UNDERSTANDING THE CONSEQUENCES OF FISHERIES-RELATED STRESSORS ON ADULT MIGRATING PACIFIC SALMONIDS

by

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ABSTRACT

Adult Pacific salmon (*Oncorhynchus* spp.) are targeted by the recreational, commercial, and First Nations fisheries during their spawning migrations through the Fraser River, British Columbia, Canada. Salmon can escape from each fishery or be released either voluntarily or due to mandate. Despite a high proportion of Pacific salmon released from each fishery, the primary (e.g., catecholamine, corticosteroid increases), secondary (e.g., metabolic, osmoregulatory, cellular responses), and tertiary (e.g., behavioural, survival outcomes) responses to fisheries-related stressors remain poorly understood. The overarching hypothesis of this thesis was that fisheries-related stressors displace fish from homeostasis, resulting in primary and secondary stress responses leading to tertiary outcomes, which in turn can be countered by facilitated recovery techniques.

A range of fisheries-related stressors resulted in physiological disturbances reflected by primary, secondary, and tertiary stress responses. Telemetry studies revealed that survival was lower for sockeye salmon released following angling compared to those released following beach seine capture. Survival was lower for sockeye salmon released from invasive gill and tangle net capture treatments relative to beach seine treatments, and this result was population-specific.

Laboratory studies investigated the time required for primary and secondary stress indices to recover following fisheries-related stressors. Biologgers showed that heart rate recovery depended on the intensity and duration of the stressor, requiring several hours. A series of indicators of primary and secondary stress, including the expression of genes related to cellular stress and cell maintenance indicated that the stress response and recovery was sex- and species-specific.

A three-pronged approach was used to investigate methods for accelerating recovery and promoting survival following capture stress by combining a laboratory-based physiology study, a field-based telemetry study, and a human dimensions survey. While facilitated recovery showed encouraging results and had general support from anglers, improved techniques are required before this approach could be implemented in freshwater release fisheries.

Together, these results support my hypothesis and provide evidence for the context-specific nature of the response and recovery to fisheries-related stressors. This thesis highlights that even a brief fisheries-related stressor can have profound consequences on Pacific salmon, as reflected by the tertiary stress response, including mortality.

PREFACE

Chapter 2: The consequences of angling and beach seine capture on the physiology, post-release behaviour and survival of adult sockeye salmon during upriver migration.

Authors: M.R. Donaldson, S.G. Hinch, D.A. Patterson, J. Hills, J.O. Thomas, S.J. Cooke, G.D. Raby, L.A. Thompson, D. Robichaud, K.K. English, and A.P. Farrell

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Chapter 3: Population-specific consequences of fisheries-related stressors on adult sockeye salmon.

Authors: M.R. Donaldson, S.G. Hinch, G.D. Raby, D.A. Patterson, A.P. Farrell, and S.J. Cooke

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Chapter 4: Physiological responses of free-swimming adult coho salmon to simulated fisheries encounters.

Authors: M.R. Donaldson, T.D. Clark, S.G. Hinch, S.J. Cooke, D.A. Patterson, M.K. Gale, P.B. Frappell, and A.P. Farrell.

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Chapter 5: Species-specifc responses and recovery of wild adult Pacific salmon to fisheries-related exercise stress

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Chapter 6: Evaluation of a simple technique for recovering Pacific salmon from capture stress: integrating comparative physiology, biotelemetry, and social science to solve a conservation problem.

Authors: M.R. Donaldson, G.D. Raby, V.N. Nguyen, S.G. Hinch, D.A. Patterson, A.P. Farrell, M. Rudd, L.A. Thompson, C.M. O'Connor, A.H. Colotelo, S.H. McConnachie, K.V. Cook, D. Robichaud, K.K. English, and S.J. Cooke

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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DEDICATION

To Lisa, who taught me to trust the process.

1 INTRODUCTION

1.1 The stress response

Darwin (1859) first described the process of adaptation through natural selection, where individual variation for adaptive traits leads to certain individuals in a population being more likely to secure fitness and pass these traits to subsequent generations. Darwin's contributions were at the forefront of Hans Selye's theoretical work on how organisms respond to noxious stimuli (Selye 1936) and the notion that the stress response is adaptive (Selye 1951). Defining stress and stressors has been problematic for researchers, even leading Selye himself to question his own definitions and rethink his conceptual framework for stress throughout his career (Selye 1973). Despite continued efforts to redefine the terminology (e.g., McEwen and Wingfield 2003), stress is typically considered to be the general, adaptive response of an organism to any demand made upon it (Selye 1973) and stressors are noxious stimuli that displace an organism from homeostasis (Selye 1936) and elicit an adaptive set of molecular, cellular, hormonal, metabolic, and behavioural responses (Romero 2004; Korte et al. 2005).

Maintaining homeostasis when faced with a stressor represents a unique intersection of physiology, behaviour and life history that is fundamental to the persistence of animal populations (Ricklefs and Wikelski 2002). If the stressor persists or if the magnitude of the stressor pushes the organism to its physiological limits such that it cannot maintain homeostasis, the stress response becomes maladaptive, leading to sublethal impairments or mortality in extreme cases (Selye 1936; Black 1958; Wood 1983). The stress response may enable the maintenance of homeostasis during a stressful event, but can also sequester energy away from anabolic processes and maintenance functions such as reproduction to catabolic activities that

mobilize energy and restore homeostasis (Wendelaar Bonga 1997). The time required to recover from the stressor will determine the frequency of maximal performance (Milligan 1996), resulting in the recovery timecourse having the potential to indirectly influence fitness.

The stress response can be triggered by environmental (e.g., temperature, oxygen), physical (e.g., capture, confinement), or perceived (e.g., startle-response, predator presence) stressors. For fish, fisheries capture may result in direct mortality through harvest but fish are commonly released either intentionally as commercial discards or through recreational catch-and-release fisheries, or inadvertently by escaping from or evading gear altogether. Fish respond to the capture stressor by mounting a stress response (i.e., primary, secondary, tertiary stress; Barton 2002; described below). For released fish, if homeostasis cannot be recovered (Schreck et al. 2000) the stressor can lead to tertiary outcomes including reduced survival (Black 1958; Wood et al. 1983). What follows is a summary of the primary, secondary, and tertiary responses to stress that will be used to develop a conceptual framework for understanding how fish respond to and recover from fisheries-related stressors.

1.2 Primary and secondary responses to fisheries-related capture and exercise stress

A general stress response to a wide range of stressors has been widely characterized at the organismal level for fish (Black et al. 1962; Wood 1991) and has a characteristic physiological response (Milligan 1996; Kieffer 2000). The primary stress response occurs when the hypothalamo-pituitary-interrenal (HPI) axis, which has received sensory input as a result of the stressor, stimulates the release of catecholamines (i.e., adrenaline and noradrenaline) and corticosteroids (i.e., cortisol; Barton et al. 2002). The surge of catecholamines in turn induces the secondary stress response, resulting in a shift from anabolic to catabolic processes to mobilize energy to respond to the stressor and to restore homeostasis (Wendelaar Bonga 1997).

The secondary stress response occurs at the tissue-level and includes cardiorespiratory, osmoregulatory, and immune system changes, as well as metabolic, ionic, and cellular responses to stress. Changes in gene expression (i.e., quantitative, qualitative, and changes in reaction coefficients) can be linked with various stressors, such as handling stress (Krasnov et al. 2005). Heat shock protein expression increases to maintain cellular homeostasis (Iwama 2004). Also at the cellular level, a suite of responses occur to temporarily tolerate or counteract stress or remove damaged cells by apoptosis (Kültz 2005). The primary and secondary stress responses can be understood in the context of aerobic and anaerobic exercise stress.

Fish activity can be either aerobic (i.e., sustained or prolonged swimming) or anaerobic (i.e., burst swimming). Sustained swimming can be maintained for long durations relative to prolonged swimming, the latter of which is higher intensity and consequently is maintained for shorter durations prior to fatigue (Brett 1964). Aerobic exercise requires that the cardiovascular system delivers oxygen from the gills to highly vascularized red musculature (Beamish 1978). For aerobic activity under unstressed conditions, metabolic demand typically matches supply and waste production is mostly balanced (Jones 1982). Circulating catecholamines, ions, metabolites, blood acid-base status, and energy stores remain largely unchanged during normal aerobic activity (Wood 1991). Stressors, such as fisheries capture, will result in a fright stress response that may begin aerobically. However, prolonged or high intensity stressors will result in conditions where tissue oxygen demands exceed supply. In such cases the stress response is initiated to temporarily maintain or increase oxygen consumption. Catecholamine release causes a number of changes in hematocrit and hemoglobin with a net effect of increasing the oxygen carrying capacity of blood to meet mounting tissue oxygen demands during the stress response. Catecholamines stimulate heart rate to increase blood flow and oxygen delivery to tissues, often

accompanied with increased ventilation rates (Pickering and Pottinger 1995). Cardiovascular responses have been observed following fisheries capture stress in many species, including smallmouth bass (Schreer et al. 2001), largemouth bass (Cooke et al. 2002a), and Atlantic salmon (Anderson et al. 1998). However, if oxidative pathways are insufficient to meet the oxygen requirements to respond to the stressor, anaerobic pathways are required.

