

Natural and Anthropogenic Factors Influencing the Pre-spawn Migration of  
Steelhead Trout

by

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## **Abstract**

Migration can result in considerable exposure to both natural and anthropogenic stressors that can impact the ability of an anadromous fish to complete this critical component of their life-history. In this thesis I use a range of physiological and behavioural measures to evaluate natural and anthropogenic factors influencing the migration of wild adult migrating steelhead in the Bulkley River, British Columbia, including pathogens and fisheries captures. Steelhead appeared to be relatively resilient to pathogens and capture within fisheries, though I observed changes to their physiology, behaviour, and survival in relation to these stressors. A key finding throughout this thesis work was the influence that water temperature had on steelhead physiology and behaviour, suggesting steelhead may be vulnerable to increases in water temperature with climate change. This work provided novel insight on the pathogens influencing wild steelhead and has provided mortality estimates that fisheries managers can incorporate into their management models.

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## **Preface**

Data in this thesis was collected under the guidelines set out by the scientific fish collection permits provided by the Government of British Columbia and in accordance with the guidelines of the Canadian Council on Animal Care administered by the Carleton University Animal Care Committee (BIO-12).

## **Co-Authorship**

### **Chapter 2: The influence of temperature, sampling location, sex, and size on disease ecology and physiology of migrating adult wild steelhead.**

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Twardek, Cooke, Beere, and Danylchuk designed the study. Twardek and Elmer completed the field work, while Twardek and Chapman completed the laboratory work. Equipment was provided by Miller, Danylchuk, and Cooke. Twardek analyzed the data and wrote the manuscript. All co-authors reviewed and provided feedback on the manuscript.

### **Chapter 3: Consequences of catch-and-release angling on the physiology, behaviour and survival of wild steelhead *Oncorhynchus mykiss* in the Bulkley River, British Columbia.**

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Twardek, Cooke, Beere, and Danylchuk designed the study. Twardek, Gagne, Elmer, Beere, and Danylchuk contributed to field work. Equipment was provided by Cooke, Danylchuk, and Beere. Twardek analyzed the data and wrote the manuscript. All co-authors reviewed and provided feedback on the manuscript.

#### **Chapter 4: Consequences of fisheries gear type and handling practices on capture-and-release of wild steelhead on the Bulkley River.**

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Twardek, Cooke, Beere, and Danylchuk designed the study. Twardek and Elmer completed the field work. Equipment was provided by Cooke, Beere, and Danylchuk. Twardek analyzed the data and wrote the manuscript. All co-authors reviewed and provided feedback on the manuscript.

## Contents

<b>Abstract.....</b>	<b>ii</b>
<b>Preface.....</b>	<b>v</b>
<b>Co-Authorship.....</b>	<b>v</b>
<b>List of Tables .....</b>	<b>x</b>
<b>List of Figures.....</b>	<b>xi</b>
<b>Chapter 1. General Introduction.....</b>	<b>1</b>
1.1 Migration.....	1
1.2 Natural stressors .....	2
1.3 Capture-and-release from fisheries .....	3
1.4 Consequences of capture-and-release .....	5
1.5 Evaluating capture-and-release in fisheries.....	7
1.6 Steelhead .....	8
1.7 Objectives.....	10
<b>Chapter 2. The influence of temperature, sampling location, sex, and size on disease ecology and physiology of migrating adult wild steelhead .....</b>	<b>14</b>
2.1 Abstract .....	14
2.2 Introduction .....	15
2.3 Methods.....	19
2.3.1 <i>Study site and collection</i> .....	19
2.3.2 <i>Sample analyses</i> .....	20
2.3.3 <i>Statistical analyses</i> .....	23
2.4 Results .....	43
2.4.1 <i>Microbe productivity</i> .....	43
2.4.2 <i>Host-gene expression</i> .....	44
2.5 Discussion .....	48
2.5.1 <i>Bacterial pathogens Ca. B. cysticola and F. psychrophilum</i> .....	48
2.5.2 <i>Sphaerothecum destruens and low prevalence microbes</i> .....	50
2.5.3 <i>Temperature and host-physiology</i> .....	52
2.5.4 <i>Sampling location</i> .....	54
2.5.5 <i>Sex and size</i> .....	57
2.5.6 <i>Conclusions</i> .....	59

<b>Chapter 3. Consequences of catch-and-release angling on the physiology, behaviour and survival of wild steelhead <i>Oncorhynchus mykiss</i> in the Bulkley River, British Columbia.....</b>	<b>61</b>
3.1 Abstract .....	61
3.2 Introduction .....	62
3.3 Methods.....	65
3.3.1 <i>Study site and collection methods</i> .....	65
3.3.2 <i>Quantification of the angling event</i> .....	66
3.3.3 <i>Reflex test</i> .....	67
3.3.4 <i>Post-release physiology</i> .....	67
3.3.5 <i>Post-release movement</i> .....	69
3.3.6 <i>Data analysis</i> .....	71
3.4 Results .....	74
3.4.1 <i>Righting reflex</i> .....	74
3.4.2 <i>Physiology</i> .....	75
3.4.3 <i>Post-release movement</i> .....	76
3.4.4 <i>Survival</i> .....	77
3.5 Discussion .....	87
3.5.1 <i>Angling-related factors</i> .....	87
3.5.2 <i>Environmental factors</i> .....	90
3.5.3 <i>Intrinsic biotic factors</i> .....	92
3.5.4 <i>Survival</i> .....	93
3.5.5 <i>Conclusions</i> .....	96
<b>Chapter 4. Consequences of fisheries gear type and handling practices on capture-and-release of wild steelhead on the Bulkley River .....</b>	<b>98</b>
4.1 Abstract .....	98
4.2 Introduction .....	99
4.3 Methods.....	102
4.3.1 <i>Study site</i> .....	102
4.3.2 <i>Capture methods</i> .....	103
4.3.3 <i>Quantification of the capture event</i> .....	105
4.3.4 <i>Post-release movement</i> .....	106
4.3.5 <i>Data analysis</i> .....	108

4.4 Results .....	110
4.4.1 Capture conditions .....	110
4.4.2 Injury.....	111
4.4.3 Righting reflex .....	112
4.4.4 Behaviour.....	112
4.4.5 Survival.....	114
4.5 Discussion .....	119
4.5.1 Fishery-specific differences .....	119
4.5.2 Capture-related variables.....	121
4.5.3 Environmental variables.....	123
4.5.4 Intrinsic biotic factors .....	125
4.5.5 Survival.....	126
4.5.6 Conclusions .....	128
<b>Chapter 5. General Conclusion .....</b>	<b>130</b>
<i>Future Research Directions</i> .....	135
<i>Final Remarks</i> .....	137
<b>References .....</b>	<b>139</b>

## List of Tables

<b>Table 2.1.</b> The prevalence of 47 pathogens in adult Bulkley River steelhead and their corresponding primer/probe sequences and efficiencies.....	25
<b>Table 2.2.</b> The biomarker assays and three reference genes evaluated by qPCR on adult Bulkley River steelhead and their corresponding primer/probe sequences and efficiencies.....	33
<b>Table 2.3.</b> Statistical outputs for multiple regression models predicting the relative load of <i>Ca. B. cysticola</i> and <i>F. psychrophilum</i> in the gill tissue of steelhead using water temperature, sampling location, sex, and size as predictor variables.....	45
<b>Table 2.4.</b> Statistical outputs for multiple regression models predicting microbe richness in the gill tissue of steelhead using water temperature, sampling location, sex, and size as predictor variables.....	45
<b>Table 3.1.</b> Statistical output for logistic regression predicting presence of the righting reflex after angling using variables measured during the capture event.....	78
<b>Table 3.2.</b> Statistical outputs for multiple regression models predicting blood physiology parameters after angling using variables measured during the capture event.....	79
<b>Table 3.3.</b> Statistical outputs for multiple regression models predicting post-release movement using variables measured during the capture event.....	80
<b>Table 4.1.</b> Statistical outputs for logistic regression models predicting intermediate-term (2-week) migration rates in steelhead after a capture event.....	115
<b>Table 4.2.</b> Statistical outputs for logistic regression models predicting peak migration rates in steelhead after a capture event.....	116

## List of Figures

<b>Figure 1.1.</b> A map of the Skeena watershed.....	12
<b>Figure 1.2.</b> Photographs showing steelhead capture by the three gear types employed on the Bulkley River, B.C.....	13
<b>Figure 2.1.</b> Differences in the relative load of: A) <i>Ca. B. cysticola</i> ; B) <i>F. psychrophilum</i> , and C) microbe richness in the gill tissue of steelhead across sampling locations.....	46
<b>Figure 2.2.</b> An NMDS plot depicting the relationships between steelhead gene expression, microbes, and extrinsic variables.....	47
<b>Figure 3.1.</b> The relationship between presence of the righting reflex and air exposure duration.....	81
<b>Figure 3.2.</b> The relationship between blood glucose level and: A) landing method; B) sex .....	82
<b>Figure 3.3.</b> The relationship between blood physiology measures and water temperature at the time of capture.....	83
<b>Figure 3.4.</b> The relationship between blood physiology measures and air exposure duration.....	84
<b>Figure 3.5.</b> The relationship between immediate post-release movement and air exposure duration .....	85
<b>Figure 3.6.</b> The relationship between 2-week relative positions and air exposure duration .....	86
<b>Figure 3.7.</b> The relationship between average daily post-release movement rate and: A) average daily water temperature; B) average daily discharge of the river.....	87
<b>Figure 4.1.</b> Differences in A) injury rates B) presence of the righting reflex C, D) migration rates across capture method.....	117
<b>Figure 4.2.</b> The relationships between migration rates and: A,C) water temperature; B,D) air exposure at the time of capture.....	118

**Figure 5.1.** The estimated population level mortality rates attributable to the recreational fishery from 1999-2015.....138

# Chapter 1. General Introduction

## 1.1 Migration

Migration is a life-history strategy that serves to benefit the individual by providing a novel environment with additional access to resources for growth or maintenance, to avoid unfavourable environmental conditions, or to facilitate reproduction (Heape, 1931). Migration is only a viable strategy if it provides fitness advantages relative to residency (Shaw and Couzin, 2013). Many species exhibit partial migration where populations are comprised of both resident and migratory individuals, highlighting that there are both costs and benefits to migration (Chapman et al., 2011; McPhee et al., 2007). Migration can be an inherently difficult period in the life of an individual (Lennox et al., 2016) as it often entails long distance movement (Wikelski et al., 2003) and physiological changes to adapt to a novel environment (Cooke et al., 2011), both of which can be energetically costly. This is further exacerbated by human threats that result in increased costs to migration including habitat destruction, barriers to movement, climate change, and overexploitation (Wilcove and Wikelski, 2008). These threats are common across all animal taxa, including the ~2.5% of fish species that undertake migration (Binder et al., 2011).

Fish migrations encompass a broad range of behaviours from the diel migrations of planktivorous fish larvae (Picapedra et al., 2015), to the transoceanic migrations of whale sharks (Hueter et al., 2013). One of the most frequently discussed examples of fish migration is anadromy, which is common in sturgeons, lampreys, osmerids, shad, and salmonids. Anadromous fishes complete the juvenile stages of their life in freshwater before entering marine environments where resources are plentiful and may facilitate

greater growth (Gross et al., 1988). Adult fish return to natal streams to spawn with presumably higher fitness than they would have, had they remained in freshwater (Kendall et al., 2015). During the migratory component of anadromy, species may travel several hundred kilometers inland (Quinn, 2011), resulting in increased exposure to both natural and anthropogenic stressors that are decreasing the value of anadromy (Thériault et al., 2008; Benjamin et al., 2013).

## **1.2 Natural stressors**

There are many natural threats that can impact fish throughout their lives, including those that undertake migration. Natural threats may be spontaneous such as the case with natural disasters and drastic environmental changes. These events can correspond to widespread mortality (Brown et al., 1983; Staudigel et al., 2006), and ecosystem perturbations that may take years to recover (Ruuhijärvi et al., 2010). However, most natural mortality is a result of both energetic and behavioural trade-offs throughout a fish's life that alter a fish's susceptibility to predation (Jørgensen et al., 2013). Maintaining an immune response to disease-causing pathogens is one such energetic trade-off where affected individuals can suffer reduced fitness (Hedrick et al., 1998).

Fish pathogens that cause disease span numerous taxa from bacterium, fungi, protozoa, metazoa, and viruses. Disease is a threat to all fish and is particularly important for fish in freshwater ecosystems where pathogens may thrive compared to marine environments (Johnson and Paull, 2011). Many cases exist where disease outbreaks have resulted in severe fish kills that impact several species (Bowater et al., 2012). However, other sublethal impacts of pathogens on wild fish also exist that may impact fitness.

Parasites for example, may alter fish behavior to increase the likelihood of predation and therefore transmission to the successive host (Crowden and Broom, 1980). Parasites have also been linked to indices of osmoregulatory and metabolic stress (Bradford et al., 2010), and can lead to changes in host prey selection, where hosts thereby prefer less profitable prey (Milinski, 1984). Ultimately these changes may result in long-term impacts such as decreases to growth (Latour et al., 2012). Although it is important to account for the consequences of natural stressors on wild fish, it is typically anthropogenic activities that can most easily be changed by humans to influence the population. Fishing is one of the most widespread anthropogenic threats to fishes, but is particularly relevant for anadromous fishes that have remained highly coveted to fishers throughout human history (Healey et al., 2009; Yu and Cook, 2015).

### **1.3 Capture-and-release from fisheries**

Fishing is an important part of human society that serves many purposes for the people that engage in the activity (Lynch et al., 2016). Fisheries can be divided into three distinct segments known as the commercial, recreational, and subsistence sectors. The commercial fishing sector operates at a large spatial scale and has a role in supporting regional economies while providing food security. Recreational fisheries are often less harvest-oriented than commercial fisheries (Pitcher and Hollingworth, 2002) though the economic benefits can be even greater than that of the commercial industry (FAO, 2012). Fishing with hooks (angling) has been an important part of human culture for at least 50,000 years, primarily as a means for people to gather food (Sahrang and Lundbeck, 1992). Angling has continued to provide social, cultural, and economic benefits to people in modern society (Lynch et al., 2016; Cooke et al., 2017), with American anglers

estimated to spend up to \$48 billion USD per year on recreational fishing (2011 estimate; American Sportfishing Association, 2013). Recreational fisheries are also expanding into remote parts of the world providing ecologically sustainable income sources to developing countries (Gupta et al., 2015). In contrast to recreational fishing that generally only supplements a fisher's diet, subsistence fishers rely on this activity as their primary source of protein. Subsistence fisheries are common in Indigenous cultures that have long-standing traditions of fishing for food (Thornton, 2001). Indigenous subsistence fisheries may employ several different fishing gears including spears, bow and arrow, various net designs, and hooks, among others. Although commercial fishing is most commonly identified as a factor contributing to declines in fish stocks (Cooke and Cowx, 2006), both recreational and subsistence fisheries can be highly consumptive and may result in the depletion of fish stocks (Post et al., 2002; Coleman et al., 2004; Albert et al., 2015), highlighting the need for effective management strategies.

One of the most common methods of resource management in fisheries is mandatory capture-and-release, with instances of this approach dating back to the middle ages (Policansky, 2002). For fish captured by hook-and-line this equates to the fish being retrieved, unhooked, and released. For fish captured in nets this equates to the fish being removed and released from the net back into the water. Capture-and-release may also occur as a voluntary action undertaken by the fisher because of their own conservation ethic, or because the catch is considered lower value. Capture-and-release is practiced across the world, with rates highly dependent on species (Policansky, 2002), and regional beliefs/policy (Arlinghaus et al., 2002; Arlinghaus et al., 2007). By the 1990's in the United States, the proportion of fish released following hook-and-line capture became

greater than that harvested (Bartholomew and Bohnsack, 2007). The presumption of releasing fish is that they will survive and suffer minimal fitness consequences from the capture event (Wydoski, 1977). Despite the many benefits of this strategy, even capture-and-release can have negative impacts on fish due to the multiple sources of stress imposed during a capture event (reviewed in Arlinghaus et al., 2007).

#### **1.4 Consequences of capture-and-release**

Capture by fisheries is considered one of the most severe acute stressors a fish may encounter during its life (Davis, 2002). Across all fisheries it is common for fish to be exercised, air exposed, and handled during a capture event (reviewed in Cooke et al., 2002; Arlinghaus et al., 2007 and Brownscombe et al., 2017). Attempting to swim free of a capture gear induces a ‘fight or flight’ response in fishes that may include primary to tertiary stress responses (Wedemeyer et al., 1990). The primary stress response associated with exercise includes the release of catecholamines and corticosteroids (Pickering, 1981). This release of hormones and neurotransmitters promotes the mobilization of glucose from the liver, into the blood, to be transported to target tissues as part of the secondary stress response (Barton and Iwama, 1991). As glucose and various amino acid stores are depleted by the muscles during anaerobic exercise, metabolic protons accumulate and are exported into the bloodstream with lactate (Milligan and Wood, 1986). The accumulation of metabolic protons in the blood leads to acidosis, which can result in mortality when severe (Wood et al., 1983). Recovery from this exercise includes returning homeostatic blood pH through increased oxygen consumption (Wood, 1991), and the restoration of muscle glycogen over the next several hours (Pagnotta and Milligan, 1991).

Following capture, fish are often handled and air exposed to facilitate removal from the capture gear (unhooking, unangling), or as part of an admiration period in the context of recreational fisheries (Lamansky and Meyer, 2016). When fish are lifted out of water their ability to transfer oxygen across the gills is impaired, extending anaerobic metabolism, and the accumulation of CO<sub>2</sub> in the bloodstream (Ferguson and Tufts, 1992; Cook et al., 2015). The physiological recovery from exercise, handling, and air exposure can result in tertiary stress responses to the individual. The tertiary stress response presents itself at the scale of the entire organism and may include decreased growth rates, inhibition of reproduction, reduced capacity to tolerate subsequent stressors, and behavioural impairment (Wendelaar Bonga, 1997). Although secondary stress responses will typically return back to normal within several hours of an angling event (Barton, 2002), tertiary stress responses such as swimming activity and habitat choice behaviour have been shown to extend for several months, particularly in the case of repeat captures (Klefoth et al., 2011). The stress response and recovery of fish is dependent on many factors including body size, temperature, nutritional status, and species (reviewed in Kieffer et al., 2000). In addition to physiological disturbance, fish may also incur direct physical damage during capture (Arlinghaus et al., 2007).

Hooking, entanglement, and handling, are all sources of injury in captured fish. Anatomical hooking damage to the gills, tongue, or esophagus can lead to substantial blood loss and is generally considered the greatest source of mortality in angled fish (Bartholomew and Bohnsack, 2005; Hühn and Arlinghaus, 2011). Hooking injuries appear to impact mortality rates relative to the type of lure/bait used (High and Meyer, 2014), and fishing method employed (Twardek et al., 2018), indicating that angling

practices can modulate the severity of these injuries. Both entanglement in nets and handling can disturb the mucous layer, damage fins, remove scales, and ultimately increase disease and pathogen susceptibility following release (Anderson, 1990). Nets made of polypropylene are typically considered most damaging, while nets made of rubber or rubberized nylon are least harmful (Barthel et al., 2003; Lizee et al., 2018). Further physical damage can result from environmental variables such as capture depth that can result in barotrauma during rapid ascension (Rummer and Bennett, 2005; Butcher et al., 2012). From a management perspective, it is important to account for the sub-lethal and lethal consequences of capture-and-release, as well as identify means to minimize these consequences on targeted fish species.

### **1.5 Evaluating capture-and-release in fisheries**

The diversity of physiological, ecological, behavioural, morphological, and life-history traits in fishes is immense (Helfman et al., 1997). Correspondingly, the ability for fish to survive from capture-and-release varies considerably (reviewed in Muoneke and Childress, 1994), so generalizing is difficult across species (Cooke and Suski, 2005). For instance, the freshwater residing largemouth bass has much lower muscle lactate after exercise than the saltwater Bonefish (*Albula vulpes*; Suski et al., 2007; White et al., 2008). Even species within the same genus may exhibit differences in hypoxia tolerance in the context of recreational fisheries (Furimsky et al., 2003). Intraspecific responses to capture-and-release may also differ between populations due to environmental parameters such as temperature (Gale et al., 2013). Studies on catch-and-release angling of Atlantic salmon show that salmon caught closer to their thermal maxima have more pronounced behavioural changes and greater mortality than salmon captured at lower temperatures

(Richard et al., 2014; Havn et al., 2015). Due to the varying sensitivities of species exposed to capture-and-release, it is suggested that species-specific protocols are developed (Cooke and Suski, 2005). Hundreds of species-specific studies have been completed, using a variety of methods including mark-recapture, holding studies, biotelemetry, and modeling (Gutowsky et al., 2015). These evaluations can be used to develop species-specific best practices, that can be employed by fishers to minimize their impacts on captured-and-released fish (Brownscombe et al., 2017). This was highlighted as one of the key principles in the government of Canada's selective fishing policy for commercial, Indigenous, and recreational fisheries in Canada (DFO, 2001).

## **1.6 Steelhead**

Steelhead are the anadromous form of rainbow trout (*Onchorynchus mykiss*) that have a natural distribution throughout the Pacific Ocean, as well as the rivers spanning the entire Pacific coast of North America and the Kamchatka Peninsula of Russia (Light et al., 1989). Steelhead have been introduced throughout the Great Lakes (Kelch et al., 2006), and many populations along the Pacific coast of North America have been supplemented by hatchery production (Tatara et al., 2017). Steelhead generally spend the first 3 to 4 years of their lives in freshwater, followed by 0 to 2 at sea, before returning to their natal stream to spawn as adults (Savvaitova et al., 2000). Unique to all other Pacific salmon species, steelhead may survive spawning and reproduce more than once in their lifetimes (Moore et al., 2014). During their freshwater spawning migrations, wild steelhead are threatened by warming temperatures, habitat degradation (Backman and Evans, 2002), hatchery operations (Katz et al., 2013), and fisheries captures (J.O. Thomas & Associates LTD., 2010) contributing to their declines across much of North America

(Good et al., 2005; Kendall et al., 2017; Smith et al., 2000). Despite their conservation status, steelhead are considered one of the most highly sought-after species to anglers around the world and the recreational fisheries they support contribute millions of dollars to local economies each year (Counterpoint Consulting, 2008). Since 1997, the B.C. Ministry of Forests, Lands, Natural Resource Operations, and Rural Development has implemented recreational fishing regulations that prohibit the harvest of wild steelhead in British Columbia, which are now common regulations amongst all states in the Pacific Northwest. In most of the rivers that wild steelhead inhabit (Fraser, Columbia-Snake, and Skeena watersheds) recreational and Indigenous subsistence fisheries coexist. The Indigenous fisheries typically target the semelparous Pacific salmon (coho, chinook, pink, chum, and sockeye salmon) for harvest, but incidentally capture steelhead as their migrations coincide temporally with the migration of all other Pacific salmon. Steelhead are less preferred than other Pacific salmon species, and are typically released when encountered in nets, aside from some ceremonial harvest (Levy and Parkinson, 2014).

The Bulkley River, British Columbia, is one such river that hosts relatively abundant wild steelhead runs (long-term mean 20,873 individuals/ year; Pers. Comm. Dean Peard, B.C. MFLNRORD), as well as an economically important recreational fishery for steelhead and an Indigenous salmon fishery. By the time steelhead reach the Bulkley River, they have already undergone physiological transformations to prepare for freshwater (Cooke et al., 2011), and have travelled over 250 rkm inland. Over the next several months, they face reduced feeding opportunities, changing water temperatures, and exposure to a suite of freshwater pathogens as they prepare for spawning. A substantial proportion of the stock is also captured by angling (59%), and Indigenous

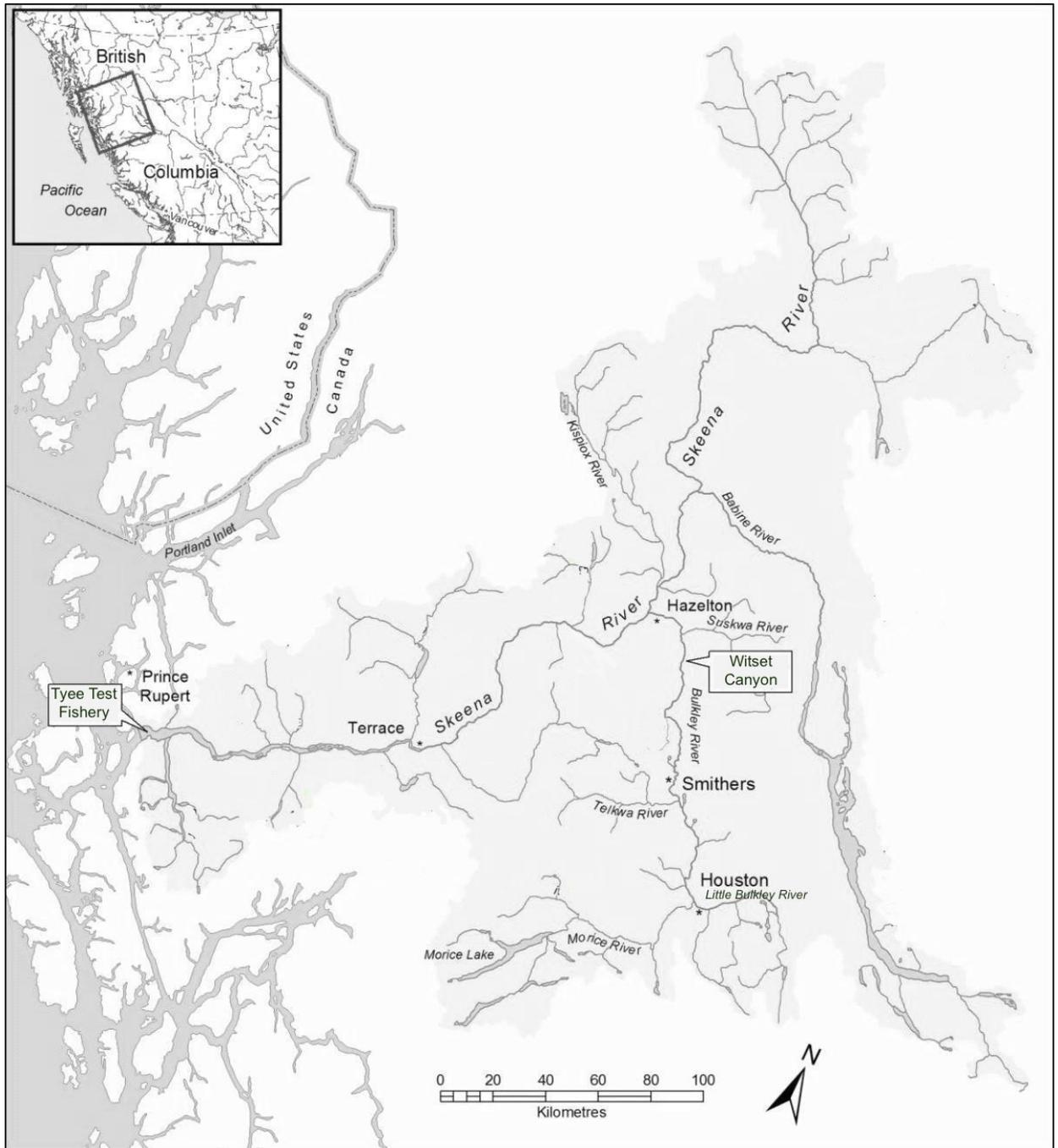
salmon fisheries (14%; MFLNRORD Data 1999-2017) that add further stress during migration. These factors make migration an inherently difficult period for steelhead, which is highlighted by the fact that in some populations individuals forego this migration and develop as the resident form of *O. mykiss* (Benjamin et al., 2013). Given that migration is declining in a rapidly changing world (Wilcove and Wikelski, 2008), it is of utmost importance that both the sub-lethal and lethal consequences of natural and anthropogenic threats are quantified, and when applicable, that science is used to mitigate these consequences on migratory populations and the fisheries they support (DFO, 2001).

## **1.7 Objectives**

The overall objective of this thesis was to evaluate the impacts of both natural and anthropogenic threats to migration in adult wild steelhead of the Bulkley River, British Columbia. Chapter 2 provides information on pathogens, a natural threat that has not previously been evaluated in wild adult steelhead. The goal of Chapter 2 was to quantify the presence and relative load of pathogens, and to link the relative load of each pathogen to steelhead physiology. I used high-throughput quantitative polymerase chain reactions to quantify the relative loads of pathogens and host gene expression in steelhead gill tissue. The subsequent chapters provide answers to specific management questions posed by stakeholders within the steelhead recreational fishery of the Bulkley River. Fishing guides were interested in understanding how various components of an angling event including their handling practices influence steelhead welfare. Further discussions with fisheries biologists from the Ministry of Forests, Lands, Natural Resource Operations, and Rural Development – Skeena Region indicated that mortality estimates were needed to account for losses in the recreational fishery, as well as the Indigenous fisheries that

operate on the Bulkley River. To address these questions, I worked with volunteer anglers, guides, and Wet'suwet'en fishers to sample fish using non-lethal blood sampling and radio-telemetry. The objective of Chapter 3 was to evaluate the physiology, behaviour, and survival of steelhead following catch-and-release angling, and to evaluate whether angling practices have an influence on these outcomes. The objective of Chapter 4 was to account for differences in the migratory behaviour, and survival of steelhead following capture-and-release by dip net, beach seine, and angling, and to assess whether capture-related variables have an influence on the outcome of a capture event. Overall, this thesis provides novel information on the pathogens present in wild adult steelhead and provides critical information needed for effective fisheries management. Results from the latter two data chapters are being used to develop species-specific recommendations for fishers to adhere to when encountering steelhead, and provide mortality estimates for provincial and state fisheries managers to incorporate into their steelhead management plans.

**Figure 1.1.** Map of the Skeena watershed highlighting the Skeena, Bulkley, and Morice Rivers, and their major tributaries. Adopted from [www.oceanecology.ca](http://www.oceanecology.ca).



