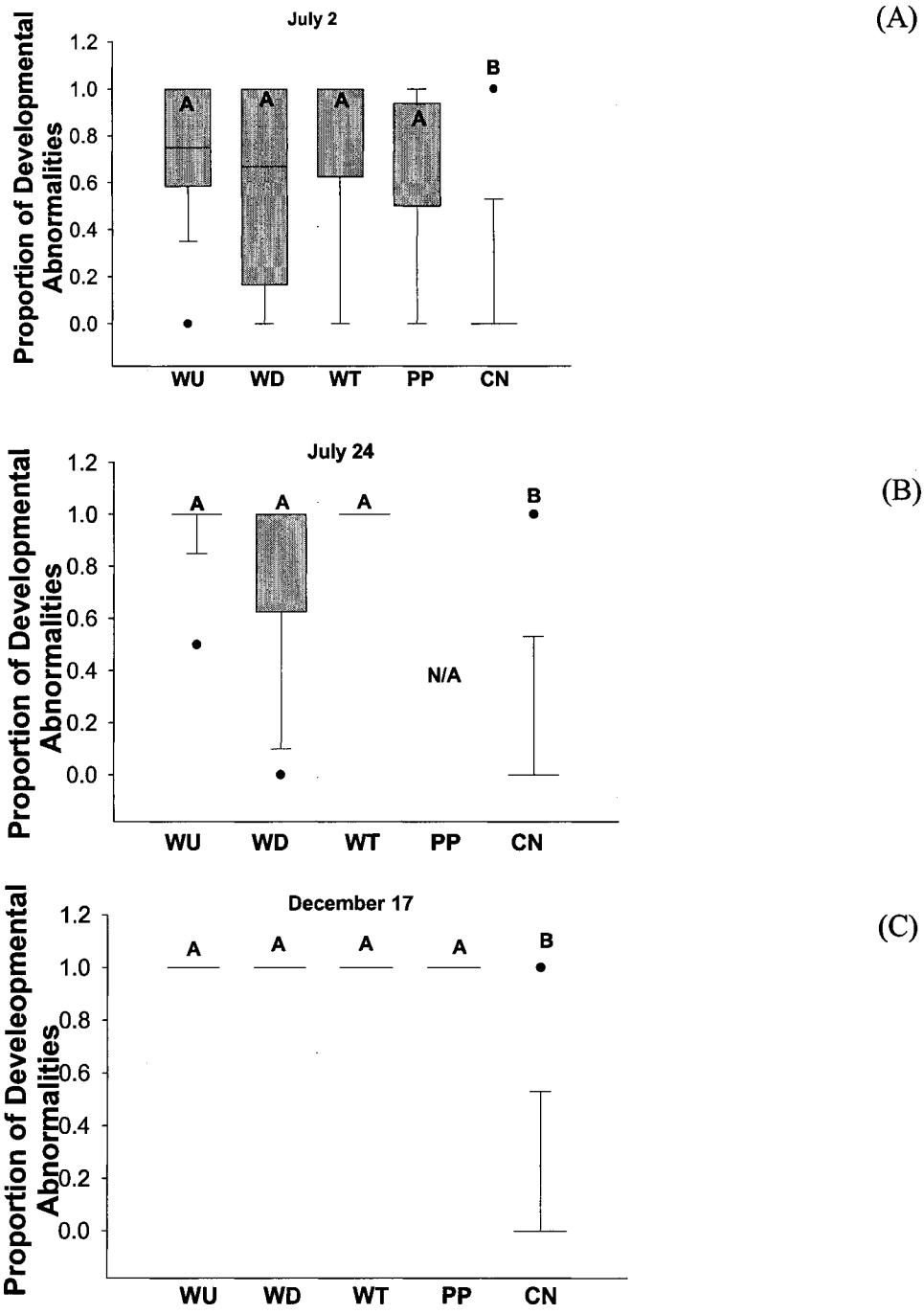

Figure 4-5 Proportion of developmental abnormalities of Japanese medaka exposed to Wilmot River sediments (WU, WD, WT) collected on July 2 (A), July 24 (B) and Priest Pond Creek sediments (PP) collected on July 2 and December 17. The control group with no sediment is indicated by CN. Different letters indicate significant differences.