Anaerobic activity can occur when the capacity for aerobic activity is exceeded or when a short duration of rapid swimming (i.e., burst swimming) is required. Burst swimming can only be maintained on the order of minutes and if prolonged, results in exhaustion and an inability to perform further burst swimming in the short-term (Jones 1982). Early work by E.C. Black set the stage for our current understanding of how fish respond to exhaustive exercise stress (Black 1955; 1957; Black et al. 1962). Black's pioneering research led to our understanding that fisheries-related stressors (e.g., hook-and-line angling or net entanglement) are examples of exhaustive exercise stressors (Wood 1991; Milligan 1996). Burst swimming may also occur under natural conditions such as predator-prey interactions or as fish navigate hydraulic barriers (Hinch and Rand 1998). Anaerobic glycolysis associated with burst swimming is largely supported by the poorly-vascularized white muscle. Burst swimming causes the anaerobic consumption of muscle phosphocreatine (PCr), adenosine triphosphate (ATP), and glycogen (Dobson and Hochachka 1987) and the accumulation of lactate in white muscle and blood (Hochachka 1991; Wood 1991). Combined metabolic (i.e., increased H^+) and respiratory (i.e., increased P_{CO_2}) acidoses result in decreased blood pH (Wang et al. 1994). This acidification can disrupt ion-osmoregulatory balance as water shifts from blood to muscle tissue resulting in temporary increases in concentrations of some plasma ions in freshwater, followed by depressed ion concentrations over the longer term (Wood 1991). A surge of catecholamines and

corticosteroids mediate much of this exhaustive exercise stress response (Milligan 1996) and cortisol plays an important role in recovery (see below; Eros and Milligan 1996; Milligan et al. 2000).

Fisheries-related stressors include perceived or physical contact with fisheries gear, followed by entanglement (e.g., net confinement) or hooking (e.g., hook and line), landing, handling and air exposure associated with removal from gear in both net and line fisheries. Previous work developed from comparative physiology studies on exercise stress has highlighted two important factors relevant to understanding how capture affects fish. First, the type and duration of stressor has consequences on the level of stress incurred by fish (Wood 1991; Kieffer 2000). For example, the duration of capture is typically proportional to the magnitude of physiological response (e.g., Gustaveson et al. 1991; Chopin et al. 1996) and the magnitude of stress, including interactive effects such as temperature and air exposure, can result in impaired ventilation, equilibrium loss, and mortality (Gingerich et a. 2007). Second, the magnitude of the physiological disturbance shows a typical recovery profile, which if severe may lead to mortality (Black 1958, Wood 1983). The general primary and secondary physiological disturbances described above in an exercise stress context have been observed in the blood and muscle of fish captured by angling (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996; 1997) and commercial gears (Parker and Black 1959; Farrell et al. 2000; 2001a,b; Skomal 2007).

In addition to the fright and exercise stress components of the capture event, fisheries-related capture and handling procedures can result in various durations of hypoxia from air exposure, ranging from seconds to several minutes and resulting in a hypoxic stress response (Ferguson and Tufts 1992; Sloman et al. 2001). Hypoxia may also occur in net fisheries if ventilation is restricted during net retrieval, sorting, and handling, or in the case of shoreline

seine fisheries, localized oxygen depletion. While exhaustive exercise alone contributes to acidosis, air exposure further reduces plasma pH (Ferguson and Tufts 1992). Post-exercise ventilation rates may be reduced at higher temperatures, resulting in reduced gas exchange (Gale et al. 2011). Air exposure can contribute to increases in blood lactate and glucose as gas exchange is inhibited due to a collapse of gill lamellae and a reduction of gill surface area (Arends et al. 1999). These events may independently elicit the general stress response but the stressors are likely cumulative, since at this stage the stress response timecourse has already begun (Ferguson and Tufts 1992; Furimsky et al. 2003).

1.3 Recovery and facilitated recovery

As described above, exhaustive exercise and air exposure leads to a profound physiological disturbance and a displacement from homeostasis. The recovery process attempts to restore homeostasis while incurring a minimally additional metabolic cost (Wood 1991). Excess post-exercise oxygen consumption (EPOC) refers to the increased oxygen consumption that occurs following exercise to repay the oxygen debt incurred during the anaerobic activity (Gaesser and Brooks 1984; Lee et al. 2003). EPOC is a critical component of recovery since it encompasses the increased oxygen required to restore oxygen and glycogen, PCr and ATP stores to tissues and to restore metabolite and ion-osmoregulatory balance. During recovery, metabolic rate can be maximal (Reidy et al. 2000; Cheng and Farrell 2007) and is supported by increased cardiac output, heart rate and cardiac stroke volume. Recovery from exhaustive exercise has ecological outcomes since swimming performance may be limited during the time required to return to routine oxygen consumption, clear blood metabolites and restore muscle energy stores (Milligan 1996).

Early work provided evidence that the physiological time-course of recovery from fatigue took over 3 h for oxygen consumption to recover (Brett 1964) and longer still for metabolites to return to routine values following exhaustive exercise (Black 1957; Turner et al. 1983). However, Milligan et al. (2000) found that rainbow trout (*O. mykiss*) that recovered in flowing water and were able to swim at a constant low velocity (i.e., 0.9 $bl \cdot s^{-1}$) following exercise had a complete metabolic recovery in ~ 2 h, earlier than individuals held in static water. Similarly, plasma cortisol levels remained relatively low in exhausted individuals that swam at constant velocity compared to those held in static water, suggesting that elevated cortisol plays a role in prolonging recovery profiles of fish held in static water. Milligan et al. (2000) suggested that the prolonged recovery from exercise stress may be caused by elevated circulating cortisol, which in turn is stimulated by post-exercise inactivity.

The results of the Milligan et al. (2000) study were applied to developing methods for facilitating and expediting the physiological recovery of non-target coho salmon (*O. kisutch*) by various fisheries gears (Farrell et al. 2000) and refined (Farrell et al. 2001a,b). Facilitated recovery using a revival box, called the Fraser Box, successfully promoted physiological recovery, rapidly restored swimming ability in 1-2 h, and resulted in high post-release survival, even for fish that appeared moribund at the time of gill net capture (Farrell et al. 2001a). This application of the results of the Milligan et al. (2000) study was further expanded to the commercial troll fishery where Farrell et al. (2001b) found that placing fish in a cage/net pen towed alongside the vessel resulted in rapid physiological recovery and no delayed mortality.

Despite the success of the Farrell et al. (2001a,b) studies on coho in the cool marine environment where salmon are hyperosmotic, no known studies of this kind have been undertaken in freshwater at warmer temperatures or on any other Pacific salmonid species.

During the freshwater phase of their migrations, environmental conditions (e.g., salinity and temperature) may influence the ability to recover from stress. Upon freshwater entry, adult salmonids are hypoosmotic and must maintain osmotic and ionic balance by producing dilute urine and actively uptaking ions at the gills (Clarke and Hirano 1995). If osmoregulatory disturbances occur in freshwater, energy is required to restore ionic gradients between blood and water (Morgan and Iwana 1991; Randall and Brauner 1991). Concomitant with this issue are river temperatures that often exceed optima for aerobic scope (Farrell et al. 2008) and can contribute to stress and disease (Gilhousen 1990). Even current river temperatures are problematic for many Pacific salmon populations (Eliason et al. 2011) and stressed fish (e.g., those released from fisheries) will have further elevated metabolic rate and reduced aerobic scope.

1.4 Tertiary consequences including survival

The tertiary response involves changes in whole-organism behaviour, performance, and disease resistance, including fitness and survivorship (Mazeaud et al. 1977). Capture and release may induce a suite of behaviours ranging from minimal changes (Whoriskey et al. 2000) to considerable departures from behavioural norms (Ryer 2004; Arlinghaus et al. 2007). Capture and release may either increase (e.g., Mäkinen et al. 2000) or decrease swimming activity (Thompson et al. 2008). Due to the exhaustive nature of capture stress, exercise to exhaustion could lead to an inability to perform further burst exercise. While minimal effects of capture stress have been observed for migrating fish in some studies (Lindsay et al. 2004), others have noted dramatic changes, including altered migration behaviour, fallback downriver, and slowed migration rates (Mäkinen et al. 2000; Thorstad et al. 2003, 2007; Donaldson 2008). Some behavioural shifts (e.g., inability to evade predators, altered migration and reproductive

behaviours) may result in direct effects on survival and fitness (Schreck et al. 1997). Acute (Maule et al. 1989) and chronic (Pickering and Pottinger 1989) stress can deleteriously affect immune function and disease resistance, potentially contributing to latent mortality (Schreck 2000). Elevated stress may have indirect effects on reproductive success and fitness (Pankhurst and Dedual 1994; Schreck et al. 2001), yet fitness consequences of fish released from fisheries gear have not been directly identified due to the difficulty of this type of study in the field (Cooke et al. 2002b; Davis 2002; Arlinghaus et al. 2007).

Reduced survival can be a tertiary outcome from fisheries-related stress. Direct and indirect mortality are common endpoints in studies designed to quantify post-release mortality from both commercial and recreational fisheries. Fisheries-related mortality is commonly categorized as immediate, short-term, or delayed (Pollock and Pine 2007). Immediate mortality is measured at the time of capture where the fish is either dead upon landing or dies prior to or during release (Pepperell and Davis 1999). Short-term mortality may be observed within hours of the capture event (usually up to 24 or 48 h) and is commonly linked with injury or an inability to recover from capture stress (Muoneke and Childress 1994). Delayed mortality occurs days or weeks following release (Pollock and Pine 2007), often making it difficult to quantify. In recreational fisheries, delayed post-release mortality is variable and context dependent, but can exceed 90% for some marine and freshwater species (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005). Likewise, discard mortality from commercial fisheries is context dependent but can range from negligible to nearly 100 % (Alverson 1994; Davis 2002). Given the variability in the proportion of fish surviving post-release, there is value in determining post-release survival for individual species (or populations) within specific contexts (e.g., under certain sets of environmental conditions or caught by particular capture methods).