**Figure 1.2.** Photographs showing steelhead capture by the three gear types employed on the Bulkley River, B.C. including (A) dip net (B) beach seine and (C) angling.



## **Chapter 2. The influence of temperature, sampling location, sex, and size on disease ecology and physiology of migrating adult wild steelhead**

### **2.1 Abstract**

Anadromous fishes such as steelhead trout are exposed to a suite of microbial pathogens during their freshwater migrations. I screened 40 adult steelhead from the Bulkley River for 45 distinct pathogens and evaluated the relationship between these pathogens and host-physiology. I also considered the role that water temperature, sampling location (above or at Witsset Falls), and sex had on pathogen loads, richness, and host gene expression groups. There were nine microbes detected in steelhead gill tissue, with high prevalence of *Candidatus Branchiomonus cysticola* (80%), *Flavobacterium psychrophilum* (95%), and *Sphaerothecum destruens* (52.5%). The relative load of *F. psychrophilum* was negatively correlated with genes related to humoral immunity suggesting the agent may have an immunosuppressive effect on this component of the immune response. Water temperature was positively correlated with genes responsive to oxygen availability and was associated with downregulation of most immune genes. Fish sampled by net at the falls had significantly greater relative loads of *Ca. B. cysticola* and *F. psychrophilum*, higher microbe richness, and differential gene expression compared to fish sampled upstream by angling. Neither sex nor the size of fish had strong relationships with host-microbes or physiology. This work highlights the sensitivity of steelhead to water temperature and presents unique differences in host-microbe dynamics across sampling locations.

## 2.2 Introduction

Pathogens are widespread in aquatic environments (Price, 1980), and play an integral role in shaping aquatic populations. Numerous pathogens can cause disease in fish, some of which have been studied for decades while others are newly emerging as disease threats (Crane and Hyatt, 2011). Fish have evolved immune mechanisms to protect against disease (Uribe et al., 2011), though ultimately there are fitness consequences associated with host-immune responses whether disease develops or not (Zuk and Stoehr, 2002). Pathogen-induced changes to physiological homeostasis (Binning et al., 2012; Miller et al., 2014; Teffer et al., 2017) result in energetic trade-offs with other demands such as metabolism or osmoregulation. These sublethal consequences may be particularly important for fish that undertake challenging, long-distance migrations. During migration, tissue energy stores tend to be depleted (e.g., Penney and Moffitt, 2014; Altizer et al., 2011) and fish often perform at levels that approach or exceed their critical swimming speeds and aerobic capacity (e.g., Hinch and Rand 1998; Burnett et al., 2014). Anthropogenic activities, infrastructure, and environmental change can further challenge migratory fish (see Lennox et al., 2016), for example, pushing fish species to the edge of their physiological limits in a warming world (Farrell et al., 2008; Crozier, 2016). Pathogen-induced disease has resulted in widespread declines in wild fish populations (Marty et al., 2003; Costello, 2009), and in some cases collapse (Pearson et al., 1999; Gibson-Reinemer et al., 2017). Consequently, understanding pathogen infection dynamics and host physiological response in wild fish populations is extremely important to provide context for monitoring any population scale changes.

Pathogen populations are dynamic and their prevalence among and within wild fish populations is highly dependent on both biotic and abiotic factors. Intrinsic biological differences related to life-history stage (Castro et al., 2015), size (Boerlage et al., 2011; Molina-Fernandez et al., 2015), age (Marty et al., 2003), condition (Rohlenova et al., 2011), and sex (Richards et al., 2010; Teffer et al., 2017) of the host species may alter individual susceptibility to infection. For example, IHN virus causes significant mortalities in juvenile salmonids, however is relatively non-pathogenic in adult fish (Traxler et al., 1997). Further, sexually mature male brown trout (*Salmo trutta*) demonstrated greater susceptibility to the trematode *Discocotyle sagitta* than females (Paling, 1965). Environmental factors can affect the physiology of both microbes and fish (Marcos-Lopez et al., 2010) and ultimately alter a fish's exposure to and susceptibility to disease-causing organisms (Wedemeyer, 1970; Rottman et al., 1992; Sepahi et al., 2013).

Water temperature is known to be a critical determinant of microbe-fish host infection dynamics given its governing role on fish physiology (Brett, 1971) and microbial proliferation and richness (Kocan et al., 2009; Thoen et al., 2016; Purcell et al., 2016). When approaching the upper limits of a species temperature tolerance, fish may exhibit temperature-induced suppression of immune function (Hardie et al., 1994; Magnadottir et al., 1999) leading to increased incidence of infection and consequent disease. While the fish host may be experiencing immunosuppression, some microbe species are known to proliferate and distribute more readily in warmer temperatures, creating a scenario where individuals vulnerable to infection are faced with increased pathogen encounters. Understanding the factors that contribute to pathogen prevalence and host-physiology is particularly important for anadromous fish species that experience

a suite of physiological changes and novel pathogens upon returning to freshwater to complete their reproductive cycle (Cooke et al., 2011; Miller et al., 2014).

Migratory pacific salmonid species typically enter rivers in large numbers to spawn after completing the marine portion of their lifecycle (Stearns, 2003). This influx of fish contributes greatly to human culture and economy, with salmon fisheries considered to be the most valuable in the Pacific Northwest (NRC, 1996; Lynch et al., 2016). As a result, salmon stocks have been subject to extensive management, monitoring, and research to understand widespread declines (Miller et al., 2014). Research has highlighted many sources of pre-spawn mortality, though warming water temperatures are considered one of the main factors contributing to declines (Hinch et al., 2012; Bowerman et al., 2018). Warm water temperature can increase the severity of other stressors such as fisheries capture (Teffer et al., 2017; Prystay et al., 2017) and pathogen dynamics (Hinch et al., 2012). Pathogens can have an influence on host stress, immunity (Miller et al., 2014; Teffer et al., 2017) and survival of wild migratory fish (Liu et al., 2011; Jeffries et al., 2014), though the study of pathogens in wild fish has been limited due to challenges associated with data collection and the spatial scale of these processes (Peeler and Taylor, 2011). Recent advances in molecular genetics (Lawson Handley, 2015) have allowed for rapid, and cost-effective quantification of gene expression, including the use of HT-qRT-PCR on the Biomark™ microfluidics platform that has recently been validated for salmon microparasites (Miller et al., 2016).

Steelhead (*Oncorhynchus mykiss*) are unique to other pacific salmonids in their potential iteroparity, and overwintering behaviour prior to spawning (Scott and Crossman, 1973). During their spawning migrations, steelhead may remain in rivers for

up to 10 months, increasing their accumulated thermal units and their exposure to pathogens relative to other anadromous salmonids. The Pacific Northwest maintains some of the last remaining entirely wild steelhead runs on Earth, with the rivers of the Skeena watershed, British Columbia supporting many of these wild runs. The consequences of warming water temperatures have been considered (Wade et al., 2013), and it is expected declines in steelhead populations will continue in the face of projected climate change (Smith et al., 1990; Good et al., 2005; Gayeski et al., 2011; Wade et al., 2013). Despite growing evidence that pathogens can have negative impacts on wild salmon populations (Miller et al., 2014), the influence of pathogens on wild steelhead remains relatively unexamined. Steelhead also provide an ideal model to study pathogen impacts on wild fish because they are iteroparous and their condition does not decline as rapid and severely as migratory Pacific salmon.

The goal of this study was to characterize the microbe load, richness, and physiology of wild adult steelhead during the freshwater phase of their spawning migration on the Bulkley River. The Fluidigm BioMark platform was used to assess the expression of 58 host-genes related to immunity, stress, osmoregulation, and metabolism, and the prevalence and load of 47 microbe species in steelhead. I evaluated the relationship between microbes and host-physiology. I then explored the influence of water temperature, sampling location, sex, and size on microbe load, richness and physiology of wild steelhead. It was predicted that biomarkers would be upregulated with warmer temperatures and at higher microbe loads, as they have previously shown signs of elevation following viral challenge studies (Miller et al., 2017) and temperature challenge studies (Jeffries et al., 2012, 2014). This work will provide the first account of pathogen

prevalence in wild steelhead of the Bulkley River and will provide insight on the factors contributing to microbe loads and host-physiology.

## **2.3 Methods**

### *2.3.1 Study site and collection*

Adult steelhead were captured September 23<sup>rd</sup> to October 29<sup>th</sup>, 2016 on the Bulkley River by dip net and angling and non-lethally sampled for gill tissue. All fish captured by dip net were from Witsset Falls (314 rkm), where the Wet'suwet'en have a long-standing salmon fishery and a mark-recapture program in collaboration with the MFLNRORD – Skeena Region. Steelhead captured by dip net were transferred in less than 5 sec to a transport sling which a runner then transported to a water-filled sampling trough (total air exposure of less than 25 sec). Steelhead were also captured by angling across the entirety of the Bulkley River upstream of Witsset Falls (325-407 rkm). Anglers used a combination of fly-fishing, spin-fishing, and centre pin fishing and various sizes of flies, inline spinners, and artificial worms. Steelhead were transferred to a flow-through tagging trough held parallel to the river's current. Steelhead were then non-lethally sampled for ~0.5 mg of gill tissue (2-3 filament tips) using sterilized diagonal cutting pliers to prevent tearing. Gill tissue was stored in 1.2 mL of *RNAlater*® solution (Qiagen, MD, USA) in 1.5 mL microtubes and stored at -20 °C for future genomic analyses. Previous work has demonstrated that the presence of pathogens and corresponding physiological changes can be detected in gill tissue even when it is not the primary infective tissue (Miller et al., 2017). All steelhead were assessed for fork length (mm) and sex and were sampled and released within 30 min of capture. Water

temperatures at the point of capture were taken using a handheld digital thermometer (Taylor Precision Digital Thermometer, #9847, Taylor USA, Oak Brook, IL, USA).

### *2.3.2 Sample analyses*

Gill samples were quantified for relative host-gene and pathogen expression at the Pacific Biological Station (Fisheries and Oceans Canada) using high-throughput nanofluidic qPCR (Fluidigm® BioMark™ Dynamic Array, CA, USA; outlined in Miller et al., 2011, 2014, 2016 and Jeffries et al., 2014). Samples were screened for 47 different pathogens that have been previously associated with disease in Canada or abroad (Miller et al., 2016; Table 1). Additional viral screening (6 viruses) was conducted for a subset of samples expressing a viral disease development state that did not test positively for any viruses in our initial panel. Taq-man assays were designed to target RNA and ultimately microparasites in an active state (Miller et al., 2016). The targeting of RNA also facilitated the screening of RNA viruses. Individual tissue samples were homogenized for 3 min at 30 hz in 600 uL Trizol (Ambion Inc., TX, USA) in microtubes with stainless steel beads and a MM301 mixer mill (Restch Inc., PA, USA). Each microtube then had 75 µl of 1-bromo-3-chloropropane added and was shaken for 1 min followed by a 5 min resting period (repeated once). Homogenized samples were then spun at 3,000 rpm for 6 min to separate genetic material from cellular debris and lipids. Approximately 100 uL of the aqueous phase was pipetted into a 96-well plate. RNA was extracted using a Biomek NXP™ automated liquid-handling instrument and MagMAX™-96 for Microarrays Total RNA Isolation Kit (Ambion Inc.) according to the manufacturer's instructions. An additional DNAase treatment was included to ensure minimal DNA contamination. The Biomek NXP™ automated liquid-handling instrument was used to complete all

extractions. The quantity (A260) and purity (A260/280) of RNA were evaluated by spectrophotometry using a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA). All samples were normalized using a Biomek NXP (Beckman-Coulter) automated liquid-handling instrument. Purified RNA was reverse transcribed to cDNA using the Invitrogen™ SuperScript™ VILO™ (CA, USA) cDNA Synthesis Kit. PCR cycling conditions included 25 °C for 10 min, 42 °C for 60 min and 85 °C for 5 min. cDNA then underwent a specific targeted amplification (STA) to provide sufficient template molecules for qPCR on the Fluidigm Biomark (Miller et al., 2016). To complete the STA step, 1.3 uL of cDNA from each sample was combined with 1.3 uL of 200 nM pooled primer mix and 2.5 uL of Taqman PreAmp MasterMix (Applied Biosystems, CA, USA). PCR cycling conditions were 95 °C for 10 min, 95 °C for 10 sec, and 60 °C for 4 min. The samples were then treated with ExoSAP-IT® PCR Product Cleanup (MJS BioLynx Inc., ON, Canada) to remove unincorporated nucleotides and primers (cycling conditions of 37 °C for 15 min then 80 °C for 15 min). Samples were then diluted 5-fold with DNA Suspension Buffer (Teknova, Hollister, California).

Controls included negative extraction controls, a negative and positive cDNA control, a negative and positive control during the STA stage, and a control that did not undergo pre-amplification. A sample mix of 5 uL was prepared using 2.5 uL 2X TaqMan® Gene Expression Master Mix (ABI PN 4369016), 0.25 uL 20X GE Sample Loading Reagent (Fluidigm, PN 85000746), and 2.25 uL pre-amplified cDNA and a 5 uL assay mix comprised of 0.9 uL 50 uM primer pairs, 1 uL of 10 uM probes, 2.5 uL 2X Assay Loading Reagent (Fluidigm, PN 85000736), and 0.6 uL of 10 uM APC probes (microbe assays only). For biomarker assays, the 0.6 uL of APC probes was replaced

with DNAase free water. Mixes were then loaded onto the Fluidigm Chip (Biomark) using an IFC Controller HX. Cycling conditions for qPCR adhered to the GE 96 X 96 Standard v1.pcl. (TaqMan®) protocol.

Samples and microbe assays were run in duplicate during the final qPCR resulting in quadruplicate measures of microbe expression while biomarker assays and reference assays (78d16.1, MrpL40 and Coil-P84) were run singly, resulting in duplicate CT values for each sample. Six serial dilutions of pooled samples (1.0, 0.2, 0.04, 0.008, 0.0016), and artificial positive constructs (APC clones) were run in singleton on the Biomark. Serial dilutions of pooled samples and APC clones provided reference levels to then estimate assay efficiencies. The Biomark Real-Time PCR analysis software was used to assign cycle thresholds (CT) for each reaction curve. Reaction curves were visually assessed for curve shape and replicate similarity. Cycle thresholds were averaged for duplicate host-biomarker assays and quadruplicate for microbe assays. Samples that failed to amplify products were excluded from calculations of biomarker averages, and the single value was applied. Samples that had two or more quadruplicates fail to amplify microbial products were treated as negative detections. Positive microbe detections were only assigned for samples with a CT below the 95% detection level, which varied for each microbe assay (Miller et al., 2016). For microbe expression, CT values were subtracted from 40 to depict the relative load (a positive scale from 0 to 40). Biomarker assays with efficiencies  $\pm 0.2$  were considered failed and were removed from analysis (Table 2). Averaged CT scores underwent an efficiency correction using the formula  $CT((\log(1+efficiency)/(\log(2))))$  as specified by the GenEx Software ([www.multid.se](http://www.multid.se)).

Biomarker data was normalized against housekeeping genes and pooled sample using the  $2^{-\Delta\Delta CT}$  method (Livak and Schmittgen, 2001).

### 2.3.3 Statistical analyses

Date and location (lowest extent of study site, lower river, middle river, upper river) were significantly different between capture methods ( $p < 0.05$ ), which represented inherent differences in the fisheries I worked with to sample migrating steelhead. The Wet'suwet'en salmon fishery operates from early August until the last week in September at Witsset Falls exclusively. The recreational fishery operates primarily between mid-September to early November and is typically completed upstream of the falls where there is greater and safer access to the river. To account for the potential influence these variables have on microbes and host-physiology, I evaluated the influence of sampling date, and location (lower, middle, upper river) on response variables using just the angled group of fish. These analyses did not reveal trends that were consistent with the hypothesis that date and location differences were driving the relationship between dip net and angled fish. These variables were therefore not included in subsequent analyses. It should be noted however, that the influence of water temperature and date could not be separated effectively given the close correlation between these variables (Pearson  $r = -0.76$ ).

Microbe data were analyzed separately for the relative load of each agent (*only F. psychrophilum* and *Ca B. cysticola* due to the low prevalence of other microbes) and microbe richness. Relative loads were modeled by multiple regression and only included samples with positive detections. Diagnostic plots suggested that the relative load models for both microbes had minimal deviations from normality. The richness response variable

was right skewed and significantly different from normal and was therefore modeled using an ANOVA generalized linear model using the Poisson distribution. All three models included water temperature (°C), sampling location, sex, and fork length as predictor variables. Pearson correlations were used to evaluate the relationships between relative loads of *Ca. B. cysticola*, *F. psychrophilum*, and *S. destruens* for all individuals with positive detections. A Kruskal's non-metric multidimensional scaling (NMDS) was used to evaluate rank order relationships between individual fish and host biomarkers. NMDS is an ordination technique that uses non-linear dimensionality reduction to visualize relationships between observations for multiple response variables. A Bray-Curtis distance matrix was created based on the gene expression values for each fish (R function *metaMDS*; package *vegan*). Dimensionality of the ordination was determined as the fewest number of axes needed to reduce the disagreement between rank orders of observed and predicted distances to appropriate levels (stress < 0.2; Kruskal, 1964). Biomarker scores were determined using weighted averages of all predicted distances. External variables including microbe productivities, microbe richness, water temperature at the time of sampling, capture method, sex, and fork length were fit into the ordination using the *envfit* function (package *vegan*). The *envfit* function fits extrinsic variables to predicted points by maximizing their correlation. Only microbes with high prevalence were fit to the ordination to reduce bias associated with microbes that had only a few positive detections. Continuous variables (microbe productivities, microbe richness, water temperature, and fork lengths) were plotted as vectors that represent maximal correlation of each variable with the ordination. Vector lengths were shortened, and biomarker labels were adjusted to increase plot comprehension. Significance was

assessed at  $\alpha < 0.05$ . All statistical analyses were conducted in R Studio Version 1.0.136 (R Core Team 2015) and figures were constructed in Sigma Plot Version 11.0.0.75.

**Table 2.1.** The prevalence of 47 viruses, as well as bacterial, fungal, and protist parasites in adult Bulkley River steelhead. All but *Kudoa thyrsites* are suspected to cause disease in salmon. For each assay the abbreviation, pathogen name, type of pathogen, prevalence, primer/probe sequences, and efficiency are presented. Assays were first run against a pooled sample, and only positive detections were included in the final run. Therefore, assays that have a prevalence of zero depict the assay efficiencies from the pooled sample run.



Assay name	Pathogen name	Type	Prevalence (%)	Primer and probe sequences	Efficiency
ae_hyd	<i>Aeromonas hydrophila</i>	Bacterium	0	F—ACCGCTGCTCATTACTCTGATG R—CCAACCCAGACGGGAAGAA P—TGATGGTGAGCTGGTTG	1.21
ae_sal	<i>Aeromonas salmonicida</i>	Bacterium	0	F—TAAAGCACTGTCTGTTACC R—GCTACTTCACCCTGATTGG P—ACATCAGCAGGCTTCAGAGTCACTG	1.14
re_sal	<i>Renibacterium salmoninarum</i>	Bacterium	0	F—CAACAGGGTGGTTATTCTGCTTTC R—CTATAAGAGCCACCAGCTGCAA P—CTCCAGCGCCGCAGGAGGAC	1.26
c_b_cys	<i>Candidatus Branchiomonas cysticola</i>	Bacterium	80	F—AATACATCGGAACGTGTCTAGTG R—GCCATCAGCCGCTCATGTG P—CTCGGTCCCAGGCTTTCCTCTCCCA	0.91
ye_ruc	<i>Yersinia ruckeri</i>	Bacterium	0	F—TGCCGCGTGTGTGAAGAA R—ACGGAGTTAGCCGGTGCTT P—AATAGCACTGAACATTGAC	1.26
fl_psy	<i>Flavobacterium psychrophilum</i>	Bacterium	92.5	F—GATCCTTATTCTCACAGTACCGTCAA R—TGTAAACTGCTTTTGCACAGGAA P—AAACACTCGGTGCTGACC	0.82
mo_vis	<i>Moritella viscosa</i>	Bacterium	0	F—CGTTGCGAATGCAGAGGT R—AGGCATTGCTTGCTGGTTA P—TGCAGGCAAGCCAACCTTCGACA	1.23
pch_sal	<i>Piscichlamydia salmonis</i>	Bacterium	7.5	F—TCACCCCAGGCTGCTT R—GAATTCCATTTCCTCTTG P—CAAACTGCTAGACTAGAGT	1.08
pisck_sal	<i>Piscirickettsia salmonis</i>	Bacterium	0	F—TCTGGGAAGTGTGGCGATAGA R—TCCCGACCTACTCTTGTTTCATC P—TGATAGCCCCGTACACGAAACGGCATA	1.18
rlo	<i>Rickettsia-like organism</i>	Bacterium	0	F—GGTCAACCCAAGAACTGCTT R—GTGCAACAGCGTCAGTGACT	1.14

sch	<i>Gill chlamydia</i>	Bacterium	0	P—CCCAGATAACCGCCTTCGCCTCCG F—GGGTAGCCCGATATCTTCAAAGT R—CCCATGAGCCGCTCTCTCT	1.29
te_mar	<i>Tenacibaculum maritimum</i>	Bacterium	0	P—TCCTTCGGGACCTTAC F—TGCCTTCTACAGAGGGATAGCC R—CTATCGTTGCCATGGTAAGCCG	1.22
vi_ang	<i>Vibrio anguillarum</i>	Bacterium	0	P—CACTTTGGAATGGCATCG F—CCGTCATGCTATCTAGAGATGTATTTGA R—CCATACGCAGCCAAAAATCA	1.23
vi_sal	<i>Vibrio salmonicida</i>	Bacterium	0	P—TCATTTGACGAGCGTCTTGTTTCAGC F—GTGTGATGACCGTTCCATATT R—GCTATTGTCATCACTCTGTTTCTT	1.16
pmcv	Piscine totivirus (CMS)	Virus	0	P—TCGCTTCATGTTGTGTAATTAGGAGCGA F—TTCCAAACAATTCGAGAAGCG R—ACCTGCCATTTTCCCCTCTT	1.25
ver	Viral encephalopathy and retinopathy virus	Virus	0	P—CCGGGTAAAGTATTTGCGTC F—TTCCAGCGATACGCTGTTGA R—CACCGCCCGTGTTTGC	1.32
vhsv	Viral haemorrhagic septicaemia virus	Virus	0	P—AAATTCAGCCAATGTGCCCC F—ATGAGGCAGGTGTCGGAGG R—TGTAGTAGGACTCTCCCAGCATCC	1.15
omv	Salmonid herpesvirus	Virus	0	P—TACGCCATCATGATGAGT F—GCCTGGACCACAATCTCAATG R—CGAGACAGTGTGGCAAGACAAC	1.21
sav	Salmon alphavirus	Virus	0	P—CCAACAGGATGGTCATTA F—CCGGCCCTGAACCAGTT R—GTAGCCAAGTGGGAGAAAGCT	1.14
				P—TCGAAGTGGTGGCCAG	

ven	Viral erythrocytic necrosis virus	Virus	0	F—CGTAGGGCCCCAATAGTTTCT R—GGAGGAAATGCAGACAAGATTTG P—TCTTGCCGTTATTTCCAGCACCCG	1.27
pspv	Pacific salmon parvovirus	Virus	0	F—CCCTCAGGCTCCGATTTTTAT R—CGAAGACAACATGGAGGTGACA P—CAATTGGAGGCAACTGTA	1.21
prv	Piscine reovirus (HSMI, CMS)	Virus	0	F—TGCTAACACTCCAGGAGTCATTG R—TGAATCCGCTGCAGATGAGTA P—CGCCGGTAGCTCT	1.2
ihnv	Infectious haematopoietic necrosis virus	Virus	0	F—AGAGCCAAGGCACTGTGCG R—TTCTTTGCGGCTTGTTGA P—TGAGACTGAGCGGGACA	1.14
ipnv	Infectious pancreatic necrosis virus	Virus	0	F—GCCAAGATGACCCAGTCCAT R—TGACAGCTTGACCCTGGTGAT P—CCGACCGAGAACAT	1.02
sav	Salmon alphavirus	Virus	0	F—CCGCACGTTGTAAGATCAGT R—CGTCCGGAATGTTGATGGA P—CTCCTGGCCCTCGAT	1.25
cr_sal	<i>Cryptobia salmositica</i>	Parasite	0	F—TCAGTGCCTTTCAGGACATC R—GAGGCATCCACTCCAATAGAC P—AGGAGGACATGGCAGCCTTTGTAT	1.16
ce_sha	<i>Ceratonova shasta</i> (formerly <i>Ceratomyxa shasta</i> )	Parasite	0	F—CCAGCTTGAGATTAGCTCGGTAA  R—CCCCGGAACCCGAAAG P—CGAGCCAAGTTGGTCTCTCCGTGAAAAC	1.33
de_sal	<i>Dermocystidium salmonis</i>	Parasite	0	F—CAGCCAATCCTTTCGCTTCT	1.19

fa_mar	<i>Facilispora margolisi</i>	Parasite	0	R—GACGGACGCACACCACAGT P—AAGCGGCGTGTGCC F—AGGAAGGAGCACGCAAGAAC R—CGCGTGCAGCCCAGTAC P—TCAGTGATGCCCTCAGA	1.32
gy_sal	<i>Gyrodactylus salaris</i>	Parasite	0	F—CGATCGTCACTCGGAATCG R—GGTGGCGCACCTATTCTACA P—TCTTATTAACCAGTTCTGC	1.18
ic_mul	<i>Ichthyophthirius multifiliis</i>	Parasite	2.5	F—AAATGGGCATACGTTTGCAA R—AACCTGCCTGAAACACTCTAATTTT P—ACTCGGCCTTCACTGGTTCGACTTGG	1.01
ku_thy	<i>Kudoa thyrsites</i>	Parasite	0	F—TGGCGGCCAAATCTAGGTT R—GACCGCACACAAGAAGTTAATCC P—TATCGCGAGAGCCGC	1.09
lo_sal	<i>Loma salmonae</i>	Parasite	10	F—GGAGTCGCAGCGAAGATAGC R—CTTTCTCCCTTTACTCATATGCTT P—TGCCTGAAATCACGAGAGTGAGACTA	0.93
my_arc	<i>Myxobolus arcticus</i>	Parasite	0	F—TGGTAGATACTGAATATCCGGGTTT R—AACTGCGCGGTCAAAGTTG P—CGTTGATTGTGAGGTTGG	1.14
my_ins	<i>Myxobolus insidiosus</i>	Parasite	0	F—CCAATTTGGGAGCGTCAA R—CGATCGGCAAAGTTATCTAGATTCA P—CTCTCAAGGCATTTAT	1.2
my_cer	<i>Myxobolus cerebralis</i>	Parasite	0	F—GCCATTGAATTTGACTTTGGATTA R—ACCATTCATGTAAGCCCGAACT P—TCGAAGCCTTGACCATCTTTTGCC	0.95
ne_per	<i>Neoparamoeba perurans</i>	Parasite	0	F—GTTCTTTTCGGGAGCTGGGAG R—GAACTATCGCCGGCACAAAAG	1.29

nu_sal	<i>Nucleospora salmonis</i>	Parasite	0	P—CAATGCCATTCTTTTCGGA F—GCCGCAGATCATTACTAAAAACCT R—CGATCGCCGCATCTAAACA	1.22
pa_ther	<i>Paranucleospora theridion</i>	Parasite	5	P—CCCCGCGCATCCAGAAATACGC F—CGGACAGGGAGCATGGTATAG R—GGTCCAGGTTGGGTCTTGAG P—TTGGCGAAGAATGAAA	0.94
pa_pse	<i>Parvicapsula pseudobranchicola</i>	Parasite	0	F—CAGCTCCAGTAGTGTATTCA R—TTGAGCACTCTGCTTTATTCAA P—CGTATTGCTGTCTTTGACATGCAGT	1.13
pa_kab	<i>Parvicapsula kabatai</i>	Parasite	0	F—GTCGGATGATAAGTGCATCTGATT R—ACACCACAACCTCTGCCTTCCA P—TGCGACCATCTGCACGGTACTGC	1.19
te_bry	<i>Tetracapsuloides bryosalmonae</i>	Parasite	2.5	F—GCGAGATTTGTTGCATTTAAAAAG R—GCACATGCAGTGTCCAATCG P—CAAAATTGTGGAACCGTCCGACTACGA	1.1
pa_min	<i>Parvicapsula minibicornis</i>	Parasite	0	F—AATAGTTGTTTGTCGTGCACTCTGT R—CCGATAGGCTATCCAGTACCTAGTAAG P—TGTCCACCTAGTAAGGC	1.21
sp_des	<i>Sphaerothecum destruens</i>	Parasite	52.5	F—GCCGCGAGGTGTTTGC R—CTCGACGCACACTCAATTAAGC P—CGAGGGTATCCTTCCTCTCGAAATTGGC	0.98
sp_sal	<i>Spironucleus salmonicida</i>	Parasite	0	F—AACCGGTTATTCGTGGGAAAG R—TTAACTGCAGCAACACAATAGAATACT P—TGCCAGCAGCCGCGTAATTC	8.6
ic_hof	<i>Ichthyophonus hoferi</i>	Parasite	0	F—GTCTGTACTGGTACGGCAGTTTC R—TCCCGAACTCAGTAGACACTCAA	1.12

na_sal	<i>Nanophyetus salmincola</i>	Fluke	0	P—TAAGAGCACCCACTGCCTTCGAGAAGA F—CGATCTGCATTTGGTTCTGTAACA R—CCAACGCCACAATGATAGCTATAC P—TGAGGCGTGTTTTATG	1.27
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**Table 2.2.** The biomarker assays and three reference genes evaluated by qPCR on adult steelhead. For each assay the abbreviation, gene name, type, primer/probe sequences, and efficiency are presented.