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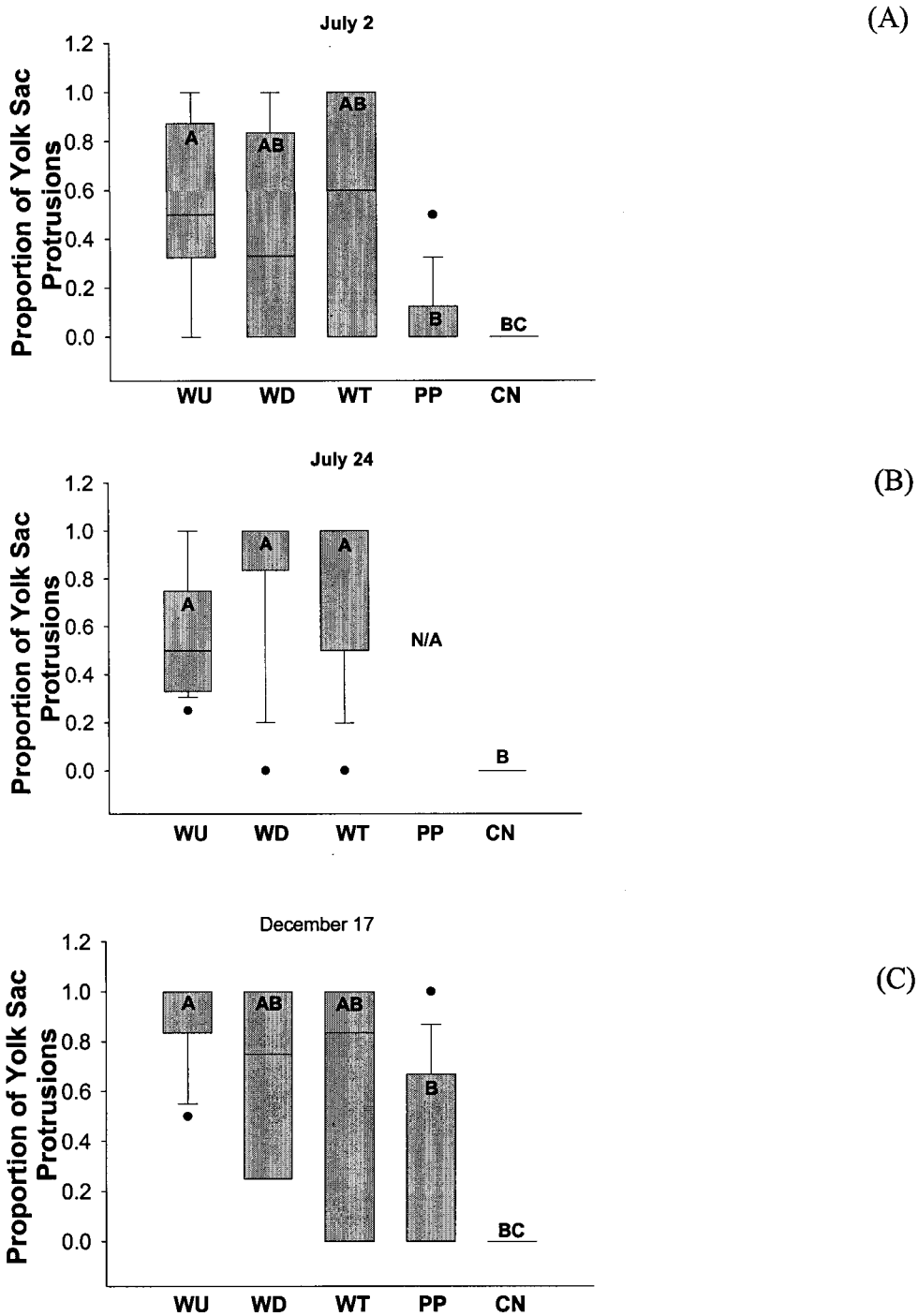

Figure 4-6 Proportion of yolk sac protrusion of Japanese medaka exposed to Wilmot River sediments (WU, WD, WT) collected on July 2 (A), July 24 (B), and December 17 (C) and Priest Pond Creek sediments (PP) collected on July 2 and December 17. The control group with no sediment is indicated by CN. Different letters indicate significant differences.

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(p's < 0.01, Mann Whitney; Fig 4-6A). Yolk sac protrusions were defined as medaka that hatched with the yolk sac first. Medaka exposed to WU sediments collected on July 2 and December 17 had significantly more yolk sac protrusions than medaka exposed to Priest Pond Creek sediments ($p < 0.01$, Mann Whitney U test, Fig 4-6A and C). Furthermore, medaka embryolarvae that were exposed to Wilmot River sediments collected on July 2, 24 and December 17 had more yolk sac protrusions than those in the control group without sediment (p's<0.01, Mann Whitney U test, Fig 4-6B and C).

