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Understanding The Physiological Effects of Suspended Material on Rainbow Trout (Oncorhynchus mykiss)

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By

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Abstract

The effect of global warming on northern environments is becoming increasingly evident. Melting of underlying permafrost is associated with widespread impacts in these environments. The loss of permafrost results in a destabilizing of underlying sedimentary layers resulting in thermokarst slumping. When this occurs on a large scale (mega-slumping) soil material becomes mobilized and is carried into local streams and rivers. The purpose of this study is to examine the sub-lethal physiological effects that suspended material has on rainbow trout (*Oncorhynchus mykiss*) in the context of the Peel River Plateau. Juvenile rainbow trout were exposed (following Environment Canada exposure guidelines) for 96h to suspended clay and field collected material of differing grain sizes: small (<90µm), medium (90-150µm) and large (150-300µm), and combine (0-300µm) at concentrations of 250, 500, 1000, and 2000mg/L. The effects of exposure were assessed by measuring plasma cortisol, plasma ion concentration (Na, Cl and Ca) as well as resting metabolic rate and swim performance. It was determined that no significant changes to the measured physiological endpoints are occurring to the model organism rainbow trout at concentrations and durations equal to or greater than those present in the natural conditions.

Authors Declaration

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

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Dedicated to My Father

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Introduction

1.1 Peel River Plateau:

The Peel River Plateau is located in the interior plains of the northern mainland sedimentary basin in the North West Territories and the Yukon (Lemieux *et al.*, 2007). The region stretches from the Beaufort Sea, along the Mackenzie, Franklin and Richardson mountains almost to the Nahanni River . The Richardson Mountains are located to the west; the Wernecke Mountains are located to the south; the Mackenzie River located to the east; and Beaufort Sea located to the North (EMR, 2012). The soil in the region is dominated by cryosols, specifically orthic and brunisolic cryosols. Cryosolic soil, which generally consists of moraine and gravelly sandy loam (EMR, 2012), is the dominant soil type in northern regions where permafrost is present. Mixing of the soil layers occurs frequently in cryosolic soils and is caused by the freezing and melting of the soil throughout the year (Trenhaile, 2010). The Peel River Plateau is an area of economic and cultural importance. It has recently been shown to have potential for oil and gas reserves (Lemieux *et al.*, 2007) and contains important transportation routes. It is also home to Inuit communities and is considered to be one of the most quickly changing areas in the country (Kokelj, 2013).

1.2 Climate Change

The Peel River Plateau is changing significantly as a result of a changing climate. Greenhouse gasses, specifically carbon dioxide, are building up in the atmosphere causing global temperatures to rise. The ground temperature in North West Territories has increased by over 0.77°C per decade since the 1970's as a result of global warming (Kokelj *et al.*, 2013). The increase in temperature is causing melting and degradation of permafrost (Frey and McClelland, 2009). Permafrost is critical for the regulation of global carbon budgets (Thienpont *et al.*, 2013). As permafrost melts it releases carbon that is stored within. This result causes an acceleration of

the positive feedback system, which then causes further melting (Frey and McClelland, 2009). Studies have found that in the last 100 to 150 years, between 30 to 65% of permafrost in the most southern regions of Canada's discontinuous permafrost zone has degraded (Jorgenson and Osterkamp, 2005).

1.3 Permafrost and Thermokarst Slumping

Permafrost is soil or rock that remains frozen (<0°C) for two or more years and is an important part of most northern ecosystems (Jorgenson and Osterkamp, 2005). Permafrost regions cover roughly a quarter of the northern hemisphere; therefore, changes in permafrost are affecting human and ecological processes (Nelson *et al.*, 2001). Permafrost loss has caused the relocation of coastal villages and the destruction of roads, pipelines, and infrastructure. The loss also results in erosion and surface subsidence or thermokarst (Rowland *et al.*, 2010). Thermokarst is the thawing of ice rich ground, which causes the ground to sink, creating depressions (Frey and McClelland, 2009). There are large variations in the way that permafrost melts; these variations result in different types of thermokarst features. There are sixteen types of thermokarst features that have been identified by Jorgenson and Osterkamp (2005). One of these types are retrogressive thaw slumps which is a slope failure as a result of the thawing of ice rich permafrost. Retrogressive thaw slumps have been estimated to affect 10% of all lakes in the Western Arctic (Theinpont, 2013). They are also the most prominent type of thermokarst on the Peel River Plateau.

Thaw slumping occurs when permafrost thaws and turns underlying ground into a slurry of mud which eventually slides to the base of the exposure (Lewkowicz, 1987, Kokelj *et al.*, 2005). Slumps are a rotational sliding of the underlying layers of sediment in an area They usually occur on curved surfaces, and require a deep percolation of water in the sediment to

occur. As water content increases in the sediment it decreases the cohesive forces between sedimentary particles. This compromises the strength of the soil (Trenhaile, 2010). When stress on the layers of sediment and soil becomes greater than the normal force keeping everything in place, a slump will occur. Slumps can occur in areas of rock but are generally found in areas with underlying deposits of glacial, lacustrine and marine clays (Trenhaile, 2010). Over a single thaw season the head wall of a slump can retreat numerous times (Lewkowicz, 1987, Kokelj *et al*. 2005). Slumping can be self-perpetuating because further permafrost loss is promoted. Slumping removes organic surface layers, allowing heat to flow into the ground more easily. This becomes amplified by greater snow accumulation in the winter; snow traps heat in the ground, heat thaws permafrost, and the loss of permafrost causes slumping to occur (Kokelj *et al*, 2009). The size of slumps is on a much larger scale across the Peel River Plateau than seen in other places. The slumps there are distinguished from smaller slumps because they are larger than 5 ha in area and greater than 4 m in height and considered to be mega-slumps (Kokelj *et al.*, 2013).

1.3.1 Impacts of Slumping

Slumping has been shown to cause significant changes in the surrounding areas. An increased ionic content and decreased dissolved oxygen content in aquatic ecosystems adjacent to slumps has been shown (Kokelj *et al.*, 2005). This is because materials trapped within the permafrost are released during slumping and are carried into the aquatic ecosystems by runoff (Kokelj and Lewkowicz, 1998). Changes in ionic concentrations as well as changes in water clarity due to slumping can greatly alter the aquatic food webs in northern areas (Kokelj *et al.*, 2009). For example, the increasing frequency of slumping events results in increased nutrient availability as well as other favorable conditions for terrestrial plant growth. This provides opportunities for the movement and colonization of local plants to occur (Lantz *et al.*, 2009).

When slumps occur next to rivers, streams and lakes, large amounts of sediment and materials are potentially released into the aquatic environment. These sediments and materials become suspended in the water column resulting in unknown effects on higher level aquatic life, specifically fish.

1.4 Suspended Sediment and Turbidity

Sediment is considered to be a solid material that was moved and deposited by ice, air, or water (Trenhaile, 2010). Suspended sediment is particulate material that is being transported within the water column and has yet to be deposited (Trenhaile, 2010). Increased suspended sediment and turbidity in inland waters are some of the most prominent consequences of mankind's changes to landscape (Bruton, 1985). Suspended sediment is made up of solid particles of silt, clay and other organic and inorganic particles that become suspended in the water column by the turbulent flow of water. The most common cause of sediment suspension is the erosion of riverbanks and runoff caused by flooding, mining, logging, dredging, construction, and agricultural activity (Kerr, 1995). The amount of sediment that can be suspended in a river or stream depends on the slope of the river, the sediment size, the velocity of the water, the amount of water flowing in the river (discharge), the roughness of the bed, and the shape of the stream channel (Trenhaile, 2010). As slope, velocity, discharge, and bed roughness increase, so will the amount of sediment that becomes suspended. The smaller the size of the sedimentary particles the easier these will become suspended. The type and concentration of suspended sediment greatly affects water clarity and turbidity (CCREM, 2008). In the case of the Peel River Plateau, the immense debris flows associated with mega-slumps are moving materials from slopes to streams and they are becoming entrained in the water column. Long-term effects can be expected because these slumps remain active for decades (Kokelj *et al*., 2013)

Turbidity in water can be caused by a number of factors including soluble organic material, plankton, microscopic organisms, and suspended matter such as clay, silt, or other fine particulates. The turbulent flow of water and Brownian motion can cause these items to stay suspended in the water column (McNeely *et al.*, 1979). The size of the particles that stay in suspension within the water column is dictated by the rate at which the water is flowing. As speed of flow increases, so does the size of particles that can stay suspended. Finer sediments such as clays and silts tend to travel with moving water and settle out of the water column only when flow becomes greatly reduced. Coarse sediment can become suspended but this occurs far less often and only when flow rates are high; otherwise, coarse sediments tend to stay on the bottom of the stream channel (Trenhaile, 2010).

Turbidity is generally measured using either a nephelometer or Jackson candle turbidimeter. The nephelometer measures turbidity in nephelometric units (NTU) whereas the Jackson candle turbidimeter measures in Jackson Turbidity Units (JTU). One JTU and one NTU are approximately even when it comes to measuring turbidity, but it is recommended to use NTUs when levels are low (< 25 JTU or NTU). This is recommended because nephlometers are more precise in their measurement (CCREM, 2008).

A nephelometer is an instrument that measures turbidity by shining light into a water sample at a 90° angle and measuring the amount of scattered light, which is directly related to the turbidity. A Jackson candle turbidimeter manually measures turbidity and consists of two parts: a flat-bottomed glass tube, and a candle. A water sample is added to the glass tube until the candle is no longer visible through the liquid. The user then reads volume markings on the glass tube where the top of the liquid is; the markings have been calibrated to correspond to amounts of suspended sediment in ppm (US EPA, 1999).

1.4.1 Factors Effecting Response to Suspended Sediment

It was determined that the effects of suspended sediment are dependent on suspended sediment concentration and length of exposure (Newcombe and Jenson, 1996). Although concentration and duration are very important when looking at the effects of suspended sediment, particle distribution, chemical composition, the presence of other contaminants in the sediment and particle shape are also key factors regarding effects response (Bilotta and Brazier, 2008). It was shown by Lake and Hinch (1999) that more angular particles show effects responses at lower concentrations than round particles.

1.5 Canadian Council of Ministers of the Environment Guidelines:

The Canadian Council of Ministers of the Environment (CCME) is an intergovernmental organization that takes action on issues that cause environmental concern (CCME, 2008). A summary of the CCME water quality guidelines for suspended solids and turbidity is below.

Table 1.1: Summary of CCME Suspended Sediment and Turbidity Guidelines

Suspended Sediment (TSS)		
	Short Term (<24 hr.)	**Long Term (>24 hr. to 30 days)**
Clear Flow	Maximum increase of 25mg/L	Maximum increase of 5 mg/L
High Flow	25 mg/L (when background is between 25 and 250 mg/L)	10% of background (when background is ≥250 mg/L
Turbidity		
	Short Term (<24 hr.)	**Long Term (>24 hr. to 30 days)**
Clear Flow	8 NTU	2 NTU
High Flow and Turbid Waters	8 NTU	10% of background (when background is ≥80 NTU

1.6 Effects of Suspended Sediment and Turbidity on Primary Producers and Invertebrates

Increased turbidity and suspended solids cause decreased light penetration through the water column. This inhibits photosynthetic efficiency of primary producers like algae, aquatic plants, and macro and microphites (Bilotta and Brazier, 2008; Bruton, 1985). The loss of primary production results in decreased food availability and plant biomass (Bruton, 1985; McCubbin *et al.*, 1990). The light decrease also changes the structure of the primary producer community (Kemp *et al.*, 2011). The number of primary producers can become decreased as suspended sediment increases because of abrasion from the sediment. The decrease results in losses of food for invertebrates and some fish (Kemp *et al.*, 2011).

Suspended sediment and turbidity can harm benthic invertebrates by causing abrasion to their exterior as well as damage their exposed organs as the particles flow past them. This results in an increased susceptibility to predators (Bilotta and Brazier, 2008). A lessening in invertebrate abundance and diversity as well as changes in community and population structure then results (Kemp *et al.*, 2011). Invertebrates thrive in course streambed habitats (Wood and Armitage, 1997), so when sediment settles out of the water column it can smother invertebrates as well as lessen their supply of oxygen (Kemp *et al.*, 2011).

1.7 Effects of Suspended Sediment and Turbidity on Fish

1.7.1 Behavioral Effects

1.7.1.1 Spawning and Migration

Various studies have shown that adverse effects can occur in fish that are exposed to increased concentrations of suspended sediment. These include both behavioral and physiological changes. The behavioral changes that have been observed include avoidance, territorial changes, as well as homing and migration changes. Suspended sediment has been shown to delay, divert and in some cases cause the avoidance of spawning in some salmon species (Spence *et al*., 1996). Within minutes of putting coho salmon (*Oncorhynchus kisutch*) into tanks with a higher suspended sediment concentration at the bottom compared to the top, the fish began to avoid the bottom of the tanks and stayed towards the surface (Servizi and Martins, 1992). Although salmon migration is not adversely effected, increased suspended sediment levels have been shown to cause delays in some cases (Spence *et al.*, 1996).

1.7.1.2 Feeding and Territory Defence

The feeding behavior of salmonids has been shown to decrease as suspended sediment concentrations increase (Berg and Northcote, 1985; Redding *et al.*, 1987). It was found that feeding rates of coho salmon decreased when exposed to both high and low concentrations of suspended sediment (Redding *et al.*, 1987). Bonner and Wilde (2002) showed that prey consumption was inversely related to the turbidity level. They found significant correlations between prey consumption and turbidity in Arkansas River shiners, emerald shiners and sand shiners, but there was no significant relationship between the two in fathead or peppered chub. In another study it was observed that in clear waters the coho salmon would travel up to 30cm to

capture food; the amount they would travel shrunk to 12 cm with increased turbidity (Berg and Northcote, 1985). They found that acts of aggressiveness decreased as they increased sediment and decreased back to pre-treatment levels when they decreased sediment. Berg and Northcote (1985) found that gill flaring increased as turbidity increased, but it was believed to be a result of gill irritation caused by the sediment, not a result of territorial defence.