<b>Assay name</b>	<b>Gene name</b>	<b>Function</b>	<b>Primer and probe sequences</b>	<b>Efficiency</b>
B2M	Beta 2-microglobulin	Immunity	F—TTTACAGCGCGGTGGAGTC R—TGCCAGGGTTACGGCTGTAC P—AAAGAATCTCCCCCAAGGTGCAGG	1.08
CD4	Cluster of differentiation 4	Immunity	F—CATTAGCCTGGGTGGTCAAT R—CCCTTTCTTTGACAGGGAGA P—CAGAAGAGAGAGCTGGATGTCTCCG	1.02
CD8a	T-cell surface glycoprotein CD8 alpha chain	Immunity	F—ACACCAATGACCACAACCATAGAG R—GGGTCCACCTTTCCCACTTT P—ACCAGCTCTACAACCTGCCAAGTCGTGC	0.95
IgMs	Immunoglobulin	Immunity	F—CTTGGCTTGTTGACGATGAG R—GGCTAGTGGTGTGAATTGG P—TGGAGAGAACGAGCAGTTCAGCA	1.07
IgT	Immunoglobulin tau	Immunity	F—CAACACTGACTGGAACAACAAGGT R—CGTCAGCGGTTCTGTTTTGGA P—AGTACAGCTGTGTGGTGCA	0.83
MHCI	Major histocompatibility complex I	Immunity	F—GCGACAGGTTTCTACCCCAGT  R—TGTCAGGTGGGAGCTTTTCTG P—TGGTGTCTTGGCAGAAAGACGG	1.02
MHCII-B	Major histocompatibility complex II $\beta$	Immunity	F—TGCCATGCTGATGTGCAG  R—GTCCCTCAGCCAGGTCCT P—CGCCTATGACTTCTACCCCAAACAAAT	1.06
TCRa	T cell receptor alpha chain	Immunity	F—ACAGCTTGCTGGCTACAGA R—TGTCCCCTTTCACTCTGGTG P—CAGCGCACACAAGGCTAATTCG	1.09
ZAP7	Tyrosine-protein kinase (ZAP-70)	Immunity	F—TCACCTCCGGACCTTTCATT R—CCATGTGGGAAGCCTTTTCTT P—TCTTGTATGGTTTTCTCC	1.02
C3	Complement component 3	Immunity	F—ATTGGCCTGTCCAAAACACA R—AGCTTCAGATCAAGGAAGAAGTTC P—TGGAATCTGTGTGTCTGAACCCC	0.95

C7	Complement factor	Immunity	F—ACCTCTGTCCAGCTCTGTGTC R—GATGCTGACCACATCAAAGTGC P—AACTACCAGACAGTGCTG	0.88
IL-15	Interleukin 15	Immunity	F—TTGGATTTTGCCCTAACTGC R—CTGCGCTCCAATAAACGAAT P—CGAACAAACGCTGATGACAGGTTTTT	0.95
IL-1B	Interleukin 1-beta	Immunity	F—AGGACAAGGACCTGCTCAACT R—CCGACTCCAACCTCCAACACTA P—TTGCTGGAGAGTGCTGTGGAAGAA	0.91
IL-8	Interleukin 8	Immunity	F—GAGCGGTCAGGAGATTTGTC R—TTGGCCAGCATCTTCTCAAT P—ATGTCAGCGCTCCGTGGGT	0.94
MMP13	Matrix Metalloproteinase 13	Immunity	F—GCCAGCGGAGCAGGAA R—AGTCACCTGGAGGCCAAAGA P—TCAGCGAGATGCAAAG	1.03
MMP25	Matrix Metalloproteinase 25	Immunity	F—TGCAGTCTTTTCCCCTTGGAT R—TCCACATGTACCCACACCTACAC P—AGGATTGGCTGGAAGGT	1.04
PCBL	Precerebellin	Immunity	F—TGGTGTGCTTTGCTGTTGT R—GCCACTTTTGGTTTGTCTC P—ATGGTTGAGACTCAGACGGAGAGTG	0.96
SRK2	Tyrosine-protein kinase FRK	Immunity	F—CCAACGAGAAGTTCACCATCAA R—TCATGATCTCATAACAGCAAGATTCC P—TGTGACGTGTGGTCCT	0.94
SAA	Serum amyloid protein alpha	Immunity	F—GGGAGATGATTCAGGGTTCCA R—TTACGTCCCCAGTGGTTAGC P—TCGAGGACACGAGGACTCAGCA	0.99
TF	Transferrin	Immunity	F—TTCAGTCTGGAAAATGTGG	1.02

TNF	Tumour necrosis factor	Immunity	R—GCTGCACTGAACTGCATCAT P—TGGTCCCTGTCATGGTGGAGCA F—CCCACCATACATTGAAGCAGATT R—GGATTGTATTCACCCCTCTAAATGGA P—CCGGCAATGCAAAA	0.93
EF2	Eukaryotic translation elongation factor 2	Immunity	F—AGGTCACAGCCGCCCTTAG R—ACACAGTCTCTGTCTGCACACACA P—CGACTGCGTCTCAGGT	1.01
IFI44A	IFN-induced protein 44-1	VDD	F—GCTAGTGCTCTTGAGTATCTCCACAA R—TCACCAGTAACTCTGTATCATCCTGTCT P—AGCTGAAAGCACTTGAG	0.89
IFIT5	Interferon-induced protein with tetratricopeptide repeats 5	VDD	F—CCGTCAATGAGTCCCTACACATT R—CACAGGCCAATTTGGTGATG P—CTGTCTCCAAACTCCCA	0.92
52Ro	52 kDa Ro protein-2	VDD	F—TGCACTATTGCCAGTAACCAT R—TGCAAGAGGAGATGCCAACA P—AGTAGGATTCACAGAGAGTT	0.98
IRF1	Interferon regulatory factor 1	VDD	F—CAAACCGCAAGAGTTCCTCATT R—AGTTTGGTTGTGTTTTTGCATGTAG P—CTGGCGCAGCAGATA	0.96
Mx	Antiviral protein	VDD	F—AGATGATGCTGCACCTCAAGTC R—CTGCAGCTGGGAAGCAAAC P—ATTCCCATGGTGATCCGCTACCTGG	0.92
RSAD	Radical S-adenosyl methionine Domain-containing protein 2	VDD	F—GGGAAATTAGTCCAATACTGCAAAC R—GCCATTGCTGACAATACTGACACT P—CGACCTCCAGCTCC	1.10
VIG10	VHSV-induced protein-10	VDD	F—GCAAACCTGAGAAAACCATCAAGAA R—CCGTCAGCTCCCTCTGCAT	0.99

DEXH	DEXH box helicase, DNA ligase-associated	VDD	P—TGTGGAGAAGTTGCAGGC F—CCATAAGGAGGGTGTCTACAATAAGAT R—CTCTCCCCCTTCAGCTTCTGT P—TGGCGCGCTACGTG	0.90
GAL3	Galectin-3-binding protein precursor	VDD	F—TTGTAGCGCCTGTTGTAATCATATC R—TACACTGCTGAGGCCATGGA P—CTTGGCGTGGTGGC	0.95
NFX	Zinc finger NFX1-type	VDD	F—CCACTTGCCAGAGCATGGT R—CGTAACTGCCCAGAGTGCAAT P—TGCTCCACCGATCG	0.95
STAT1	Signal transducer and activator of transcription 1-alpha/beta	VDD	F—TGTCACCGTCTCAGACAGATCTG R—TGTTGGTCTCTGTAAGGCAACGT P—AGTTGCTGAAAACCGG	0.85
VAR1	Mitochondrial ribosomal protein (VAR1)	VDD	F—CCACCTGAGGTAAGATAAGACA R—TTAAGTCCTCCTTCCTCATCTGGTA P—TCTACCAGGCCTTAAAG	0.99
HBA	Hemoglobin subunit alpha	Stress	F—GCCCTGGCTGACAAATACAGA R—GAGCAGGAACTGGAGTCCAATG P—ACCATCATGAAAGTCC	1.12
CA4	Carbonic anhydrase 4	Stress	F—GGTCATTTTGGTTTTGTACACAGTCT R—CCTAGATATAGCTATCCACGTAACCTA P—TGATACGTGGTATAGAAAAG	0.92
HIF1A_3	Hypoxia-inducible factor 1-alpha	Stress	F—CACTACAACCTCTCCTCACTCACTCTGT R—AGCAGCCAAACTATAAGATCACTGATAC P—CTGCCCTTTATTTGTCTC	1.11
HIF1A_7	Hypoxia-inducible factor 1-alpha	Stress	F—TGGCAAATCTGCCTACGAATT R—GCAGGCTCTTGGTCACATGA	0.99

HSC70	Heat shock cognate 70	Stress	P—ATCATGCCCTGGACTC F—GGGTCACACAGAAGCCAAAAG R—GCGCTCTATAGCGTTGATTGGT P—AGACCAAGCCTAAACTA	0.88
HSP90a	Heat shock protein 90-alpha	Stress	F—AGTACCCTGTTGCACTGAGTTTTAAA R—GAATGTTTCATTTCCATTGTTCA P—ATTGGACTGGTAGATGTGT	0.88
HSP90 (alike)	Heat shock protein 90-alpha (alike)	Stress	F—TTGGATGACCCTCAGACACACT R—CGTCAATACCCAGGCCTAGCT P—CCGAATCTACCGGATGAT	1.05
MAP3K	Mitogen-activated protein kinase kinase kinase 14è Mapk14	Stress	F—GCTCCCTGGGTTTCATGGAT R—GCCTCCCTTCAGCAGAGACA P—CCAGCAATAGCTTATG	1.08
PARK7	Parkinson disease (autosomal recessive, early onset) 7; SP22	Stress	F—ACTGCAAGCAGCATGATCAACT R—TTGGCCTGTGTATCATAATGAACA P—CCCCACCTACTCAGC	1.02
SEPW1	Selenoprotein W	Stress	F—TGAGGATGAATTCCCAGGTGAT R—AAACCACCCAGAGGTTGAAGGT P—TTGAGATTACTGGTGAAAGC	1.12
SERPIN	Serpin H1-Precursor (heat shock protein 47)	Stress	F—ACTATGACCACTCGAAGATCAACCT R—CCCATTCGTTGATGGAGTTCA P—AGGGACAAGAGGAGC	0.86
COMMD7	COMM domain-containing protein 7	MRS	F—CAAAGCCAGTATGGACTGTTTCAG R—TTGTTTTCTGCTGCCCCCTCA P—ACCTGATCGCCAGTAGCATGAGCATGTAC	0.89
FYB	FYN-binding protein	MRS	F—TGAGATGAGCTTGTGTCTACAG R—GCAGTAAAGATCTGCCGTTGAGA P—CTCAACGATGACATCCACAGTCTCCCC	1.01

HTA	Histone acetyltransferase	MRS	F—CTTGTAACAGTTCGACATGGCTTATT R—TGGTGAAGCATTCTGTATGTCAA P—TCTGTACTGAGCATCCCCGCACATTACA	0.84
KRT8	Keratin, type II cytoskeletal 8	MRS	F—CGATTGAGCGGCTGGATAA R—GCATTGTTTACCTTTGACTTGAATTG P—CCCCCTTCTACTCTCTTGCTCACCATTC	1.04
SCG	Secretogranin II	MRS	F—GGATGTGAAGAATCCAACACTGAT R—ACACCACTTCAAAGTAGCCATACATT P—CGGCTGTATGTGCACTG	0.87
NKAa1a	Na/K ATPase $\alpha$ -1a (freshwater)	Osmoregulation	F—TGGAATCAAGGTTATCATGGTCACT R—CCCACACCCTTGGCAATG P—ATCATCCCATCACTGCGA	1.00
NKAa1b	Na/K ATPase $\alpha$ -1b (saltwater)	Osmoregulation	F—GCCTGGTGAAGAATCTTGAAGCT R—GAGTCAGGGTCCGGTCTTG P—CCTCCACCATTGCTCA	1.13
ALDOA	Aldolase A, fructose-bisphosphate	Metabolism	F—CGTGATTCAAGTGTGTCATCTTGA R—TTCCTCCAGTGTTCCTTCAGTCA P—AAGTACATGTGCCTTCTT	0.94
COX6B1	Cytochrome C oxidase	Metabolism	F—GCCCCGTGTGACTGGTATAAG R—TCGTCCCATTCTGGATCCA P—TCTACAAATCACTGTGCC	0.86
IDH3b	Isocitrate dehydrogenase 3 (NAD <sup>+</sup> )-beta	Metabolism	F—TCGTGTTTGGCTGTTTCAGTCA R—AGTGGCTTGTTCGTTTGCAA P—CAAAGCTCTTTCATCATT	0.80
LDHB	Lactate dehydrogenase B	Metabolism	F—GTCACTGCTCCATTTTACACTCTAG R—CCCAAAGTCCCCTCCCAGATAAC P—CTGTTCTTAGCTTCCC	0.85
MPDU1		Metabolism	F—TGCTTGACCCCTTGATTATAGCTA	1.05

	Mannose-P-Dolichol Utilization Defect 1 Protein (alike)		R—GACCATAATCTAGAATGAAAACGCATT P—CTTCCTGGTTGTGTTCTG	
PGK3	Phosphoglycerate kinase	Metabolism	F—GGCAAAGTGCTCCCTAAGTTTC R—TAGAGAGCAGGGCTGGTGCTA P—CACCTGCGCTTGT	1.12
sema4ab	Sema domain, immunoglobulin domain, transmembrane domain, and short cytoplasmic domain, (semaphorin) 4Ab	Reference	F—GTCAAGACTGGAGGCTCAGAG R—GATCAAGCCCCAGAAGTGTGTTG P—AAGGTGATTCCCTCGCCGTCCGA	0.98
COIL-P84-2	Coiled-coil domain-containing protein 84	Reference	F—GCTCATTTGAGGAGAAGGAGGATG R—CTGGCGATGCTGTTCTGAG P—TTATCAAGCAGCAAGCC	0.98
MRPL40	39S ribosomal protein L40, mitochondrial precursor	Reference	F—CCCAGTATGAGGCACCTGAAGG R—GTTAATGCTGCCACCCCTCTCAC P—ACAACAACATCACCA	0.94
ACTB	Beta-actin	Excluded	F—GAAATCGCCGCACTGGTT R—CGGCGAATCCGGCTTT P—TTGACAACGGATCCGGT	1.38
C1Qc	Complement C1q subcomponent subunit C	Excluded	F—CGCCGGTGAGTGGAATCTA R—CTTCTCCATCATGTGGTGTGCTA P—ACCTCCAAACATAGAAGAG	0.69
CCL4	Chemokine (C-C motif) ligand 4	Excluded	F—TCTCTTCATTGCAACAATCTGCTT R—ACAGCAGTCCACGGGTACCT P—CTACGCAGCAGCATT	1.04
Cd83	Cluster of Differentiation 83	Excluded	F—GTGGCGGCATTGCTGATATT R—CTTGTGGATACTTCTTACTCCTTTGCA P—CACCATCAGCTATGTCATCC	Failed
CIRBP	Cold-inducible RNA-binding protein	Excluded	F—TGATTGACTGTTTTGCCAACTGA R—TCAGACCTTTGTGTGCATTTACCT	Failed

FK506	FK506-binding protein 10 precursor	Excluded	ATGGTGATGAGCCTGAAT F—ACTATGAGAAATGCCCCCATCAC R—CTCGTCCAGACCCTCAATCAC P—CCTGGGAGCCAACAA	Failed
Glut2	Solute carrier family 2, facilitated glucose transporter member 2-like	Excluded	F—GGAACCTTACATCAACTGGCTACA R—GCAGTGGCCAGTAGTAGTCATTACC P—CTGGTATACTACTGAGTCAGG	Failed
Hep	Hepcidin	Excluded	F—GAGGAGGTTGGAAGCATTGA R—TGACGCTTGAACCTGAAATG P—AGTCCAGTTGGGGAACATCAACAG	1.28
HERC6	Probable E3 ubiquitin-protein ligase	Excluded	F—AGGGACAACCTGGTAGACAGAAGAA R—TGACGCACACACAGCTACAGAGT P—CAGTGGTCTCTGTGGCT	1.24
HIF1A-6	Hypoxia-inducible factor 1-alpha-like	Excluded	F—AGAGGAGGCAGTGCTGTATTCAA R—GGGACAAGGCCCTCCAAT P—AGGGCCCTGACCATG	Failed
IGFBP1	Insulin-like growth factor binding protein-1	Excluded	F—AGATAACCAGCTCTCAGCAGGAA R—ATGTTTGTACAGTTGGGTAGGTAGA P—TAGGAGAGAAGTTCACCAAC	Failed
IL-11	Interleukin 11	Excluded	F—GCAATCTCTTGCCCTCCACTC R—TTGTCACGTGCTCCAGTTTC P—TCGCGGAGTGTGAAAGGCAGA	0.79
JUN	AP-1 Transcription Factor Subunit	Excluded	F—TTGTTGCTGGTGAGAAAACCTCAGT R—CCTGTTGCCCTATGAATTGTCTAGT P—AGACTTGGGCTATTTAC	Failed
LdhaL	L-lactate dehydrogenase A chain-like	Excluded	F—TTTGTTTAGTGTGTGCGAGAGTTG R—TCCGTGCACTTACGGTTAGTTTT P—CCAGAGCCATTTCAGT	Failed

SHOP21	Salmon hyperosmotic protein 21	Excluded	F—GCGGTAGTGGAGTCAGTTGGA R—GCTGCTGACGTCTCACATCAC P—CCTGTTGATGCTCAAGG	1.29
TCRb	T cell receptor beta chain	Excluded	F—TCACCAGCAGACTGAGAGTCC R—AAGCTGACAATGCAGGTGAATC P—CCAATGAATGGCACAAACCAGAGAA	4.02
VIG4	VHSV-inducible protein-4	Excluded	F—TGGCTTCCCACATTGCAA R—CCTCCTCCCCCTGCAT P—AGATGGAGACAGGAATG	0.80

## 2.4 Results

Fifteen steelhead ( $598 \pm 62$  mm; 53% female) were captured by dip net at Witset Falls (314 rkm) between September 23-29, 2017. An additional 25 steelhead ( $688 \pm 16$  mm; 68% female) were captured by angling (rkm 325-407) between September 23-October 29, 2017.

### 2.4.1 Microbe productivity

*Candidatus Branchiomonas cysticola* (80%), *Flavobacterium psychrophilum* (95.0%), and *Sphaerothecum destruens* (52.5%) were the most prevalent microbes in Bulkley River steelhead gill tissue (Table 1). The relative load of *Ca. B. cysticola* was not significantly correlated with water temperature ( $p=0.25$ ), sex ( $p=0.97$ ), or fork length ( $p=0.33$ ) but was significantly greater for fish caught at the falls than above ( $t$ -value=2.86,  $p<0.01$ ; Figure 1A). The relative load of *F. psychrophilum* was not significantly correlated with water temperature ( $p=0.94$ ) or sex ( $p=0.07$ ), or fork length ( $p=0.31$ ) but was significantly greater for fish caught at the falls than above ( $t$ -value=4.37,  $p<0.01$ ; Figure 1B). The relative loads of *Ca. B. cysticola* and *F. psychrophilum* were not closely correlated (Pearson  $r=0.23$ ), neither were *Ca. B. cysticola* and *S. destruens* (Pearson  $r=0.33$ ), or *F. psychrophilum* and *S. destruens* (Pearson  $r=0.17$ ). Water temperature ( $p=0.19$ ), sex ( $p=0.12$ ), and fork length ( $p=0.94$ ) were not significant predictors of microbe richness. Microbe richness was also significantly greater for fish caught at the falls compared to those caught above ( $\chi^2=11.79$ ,  $df=1$ ,  $p<0.01$ ; Figure 1C).

#### 2.4.2 Host-gene expression

Gene expression data was successfully reduced into two-dimensional ordination (stress=0.18; Figure 2). The genes most positive on NMDS1 included RSAD, IFIT5, Mx, NFX, DEXH, VAR1, X52Ro and IFI44A, which are indicative of a viral disease development response when co-expressed (Miller et al., 2017). Five individuals had a value greater than 0.2 on NMDS1, suggesting they were in a state of viral disease development.

Fitted relative loads of *F. psychrophilum*, and *Ca. B. cysticola*, as well as microbe richness were close in ordination space, positive on NMDS1 and NMDS2. Only the relative load of *F. psychrophilum* ( $p=0.04$ ) and pathogen richness ( $p=0.03$ ) were significantly associated with the ordination gradient, while the relative load of *Ca. B. cysticola* was not ( $p=0.07$ ). These microbe parameters shared similar ordination space to HBA, SAA, and SRK2, and were opposite in ordination space to genes related to humoral immunity (IgMs, IgT) and MHCI.

Water temperature was correlated with the ordination gradient ( $p=0.04$ ) being slightly negative on NMDS1 and positive on NMDS2. Water temperature was close in ordination space to genes responsive to oxygen availability such as CA4, SEPW1 and HBA, while opposite in ordination space to immune genes such as CD4, IL17D, and MMP13. Capture method was significantly associated with the ordination gradient ( $p<0.01$ ). Fish sampled at the falls were closer in ordination space to genes responsive to oxygen availability including CA4, SEPW1, and HBA, while fish upstream of the falls were closer in ordination space to genes related to stress and immunity such as IL17D,

IL8, MMP13, particularly humoral immune genes (IgMs, IgT). Sex was not correlated with the ordination gradient ( $p=0.81$ ), nor was fork length ( $p=0.29$ ).

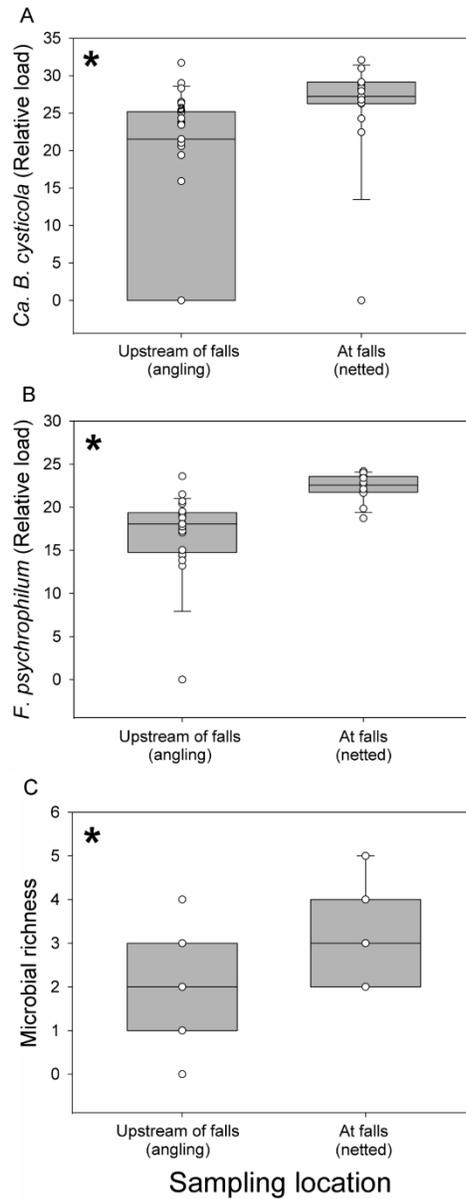
**Table 2.3.** ANOVA output predicting the relative load of *Ca. B. cysticola*, and the relative load of *F. psychrophilum* in the gill tissue of steelhead captured in the Bulkley River, B.C. The model includes sampling location and sex as categorical variables and water temperature ( $^{\circ}\text{C}$ ) and fork length (mm) as continuous variables. Estimates for the factors are presented relative to a reference level which is fish that were angled and female. Significant predictors are emphasized with boldface font.

Variable	Relative load: <i>Ca. B. cysticola</i>			Relative load: <i>F. psychrophilum</i>		
	Estimate $\pm$ SE	t-value	P	Estimate $\pm$ SE	t-value	P
(Intercept)	32.43 $\pm$ 6.12	5.30	<0.01	14.53 $\pm$ 3.43	4.24	<0.01
Water temperature	-0.47 $\pm$ 0.40	-1.18	0.25	0.02 $\pm$ 0.22	0.07	0.94
<b>Sampling location: Falls</b>	<b>4.79 <math>\pm</math> 1.68</b>	<b>2.86</b>	<b>&lt;0.01</b>	<b>4.07 <math>\pm</math> 0.93</b>	<b>4.37</b>	<b>&lt;0.01</b>
Sex: Male	0.05 $\pm$ 1.39	0.04	0.97	1.46 $\pm$ 0.79	1.85	0.07
Fork length	-0.01 $\pm$ 0.01	-1.00	0.33	0.01 $\pm$ 0.01	1.03	0.31

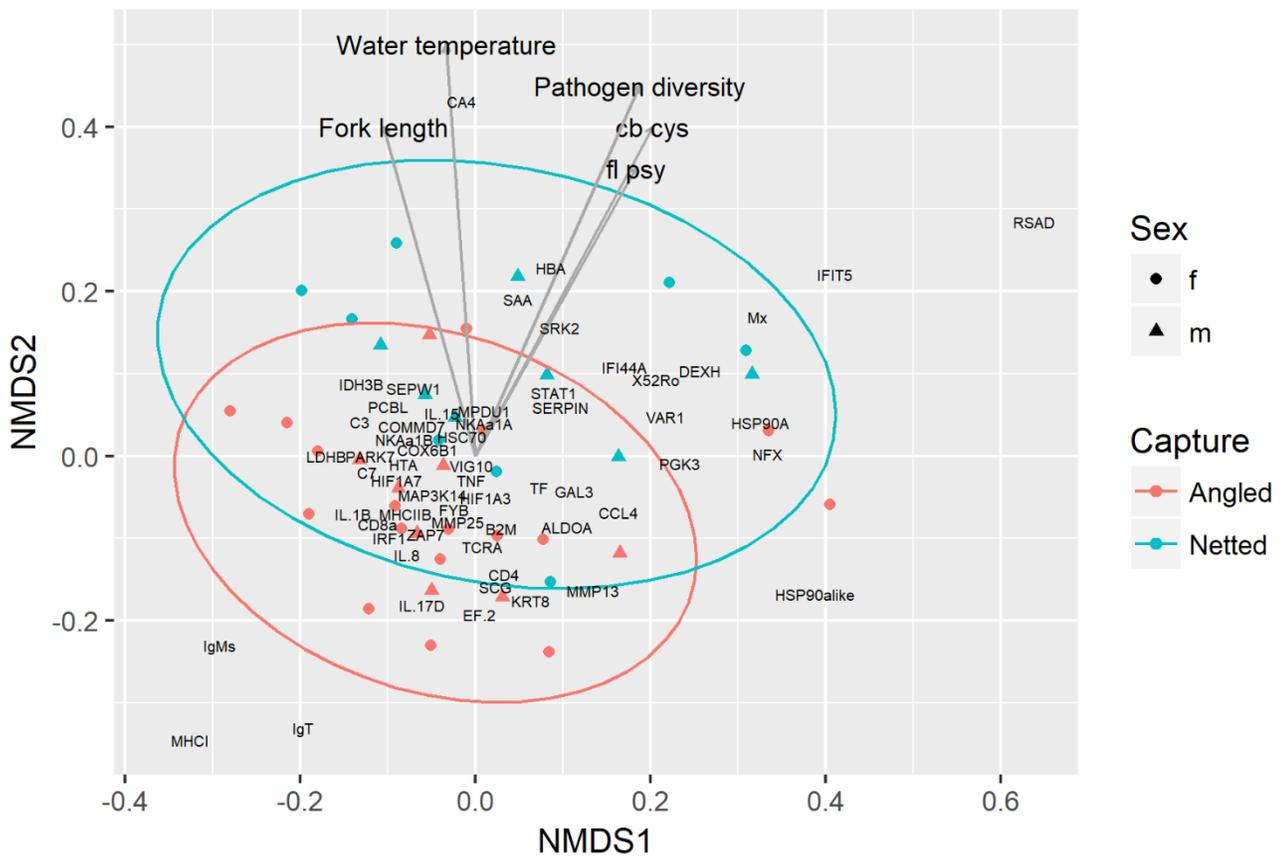
**Table 2.4.** ANOVA output predicting microbe richness in the gill tissue of steelhead captured in the Bulkley River, B.C. The model includes sampling location and sex as categorical variables and water temperature ( $^{\circ}\text{C}$ ) and fork length (mm) as continuous variables.

Variable	Microbe richness		
	$\chi^2$	df	P
Water temperature	1.74	1	0.19
<b>Sampling location</b>	<b>11.79</b>	<b>1</b>	<b>&lt;0.01</b>
Sex	2.51	1	0.11
Fork length	0.01	1	0.94

**Figure 2.1.** The relative load of A) *Candidatus Branchiomonas cysticola* B) *Flavobacterium psychrophilum* and C) microbe richness in the gill tissue of steelhead captured by angling upstream of the falls and netting at the falls. Asterisks denote a statistically significant difference ( $p < 0.05$ ).



**Figure 2.2.** An NMDS plot of host gene expression in the gill tissues of Bulkley River steelhead (n=40). The shape of each point reflects sex (circle=female, triangle=male), and the colour represents sampling location (red=upstream of falls, blue=at falls). Vectors represent extrinsic variables fit into the ordination including the relative loads of *Candidatus Branchiomonas cysticola* and *Flavobacterium psychrophilum*, microbe richness, water temperature, and fork length.



## 2.5 Discussion

Adult steelhead migrating to Witsset Falls of the Bulkley River had already travelled over 300 rkm inland and have therefore had considerable exposure to freshwater pathogens that could have implications for their migration. Our work has characterized the relationship between pathogen burdens and host-physiology in steelhead, and evaluated whether external factors related to the environment, sampling location, or individual, influence pathogen and host dynamics. I found that genes previously indicative of viral disease development (Miller et al., 2017) were similarly co-expressed in Bulkley River steelhead providing validation for the VDD panel of genes in the species. Our results indicate that greater microbe richness and relative loads of *F. psychrophilum* correspond to changes in gene expression, including suppression of genes related to the humoral immune response. This study also found elevated expression of genes responsive to oxygen availability at warmer water temperatures, highlighting the potential for changes to steelhead physiology with future climate change (Wade et al., 2013). Interesting differences existed between steelhead sampled at Witsset Falls compared to those upstream. It is suggested Witsset Falls may act as a migratory culling point (Risley et al., 2018), whereby the most pathogen-rich fish are less likely to surpass the falls. Our work identified 9 distinct pathogens in Bulkley River steelhead, though only two were sufficiently prevalent to evaluate their influence on host-physiology.