The most common developmental abnormalities found in medaka exposed to sediments collected on July 2, July 24, and December 17 are provided in Table 4-2 (see also Fig 4-7). Medaka exposed to WU sediments collected on July 2 and December 17 and WT sediments collected on December 17 had noticeably more spinal deformities than any other groups (Table 4-2). In total, embryolarvae exposed to WU sediments collected on July 2 and July 24 had noticeably more abnormalities than any other groups.

4.3.2 Comparing the seasonal toxicity of Wilmot River Sediments

Medaka that were exposed to sediments collected in December exhibited greater mortality than those exposed to sediments in July. The mortality of medaka exposed to upstream (WU) and downstream (WD) December 17 sediments was significantly greater than those exposed to sediments collected from the same sites in July 2 and July 24 and July 2, respectively ($p < 0.01$, Mann Whitney U test; Fig 4-2). Medaka exposed to July 24 sediments hatched earlier than those exposed to July 2 and December 17 sediments. These differences were significant for WT and WU sediments collected on July 24 and

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Table 4-2 The total number of cases of the most common abnormalities found in Japanese medaka exposed to Wilmot River sediments (WU, WD, WT), Priest Pond Creek sediments (PP) collected on July 2, July 24, and December 17, and the control group with no sediment (CN).

	Kyphosis	**Lordosis**	**Scoliosis**	**Pericardinal edema**	**enlarged or deformed yolk sac**	**eye deformity**	**Pectoral Fin abnormality or loss**	**Total**
July2								
PP	5	1	0	13	12	1	1	33
WU	1	5	6	17	22	1	2	54
WD	0	1	0	8	13	0	1	23
WT	2	2	0	10	12	1	1	28
July24								
WU	3	0	0	23	22	0	2	50
WD	1	2	0	7	11	1	0	22
WT	1	1	0	10	10	0	2	24

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	Kyphosis	Lordosis	Scoliosis	Pericardinal edema	enlarged or deformed yolk sac	eye deformity	Pectoral Fin abnormality or loss	Total
Dec. 17								
PP	3	0	2	17	15	0	4	41
WU	4	5	0	7	9	0	4	29
WD	1	0	0	4	4	1	2	12
WT	3	5	4	12	13	1	3	41
CN								
	0	1	0	1	0	0	0	2