1.7.1.3 Predator Defence

Some work suggests that waters with substantial amounts of turbidity or suspended sediment concentrations may not bother certain species of fish. Reid *et al.* (2002) showed that there was no identifiable avoidance by radio labeled arctic grayling and mountain white fish when sediment from road construction was released into their habitat. Gregory and Northcote (1993) suggested that some juvenile forms of fish actively seek out turbid waters for protection and cover. Turbid waters can also help fish hunt by providing them cover in which to stalk prey (Bruton, 1985). In a study looking at the response of juvenile chinook to predators it was found that the fish's response was shorter and less marked in turbid conditions. This suggests that turbidity reduces the amount of risk from predication the individual perceives (Gregory, 1993).

1.7.2 Physiological Effects

1.7.2.1 Effects on the Gill

Studies have shown that suspended sediment can cause adverse effects to gills and respiration. Sediment can become directly bound to the gills of fish, causing direct physiological effects and impaired respiration (Newcombe and Flagg, 1983). Arctic grayling exposed to suspended sediment (concentrations averaging 1205 mg/l) showed increased mucus production by the gills after 24 hours. After 48 hours of exposure, gill damage was present, and after 96

hours of exposure, extensive epithelial hyperplasia and hypertrophy of the gill lamella was observed (Reynolds *et al.*, 1989). Another study using juvenile *Epinephelus coioides* gill tissue was found to have both hypertrophy and hyperplasia present after exposure to suspended sediment (Au *et al*, 2004). In two species of minnows (*Cyprinella galactura,* and *Erimonax monachus*) it was found that as suspended sediment concentrations increased, the gill condition of the exposed fish worsened. At lower concentrations of suspended sediment (>100mg/L), gill filaments were less discernable and in the highest test concentration (500mg/L), gill cavities were filled with mucus and sediment particles, and gill arches were fused together (Sutherland and Meyer, 2007). The fusing of gill arches and excessive buildup of mucus and sediment between the gill filaments greatly affects the efficiency of gas exchange occurring in the fish (Henley *et al.*, 2000). In a study using juvenile *Epinephelus coioides,* significant thinning of the gill epithelium occurred; it was concluded that there is a linear relationship between thickness of epithelial thinning and suspended sediment concentration (Au *et al*, 2004). Increased levels of suspended sediment have also been shown to reduce the oxygen content of the water (Henley *et al.*, 2000). Cough frequency in salmon was found to increase with elevated levels of suspended sediment but not significantly (Servizi and Martins, 1992). The number of chloride cells present on the gill surface increased and Na+/K+ ATPase activity decreased in juvenile *Epinephelus coioides* when exposed to suspended sediment concentrations of 2000 mg/L. Both changes inhibit the ability of the fish to osmoregulate properly (Au *et al*, 2004). In a test comparing fish upstream and downstream of a construction site releasing suspended sediment into the environment, oxygen consumption rates were significantly greater in the downstream fish (Reid *et al.*, 2003).

1.7.2.2 Cardiovascular Response

Riverine and lacustrine lake bass exposed to silt concentrations as low as 10 NTU were shown to have cardiovascular responses that included both increased and decreased stroke volume and heart rate, resulting in cardiac output changes (Bunt *et al.*, 2004). The responses were not uniform, but lacustrine rock bass were found to have a more extreme response to exposure than the riverine rock bass. This discrepancy is thought to occur because the riverine fish are accustomed to fluctuations in turbidity in their native habitat (Bunt *et al*., 2004).

1.7.2.3 Stress Physiology

Suspended sediment has also been shown to cause numerous affects to blood physiology of fish, specifically to blood cortisol levels, blood glucose levels, and hematocrit. Suspended sediment has been shown to cause changes in cortisol levels in many species of fish. It was observed that elevating suspended sediment concentrations caused an elevation in stress hormone (cortisol) levels in both wild as well as high and low inbred strains of Ayu (*Plecoglossus altivelis*) (Awata *et al*., 2011). Two species of salmon (*Oncorhynchus kisutch*, and *Salmo gairdneri*) were shown to have significantly higher levels of cortisol present after acute exposures to high (2000 to 3000mg/l) and low (400 to 600 mg/l) concentrations of suspended sediment (Redding *et al.*, 1987). Hematocrit was shown to rise consistently in both coho and steelhead salmon as suspended sediment concentrations were raised. This is believed to be a result of the fish struggling to compensate for the respiration problems plaguing the gills by increasing their oxygen carrying capacity via a higher number of red blood cells (Redding *et al.*, 1987). Suspended sediment exposure has been known to cause changes in blood glucose levels. It was observed that glucose levels were all elevated above control levels, but not significantly,

when tested using ANOVA. However, when the regression between glucose levels and suspended sediment was done, it was found to be significant (Servizi and Martins, 1992).

1.7.3 Cellular Effects

The effects of suspended sediment have also been found at cellular levels. Rainbow trout (*Oncorhynchus mykiss)* were exposed to pulses of suspended sediment daily for 8 and 24 days. The concentrations of suspended sediment used were 300, 1300, 5000 mg/L. Results showed that no gill damage or physiological effects had occurred, but they did find cellular effects within the spleen and kidney, as well as metabolic changes. It was hypothesized that these cellular changes could be representing an adaptive response (Michel *et al.*, 2013). Tse *et al.* (2010) found that grouper exposed to suspended sediment concentrations as low as 32mg/L exhibited significant DNA damage, specifically increased DNA tail length, after 5 days of exposure. They found that after a 10-day recovery period, DNA tail length returned to normal control levels.

1.7.4 Effects on Growth and Reproduction

Effects of growth and reproduction as a result of increased suspended sediment concentrations have been observed in different species of fish. Sediment in low concentrations is needed for proper spawning and incubation of eggs in salmon, but may block the emergence of fry, and decrease the flow of water around the eggs before hatching. Increased sediment can also cause mortality in embryos because of oxygen stress; decreased amounts of oxygen available for embryos causes death (Spence *et al.*, 1996). A significantly reduced growth rate was found in two species of minnows (*Cyprinella galactura*, and *Erimonax monachus*) exposed to high concentrations of suspended sediment (500 mg/L) and, in general, there is an inverse relationship between growth rate and suspended sediment concentration (Sutherland and Meyer, 2007). It is also believed that increased sediment deposition can change stream makeup, causing a decline in

the quality of the spawning habitat. This reduced quality may result in an impairment of egg and larval development as well as decreased fry emergence (Levesque and Dube, 2007). The settling of sediment can also restrict waste exchange, decrease dissolved oxygen levels, and physically damage eggs (Bruton, 1985; Scheurer *et al*, 2009). This fosters stress, degrades disease resistance and inhibits growth and abundance (Levesque and Dube, 2007). When walleye eggs were exposed to elevated concentrations of suspended sediment (up to 500 mg/L), there was a slight but insignificant decrease in hatching rate, however, they found that all of the hatched individuals where healthy and had no malformations or abnormalities (Suedel *et al*, 2012). In waters with concentrations of suspended sediment 1000-6000 mg/L, reduction in fish population size was found. *S.trutta* population in the turbid waters was $1/7^{th}$ the size of the population in the clear waters (Bruton, 1985). Furthermore, it was found that clear ponds produced 1.7 to 5.5 times more fish on a total weight basis than turbid ponds (Bruton, 1985).

Table 1.2: Effects of Suspended Material on Fish Summary

Area Of Effect	Effect/ Finding	Effect []	Duration	Species	Reference
Behavior					
Avoidance and migration	Avoidance of Increased SS []	0 – 6.78 g/L	96 hr	*Oncorhynchus kisutch*	Servizi and Martins, (1992)
	Use of turbid waters for protection from predators	0- 810 NTU	10 min	*Oncorhynchus tshawytscha*	Gregory and Northcote (1993)
Feeding	Decreased feeding rate with increases SS []	0.4 – 0.6 g/L and 2-3 g/L	7-8 days	*Oncorhynchus kisutch*	Redding and Schreck, (1987)
	Prey consumption inversely related to turbidity	0 to 4000 NTU	10 min	*Notropis atherinoides, Cyprinella lutrensis, Notropis stramineus, Notropis girardi, and Pimephales promelas*	Bonner and Wilde (2002)
	Decreased distance traveled for food	0- 20 NTU		*Oncorhynchus kisutch*	Berg and Northcote, (1985)
	No avoidance response to increased SS []	0 – 200 mg/L	22 days	*Thymallus arcticus, and Prosopium williamsoni*	Reid *et al.*, (2002)
	Decreased length of perceived predator danger	~ 23 NTU	12 days	*Oncorhynchus tshawytscha*	Gregory, (2003)
Physiology					
Gill Physiology	Direct binding of sediment to gills	82-87 g/L	24 hr	*Oncorhynchus tshawytscha and Oncorhynchus nerka*	Newcombe and Flagg, 1983
	Increased gill mucus after 24 hr exposure	Sediment [] averaging 1205 mg/L	3-4 months	*Thymallus arcticus*	Reynolds, (1989)
	Gill damage	Sediment [] averaging 1205 mg/L	3-4 months	*Thymallus arcticus*	Reynolds, (1989)

Area Of Effect	Effect/ Finding	Effect []	Duration	Species	Reference
Physiology					
Gill Physiology	Hyperplasia and Hypertrophy of gill epithelial	Sediment [] averaging 1205 mg/L	3-4 months	*Thymallus arcticus*	Reynolds, (1989)
	Hyperplasia and Hypertrophy of gill tissue	2000 mg/L	6 weeks	*Epinephelus coioides*	Au *et al*., (2004)
	Gill condition decreases as SS[] increases	500mg/L	21 days	*Cyprinella galactura and Erimonax monachus*	Sutherland and Meyer, 2007
	Less discernable gill filaments	100mg/L	21 days	*Cyprinella galactura and Erimonax monachus*	Sutherland and Meyer, 2007
	Fused gill arches, and gill cavities filled with mucus	500mg/L	21 days	*Cyprinella galactura and Erimonax monachus*	Sutherland and Meyer, 2007
	Decreased gill epithelium with increased SS []	2000 mg/L	6 weeks	*Epinephelus coioides*	Au *et al*, 2004
	Increased gill flaring	0-20 NTU		*Oncorhynchus kisutch*	Berg and Northcote, 1985
	Increases cough frequency	0 – 6.78 g/L	96 hr	*Oncorhynchus kisutch*	Servizi and Martins, 1992
	Increased number of chloride cells on the gill	2000 mg/L	6 weeks	*Epinephelus coioides*	Au *et al*, 2004
	Decreased Na+/K+ ATPase activity	2000 mg/L	6 weeks	*Epinephelus coioides*	Au *et al*, 2004
	Significantly increased oxygen consumption rates	Over 450 mg/L	30 hrs	*Oncorhynchus mykiss*	Reid et al. 2003

Area Of Effect	Effect/ Finding	Effect []	Duration	Species	Reference
Physiology					
Cardiovascular Physiology	Increased Cardiac output as a result of increased stroke volume and heart rate	Up to 600 NTU	8 hr	*Ambloplites rupestris*	Bunt et al, 2004
Cellular level	Cellular changes in spleen, kidney and metabolism	300, 1300, 3000 mg/L	Daily pulse exposures for 8 days	*Oncorhynchus mykiss*	Michel *et al.*, 2013
	DNA damage, specifically increased tail length	32 mg/L	10 days	*Epinephelus coioides*	Tse *et al.* 2010
Growth and Reproduction	Significantly decreased growth rate	500 mg/L	21 days	*Cyprinella galactura and Erimonax monachus*	Sutherland and Meyer, 2007
	Slightly decreased hatching rate	500 mg/L	72 hr	*Sander vitreus*	Seudel *et al*, 2012
Blood Physiology	Increased cortisol levels	200 mg/L	3h	*Plecoglossus altivelis*	Awata *et al.*, 2011
	Increased cortisol levels	0.4 – 0.6 g/L and 2-3 g/L	7-8 days	*Oncorhynchus kisutch*	Redding and Schreck, (1987)
	Increased hematocrit	0.4 – 0.6 g/L and 2-3 g/L	7-8 days	*Oncorhynchus kisutch*	Redding and Schreck, (1987)
	Increased blood glucose	0.53 g/L and 1.36 g/L	96 hr	*Oncorhynchus kisutch*	Servizi and Martins, 1992

1.8 Endpoints

The endpoints which have been chosen for this study are plasma ion balance, stress response, resting metabolic rate and swim performance. These endpoints were chose because we were hoping to observe secondary effects resulting from changes to gill physiology as a result of exposure to suspended material.

1.8.3 Plasma Ion Balance

Changes in plasma ion concentrations are often associated with responses to outside challenges such as environmental changes or toxicants. Therefore by measuring changes to plasma ion concentrations we can determine if challenges to internal physiological mechanisms are occurring (Al-Jandal and Wilson, 2011). Normal resting plasma levels measured in rainbow trout are: Na 150 mmol/L (Morisawa, 1983), Cl 130 mmol/L (Davie, 1983) and Ca is generally accepted to be near 2.5 mmol/L (Andreasen, 1985) and our results are within these normal levels.

1.8.2 Stress Response (Plasma Cortisol)

Cortisol is a glucocorticoid, and is released from the adrenal cortex in mammals. It is released in times of stress, resulting in increased glucose production, increased breakdown of proteins, increased release of fatty acids, and regulation of the immune system (Moyes and Schulte, 2008). The stress axis is rapidly activated in fish as in other vertebrates by exposure to any form of disturbance that is perceived as a threat. The subsequent elevation of blood cortisol levels is a key element of stress response and is widely employed as an index of stress (Barton, 2002). The threshold for highly stressed rainbow trout is considered to be a plasma cortisol concentration of over 150ng/mL (Barton *et al*. 1980, Gregory and Wood 1999, Pagnotta *et al*. 1994).