### 2.5.1 Bacterial pathogens *Ca. B. cysticola* and *F. psychrophilum*

Steelhead trout migrating up the Bulkley River had high observed prevalence of bacterial pathogens *Candidatus Branchiomonas cysticola* and *Flavobacterium*

*psychrophilum*. Both pathogens have been found at high prevalence in wild salmon across British Columbia (Bass et al., 2017; Teffer et al., 2017). The low temperature of the Bulkley River during sampling (7.1±0.3 °C) may explain the higher prevalence of *F. psychrophilum*, which is typically most prevalent and severe at temperatures below 10 °C (Holt 1987). *F. psychrophilum* was also the most prevalent agent identified in rainbow trout from the Babine River (Skeena watershed) where temperatures were even cooler at the time of sampling (Wellband and Heath, 2009). This agent is responsible for bacterial cold-water disease that is characterized by necrosis of the fins and caudal regions, organs, and musculature (Starliper 2011). The prevalence of *Ca. B. cysticola* in Pacific salmon species is generally higher in warmer water temperatures exceeding 16 °C (Bass et al., 2017; Teffer et al., 2017). Although there has been little evidence to suggest *Ca. B. cysticola* increases mortality in captured salmon (Teffer et al., 2017; Gunnarsson et al., 2017), there can be sublethal consequences of *Ca. B. cysticola* such as proliferative gill formation and bacterial filled cysts in the gills (Toenshoff et al., 2012; Mitchell et al., 2013). No visual symptoms of disease were present in steelhead, but both agents were generally associated with negative or neutral expression of most immune genes (e.g. CD8a, Il-8, MHCI, IgT, and IgMs). This relationship between the productivity of *F. psychrophilum* and downregulation of immune genes has also been observed in adult sockeye salmon, though *Ca. B. cysticola* had opposite effects of *F. psychrophilum* in that study (Teffer et al., 2017). Similarly, *F. psychrophilum* was negatively associated with immune genes (TNF, IFN, and CXC8) in immune challenged wild rainbow trout (Wellband and Heath, 2009). Downregulation of these genes may reflect a relationship between immunosuppression and microbe productivity. The greatest downregulation was

observed in genes involved in the humoral immune response (IgT and IgMs), which are known to be suppressed by *F. psychrophilum* (Barnes and Brown, 2011; Teffer et al., 2017). Other indices related to metabolic stress (plasma lactate and cortisol) have shown positive associations with *F. psychrophilum*, while osmoregulatory measures (plasma osmolality, chloride, and sodium) have shown negative associations with the microbe (Hruska et al., 2010; Bass et al., 2017; Teffer et al., 2017). In addition to these bacterial agents, pathogens from other taxa were also detected in Bulkley steelhead at lower prevalence.

#### 2.5.2 *Sphaerothecum destruens* and low prevalence microbes

Approximately half of the Bulkley River steelhead tested positively for *Sphaerothecum destruens*; a eukaryotic generalist parasite that can infect fish in both freshwater and marine ecosystems (Kent, 2011; Andreou and Gozlan, 2016). *S. destruens* has resulted in sub-lethal consequences for infected cyprinids including inflammation, lesions, and cell death (Andreou et al., 2011). *S. destruens* was also associated with mortality in winter-run juvenile chinook salmon, though juvenile rainbow trout seemed to be more resilient to infection (Arkush et al., 1998). Of the three microbes that had high prevalence in steelhead, there were no strong correlations in their relative loads to suggest similar infection requirements. This is contrary to previous studies on salmon highlighting a moderate-strong relationship between *Ca. B. cysticola* and *F. psychrophilum* (Bass et al., 2017; Teffer et al., 2017). These surveys were undertaken in the Fraser River watershed of southwestern, B.C., which is several hundred kilometres south of the Skeena River watershed where the current study was completed. Differences between the watershed environments (temperature, water chemistry), terrestrial

surroundings, community assemblage, and site-specific stressors are likely to have influenced pathogen transmission among host-species (Ferguson et al., 2003; Hullar et al., 2006). These studies also focused on semelparous species whose condition declines severely in preparation for spawning relative to the iteroparous steelhead in the present study (e.g. Bass et al., 2017; Teffer et al., 2017).

All other microbes had low prevalence in Bulkley River steelhead (Table 1), and thus no attempts were made to quantify their influence on host-gene expression despite previous evidence that they can be pathogenic and result in mortality (Gunnarsson et al., 2017). *Ichthyophthirius multifiliis*, a common agent in many fish populations, is the causative agent of white-spot disease, or “ich” (Schäperclaus, 1991). This agent was suggested to result in pre-spawn mortality as high as 80% in sockeye salmon on spawning grounds of the Babine River within the Skeena watershed (Traxler et al., 1998). *Piscichlamydia salmonis* and *Loma salmonae* are sources of gill disease (Schmidt-Posthaus et al., 2012; Speare and Lovy, 2012) while *Paranucleospora theridion* can result in proliferative gill inflammation (Nylund et al., 2010; Nylund et al., 2011). *Tetracapsuloides bryosalmonae* is the causative agent of proliferative kidney disease (Hedrick et al., 1993).

Low prevalence of these agents in Bulkley River steelhead may be a result of the relatively pristine nature of this water body compared to other salmon rivers in British Columbia. There is minimal habitat loss or alteration to increase fish stress, considerable distance from aquaculture operations where disease prevalence can be high, and cool temperatures that may limit disease development (Harvell et al., 2002). The optimal temperature ranges for *Ichthyophthirius multifiliis* (Aihua and Buchmann, 2001),

*Piscichlamydia salmonis* (Schmidt-Posthaus et al., 2012), *Loma salmonae* (Kent and Speare, 2005), *Tetracapsuloides bryosalmonae* (Foott and Hedrick, 1987), and *Ichthyophonus hoferi* (Kocan et al., 2009) are warmer than those recorded during our sampling period (mean=7.1 °C, 3.0-11.5 °C) suggesting they could increase in prevalence and pathogenicity with warming water temperatures in the future.

Patterns in the expression of a suite of genes associated with viral disease response in salmon were identified in this population. This response is characterized by strong up-regulation of RSAD, IFIT5, Mx, NFX, DEXH, VAR1, X52Ro, and IFI44A (Miller et al., 2017). However, the disease panel used in this study did not indicate a viral infective agent was present. Consequently, there is a distinct possibility that there are viruses present in the steelhead population that were not included in our screen. Samples from fish with this viral signature will undergo high throughput sequencing in the future to identify potential viral agents causing this unique gene profile.

### 2.5.3 Temperature and host-physiology

Water temperature at the time of capture was most positively associated with the expression of genes related to acid-base regulation (CA4), oxygen transport (HBA), inflammation (SAA), and oxidative stress (SEPW1). Expression of these genes is likely a response to the availability of oxygen in the water in relation to temperature. As water temperatures increase, dissolved oxygen decreases and CO<sub>2</sub> accumulates in the bloodstream. In the gills, CA4 would be activated to catalyze the hydration of CO<sub>2</sub> to H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> for export to the water (Gilmour and Perry, 2009). This corresponds to the observed increase in blood acidity in *O. mykiss* during warmer water temperatures (Kieffer et al., 1994; Chapter 3). Lactacidosis also increases with warmer temperatures in

rainbow trout and steelhead (Kieffer et al., 1994; Chapter 3), which would have suggested higher expression of LDHb, but this was not the case. Decreased availability of oxygen would also call for greater expression of HBA to increase the capacity for oxygen transport in the gills, which has also been observed in rainbow trout and sockeye salmon (Jewett et al., 1991; Evans et al., 2011). Water temperature also showed a positive association with SEPW1 that functions as an antioxidant (Whanger 2009). Expression of SEPW1 was also elevated in adult sockeye held at water temperatures above 19 °C compared to 13-14 °C (Jeffries et al., 2014). Despite evidence that temperature may have influenced oxygen uptake in Bulkley River steelhead, water temperature did not result in marked changes in the expression of hypoxia-inducible factors (HIFAs) that were responsive to temperature in the crucian carp (*Carassius carassius*; Rissanen et al., 2006). Temperature was also found to correlate with metabolic genes involved in the tricarboxylic acid cycle (IDH3B), and the electron transport chain (COX6B), although these genes accounted for a relatively small amount of variation in Bulkley River steelhead. Wild rainbow trout in similar temperature conditions as the Bulkley River showed increased resting state expression of glycolytic genes (PK and PEP3K) with temperature (Wellband and Heath, 2009), but glycolytic genes were generally not upregulated with temperature in Bulkley steelhead. Perhaps most relevant to steelhead during migration are changes to active metabolic rates, which also increase with water temperature in *O. mykiss* (MacLeod and Pessah, 1973).

Although water temperature appeared to upregulate genes responding to oxygen availability, it was associated with neutral or down-regulation of most immune genes (e.g. IL17 and MMP13), despite most research suggesting suppressed immune function

with colder temperatures (Bly et al., 1997). Heat shock proteins also had a generally neutral or negative relationship with water temperature. Previous work evaluating the influence of thermal stress on the transcriptome of sockeye and pink salmon suggests upregulation of heat shock proteins (HSP90AA1, HSPAB1, SERPINH1) with warm temperatures (Miller et al., 2009; Evans et al., 2011; Jeffries et al., 2014). Water temperatures were lower on the Bulkley River and were likely not altering protein conformation or protein aggregation in steelhead to a degree that would necessitate changes in the expression of this family of chaperone proteins.

#### 2.5.4 Sampling location

Wild steelhead captured by dip net at Witset Falls were found to have greater microbe species richness, and relative loads of *Ca. B. cysticola*, *F. psychrophilum*, and *S. destruens*, and differential gene expression compared to fish captured by angling. Angled fish had greater expression of immune genes, particularly those related to humoral immunity such as IgT and IgMs. This could be reflective of greater loads of *F. psychrophilum* in netted fish or reflect changes in immunity over the course of migration (Teffer et al., 2017). These unexpected differences between fish captured at Witset falls vs. upstream of the falls may be explained by differences in the stage of migration, or sampling method, and may provide some evidence that Witset Falls is a natural barrier influencing pathogen dynamics during steelhead migration.

Witset Falls constitutes the most difficult hydrological stretch of the Bulkley River. Steelhead are known to delay their migration at this point (Chapter 4; Kintama et al., 2009), resulting in large densities of steelhead below the falls. Confinement of steelhead below this migratory corridor may increase exposure to infected individuals and

correspondingly increase pathogen prevalence and loads at this location (Gilhousen, 1990). Although not empirically tested here, higher pathogen loads at the falls suggest it may act as a barrier to fish with greater pathogen burdens (i.e. migratory culling; Bradford et al., 2010; Altizer et al., 2011). Telemetry work indicated that fish captured from Witsset Falls are in the active stage of upstream migration, while steelhead angled upstream of the falls are primarily in the final holding stages of migration (Chapter 4; Økland et al., 2001). Fish captured at the falls had greater expression of CA4, HBA, and SEPW1 that would be expected for actively migrating fish with greater oxygen requirements than holding fish. Fish captured upstream of the falls also had greater expression of humoral immune genes (IgMs, IgT) and B2M, MHCIIb, and IRF1 that tended to increase over the course of migration in Sockeye salmon (Teffer et al., 2017). Steelhead upstream of Witsset Falls may therefore represent successful migrants that are at or near spawning sites. This notion is partly supported by telemetry work that was completed concurrent to the present study.

A concurrent telemetry study evaluating migratory survival following angling suggests that pathogen-attributed mortality during fall migration is low for steelhead captured upstream of Witsset Falls. This study found that just 4.5% of steelhead died (three fish) following angling. Mortality for two of these fish was a direct result of hooking injury and extensive bleeding (Chapter 3) though the source of mortality for the other fish was unclear. If this mortality was related to pathogen burden, it would still suggest that pathogen-induced mortality does not exceed 1.5% for angled steelhead during fall migration. Previous telemetry work at Witsset Falls also provides some support for the theory that Witsset Falls may cull fish with greater pathogen burdens. This work

indicated that approximately 8-12% of steelhead that enter the canyon/falls area do not successfully migrate upstream of the falls (Kintama et al., 2009; Chapter 4). As increased pathogen loads (including *Ca. B. cysticola* and *F. psychrophilum*) entail reduced swimming capabilities, lethargy, negative effects on movement, and decreased survival (Schachte 1983; Kent et al., 1989; Barber et al., 2000; Wagner et al., 2003; Risley et al., 2018), successful migrants (angled fish) may be biased towards fish with lower pathogen loads relative to the higher pathogen loads observed in actively migrating fish caught by dip net. Given the confounding nature of our sampling method (dip net at the falls and angling above the falls) it is unclear whether observed differences might be attributed to sampling locations, or whether pathogen loads and host-physiology altered steelhead vulnerability to capture gears.

Previous research has highlighted that various fishing gears have sampling bias that may select for fish with phenotypes that make them more vulnerable to capture (Philipp et al., 2015). Angling is suggested to capture fish with greater aggression/boldness (Arlinghaus et al., 2016), higher metabolic rate (Hessenauer et al., 2015), lower metabolic stress (Louison et al., 2017), and ultimately fish with the highest fitness levels (Sutter et al., 2012). Angled fish had lower expression of genes responsive to oxygen availability and metabolism, which may provide some support for this notion of capturing fish with lower metabolic stress. Simulation models indicate that active individuals are generally considered the most vulnerable to capture by angling (Alós et al., 2012), which would suggest holding steelhead are less likely to be captured. However, these models are typically based off a relatively random distribution of anglers within a habitat. Steelhead anglers almost exclusively target slow moving water or refuge

sites where steelhead are known to hold, and the water is conducive to ‘swinging a fly’. Keefer et al., (2009) found that steelhead holding near tributary mouths were the most vulnerable to the recreational fishery. Unlike other Pacific salmon that attack lures and flies out of aggression, steelhead will feed opportunistically during spawning migrations (Moyle, 2002). Pathogens can reduce feeding behaviour in fish and therefore a steelhead’s vulnerability to angling (Schachte 1983; Brown et al., 1987; Damsgard et al., 1998; Ajuzie et al., 2008; Henriksen et al., 2013). If angling actively selects for ‘healthier’ individuals, then recreational harvest could influence pathogen dynamics given that the ‘healthiest’ individuals would be removed from the population. Future research should consider both possibilities of migratory culling at natural barriers, and pathogen-related sampling bias associated with angling vs. net capture.

#### *2.5.5 Sex and size*

Sex-specific differences exist in energy allocation during the spawning migrations of salmonid fishes. Female salmon invest expansive amounts of energy into egg production (~20% body mass; Hendry et al., 2000), as well as competing, clearing, and defending nest sites (Foote 1990; Steen and Quinn, 1999; McPhee and Quinn, 1998). Males contribute less towards their gonads (Hendry et al., 2000), but invest substantially into secondary sexual characteristics (Järvi et al., 1990) and direct competition for females (Quinn et al., 1996). These sex-specific differences in energy allocation could result in sex-specific differences in infection, stress, metabolism, and mortality during migration. Mortality of sockeye salmon held at 19 °C was greater than 98% in females compared to just 54% in males (Jeffries et al., 2012b). Female salmon have also shown higher indices of stress in plasma compared to males (Donaldson et al., 2014; Teffer et

al., 2017), but there was little support for this in Bulkley River steelhead (Chapter 3). Despite evidence of sex-specific migratory physiology at the organismal level, these differences are often not observed at the level of the transcriptome in most studies (Miller et al., 2009; Jeffries et al., 2012a; Jeffries et al., 2014). However, Teffer et al., (2014) found greater expression of immune-related gene transcripts in the gills of female sockeye salmon compared to males. There was no clear difference in gene expression for male and female steelhead in the Bulkley River, nor were there differences in pathogen loads or richness. These differences would only be expected if energy allocation differed during early migration, altering individual susceptibility to pathogens. A study on the Yakima River (Columbia Basin) suggests that summer-run steelhead females have already begun reproductive maturation several months prior to spawning (Pierce et al., 2016). Similarly, males had already developed observable secondary sexual characteristics (kypes) during sampling in the fall. Both sexes may have therefore contributed similar amounts of energy to reproduction, explaining the lack of sex-specific physiological differences in Bulkley River steelhead. This hypothesis is consistent with Penney and Moffitt (2014) that found similar energy density, lipid, and protein storage during early migration in male and female steelhead from the Snake-Columbia River.

The availability of resources can also influence the energetics and corresponding ability to reproduce successfully while maintaining critical functions such as immunity (Cox et al., 2010). Larger fish typically begin with more resources than smaller ones (Shuter and Post, 1990; Breck, 2014), and also have lower specific metabolic rates, and swim speed requirements which can decrease their relative energy consumption during migration (Weatherley and Gill, 1995). Fork length was close in ordination space with

water temperature and appeared to be associated with down regulation of genes involved in tissue repair (KRT8) and the inflammatory process (IL17D, MMP13) but its relationship with the ordination was not significant. Similarly, expression of immune and metabolic genes was not correlated with body size in wild rainbow trout from the Babine River of the Skeena watershed (Wellband and Heath, 2009), suggesting size may not influence expression of these gene groups within *O. mykiss*.

### 2.5.6 Conclusions

Positive detections of nine microbe species of bacterial, viral, fungal, myxozoan, and other eukaryotic origin were found in steelhead trout from the Bulkley River. The majority of fish were infected with *Ca. B. cysticola*, *F. psychrophilum*, and *S. destruens*. The relative loads of *Ca. B. cysticola*, and *F. psychrophilum* tended to have a neutral or negative effect on stress and immune genes, with the strongest suppression of genes related to the humoral response. As most microbes at low prevalence in Bulkley River steelhead have optimum temperatures higher than those observed here, these pathogens may become more severe with warming temperatures arising from climate change (Marcogliese, 2008). Water temperature at the time of capture was positively correlated with genes responsive to oxygen availability and was also associated with neutral or down-regulation of most immune genes. Microbe richness and loads were greater in fish captured at vs. upstream of the falls, and fish at the falls had generally lower expression of stress and immune genes. Numerous explanations could account for the higher microbe loads and richness at Witset Falls, related to the sampling location, stage in migration, sampling bias, among others. I encourage future research to consider the potential for migratory culling at similar natural or anthropogenic barriers to migration.

This study indicated little influence of sex or size on pathogen susceptibility and host-physiology during the early component of steelhead migration. Results from this study will provide an important baseline when evaluating microbe prevalence in the future which is particularly salient for steelhead given the threats they face across their native range. Future work could combine tools in molecular genetics and biotelemetry (see Miller et al., 2011 as example) to evaluate the influence of various pathogens on steelhead survival closer to the spawning period when energy stores have been depleted and steelhead may be more vulnerable to infection.

# **Chapter 3. Consequences of catch-and-release angling on the physiology, behaviour and survival of wild steelhead *Oncorhynchus mykiss* in the Bulkley River, British Columbia**

## **3.1 Abstract**

Steelhead, the anadromous form of rainbow trout (*Oncorhynchus mykiss*), is one of the most coveted recreationally targeted salmonids worldwide, and catch-and-release (C&R) is commonly used as a conservation strategy to protect wild stocks. Nevertheless, little research has examined how wild steelhead respond to capture and handling. During a summer-run recreational fishery on the Bulkley River in British Columbia, I used non-lethal blood sampling and radio telemetry to assess the physiological stress response, post-release behaviour, and survival of wild steelhead exposed to either 0 sec, 10 sec, or 30 sec of air exposure, over a range of water temperatures, fight times, and landing methods. Steelhead that were air exposed following landing had greater reflex impairment and moved further downstream immediately following release than fish kept in the water, though there was no observed difference in movement two weeks after capture. Overall, angled fish had significantly greater blood lactate levels than baseline levels (obtained from a subsample of fish dip netted from the river) suggesting a general stress response to angling and handling. Regardless of air exposure treatment, water temperature was positively associated with blood lactate and negatively associated with blood pH. Other variables such as fish body size (mm) and fight time (sec) had little influence on any of the physiological or behavioural variables. Estimated 3-day survival of steelhead was 95.5%, with deep-hooking as the primary source of mortality. Over-winter mortality of caught-and-released fish was estimated at 10.5%, with an estimated

total pre-spawn mortality of 15.0%. This study is the first to evaluate the factors that influence C&R outcomes in wild steelhead in a recreational fishery. Findings suggest that steelhead anglers should not remove fish from the water, and that anglers should be cautious (minimize handling and air exposure) when water temperatures are warmer.

### **3.2 Introduction**

*Oncorhynchus mykiss* are an iteroparous fish species that maintain populations with a range of life-history strategies (Moore et al., 2014). These life-history strategies are typically described in terms of the freshwater resident forms (rainbow trout) and the anadromous forms (steelhead). Unlike other iteroparous salmonids with anadromy (e.g. Atlantic salmon; *Salmo salar*), steelhead over-winter in freshwater and spawn in tributaries during the spring, prior to emigrating back to the ocean as kelts (Quinn, 2005). Their spawning migrations often span hundreds of kilometres resulting in considerable energy expenditure (94% loss in lipid content of white muscle tissue; Penney and Moffitt, 2014). This severe energy depletion coupled with down-regulation of feeding hormones during their reproductive cycle may make steelhead particularly susceptible to anthropogenic stressors that result in additional energetic costs (Fenkes et al., 2016). Previous research has highlighted the negative influences of warm water temperatures (Wade et al., 2013), habitat degradation (NRC, 1996), water pollution (Suttle et al., 2004) and fisheries interactions (Andrews and McSheffries, 1976; Stewart and Lewysnky, 1988) on steelhead populations. Given the multifaceted stressors steelhead face during their upstream migration, Kendall et al., (2015) suggested the need to further evaluate the role of anthropogenic impacts (including fisheries) on steelhead.

Capture by fisheries can be one of the most severe acute stressors imposed on fish throughout their lives (Davis, 2002). Previous work investigating commercial net fisheries for salmon has shown significant by-catch mortality of wild steelhead following discard (J.O. Thomas & Associates LTD., 2010). Discard may also occur in recreational fisheries (typically termed catch-and-release; herein, C&R) either voluntarily or to comply with regulations (Arlinghaus et al., 2007). Comparatively little is known about recreational fisheries discard mortality (Cooke and Suski, 2005), and even less is known about the impacts of recreational angling practices on steelhead physiology, behaviour, and survival (but see Nelson et al., 2005 for the general effects of angling on winter-run steelhead). Given the widespread decline and conservation status of wild steelhead populations (Gayeski et al., 2011; Good et al., 2005; Smith et al., 1990), recreational fisheries for steelhead have been established as primarily C&R in hopes of conserving wild populations. The efficacy of C&R as a conservation tool, however, is contingent on released fish surviving and incurring negligible fitness consequences (Cooke and Schramm, 2007; Wydoski, 1977).

Fish may be subject to considerable stress and even mortality during a capture event, and this may be related to environmental variables, intrinsic biotic factors (e.g. fish condition, disease presence, size, age, sex), and angler behaviour (e.g. gear choice, fight time, and air exposure; reviewed by Cooke and Suski, 2005). Prolonged fight times, air exposure, and hooking injury can lead to blood acidosis, hypercapnia, and injury, respectively (Wood et al., 1983; Ferguson and Tufts, 1992; Meka, 2004). Previous work has suggested general guidelines for anglers to adhere to including the minimization of fight times, air exposures, and proper gear choice (Brownscombe et al., 2017). Although

generalities can be made, differences exist in morphology, life-history, and surrounding environments creating the need for species- and context-specific evaluations (Cooke and Suski, 2005). Salmonid species are considered some of the most valuable and coveted species to recreational anglers, and have been subject to numerous studies assessing the extent of sublethal consequences and mortality following recreational fisheries encounters (Gjernes et al., 1993; Schreer et al., 2005; Pope et al., 2007; Boyd et al., 2010; Lennox et al., 2015). Albeit, even amongst salmonids the response to fisheries capture is consistently context- and fishery-specific (Raby et al., 2015; Patterson et al., 2017). As anglers have a considerable role on the outcome of an angling event (Cooke et al., 2017), it is relevant for recreational fisheries to have scientifically based best practices for anglers to follow to minimize harm on released fish. Despite the popularity of steelhead as a recreational fish species (Kelch et al., 2006), little to no research has evaluated the response of wild steelhead to various C&R angling practices. An exception is a study on the Chilliwack River of British Columbia which investigated the movement and survival of winter-run steelhead following catch-and-release, but focused primarily on the differences between fish of hatchery and wild origin (Nelson et al., 2005).

The objectives of this study were to evaluate the sub-lethal impacts and survival of wild steelhead following C&R angling. Steelhead were assessed for the presence of the righting reflex (an effective and non-invasive proxy for stress and mortality following fisheries interactions; Danylchuk et al., 2007b; Davis, 2010) and for physiological indicators, specifically glucose, lactate, and pH (Barton et al., 1998). A separate group of steelhead was monitored for immediate post-release movement, long-term migration, and mortality. It was anticipated that air exposure would impair reflexes, increase

physiological alterations, and promote downstream post-release movement of angled wild steelhead. Findings from this research will help refine management strategies and identify best handling practices for wild steelhead.

### **3.3 Methods**

#### *3.3.1 Study site and collection methods*

Steelhead were sampled from the Bulkley River, British Columbia from September 17<sup>th</sup> to November 7<sup>th</sup>, 2016. The Bulkley River is located ~250 rkm inland, stretching an additional 141 km to the Bulkley-Morice confluence (Ministry of Forests, Lands, Natural Resource Operations and Rural Development (FLNRORD), 2013). The Bulkley River watershed drains an area of approximately 12,000 km<sup>2</sup>, making it the largest tributary of the Skeena River. The river is considered relatively pristine with no manmade barriers (i.e. dams) to fish migration. As a result, the river has maintained an entirely wild summer-run steelhead population averaging 21,520 (9,735 to 41,428) individuals from 1999 to 2016 (Witset [formerly Witset] Mark-Recapture, 2017) and contributes greatly to the world-renowned Skeena recreational fisheries that are estimated to be worth nearly \$53 million CAD annually (Counterpoint Consulting, 2008). Recreational anglers can access the river by jet boats, pontoon boats, or walk-in sites and are permitted to use both fly fishing and spin-cast equipment. Starting in 1991, the mandatory C&R of wild steelhead has become part of the B.C. FLNRORD's fishing regulations for the entire Skeena Watershed; the rule was expanded to all of British Columbia in 1997.

Steelhead were captured by approximately 30 different recreational anglers using fly fishing (spey rods, flies size #8+), spin-cast fishing (various sizes of inline spinners and artificial worms), and by centre pin (a free spooling reel, various sizes of inline spinners and artificial worms) while wading from shore. Anglers represented all levels of experience, from first time steelhead anglers to anglers that fish every day for the entire steelhead season. Most fish were captured by experienced steelhead anglers, which is representative of the general angling public of the Bulkley River. All fish were captured upstream of Witsset Falls from rkm 314 to 407 (Figure 1), using single barbless hooks as per the recreational fishing guidelines in the Skeena region (FLNRORD, 2017). Fish were landed with the assistance of another angler, who either netted the fish using a rubber or nylon landing net or grabbed the caudal peduncle of the fish to secure it near shore (tail-grab). It was determined before the angling event which method would be used based on whether the angler had brought a net along. These conditions allowed anglers to unhook steelhead without lifting them out of the water at any point. Steelhead were selected randomly to be measured for blood physiology parameters, or radio-tagged following capture, thus creating two separate groups from here on referred to as the ‘physiology’ group and the ‘movement’ group, respectively.

### *3.3.2 Quantification of the angling event*

For each capture event, the fight duration (sec), anatomical hooking location, hook removal difficulty, water temperature (°C), fork length (cm), and sex were recorded. The fight duration was considered the time from hooking to landing by either tail-grab or landing net. Hook removal difficulty was determined by a 1-5 ordinal ranking system that reflected the effort needed by the angler or guide to remove the hook from the fish. A

score of '1' indicated the hook was removed with no effort (hook fell out once the fish was landed), while a score of '5' indicated that substantial effort was needed to remove the hook (pliers were required to remove the hook). Water temperatures at the time of capture were taken using a handheld digital thermometer (Taylor Precision Digital Thermometer, #9847, Taylor USA, Oak Brook, IL, USA). Water temperature throughout the season was taken using a data logger (HOBO Water Level Data Logger, #U20L-01, Bourne, MA, USA) that recorded every 15 min. Discharge rates were taken from the Bulkley River at Smithers using Environment and Climate Change Canada's Real Time Hydrometric Data search engine.

### *3.3.3 Reflex test*

A righting reflex test (equilibrium) was used as an indication of reflex ability after angling and air exposure. To assess the righting reflex, fish were flipped ventral side up and monitored for their ability to return to normal orientation within 3 sec (outlined in Raby et al., 2012). To minimize handling of captured steelhead, only the righting reflex test was evaluated. This reflex is typically the one that is most "responsive" when salmonids are exhausted (Raby et al., 2012). Reflex tests were completed immediately following capture for fish that would be included in the physiology group, and immediately before release for fish in the movement group due to logistical constraints.