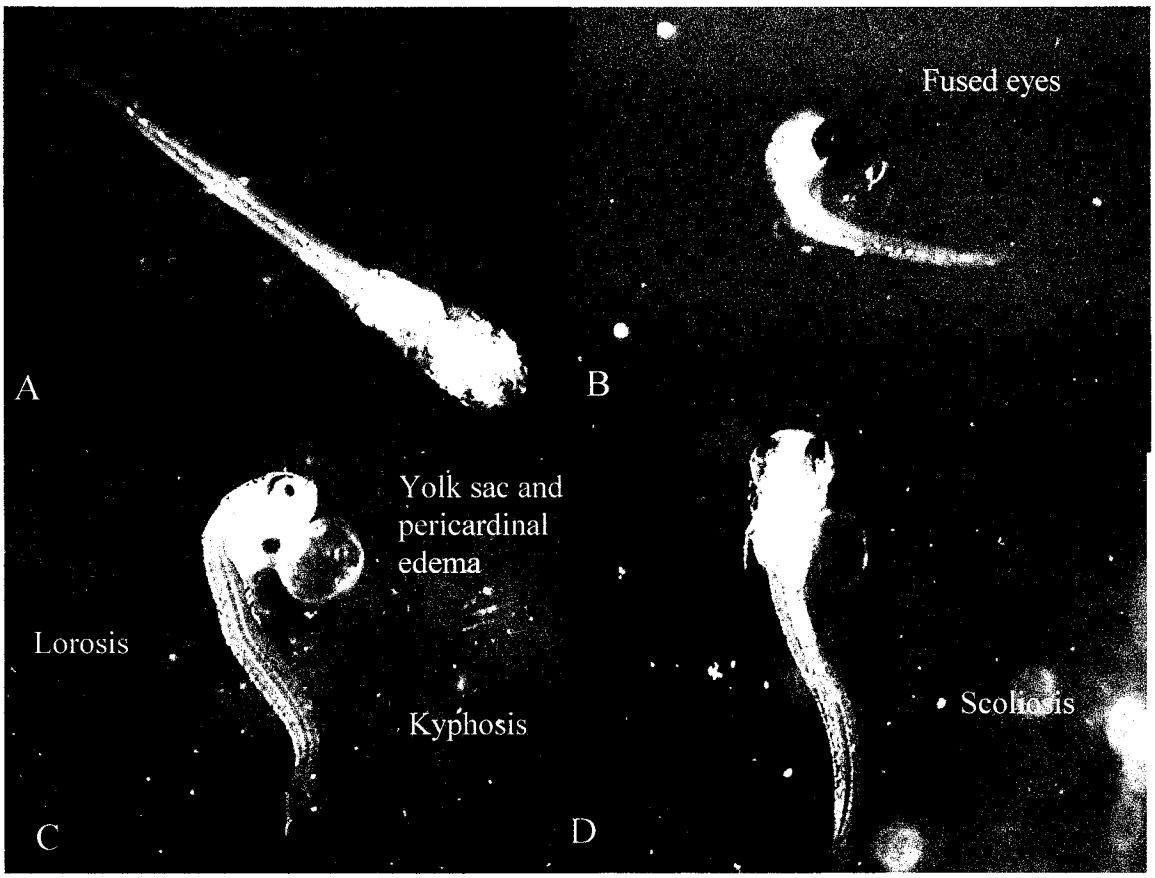

Fig 4-7 Normal fry (A), fused eyes (B), kyphosis, lordosis, yolk sac and pericardinal edama (C), and scoliosis (D).

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sediment collected from the same sites on December 17 and also between WT sediments collected on July 24 and WT sediment collected on July 2 (p's<0.05, Tukey's post hoc multiple comparison; Fig 4-3). Medaka exposed to WT sediments collected on July 24 were significantly smaller than those exposed to July 2 sediments (p<0.01, Tukey's post hoc multiple comparison; Fig 4-4). Furthermore, the proportion of developmental abnormalities were significantly greater in medaka exposed to WU sediments collected on December 17 than those exposed to July 2 sediments (p<0.017, Mann Whitney U test; Fig 4-5).

4.3.3 Sediment Analysis

All sediments from the Wilmot River and Priest Pond Creek could not be analyzed in time for the preparation of this thesis. Unforeseeable delays at the Aquatic Ecosystem Protection Research Branch at the National Water Research Institute in Burlington, Ontario made it impossible to have sediments analyzed in time for submission of this thesis.

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4.4 DISCUSSION

4.4.1 Comparing the toxicity of Wilmot River sediments to controls

The objective of this part of the study was to compare the toxicity of sediments taken from a river heavily impacted by agriculture with those taken from a relatively clean river. The Wilmot River watershed is dominated by agriculture with 78% of the watershed devoted to farming and only 11% in forest (Department of Agriculture and Forestry 2000). There were two pesticide related fish kills in the Wilmot River in 2002 (Gormley *et al.* 2005) and pesticides have been detected in Wilmot river water and sediment every year since that time (Murphy and Mutch 2005). Thus, this river was selected as a preliminary test river to develop and evaluate a vertebrate sediment toxicity bioassay and provide information on the possible toxicity of sediments. In contrast to the Wilmot River, Priest Pond Creek has little agricultural activity with only 11% devoted to farming and 83% in forest (Department of Agriculture and Forestry 2000). There have been no known fish kills in Priest Pond Creek.

Although sediment analysis was scheduled to be completed well before the completion of this project, unforeseen problems made this impossible. It is possible that agricultural contaminants are responsible for the observed effects on Japanese medaka but, without the sediment analysis, this remains speculative. Plausible contaminants include pesticides, high levels of nutrients, agricultural related metals, and other anthropogenic materials. However, the observed effects in this study may have been due to sublethal or lethal concentrations of substances from natural, agricultural, or other anthropogenic sources.