1.5 Pacific salmon migrations, fisheries and implications

Reproductive migrations represent some of the most challenging life history stages for organisms across multiple taxa (Dingle 1996; Dingle and Drake 2007). Pacific salmon exemplify the complex interplay between physiology and behaviour throughout their spawning migrations, from the perception of the cues that initiate migration to the factors that affect mating systems at the spawning grounds (Quinn and Adams 1996; Hinch et al. 2006; Ueda et al. 2007). Anadromous migrations require individuals to transition between marine and freshwater in a cyclical and predictable manner, increasing their vulnerability to capture by multi-sectoral fisheries (Donaldson et al. 2011). As a consequence, Pacific salmon are targeted by commercial, recreational and First Nations fisheries throughout their reproductive migrations. Fisheries interactions can occur during their coastal approach in the marine environment and estuaries (e.g., purse seine, gill net, and trolling) or upon freshwater entry (e.g., gill net, beach seine, dip net, and rod-and-reel). Despite the diverse capture methods used, there are many similarities between sectors, including the fact that each sector can release fish following capture (Cooke and Cowx 2006). While the role of release fisheries has been speculated as a potential contributor to freshwater migration mortality for Pacific salmonids, the studies conducted thus far are fragmented and offer a range of results.

Pacific salmon released from different marine and freshwater fisheries gears has yielded highly variable results, depending on the species, gear, environment, and timecourse of study. Gill net survival differs considerably depending on the species being targeted, and can range from 35 to > 90 % for coho salmon (Parker et al. 1959; Farrell et al. 2000; 2001a; Buchanan et al. 2002), 15 to > 90 % for Chinook salmon (*O. tshawytscha*, Parker et al. 1959; Vander Haegen et al. 2004), and between 0 and 60 % for sockeye salmon (Thompson et al. 1971; Thompson and

Hunter 1973). Survival of released Chinook salmon from troll fisheries ranges between 70 and 90 % (Wertheimer 1988) and > 95 % for coho salmon when recovery is facilitated by a recovery box (Farrell et al. 2001b). Purse seine captured and released Chinook salmon survival can exceed 75 % (Candy et al. 1996) but has been highly variable for sockeye salmon, apparently dependent on stock, location, and tagging date (English et al. 2005; Crossin et al. 2009). For release from freshwater recreational fisheries, a range of 70 to 99 % has been observed (Bendock and Alexandersdottir 1993; Vincent-Lang et al. 1993; Cowen et al. 2007). Given this variability between species, gears, and context of study, quantifying post-release survival can be difficult (Chopin and Arimoto 1995; Davis 2002; Gale et al. 2011). Further complicating this interpretation is the fact that the methods used to quantify mortality are likewise variable, and often rely on holding fish in pens, tanks, or cages (Davis 2002) rather than releasing fish back to their natural environments where they may encounter predators and naturally variable environmental conditions. Biotelemetry is being increasingly used as a methodological approach to link long-term post-release survival in the natural environment with release from capture fisheries (Donaldson et al. 2008).

The range of survival observed following fisheries release reflects the diversity among the species and populations of Pacific salmon. Although Pacific salmon species share semelparous and anadromous migration strategies, they can differ in many respects including life history, morphology, physiology, performance, behavior, and thermal tolerance (Williams and Brett 1987; Standen et al. 2002; Crossin et al. 2003; Lee et al. 2002; MacNutt et al. 2006). Even at the population level, Pacific salmon are remarkably diverse. For example, sockeye salmon have over 100 genetically distinct populations in the Fraser River watershed (Beacham et al. 2005). Sockeye salmon populations have unique physiological adaptations (Lee et al. 2003;

Farrell et al. 2008; Crossin et al. 2004; Eliason et al. 2011), body morphologies, gross somatic energy reserves, egg number, and migration behaviours and migration timing (Hinch and Rand 2000; Crossin et al. 2004). With such population diversity, certain populations are more vulnerable to predicted changes in climate (Eliason et al. 2011; Martins et al. 2011). Fisheries-related stressors may differently influence sockeye salmon (Donaldson et al. 2010a) but it remains unclear if post-release survival is indeed population-specific.

In recent years, some Fraser River salmonid populations, including wild interior Fraser River coho salmon as well as Cultus and Sakinaw sockeye salmon populations have been listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and while the global population level for sockeye salmon populations is listed as least concern, some populations are listed as threatened by the International Union for the Conservation of Nature (IUCN; Rand 2011). Since these imperilled populations co-migrate with other commercially harvested salmonid populations, understanding the consequences of fisheries capture and release is high priority. Canada's Wild Salmon Policy (DFO 2005) calls for a focus on species and stock-specific objectives for making predictions on migration mortality.

1.6 Conceptual framework to understand fisheries-related consequences

The primary, secondary and tertiary responses to stress can be integrated to provide a foundation to contextualize the consequences of capture on the physiology, behaviour, and survival of fish (Figure 1). In Figure 1, the thick black solid line provides an example of a typical primary or secondary stress response of a physiological variable such as plasma cortisol, to a fisheries-related stressor (see chapters 2-5). The stressors, indicated by a bracket along the general response line, exemplify the multiple, interactive, and potentially cumulative stressors involved in a fisheries capture event. These stressors result in the organism mounting a general

stress response that depends on environmental conditions and the initial condition of the individual fish. Following the initial primary and secondary response, a negative feedback typically occurs and recovery is initiated. The thick black broken line represents a disrupted negative feedback, where recovery to routine values does not occur, resulting in a tertiary response that could result in tertiary outcomes and life history consequences (see chapters 2 and 3). The grey broken line represents an example recovery profile for individuals held in facilitated recovery gear, illustrating a reduced stress response and accelerated recovery (see chapter 6). The strength of this conceptual framework is that since it integrates the primary, secondary and tertiary stress responses it enables a complete assessment of the physiological mechanisms that contribute to fisheries-related mortality. The framework enables specific questions to be asked about how fish respond to and recover from stress, which can in turn be used to understand the context-, species-, population-, and sex-specific nature of the tertiary outcoms of fisheries-related stressors.

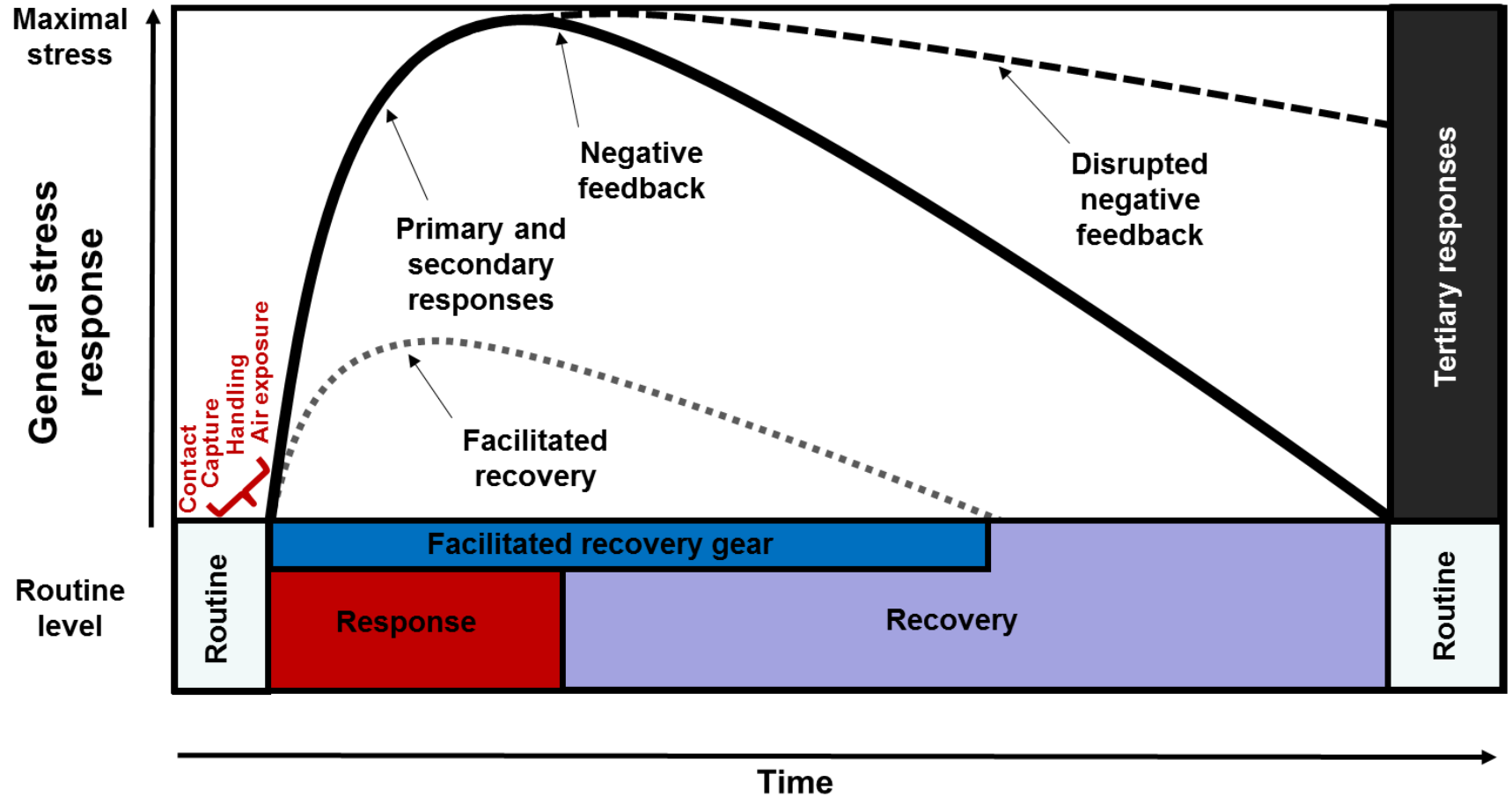

Figure 1. Schematic of the conceptual framework for understanding the primary, secondary, and tertiary consequences of fisheries-related capture and handling on Pacific salmon.