### *3.3.4 Post-release physiology*

Following landing, fish to be included in the physiology group were transferred to a recovery bag and included into one of three air exposure treatment groups; 0 sec (N=18), 10 sec (N=12), and 30 sec (N=15) by lifting the bag out of the water. Treatment

groups were selected to be representative of the range of common practices; final selection was based on regional consultation (personal communication with regional anglers and guides, 2016). Fish were then held in the recovery bag for approximately 20 min to obtain elevated values for the blood physiology stress indices. It should be noted that these values peak between 5-15 min for lactate and 120-240 min for glucose in hatchery rainbow trout plasma, although glucose is still significantly elevated compared to baseline values 15 min after stress (Perrier et al., 1978; López-Patiño et al., 2014). As holding steelhead for 120 min was not feasible while working with anglers, I opted to hold fish for 20 min to capture peak lactate, and elevated glucose levels. 2 mL of blood were non-lethally sampled from the caudal vasculature using a 21 g x 1.5” needle (BD Vacutainer needles, #360213, Mississauga, ON, Canada), and a 4 mL Vacutainer (59 USP units of Lithium Heparin, Mississauga, ON, Canada). Blood was analyzed for glucose ( $\text{mmol}\cdot\text{L}^{-1}$ , Accu-Check Compact Plus, Roche Diagnostics, Basel, Switzerland), lactate ( $\text{mmol}\cdot\text{L}^{-1}$ , Lactate Plus, Nova Biomedical Corporation, Waltham, MA, USA), and pH (HI-99161 w/automated temperature compensation, Hanna Instruments, Woonsocket, Rhode Island, USA) using point-of-care devices that have been previously validated for use on fish (Stoot et al., 2014). The stress responsive values of angled fish were compared to baseline physiology values obtained from steelhead sampled within 3 min of an acute stressor (Cooke et al., 2013; Lawrence et al., In press). This was not feasible for fish captured by angling (5 min fight times), so opportunistic sampling was completed at the Wet’suwet’en salmon fishery/steelhead mark-recapture program at Witsset Falls (rkm 314). Here, steelhead captured by dip net were immediately ( $<15$  sec) transported to the sampling trough and measured, as part of the long-term mark-recapture

project (<30 sec of handling). Baseline samples were obtained between September 22-29 ( $8.6 \pm 0.5$  °C) during the final week of the Wet'suwet'en fishing operations, while samples from angled fish were obtained September 20 to October 29, 2016 ( $7.0 \pm 0.3$  °C).

### *3.3.5 Post-release movement*

Following C&R, landed steelhead included in the movement group were secured in the river in a flow through tagging trough and had a radio-transmitter (4.7 g weight in air, 220-441 day battery life, 33 pulses per minute; Series F1970, Advanced Telemetry Systems, Isanti, MN, USA) attached externally (Jepsen et al., 2015). Transmitters were attached using two stainless steel 18 g surgical needles inserted into the dorsal musculature posterior to the dorsal fin. Wire attached to the tag was then threaded through the needles, and the needles were removed. The wire was then secured to the fish using steel crimps, with small plastic backing plates separating the crimps from the body of the fish to minimize tissue irritation. Fish were then included into one of three air exposure treatment groups; 0 sec (N=22), 10 sec (N=25), or 30 sec (N=21). To conduct air exposures, tagged fish were lifted out of the water for the duration of the air exposure treatment. Fish were returned to the water and were assessed for the righting reflex and released. If a fish did not swim away immediately, it was held loosely by the caudal peduncle until it was able to swim away on its own (all <15 sec). Presence of an externally-attached telemetry tag (7.0 g) had little to no influence on swimming performance or blood physiology of Atlantic salmon (450-590 mm; Thorstad, 2000) so it was expected that the tag effect (4.7 g) was negligible for the wild steelhead in our study (508-870 mm). Further, tags weighed approximately 0.1% of average steelhead body

mass and were therefore well below the recommended tag to fish weight ratio of 2.0% (Smircich and Kelly, 2014).

Fish were tracked manually using a radio telemetry receiver and a 3-element Yagi antenna (Lotek Biotracker, Lotek Wireless, Ontario, Canada). Fish were located using zero-point tracking (successive gain reductions; Cooke et al., 2012), and had their positions saved using a handheld GPS instrument (Garmin GP 60 Handheld GPS Device, 010-00322-00, Garmin, Olathe, KS, USA) set to Universal Transverse Mercator projection (UTM). The location of fish 20 min after release was recorded as an indication of immediate post-release movement. Tracking was completed opportunistically by jet-boat and raft depending on the stretch of river anglers visited that day. Most sections of the river were tracked weekly (September 17<sup>th</sup>-November 6<sup>th</sup>). The entirety of the Bulkley and lower 50 km of the Morice River were tracked by raft between October 23<sup>rd</sup>-27<sup>th</sup>, 2016 and November 2<sup>nd</sup>-6<sup>th</sup>, 2016. For fish that were found twice within a 3-day period, average daily movement rate was calculated as the absolute movement rate regardless of up or downstream direction (Richard et al., 2014). A post-winter season relocation was conducted from April 6<sup>th</sup> -12<sup>th</sup>, 2017 by rafting, hiking, driving, and aircraft (Cessna-185 fixed wing telemetry attachment) to identify mortalities. Tracking spanned the entire Bulkley River study site and the lowermost 70 km of the Morice River, as well as the first 30 km of the Telkwa River (a tributary of the Bulkley River). A final tracking was completed by raft between August 21<sup>st</sup>-30<sup>th</sup>, 2017 to identify in-river mortalities and emigration rates from the 141 km study site.

Survival was estimated at four time intervals; 3-day survival, survival to winter, overwinter survival, and survival to emigration. Survival estimates were adjudged based

on individual fish movement patterns (Donaldson et al., 2008). If a fish moved upstream at any point it was considered alive at that point and all previous points in time. If a fish moved upstream and maintained its position in the river it was considered alive at that point and all previous points in time. If a fish moved multiple kilometers downstream within the first few days after capture and never moved back upstream it was considered a mortality. These mortality designations were confirmed by the presence of a radio-transmitter in the study site during tracking in August, 2017, when surviving steelhead would have emigrated to the ocean. Fish that were not located at a certain time point were considered inconclusive as the potential outcomes of long distance migrations, tag malfunction, and predation events could not be discerned. As some fish could not be designated as true mortalities (Bird et al., 2017), survival rates are presented as estimated (assumes equal proportions of dead and alive fish among those not located in the spring), maximum (assumes all fish not located in the spring emigrated the system and survived), minimum (assumes all fish not located in the spring died) survival (example Gagne et al., 2017). Based on the wide-ranging movements of steelhead (including downstream movement) during migration on the Bulkley River (Kintama et al., 2009), it seems likely that most undetected fish would be survivors that emigrated from the study area. If they were dead fish they would have still been detected in the river after the spawning period, given that movement rates of dying/dead fish were low (<10 km downstream).

### *3.3.6 Data analysis*

Separate multiple regression models were created for the blood glucose, blood lactate, and blood pH response variables that included air exposure, fight time, sex, fork length, landing method, and water temperature as predictor variables. A logistic

regression model was used to predict the presence of the righting reflex with the same variables listed above (R function *glm*, specifying *family = 'binomial'*; R Core Team 2015). Only angled fish (0, 10, and 30 sec air exposure groups) were included for the reflex impairment and blood physiology candidate models as many angling-related variables were not relevant to fish collected via dip net (e.g. fight time, air exposure, landing method). In addition, only reflex assessments conducted on 'physiology' fish were included as a response variable in the righting reflex model. To compare physiology values across air exposure groups and baseline fish, a one-way Analysis of Variance (ANOVA) was conducted for each blood parameter, while the Chi-square test was used to evaluate the righting reflex.

ARCMAP GIS 10.0 was used to plot all location data onto a river line of the Bulkley and Morice Rivers, and potential spawning tributaries. Fish locations that fell adjacent to the river line were snapped to the nearest edge of the river using the 'near' function. The distances from the release sites to successive fish locations were determined using the 'create routes' and 'distance along a route' functions in the 'linear referencing tools' menu. All data were first projected into the UTM Zone\_9 projection.

A multiple regression model was used to evaluate the influence of air exposure, fight time, sex, fork length, landing method, and water temperature on immediate post-release movement. The relative position of fish approximately 2 weeks after capture (10-19 days) was available for most fish and was treated as a categorical variable with three levels of either upstream (>500 m up), no change ( $\pm$  500 m), or downstream (>500 m down) movement from the capture site. This variable was modeled using ordinal logistic regression with the *rms* package and included the same predictor variables used for the

immediate movement model (R function *lmr*; R Core Team 2015). This model was created to evaluate any longer-term impacts of catch-and-release practices on behaviour, and only included surviving fish. Ordinal logistic regression was used to evaluate the relationship between immediate post-release movement and relative position after 2 weeks. Reflex tests completed on ‘movement’ fish were used to predict future movement using one-way ANOVAs and Chi-square tests. Daily movement rate was modeled using a generalized linear model and the Poisson distribution for right skewed count data and included average daily water temperatures and discharge as explanatory variables. Given the number of variables that varied across each angling event, I conducted Chi-square tests and ANOVAs across air exposure treatments to evaluate whether there were significant differences in fork lengths, fight times, water temperatures, anatomical hooking locations, sex proportions, capture methods, capture dates, or landing methods across air exposure treatments for both physiology and post-release movement groups. Our final models were restricted to include the variables considered most relevant to the outcome of an angling event, while minimizing the number of variables per observation (Austin and Steyerberg, 2015; Brownscombe et al., 2017). Further, variance inflation factors were used to evaluate the extent of collinearity between variables but little evidence of collinearity existed (all VIF <3.0). Model assumptions were evaluated by analyzing diagnostic plots of residuals (standardized residuals verse theoretical quartiles, residual verses fitted values, variance of residuals, and Cook’s distance). Based on Cook’s distance values, and the fact that deeply hooked fish died shortly after release and floated downstream, the two deeply hooked radio-tagged fish were removed from further analyses on post-release movement. One fish that died a few days after release was only

included for immediate post-release movement. One fish was dragged onto shore during capture and was removed from behavioural assessments, but was included for post-release mortality. One fish was only detected upon release, and was therefore included for the immediate movement model, but was excluded for assessment of longer-term movement and post-release mortality. An additional two fish were excluded that had faulty tags producing unreliable location data. Significant differences between factored levels were evaluated using the Tukey post-hoc test at an alpha level of 0.05.

### **3.4 Results**

A total of 129 wild steelhead ( $687 \pm 7$  mm FL) were captured by fly angling (n=92), spin-casting/centre pin angling (n=23), and dip net (n=14). Fight time ranged from 100 to 960 sec ( $299 \pm 15$  sec), and was positively correlated with the size of the fish ( $p < 0.01$ ). Fish were primarily hooked in the corner of the mouth (61%) but were also hooked in the interior of the mouth (17%), snout (8%), tongue (4%), tail (<1%), and under the jaw (<1%). Seven percent of hooks fell out prior to visual assessment so no location could be determined. No fish were hooked in the gills or esophagus. Three of five fish hooked in the tongue were considered deep hooked due to the extent of bleeding from the hooking site.

#### *3.4.1 Righting reflex*

Presence of the righting reflex in physiology fish was significantly lower for 10 sec ( $0.58 \pm 0.15$ ;  $p = 0.03, 0.01$ ) and 30 sec ( $0.47 \pm 0.13$ ;  $p < 0.01, < 0.01$ ) air exposed groups, than for 0 sec ( $0.94 \pm 0.06$ ), and baseline groups ( $1.0 \pm 0$ ; Figure 3.1). No other variable of fork length, fight time, water temperature, sex, or landing method had a strong influence on reflex ability (df=39, all  $p > 0.05$ ; Table 3.1). Radio-tagged steelhead that

failed the righting reflex test tended to have further immediate movement downstream than steelhead that passed the reflex test (F-value=4.66, df=65, p=0.03). However, this reflex test was not correlated with blood glucose, lactate or pH, or relative position after 2 weeks.

### 3.4.2 Physiology

The timing of blood sampling after the angling event was similar across the 0 sec ( $1116 \pm 49$  sec), 10 sec ( $1148 \pm 45$  sec), and 30 sec ( $1133 \pm 63$  sec) air exposed groups. Blood samples were taken in less than 3 min for the baseline group ( $151 \pm 24$  sec; N=14). There was no significant difference in fork lengths, fight times, water temperatures, anatomical hooking location, sex proportions, capture method, capture date, or landing method across air exposure treatments (all  $p > 0.05$ )

There was no significant difference in the blood glucose levels among baseline ( $2.5 \pm 0.3$  mmol•L<sup>-1</sup>), 0 sec ( $2.5 \pm 0.2$  mmol•L<sup>-1</sup>), 10 sec ( $2.2 \pm 0.3$  mmol•L<sup>-1</sup>), and 30 sec ( $2.5 \pm 0.3$  mmol•L<sup>-1</sup>) air exposed fish (F-value=0.32, df=3, p=0.81). Blood glucose levels were significantly higher for steelhead that were tail-grabbed ( $2.6 \pm 0.2$  mmol•L<sup>-1</sup>) compared to those landed by net ( $2.2 \pm 0.2$  mmol•L<sup>-1</sup>; t-value=2.68, p=0.01; Table 3.2, Figure 3.2A). Males ( $2.7 \pm 0.1$  mmol•L<sup>-1</sup>) also had significantly higher blood glucose levels than females ( $2.2 \pm 0.2$  mmol•L<sup>-1</sup>) regardless of treatment (t-value=2.25, p=0.03, Figure 3.2B). Blood lactate levels of fish that were angled and air exposed for 0 sec ( $5.1 \pm 0.5$  mmol•L<sup>-1</sup>; p<0.01), 10 sec ( $5.0 \pm 0.4$  mmol•L<sup>-1</sup>; p=0.01), or 30 sec ( $5.3 \pm 0.4$  mmol•L<sup>-1</sup>; p<0.01) were significantly higher than baseline lactate levels ( $3.0 \pm 0.4$  mmol•L<sup>-1</sup>). There was no significant difference in blood lactate levels among air exposure groups (F-value=0.09, df=42, p=0.92, Table 3.2, Figure 3.4A). Blood lactate was

positively correlated with water temperature ( $t$ -value=2.64,  $df$ =37,  $p$ =0.01; Table 3.2, Figure 3.4A). There was no significant difference in blood pH between the baseline ( $7.40 \pm 0.03$ ), 0 sec ( $7.53 \pm 0.04$ ), 10 sec ( $7.57 \pm 0.03$ ), or 30 sec ( $7.46 \pm 0.05$ ) air exposure groups ( $F$ -value=2.76,  $df$ =3,  $p$ =0.05, Figure 3.3C), although pH was negatively correlated with water temperature ( $t$ -value=-3.52,  $df$ =37,  $p$ <0.01; Table 3.2, Figure 3.4B).

### 3.4.3 Post-release movement

The average tagging time from landing to tag attachment was  $283 \pm 16$  sec, and did not differ significantly between treatments ( $p$ =0.20). There was no significant difference in fork lengths, fight times, water temperatures, anatomical hooking location, sex proportions, capture method, capture date, or landing method, across air exposure treatments (all  $p$ >0.05). Movement of fish 20 min after release (immediate post-release movement) ranged from 149 m downstream to 99 m upstream. Fish air exposed for 10 sec ( $-13.8 \pm 7.2$  m; movement  $t$ -value=-2.64,  $p$ =0.01) and 30 sec ( $-14.6 \pm 4.6$  m;  $t$ -value=-2.47,  $p$ =0.02) moved downstream significantly further than 0 sec fish ( $8.8 \pm 6.3$  m) during their immediate post-release movement (Table 3.3, Figure 3.5). No other variables (fight time, landing method, sex, fork length, water temperature) had a significant influence on immediate movement following release (Table 3.3). Long-term (7 month) migration of steelhead ranged from 11.3 rkm downstream to 60.1 rkm upstream. The relative position of steelhead after 2 weeks was not significantly influenced by air exposure, fight time, sex, fork length, landing method, or water temperature (Table 3; Figure 3.6). Immediate post-release movement was not significantly correlated with the relative position of steelhead after 2 weeks ( $F$ -value=1.00,  $df$ =51,  $p$ =0.38).

The average daily movement rate of fish during the fall was significantly influenced by mean daily water temperature ( $z$ -value=-43.36,  $df$ =118,  $p$ <0.01), and tended to increase from 4.5 to 6.5 °C and remain low and constant from 6.5 to 11.7 °C (Figure 3.7). Average daily movement rate also had a significant relationship with mean discharge ( $m^3 \cdot s^{-1}$ ;  $z$ -value=5.14,  $df$ =118,  $p$ <0.01; Figure 3.7), and appeared to peak at intermediate discharge rates.

#### *3.4.4 Survival*

Steelhead survival within the first 3 days of capture was estimated at 95.5% ( $N$ =67). Estimated steelhead survival to winter was slightly lower than 3-day survival at 94.0% ( $N$ =67). Steelhead overwinter survival was estimated at 85% with a maximum survival of 86.7% and a minimum survival of 75%. Similarly, emigration from the study site following spawning was estimated at 85% with a maximum emigration rate of 86.7% and a minimum estimate of 75%.

**Table 3.1.** Logistic regression output predicting presence of the righting reflex in steelhead (n=45) immediately after an angling event. The model includes air exposure, sex, and landing method as categorical variables. Fight time, fork length, and water temperature were included as continuous variables. Significant variables are emphasized with boldface font.

Parameter	$\chi^2$	df	P-value
<b>Air exposure (sec)</b>	<b>10.42</b>	<b>39</b>	<b>&lt;0.01</b>
Fight time (sec)	1.82	39	0.18
Sex	1.23	39	0.27
Fork length (mm)	0.80	39	0.37
Landing method	2.33	39	0.13
Water temperature (°C)	0.82	39	0.36

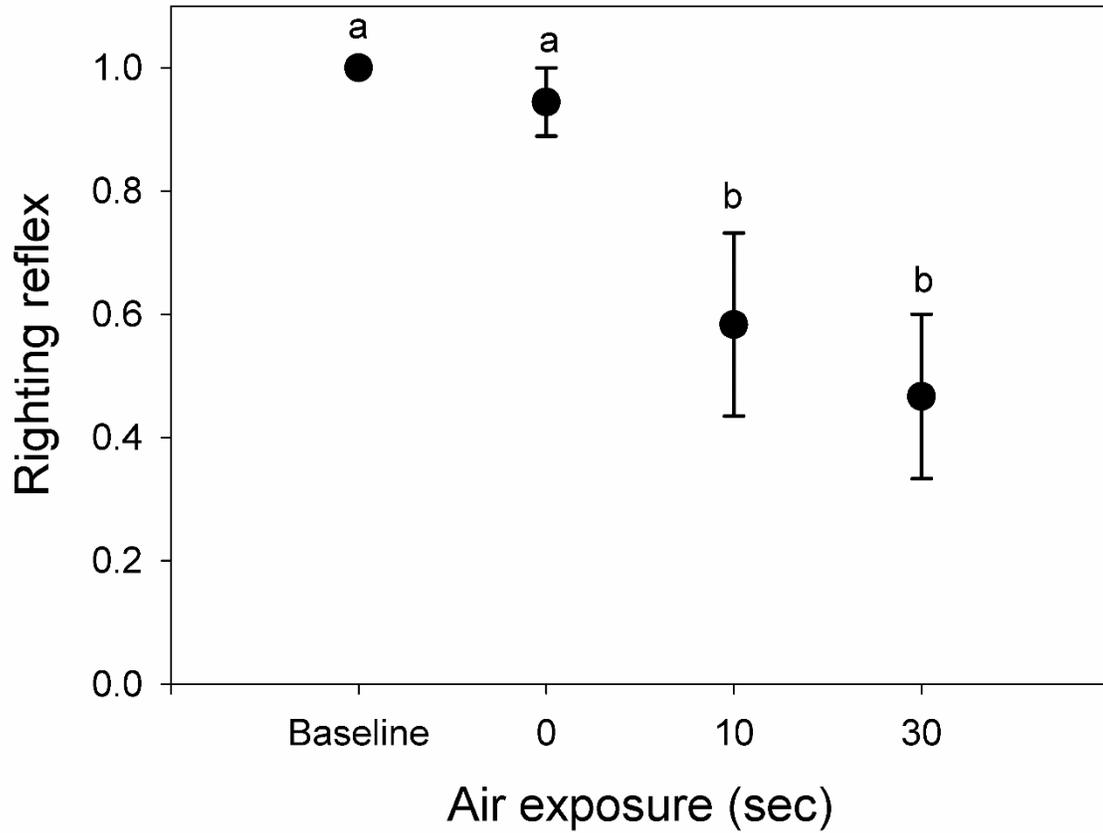
**Table 3.2.** Multiple regression model output predicting steelhead (n=45) blood glucose, lactate, and pH after approximately 20 min of holding following the angling event. The model includes air exposure, sex, and landing method as categorical variables. Inferences for factors are presented relative to reference levels, which were 0 sec for air exposure, female for sex, and netted for landing method. Fight time, fork length, and water temperature were included as continuous variables. Significant variables are emphasized with boldface font.

Variable	Glucose			Lactate			pH		
Parameter	Estimate ± SE	t-value	p	Estimate ± SE	t-value	p	Estimate ± SE	t-value	p
(Intercept)	0.44 ± 1.67	0.26	0.79	<b>6.16 ± 2.91</b>	<b>2.12</b>	<b>0.04</b>	<b>7.69 ± 0.29</b>	<b>26.93</b>	<b>&lt;0.01</b>
Air exposure: 10 sec	-0.35 ± 0.33	-1.06	0.29	0.39 ± 0.58	0.68	0.50	-0.02 ± 0.06	-0.29	0.77
Air exposure: 30 sec	-0.02 ± 0.32	-0.05	0.96	0.14 ± 0.55	0.26	0.80	-0.06 ± 0.05	-1.19	0.24
Fight time (sec)	0.01 ± 0.01	-0.30	0.77	0.01 ± 0.01	1.25	0.22	-0.01 ± 0.01	-0.09	0.93
Sex: Male	<b>0.62 ± 0.28</b>	<b>2.25</b>	<b>0.03</b>	-0.34 ± 0.48	-0.71	0.48	-0.01 ± 0.05	-0.21	0.83
Fork length (mm)	0.01 ± 0.01	0.10	0.92	-0.01 ± 0.01	-2.00	0.05	-0.01 ± 0.01	1.14	0.26
Landing method: Tail-grab	<b>0.88 ± 0.33</b>	<b>2.68</b>	<b>0.01</b>	0.46 ± 0.57	0.81	0.42	-0.04 ± 0.06	-0.63	0.53
Water temperature (°C)	0.18 ± 0.10	1.87	0.07	<b>0.46 ± 0.17</b>	<b>2.64</b>	<b>0.01</b>	<b>-0.06 ± 0.02</b>	<b>-3.52</b>	<b>&lt;0.01</b>

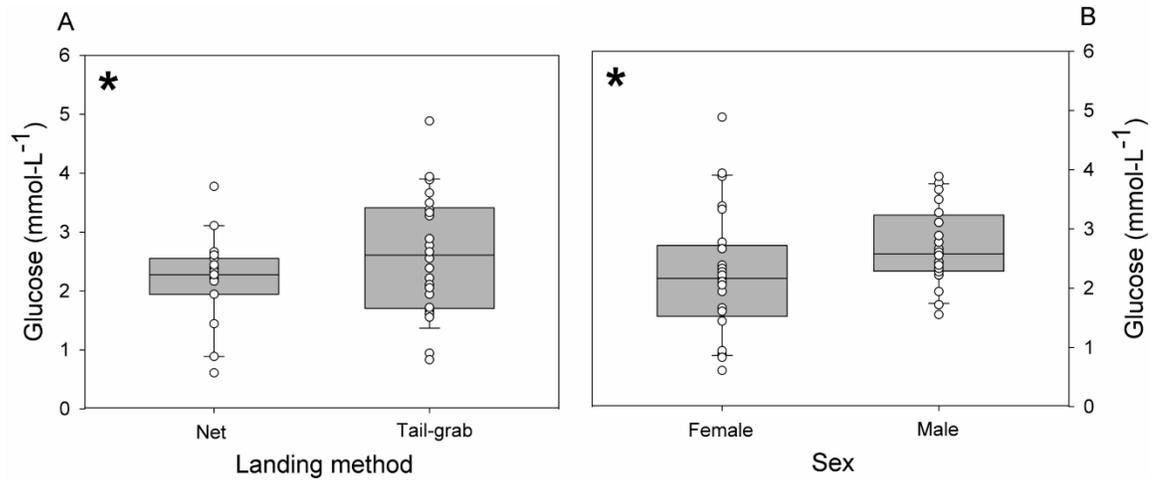
**Table 3.3.** Multiple regression model output predicting immediate post-release movement (n=67), and 2-week relative position of steelhead (n=54). The model includes air exposure (sec), sex, and landing method as categorical variables. Inferences for factors are presented relative to reference levels, which were 0 sec for air exposure, female for sex, and netted for landing method. Fork length and water temperature (°C) were included as continuous variables. Significant variables are emphasized with boldface font.

Variable	Immediate post-release movement			2-week relative position		
	Estimate ± SE	t-value	P	Estimate ± SE	t-value	P
(Intercept)	3.41 ± 40.83	0.08	0.94	-	-	-
Air exposure: 10 sec	<b>-24.10 ± 8.91</b>	<b>-2.64</b>	<b>0.01</b>	0.03 ± 0.67	0.05	0.96
Air exposure: 30 sec	<b>-23.95 ± 9.60</b>	<b>-2.45</b>	<b>0.02</b>	0.25 ± 0.74	0.33	0.74
Fight time (sec)	0.01 ± 0.03	0.03	0.71	-0.01 ± 0.01	-0.85	0.40
Sex: Male	-5.78 ± 7.77	-0.74	0.46	-0.77 ± 0.57	-1.34	0.18
Fork length (mm)	0.02 ± 0.06	0.31	0.76	0.01 ± 0.01	0.08	0.94
Landing method: Tail-grab	-9.61 ± 9.29	-1.03	0.31	0.19 ± 0.70	0.27	0.79
Water temperature (°C)	-0.64 ± 1.59	-0.41	0.69	0.20 ± 0.39	1.78	0.08

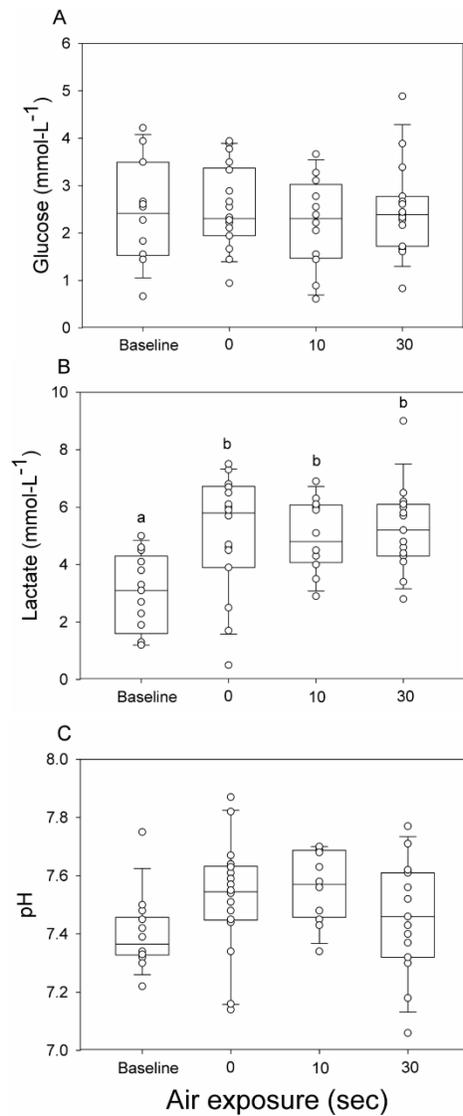
**Figure 3.1.** Presence of the righting reflex in baseline (N=14), 0 sec (N=18), 10 sec (N=12), and 30 sec (N=15) air exposed steelhead following angling. Different letters denote a statistically significant difference ( $p < 0.05$ ).



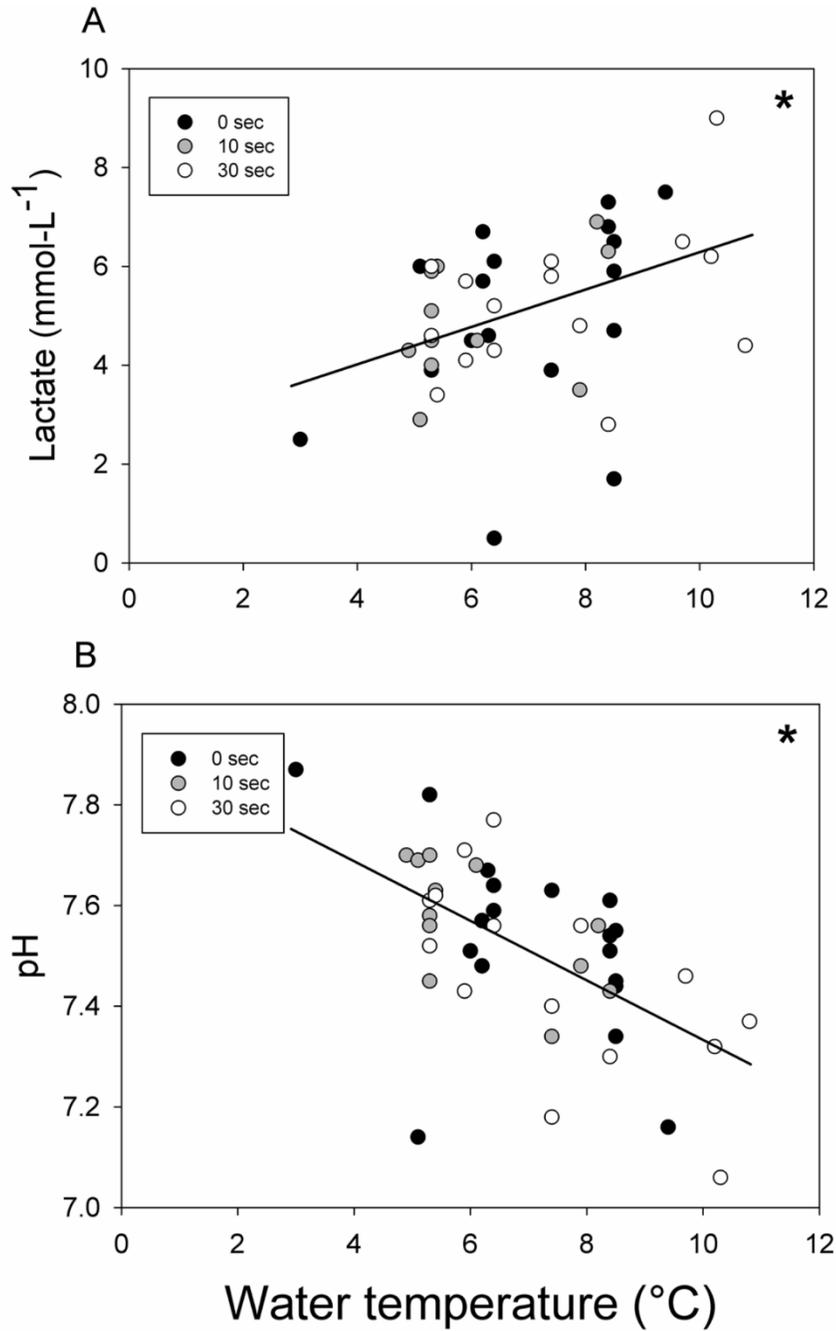
**Figure 3.2.** Mean blood glucose levels of wild steelhead that were angled and A) landed by net (n=19), or tail-grab (n=26) B) female (n=25), or male (n=20). Blood samples were taken approximately 20 min after angling. Asterisks denote a statistically significant difference ( $p < 0.05$ ).