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4.4.1.1 Overview of observed endpoints

In this study, fish exposed to Wilmot River sediments collected on July 2 and December 17 exhibited earlier hatching times, increased numbers of developmental abnormalities, increased mortality, and reduced growth when compared to the control group with no sediment. Although medaka exposed to control sediments from Priest Pond Creek had similar amounts of developmental abnormalities, those that were exposed to Wilmot River hatched earlier, exhibited higher mortality, and were noticeably smaller. Furthermore, Wilmot River downstream and upstream sediments collected on December 17 were the only sediments that resulted in a mortality rate greater than 50% in exposed medaka. In general, these results suggest that Wilmot River sediments were generally more toxic to medaka than one or both of the control groups. In some cases, fish exposed to Priest Pond Creek sediments also differed when compared to the control group with no sediment. For example, medaka exposed to Priest Pond Creek sediments consistently had more developmental abnormalities and were smaller than medaka in the control group with no sediment. These results indicate that there also appeared to be a degree of toxicity in the Priest Pond Creek control sediments. Although control sediments were expected to have minimal amounts of contaminants, it is not uncommon for reference sites to have some contamination (Burton 1991). Alternatively, some natural component of sediments, such as oxygen loss due to the activity of microorganisms, may hinder medaka development in these experiments.

A number of investigations have been conducted on the effects of contaminated sediments to fish. Many of these studies documented responses to contaminated

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sediment that were similar to those observed in the present study. For example, fish exposed to contaminated sediments have been found to have reduced hatching times (Strmac *et al.* 2002, Cooper *et al.* 1993), higher mortality (Strmac *et al.* 2002, Hopkins *et al.* 2000, Cooper *et al.* 1993, Ankley *et al.* 1990, Dawson *et al.* 1988, Hoke and Prater 1980), decreased size (Rowe 2003, Mondon *et al.* 2001, Hopkins *et al.* 2000), and an increased number of developmental abnormalities (Strmac *et al.* 2002, Mondon *et al.* 2001, Cooper *et al.* 1993, Dawson *et al.* 1988). In addition to information from these studies, other investigations where fish were exposed to non sediment bound contaminants will be compared with the findings in this study.

A significant decrease in survivorship indicates that contaminants could be present in study sediments; however, natural components in the sediments may also have been the cause of increased mortality. Many researchers have found decreased survivorship in fish exposed to agricultural related contaminants (e.g., Samson *et al.* 2001, Francis *et al.* 1984, Seim *et al.* 1984, Kincheloe *et al.* 1979, Rubin and Elmaraghy 1977, Carlson 1971). Nevertheless, high concentrations of almost any contaminant would cause mortality in fish; therefore, it would be difficult to use survivorship alone as a method in determining the presence of potential contaminants.

Although medaka were found to hatch prematurely in this study, the majority of researchers have found that exposure to contaminants is usually associated with delayed hatching times (e.g., González-Doncel *et al.* 2003, Todd and Van Leeuwen 2002, Rach *et al.* 1998, Fent and Meier 1994). Little research has been conducted on the causes of altered hatching times; thus, the reasons why these alterations occur in the presence of

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pollutants are speculative. One possibility, as yet unexplored, is that changes in hatching time are associated with the ability of the contaminant to pass through the egg chorion. For example, pollutants that pass easily through the chorion may trigger early hatching to allow fry to escape the area. Conversely, if the contaminant does not pass through the chorion, a better strategy might be to delay hatching time until the surrounding environment improves. Although premature hatching is less common than delayed hatching, it has been noted in a few studies. For example, Dave and Xiu (1991) found that zebrafish (*Brachydanio rerio*) exposed to low concentrations of mercury hatched earlier than controls. In addition, Grande and Anderson (1983) found that the hatching times of Atlantic salmon exposed to 5000 µg/l of chromium hatched significantly earlier than controls. Strmac *et al.* (2002) found that the hatching times of zebrafish eggs were significantly decreased after exposure to Körsch river acetone based sediment extracts contaminated with heavy metals, pesticides, and polyaromatic hydrocarbons (PAHs). Furthermore, Japanese medaka hatched prematurely after exposure to sediment contaminated with furans and dioxins (Cooper *et al.* 1993).

A reduction in body size can have critical repercussions on the survival and reproduction of fish. For instance, larger individuals have a higher chance of survival than their smaller conspecifics (Hutchings 1991) and they may be selected for mating before smaller individuals (Howard *et al.* 1998). A significant reduction in length is often associated with exposure to contaminants such as pesticides and metals. For example, Teather *et al.* (2005) found that exposure to environmentally relevant concentrations of the insecticide azinphos-methyl resulted in significantly smaller