1.8.1 Resting Metabolic Rate

Metabolic rate is a direct representation of energy consumption and overall animal health. Metabolic rate can be determined by measuring oxygen consumption rates (Weir, 1948). Oxygen consumption rates have been shown to have a clear dose response for many chemicals and are often used to measure metabolic rate (McKim and Erickson, 1991). There are three ways to measure oxygen consumption rates using a respirometer: open system, closed system, and intermittent. Open system respirometry measures the difference between gas content in the water and the rate of water movement through the respirometry chamber containing a test subject. Closed system respirometry measures the oxygen content of the water in a closed chamber containing a test subject (Steffensen, 1989). Intermittent respirometry combines closed and open systems; it measures oxygen content in a closed circuit for a fixed amount of time and then cycles new water into the system. It consists of a measurement phase, flush phase and a lag phase (Schurmann and Steffensen, 1997). Oxygen consumption per unit of body mass is calculated using the formula:

$$MO_2 = Vr \times \left(\frac{\Delta CwO2}{\Delta t \times bw}\right)$$

Where MO_2 is the oxygen consumption rate per unit body mass, Vr is volume of water in the system, ΔCwO_2 is oxygen conc. in the water, Δt is time period, and bw is weight of the animal (Steffensen, 1989). Intermittent respirometry is the preferred method of measurement because closed systems can have large changes in oxygen levels as well as buildup of excretory metabolites, and open systems can be troublesome because it is difficult to account for animal

activity (Steffensen, 1989). Normal values for resting oxygen consumption rates for rainbow trout have been measured to be between 8.5 and 15μmol/g/hr (Miller et al. 1995)

1.8.4 Swim Performance (U_{crit})

Measuring swim performance is one way to measure the effects related to pollution and environmental changes. One of the most common ways to do this is through measuring sustained swim velocity.The sustained swim velocity was calculated (U_{crit}) as given in Jain et al. (1997) using the formula

$$Ucrit = Ui + \left(\frac{Ti}{Tii} \times Uii\right)$$

Where U*i* is the highest velocity maintained for the whole interval (BLs^{-1}), U*ii* is the velocity increment, T*i* is the time elapsed at fatigue velocity (min) and T*ii* is the interval length. Normal ramped U_{crit} values for rainbow trout are and 4.5 BLs^{-1} (Wood, 1997)

Objectives:

The purpose of this study is to identify the effects of increased suspended material on freshwater fish in the context of permafrost degradation and thermokarst activity (slumping). First the exposure-response relationships will be investigated using rainbow trout as the model organism and defined suspended material (at three grain sizes). The exposure-response relationships will then be applied using material collected from thermokarst sites in order to investigate the response to natural conditions.

The objectives of this study are as follows:

i) Determine the effect of exposure to increased concentrations of suspended material on juvenile rainbow trout through measurement of:

a) Metabolic rate (oxygen consumption),

b) Stress response (cortisol).

c) Ion balance (specifically focusing on Na^+, Cl^- and Ca^+ in plasma).

ii) Determine the potential impact of Peel Plateau slumping on fish.

a) Examine effect levels from objective i in the context of field measurements
b) Compare physiological effects of field collected suspended material on rainbow trout to effect of the model material used in objective i).
c) Identify effects thresholds for suspended material in northern environments

Hypothesis:

It is hypothesized that:

1) Metabolic activity will be significantly increased in fish exposed to increased suspended sediment concentrations. This is based on the assumption that as the fish attempt to cope with the increased sediment concentrations they will expend significantly more energy

2) The cortisol levels of rainbow trout will show statistically significant increases as a result of exposure to increasing suspended sediment concentrations. It is assumed that the stress response will be measurably activated by suspended sediment.

3) Ion balance in rainbow trout will be impaired (or altered) in fish exposed to suspended sediment. This is based on the observation that suspended sediment can clog gills and impair gill function.

4) Smaller grained sediments will show statically different physiologically effects than larger grained sediments do at the same exposure concentrations. This is based on the assumption that smaller sediments can more easily become lodged in gills and stay suspended more easily.

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Chapter 2

Understanding the Effects of Suspended Material on Rainbow Trout (Oncorhynchus mykiss)

2.1 Abstract

The effect of climate warming on northern environments is becoming increasingly evident. Melting of underlying permafrost is associated with widespread impacts in these environments. The loss of permafrost results in a destabilizing of underlying sedimentary layers resulting in thermokarst slumping. When this occurs on a large scale (mega-slumping) soil material becomes mobilized and is carried into local streams and rivers. The purpose of this study is to examine the sub-lethal physiological effects that suspended material has on rainbow trout (*Oncorhynchus mykiss*) in the context of the Peel River Plateau. Juvenile rainbow trout were exposed (following Environment Canada exposure guidelines) for 96h to suspended clay and field collected material of differing grain sizes: small (<90µm), medium (90-150µm) and large (150-300µm), and combine (0-300µm) at concentrations of 250, 500, 1000, and 2000mg/L. The effects of exposure were assessed by measuring plasma cortisol, plasma ion concentration (Na, Cl and Ca) as well as resting metabolic rate and swim performance. It was determined that no significant changes to the physiological endpoints measured are occurring to the model organism rainbow trout at concentrations and durations equal to or greater than those present in the natural conditions.

2.2 Introduction

As scientists continue to study our global climate more and more alarming results are being discovered. Gasses specifically carbon dioxide, are building up in the atmosphere causing global temperatures to rise. Ground temperatures in northern Canada have increased by 0.77°C every decade since the 1970's (Kokelj *et al.*, 2013); this is resulting in the degradation of permafrost (Frey and McClelland, 2009). In the last 100 to 150 years the southern most regions of Canada's discontinuous permafrost zone have degraded by 30 to 65% (Jorgenson and Osterkamp, 2005). Permafrost loss has caused the relocation of coastal villages and the destruction of roads, pipelines, and infrastructure. The loss also results in erosion and surface subsidence or thermokarst (Rowland *et al.*, 2010). Thermokarst is the thawing of ice rich ground, which causes the ground to sink, creating depressions (Frey and McClelland, 2009). Retrogressive thaw slumps are one common type of thermokarst that have been estimated to affect 10% of all lakes in the Western Arctic (Theinpont, 2013).

Thaw slumping occurs when permafrost thaws and turns underlying ground into a slurry of mud which eventually slides to the base of the exposure (Lewkowicz, 1987, Kokelj *et al*., 2005). Slumps require a deep percolation of water in the sediment to occur. As water content increases the cohesive forces between sedimentary particles decreases compromising soil strength (Trenhaile, 2010). Eventually a point is reached where the normal forces holding the layers together can no longer with stand the gravitational stress and a slump occurs. This is an active process and can occur multiple times over a single thaw season and can be self-perpetuating because further permafrost loss is promoted (Lewkowicz, 1987, Kokelj *et al*. 2005). Slumping removes organic surface layers, allowing heat to flow into the ground more easily. This becomes amplified by greater snow accumulation in the winter; snow traps heat in the ground,

heat thaws permafrost, and the loss of permafrost causes slumping to occur (Kokelj *et al*, 2009). The Peel River Plateau is one area in northern Canada where thaw slumps are occurring at a high rate. Furthermore the size of slumps is on a much larger scale across the Peel River Plateau than seen in other places. The slumps there are distinguished from smaller slumps because they are larger than 5 ha in area and greater than 4m in height and considered to be mega-slumps (Kokelj *et al.*, 2013).

Thaw slumping has been shown to cause significant changes to adjacent aquatic ecosystems. Specifically increased ionic content dissolved oxygen and suspended material concentrations are shown to occur (Kokelj and Lewkowicz, 1998). These changes as well as changes in water clarity due to slumping can greatly alter the aquatic food webs in northern areas (Kokelj *et al.*, 2009). When thermokarst slumps especially mega-slumps occur next to rivers large amounts of sediment and ground material is released into the aquatic environment. This greatly increases the turbidity and concentration of material suspended in the water column potentially resulting in effects on aquatic life, especially fish.

Increased suspended solids are known decrease primary production of aquatic ecosystems by limiting the amount of light that can penetrate the water column (Bilotta and Brazier, 2008; Bruton, 1985). The loss of primary producers also decreases food availability and plant biomass (Bruton, 1985; McCubbin *et al.*, 1990). Furthermore, increased suspended material also harms the invertebrate communities of the aquatic ecosystems. The loss of primary producers decreases food availability for invertebrates (Kemp *et al.*, 2011). Additionally, increased suspended material increases predator susceptibility of invertebrates (Bilotta and Brazier, 2008) and can smother them when the material settles (Kemp *et al.*, 2011).

The effect of increased suspended material has been studied in different contexts and on multiple fish species looking at behavioural, physiological and reproductive responses. Many effects to fish behaviour as a result of increased suspended material exposure have been documented. Suspended material has been shown to delay, divert and in some cases cause the avoidance of spawning in some salmon species (Spence *et al*., 1996). Furthermore, a general avoidance of areas of suspended material has been found, salmon (*Oncorhynchus kisutch*) began to avoid the bottom of the tanks and stayed towards the surface when placed into tanks with a higher suspended sediment concentration at the bottom compared to the top (Servizi and Martins, 1992).Both high and low suspended material increases are also known to decrease feeding rates as well as feeding behaviour (Berg and Northcote, 1985; Redding *et al.*, 1987), and consumption of prey was shown to have an inverse relationship with turbidity (Bonner and Wilde, 2002).

Physiology has also been shown to change as a result of suspended material exposure. Numerous effects on the gills and respiration of exposed fish have been shown to occur. These changes include: impairment of respiration (Newcombe and Flagg, 1983), increased mucous production and gill damage in arctic grayling (Reynolds *et al.*, 1989), fusion of gill arches and increased mucous in gill cavities in two species of minnows (Sutherland and Meyer, 2007), and changes in osmoregulatory ability of juvenile *Epinephelus coioides* as a result of an increased number of chloride cells on the gill and a decreased Na+/K+ ATPase activity (Au *et al*, 2004). Suspended material has also been shown to cause affects to blood physiology of fish. Increased blood cortisol levels were found in two species of salmon (*Oncorhynchus kisutch*, and *Salmo gairdneri*) (Redding *et al.*, 1987), as well as in both wild and inbred strains of Ayu (*Plecoglossus altivelis*) (Awata *et al*., 2011). Servizi and Martins (1992) observed increases in blood glucose

levels as a result of suspended material. Moreover, it was found that suspended material resulted in steadily rising hematocrit levels in steel head salmon (Redding *et al.*, 1987).

Changes on a cellular level have also been shown to occur as a result of exposure to suspended material. Rainbow trout were found to have cellular changes within the kidney specifically changes to the degradation of tubule cells in the kidney as well as metabolic changes after exposure to suspended material (Michel *et al.*, 2013). Tse *et al.* (2010) found that grouper exposed to suspended sediment concentrations as low as 32mg/L exhibited significant DNA damage, after 5 days of exposure.

Growth and reproduction is another area that has been shown to be affected by exposure to suspended material. Increased sediment can cause mortality in embryos because of oxygen stress (Spence *et al.*, 1996). It was determined that there is an inverse relationship between growth rate of two minnow species (*Cyprinella galactura*, and *Erimonax monachus)* and suspended sediment concentration (Sutherland and Meyer, 2007). As the material settles it changes stream makeup, causing a decline in spawning habitat. This decline results in an impairment of egg and larval development as well as decreased fry emergence (Levesque and Dube, 2007). The settling of sediment can also restrict waste exchange, decrease dissolved oxygen levels, and physically damage eggs (Bruton, 1985; Scheurer *et al*, 2009).

Although these previous studies have been important for gaining understanding of what kind of effects can occur and much knowledge was gained from them none of these tests looked at the effects of suspended material on fish in the context of the Peel River Plateau. The studies looked at the affect that increased suspended material as a result of construction, volcanic eruptions and other sources had on fish. However none of the studies looked at the effect that the

parameters found in the Northwest Territories and on the Peel River Plateau as a result of thermokarst and mega-slumping are having on fish

Objectives:

The purpose of this study is to identify the effects of increased suspended material on freshwater fish in the context of permafrost degradation and thermokarst activity (slumping). First the exposure-response relationships will be investigated using rainbow trout as the model organism and defined suspended material (at three grain sizes). The exposure-response relationships will then be applied using material collected from thermokarst sites in order to investigate the response to natural conditions.

The objectives of this study are as follows:

i) Determine the effect of exposure to increased concentrations of suspended material on juvenile rainbow trout through measurement of:

a) Metabolic rate (oxygen consumption),

b) Stress response (cortisol).

c) Ion balance (specifically focusing on Na^+, Cl^- and Ca^+ in plasma).

ii) Determine the potential impact of Peel Plateau slumping on fish.

a) Examine effect levels from objective i in the context of field measurements
b) Compare physiological effects of field collected suspended material on rainbow trout to effect of the model material used in objective i).
c) Identify effects thresholds for suspended material in northern environments

Hypothesis:

It is hypothesized that:

1) Metabolic activity will be significantly increased in fish exposed to increased suspended sediment concentrations. This is based on the assumption that as the fish attempt to cope with the increased sediment concentrations they will expend significantly more energy

2) The cortisol levels of rainbow trout will show statistically significant increases as a result of exposure to increasing suspended sediment concentrations. It is assumed that the stress response will be measurably activated by suspended sediment.

3) Ion balance in rainbow trout will be impaired (or altered) in fish exposed to suspended sediment. This is based on the observation that suspended sediment can clog gills and impair gill function.

4) Smaller grained sediments will show statically different physiologically effects than larger grained sediments do at the same exposure concentrations. This is based on the assumption that smaller sediments can more easily become lodged in gills and stay suspended more easily.