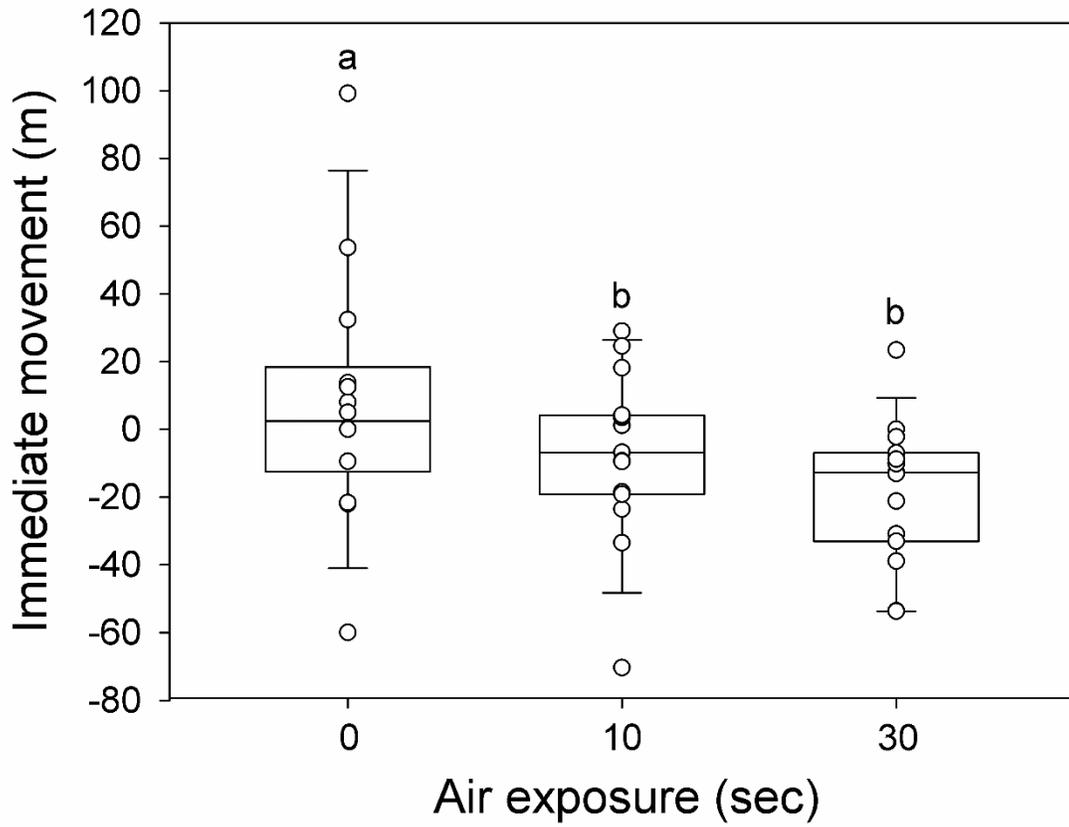
**Figure 3.3.** Blood parameter values of air exposed steelhead following angling and approximately 20 min of holding. A) Blood glucose levels of baseline (n=14), 0 sec (n=18), 10 sec (n=12), and 30 sec (n=15). B) Blood lactate levels of baseline (n=13), 0 sec (n=18), 10 sec (n=12), and 30 sec (n=15). C) Blood pH of baseline (n=14), 0 sec (n=18), 10 sec (n=12), and 30 sec (n=15). Different letters denote a statistically significant difference ( $p < 0.05$ ).



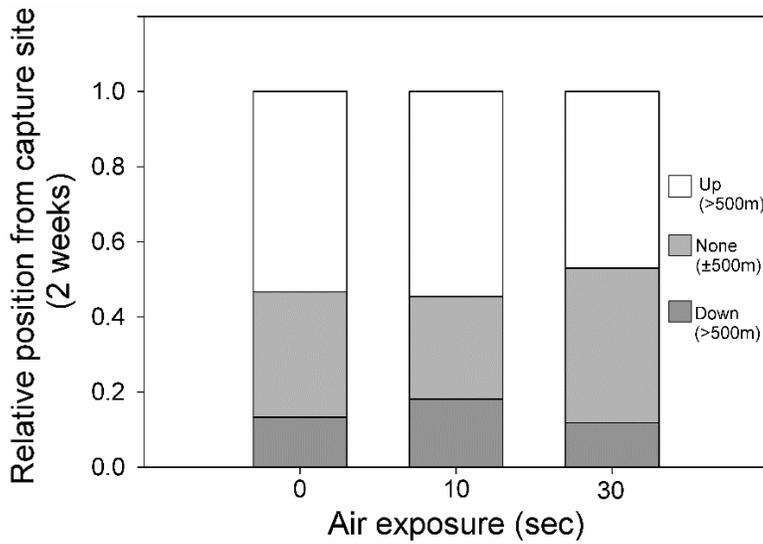
**Figure 3.4.** The relationship between water temperature ( $^{\circ}\text{C}$ ) and A) blood lactate levels and (B) blood pH of steelhead following angling and approximately 20 min of holding (n=45). Asterisks denote a statistically significant relationship between parameters ( $p < 0.05$ ).



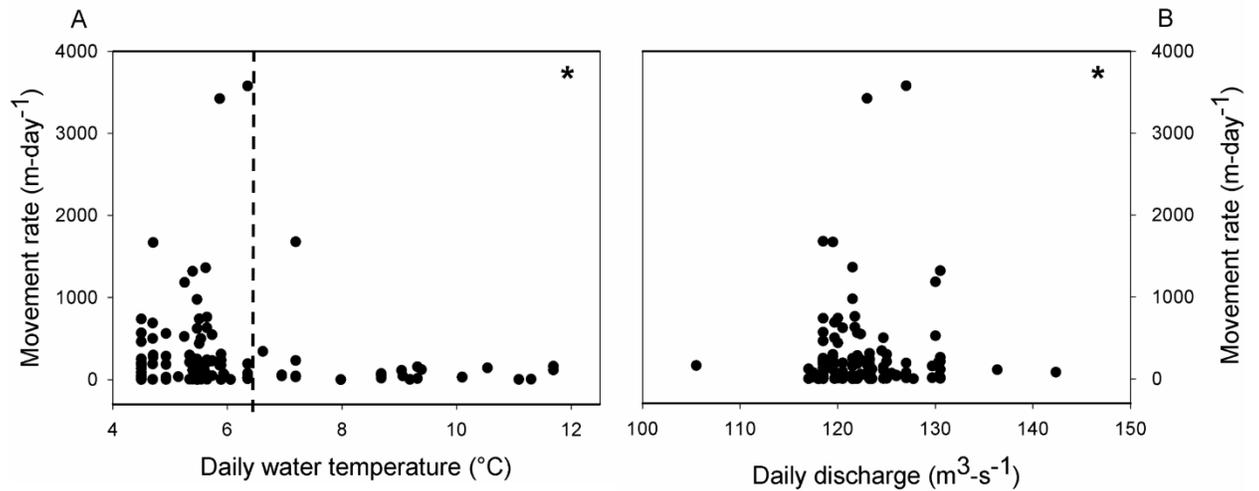
**Figure 3.5.** Immediate post-release movement of steelhead following angling and air exposures of 0 sec (n=22), 10 sec (n=25), and 30 sec (n=21). Different letters denote a statistically significant difference ( $p < 0.05$ ).



**Figure 3.6.** The 2-week relative position of steelhead following angling and air exposures of 0 sec (N=15), 10 sec (N=22), and 30 sec (N=17). Relative positions of each fish were categorized 2 weeks after capture as down (>500 m movement downstream of the capture site; dark grey), none (less than 500 m movement from the capture site; grey), up (>500 m movement upstream of the capture site; white).



**Figure 3.7.** The relationship between average daily movement rate of steelhead and (A) average daily water temperature (°C) and (B) average daily discharge following angling (n=121). The dashed line in (A) indicates the location of 6.5 °C, a potential thermal threshold to movement. Asterisks denote a significant correlation ( $p < 0.05$ ).



### 3.5 Discussion

#### 3.5.1 Angling-related factors

During an angling event fish are forced to exercise that requires the use of anaerobic metabolism (Ferguson and Tufts, 1992). Longer fight times (angling durations) typically increase blood acidosis as secondary metabolites such as lactate are produced and free protons (H<sup>+</sup>) accumulate in the blood stream (Milligan and Wood, 1986; Wood, 1991). Indices of metabolic stress (glucose, lactate, pH) were not influenced by fight times in the steelhead caught-and-released on the Bulkley River, but blood lactate values were significantly greater in fish that were angled compared to baseline levels. This result is consistent with Meka and McCormick (2005), who found little relationship between

blood glucose and fight time, but found lactate values to be greater in wild *O. mykiss* experiencing extended (>2 min) angling events. Fight times also had little influence on immediate movement, or 2-week position, suggesting that steelhead are relatively resilient to angling-induced exercise. This resilience likely stems from their capacity to complete several hundred-kilometer freshwater migrations (Penney and Moffit, 2014), while burst swimming to traverse high velocity flow segments (waterfalls, rapids, creeks). Relative to other anadromous salmonids, steelhead are considered strong swimmers with the highest burst velocity (m/s), prolonged velocity (m/s) and maximum jumping height (m; Reiser et al., 2006). Offspring survival to the eyed egg stage in hatchery steelhead did not differ greatly between steelhead that were angled and released compared to controls (Pettit, 1977). It is possible however, that there were sub-lethal consequences of exercise on our wild steelhead that were not measured in this study.

Following exercise, steelhead in our study were landed by a fishing net designed for anglers or by hand (tail-grab). Few studies consider landing method as a potential contributor to angling-related outcomes, yet the steelhead in our study had greater blood glucose levels when tail-grabbed. Glucose and other carbohydrates are not major sources of energy in the white muscle of salmonids (Brett, 1995). Nonetheless, glucose has been shown to respond to acute stressors such as stocking density and hypoxia in farmed rainbow trout (Polakof et al., 2012). Landing by tail-grab resulted in longer fight times than netting, but the increase in glucose was not influenced by fight time, suggesting it was likely greater handling stress that resulted in the elevated glucose (Liu et al., 2014).

Air exposure is an angling-related stressor that occurs in virtually all recreational fisheries even though in most cases it can be avoided. Immediately following the angling

and air exposure event, fish are subject to decreased pH in the blood as CO<sub>2</sub> and additional lactate accumulate, and the blood's ability to retain oxygen declines by over 80% (Ferguson and Tufts, 1992). It is suggested that intracellular acidosis of the muscles is responsible for mortality within the hours following exhaustive exercise and air exposure (Wood et al., 1983). The sensitivity of fish to removal from the water is highly dependent on both the species and environmental conditions (e.g. water temperature; Cook et al., 2015; Gingerich et al., 2007). For example, black bass *Micropterus* spp. can survive 10-minute air exposure periods (White et al., 2008), while hatchery rainbow trout have shown considerable mortality after just 30 and 60 sec air exposures in controlled settings, albeit those fish were also cannulated (Ferguson and Tufts, 1992).

In addition to physiological stress, air exposure can also result in behavioural changes. The steelhead in our study that were air exposed for 10 and 30 sec had greater immediate post-release fallback and reflex impairment than fish kept wet, though no considerable differences were evident between the 10 and 30 sec groups. Similar results have been observed in brook trout (*Salvelinus fontinalis*; Schreer et al., 2005), Coho salmon (*Oncorhynchus kisutch*; Raby et al., 2012), and bonefish (*Albula vulpes*; Danylchuk et al., 2007a) that had impaired swimming capabilities immediately after short air exposures. This immediate fallback may have been greater due to the additional stress associated with the tagging procedure, but evaluation of similar tagging procedures on Atlantic salmon suggested little difference in blood physiology or short-term swimming performance compared to untagged controls (Thorstad et al., 2000). Although these differences in immediate movement are likely minimal on the scale of a several hundred kilometer migration, they still provide an indication of acute post-release impairment

over a short-term period, similar to other immediate measures such as the reflex action mortality predictors (RAMP; Davis, 2010). Impairments from air exposures appear to return to normal over longer-term monitoring periods as air exposure was not related to relative position after 2 weeks in steelhead. In addition, fish that fell back further immediately after capture had no changes in their relative position after 2 weeks compared to fish that held position or moved upstream after release. It should be noted that air exposures could have had sub-lethal effects on steelhead that were undetected based on their movement. Richard et al., (2014) found no relationship between air exposures of 0-30 sec and movement in wild Atlantic salmon but found that air exposures of just 10 sec decreased offspring production, with even greater reductions as air exposure duration increased (Richard et al., 2013). As C&R practices were highly responsible in the Bulkley River, it is likely that environmental and intrinsic factors may have had greater influences on physiology and movement.

### *3.5.2 Environmental factors*

Temperature is often considered the ‘master factor’ for fishes due to its highly influential role on physiological processes (Brett, 1971). As temperatures increase, physiological indices of stress such as glucocorticoids also tend to increase (Wendelaar and Bonga, 1997; Barton, 2002). As a result, water temperatures have been a major consideration in C&R studies. A meta-analysis on the capture-and-release of fish indicated that warmer water temperatures increased sub-lethal stress and/or mortality in 70% of reported articles (Gale et al., 2013). For Bulkley River steelhead, water temperature at the time of capture had a positive correlation with blood lactate and negative correlation with blood pH. Numerous C&R studies conducted on wild and

hatchery rainbow trout have found greater stress (Wydoski et al., 1976; Kieffer et al., 1994; Meka and McCormick, 2005), impaired behaviour (Simpkins et al., 2004), and enhanced mortality rates (Dotson, 1982; Schisler and Bergersen, 1996) with warmer water temperatures. Steelhead angled in 8-23 °C water had a mortality rate of 9.6% approximately 36-hours post-release, with 83% of mortalities occurring in temperatures above 21 °C (Taylor and Barnhart, 1997). This sensitivity to warmer water temperature, may be particularly salient in the face of global climate change. Neither reflex impairment, nor immediate post-release movement was affected by water temperatures. This suggests that immediate behavioural and reflex impairment may be driven primarily by the angling-related factors discussed previously. Water temperatures also appear to be important in the daily movement of steelhead.

Water temperatures were correlated with the average daily movement rate of steelhead during the fall, with movement considerably higher from 4.5 to ~6.5 °C, compared to 6.5 to 12 °C. Modeling suggested that stocked steelhead movement increases past a threshold temperature averaging 3.8 °C in Lake Michigan tributary streams (Workman et al., 2002). While Columbia-Snake River steelhead (wild and hatchery) halted their upstream movement at 4 °C and re-initiated upstream movement at about 7 °C, with potential impacts of both photoperiod and discharge (Keefer et al., 2008). Perhaps most relevant, Skeena River (mainstem of the Bulkley River) wild steelhead resumed migrating at temperatures of 2-5 °C (Lough, 1980), suggesting that Bulkley and Skeena River steelhead may have similar temperature thresholds. Average daily movement was also correlated with average daily discharge, with movement appearing to peak at approximately 120 m<sup>3</sup>.s<sup>-1</sup> and 130 m<sup>3</sup>.s<sup>-1</sup>. Similarly, Atlantic salmon

movement was also significantly influenced by both water temperature and discharge (negative correlation), but not C&R (Richard et al., 2014). Although environmental factors such as temperature and discharge can be important determinants of fish physiology and movement, intrinsic biological properties related to the individual can also be highly influential.

### *3.5.3 Intrinsic biotic factors*

Intrinsic biotic factors such as size and sex can have impacts on fish physiology and movement during migration (Jeffries et al., 2014; Penney and Moffit, 2014). Although there was no difference in blood lactate and pH between sexes, males had significantly higher blood glucose levels than females. Previous work on juvenile chinook salmon indicated that males had greater levels of plasma glucose and cortisol following 30-day exposure to mill effluent (Afonso et al., 2003). However, the angling-related stressors (aside from landing method) had little influence on blood glucose values, suggesting values observed here may be similar to their free-swimming physiological levels during migration, rather than a response to the acute angling stressor. Penney and Moffitt (2014) found that sexually mature steelhead males had greater protein content than females, suggesting differences in energy allocation prior to reproduction. It is therefore possible that males rely more on glycogen/glucose as an energy source, though the contribution of carbohydrates to energy in salmonids is generally considered low (Brett, 1995; Polakof et al., 2012). In Bulkley River steelhead, sex did not predict post-release movement nor was survival different across sexes. Sex also had little influence on the movement of wild rainbow trout during their spawning migrations in the Tongariro River, New Zealand, migration rate of caught and released Atlantic salmon in the River

Klaralven, Sweden (Dedual, 1999; Hagelin et al., 2016), or spawning success of Chilliwack River steelhead (Nelson et al., 2005). For the steelhead in our study, fork length showed no relationship with any of the blood physiology, or movement variables. Although size was not a predictor of migration distance in Bulkley River steelhead, it may have advantages for caught-and-released fish during kelt emigration, when larger sized individuals have higher protein content and energy density (Penney and Moffitt 2014).

#### 3.5.4 Survival

Steelhead survival within the first three days and several weeks following C&R was high at an estimated 95.5, and 94.0% respectively. Two fish that were deeply-hooked appeared to die within the first three days of capture, while one other fish that was air exposed for 30 sec seemed to die shortly after three days. Deep-hooking rupturing vital organs (tongue) was the primary source of short-term mortality for wild steelhead captured and released on the Bulkley River. The rate of deep-hooking mortality was 3.0% which is comparatively low relative to the average hooking mortality of 16% estimated across the Salmonidae (Hühn and Arlinghaus, 2011). *O. mykiss* alone have been subject to countless C&R evaluations with hooking mortality estimates ranging from 0-88.5% depending on the hook type, use of bait, fish length, and water temperature (Hühn and Arlinghaus, 2011). Immediate hooking mortality estimates for bait-angled steelhead range from 0.31-11.00% with a total mean across studies of  $4.06 \pm 0.26\%$  (Mongillo, 1984; Hooton, 1987; Lirette, 1988, Lirette, 1989; Thomas, 1995; Ministry of Lands, Forestry, and Natural Resources, unpublished data; Nelson et al., 2005). Most of these are likely an underestimate of total C&R mortality as delayed mortalities were not accounted

for. Nelson et al., (2005) may provide the most comparable C&R evaluation despite studying wild winter-run steelhead from the Chilliwack River. These steelhead were of similar size (782-940 mm vs. 508-870 mm), and water temperatures were similar in range (4.0-7.0 °C vs. 4.5 to 11.7 °C) and had a correspondingly similar short-term mortality of 5.0% vs. 4.5% in our study (Nelson et al., 2005). Interestingly, none of the Chilliwack River steelhead that died from catch-and-release were deeply-hooked despite this being the primary source of mortality in Bulkley River steelhead. The high catch-and-release angling survival may be attributed to the strict angling regulations imposed on terminal tackle that have historically been associated with greater hooking mortality (Hooton, 2001).

Anglers fishing on the Bulkley River, B.C. are restricted to C&R only using single, barbless hooks, without the use of natural baits (B.C. Ministry of Forests, Lands, and Natural Resource Operations, 2017). Alternative hook types such as treble hooks are typically assumed to cause greater anatomical damage and mortality although evidence suggests little difference compared to single hooks (Bartholomew and Bohnsack, 2005; Kerr et al., 2016). Barbed hooks however, have been shown to increase hooking injury in wild rainbow trout compared to barbless hooks (Meka, 2004; but see Dubois and Debiezlig, 2004). The use of live-bait is also generally implicated with higher rates of deep-hooking in steelhead (Hooton, 2001). Live bait provides both visual and chemical stimuli that excite the central nervous system and increase feeding behaviour (increased swimming and biting/snapping actions) in farmed rainbow trout that feed using both visual and olfactory systems (Valentinčič and Caprio, 1997). Live bait angling in an Idaho stream resulted in 17% of hatchery-reared rainbow trout being hooked in the gills

or esophagus and a 16% mortality rate (Schill, 1996), while stocked rainbow trout angled by live bait in a South Carolina reservoir had 39% hooking mortality, compared to just 5% mortality for fish caught by artificial bait (Barwick 1985). Cool water temperatures (4.5-12 °C) during the angling season could also be maintaining high pre-winter survival of steelhead in the Bulkley River. Wild rainbow trout captured at maximum daily water temperatures greater than 23 °C had up to 16% mortality while mortality on days with maximum temperatures of 20.8 °C was 0% (Boyd et al., 2010).

Mortality of caught-and-released steelhead over-winter was approximately 10.5% (one of these appeared to die several weeks after capture but prior to the onset of winter). This rate is consistent with the 11% over-winter mortality of Sustut River steelhead, (Skeena system), and lower than the 18-38% mortality of Columbia-Snake River steelhead prior to spawning (Keefer et al., 2008). Survival to spawning was higher in Chilliwack River wild winter-run steelhead at 95% (Nelson et al., 2005) compared to the 85% pre-spawn survival for steelhead in the Bulkley River recreational fishery but Bulkley River fish travel significantly further inland and have to survive several extra months in freshwater. Our estimates are based on the assumption that there was no tag loss throughout the 7-month study period. Mortality estimates may therefore include any fish that shed their tags throughout their migration, causing the tag to remain stationary within the river. However, based on the high number of fish observed to emigrate from the river, it is clear that tag loss was not common. Previous work with externally attached tags on adult brown trout (Økland et al., 1996; Aarestrup and Jepsen, 1998; Thorstad et al., 2014) and Atlantic salmon (Aarestrup et al., 2000) in low-vegetation rivers (similar to the Bulkley) suggests little to no tagging-related mortality or tag loss. Although mortality

related to deep hooking can be directly attributed to the angling event, other mortalities may also be partly explained by the stress imposed during catch-and-release. Of the 7 non-deeply hooked fish that died, some were subject to an extreme component of an angling event that may explain their delayed mortality. One dead fish was air exposed for 30 sec, one was fought for nearly 15 min and air exposed for 30 sec, one was caught at the upper temperature limit for our study (13 °C), and one was caught at the upper temperature limit and dragged onto shore during capture. Despite a low number of mortalities, they provide some evidence that various components of an angling event may contribute to long-term survival. Although precautionary angling restrictions and cool water temperatures during the majority of the angling season are likely reducing short-term mortality of steelhead in the Bulkley River C&R recreational fishery, it is clear that sub-lethal stress still occurs as a result of other angling-related factors.

### *3.5.5 Conclusions*

Angling-related factors such as air exposure, increased reflex impairment and immediate downstream movement, while angling in general increased blood lactate values compared to baseline levels. None of the angling-related variables had any apparent long-term consequences on migration rates. It is still possible however, that angling-related variables could have sub-lethal impacts at the time of spawning. Water temperature had a strong correlation with metabolic indices of anaerobiosis (lactate and pH) and was correlated with average daily movement rates during fall migration. Findings from this study suggest the C&R regulations on the Bulkley River are an effective conservation strategy for steelhead given high post-release survival to spawning (~85%). The minimal amount of short-term mortality I observed tended to be associated

with deep hooking to the tongue. Results from this study suggest that anglers should not remove fish from the water, and that anglers should be conscious of water temperature while they are angling. I will communicate these findings to the Bulkley River angling community based on their existing perceptions and knowledge on catch-and-release angling for steelhead (Guckian et al., In press), in partnership with Keepemwetfishing, an emerging social brand to communicate best angling practices (Danylchuk et al., 2018). Fisheries managers may consider implementing a regulation similar to Washington that prohibits the removal of wild steelhead and salmon from the water.

## **Chapter 4. Consequences of fisheries gear type and handling practices on capture-and-release of wild steelhead on the Bulkley River**

### **4.1 Abstract**

Wild steelhead are captured-and-released during spawning migrations in commercial, Indigenous, and recreational fisheries, though the consequences of these capture events on steelhead migration have been poorly studied. This study evaluated injury, reflex impairment, behavioural alterations, and survival of wild adult steelhead following capture-and-release in the Indigenous dip net, Indigenous beach seine, and recreational angling fisheries of the Bulkley River, British Columbia. Steelhead were captured using common handling practices employed in each fishery and were monitored for post-release movement using radio telemetry. A greater proportion of steelhead captured by dip net and seine had impaired righting reflexes compared to angled fish, but only dip net fish had notably higher incidence of injury (i.e., net marks, torn fins, flesh wounds, scale loss). Fish captured by dip net and seine had considerably faster peak migration rates ( $>4000 \text{ m-day}^{-1}$ ) than angled fish ( $<1000 \text{ m-day}^{-1}$ ) which likely reflects when the steelhead are encountered during their migration (active vs. holding) rather than a response to capture. Air exposure (15-74 sec) and water temperature (9.2-15.1 °C) at the time of capture had significant negative relationships with immediate behaviour (temperature only), intermediate-term (10-20 days after capture) migration rates, and peak migration rates in dip net captured steelhead. There were no significant effects of capture duration (sec) or fish length (mm) on injury, reflexes, or migratory behaviour. The 3-day survival was 80-97% for dip net caught steelhead, 84-100% for seine caught steelhead, and 54-100% for angled steelhead. Findings from this work suggest capture

conditions and handling practices can influence wild steelhead migration following release. Survival estimates will provide important data for fisheries managers accounting for steelhead discard mortality across each of these gear types.

## **4.2 Introduction**

Fisheries are often a complex blend of motivations and human-fish interactions (Cooke and Cowx 2006; Young et al., 2016). In many cases, modes of capture are relatively indiscriminate, with fishing gears such as hook-and-line and nets, being able to capture a wide range of species in the same location (Gray and Kennelly, 2017; Northridge et al., 2017). Many fish are released to comply with harvest restrictions, or as a voluntary behaviour on behalf of the fisher for conservation purposes or because the bycatch is considered low value to the fisher (Arlinghaus et al., 2007; Davies et al., 2009). The underlying assumption of releasing captured fish is that these individuals will survive with minimal fitness consequences from the capture event. This is not always the case however, as capture by fisheries can be one of the most severe acute stressors imposed on fish throughout their lives (Davis, 2002). As a result, a considerable amount of research has evaluated both the lethal and sub-lethal effects of capture-and-release in commercial, subsistence, and recreational fisheries (Alverson, 1994; Bartholomew and Bohnsack, 2005; Wilson et al., 2014).

Differences exist across species, population, and fisheries that can influence the severity that capture has on an individual (Cooke and Suski, 2005; Patterson et al., 2017; Raby et al., 2015). Fish captured in net fisheries (gillnet, seine net, tangle net, fyke net) may experience physical damage to organs, flesh, scales, and the mucous layer due to entanglement (Vander Haegan et al., 2004; Smith and Scharf, 2011; Colotelo et al., 2013;

Raby et al., 2015; Bell and Lyle, 2016). Physical damage may also occur for fish captured by angling due to damage at the hooking location (Hühn and Arlinghaus, 2011) and handling (Colotelo and Cooke, 2011). In most fisheries there is a degree of exhaustion resulting from anaerobic exercise that can result in physiological changes in the captured fish (Kieffer, 2000) and even mortality (Wood, 1983). It is also common for captured fish to be air exposed before returning to the water (Cook et al., 2015). In commercial fisheries air exposures can exceed 60 min due to large catches and the time needed to process fish (Davis, 2002). In recreational fisheries air exposures are related to unhooking, and admiration of captured fish, but typically do not exceed 60 sec for salmonid species (Lamansky and Meyer, 2016). Air exposure can lead to physiological and behavioural changes in fish (Rapp et al., 2014; Thompson et al., 2008), which can be further exacerbated during certain environmental conditions such as warm water temperatures (Gingerich et al., 2007). There are multiple factors that can influence capture-and-release outcomes, and their relative impact can vary on a fishery- and species-specific basis suggesting the need for context specific evaluations (Brownscombe et al., 2017; Cooke and Suski, 2005; Raby et al., 2015). Thus far, few studies have compared the capture-and-release outcomes of beach seine and angling gear within a single system (but see Donaldson et al., 2011), and no studies have evaluated the consequences of dip net capture (Figure 1A). Dip nets are commonly used to capture Pacific salmon (*Oncorhynchus* spp.) in the Pacific Northwest (e.g. Fraser and Thompson Rivers of British Columbia and the Copper, Kenia, Kasilof Rivers, Fish and Sweathart Creeks of Alaska) but often have steelhead bycatch that must be released. These species-specific evaluations are particularly important for species such as steelhead that are in

decline (Northwest Fisheries Science Centre, 2015; Smith et al., 2000), and are exploited by multiple fisheries during critical periods of their lives.