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Japanese medaka fry. Similarly, exposure to sublethal concentrations of the fungicide Acrobat significantly reduced the growth of medaka fry (Teather *et al.* 2001). Japanese medaka exposed to bis (Tri-n-butyltin) oxide, a compound used in pesticides, were significantly shorter than those in the control group (Walker *et al.* 1989). Exposure to cadmium significantly reduced the growth of Atlantic salmon fry (*Salmo salar*) (Peterson *et al.* 1983), alevins (Rombough and Garside 1982), and bull trout *(Salvelinus confluentus*) (Hansen *et al.* 2002). Additionally, Rowe (2003) found that the growth of sheepshead minnow (*Cyprinodon variegatus*) was significantly reduced after exposure to sediments contaminated with a wide range of trace metals such as cadmium, copper, and selenium. Similarly, Mondon *et al.* (2001) also found a reduction in the length of greenback flounders (*Rhombosolea tapirina*) after exposure to sediments contaminated with various metals and a metal contaminated diet.

Developmental abnormalities in fish can result in impaired growth and may affect their survivorship (Hilomen-Garcia 1997, Koumoundouros *et al.* 1997). Although, developmental abnormalities can occur naturally (Andrades *et al.* 1996), some deformities are quite frequent in fish after exposure to pollutants (e.g., Teather *et al.* 2001, Villalobos *et al.* 2000, Strmac and Braunbeck 1999). Common developmental abnormalities found in medaka exposed to sediments in this study included pericardial edema, fin and eye deformities, yolk sac edema, and spinal deformities such as lordosis, scoliosis, and kyphosis. A significant number of developmental abnormalities, as observed in exposed individuals in the present study, provides stronger evidence that anthropogenic contaminants may have been present in study sediments. Some

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researchers have found that certain contaminants can induce specific abnormalities in fish (Couch *et al.* 1977) and thus may be useful in identifying potential contaminants. Unfortunately, different contaminants often cause the same developmental abnormalities in fish, which can make pollutant identification difficult.

Pesticides can induce developmental abnormalities in fish consistent with those found in this study. For example, Villalobos *et al.* (2000) found that sublethal concentrations of the herbicide thiobencarb® caused pericardial edema, skeletal deformities, and weak resorption of the yolk sac in Japanese medaka. The fungicide Acrobat®, containing the active ingredients dimethomorph and mancozeb, caused pericardial edema and spinal deformities in Japanese medaka (Teather *et al.* 2001). Pericardial edema was also observed in Japanese medaka exposed to the insecticide permethrin (González-Doncel *et al.* 2003). In addition, the fungicide triphenyltin was found to cause spinal deformities such as lordosis, heart edema, yolk sac edema, and opaque eyes (Strmac and Braunbeck 1999). More generally, organochlorine pesticides may induce scoliosis in fish. For example, Couch *et al.* (1977) discovered that kepone induced scoliosis in sheepshead minnows (*Cyprinodon variegatus*). In this study, scoliosis occurred in medaka exposed to some Wilmot River sediments collected in July and to sediments from both the Wilmot River and Priest Pond Creek in December. This suggests that the sediments used in this study may have accumulated organochlorine pesticides. One of the most frequently used pesticides on Prince Edward Island is the organochlorine fungicide chlorothalonil (Savard *et al.* 1999). The fungicide has previously been detected in PEI stream sediments (Mutch *et al.* 2002) and in Wilmot

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River water (Murphy and Mutch 2005).

Researchers have found that metals can also cause developmental abnormalities consistent with those observed in medaka exposed to sediments in this study. For example, sediment extracts containing zinc resulted in pericardial edema, gut edema, and fin abnormalities in fathead minnows (Dawson and Stebler 1988). Exposure to low concentrations of lead caused lordoscoliosis in exposed Altantic salmon (Grande and Anderson 1983) and scoliosis in brook trout alevin hatchlings (Holcombe *et al.* 1976). Similarly, Razorback suckers (*Xyrauchen texanus*) exposed to selenium exhibited scoliosis and eye deformities (Hamilton *et al.* 2005). Furthermore, Weis and Weis (1977) found that killifish (*Fundulus heteroclitus*) exposed to low concentrations of methylmercury exhibited various eye deformities.

Other anthropogenic contaminants can cause the abnormalities observed in this study. For example, low concentrations of the alkylating agents methylnitrosourea, ethylnitrosourea, methyl methanesulfonate, and ethyl methanesulfonate caused eye deformities, kyphosis, and bent tails in Japanese medaka embryos and larvae (Soloman and Faustman 1987). Alkylating agents can be found in water and air due to the high abundance of these compounds in pollutants (Soloman and Faustman 1987). Gray *et al.* (1999) discovered that Japanese medaka exposed to low concentrations of 4-tert-octylphenol had pericardial edema, bent tails, and eye deformities. Gray and Metcalfe (1999) also found that 4-tert-octylphenol caused developmental abnormalities such as yolk sac edema, developmental arrest, and hemorrhaging in Japanese medaka embryos.