1.7 Thesis objectives and chapter organization

Within the context of the conceptual framework presented above, the objective of this thesis was to understand how Pacific salmon respond to and recover from fisheries-related capture stress. My overarching hypothesis was that fisheries-related stressors displace fish from homeostasis, resulting in a primary and secondary stress response that would have tertiary outcomes, which could be countered by facilitated recovery techniques.

Chapter 1 has provided a conceptual framework linking the stress response with fisheries capture and release.

Chapters 2 and 3 are field studies that focused on understanding how the primary and secondary stress responses relate to tertiary outcomes for survival following release from different capture methods. The objective of chapter 2 was to determine if capture methods differently influence the stress response and post-release survival of sockeye salmon. To test this hypothesis, sockeye salmon were biopsied or radio telemetry tagged and released following angling, beach seine, or 24 h holding in a net pen. Chapter 3 tested the hypothesis that different capture methods would have population-specific outcomes for stress and survival. To test this hypothesis, two populations of sockeye salmon were biopsied or acoustic telemetry tagged and released following beach seine capture or tangle net and gill net simulations.

With the results of chapters 2 and 3 pointing to profound effects of fisheries capture and release on survival, chapters 4 and 5 took a laboratory approach to investigate how Pacific salmon recover from stress following fisheries-related stressors under controlled conditions. Chapter 4 tested the hypothesis that the intensity and magnitude of fisheries-related stressors influence the timecourse of recovery. To address this hypothesis, heart rate loggers were used to

determine the time required for individuals from a single population of coho salmon to recover following seine net simulations and fisheries-related exhaustive exercise. With chapters 2, 3, and 4 identifying that the type of fisheries stressors affects the stress response, survival, and the duration of recovery, chapter 5 tested the hypothesis that the recovery processes is sex- and species-specific. To test this hypothesis, the recovery patterns of one sockeye salmon population versus one pink salmon population were compared following a controlled stressor using a series of indicators of primary and secondary stress, including the expression of genes related to cellular stress and cell maintenance.

With chapters 2 and 3 finding that fisheries capture stressors influence the stress response and survival and chapters 4 and 5 identifying long durations required for recovery, the objective of chapter 6 was to test the hypothesis that facilitated recovery methods can influence the rate of physiological recovery and the likelihood of survival. Chapter 6 tests the predictions that the recovery of primary and secondary stress indices can be expedited and that survival can be improved using facilitated recovery methods. Chapter 6 takes a three-pronged approach to test methods for facilitated recovery; (i) a laboratory-based case study to identify effective recovery gear design and durations, (ii) a telemetry study to determine post-release survival in relation to facilitated recovery gear, and (iii) a human dimensions survey to assess angler's attitudes towards recovery bag use.

Chapter 7 integrates the results of chapters 2 through 6 to place the findings in an applied management and conservation context and suggest directions for future research.

2 THE CONSEQUENCES OF ANGLING AND BEACH SEINE CAPTURE ON THE PHYSIOLOGY, POST-RELEASE BEHAVIOUR AND SURVIVAL OF ADULT SOCKEYE SALMON DURING UPRIVER MIGRATION

2.1 Introduction

For adult migrating Pacific salmon, the transition into freshwater requires a series of changes in osmoregulatory and ionoregulatory systems (Shrimpton et al. 2005) and physiological and morphological changes associated with the development of secondary sexual characteristics (Brett 1995; Hendry and Berg 1999). Environmental conditions in freshwater, particularly high river temperatures, can result in a number of physiological costs, including elevated indices of stress (Macdonald 2000) and stock-specific collapse of aerobic scope (Farrell et al. 2008). Fisheries interactions are overlaid on these inherent migration-related challenges. As Pacific salmon enter freshwater, they are targeted by recreational (e.g., hook and line), commercial (e.g., gill net) and First Nations (e.g., gill net, beach seine) fisheries.

In the Fraser River, the sockeye salmon recreational fishery has traditionally been regulated as a retention fishery with a bag limit of two fish. However, catch-and-release is common among anglers through either mandated (e.g., non-retention recreational fishery during low abundance years) or voluntary means (e.g., by anglers who reach their bag limit or choose to release undersized or undesirable fish, non-target species, or fish that are beginning to display secondary sexual characteristics; see Kristianson and Strongitharm 2006). Fisheries and Oceans Canada creel surveys estimated that over 100 000 recreationally caught sockeye salmon were released between 2004 and 2006, along with over 220 000 harvested fish (Mahoney 2005; 2006), representing an almost 2:1 harvest to release ratio. Similarly, beach seining is traditionally a

retention fishery used by First Nations in the Fraser River, but captured non-target species are often released. While the fundamental assumption of release fisheries is a minimal sublethal impairment and minimal effect on survival (Cooke and Schramm 2007), little is known about the effects of catch-and-release on physiological condition, post-release behaviour and survival for any anadromous Pacific salmon species. A number of methods for promoting physiological recovery and survival following a capture event have been tested, including the use of recovery boxes (Farrell et al. 2001a; 2001b). The 'Fraser box', which promotes survival of Pacific salmon released from commercial gear, has been validated in the marine environment, but no such method has been reported for use in freshwater.

The limited studies on the consequences of fisheries interactions on migrating Pacific salmon in freshwater reflect the logistic constraints associated with the capture, handling and holding of actively migrating fish and the cost of carrying out large-scale field studies. Recently, there has been a call for more experimental and integrative approaches to catch-and-release research, particularly for studies that use telemetry as a tool to assess behaviour and survival (Donaldson et al. 2008). While field-based fisheries capture studies often preclude the ability to use true 'controls' (Pollock and Pine 2007), experiments that contrast different capture techniques have been conducted previously (Vander Hagen et al. 2004; Gingerich et al. 2007) and enable the assessment of relative effects of different capture methods.

This chapter addressed one such knowledge gap for sockeye salmon by using previously established protocols for physiological biopsy and radio telemetry (Cooke et al. 2005) to compare the survival consequences of release from hook-and-line angling, beach seine and net pen holding in freshwater. This chapter links the primary, secondary and tertiary stress responses by comparing physiological condition, post-release behaviour and survival to natal

sub-watersheds for sockeye salmon that were either immediately captured and released by angling or beach seine with angled fish that were recovered in a net pen for 24 h. This recovery period was chosen because salmonids generally recover physiologically within this time following a stressful encounter (Milligan et al. 1996; Donaldson et al. 2010a).

2.2 Methods

2.2.1 Study site and fish capture methods

Experimental procedures were conducted on the Fraser River at Grassy Bar, near Chilliwack, British Columbia, Canada over a 4-week period in August 2009. This location has become one of the more popular locations in the lower Fraser River mainstem for angling sockeye salmon in recent years (Mahoney 2006). This study was developed as part of a synergistic study investigating the short-term survival of sockeye salmon caught by recreational anglers in 2008 and 2009 by holding salmon in net pens for 24 h (J.O. Thomas and Associates, 2009a; 2009b).

Three treatment groups were established which involved biopsy or telemetry tagging and release following: 1) angling, 2) beach seining, and 3) angling followed by a 24-h holding period in a net pen. Individuals were biopsied or tagged and released from all treatment groups in equal proportions during each week.

For the angling group, volunteer anglers of varying experience and skill captured sockeye salmon using standard bottom-bouncing gear from either shore or boats anchored near shore. Their angling setup included long leaders (i.e., > 3 m), barbless J-shaped hooks sized 1 to 3/0 that were generally bare, and a heavy weight that bounced along the riverbed but suspended the hook in the water column. Capture durations ranged between 1 and 5 min (see results). Fish

were generally landed in a long-handled knotless landing net, unhooked, and transferred by hand into a submerged black, flow-through mesh and hypolon fish transport bag (1.0 m x 0.2 m). Air exposure durations ranged from < 15 s to ~ 1 min, depending largely on the ease by which the barbless hook was removed. Anglers endeavoured to minimise the impact of the capture event on the fish by keeping angling durations short, rapidly removing the hook, and minimizing air exposure durations, factors that can exacerbate stress (Arlinghaus et al. 2007).

Capture by beach seine occurred at the study site using a 64 m x 7.5 m x 5 cm mesh beach seine net that was tied off on shore and dragged in an arc into the middle of the river and drawn closed using a power boat. The seine was then brought to shore and fish were rapidly removed from the net. Throughout the entire process, the net remained completely submerged in water, resulting in only 2-3 s of air exposure as fish were transferred by hand from the net to an individual fish bag. Technicians continually monitored the catches from both capture methods and recorded qualitative information about angling durations, air exposure durations, injuries (e.g., hooking location and degree of bleeding), and general condition descriptions. Again, fish were either biopsied or tagged and released immediately as described below.