2.3 Materials and Methods

2.3.1 Fish Culture

Juvenile rainbow trout (*Oncorhynchus mykiss,* 90.37±44.13 (mean±SD), n=364) were purchased from a local commercial supplier (Rainbow Springs Trout Hatchery, Thamesford, ON). They were housed in 220L polyethylene tanks supplied with flowing water which was a mix of de-chlorinated city water and reverse osmosis generated water. Water temperature was 13.5 °C (±1.5) and water conductivity was maintained at 230 µS (±30 µS) with water hardness of 84 mg $CaCO_3$ L^{-1} (with 546 ± 8 µM Ca, 293 ± 5 µM Mg and 527 ± 12 µM Na). Trout were monitored daily and fed commercial trout food (Skretting NutraFry 2.3, Skretting, St. Andrews, NB) at 2% of their body weight per day. Housing and experimentation of fish was reviewed and approved by the Wilfrid Laurier University Animal Care Committee in accordance with the Canadian Council for Animal Care.

2.3.2 Exposure to Suspended Material

2.3.2.1 Exposure System

Glass aquaria (50 L) were used for the static exposures. In order to keep the test material suspended during exposures an aeration apparatus was build using the design of Sutherland, 2006 and can be seen in Figure 2.1. The system used one central reciprocating shaft driven by compressed air and connected to four individual arms which extended into each of the exposure tanks. Each of the arms delivered aeration to the bottom of the tanks and as it swept back and forth so any settling material was re-suspended. The drive shaft uses a pneumatic air cylinder connected to a solenoid valve that automatically extended and retracted at a distance set to equal the length of the exposure tanks.

2.3.2.2 Acute Exposure to Suspended Material

Rainbow trout were exposed to suspended material at nominal concentrations of 250, 500, 1000, or 2000 mg/L for 96 h following standard exposure methods (test method EPS1/RM/13, Environment Canada (2007)). Exposures were static and for each exposure series two control groups (no added material) were included, one group with the exposure apparatus to keep material suspended and the other one without. Test concentrations were prepared by adding appropriate amounts dried and sieved material to 40L of water. Fish (n=7 per treatment) were netted from the rearing tank and non-selectively distributed to treatment tanks. In the first set of exposures the n of 7 fish per treatment was split into two tanks with three and four fish respectively. The second set of exposures did not split the fish between two tanks. During tests, water quality was checked daily including temperature, conductivity, dissolved oxygen (DO), and pH were measured daily (YSI Pro Plus, YSI Inc, Yellow Springs, OH).Measurements are available in Table 2.1.

2.3.2.3 Exposure Materials

The effects on fish of three different materials were compared, one of known characteristics and two naturally occurring material. The known material was a commercially available kaolin clay (Hawthorn Bond Clay, Christy Minerals, High Hill, MO) while the two natural sources were collected from a site near Yellowknife in the North West Territories (Birch site) and a site near Fort Smith, also in the NWT (Fort Smith site). Birch site material was collected at a thermokarst slump (GPS location: 12.506 N, 114.278 W) while the Fort Smith site was upstream of the municipal boat launch where a landslide had occurred in 1968 (GPS Location 60.018496, -111.894013).

Each material was separated into three different size fractions based on those used by Servizi and Martins (1987). The fractions used were 150 to 300 µm (large grain size), 90 to 150 µm (medium grain size) and <90 µm (small grain size). Tests were also conducted with the combined fractions (0 to 300 µm). Fractions were created by sieving using a WS Tyler ROTAP sieve shaker (Model RX-29) for 5 min to separate the material into the size fractions. The effect of grain size was tested by exposures to small, medium or large grain sizes. The grain size distributions for each of the sample materials are given in Table 2.2.

2.3.3 Chase Test

The ability of exposed fish to mount a stress response was tested by conducting a chase test. The test was done according to methods given in Vijayan and Moon (1992) and involved exposing the a group to 2000mg/L of <90 µm material from Fort Smith for 96h and then chasing them for 3 min using a dip net, and then sampling after 30min, and comparing the cortisol levels to the control groups (Vijayan and Moon, 1992). In the test we used three control groups: two groups with no added material, one with the exposure apparatus to keep material suspended and the other one without, and one group with 2000mg/L of <90 µm suspended material from the Fort Smith site.

2.3.4 Sample Collection and Measurements

The effect of suspended material on plasma ions (Na, Ca and Cl) and cortisol was measured after the 96h exposure. Blood samples were collect via caudal puncture from fish after removing them from the exposure system, and anesthetizing with 0.2 g/L of tricaine methanesulfonate (MS 222) buffered with 0.2 g/L of sodium bicarbonate. Blood was centrifuged at 13000 rpm for 4 min at 3˚C, plasma separated and stored at -80C.

2.3.4.1 Measurement of Plasma Endpoints

Plasma samples were thawed and purified to extract the cortisol using the triple extraction method (Meina *et al*., 2013). After purification, ten samples were chosen at random and 100 µL was taken from each sample to create a pooled a plasma sample. This pooled plasma sample was used to run a dilution range finder test. After the range finder test it was determine that a 50x sample dilution was ideal for cortisol measurement because it showed the best match to the slope of the standard curve. All samples were diluted 50x using EIA buffer and measured using the commercially available kit (Cortisol Express EIA kit, Cayman Chemical, Ann Arbor, MI), and a read on a micro plate reader (SpectraMax 384 Plus, Molecular Devices, Sunnyvale, CA). The cortisol concentrations were then determined following the methods given in the kit.

After cortisol measurement plasma samples were diluted for measurement of calcium and sodium which was done by flame atomic absorption (AAS) (SpectrAA 880, Varian INC, Walnut Creek, CA) using the recommended instrument parameters. Plasma chloride concentrations were measured by coulombmetric titration using a chloride meter (926S Chloride Analyzer, Nelson-Jameson Inc., Marshfield, WI).

2.3.4.2 Resting Metabolic Rate and Swim Performance (Ucrit)

Resting metabolic rate (oxygen consumption rate) and swim performance were also measured after exposure. Metabolic rate determinations were done in 72mm diameter by 400mm clear acrylic static respirometry chambers (Loligo systems Tjele, Denmark) with the Loligo Systems Automated Respirometry system running AutoResp software (Loligo Systems, Tjele, Denmark). Fish were removed from the exposure system, weighed, transferred to chambers and then acclimated to the chambers for 30 min before the measurement period began. The

intermittent measurement cycle was adapted from (Steffensen, 1989) and consisted of three phases a flush phase (4 min), a wait phase (1 min) and a measurement phase (5 min). Measurement was repeated for 5 cycles and the oxygen consumption rates were determined by taking the average of all five measurements.

To measure swim performance, fish were removed from the exposure system after 96h, measured for length and moved into a 30 L swim flume (D30, Loligo Systems; Tjele, Denmark). Flow in the swim chamber of the flume was calibrated using a flow meter (U278- HFA, Hüntzsch; Waiblingen, Germany). To measure swim performance a ramp U_{crit} procedure was adapted from the protocol used in Jain et al. (1997). Specifically, fish were given 40 min to acclimate to the swim flume at a velocity of 0.4 body lengths per second (BLs^{-1}). After the acclimation period water velocity was increased to 1.0 BLs^{-1} and then ramped to 3.0 BLs^{-1} over the next 10 min. Ramping was done by increasing velocity at a rate of 0.4 BLs^{-1} every 2 min until the velocity of 3.0 BLs^{-1} was reached. The velocities were then increased by 0.75 BLs^{-1} every 20 min until the fish became exhausted. Exhaustion occurred when a fish could no longer sustain swimming in the water column after being returned to the water column 2 times. At the time of exhaustion the fish was removed and the length of successful swimming time at the given velocity was recorded.

2.3.5 Calculations and Data Analysis

The sustained swim velocity was calculated (U_{crit}) as given in Jain et al. (1997) using the formula

$$Ucrit = Ui + \left(\frac{Ti}{Tii} \times Uii\right)$$

Where U*i* is the highest velocity maintained for the whole interval (BLs^{-1}), U*ii* is the velocity increment, T*i* is the time elapsed at fatigue velocity (min) and T*ii* is the interval length.

Resting metabolic rate was determined through the measurement of oxygen consumption per unit of body mass is calculated using the formula:

$$MO_2 = Vr \times \left(\frac{\Delta CwO2}{\Delta t \times bw}\right)$$

Where MO_2 is the oxygen consumption rate per unit body mass, Vr is volume of water in the system, ΔCwO_2 is oxygen conc. in the water, Δt is time period, and bw is weight of the animal (Steffensen, 1989).

Plasma ions (Ca^{2+}, Cl^-, Na^+), plasma cortisol, resting metabolic rate, and U_{crit} were analyzed on a concentration and grain size basis using the program SPSS (IBM, Armonk, NY) to determine if there were statistically significant differences between the control and treatment groups. The data was first checked for normal distribution and homoscedasticity. Once those parameters were met an ANOVA test was used to determine if significant differences were present ($P \leq 0.05$), in which case Dunnett's post hoc test was used to determine which treatments were significant from controls.

2.4 Results

2.4.1 96h Exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes and Concentration

2.4.1.1 Plasma Ions

Exposure to suspended clay particles of three grain sizes (<90, 90-150 , 150-300µm) at concentrations of 250, 500, 1000 and 2000 mg/L for 96 h showed significant changes in plasma ion concentrations compared to controls (Figure 2.1). Specifically plasma calcium concentrations (Table 2.3) were found to be significantly higher in the 2000mg/L treatment group of each of the grain sizes used, and plasma chloride concentrations (Table 2.4) were found to be significantly lower than that of the control group in the 2000mg/L treatment group of each grain size used. However, it was shown that the same exposure did not have any effects on plasma sodium concentrations (Table 2.5).

2.4.1.2 Plasma Cortisol and Resting Metabolic Rate

After exposure to suspended clay particles of three grain sizes (<90, 90-150 , 150-300µm) at concentrations of 250, 500, 1000 and 2000mg/L for 96h it was determine that plasma cortisol concentrations were significantly changed. Specifically plasma cortisol concentrations (Table 2.6) were found to be significantly increased in highest treatment concentration (2000mg/L) of all three grain sizes used the when compared to the control group. However, it was determined that the same exposure conditions had no effect on resting metabolic rate (Table 2.7). Both of these results are summarized in (Figure 2.3).

2.4.2 96h Exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Field Collected Material and Clay of Differing Grain Sizes

2.4.2.1 Plasma Ions

After exposure to suspended clay and field collected material of grain sizes (<90 and 0-300µm) at a concentration of 2000mg/L for 96h it was determine that plasma ion concentrations in all three ions measured (Na, Ca, Cl) were not affected (Figure 2.4). The means for all three ions can be found in Tables 2.10 (Na), 2.8 (Ca), and 2.9 (Cl).

2.4.2.2 Plasma Cortisol Concentration, Resting Metabolic Rate, and Swim Performance (U_{crit})

After exposure to suspended clay and field collected material of grain sizes (<90 and 0-300µm) at a concentration of 2000mg/L for 96h it was found that no significant differences were present in plasma cortisol concentrations (Table 2.12), and resting metabolic rate (Table 2.11), these results are summarized in Figure 2.5. Furthermore, it was determined that after exposure to material from the Fort Smith collection site at a grain of <90µm and a concentration of 2000mg/L that swim performance was unaffected (Figure 2.6). The means for swim performance can be found in Table 2.13.

2.4.2.3 Chase Test

Significant differences were found between the group of fish chased after the 96h exposure period and the control groups that were not (Figure 2.7). The control groups had an average cortisol value of 19.95 ±2.51 ng/mL and the chase group had an average of 35.75 ±4.83 ng/mL. The significant difference present between the control groups and chased group shows that fish are still able to mount a stress response after 96h of exposure to suspended material.

2.5 Discussion

We hypothesized that exposure to suspended material would induce physiological changes (ion regulation, stress hormones (cortisol)) and these would link to whole organism effects (oxygen consumption rate and swim performance) following the model proposed by Beitinger and McCauley (1990), where stress responses are integrated into ecologically relevant whole body responses such as growth, reproduction, and swim performance. Previous studies have shown that suspended material can cause damage to fish gills, resulting in changes to their osmoregulatory ability (Au *et al*, 2004). If gill damage occurred in response to suspended material exposure it might induce a significant physiological disruption, detectable by assessment of ion balance, and an associated stress response. These and other impacts may cause tertiary whole body performance reductions; we looked for these using metabolic rate (oxygen consumption) and swim performance (ramped Ucrit). The context for these studies is to help contribute to the understanding of retrogressive thermokarst mega-slump disturbances on aquatic ecosystems in the lower Peel watershed of the North West Territories Canada. (Kokelj, 2013) There is concern for the potential effects that the suspended material released by these mega slump disturbances could have on fish. We approached this problem by exposing a sensitive model organism (rainbow trout), to the conditions typically occurring downstream of mega-slump disturbances.

2.5.1 Environmental Context

In designing our exposures we considered the conditions that fish could experience downstream of thermokarst disturbances in terms of concentration as well as duration. During the

warm summer months on the Peel River Plateau mega-slump activity varies according the amount of solar radiation (Kokelj et al., 2013). Throughout the day elevated amounts of material are released and then during the shortened night, it decreases. This means that the amount of suspended material that native fish are being exposed to fluctuates and measured turbidity values can vary greatly from<100 to 1200 NTU (Kokelj et al., 2013). While it is has been shown that fish avoid areas of increased turbidity (Servizi and Martins, 1992), fish native to the Peel River (ie Arctic char and Dolly varden) are subjected to turbid areas during migration. The duration the exposure to migrating fish is not known, but it is assumed to be in the magnitude of hours or days. Therefore we chose to use a standard 96 h acute exposure period and a constant loading level to mimic peak worst-case exposure conditions. Measured turbidity values in our exposures were similar to or in some cases exceeded measured field values (see Figure 2.8). It is worth noting that the only exposures that resulted in significant physiological effects (clay at 2000mg/L) had measured NTUs exceeding field values (except for the 150-300µm clay, Figure 2.8).