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4.4.1.2 Potential contaminants in study sediments

Sediments in the Wilmot River were expected to contain pesticides or pesticide metabolites due to the documented pesticide related fish kills and the high percentage of agriculture within the watershed. Two fish kills occurred in the Wilmot River during July, 2002 (Gormley et al. 2005). Both events were likely due to the pesticide azinphos-methyl since it was detected in Wilmot River water immediately after the fish kills. Azinphos-methyl is highly toxic to fish (Department of Fisheries and Oceans 2003) and sublethal concentrations are known to significantly reduce the growth of Japanese medaka fry (Teather *et al.* 2005). In addition, Murphy and Mutch (2005) stated that the pesticides chlorothalonil and metalaxyl were detected in Wilmot River water in October 2003, although no pesticides were detected in sediment samples at that time. They indicated that the concentrations of chlorothalonil, a fungicide that is highly toxic to fish (USEPA 1999b), found in river water were likely hazardous to the aquatic environment. In addition, the fungicide Dithiocarbamate was detected three times in Wilmot River sediment during 2004. Priest Pond Creek control sediments may have also contained small amounts of pesticides since the watershed was not completely devoid of agriculture.

Metals such as cadmium, lead, copper, and zinc can enter agricultural systems through fertilizers, animal manure, animal feed, roughage, and the atmospheric deposition of metals (Haygarth 2002). Mercury is a metal that is released into the air though industrial sources such as chlorine production, hazardous and medical waste incineration, industrial boilers, municipal waste combustors, and utility coal boilers (Evers 2005). Airborne mercury can eventually be deposited in watersheds, potentially harming aquatic

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organisms (Evers 2005). For example, low concentrations of mercury have been detected in sediment samples from New Brunswick, Nova Scotia, and Newfoundland (Kamman *et al.* 2005). In addition, Nova Scotia had some of the highest mercury counts in a surface water sampling study across eastern North America (Dennis *et al.* 2005).

Sediments from the Wilmot River could have accumulated high levels of nutrients since the watershed is surrounded by agricultural farmland. Recent water chemistry analysis revealed that water from the three Wilmot River sites studied in this experiment contained 7.65 mg/L (WU), 6.63 mg/L (WD), 8.70 mg/L (WT) of nitrates respectively (Gormley 2003). These values are quite high considering that the freshwater nitrate guidelines for the protection of aquatic life is 13 mg/L (Environment Canada 2003). Nutrient input into Priest Pond Creek is expected to be much lower given the small amount of agriculture in its watershed. This is supported by water chemistry analysis indicating a nitrate concentration of 0.10 mg/L (Gormley 2003).

4.4.2 Comparing the Seasonal Toxicity of Wilmot River Sediments

The second objective of this study was to compare the toxicity of Wilmot River sediments collected at different times of the year. Sediments collected during July were expected to be more toxic than December sediments, since July sediments were collected during the growing season when pesticides are commonly applied. Prince Edward Island farmers will apply herbicides when the potatoes are planted, which is usually in June (Rachael Cheverie, Integrated Pest Management Specialist, PEI Department of Agriculture and Forestry, June 3, 2005). Fungicides are initially applied at the end of

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June, and are used every one to two weeks until September, depending on the weather. Insecticides are generally used in the middle or at the end of July and possibly once in the middle of August. In September, two applications of herbicides are also used in order to kill the tops of the potato plants prior to harvest (James Mutch, PEI Department of Environment, Energy and Foresty, March 9, 2005). However, many pesticide applications are dependent on the weather, the variety of the potato used, and the number and type of agricultural pests.

Contrary to what was expected, December sediments were generally more toxic to medaka than those collected on July 2 and those collected on July 24 were generally more toxic than July 2 sediments. Wilmot River sediments may have accumulated pesticides due to agricultural runoff throughout the year. According to Rachael Cheverie (Integrated Pest Management Specialist, PEI Department of Agriculture and Forestry, June 3, 2005), only a few applications of pesticides would have been applied on the potato fields before July 2. Additionally, more pesticides would have been sprayed on the fields by July 24 and noticeably more would have been applied by December 17. Therefore, it is possible that pesticides accumulated in aquatic sediments over this period.