For the net pen recovery group, angled fish in Hypolon bags were slowly walked in-river from the point of landing to one of three large net pens for holding (4 m x 4 m x 3 m). Before transfer, each fish was first tagged in the dorsal musculature while inside the Hypolon bag with a uniquely numbered anchor tag (Floy Tag & Mfg., Inc., Seattle, Washington, U.S.A.) and then released into the net pen with the transport bag immersed in the net pen. Each fish swam freely into the pen without any further handling. Net pens were constructed of 125 mm diameter, foam-filled, PVC piping fitted with 25 mm knotless mesh netting. The net pens were located in a low flow (< 0.5 $m \cdot s^{-1}$) side channel and the bottom corners were anchored with four 14 kg

anchors. Sheets of floating Styrofoam and an anti-predator frame were used on the water surface of each net pen to deter predators from entering the pen and prevent fish from jumping out of the pen. To minimize crowding stress, holding capacities in each net pen were restricted to a maximum of 35 fish (i.e., approximately 1 sockeye salmon per 1.4 m^3 of water; J.O. Thomas and Associates 2009b). After a 24-h period in the net pen fish were either biopsied or tagged and released.

2.2.2 Biopsy and tagging methods

Fish were randomly selected for either biopsy or gastric radio telemetry tagging (i.e., tagged fish were not biopsied). Established protocols were used for gastric tagging adult salmon without anaesthetic, since fish that could be harvested for human consumption cannot be anaesthetized (Cooke et al. 2005; Young et al. 2006). All protocols were approved by the University of British Columbia and Carleton University Animal Care committees in accordance with the Canadian Council of Animal Care.

The entire biopsy and tagging procedures were always completed in ≤ 2.5 min. For the immediate release groups, fish were either biopsied or tagged while in a Hypolon bag, which was constantly supplied with clean flowing river water that passed through the fish's gills. Biopsy involved a rapid 2 mL blood sample collected using caudal venipuncture with a 3.8 cm, 21-gauge needle and a vacutainer (lithium heparin, 3 mL, Becton-Dickson, NJ) to assess plasma physiological indices. Individuals from the recovery group were randomly dip-netted from the net pen and placed into a Hypolon bag for biopsy or tagging. Netting individuals for sampling necessitated the possible disturbance of other individuals in the net pen. Dip net capture generally took < 30 s per individual. Tagging or biopsy procedures were completed in < 3 min. To test for effects of sampling order on physiological variables, a linear regression of each

plasma variable was conducted by sample order on each day (see results). For all fish, a scale sample and a 0.5 g adipose fin clip were taken for identification of stock complexes, and fork length (FL) measurements were made.

A total of 99 sockeye salmon were radio-tagged for all the three treatment groups. A coded radio transmitter (MCFT-3A-3V, Lotek Wireless Inc., Newmarket, ON) was inserted gastrically with the trailing end of the antenna exiting the mouth and crimped to drift laterally along the individual's body. Coded transmitters enabled the identification of individual fish as they were detected at receiver stations. Transmitters were 16 mm in diameter, 46 mm long with a 460 mm long antenna. As part of another study, a thermal logger was attached to each transmitter and waterproofed using Plasti Dip multi-purpose rubber coating (Plasti Dip International, Blaine, MN), adding ~10 mm in length (Donaldson et al. 2009). The transmitter/thermal logger complex weighed 17 g in air and 7 g in freshwater. Tags transmitted on the 150 MHz band on six unique frequencies (320, 360, 440, 460, 600 and 800 kHz) with three pulse intervals per frequency (4.5, 5.0, and 5.5 s) to reduce the occurrence of signal collisions.

2.2.3 Laboratory assays and calculation of physiological and energetic variables

Individual population origin was determined from DNA analysis of fin clips and scales (Beacham et al. 2004). Plasma cortisol, ions (K^+, Cl^- and Na^+), glucose, lactate and osmolality, were quantified from blood samples based on procedures described in Farrell et al. (2001b), except osmolality analyses were conducted using an AI 3320 Freezing Point Osmometer (Advanced Instruments Inc, Norwood, MA) and K^+ and Na^+ were conducted using a Model 410 Single Channel Flame Photometer (Cole Parmer, Montreal, QC).

2.2.4 Telemetry methods and determination of survival and migration rate

Fixed radio-telemetry receiver stations (SRX400 or SRX400A, Lotek Wireless Inc., New Market, ON) used 3-element or 4-element Yagi antennas (Maxrad Inc., Hanover Park, IL, or Grant Systems Engineering Inc., King City, ON) and were strategically positioned both down-river and up-river of the release site (Figure 2) and described in Robichaud and English (2006; 2007). Twenty one receivers were distributed at 18 locations throughout the Fraser River mainstem and into tributaries that lead to natal sub-watersheds (Figure 2).

Arrival at natal sub-watershed was determined by detection with fixed station telemetry receivers located in tributaries en-route to spawning grounds. Failure of an individual to reach a subsequent receiver location was termed en-route mortality (Robichaud and English 2006; 2007). Individuals that were reported as fisheries harvest were excluded from this study. For statistical analyses, I evaluated percentage survival after 24 h, 48 h, 96 h and ultimate survival to reach spawning areas (Table 1).

Migration rates were calculated from release to detection at the Thompson River Confluence. Some populations have divergent migration paths after passing this location, which resulted in sample sizes that were too low for such analysis for receivers located further up-river. Migration rate was calculated by dividing the distance between two fixed receiver sites by the difference in time from the first detection at a downriver receiver or release site to the first detection at the subsequent upriver site. In instances where an individual failed to be detected at a particular site, that individual was excluded from the migration rate calculation for that section of the river.

2.2.5 River temperature monitoring

A temperature logger (resolution ± 0.1 ºC, accuracy ± 0.2 ºC, Onset Computer Corporation, Pocasset, MA), programmed to record temperatures every 15 min, was deployed in the net pen offshore at the angling site. An additional temperature logger (resolution ± 0.1°C, accuracy ± 0.2°C, Vemco-Amirix Systems, Inc., Halifax, NS; resolution ± 0.1 ºC, accuracy ± 0.2 ºC, Onset Computer Corporation, Pocasset, MA), programmed to record temperature every hour, was deployed in-river near the release site at Whonnock (Figure 2). This location is representative of the range of temperatures encountered by all individuals during the capture and tagging procedure, as the Fraser River is a large, well-mixed system (Patterson et al. 2007) and temperatures in the lower river are generally correlated with upper river temperatures (Hague et al. 2008). However, I cannot exclude minor variations in temperature exposures that may have been behaviourally exploited by fish but unresolved by my telemetry array (Donaldson et al. 2009).

2.2.6 Statistical analyses

Normality was assessed using Shapiro-Wilk tests and homogeneity of variance was assessed using Levene's test. One-way analysis of variance (ANOVA) was used to test for differences in physiological variables for each treatment group (beach seine, angling, 24 h net pen holding). Where significant differences were found, Tukey-Kramer post-hoc tests were used (Zar 1999). Pearson chi-square analysis was used to test for differences in post-release survivorship between groups. Wilcoxon univariate survival analysis was used to determine the time to mortality and one-way ANOVA was used to determine longevity between groups. Wilcoxon/Kruskal-Wallis tests were used to compare migration rates between groups at each receiver location, followed by pairwise comparisons for significant relationships. Additional

statistical tests are summarized in the results section. All values presented here represent means ± S.E., unless otherwise noted. Statistical analyses were conducted using JMP v. 8.0. (SAS Institute Inc., Cary, N.C.).

2.3 Results

2.3.1 Fish characteristics and capture details

All sockeye salmon were silver-coloured and without overt signs of secondary sexual characteristics to visually determine individual sex. DNA stock analysis of tagged individuals revealed that the majority were destined for mid- and upper-Fraser River spawning areas. Mean fork length was 58.6 ± 0.4 cm.

Capture time with angling, from hooking to landing was 2.7 ± 0.1 min. Capture time was not associated with fork length (Pearson's correlation analysis, $p > 0.05$) or with survival to reach natal subwatersheds (logistic regression, $p > 0.05$). Fish captured by anglers were generally hooked in or around the mouth. However, two fish were foul-hooked in the body or head, and some individuals bled from their hook wounds or from the fishing line tangling around their gill arch.

The time for the beach seine to be closed and brought to shore took 6.0 ± 0.1 min. The required time to remove an individual from the seine and complete all biopsy or tagging procedures took up to 4 min. Fish captured by beach seine appeared to be in good physical condition, although some individuals had visible scale loss and fresh net mark bruising.

For the net pen treatment, pairwise correlations revealed that plasma cortisol increased with sampling order ($r = 0.317$, d.f. = 56, $p = 0.015$), but this was not the case for any other

plasma variable (all $p > 0.05$). The length of time to mortality was not influenced by sampling order for the net pen group (linear regression, $r^2 = 0.002$, SS = 7375.3, d.f. = 34, $p = 0.815$). Migration rates between receiver locations were not influenced by sampling order for the net pen group (linear regression, all $p > 0.05$). Low survival of the net pen group (1 of 35) precluded the ability to make statistical comparisons between sampling order and survival to reach natal sub-watersheds.

Both capture gears resulted in fish showing signs of exhaustion (e.g., difficulty maintaining equilibrium, few attempts to burst swim away from the sampling team). Fish released immediately were in these states. In contrast, fish released from the net pen after 24 h were typically vigorous and were able to burst swim away from the sampling team. It was difficult to assess whether or not the fresh scale loss observed on several individuals was due to net pen holding, initial capture, or pre-capture conditions.