This research was conducted as two linked studies; the first looked at how exposure to a model material (clay) of known size composition affected physiological parameters in juvenile rainbow trout. The second study built on the understanding gained from the first and used field collected material for exposure. For all of the studies exposure concentrations were confirmed by sampling, filtering and weighing the amount of dried material, which ranged from 78 to 100% of nominal concentration. The first study showed that suspended clay of all grain sizes up to concentrations of 1000mg/L did not cause significant change: specifically, in ion balance, stress response, and resting metabolic rate. However concentrations of 2000 mg/L at all grain sizes resulted in significantly increased plasma calcium, decreased plasma chloride (Figure 2.2) and

increased plasma cortisol concentrations (Figure 2.3). In the second study, using field collected material at the highest exposure concentration (2000 mg/L) there were no significant changes in plasma ions, cortisol, resting metabolic rate or U_{crit} compared to controls (Figure 2.4, 2.5, and 2.6). Additionally a repeated exposure to clay at 2000mg/L showed no significant impacts (for all grain sizes).

Significant changes were only found at the highest concentration (2000mg/L) of exposure to clay in the first study. When we repeated this exposure to confirm this effect (and also compare effects directly to natural material) we found no significant effects. The lack of effects from 2000 mg/L exposure in the second study caused us to reexamine the initial result from the first study. The reason for the differing results is not clear as there were few differences between the two groups. Differences include the fact that the two tests were carried out at different times of year (study 1 in the winter study 2 in summer). As well, while we purchased fish from the same commercial supplier, the trout were brought into the Laurier Animal Care Facility for study 1 and 2 were purchased at different times. As well, in study 1 there was a building power systems failure due to inclement weather during the test and although the systems in the Animal Care Facility were connected to a working backup power system it is possible that some aspect of the event could have contributed to the measured physiological disruption. While no clearly identifiable reason for the opposing results is known, the weight of the evidence from the other tests conducted supports the lack of effect at 2000 mg/L clay. The same concentration of the other materials also produced no effects.

2.5.2 Discussion of Endpoints

2.5.2.1 Plasma Ions

Changes in plasma ions are often associated with a response to external environmental challenges (Al-Jandal and Wilson, 2011); this includes exposure to suspended material. For example the study done by Au *et al* (2004) exposed *Epinephelus coioides* for 6 weeks to 5 different concentrations (50, 100, 200, 1000, 2000 mg/L) of dried sediment collected from a local riverbed. The results showed that changes to the osmoregulatory capacity, increased number of chloride cells on the gill and a decreased Na+/K+ATPase activity in juvenile *Epinephelus coioides*. These changes were associated with impacts to the gill, specifically lifting of gill epithelium and hyperplasia. We found no changes to plasma ion concentrations after 96h of exposure to similar concentrations. Direct comparison of our results with rainbow trout and those of Au *et al* (2004) are not warranted as the latter was done on a marine fish in marine waters and over a much longer period of exposure. The longer duration allows for a greater exposure of the gills to suspended material and therefore greater chances that osmoregulation will be affected. The osmoregulatory responses of marine fish are likely very different to those of freshwater fish. We found that no significant changes occurred to plasma ions, this could suggest that there was no impact on the gill (except for at the highest concentration of clay Figure 2.2) or that impacts occurred but the fish were able to maintain plasma ions. Subsequent investigation into the time course of osmoregulatory changes during exposure and gill histology could provide insights and improve the understanding of suspended materials on ion balance.

2.5.2.2 Cortisol

Changes to stress (plasma cortisol concentration) were expected because cortisol is one of the most important hormones for controlling osmoregulation (Heath, 1995). Other studies found

increases to plasma cortisol as a result of exposure to suspended material. For example Redding *et al.* (1987) showed increased plasma cortisol in two salminoid species (*Oncorhynchus kisutch*, and *O. mykiss*). Exposures in that study were done using clay, topsoil, or volcanic ash at either low (400-600mg/L) or high (2000-4000mg/L) concentrations for up to 8 days. Increases in plasma cortisol were measured for up to 24h but then returned to normal for the remainder if the exposure. The study by Awata *et al*. (2011) exposed wild and inbred strains of Ayu (*Plecoglossus altivelis*) to 200mg/L of suspended kaolin clay and observed increased plasma cortisol concentrations after 3h of exposure. We found that no changes to plasma cortisol concentration occurred after 96h

Redding *et al.* (1987) proposed that the initial exposure to suspended material produces a temporary stress response that subsequently dissipates resulting in a fall back to normal cortisol levels over time. The differences present between our study and the others are likely a result of exposure duration. Redding *et al.* (1987) measured the increase in cortisol concentration at 24h or less, and Awata *et al*., 2011 measured the change at 3h, both of which are far earlier time point than when our measurements were taken. We only sampled at the 96h time point so it is possible that there was an initial increase cortisol levels but after the initial increase levels returned to normal over time. We did not see a response since our measurements were taken long after the time where you would see the initial response. We did not quantify the initial transitory stress response because initiation of tests involved capture from holding tanks and transfer, it would have been impossible to separate the potential suspended material stress response from the handling stress. Additionally we only sampled at 96 h because we were interested in the possibility of sustained stress induced by exposure.

An additional interpretation of the lack of elevated cortisol at 96 h is that fish were overly stressed throughout our exposures and by 96 h had exhausted the cortisol stress response. To test this possibility we ran supplementary exposure at 2000 mg/L of <90 µm Fort Smith material and after 96 h we subjected one of the exposure group to a standard stress test (Vijayan and Moon 1992). The chased group was significantly elevated compared to the un-chased control groups (see Figure 2.7). This indicates that after 96 h fish were relatively unstressed (and not stressed to the point of being unable to mount a stress response). Three things further support this conclusion:

1) All of the cortisol concentrations measured were under the 150ng/mL which is considered to be the threshold for high stress level in rainbow trout (Barton *et al*. 1980, Gregory and Wood 1999, Pagnotta *et al*. 1994).

2) If the fish were stressed they could have possibly also shown other indications, including plasma ion changes but no other changes occurred. Cortisol is one of the most important hormones for controlling osmoregulation (Heath, 1995) but levels were normal. Normal resting plasma levels measured in rainbow trout are: Na 150 mmol/L (Morisawa, 1983), Cl 130 mmol/L (Davie, 1983) and Ca is generally accepted to be near 2.5 mmol/L (Andreasen, 1985) and our results are within these normal levels.

3) When stress occurs it does not affect one physiological process; it affects many and causes changes from the cellular level all the way up to the whole body level. Therefore increases in stress should be able to be measured through changes in things like swim performance and metabolic rate (Beitinger and McCauley, 1990). But we measured no changes to measured metabolic rate and swim performance after exposure to suspended material. In addition our

measurements of resting oxygen consumption rate and U_{crit} are at or near what the normal values are considered to be (between 8.5 and 15µmol/g/hr for oxygen consumption (Miller et al. 1995) and 4.5 BLs^{-1} for ramped U_{crit} (Wood, 1997)).

2.5.2.3 Mo2 and Swim Performance

Changes to small scale aspects of the fish physiology such as ion balance/osmoregulation which then result in whole body stress response would cause changes to whole body physiology mechanisms such as metabolic rate (oxygen consumption) and swim performance (Beitinger and McCauley, 1990). When osmoregulation is affected by external environmental changes to compensate the fish will use chloride cells and Na+/K+ATPase activity to restore the balance (Heath, 1995). The use of these mechanisms would result in an increased energy use, an increased need for energy production, and therefore an increase in metabolic rate. Furthermore, other studies showed exposure to increased suspended material resulted in changes to the gills including: impairment of respiration (Newcombe and Flagg, 1983), increased mucous production and gill damage in Arctic grayling (Reynolds et al., 1989), fusion of gill arches and increased mucous in gill cavities in two species of minnows (Sutherland and Meyer, 2007). If these changes had occurred in the trout in our experiment the decreased diffusion of oxygen across the gill membrane may have caused hyperventilation, which would result in an increased resting metabolic rate and therefore oxygen consumption rate. But there were no changes to resting metabolic rate.

Since no changes to resting metabolic rate occurred we decided to measure swim performance instead of further pursuing resting metabolic rate. By measuring swim performance we examined whether the potential effects of suspended sediments, which were not observable at

rest became apparent during forced activity. Berli *et al.* (2014) swam rainbow trout in a swim chamber with suspended calcium carbonate at concentrations of 110, 220, and 440 mg/L and found that rainbow trout exhibited significant decreases in the swim performance compared to unexposed controls in clean water. We found that swim performance was not affected by exposure to suspended material at concentrations of 2000mg/L. Berli *et al.* (2014) used calcium carbonate suspended in water to create conditions of increased turbidity, where in contrast we used actual suspended ground material to increase turbidity. We exposed our fish to suspended material and then swam them in clean water where Berli et al. (2014) swam them in water with suspended calcium carbonate. As discussed earlier, stress levels have been shown to increase during the initial exposure to suspended material and then slowly return to normal over time (Redding et al. 1987). Since, Berli et al. (2014) didn't expose the fish until they swam them, their swim capacity reduction could have resulted from the short term stress during the initial exposure. Our swim performance test was done in clean water and after 96h the fish may have become acclimated to the conditions and stress levels were back down to normal by the time we swam them. We measured U_{crit} (and oxygen consumption rates) in clean water was because it was not possible to maintain consistent/constant exposure conditions in either the respirometry chambers and swim flume. Further, we were interested to understand if disruptions induced by suspended material would persist in non-turbid waters.

2.5.3 Conclusion

Our results suggest that exposure to suspended material in the context of the Peel River Plateau does not cause significant effects to rainbow trout (specifically to plasma ion concentration, plasma cortisol concentration, resting metabolic rate, and swim performance). This conclusion was drawn from the fact that the exposure concentrations that we used matched and in

most cases exceeded the concentrations of suspended material present in the Peel River plateau but still did not cause significant changes to the endpoints measured. Furthermore, the exposures used in this study involved a greater duration of exposure to maximum or greater than maximum suspended material concentrations, and still did not show significant affects as a result. In short no significant changes to the physiological endpoints measured are occurring at concentrations and durations far greater than are present in the natural conditions. However, it is essential to note that rainbow trout are not native to the Peel River. Also, the natural material that we tested was not directly collected from mega slump disturbances so this study leaves open the possibility that effects could be seen if the same tests were done using native species and or site specific material.

2.5.4 Future Directions

There are a number of future directions that could be beneficial for the project. First of all using material that is native to the Peel River Plateau collected from areas of mega-slump disturbance would be useful. This material could show different results due to potential differences in chemical composition, grain size distributions, or particle shape. Moreover, conducting exposures using fish native to the area would also be a good next step since we only exposed a model organism that is not present in the area of interest of the project. Lastly, if the duration of exposure in the wild could be determined it would be beneficial to tailor a new set of exposures using the durations of exposure experienced by migrating fish in the wild, this would allow for a more realistic representation for potential physiological affects to native fish.

2.6 Figures

Figure 2.1

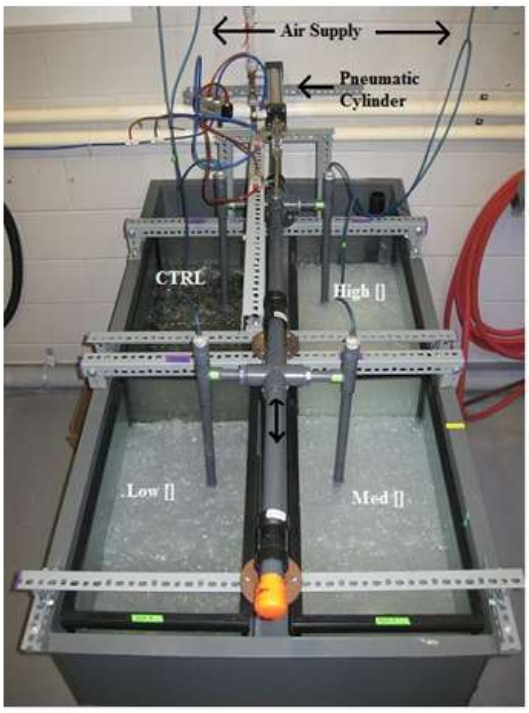

Figure 2.1 Exposure set up used for all exposures, based on the design proposed in Sutherland 2006.