Wilmot River sediments may also have accumulated high levels of nutrients or agricultural related metals. Agricultural fertilizers which may contain metals (Haygarth 1998) are sprayed on fields throughout the growing season. Therefore, the concentration of nutrients and metals from agricultural fertilizers in the sediments may have also accumulated throughout the year. Thus, the observed increase in toxicity in July 24 and December 17 sediments compared to July 2 sediments may have been due to increased

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levels of nutrients or agricultural related metals entering the river.

4.4.3 Conclusion

Medaka exposed to Wilmot River sediments exhibited reduced survivorship and a number of sublethal responses. These sediments may have contained agricultural related contaminants such as pesticides, high levels of nutrients, and agricultural related metals, due to the high percentage of agriculture on the Wilmot River watershed. However, natural processes within the sediment may also have caused these responses. Wilmot River sediments also appeared to increase in toxicity between July and December. This may indicate that the sediments were accumulating toxic levels of pesticides, nutrients, metals, or other contaminants during this period. Exposure to Priest Pond Creek control sediments also resulted in some sublethal responses, similar to those found in medaka exposed to sediments from the Wilmot River. This suggests that either a) the small amount of agriculture in the Priest Pond Creek watershed is impacting sediments or b) some other component of the sediment is affecting medaka development. In general, Wilmot River sediments and, to a certain degree, Priest Pond Creek sediments, were toxic to exposed medaka. Since Japanese medaka are less sensitive to certain contaminants than native salmonids on PEI, the same sediments used in this study could be negatively impacting native salmonid populations in PEI streams.

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CHAPTER 5:

GENERAL DISCUSSION

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The first experiment compared the toxicity of refrigerated and frozen sediment from the Wilmot and West River using a vertebrate model, the Indian medaka. Results from this experiment suggested that freezing Wilmot and West River sediment increased its toxicity to medaka. The cause of increased toxicity in this study is speculative: freezing may have created or released toxicants, cells of microorganisms and invertebrates could have ruptured upon freezing and released contaminants, increased storage time of frozen sediments may have allowed for microorganisms to degrade contaminants and produce toxins that were more potent than the parental contaminant, or a decrease in microbial activity within the sediment may have resulted in increased levels of ammonia. This experiment suggests that future studies using vertebrate models should not freeze sediments used in aquatic sediment toxicity tests. Refrigerating sediments at approximately 4 or 5 °C for short time periods is recommended for aquatic sediment toxicity tests using vertebrate models.

The first part of the second experiment assessed the toxicity of Wilmot River sediments using Japanese medaka embryos. A control site on Priest Pond Creek and a control group without sediment were used for comparison. Wilmot River sediments were associated with decreased survivorship, prolonged hatching times, stunted growth, and more developmental abnormalities of exposed medaka embryos when compared to the control group with no sediment. Medaka exposed to Priest Pond Creek sediments differed in some, but not all endpoints when compared to those exposed to Wilmot River sediments. These results suggest that Wilmot River sediments and Priest Pond Creek sediments accumulated contaminants. Sediments may have contained agricultural related

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contaminants such as pesticides, nitrogen, and agricultural related metals. Nevertheless, natural processes within the sediment may have also caused these responses. The second part of this experiment tested the seasonal toxicity of Wilmot River sediments. I predicted that sediments collected in July would be more toxic than those collected in December. Contrary to my prediction, December sediments were generally more toxic to medaka than those collected in early July and sediments collected on July 24 were generally more toxic than July 2 sediments. This may indicate that the sediments were accumulating toxic levels of contaminants during the year. In conclusion, this study revealed that Wilmot River sediments and to a certain degree, Priest Pond Creek sediments, were toxic to exposed medaka. However, the direct causes for this toxicity are unknown. A literature review showed that Japanese medaka are less sensitive to certain contaminants than native salmonids on PEI; thus, the sediments collected in this study could be negatively impacting native salmonid populations in PEI streams. Future studies investigating the toxicity of stream sediments should assess the toxicity of sediments from more test and control sites on PEI. Suggested test rivers would include rivers which are highly impacted by agriculture, such as the Wilmot River, the Dunk River, and the Bradshaw River. Control rivers should include rivers that have little agricultural impact such as Cow Creek, Bear River, Cross River, Priest Pond Creek, and the Mitchell River.

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CHAPTER 6:

LITERATURE CITED

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