At the time of telemetry tagging, river temperatures were stable throughout the duration of the study (17.4 to 18.9 °C; mean = 18.3 °C). There was no relationship between tagging temperature and survival to reach natal sub-watersheds for any of the treatment groups (Student's *t*-test, all $p > 0.05$). Water temperatures measured in the net pen were similar to those measured in river (17.6 to 19.6 °C; mean = 18.6 °C).

2.3.2 Physiological responses to capture and net pen recovery

Significant relationships were detected among treatment groups for plasma cortisol ($F_{2,108} = 99.0$, $p < 0.001$; Tukey-Kramer HSD test, $p < 0.05$), glucose ($F_{2,108} = 16.2$, $p < 0.001$; Tukey-Kramer HSD test, $p < 0.05$), sodium ($F_{2,108} = 16.3$, $p < 0.001$; Tukey-Kramer HSD test, $p < 0.05$), chloride ($F_{2,108} = 37.3$, $p < 0.001$; Tukey-Kramer HSD test, $p < 0.05$), and osmolality

($F_{2,108}$ = 10.3, $p < 0.001$; Tukey-Kramer HSD test, $p < 0.05$), but not lactate ($F_{2,108}$ = 3.0, p = 0.051). For each of these significant relationships, Tukey-Kramer post-hoc tests revealed that the net pen group contributed significantly to the relationship (denoted by dissimilar letters in Figure 3), but that the angling and beach seine groups were not significantly different (all $p > 0.05$). Relative to fish sampled immediately after capture, individuals that were held in the net pen for 24 h had significantly elevated plasma cortisol and glucose levels, and sodium and chloride ions and osmolality were all significantly decreased relative to fish sampled immediately after beach seining and angling (Figure 3).

2.3.3 Behaviour and survival

Migration rate from the release site to Harrison River confluence site (Figure 4), the first up-river detection location, was significantly different between groups (χ^2 = 7.89, d.f. = 2, p = 0.019), with the post-angling net pen recovery group traveling faster relative to those released immediately (χ^2 = 7.87, d.f. = 1, p = 0. 005), but not those released immediately after beach seining (χ^2 = 1.52, d.f. = 1, p = 0.217). No significant differences in migration rates between groups were detected in passage between the following sites: Harrison to Hope (χ^2 = 1.57, d.f. = 2, p = 0.457), Hope to Qualark (χ^2 = 3.79, d.f. = 2, p = 0.150), Qualark to Sawmill (χ^2 = 1.13, d.f. = 2, p = 0.568), and Sawmill to Hell's Gate (χ^2 = 1.33, d.f. = 2, p = 0.512). Migration rates between the Hell's Gate and Thompson River confluence sites differed (χ^2 = 9.07, d.f. = 2, p = 0.011), with the angling group travelling faster relative to the beach seine group (χ^2 = 7.79, d.f. = 1, p = 0.005) but not the net pen group (χ^2 = 3.79, d.f. = 1, p = 0.052; Figure 4).

In total, 8 individuals (8.8 %) were never detected at up-river fixed station receivers and were considered to be mortalities. The net pen holding had significantly more individuals that

were not detected up-river (18.9 %) relative to either beach seine or angling (0.0 % and 2.7 %, respectively; Pearson chi-square analysis; $\chi^2 = 9.49$, d.f. = 2, $p = 0.009$). Within the first 24 h after release, net pen recovery resulted in lower survival (80.6 %; Table 1) compared with immediate release (> 95 %). For fish that were detected at up-river fixed stations, survivorship analysis revealed a significant difference in the length of time to mortality among the treatment groups (Wilcoxon univariate survival analysis; $\chi^2 = 11.33$, d.f. = 2, $p = 0.004$). Pairwise comparisons revealed that the net pen group succumbed to mortality faster than the beach seine group ($\chi^2 = 8.74$, d.f. = 1, $p = 0.003$) and angling group ($\chi^2 = 4.35$, d.f. = 1, $p = 0.037$) but there was no difference between the angling and beach seine groups ($\chi^2 = 3.41$, d.f. = 1, $p = 0.065$).

Survival to natal sub-watersheds after net pen recovery was only 2.9 %. In contrast, survival to natal sub-watersheds after immediate release was over 10-times higher, being 52.2 % and 36.3 %, respectively, for beach seining and angling (Table 1).

2.4 Discussion

2.4.1 Immediate release after capture

Capture by beach seine and angling resulted in a similar physiological response, likely due to the comparable, rapid duration between capture and sampling for both groups. Plasma values were characteristic of previous results for salmonids sampled immediately following exercise and capture-related stressors (Hinch et al. 2006; Young et al. 2006). Many of the plasma parameters measured here typically continue to increase with time after a stress, and would likely reach peak values 0.5 - 2 h following the stressor (e.g., plasma cortisol; Barton et al. 2002).

The short-term survival of fish in each treatment reflected the general trends that emerged from the plasma physiology results. Over 95 % of individuals released from the beach seine and angling groups survived for an additional 24 h following release. Parallel studies were conducted in 2008 (J.O. Thomas and Associates 2009a) and 2009 (J.O. Thomas and Associates 2009b) that investigated the 24 h survival of sockeye salmon captured by both angling and beach seine by monitoring fish in net pens, where adjusted survival was 98.8 % and 98.3 % in 2008 and 2009, respectively. Adult sockeye that were held in pens in a saltwater harbour for 24 h following capture by purse seine showed similar survival trends, where 100 % survival was observed (Cooke et al. 2005). Survival was similar for the angling and beach seine groups over a 24 h period, but I observed general decreasing survival over time in each of the treatment groups after 24 h.

The trend in decreasing survivorship over time was reflected in ultimate survivorship to reach natal sub-watersheds, where 52.2 % and 36.3 % of fish immediately released by beach seine and angling, respectively, reached spawning areas. Martins et al. (2010) modelled survival of radio-tagged adult migrating Fraser River sockeye salmon released from purse seine in the marine environment and from tangle net (i.e., a fine mesh gill net) or fishwheel in the freshwater environment between 2002 and 2007. They found >70 % survival to spawning areas for marine-tagged fish, whereas river-tagged survival ranged between 30 and 50 %. These marine-tagged fish may represent the best available telemetry survival data that approach true baseline survivorship values for the freshwater migration since they exclude capture and handling effects that occur in freshwater. Based on this 70 % survival value, and assuming that survival is similar among populations, my results show 20-35 % mortality associated with capture and immediate release by either beach seine or angling in the lower Fraser River.

Capture and handling at warm river temperatures (relative to cooler marine temperatures) is proposed to account for much of the difference in survival, compounded by the possibility that individuals are still undergoing physiological changes associated with osmoregulatory preparedness for freshwater during their transition through the lower Fraser River (Shrimpton et al. 2006; Martins et al. 2010). Interestingly, post-release survival was not correlated with river temperatures in this study despite previous findings that high water temperatures may contribute to stress and disease for migrating sockeye salmon (Macdonald 2000; Wagner et al. 2005). Throughout the tagging period, river temperatures were consistently moderate to high for the mainstem Lower Fraser River (Patterson et al. 2007) yet they did not vary appreciably throughout the study, which may explain why a temperature effect on survival was not detected for any treatment group.

Catch-and–release could be a viable in-river practice provided that best practices as exemplified in this study are used in the capture, handling, and release of Pacific salmon. Rapid angling durations (i.e., ~ 3 min in this study), careful handling that minimizes physical injury and rapid release are likely all factors that reduce the sublethal and potentially lethal consequences of catch-and-release. While it is not possible to account directly for the effects of tagging or sampling procedures based on my study design, these factors did contribute to handling time.

2.4.2 Net pen recovery post-angling

Rather than enabling fish to recover from capture, the net pen treatment resulted in a significantly greater physiological disturbance relative to the beach seine and angling groups. Net pen holding for 24 h resulted in depressed plasma osmolality and ions, a ~4-fold elevation of plasma cortisol and a ~2-fold elevation of plasma glucose relative to both the beach seine and angling groups, similar patterns to those observed for juvenile coho salmon subjected to a

confinement stressor and transferred to either seawater or freshwater (Redding and Schreck 1983). The elevated plasma stress could be a consequence of either the net pen itself contributing to chronic stress, or fish being stressed further when they were dip-netted from the net pen prior to sampling or release. I found that sampling order did influence plasma cortisol values, but had no effect on the other plasma variables or on post-release behaviour or short-term survival. Even so, lowest plasma cortisol value recorded for the net pen group (92.2 $ng \cdot mL^{-1}$), which was one of the first fish sampled on that day, was still higher than the mean plasma cortisol values of the freshly caught angled (89.1 ± 73.0 $ng \cdot mL^{-1}$) or beach seine (64.2 ± 44.3 $ng \cdot mL^{-1}$) fish, suggesting that the net pen contributed to the physiological disturbance but that the secondary netting further elevated this variable. These results make it difficult to draw specific conclusions on whether the plasma cortisol response was caused by the net pen itself, the secondary dip-netting of fish from the net pen, or more likely a combination of the two stressors. Short-term confinement typically results in some degree of stress (Portz et al. 2006). The stress response observed here could be related to the fact that sockeye salmon were artificially prevented from resuming their normal spawning migrations, and unable to maintain normal up-river swimming behaviour.