2.6.1 96h Exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes and Concentrations

Figure 2.2

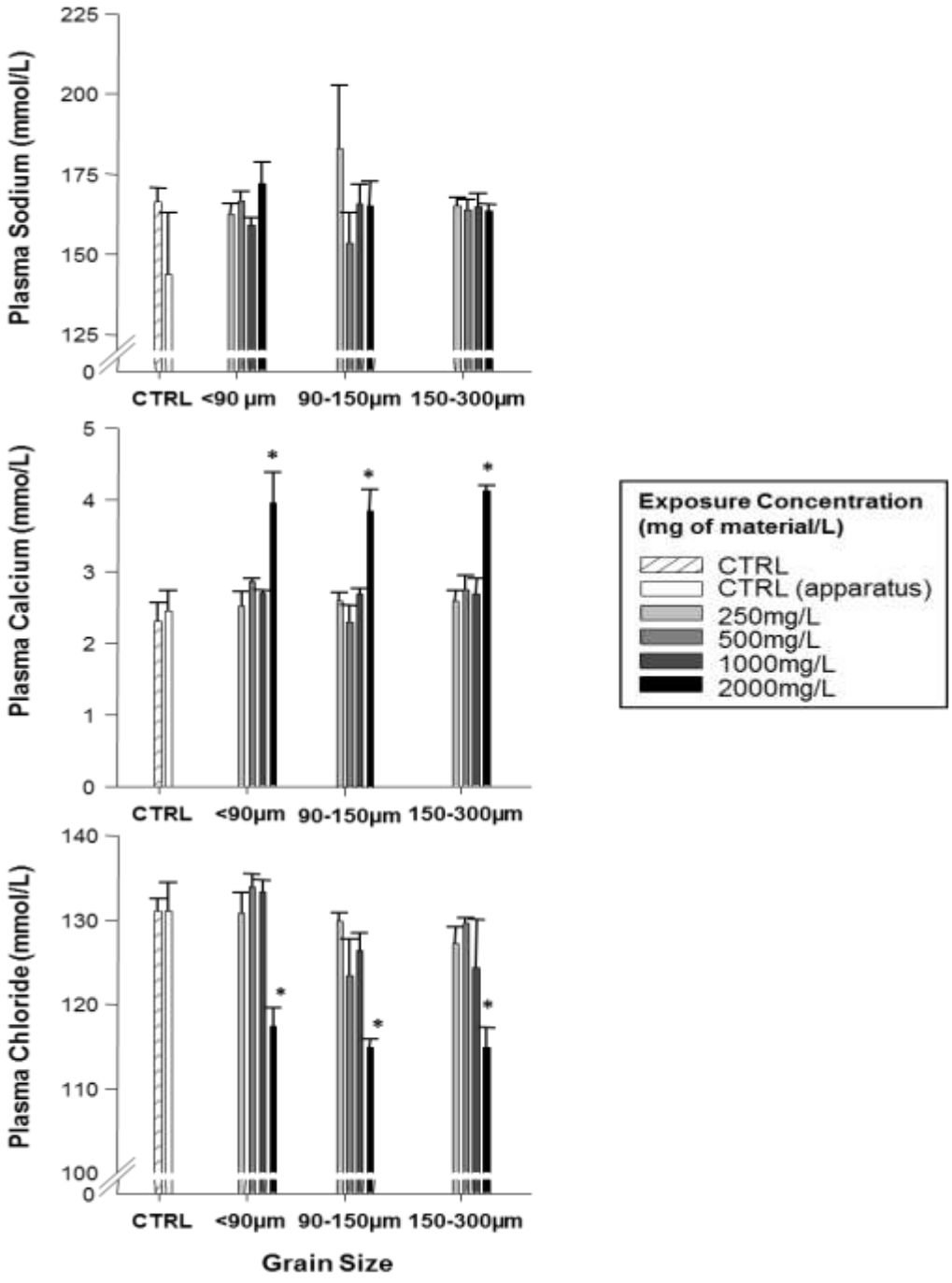

Figure 2.2: The Effect of 96h Exposure to Suspended Clay Particles of Differing Grain Sizes and Concentration on Plasma Ion Concentrations in Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

Plasma ion concentrations (mean±SEM, n=7) of juvenile rainbow trout (Oncorhynchus mykiss) after 96 hr exposure to suspended clay particles of different grain sizes and concentrations. The hashed bar represents the unexposed control, the open bar represents the unexposed control with the exposure apparatus present, and the shaded bars represent the treatment concentrations. A * denotes where significant differences were found to be present between the control and treatment group (Dunnett's test, $p \leq 0.05$). The two controls were not significantly different from one another, and all treatments were compared to the control that included the exposure apparatus. Significant differences were found to be present between the control group and the 2000mg/L concentration of all grain sizes for plasma calcium and chloride concentrations, but no differences were found to be present in plasma sodium concentrations. Specifically calcium was significant increased, and chloride was significantly decreased.

Figure 2.3

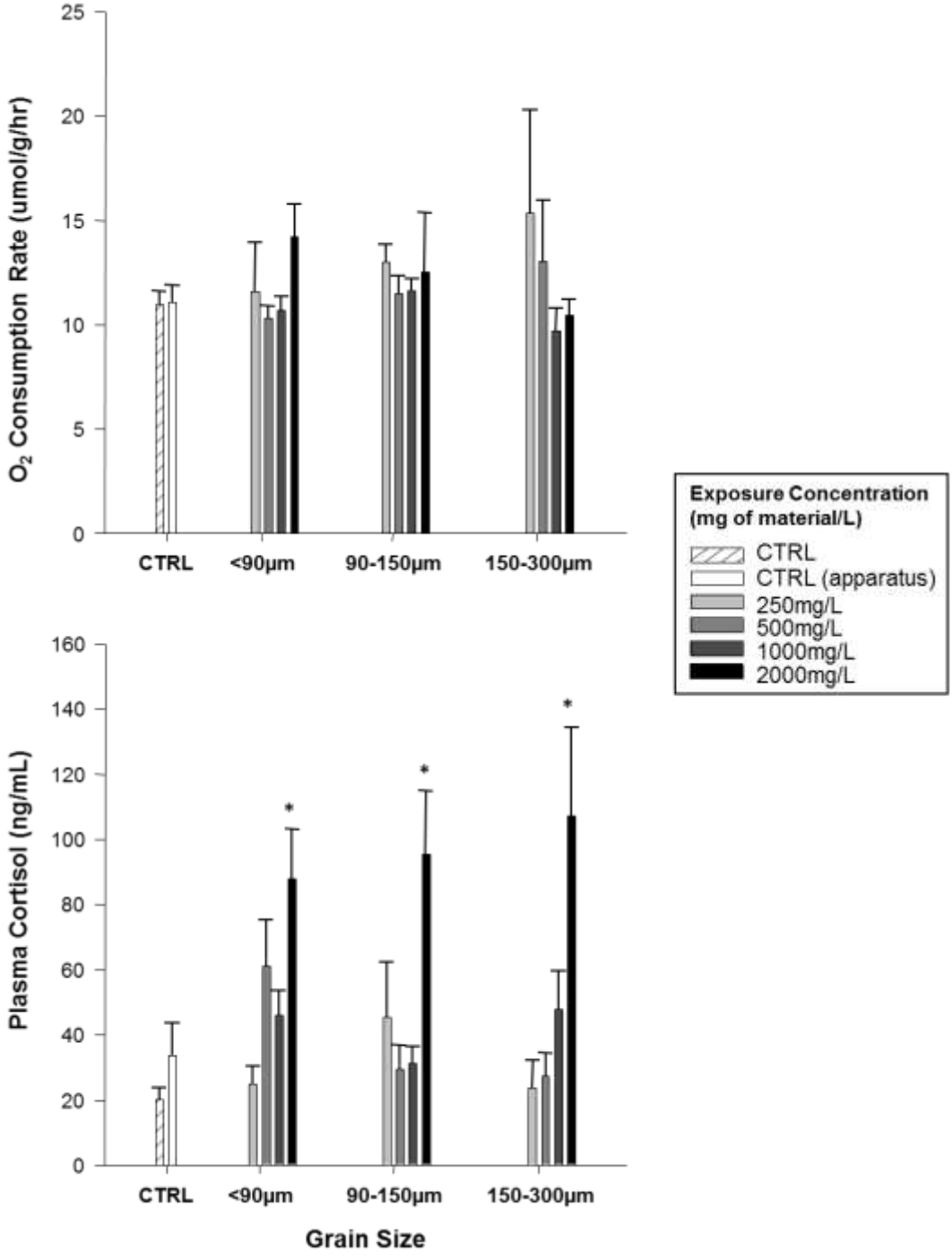
O_2 Consumption Rate (umol/g/hr)
25
20
15
10
5
0
CTRL
<90µm
90-150µm
150-300µm
Exposure Concentration (mg of material/L)
CTRL
CTRL (apparatus)
250mg/L
500mg/L
1000mg/L
2000mg/L
Plasma Cortisol (ng/mL)
160
140
120
100
80
60
40
20
0
*
CTRL
<90µm
90-150µm
150-300µm
Grain Size

Figure 2.3: The Effect of 96h Exposure to Suspended Clay Particles of Differing Grain Sizes and Concentration on Plasma Cortisol Concentration and Resting Metabolic Rate of Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

Plasma cortisol concentration (mean±SEM, n=7) and resting metabolic rate (as estimated by weight adjusted resting oxygen consumption rates, mean±SEM, n=7) of juvenile rainbow trout (*Oncorhynchus mykiss)* after 96 hr exposure to suspended clay particles of different grain sizes and concentrations. The hashed bar represents the unexposed control, the open bar represents the unexposed control with the exposure apparatus present, and the shaded bars represent the treatment concentrations. A * denotes where significant differences were found to be present between the control and treatment group (Dunnett's test, $p \leq 0.05$). The two controls were not significantly different from one another, and all treatments were compared to the control that included the exposure apparatus. Significant differences were found between the control group and the 2000mg/L concentration of all grain sizes of the plasma cortisol concentrations, but no differences were found to be present in the resting metabolic rate.

2.6.2 96h Exposure of Juvenile Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Field Collected Material and Clay of Differing Grain Sizes

Figure 2.4

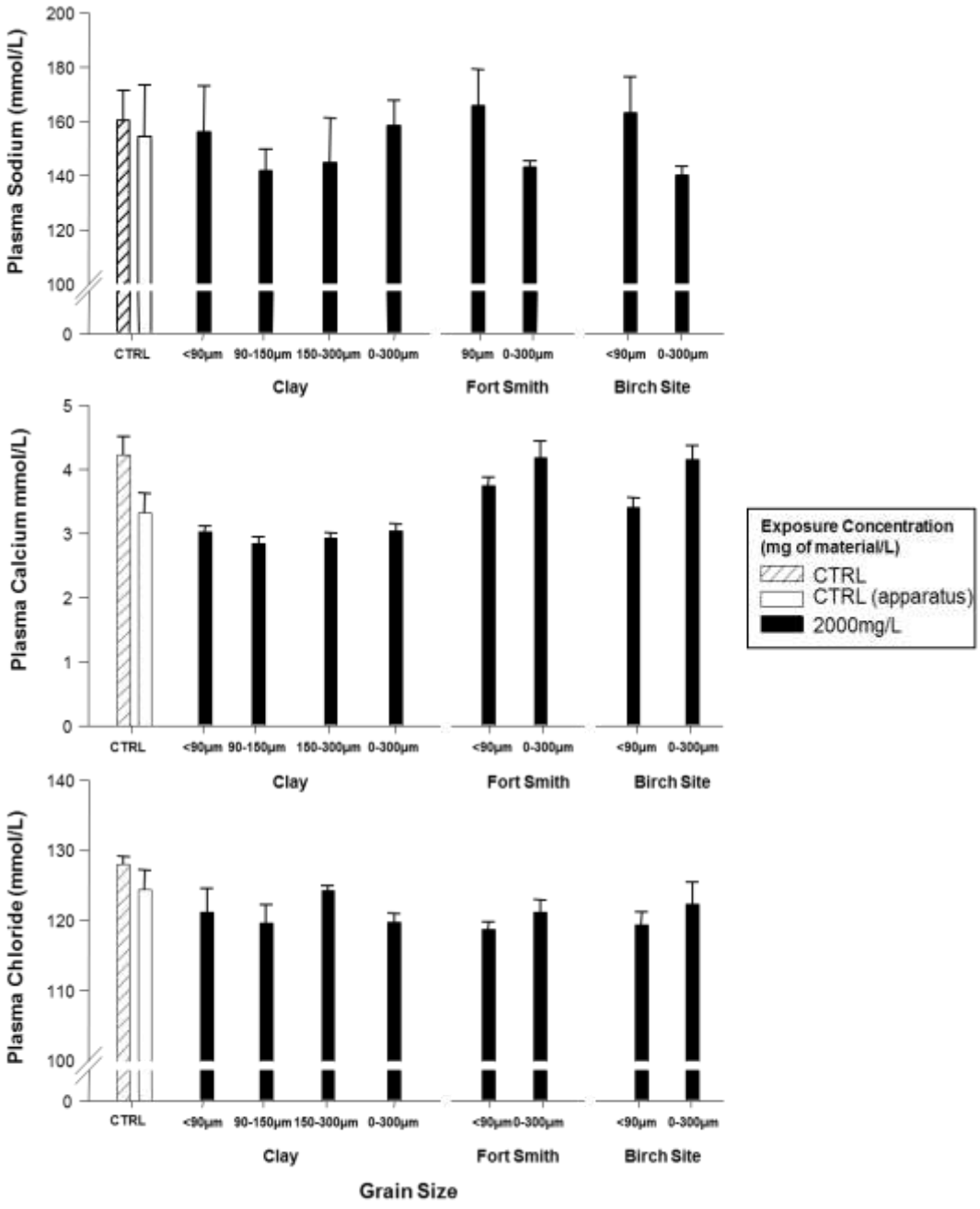

Figure 2.4: The Effect of 96h Exposure to Suspended Field Collected Material and Clay Particles of Differing Grain Sizes at a Concentration of 2000mg/L on Plasma Ion Concentration in Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

Plasma ion concentration of juvenile rainbow trout (*Oncorhynchus mykiss)* after 96 hr exposure to suspended field collected material and clay particles of different grain sizes at a concentration of 2000mg/L. The hashed bar represents the unexposed control; the open bar represents the unexposed control with the exposure apparatus present, and the shaded bar represent the treatment concentration. The two controls were not significantly different form one another and all treatments were compared to the control that included the exposure apparatus. None of the plasma ions measured were shown to have significant differences between the control and treatment group, and this was the case for all material sources and grain sizes (Dunnett's test, $p \leq 0.05$).