Steelhead trout are one of the world's most iconic salmonid species, serving a keystone function in freshwater ecosystems (Willson and Halupka, 1995), while contributing to human culture, economy, and recreation (Counterpoint Consulting, 2008). Due to their declining populations across their distribution they are generally not targeted in harvest-oriented fisheries, though some ceremonial harvest still occurs in Indigenous fisheries (e.g. Levy and Parkinson, 2014). Despite minimal legal harvest of wild steelhead, the existence of salmon fisheries for pink (*Oncorhynchus gorbuscha*), coho (*Oncorhynchus kisutch*), chinook (*Oncorhynchus tshawytscha*), and sockeye (*Oncorhynchus nerka*) salmon results in a considerable amount of steelhead bycatch (J.O. Thomas, 2010) as salmon and steelhead runs co-occur. For example, in the Skeena River system it is estimated that 1.5% of Skeena summer-run steelhead may be captured each year in the test fishery, with apparent short-term mortality rates of 49% (J.O Thomas, 2010; Tye Test Fishing Index 1998-2017). A substantial proportion of this steelhead stock is then captured in the Bulkley River, as approximately 4% of individuals are captured by seine, 10% by dip net, and 59% captured by angling (MFLNRORD Data 1999-2017). Capture within commercial, Indigenous, and recreational sectors is common throughout most watersheds in the Pacific Northwest (e.g. Fraser and Columbia Rivers), yet the response of steelhead to different modes of capture-and-release has received little attention.

The purpose of this study was to quantify the sub-lethal impacts and mortality of wild steelhead following capture-and-release by dip net, beach seine, and angling on the

Bulkley River, British Columbia. Steelhead were assessed for injury, reflex ability, migratory behaviour, and survival following fisheries captures during their spawning migrations. This study also evaluated the key drivers of migratory stress and mortality in each of these fisheries to help refine handling practices and management of wild steelhead.

## **4.3 Methods**

### *4.3.1 Study site*

The Bulkley River is the largest tributary of the Skeena River, draining an area of approximately 12,000 km<sup>2</sup>. The river begins approximately 266 river kilometers (rkm) inland and extends 141 km to the Morice River which then spans an additional 74 km to Morice Lake. Along the Bulkley River there are three relatively larger tributaries that steelhead can migrate into including the Suskwa River (rkm 15), the Telkwa River (rkm 100), and the Little Bulkley River (rkm 141), as well as a number of smaller ones that steelhead may enter during spring runoff. The river is free of manmade barriers, with Witsset (formerly Moricetown) Canyon as the greatest hydrological barrier (15 m change in altitude) to fish migration. At Witsset canyon, there is a long-standing Wet'suwet'en Indigenous subsistence fishery. The Wet'suwet'en fishers target migrating salmonids (primarily coho, pink, and chinook salmon) with the majority of steelhead being released after capture. As of 1999 the MFLNRORD and the Wet'suwet'en have collaborated to conduct a mark-recapture program (using conventional Anchor-T tags) for all steelhead captured-and-released in the beach seine and dip net fisheries. In addition to these subsistence salmon fisheries, the river also maintains one of the most renowned steelhead

recreational fisheries on Earth with the largest steelhead capture of any stream in British Columbia. Each year an average of 20,873 summer-run steelhead migrate up to the Bulkley River, although the 2017 run was estimated at just 9,234 fish (Witset [formerly Moricetown] Mark-Recapture, 2017). Recreational anglers can access the majority of the river by jet boats, pontoons, and walk-in sites, and use both fly fishing and conventional fishing gear. Since 2010, recreational angling effort has averaged 12,524 angler days during the steelhead season (MFLNRORD Data 2010-2015). All steelhead must be released following recreational capture as per the MFLNRORD's fishing regulations in the province of British Columbia.

#### *4.3.2 Capture methods*

Steelhead sampling was completed on the Bulkley River, British Columbia from August 24<sup>th</sup> to October 10<sup>th</sup>, 2017 (Figure 1.1). Wet'suwet'en fishers sampled steelhead from Widzin-Kwah Canyon by dip netting and beach seining. Dip net sampling took place above the canyon just below the top of the falls using traditional dip net gear (a ~600 cm aluminum pole with 4 cm x 4 cm square nylon mesh) between August 25<sup>th</sup>-September 30<sup>th</sup>, 2017. Steelhead were brought to surface by the fisher and were placed into a transport cradle that was carried by a runner to the sampling station approximately 20 m away. Steelhead were placed by the runner into a water-filled storage tub (~150 L) that was used to hold the steelhead while other fish were being processed. Steelhead were dip netted from the storage tub into a sampling trough where they were measured, sexed, and visually assessed for injuries, tags and scars. As most other species were harvested after capture, dip netters would typically sort and release steelhead before sorting and harvesting other species of fish. Fish were released by samplers into slow-moving water

on the edge of the falls through a plastic slide or were placed over the edge depending on the water level.

Beach seine sampling (~90 m x 8 m x 5 cm stretched mesh opening size) was conducted in the canyon by jet boat between August 29<sup>th</sup>-September 22<sup>nd</sup>. One end of the seine was anchored to shore while the other was pulled by jet boat into an arc and corralled back to shore into an area of approximately 5 m<sup>2</sup>, with a mean depth of approximately 20 cm. The beach seine crew sorted fish species in a sampling trough held within the river. Steelhead were sorted before all other species in the net as fishers had previously observed that steelhead were particularly sensitive to long-term confinement and were aware that steelhead will not be spawning until the following spring, unlike the salmon captured. Steelhead were measured, sexed, and assessed for injuries, tags, and scars by workers as part of the MFLNRORD monitoring program.

Recreational anglers captured steelhead throughout the entirety of the Bulkley River upstream of Witsset Falls to the confluence of the Morice-Bulkley rivers. These fish were generally closer to potential spawning sites than fish captured at Witsset Falls. Anglers targeted steelhead by fly fishing (spey rods #7, 8, and 9), centre-pin fishing (a free-spooling reel), and spin-casting (Ugly Stik, 8'6", Medium). Anglers used various sizes/colours of flies, inline spinners, spoons, and artificial worms on single barbless hooks. Flies were tied to size 1 hooks and smaller, while heavy tackle was used in conjunction with hooks as large as 3/0. Fish were landed in rubberized landing nets, or were secured by the caudal peduncle with a bare hand (tail-grab). In some cases, anglers lifted steelhead out of the water for an admiration period.

### *4.3.3 Quantification of the capture event*

Elements of the capture events were recorded that were common across all three fisheries. Capture-related variables included capture duration (sec), air exposure duration (sec), and the time to attach the transmitter (sec). For dip netted and beach seined fish, capture duration was considered the elapsed time from netting the fish to the point that the fish was secured in the sampling trough. Capture duration was taken as the time elapsed between hooking and landing for angled fish. Air exposure was taken as the time the fish was lifted out of the water prior to entering the sampling trough. Each fish captured was assessed for fork length (mm), sex, scale loss (presence vs. absence), and injury at the time of capture. Injuries were classified as the presence or absence of flesh wounds, net marks, fin and tail damage, and organ damage. Scars or predator wounds that were inflicted before the capture event were also recorded but were not included as a capture-related injury. I was unable to isolate capture injuries from those incurred naturally. This may be particularly important for dip net captured fish that are actively trying to ascend steep falls and may collide with canyon walls and rocks. Anatomical hooking location was noted for fish captured by angling. Water temperature (°C) was recorded following each capture using a handheld digital thermometer (Taylor Compact Waterproof Digital Thermometer).

#### *4.3.4 Post-release movement*

Following capture and processing in each of these fisheries, steelhead were secured in a water filled (flow-through) v-shaped sampling trough (100 cm length x 25 cm width x 20 cm height) or recovery bag (100 cm length x 20 cm diameter). A radio-telemetry transmitter (4.7 g weight in air, 220-441 day battery life, 33 pulses per minute; Series F1970, Advanced Telemetry Systems, Isanti, MN, USA) was attached externally behind the dorsal fin using two stainless steel 18 gauge surgical needles inserted through the dorsal musculature. Wires were secured to the dorsal musculature on the opposite side of the transmitter using steel crimps and plastic backing plates to reduce tissue irritation. Tagged fish were removed from the sampling trough/recovery bag and were assessed for presence of the righting reflex prior to release. To assess this reflex, fish were rotated ventral side up and were monitored for their ability to regain normal orientation within 3 sec (as per Davis, 2010). A previous review on externally-attached telemetry tags suggested little impact to physiology and behaviour in salmonids (Jepsen et al., 2015), so it is expected that the influence of the 4.7 g tags attached to the wild steelhead in our study would be minimal.

Fish were located by zero-point manual tracking (Cooke et al., 2012) using a radio telemetry receiver and a 3-element Yagi antenna (Lotek Biotracker, Lotek Wireless, Ontario, Canada). The positions of each fish were recorded using a handheld GPS instrument (Garmin GP 60 Handheld GPS Device, 010-00322-00, Garmin, Olathe, KS, USA) set to Universal Transverse Mercator (UTM). Angled fish were located 20 min after release as an indication of immediate post-release movement following capture. Precise tracking locations could not be determined for beach seine and dip net fish due to

logistical constraints working along a waterfall and canyon. Dip net fish were therefore evaluated for their presence above or below the falls 24 hours following release. Both dip netted and beach seined fish were monitored for their emigration from Witsset Falls, defined as movement greater than 500 m above or below the falls. Successive tracking was completed opportunistically by jet boat and raft, based on the section of river that angler volunteers planned to fish that day. The entirety of the Morice-Bulkley system between Morice Lake (headwaters of the Bulkley River) and the Suskwa-Bulkley confluence (20 km below the most downstream tagging site), and the two major tributaries (Telkwa River, Little Bulkley River) were tracked between October 10<sup>th</sup>-19<sup>th</sup>, 2017. Tracking data from this period were used to calculate intermediate-term migration rates ( $\text{m-day}^{-1}$ ), defined as the displacement of each fish from its release site 10-20 days after release, divided by the number of elapsed days (10-20 days) since capture. This tracking data was also used to determine the peak migration rate ( $\text{m-day}^{-1}$ ) of each fish, taken as the fastest migration rate observed between successive detections in the Fall of 2017. A secondary tracking event was completed between April 7<sup>th</sup>-19<sup>th</sup>, 2018 by rafting, hiking, and aircraft. Tracking spanned the entirety of the Morice and Bulkley rivers (rkm 15). Previous work suggested that steelhead only infrequently enter the Little Bulkley and Telkwa rivers prior to spawning so these sections were not tracked in April. I extended our tracking area to 30 km below our most downstream release site to account for active downstream movement and potential drift of carcasses, as previous experimental release of dead European silver eels (*Anguilla anguilla*) found downstream movement up to 30.1 km, though it was typically much less (Havn et al., 2017).

Steelhead survival rates were estimated for the first three days following capture, and prior to winter. If a fish moved upstream after any of these time points, it was considered alive at that point in time and all previous points in time. Mortalities were designated as the earliest point in time (3-day, pre-winter) that a transmitter was found at the same location as in April 2018. Fish that were not detected after a certain time point were excluded from that point in time onwards as the possibilities of tag malfunction, emigration from the study system, and missed detections could not be discerned.

#### 4.3.5 Data analysis

Spatial analyses were conducted in ARCMAP GIS 10.5.1 for all location data. Location data was projected into UTM Zone 9 and was snapped to river lines of the Bulkley and Morice rivers using the ‘near’ function. Distances between fish locations were quantified using the ‘create routes’ and ‘distance along a route’ functions in the ‘linear referencing tools’ menu.

Differences in fork length, sex ratio (% male and female), water temperature, air exposure duration, and tagging time across each capture group were evaluated using analysis of variance models (ANOVA; R function *aov*; R Core Team 2015), and a chi-square test (R function *chisq.test*; R Core Team 2015). Injuries, scale loss, and maintenance of the righting reflex were classified as a binomial categorical response (presence vs. absence) and were compared across capture groups using chi-square tests, while differences in intermediate-term, and peak migration rates across capture groups were modelled using ANOVA’s. A post-hoc Tukey test for chi-square tests (R function *chisq.post.hoc*; package *fifer*) and general linear hypotheses (R function *TukeyHSD*; R Core Team 2015) was used when statistically significant differences existed across

capture groups. Each capture group was then analyzed separately for each response variable. All models included capture duration, water temperature, and fork length as explanatory variables with additional variables included when relevant to the fishery. These additional variables included air exposure for models restricted to dip netted fish and gear choice (fly vs. tackle) for models restricted to angled fish. Incidence of injury, and reflex impairment were modelled by logistic regression (R function *glm* specifying *family=binomial*; R Core Team 2015) for dip netted fish only, as the sample sizes for these variables were too low to facilitate further analysis in the other capture groups. Logistic regression was also used to model presence above or below the falls for dip netted fish. The relationship between presence above and below the falls for dip netted fish and emigration timing was modelled using a chi-square test. Multiple regression (R function *lm*; R Core Team 2015) was used to model immediate post-release movement of angled fish, and the intermediate-term (10-20 days post-capture) and peak migration rates of all fish. An intermediate-term migration rate model was not created for seine-caught fish due to low sample size for this response variable.

Sample sizes were sufficient to complete chi-square tests. All logistic regression models were assessed using the Hosmer-Lemeshow goodness-of-fit test in R with the *sjstats* package function *hoslem\_gof* (Lüdecke 2017) that indicates whether observed values are significantly different from expected values ( $\alpha > 0.05$ ). Residuals for each level of a response variable in an ordinal logistic regression model were assessed using a goodness-of-fit test (R function *resid*, specifying “gof”; Harrell, 2018) that employs the Cressie-van Houwelingen normal test statistic. Normality of multiple linear regression models was assessed by visual examination of diagnostic plots of the residuals

(standardized residuals versus theoretical quartiles, residual versus fitted values, variance of residuals, and Cook's distance). One fish was removed from movement analyses as it died immediately after release in the dip net fishery. Two fish that had evidence of a previous capture event (gill net, dip net, or general net marks) were also removed from analyses on injury, reflex ability, and movement as the influence of previous capture events could not be discerned from the most recent tagging event. Fish recaptured within any of the three fisheries following tagging and release were included in all movement analyses up until the time point that the fish was recaptured and was then excluded from all further movement analyses. Where appropriate, descriptive statistics are reported as means  $\pm$  SE. Statistical significance was considered at  $\alpha < 0.05$ .

#### **4.4 Results**

A total of 94 wild steelhead were captured during this study, distributed among dip net (n=35), beach seine (n=25), and angling (n=34) capture. There were no significant differences in the fork lengths of fish captured by dip net ( $633 \pm 16$  mm), beach seine ( $671 \pm 21$  mm), or angling ( $664 \pm 18$  mm), or the proportion of females captured across dip netted (73%), seined (72%), and angled (65%) groups (both  $p > 0.05$ ).

##### *4.4.1 Capture conditions*

Differences in environmental and capture conditions were examined across each capture group. Water temperature was higher for fish caught by dip net ( $10.9 \pm 0.4$  °C) and seine ( $11.6 \pm 0.4$  °C), than angling ( $9.3 \pm 0.3$  °C), and angled fish were caught later in the season. The trough water temperature ( $11.4$  °C) that dip netted steelhead were processed in at Witsset Falls was consistently warmer than the river temperature ( $11.0$  °C). Dip net fish were captured and processed within 25-840 sec ( $261 \pm 42$  sec), while seine

fish were captured and processed within 270-1020 sec ( $530 \pm 41$  sec). Angled fish were caught by both fly fishing ( $n=21$ ) and spin-casting/centre pin angling ( $n=13$ ). Angling duration ranged from 85-835 sec ( $230 \pm 28$  sec). Air exposure was higher for dip netted fish ( $41 \pm 7$  sec), than seined fish ( $<1$  sec), and angled ( $4 \pm 1$  sec) fish. Dip net ( $199 \pm 14$  sec), and seine-caught ( $173 \pm 15$  sec) fish had longer tagging times than angled fish ( $86 \pm 4$  sec).

#### 4.4.2 Injury

A significantly higher proportion of dip netted fish (48%) were injured (flesh wounds, net marks, fin and tail damage) compared to seined (8%;  $\chi^2=12.50$ ,  $df=89$ ,  $p<0.01$ ) and angled fish (6%;  $\chi^2=17.01$ ,  $df=89$ ,  $p<0.01$ ; Figure 4.1A). Flesh wounds were present in 3% of dip netted fish, and 4% of seined fish. Net marks were present in 18% of dip netted fish and 4% of seine fish, while damage to the fins and tails was also present in dip netted (42%) but not seined fish (0%). There was no incidence of organ damage in dip netted or seined fish. Angled fish had no incidences of flesh wounds, net marks, or fin damage, but one fish (3%) had an organ wound (gill). Fish were hooked almost exclusively in the corner of the mouth (97%) but one fish was hooked in the interior of the mouth (3%). No fish was classified as deeply-hooked in the gills, esophagus, or tongue. Neither capture duration ( $z$ -value=0.01,  $df=28$ ,  $p=1.0$ ), air exposure duration ( $z$ -value=1.33,  $df=28$ ,  $p=0.19$ ), water temperature ( $z$ -value=0.90,  $df=28$ ,  $p=0.37$ ), or fork length ( $z$ -value=0.97,  $df=28$ ,  $p=0.33$ ) had a significant influence on the occurrence of injury in dip netted fish. The occurrence of scale loss was also significantly greater in dip netted fish (73%) than seined (24%;  $\chi^2=14.50$ ,  $df=89$ ,  $p<0.01$ ) and angled fish (18%;  $\chi^2=24.63$ ,  $df=89$ ,  $p<0.01$ ).

#### 4.4.3 Righting reflex

A significantly lower proportion of dip netted fish (73%;  $\chi^2=7.91$ ,  $df=89$ ,  $p=0.02$ ) and seined fish (76%;  $\chi^2=6.01$ ,  $df=89$ ,  $p=0.05$ ) maintained the righting reflex after capture compared to angled fish (97%; Figure 4.1B). There was no significant influence of capture duration (z-value=0.17,  $df=28$ ,  $p=0.86$ ), air exposure duration (z-value=-1.28,  $df=28$ ,  $p=0.20$ ), water temperature (z-value=-1.01,  $df=28$ ,  $p=0.31$ ), or fork length (z-value=1.06,  $df=28$ ,  $p=0.29$ ) on the proportion of dip netted fish that maintained the righting reflex.

#### 4.4.4 Behaviour

Dip netted fish were monitored for their presence above or below the falls within 24 hours. Approximately 44% of dip netted steelhead dropped back below the falls, while 12.5% of fish tagged below the falls migrated above the falls and then dropped back down. Whether a fish fell down the falls or remained above the falls was not correlated with their emigration time from Witset Falls ( $\chi^2=0.05$ ,  $df=2$ ,  $p=0.98$ ). Steelhead that fell down the falls were captured at warmer water temperatures ( $11.6 \pm 0.6$  °C) than those that remained above ( $10.1 \pm 0.3$  °C; z-value=-2.14,  $df=31$ ,  $p=0.03$ ). No other variables including capture duration (z-value=-1.01,  $df=31$ ,  $p=0.32$ ), air exposure duration (z-value=0.48,  $df=31$ ,  $p=0.63$ ), or fork length (z-value=-1.10,  $df=31$ ,  $p=0.27$ ) had a significant effect on the presence of steelhead above or below the falls within 24 hours of capture. Approximately 48% of dip netted steelhead left the falls ( $\pm 500$  m displacement) in 2 days or less following capture, while 38% of steelhead left between 3-9 days, and 14% of fish took greater than 10 days to leave the falls. Seined fish had similar

emigration rates from Witsset Falls with 60% leaving in 2 days or less, 25% leaving between 3-9 days, and 15% taking greater than 10 days. Approximately 9% of dip net and 12% of beach seine steelhead did not migrate upstream of Witsset Falls following capture. Immediate movement (20 min following release) of angled steelhead ranged from 28 m downstream to 33 m upstream. Immediate movement of angled fish was not significantly influenced by capture duration (t-value=1.40, df=13, p=0.19), gear choice (t-value=-0.05, df=13, p=0.96), water temperature (t-value=0.76, df=13, p=0.46), or fork length (t-value=-1.17, df=13, p=0.26).

Steelhead movement ranged from 38 rkm downstream to 145 rkm upstream during the study period. The migration rate of steelhead ranged from 1562 m-day<sup>-1</sup> downstream to 18460 m-day<sup>-1</sup> upstream. The intermediate-term (10-20 day) migration rate of dip netted fish (2856 ± 575 m-day<sup>-1</sup>; t-value=3.53, df=48, p<0.01), and seine-caught fish (3025 ± 518 m-day<sup>-1</sup>; t-value=2.96, df=48, p<0.01) was significantly greater than that of angled fish (285 ± 144 m-day<sup>-1</sup>; Figure 4.1C). Air exposure duration (t-value=-3.16, df=14, p<0.01), and water temperature at the time of capture (t-value=-2.86, df=14, p=0.01) were significantly and negatively correlated with intermediate-term migration rate of dip netted fish (Figure 4.2A, 4.2B). Neither capture duration or fork length had significant correlations with intermediate-term migration rates in the dip net or angling groups (all p>0.05; Table 4.1). Neither was there a significant influence of gear choice (fly vs. spinning or centre pin rod; p=0.20) or water temperature (p=0.91) on the intermediate-term migration rate of angled fish.

Peak migration rates were significantly faster for dip net (4149 ± 605 m-day<sup>-1</sup>) and seine fish (4460 ± 880 m-day<sup>-1</sup>) than angled fish (755 ± 419 m-day<sup>-1</sup>; dip net vs.

angled; t-value=3.87, df=78,  $p<0.01$ ; seined vs. angled t-value=3.89, df=78,  $p<0.01$ ; Figure 4.2C, D). The peak migration rate of dip netted fish was negatively correlated with air exposure duration (t-value=-3.94, df=23,  $p<0.01$ ), and water temperature at the time of capture (t-value=-3.14, df=23,  $p<0.01$ ), but these variables were not correlated with peak migration rates of beach seine and angled steelhead (all  $p>0.05$ ; Table 4.2). Neither capture duration or fork length had a significant influence on the peak migration rate of fish in any of the three capture groups (all  $p>0.05$ ). Gear type was not significantly correlated with the peak migration rate of angled fish ( $p=0.58$ ).

#### *4.4.5 Survival*

The 3-day survival of dip net steelhead was 80-97%, and 84-100% for seine steelhead, compared to 54-100% for angled steelhead. Uncertainty in these survival estimates account for fish that were never detected after release or were never detected making an upstream movement. Approximately 9% of dip net, 8% of beach seine, and 9% of angled steelhead were never detected again shortly after release.

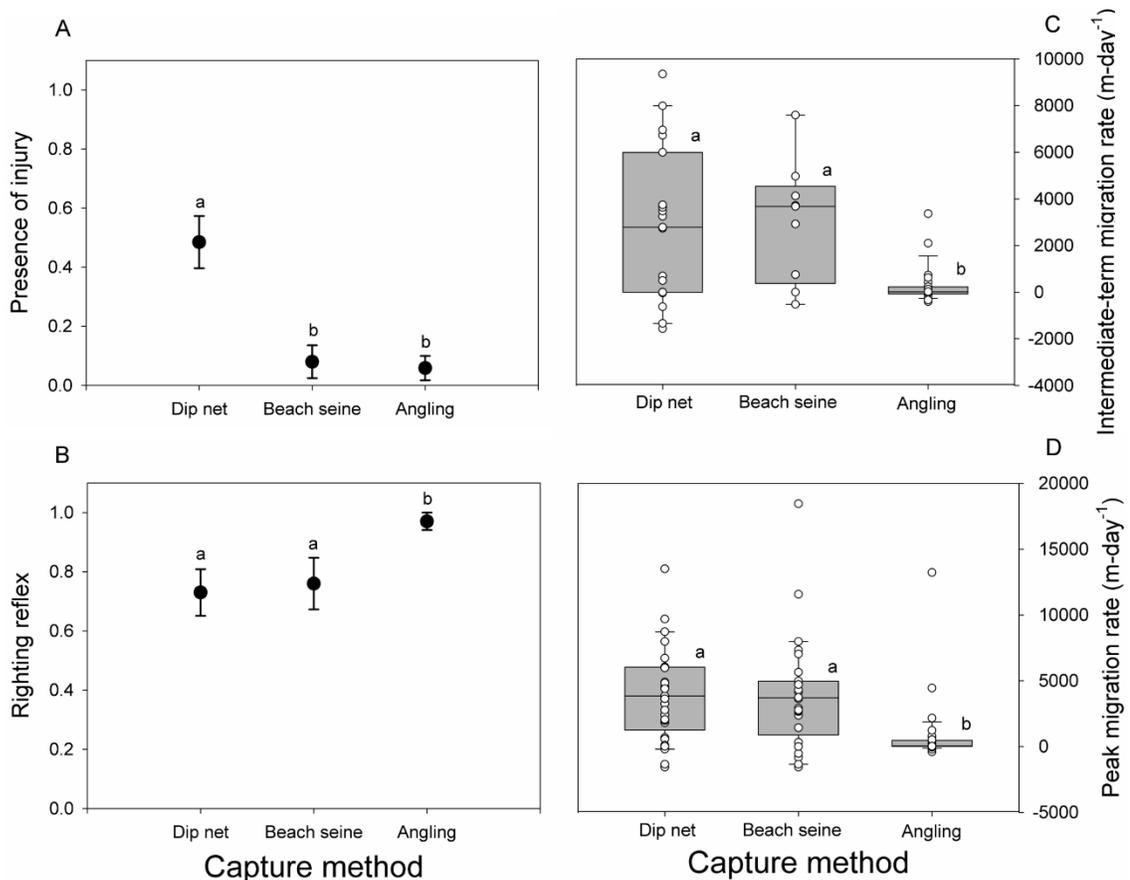
**Table 4.1.** Logistic regression outputs predicting intermediate-term (2-week) migration rates in steelhead (n=50) after a capture event. Models were developed for the dip net and angling capture groups and included capture duration, water temperature, and fork length as continuous variables. In the dip net capture group air exposure was included as a continuous variable and in the angling group gear choice (fly fishing vs. spinning or centre pin rod) was included as a categorical variable. A model for fish captured by beach seine was not included due to insufficient sample size. Significant variables are emphasized with boldface font.

Parameter	t-value	df	p-value
Dip net			
<b>Air exposure (sec)</b>	<b>-3.16</b>	<b>14</b>	<b>&lt;0.01</b>
Capture duration (sec)	-1.85	14	0.09
<b>Water temperature (°C)</b>	<b>-2.86</b>	<b>14</b>	<b>0.01</b>
Fork length (mm)	0.21	14	0.84
Angling			
Capture duration (sec)	-1.11	18	0.28
Gear choice	-1.34	18	0.20
Water temperature (°C)	-0.12	18	0.37
Fork length (mm)	1.12	18	0.36

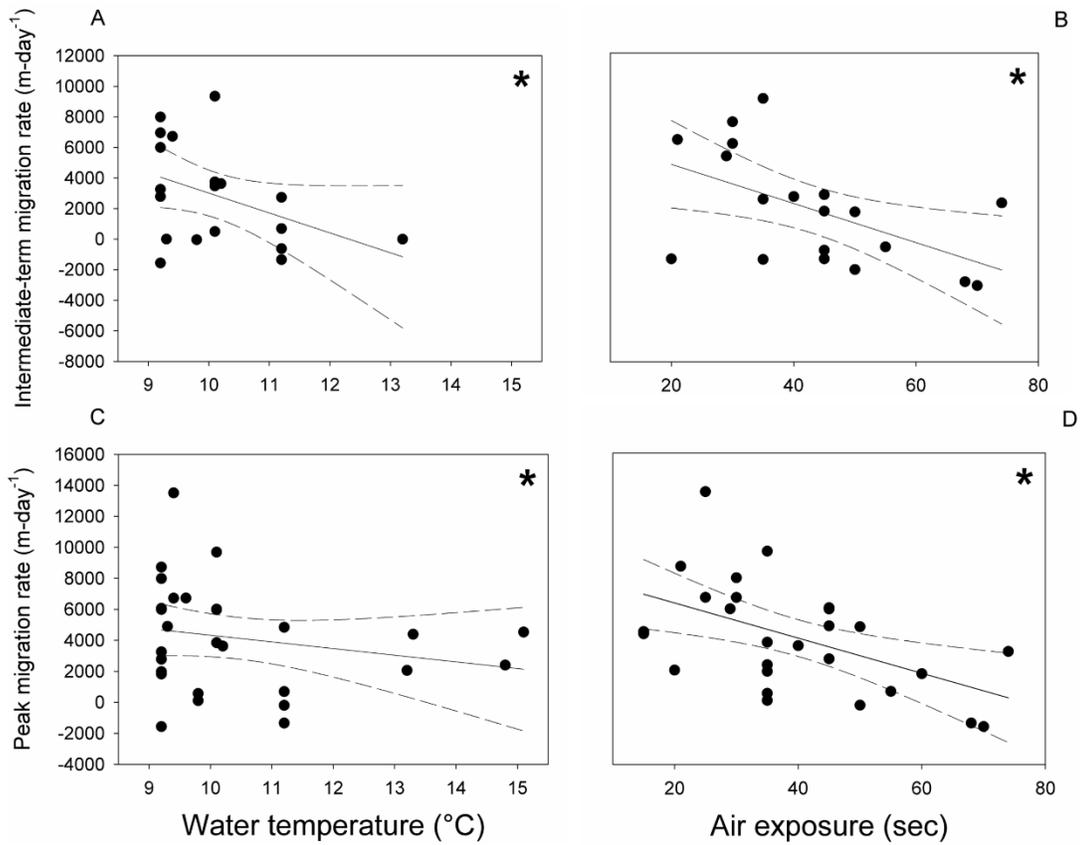
**Table 4.2.** Logistic regression outputs predicting peak migration rates in steelhead (n=80) after a capture event. Models were developed for each capture group and included capture duration, water temperature, and fork length as continuous variables. In the dip net capture group air exposure was included as a continuous variable and in the angling group gear choice (fly fishing vs. spinning or centre pin rod) was included as a categorical variable. Significant variables are emphasized with boldface font.