In contrast to the high survival of the beach seine and angling groups in the first 24 h post-release, only 80 % of the individuals released from the net pen survived the same time period and only 2.9 % successfully reached natal sub-watersheds. The survival analysis similarly revealed that the net pen group succumbed fastest with no differences in the time to mortality found for the immediately released groups. A trend emerged in survival between the immediately released and net pen angling groups, where the net pen group survival pattern appeared offset by 24 h, due to the fact that they were held in the net pen for that time. If

examined on the same time trajectory, nearly all individuals in both of these groups survived the first 24 h from both treatments, then by 48 h survival was ~ 80 %, and by 96 h survival was ~ 60 % (Table 1). However, a large difference in ultimate survival to reach spawning areas was observed, where mortality was nearly absolute for the 24 h net pen group. The trend in decreasing survival over time is likely due to natural mortality rates as found by previous telemetry studies on adult migrating sockeye salmon (e.g., English et al. 2005; Cooke et al. 2006; Crossin et al. 2009), but latent mortality associated with the stress of the capture and/or net pen holding treatments is possible and has been suggested previously (Donaldson et al. 2010a).

While the survivorship of the beach seine and angling groups is similar to other sockeye salmon studies conducted in freshwater, survival of the net pen holding group is much lower than observed previously (Martins et al. 2010). Fish in the net pen treatment clearly had higher elevations of physiological indices of stress, which can be linked with an increase in mortality due to bacterial or fungal disease development long after the stressor occurred and may have contributed at least in part to the high mortality rates observed here (Mazeaud et al. 1977; Pickering and Pottinger 1989; Schreck 2000; Budy et al. 2002). While not replicated for the beach seine group, results could have been similar given the remarkable post-capture similarities with immediate release both for survival and physiology.

The net pen group migrated significantly faster than either of the immediately released groups from the release site to the next-closest up-river receiver station at the Harrison River confluence, and in general migration rates were slowest through this region. In response to fisheries capture stress, both increased swimming activity for Atlantic salmon (*Salmo salar*, Mäkinen et al. 2000), sulking and deep-diving behaviour in sockeye salmon (Quinn and terHart 1987) and decreased swimming activity for largemouth bass (*Micropterus salmoides*, Thompson

et al. 2008) have been reported. Being unable to seek cooler water at depth may have in fact have contributed to the stress observed here post-capture (Quinn and terHart 1987). In salmonids, decreased activity has been suggested as a result of compromised performance while fish recover physiologically from the stressor (Milligan 1996); however increased activity has been proposed as a behavioural response to escape from a stressor (Mäkinen et al. 2000). At subsequent locations, migration rates did not vary between groups and were consistent with previous studies on Fraser River sockeye salmon (e.g., Hanson et al. 2008). The only subsequent location where migration rate differed between treatment groups was in the reach leading from Hell's Gate to the Thompson River confluence, where the angling group traveled at faster speeds than the other groups. Hell's Gate is a river constriction with high flows and difficult areas of passage that are energetically and physiologically costly to migrating salmon (Hinch and Bratty 2000; Hinch and Rand 1998). A previous radio-telemetry study on adult sockeye salmon found that co-migrating individuals retain their rank in swimming speed throughout much of the migration through the lower Fraser River, but the passage through Hell's gate results in a reshuffling of ranks (Hanson et al. 2008). The finding that the angling group migrated faster as they moved past this migration segment is unexpected, but the differences between groups suggests potential latent treatment effects that emerged following passage through this difficult segment of the river.

2.4.3 Conclusions and future directions

This chapter found that differences in physiological condition between treatments were reflected by both short-term survival and survival to reach natal sub-watersheds. A small, but important difference in survivorship was observed between the beach seine and angling groups, where angled fish had 15.9 % lower survival to reach natal sub-watersheds. Relative to adult

sockeye salmon tagged in the marine environment, which is the best telemetry data I have to assess baseline mortality, handling and releasing fish in the lower Fraser River is associated with mortality in the range of 20 to 35 %; however I recommend that this finding be tested empirically by tagging fish in the marine and freshwater environments concurrently. While 24 h holding of migrating salmon in net pens is useful for documenting initial and short-term mortality, there may be considerable mortality in the days and weeks following release, which cannot be assessed directly using net pen holding studies and requires approaches such as biotelemetry (also see chapter 3). The lower survival of the angling group relative to the beach seine suggests that future research is required to improve methods for promoting recovery from capture stress in freshwater, particularly ones where fish can be released without the necessity of secondary handling (see chapter 6).

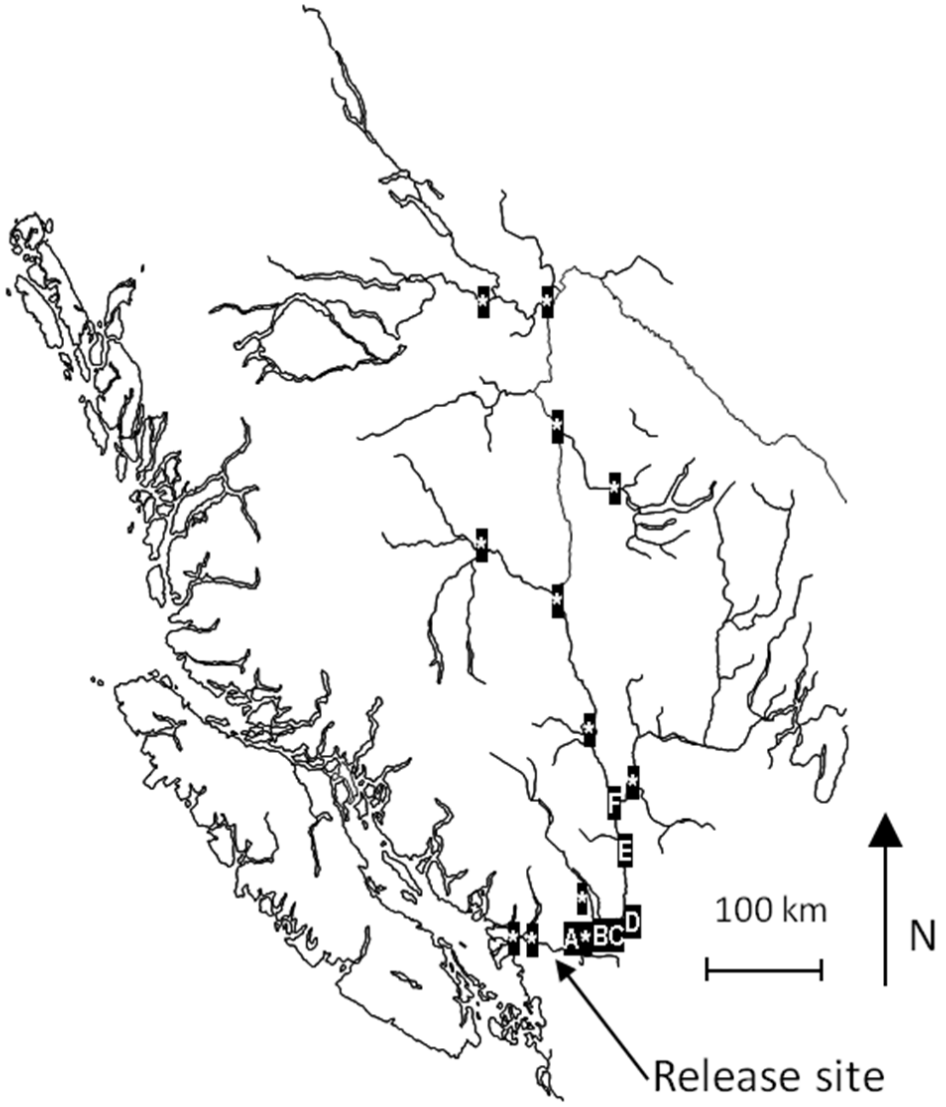

Figure 2. Map showing the Fraser River, British Columbia, Canada watershed and the study and release site. Asterices denotes radio receiver stations distributed throughout the Fraser River mainstem and into tributaries throughout the watershed. Letters represent receiver locations used in the calculation of migration rates, as follow: A (Harrison River confluence), B (Hope), C (Qualark), D (Sawmill), E (Hell's Gate), and F (Thompson River confluence).