Figure 2.5

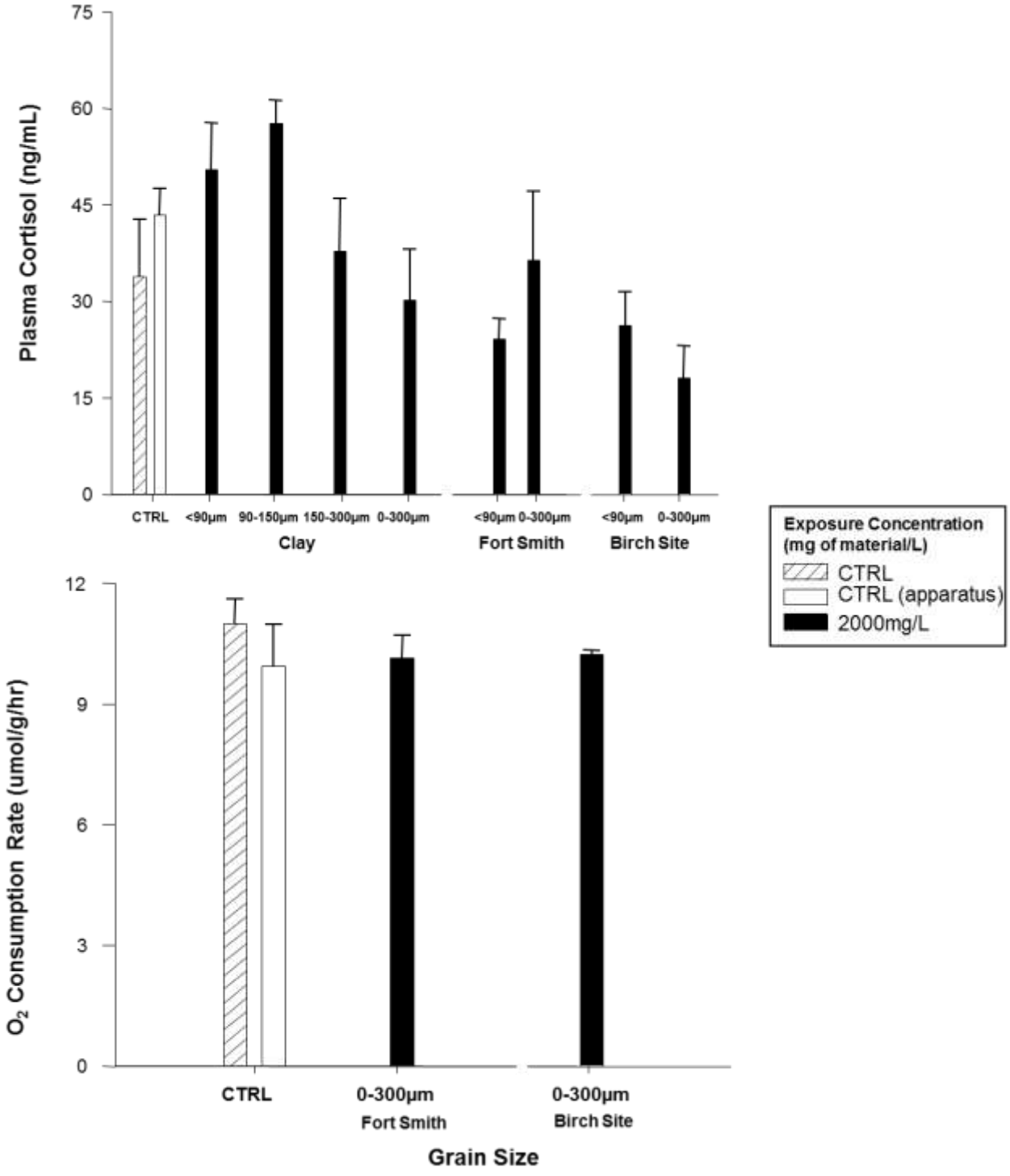
Plasma Cortisol (ng/mL)
75
60
45
30
15
0
CTRL
<90µm
90-150µm
150-300µm
0-300µm
Clay
<90µm
0-300µm
Fort Smith
<90µm
0-300µm
Birch Site
Exposure Concentration (mg of material/L)
CTRL
CTRL (apparatus)
2000mg/L
O2 Consumption Rate (umol/g/hr)
12
9
6
3
0
CTRL
0-300µm
Fort Smith
0-300µm
Birch Site
Grain Size

Figure 2.5: The Effect of 96h Exposure to Suspended Field Collected Material and Clay Particles of Differing Grain Sizes at a Concentration of 2000mg/L on Plasma Cortisol Concentration and Resting Metabolic Rate of Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

Plasma cortisol concentration and resting metabolic rate (as estimated by weight adjusted resting oxygen consumption rates) of juvenile rainbow trout (*Oncorhynchus mykiss)* after 96 hr exposure to suspended field collected material and clay particles of different grain sizes at a concentration of 2000mg/L. The hashed bar represents the unexposed control, the open bar represents the unexposed control with the exposure apparatus present, and the shaded bar represent the treatment concentration. The two controls were not significantly different form one another and all treatments were compared to the control that included the exposure apparatus. None of the material sources were shown to cause significant differences between the control and treatment group, this was the case for both plasma cortisol concentration and resting metabolic rate (Dunnett's test, $p \leq 0.05$).

Figure 2.6:

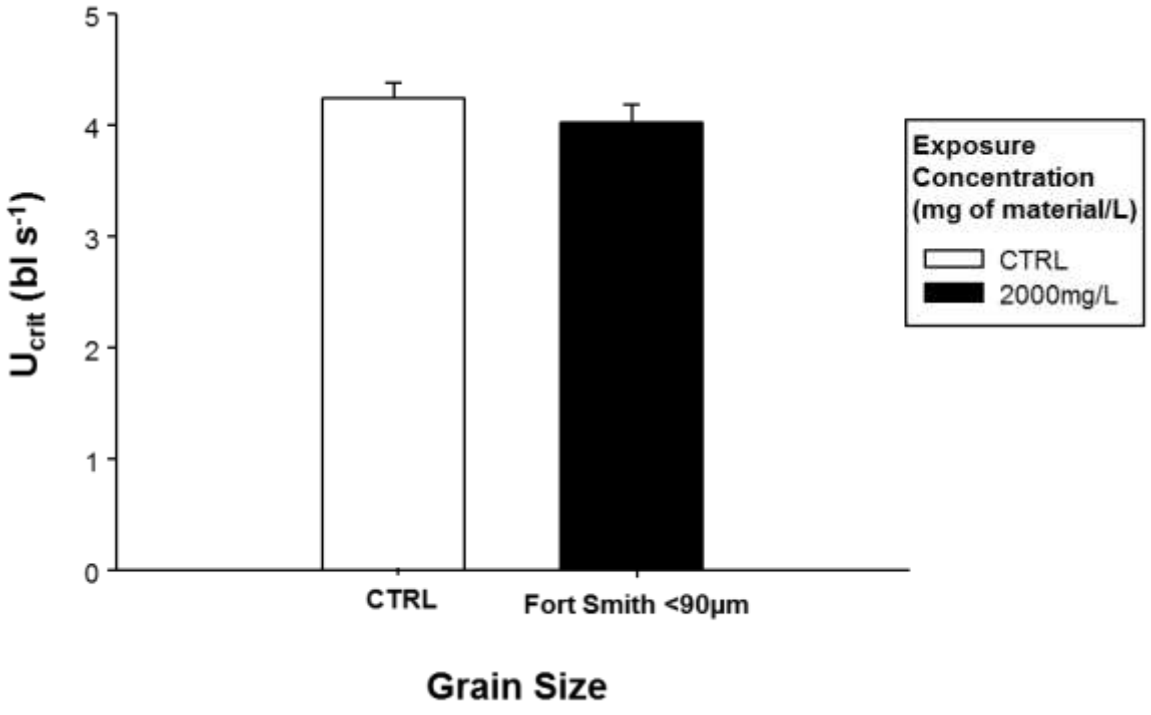
5
4
3
2
1
0
U_{crit} (bl s^{-1})
CTRL
Fort Smith <90µm
Grain Size
Exposure Concentration (mg of material/L)
CTRL
2000mg/L

Figure 2.6: The Effect of 96 h Exposure to Suspended Field Collected Material of Grain size <90um at a concentration of 2000mg/L on Swim performance of Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

Swim Performance (U_{crit}) after 96h exposure to Fort Smith field collected material of grain size <90um at a concentration of 2000mg/L (mean±SEM, n=6). The open bar represents the unexposed control and the shaded bar represent the U_{crit} after treatment. No significant difference between control and treatment group was present (Dunnett's test, $p \leq 0.05$).

Figure 2.7

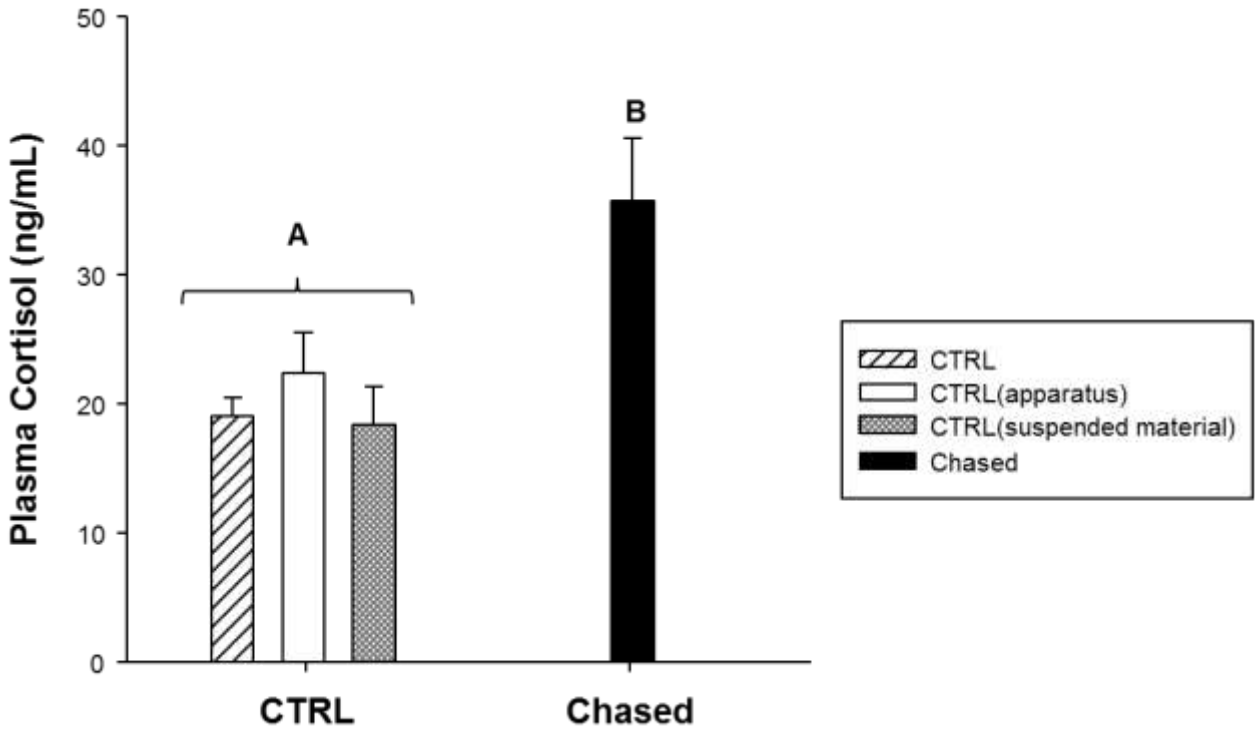
Plasma Cortisol (ng/mL)
50
40
30
20
10
0
A
B
CTRL
Chased
CTRL
CTRL(apparatus)
CTRL(suspended material)
Chased

Figure 2.7: The Effect of Chasing Juvenile Rainbow Trout (*Oncorhynchus mykiss)* after 96h Exposure to Suspended Field Collected Material of Grain size <90um at a concentration of 2000mg/L on Plasma Cortisol.

Plasma Cortisol (ng/mL) after 96h exposure to Fort Smith field collected material of grain size <90um at a concentration of 2000mg/L (mean±SEM, n=7). The hashed bar represents the unexposed and un-chased control; the open bar represents the unexposed and un-chased control with the exposure apparatus present, the dotted bar represents the control group exposed to suspended material but not chased and the shaded bar represent the group exposed to suspended material and chased after exposure. The three controls were not significantly different form one another (represented by **A,** Dunnett's test, $p \leq 0.05$) but the chased group was significantly different from all three of the un-chased controls (represented by **B,** Dunnett's test, $p \leq 0.05$).

Figure 2.8

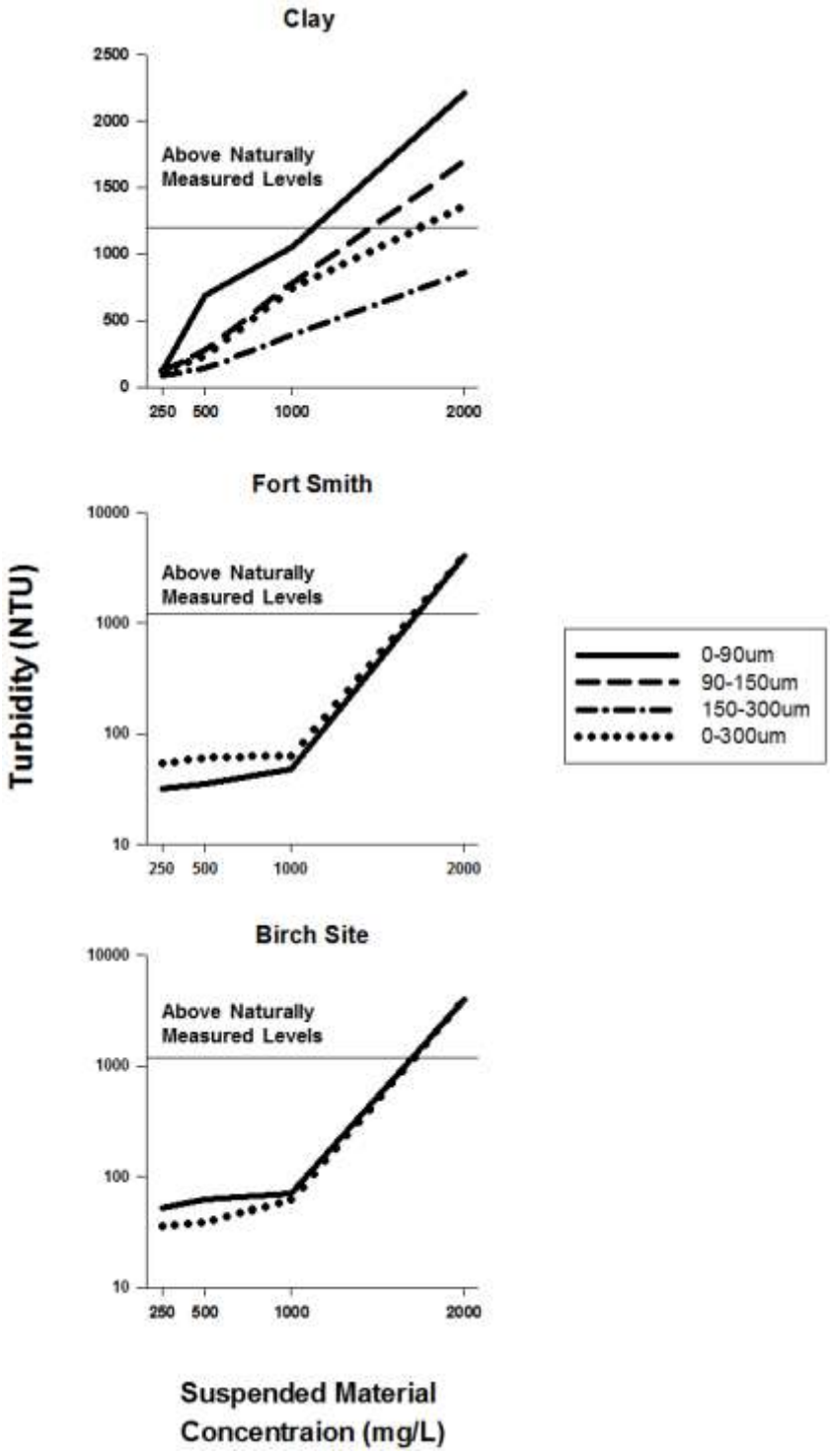
Clay
2500
2000
1500
1000
500
0
Above Naturally Measured Levels
250
500
1000
2000
Fort Smith
10000
1000
100
10
Above Naturally Measured Levels
250
500
1000
2000
Birch Site
10000
1000
100
10
Above Naturally Measured Levels
250
500
1000
2000
Turbidity (NTU)
0-90um
90-150um
150-300um
0-300um
Suspended Material Concentraion (mg/L)

Figure 2.8: Relationship between turbidity and exposure concentration

Measured turbidity values for each exposure concentration, each plot represents the different exposure material used and is broken down by grain size. The horizontal line on each plot represents the maximum turbidity measured downstream of active thermokarst slump sites on the Peel River Plateau.