Parameter	t-value	df	p-value
Dip net			
<b>Air exposure (sec)</b>	<b>-3.94</b>	<b>23</b>	<b>&lt;0.01</b>
Capture duration (sec)	-1.79	23	0.09
<b>Water temperature (°C)</b>	<b>-3.14</b>	<b>23</b>	<b>&lt;0.01</b>
Fork length (mm)	0.06	23	0.95
Beach seine			
Capture duration (sec)	-0.91	17	0.18
Water temperature (°C)	-0.28	17	0.78
Fork length (mm)	-1.30	17	0.21
Angling			
Capture duration (sec)	-0.83	27	0.41
Gear choice	-0.60	27	0.58
Water temperature (°C)	-0.32	27	0.75
Fork length (mm)	0.60	27	0.56

**Figure 4.1.** Differences in (A) the proportion of fish with external injuries, (B) the proportion of fish maintaining the righting reflex, (C) intermediate-term (solid fill) and peak migration rates (no fill) following capture-and-release by dip net (n=33, 33, 19, 29, respectively), beach seine (n=25, 25, 9, 21, respectively), and angling (n=34, 34, 23, 32, respectively). Different letters denote a statistically significant difference ( $p < 0.05$ ).



**Figure 4.2.** Influence of water temperature ( $^{\circ}\text{C}$ ) and air exposure (sec) on intermediate-term migration rates (A, B,  $n=19$ ) and peak migration rates (C, D,  $n=29$ ) for dip net-captured steelhead. Asterisks denote a significant relationship between parameters ( $p<0.05$ ).



## 4.5 Discussion

### 4.5.1 Fishery-specific differences

Fishery-specific conditions varied across capture groups which influenced the occurrence of injury, reflex impairment, migratory behaviour, and survival of migrating steelhead. Dip net fish had a greater incidence of injury (48%) compared to seined (8%) and angled fish (6%), which may be related to difficult handling conditions and gear type (net type) but may reflect natural injury while surpassing a 15 m cascade. Dip net fish were entangled and in some cases were dropped or scraped along rocks during capture, which typically did not occur with the other capture groups. Seine fish had the same incidence of net mark injuries (4%) as steelhead caught by seine in the Columbia River (3.5%; Rawding et al., 2016), suggesting consistently low levels of injury from this capture method. Angled fish suffered no injuries aside from one fish with a gill filament tear by a single hook (2.9%). This rate of hooking injury is consistent with the rate reported for angled Bulkley River steelhead at 2.3% (Chapter 3).

Impairment of the righting reflex was similar in both dip net (27%) and seine fish (24%) at the time of capture, but considerably lower than that of angled fish (3%). Dip net and seine fish experienced similar impairment of the righting reflex as coho salmon caught in a Fraser River Indigenous beach seine fishery (26%; Raby et al., 2012). Impairment may be explained by greater incidence of injury and longer air exposure durations at the time of capture in the dip net group, while the seine fish may have experienced hypoxia due to crowding in the net.

Despite elevated water temperature in both dip net and beach seine groups, and greater air exposure durations for dip netted steelhead, the migration rates of these fish were significantly greater than that of angled fish. Slow migration rates following angling catch-and-release is consistent with findings on Bulkley River steelhead (Chapter 3), River Alta Atlantic salmon (Thorstad et al., 2007), and Upsalquitch River Atlantic salmon (Tufts et al., 2000). However, given minimal physiological disturbance and high survival of caught-and-released steelhead (Chapter 3), it is likely that differences in migration rates are related to the migratory phase steelhead were undertaking at the time of capture (Økland et al., 2001). Steelhead captured in the net fisheries were sampled from a high velocity hydrological section of river during the active migratory phase, while those captured by angling were targeted in holding water typically near spawning tributaries.

Steelhead captured by angling may have been closer to migratory destinations than fish captured by dip net, which may explain the observed differences in migration rates between the capture groups. This difference was absent for angled and beach seined sockeye salmon captured in the active migratory phase (Donaldson et al., 2011). Kintama et al., (2009) assessed the migration rate of steelhead captured by seine at Witset Falls, and their downstream movement rate of 8% following capture was similar to what I observed for seine (12%) and dip net fish (8%) in our study. Further, Kintama et al., (2009) found the average upstream migration rate of steelhead leaving Witset Falls to be approximately double (6080 m/day) the rate of steelhead captured by beach seine (3025 m/day) and dip net (2856 m/day) in our study (though our estimates include downstream migrants). There are many factors that can contribute to the observed differences in

injury, reflex impairment, and migration rates of fish captured in each fishery related to the inherent characteristics of the capture method, environmental variables, and intrinsic biotic factors.

#### 4.5.2 Capture-related variables

For the dip net and beach seine fisheries, differences in capture duration were primarily related to differences in the time taken to process fish after capture. In both fisheries, steelhead were confined to a small, shallow area that was likely hypoxic given moderate to high levels of excess post-exercise oxygen consumption in Pacific salmon (Farrell et al., 2003; Raby et al., 2014). Air saturation went from 90% to less than 60% after ten minutes in a crowded beach seine net (*Oncorhynchus* spp.) on the Fraser River, which corresponded to physiological changes in coho salmon (Raby et al., 2014; 2015). Following capture, oxygen consumption may not return to baseline levels for several hours in coho salmon (Raby et al., 2015) while heart rate can be elevated greater than 15 hours in 8-10 °C water for adult Atlantic and coho salmon (Anderson et al., 1998; Donaldson et al., 2010; Raby et al., 2015). Nonetheless, capture duration (i.e. time spent in these confined and likely hypoxic areas) was not correlated with reflex impairment or migratory behaviour in net captured steelhead.

The duration of capture is one of the primary factors considered when evaluating the response of fish to catch-and-release angling (Brownscombe et al., 2017). Longer capture durations correspond to greater anaerobic exercise and physiological disturbance including metabolic, acid-base, and ionic changes (Wood et al., 1983; Holeton et al., 1983). Angling-induced exercise increased plasma cortisol and lactate in Alagnak River, AK, rainbow trout (Meka and McCormick, 2005), though this was not observed in angled

Bulkley River steelhead (Chapter 3). Angling-induced exercise was also found to have minimal long-term consequences to growth and survival of hatchery rainbow trout relative to controls (Pope et al., 2007). The most convincing evidence that angling durations have a negligible impact on steelhead fitness comes from Pettit et al., (1977). In their study, hatchery steelhead were angled to exhaustion in a hatchery, marked, and released to the wild in the fall (Pettit et al., 1977). The following spring marked steelhead returned to the hatchery to spawn and there was no significant difference in the number of offspring reaching the eye-up stage (86.5 vs. 86.2%) for angled vs. control females (Pettit et al., 1977). Similarly, there was no influence of capture duration on the reflex ability or migration rate of steelhead in the current study. Although capture duration had little influence on migratory behaviour, it is likely beneficial to capture, process, and release steelhead from these fisheries promptly (Raby et al., 2014).

Following capture, fish may be lifted out of water to facilitate hook removal or processing before release. This air exposure period can lead to physiological changes in fish as carbon dioxide and corresponding acidosis in the blood increases due to the collapse of gill lamellae (Boutilier, 1990). Air exposure can also induce metabolic changes as cortisol, glucose, and lactate are released into the blood stream (Arends et al., 1999), although blood glucose and lactate concentrations were not different for steelhead air exposed for 0, 10, or 30 sec on the Bulkley River (Chapter 3). In the current study, both the beach seine and angling fisheries had average air exposure durations of less than 5 sec which likely had little influence on steelhead physiology and had no significant impact on migration rates (as per Donaldson et al., 2011 with sockeye salmon). Fish captured by dip net had longer air exposures that averaged 41 sec and extended up to 90

sec. Air exposure had no significant influence on reflex impairment or immediate movement around the falls for dip netted steelhead, while 30 sec of air exposure increased reflex impairment (Ward et al., In prep), but did not impair swimming ability of brook trout (Schreer et al., 2005). Despite no immediate impacts, air exposure appeared to decrease the intermediate-term and peak migration rates of dip netted steelhead during the fall. Little to no research has assessed whether changes in migration rate impact reproductive success of salmonid fishes. Migratory delay may provide an indication of impairment, which could have energetic consequences at the time of spawning when energy is limited (Penney and Moffit, 2014; Raby et al., 2015). Offspring production in Atlantic salmon (*Salmo salar*) was decreased after just 10 sec of air exposure with further reductions as air exposure increased (Richard et al., 2013). It should be noted however, that the influence of air exposure duration can not be completely discerned from the influence of entanglement in the dip net as both stressors occurred concurrently before transfer to the transport cradle.

#### *4.5.3 Environmental variables*

Environmental conditions at the time of capture can modulate the severity of a capture event (Gale et al., 2013). Steelhead are a cold-water species that are particularly sensitive to warm water temperatures (Wade et al., 2013). Water temperatures above thermal optima can reduce cardiac function and aerobic scope (Rummer et al., 2014), increase oxygen consumption (Claësson et al., 2016), and elevate indices of metabolic stress (Wendelaar Bonga, 1997). Water temperature has been correlated with physiological indices of metabolic stress in angled fishes including steelhead (Chapter 3), rainbow trout (Meka and McCormick, 2005), bonefish (*Albula vulpes*; Brownscombe et

al., 2015), and largemouth bass (*Micropterus salmoides*; Brownscombe et al., 2014). Physiological constraints imposed by water temperature can result in changes to fish reflex ability and movement (McLean et al., 2016; Wootton et al., 1998). Temperature at the time of capture was not linked to increased reflex impairment in steelhead, although it tended to increase reflex impairment following angling and air exposure in brook trout caught in 12-23 °C (Ward et al., in prep). Temperature did increase the rate of fallback to below the falls in dip net-captured steelhead attempting to surpass the most challenging hydrological stretch of the Bulkley River. This fallback rate of 44% is higher than that recorded for steelhead ascending hydroelectric dams on the Columbia and Snake River system (21%; Boggs et al., 2004). The consequences of this fallback may be minimal, as there was no significant delay in the time to emigrate the Witsset Falls area for fish that fell below the falls relative to those that remained above. Similarly, Columbia/Snake River sockeye that fell down a hydroelectric dam experienced the same survival to spawning grounds as those that did not fall back (Naughton et al., 2006). It should be noted however that 9% of dip netted steelhead fell down the falls and never migrated back up the falls. This behaviour may reflect a stress response to dip net capture (e.g. Makinen et al., 2000) or may reflect natural searching behaviour that is common in anadromous salmonids (Økland et al., 2001; Richard et al., 2014).

Temperature at the time of capture also had an influence on the intermediate-term and peak migration rates of steelhead following capture. Physiological status following capture is related to water temperature in both steelhead (Chapter 3) and Coho salmon though most physiological changes return to baseline levels within 24 hours (Raby et al., 2015). Further, long-term survival was not related to beach seine and angling capture

temperatures in sockeye salmon (Donaldson et al., 2011). Steelhead caught in warmer temperatures earlier in the season would have experienced warmer temperatures following capture as well. This makes it difficult to discern whether it is indeed the temperature at the time of capture driving this relationship through an exacerbated stress response (Gale et al., 2013), or simply greater thermal exposure following capture. Accumulated thermal units were correlated with slower migration rates in chinook salmon, and water temperature in the main-stem Columbia River was correlated with staging behaviour in cool-water tributaries and delayed migration rates in steelhead (High et al., 2006). The opposite relationship was observed in Atlantic salmon however, that had faster migration rates in 22 °C than 14 °C (Richard et al., 2014). As water temperature was not a significant predictor of movement in angled or beach seined fish, it is suggested the temperature-related movement in dip net steelhead is a response to increased capture stress rather than accumulated thermal units.

#### *4.5.4 Intrinsic biotic factors*

Steelhead body size had little influence on injury, reflex impairment, or movement following capture in any of the three fisheries. Smaller fish captured in net fisheries generally suffer greater injury and discard mortality from entrapment in mesh (Sangster et al., 1996; Davis and Olla, 2002). Mesh sizes in both the seine and dip net fisheries of the Bulkley River were likely sufficiently small that most fish were not trapped within mesh during capture. Fish size also had little influence on injury, reflex impairment, or movement of steelhead following catch-and-release angling, which mirrors the findings of Chapter 3, that found no influence of size on the physiology and behaviour of angled steelhead. Intersexual differences were not considered in this study,

as the majority of steelhead intercepted in these fisheries were female (70%). This suggests that the steelhead population may be female skewed or that females are more vulnerable to capture in these fisheries. Female Pacific salmon have consistently higher mortality rates than males following capture (Gale et al., 2014; Jeffries et al., 2012; Teffer et al., 2017), though these fish were closer to spawning than the Bulkley River steelhead. Previous research on angled steelhead (Chapter 3), angled rainbow trout (Dedual et al., 1999), and trapped Atlantic salmon (Hagelin et al., 2016) found no differences in migratory movements between sexes. In addition to sublethal consequences of capture on steelhead migration, these capture events may also result in immediate and delayed mortality.

#### *4.5.5 Survival*

Survival of dip net fish within 3 days of capture was 80-97% seine fish survival was 84-100%. Mortality in the net fisheries may be related to the capture method, but may also reflect warmer temperatures faced by steelhead during dip net and seine capture that operate earlier in the season on the Bulkley River. There were also site-specific differences in the locations that steelhead were caught, though this has not previously been identified as a factor contributing to mortality in captured fishes. Previous beach seine survival estimates for steelhead have been conducted in the Columbia River (97% survival over 11 days; Rawding et al., 2016) and Rogue River (96% immediate survival; Everest 1973). Previous short-term minimum survival estimates for steelhead captured and released by beach seine at Witsset Falls was 78%, though this estimate did not include the fate of fish moving downstream or those that were not detected again (Kintama et al., 2008). Beach seine capture-and-release on other *Oncorhynchus* species such as coho

salmon (12% over 4 days; Raby et al., 2012), and sockeye salmon (<5% over 1-day; Donaldson et al., 2011), generally indicate low mortality rates shortly after capture. However, life-history differences between Pacific salmon (semelparity) and steelhead (iteroparity) make survival comparisons difficult. To date, there are no studies that have evaluated catch-and-release mortality in dip net fisheries for adult fish.

Survival of angled steelhead 3 days after release (54-100%) was variable compared to previous catch-and-release investigations on the Bulkley River that found approximately 5% mortality over these periods (Chapter 3). Hooking mortality across both years of evaluation was just 2% which is notably lower than the 16% average hooking mortality across the Salmonid family (Hühn and Arlinghaus, 2011). High survival in these fisheries is a likely result of the angling restrictions imposed by the MFLNRORD for Skeena Region 6 that only permit the use of artificial baits on single, barbless hooks, as well as specialized anglers that implement responsible angling practices (Nguyen et al., 2013; Lamansky and Meyer, 2016). Moreover, water temperatures during the angling season are cool which may decrease mortality relative to other steelhead recreational fisheries (Taylor and Barnhart, 1997). Although a future carcass survey is needed to confirm mortalities, the mortality estimate of 4.5% from Chapter 3 is comparable to the average catch-and-release angling mortality rate for steelhead reported across other studies at 4.1% (Mongillo, 1984; Hooton, 1987; Lirette, 1988, Lirette, 1989; Thomas, 1995; Ministry of Lands, Forestry, and Natural Resources, unpublished data; Nelson et al., 2005).

Future carcass surveys will refine over-winter mortality estimates of dip net, angled, and beach seined steelhead. A capture-and-release study on sockeye salmon

found that both angling and beach seine survival was greater than 95% within 24 hours of release, although delayed mortality was greater following angling (64%) than beach seine (48%; Donaldson et al., 2011). It should be noted however, that our mortality estimates do not discern capture-related mortalities from tagging-related mortality or tag loss. Previous work with externally attached tags on adult brown trout (Thorstad et al., 2014; Økland et al., 1996; Aarestrup and Jepsen, 1998) and Atlantic salmon (Aarestrup et al., 2000) in low-vegetation rivers (similar to the Bulkley) suggests little to no tagging-related mortality and tag loss. Furthermore, the combined mortality and tag loss of Bulkley River steelhead using the same tagging method and model of tag was 0-4.5% in 2016 (Chapter 3). Mortality estimates could be higher than reported here if the eight fish that were never detected again died, but this cannot be distinguished from tag malfunction, harvest, and emigration from the study site.

#### *4.5.6 Conclusions*

Inherent differences exist across fisheries that can result in varying levels of sublethal stress and survival for captured fish. Steelhead captured by dip net experienced greater levels of entanglement, air exposure, and difficult handling (dropped and scraped along rocks) that likely increased the occurrence of injury and reflex impairment relative to the other capture groups. Fish caught in the dip net and beach seine fisheries were actively migrating and had faster migration rates than angled steelhead that moved very little from release sites. Migration rates in dip net-captured fish tended to decrease with greater air exposure durations and warmer water temperature at the time of capture, suggesting that handling practices and capture conditions within the fishery can have sublethal consequences on steelhead migration. Dip net fishers may increase the welfare of

captured steelhead by taking extra precaution earlier in the season when temperatures are warmer, and by reducing air exposure during capture. Nonetheless, given the high survival across all fisheries (>80%), it is suggested that these fisheries may coexist without instilling considerable harm to the steelhead population although such determinations are best made by fisheries managers with information on the population status of a given stock.

## Chapter 5. General Conclusion

The principal objective of this thesis was to evaluate the factors influencing the upriver migration of wild adult steelhead. The second chapter of my thesis considered the impacts of pathogens on host-physiology, and it was found that certain pathogens such as *F. psychrophilum* occur at high prevalence in steelhead and tend to suppress function of the humoral immune system during migration. Overall, the prevalence of pathogens in steelhead was generally lower than in previous evaluations conducted on migratory salmonids in B.C., although those species were semelparous (Bass et al., 2017; Teffer et al., 2017). The third and fourth chapters of my thesis evaluated the consequences of capture events on steelhead migration. Capture appeared to have sublethal consequences on reflex ability, physiology, and short-term behaviour, though short-term survival was high following capture by all three gear types, and was high compared to other salmonid species (Hühn and Arlinghaus, 2011). Nonetheless, if I apply the mortality rates derived from this thesis to the catch rates recorded by the MFLNRORD, I can estimate that recreational fisheries remove approximately 2.7% of the steelhead population in the Bulkley River (Figure 5.1).

The second objective of my thesis was to determine which capture-related, environmental, or intrinsic biotic variables have an influence on steelhead migration in the context of pathogens and fisheries capture. This information will help to better understand pathogen dynamics in migrating steelhead and inform species-specific best practices for fishers to implement with the aim of reducing sublethal and lethal consequences of capture on migrating steelhead. In Chapters 3 and 4, I considered the

influence of two capture-related variables, and in all chapters I considered the influence of water temperature, and intrinsic biotic variables (sex and size) on measured outcomes.

The variables of air exposure and capture duration are commonly associated with negative outcomes during capture events (Brownscombe et al., 2017). In Chapter 3, I found that air exposures of just 10-30 sec resulted in greater reflex and swimming impairment immediately after capture compared to fish kept submerged. Although the influence of 30 sec or less of air exposure appeared to have a negligible effect shortly after capture, results from Chapter 4 indicate that longer air exposures ranging from 15-90 secs may have lasting effects weeks after the capture-and-release event. Most studies evaluating air exposure are conducted at much longer air exposure durations than those considered in this thesis (e.g. Gagne et al, 2017; Suski et al., 2007; Schreer et al., 2005), and 10 sec of air exposure is generally not considered harmful and has even been recommended as a reasonable amount of time for anglers to remove fish from the water (Cook et al., 2015). Further, few studies have identified long-term consequences of air exposure less than 60 sec in duration (as identified in Chapter 3), suggesting that steelhead may be particularly sensitive to impaired respiration caused by the collapse of gill lamellae when removed from the water (Cook et al., 2015). This notion is supported by the observed upregulation of genes responsive to oxygen availability (CA4, HBA, SEPW1) with increasing temperatures when dissolved oxygen levels would be lower (Chapter 2). Sensitivity to impaired respiration has also been reported in Atlantic salmon that maintain similar life-history characteristics to steelhead. Richard et al. (2013), found that just 10 sec of air exposure decreased an individual's reproductive success (Richard et al., 2013). Air exposure appears to have a greater influence on steelhead, than capture

duration, which had no strong correlations with physiology, behaviour, or survival across any of the capture gears evaluated in this thesis. Steelhead can migrate several hundreds of kilometers inland, which requires the use of burst swimming similar to that experienced during a capture event (Ruggerone, 2006). As steelhead are of the strongest swimmers within the *Oncorhynchus* genus (Reiser et al., 2006), it is not surprising that capture duration had minimal influence during capture events, which is consistent with previous work evaluating exercise stress on steelhead (Pettit et al., 1977).

Common across all chapters was the inherent influence that water temperature had on physiological and behavioural processes. Temperature is considered to have a ‘controlling’ influence on key processes in fish such as maintenance and growth (Brett, 1969). Fish adjust their physiological processes in response to temperature changes as a means to provide sufficient scope for activity (Brett, 1969). These adjustments can be energetically costly which is particularly important for migratory fish species such as steelhead that undergo long distance migrations under fixed energy constraints (Brett and Glass, 1973). Steelhead are a cold-water species and migration can be hindered at temperatures above 16-17 °C (Hicks, 2000; Salinger and Anderson, 2005). The temperatures in the Bulkey River were cool (4.5-15.1 °C at the time of capture), but indices of physiological stress still showed signs of elevation with temperature. In Chapter 2, water temperature was positively correlated with the expression of genes responsive to oxygen availability, and in Chapter 3 with indices of metabolic stress in the blood. Even within preferred thermal limits, species can experience increases in stress and mortality following an acute stressor (Gale et al., 2013). Temperature did not increase reflex impairment despite its close link with physiological stress (McLean et al.,

2016; Raby et al., 2015) but it did increase immediate fallback for fish caught in the dip net fishery (Chapter 4). This fishery was associated with warmer water temperatures, and release of fish into an area with high flow rates which may explain why this relationship with temperature was not observed in angled fish (Chapter 3). Warmer water temperatures would likely increase mortality in all Bulkley River fisheries, given that previous work reported twice the rate of catch-and-release angling mortality in water temperatures of 8-23 °C (Taylor and Barnhart, 1997). In addition, most pathogens detected in steelhead have optimal temperatures greater than those observed in the Bulkley, suggesting that disease dynamics will change as temperatures warm. Findings from this thesis provide further evidence that steelhead will be sensitive to warming water temperatures in the face of climate change (Wade et al., 2013). This may include changes in their migratory physiology, responses to fisheries captures, and vulnerability to disease.

There is very little evidence in this thesis to suggest that the intrinsic biotic factors of sex and size were influencing steelhead migration in the context of pathogens, physiology, behaviour, or survival. The only difference observed between male and female steelhead was in the concentration of blood glucose following angling. However, glucose was not responsive to air exposure treatments, and is not considered an important source of energy in salmonids (Brett, 1995), suggesting the biological significance of this difference is minimal. It may be expected that differences in pathogen susceptibility, physiological stress, behaviour, and survival would exist if energy allocation in reproduction differed across sexes during early migration (Reznick, 1985). However, Penney and Moffitt (2014) found similar energy density, lipid, and protein storage during

early migration in male and female steelhead from the Snake-Columbia River. On the Bulkley River, egg production in females and development of secondary sexual characteristics in males may have resulted in similar energetic contributions to reproduction, and the lack of sex-specific differences observed in Bulkley River steelhead. Size also had little influence on pathogen dynamics and capture responses in migratory steelhead. It may be that size has a greater effect towards the end of migration when resources are limited and smaller steelhead show worse condition (less protein content and energy density; Penney and Moffit, 2014).

Information on the factors contributing to catch-and-release outcomes can be used to develop species-specific best practices for fishers to implement when encountering steelhead. Results from my study suggest that fish should be kept wet following capture, and that special care should be taken by fishers when temperatures are warm earlier in migration. Steelhead anglers and Indigenous fishers are generally aware that steelhead are sensitive to anoxia resulting from being lifted out of the water or confined in nets and tend to reduce the extent to which steelhead are exposed to these conditions (Lamansky and Meyer, 2016; Roth et al., 2018; Guckian et al., In press). Nonetheless, there is a need to communicate this information to steelhead anglers given that only half are confident they know and are using 'best practices' while angling (Guckian et al., in press). This study determined that most best practices information is self-taught, learned from others, or gained online (Guckian et al., in press). I used the results of this survey to inform the communication channels used to disseminate these best practices to the fishing community, which included an informative postcard developed in collaboration with

Keepemwet Fishing, a growing social media movement being used to communicate responsible angling practices (Danylchuk et al., 2018).

### *Future Research Directions*

Although these capture-related variables have been shown to have sub-lethal consequences on steelhead, they may also have consequences on reproductive success and survival that were not accounted for in this thesis. This thesis determined whether fish survived to the spawning period, but the rate of steelhead mortality was too low to evaluate which components of a capture event may contribute to increased mortality. In both Chapters 3 and 4 a number of steelhead went undetected though the reasons for this are unclear. Automated data logging stations at the upper and lower ends of the study site could identify if any of these undetected fish left the study area and would help refine mortality estimates. Further, this thesis did not evaluate whether fish successfully spawned. Future work is needed to evaluate whether capture in general, as well as the components of a capture event, contribute to these fitness outcomes during the final stages of the steelhead spawning migration. Spawning success could be evaluated through parentage analysis (e.g. Baumsteiger et al., 2008) in a river system that provided the opportunity to intercept and genotype upstream migrating adults, apply treatments, and then collect and genotype the progeny of these individuals. This would be feasible in a river system with a fish weir, fishway, or counting fence (e.g. Babine River), which would also provide an effective control group for mortality assessments (e.g. Lindsay et al., 2004).

Recent technological advancements in molecular genetics are now providing novel opportunities to non-lethally evaluate the impact of host-pathogens and

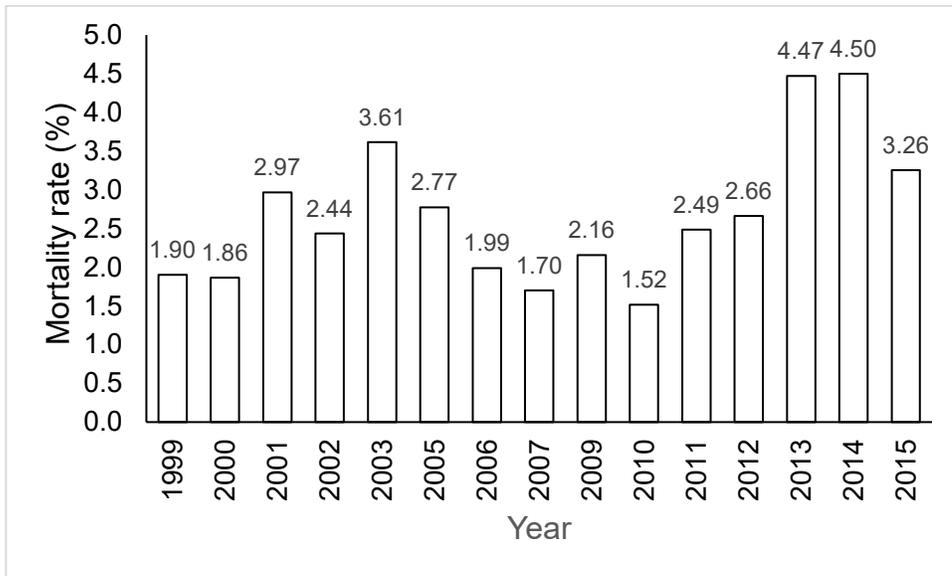
transcriptome level physiology on the fitness of fish in the wild (Miller et al., 2016). We evaluated the use of a suite of biomarkers to characterize steelhead physiology during upstream migration. A number of genes were correlated with water temperature (presumably in relation to oxygen availability) and may be of interest to other researchers looking at the impacts of water temperature on fish physiology (CA4, SEPW1, HBA). Future work could combine the molecular genetics tools used in Chapter 2, with the physiological measures, and biotelemetry tools used in Chapters 3 and 4. This method could be used to further investigate differences between fish captured by dip net at Witset Falls and angling upstream of the falls. As discussed in Chapter 2, I suggest this could be related to inherent differences in the sampling locations (a difficult hydrological section of the river, compared to slow moving holding water), or sampling bias associated with the capture gear. Future work could evaluate which of these explanations is more likely by sampling fish below the falls using both capture methods, attaching telemetry transmitters, and linking successful migration above the falls to individual pathogen burdens.

Recent work has linked host-gene expression and microbe productivity to indices of osmoregulatory and metabolic stress (Bass et al., 2017; Teffer et al., 2017), as well as pre-spawn mortality in migratory salmonids (Evans et al., 2011). In Chapter 2 I identified differences in the relative load of various pathogens in migrating steelhead, and in Chapter 3 I determined that there is approximately 10% mortality of steelhead over winter. The direction of future work could evaluate whether there is a link between host-pathogen productivities, and steelhead survival throughout migration, particularly in winter as fish approach spawning.

### *Final Remarks*

The body of work within this thesis provides novel information on the pathogens present in a wild steelhead population, and the influence that these pathogens have on steelhead physiology. This pathogen survey may serve as an important baseline or comparator when completing similar surveys on steelhead in the future. Furthermore, my work has provided mortality estimates for fish captured by angling, beach seine, and dip net, which will provide necessary information for the effective management of wild steelhead populations in rivers that support recreational and subsistence fisheries. This work has also identified best-practices for fishers to implement when intercepting steelhead to increase fish welfare upon release. Overall, this thesis has increased the body of knowledge on natural and anthropogenic factors that influence the migration of this iconic salmonid species.

**Figure 5.1.** The estimated population level mortality rates attributable to the recreational fishery from 1999-2015. These mortality rates are generated by applying the 4.5% mortality rate (Chapter 3), to the population (Witset Mark-recapture program) and catch rate (MFLNRORD, Angler surveys) data for the Bulkley River.



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