2.7 Tables

Table 2.1: Mean Exposure Water Quality Parameters Across all Exposures

	Test Parameter			
	Temp (°C)	**Conductivity (**uS)	**Dissolved Oxygen (%)**	**pH**
Mean	14.97	266.6	96.34	8.02
Standard Error	0.018	1.85	3.55	0.012

Table 2.2: Breakdown of Exposure Materials by Grain Size

Material	**Grain Size Designation**		
	Small (<90 µm)	**Medium (90-150 µm)**	**Large (150-300 µm)**
Clay	36.2%	33.9%	29.9%
Birch Site	83.03%	12.36%	4.61%
Fort Smith	62.69%	19.16%	18.15%

Table 2.3: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Calcium Concentration in mmol/L. A * Denotes where a significant difference is present between treatment and the control (Dunnett's, P≤0.05).

Exposure Material	**Concentration (mg/L)**											
Clay	**Ctrl**		**Ctrl (apparatus)**		**250**		**500**		**1000**		**2000**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	2.31	±0.25	2.44	±0.29	2.52	±0.20	2.86	±0.04	2.70	±0.04	3.95*	±0.42
90-150µm	2.31	±0.25	2.44	±0.29	2.61	±0.12	2.32	±0.23	2.72	±0.07	3.87*	±0.30
150-300µm	2.31	±0.25	2.44	±0.29	2.59	±0.15	2.75	±0.20	2.69	±0.22	4.12*	±0.08

Table 2.4: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Chloride Concentration in mmol/L. A * Denotes where a significant difference is present between treatment and the control (Dunnett's, P≤0.05).

Exposure Material	**Concentration (mg/L)**											
Clay	**Ctrl**		**Ctrl (apparatus)**		**250**		**500**		**1000**		**2000**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	131	±1.49	131	±3.36	130.79	±2.41	133.93	±1.49	133.29	±1.43	117.33*	±2.23
90-150µm	131	±1.49	131	±3.36	129.79	±1.04	123.43	±4.26	126.36	±2.09	114.83*	±1.05
150-300µm	131	±1.49	131	±3.36	127.14	±2.03	129.57	±0.69	124.36	±5.67	114.80*	±2.40

Table 2.5: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Sodium Concentration mmol/L. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration (mg/L)											
Clay	Ctrl		Ctrl (apparatus)		250		500		1000		2000	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	166.36	±4.16	143.50	±19.32	162.34	±3.36	166.43	±2.96	159.04	±2.17	172.05	±6.48
90-150µm	166.36	±4.16	143.50	±19.32	183.01	±19.51	153.26	±9.64	165.53	±6.04	164.98	±7.60
150-300µm	166.36	±4.16	143.50	±19.32	165.09	±2.54	163.68	±3.15	164.53	±4.19	163.38	±2.09

Table 2.6: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Cotrisol Concentration in ng/mL. A * Denotes where a significant difference is present between treatment and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration (mg/L)											
Clay	Ctrl		Ctrl (apparatus)		250		500		1000		2000	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	20.01	±3.79	33.71	±10.02	24.94	±5.37	61.22	±14.02	46.17	±7.47	87.80*	±15.34
90-150µm	20.01	±3.79	33.71	±10.02	45.53	±16.90	29.63	±7.30	31.48	±5.08	95.57*	±19.32
150-300µm	20.01	±3.79	33.71	±10.02	23.66	±8.63	27.43	±7.08	47.76	±12.02	107.16*	±27.14

Table 2.7: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay of Differing Grain Sizes on Mean (±SEM, n=7) Resting Metabolic Rate in µmol/g/h. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration (mg/L)											
Clay	Ctrl		Ctrl (apparatus)		250		500		1000		2000	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	10.99	±0.62	11.05	±0.85	11.59	±2.35	10.29	±0.62	10.68	±0.68	14.19	±1.57
90-150µm	10.99	±0.62	11.05	±0.85	12.98	±0.85	11.50	±0.85	11.64	±0.58	12.51	±2.86
150-300µm	10.99	±0.62	11.05	±0.85	15.34	±4.96	13.06	±2.90	9.71	±1.08	10.44	±0.78

Table 2.8: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay and Field Collected Material of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Calcium Concentration in mmol/L. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration					
Clay	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	4.22	±0.28	3.32	±0.30	3.03	± 0.08
90-150µm	4.22	±0.28	3.32	±0.30	2.84	±0.09
150-300µm	4.22	±0.28	3.32	±0.30	2.93	±0.07
0-300µm	4.22	±0.28	3.32	±0.30	3.04	±0.11
Fort Smith	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	4.22	±0.28	3.32	±0.30	3.74	±0.13
0-300µm	4.22	±0.28	3.32	±0.30	4.18	±0.26
Birch Site	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	4.22	±0.28	3.32	±0.30	3.40	±0.15
0-300µm	4.22	±0.28	3.32	±0.30	4.15	±0.22

Table 2.9: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay and Field Collected Material of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Chloride Concentration in mmol/L. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration					
Clay	**Ctrl**		**Ctrl**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	128	±1.13	124.43	±2.75	121.14	±3.41
90-150µm	128	±1.13	124.43	±2.75	119.67	±2.51
150-300µm	128	±1.13	124.42	±2.75	124.29	±0.61
0-300µm	128	±1.13	124.43	±2.75	119.71	±1.29
Fort Smith	**Ctrl**		**Ctrl**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	128	±1.13	124.43	±2.75	118.7143	±1.11
0-300µm	128	±1.13	124.43	±2.75	121.143	±1.79
Birch Site	**Ctrl**		**Ctrl**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	128	±1.13	124.43	±2.75	119.43	±1.72
0-300µm	128	±1.13	124.43	±2.75	122.29	±3.11

Table 2.10: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay and Field Collected Material of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Sodium Concentration in mmol/L. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration					
Clay	Ctrl		Ctrl		2000(mg/L)	
	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	160.35	±10.87	154.18	±19.25	156.10	±16.95
90-150µm	160.35	±10.87	154.18	±19.25	141.73	±7.93
150-300µm	160.35	±10.87	154.18	±19.25	144.59	±16.63
0-300µm	160.35	±10.87	154.18	±19.25	158.37	±9.44
Fort Smith	Ctrl		Ctrl		2000(mg/L)	
	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	160.35	±10.87	154.18	±19.25	165.55	±13.64
0-300µm	160.35	±10.87	154.18	±19.25	143.03	±2.30
Birch Site	Ctrl		Ctrl		2000(mg/L)	
	Mean	SEM	Mean	SEM	Mean	SEM
<90µm	160.35	±10.87	154.18	±19.25	163.13	±13.12
0-300µm	160.35	±10.87	154.18	±19.25	139.93	±3.36

Table 2.11: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Field Collected Material of Differing Grain Sizes on Mean (±SEM, n=7) Resting Metabolic Rate in Rate in µmol/g/h. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration (mg/L)					
Fort Smith	Ctrl		Ctrl		2000	
	Mean	SEM	Mean	SEM	Mean	SEM
0-300µm	10.99	±0.62	9.95	±1.02	10.15	±0.56
Birch Site	Ctrl		Ctrl		2000	
	Mean	SEM	Mean	SEM	Mean	SEM
0-300µm	10.99	±0.62	9.95	±1.02	10.22	±0.11

Table 2.12: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Clay and Field Collected Material of Differing Grain Sizes on Mean (±SEM, n=7) Plasma Cortisol Concentration in ng/mL. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05)

Exposure Material	Concentration					
Clay	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	33.87	±8.84	43.33	±4.17	50.38	±7.32
90-150µm	33.87	±8.84	43.33	±4.17	57.61	±3.62
150-300µm	33.87	±8.84	43.33	±4.17	37.82	±8.11
0-300µm	33.87	±8.84	43.33	±4.17	30.12	±7.99
Fort Smith	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	33.87	±8.84	43.33	±4.17	24.12	±3.19
0-300µm	33.87	±8.84	43.33	±4.17	36.42	±10.67
Birch Site	**Ctrl**		**Ctrl (apparatus)**		**2000(mg/L)**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
<90µm	33.87	±8.84	43.33	±4.17	26.28	±5.17
0-300µm	33.87	±8.84	43.33	±4.17	18.14	±4.95

Table 2.13: The effect of a 96h exposure of Rainbow Trout (*Oncorhynchus mykiss)* to Suspended Field Collected Material on Mean (±SEM, n=6) Swim Performance in BL/s. No significant differences were present between any treatment groups and the control (Dunnett's, P≤0.05).

Exposure Material	Concentration (mg/L)			
Fort Smith	**Ctrl**		**2000**	
<90µm	4.24	±0.14	4.02	±0.16

Table 2.14: Corresponding Measured Turbidity Values in NTU for Each Exposure Grain Size and Concentration.

Exposure Material	Concentration (mg/L)			
Clay	**250**	**500**	**1000**	**2000**
<90µm	127	688	1050	2213
90-150µm	124	136	778	1696
150-300µm	85	141	142	861
0-300µm	122	151	740	1358
Fort Smith	**250**	**500**	**1000**	**2000**
<90µm	34	36	48	4000
0-300µm	61	54	64	4000
Birch Site	**250**	**500**	**1000**	**2000**
<90µm	53	62	70	4000
0-300µm	36	39	62	4000

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Chapter 3
Integrative Aspects

Integrative Aspects

My project is one of an integrative nature because has been established that the climate is going to continue changing, and it is past the point where we can reverse the changes that have occurred. This means that we need to develop strategies to adapt to the change instead of trying to reverse it. If we want to develop effective strategies we need to study our changing environment and that is exactly what is being done by my project through the Cumulative Impact Monitoring Program (CIMP) from Aboriginal Affairs and Northern Development Canada.

The overall goal of the program that my project was part of is to investigate impacts of mega-slump disturbances on terrestrial and aquatic ecosystems in the lower Peel watershed of the North West Territories. The project involves collaboration between thirteen teams from different universities, labs, departments, aboriginal and community groups, as well as government departments, institutes and agencies. The specific things being done are:

- The tracking of changes in large scale landscape disturbances; studying the impacts of natural and anthropogenic disturbances on the physical, chemical, and biological characteristics and ecology of rivers and streams;
- Studying impacts on aquatic systems by assembling geospatial data from disturbance layers and portraying this data on a watershed basis
- Studying the impacts of the increased suspended material to determine the disturbance thresholds related to the health of local fish
- Assembling a community based monitoring program involving local communities and utilizing traditional knowledge to describe the changing conditions of the area

The knowledge gained from this study will be combined with understanding and data from other facets of scientific investigation including ecology, geomorphology and environmental science to develop a broad understanding of how mega-slumps, increased suspended material concentrations, and warming are affecting the environment in the Peel River Plateau and arctic. The understanding gained from these projects will allow us to develop adaptive strategies for climate change so we can preserve our northern ecosystems. For example:

- Baseline data has been compiled through the tracking of changes and collection of geospatial measurements which will allow us to measure the occurring changes as well as understand where the slumps are occurring and hopefully allow us to better predict where they may occur.
- New techniques to track and determine the extent of impacts to stream health as a result of permafrost degradation have been developed. These techniques are helping us understand the full effect the changing environment is having on overall stream health and therefore take the proper measures to conserve and manage the remaining streams and rivers.
- The creation of maps showing slump disturbances on a watershed basis has provided important information for fisheries research, allowing areas of high vulnerability for fish to be identified.
- Community groups have been observing and recording changes to local habitats and species to help identify local changes which are occurring.
- The study of the relationship between water quality and stream health along with the understanding gained from my study will allow for the establishment of water quality thresholds to begin.

The techniques we developed in the lab to measure the effects of suspended material on fish can be adapted for field use allowing them on native fish species, as well as effective tools for risk assessment and characterization. By integrating the knowledge and understanding that comes from my project as well as the projects of my collaborators we will hopefully be able to produce adaptive strategies to help the Peel Region and other northern areas to deal with the changes occurring.