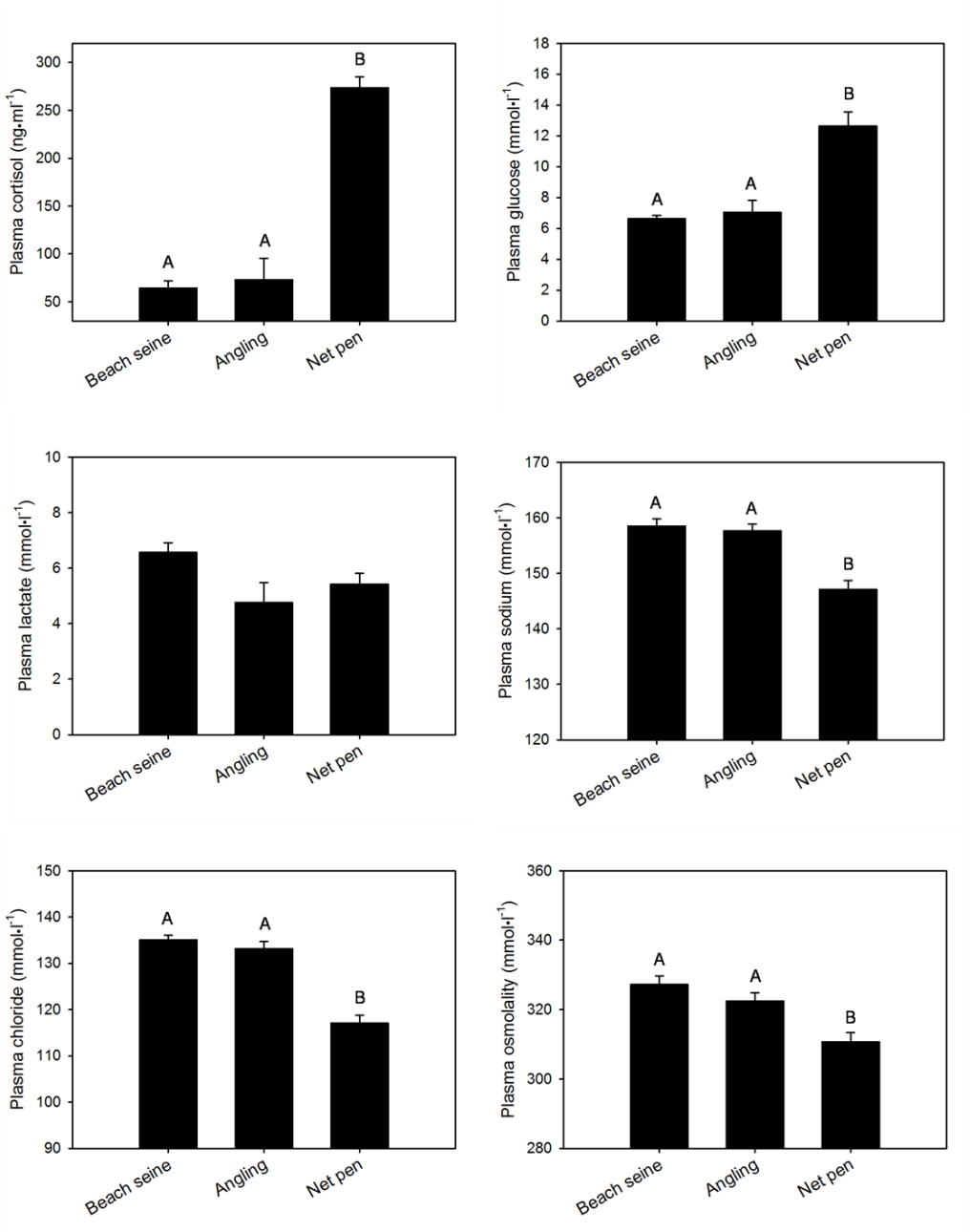

Figure 3. Mean (±SE) for plasma indices by beach seine, angling, or net pen confinement treatment group for adult Fraser River sockeye salmon. Sexes and populations pooled. Statistical details for one-way analysis of variance (ANOVA) and Tukey-Kramer post-hoc tests are presented above each panel.

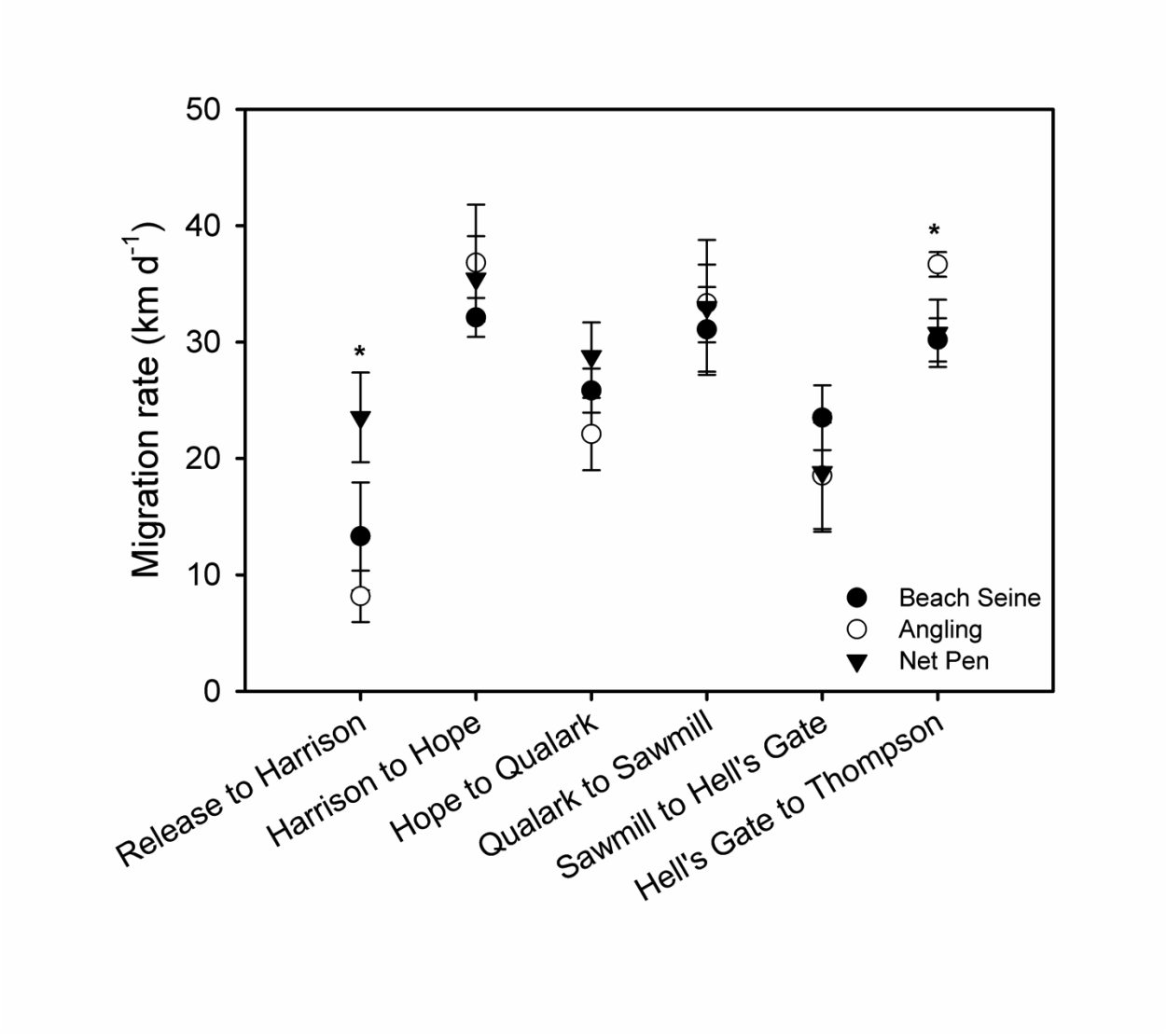

Figure 4. Migration rates (km·d^{-1}) of adult sockeye salmon detected at each radio telemetry receiver location through the Fraser River mainstem across three treatment groups (beach seine, angling, and 24 h net pen recovery).

Table 1. Survival of sockeye salmon captured by beach seine or angling and immediately released or confined in a net pen for 24 h.

Capture method	Survived > 24 h	Survived > 48 h	Survived > 96 h	Reached natal subwatershed*
Beach seine (immediate release)	21 of 22 (95.5 %)	20 of 22 (90.9 %)	16 of 22 (72.7 %)	12 of 23 (52.2 %)
Angling (immediate release)	31 of 32 (96.9 %)	25 of 32 (78.1 %)	19 of 32 (59.4 %)	12 of 33 (36.3 %)
Net pen recovery (holding for 24 h)	29 of 36 (80.6 %)	22 of 36 (61.1 %)	12 of 36 (33.3 %)	1 of 35 (2.9 %)

*Survival to reach natal subwatersheds represents only individuals that were detected by fixed station receivers at terminal areas. Total numbers do not include unreported fisheries harvest.

3 POPULATION-SPECIFIC CONSEQUENCES OF FISHERIES-RELATED STRESSORS ON ADULT SOCKEYE SALMON

3.1 Introduction

The fate of fish and other animals released from capture is an increasing conservation concern (Davis 2002; Lewison and Crowder 2003; 2007; Read et al. 2006). However, any lethal or sublethal consequences of fisheries release are context-, species- and perhaps population-specific, making generalities particularly difficult to establish (Muoneke and Childress 1994). In freshwater, there is the added difficulty of a paucity of research (Raby et al. 2011). Commercial fisheries use drift gill nets and aboriginal fisheries use drift and set gill nets as well as beach seines to capture river migrating sockeye salmon. Tangle nets, which are small-mesh gill nets are also used in fisheries, including research fisheries (Vander Haegen et al. 2004; Donaldson et al. 2010a). Delayed mortality (i.e., occurring up to several days) following release or escape from fisheries can occur days or weeks after net disentanglement (Davis 2002; Broadhurst et al. 2008; Donaldson et al. 2010a), and has been suggested to be stress-related for salmonids (Black 1958; Wood et al. 1983; Kieffer et al. 2002).

Sockeye salmon have a discrete population structure, where returning adults have high fidelity to natal spawning areas, resulting in over 100 genetically distinct populations in the Fraser River watershed alone (Beacham et al. 2005). Differences in migration timing, upriver migration conditions and spawning locations have resulted in a suite of population-specific physiological adaptations (Lee et al. 2003; Farrell et al. 2008; Crossin et al. 2004; Eliason et al. 2011). Eliason et al. (2011) showed that cardiorespiratory physiology varies at the population level for Fraser River sockeye salmon, where populations that undergo more challenging

migratory conditions are better adapted to cope with these challenges, including having greater aerobic scope, a better coronary supply and more ventricular β-adrenoceptors compared with populations with less physiologically demanding migrations. Body morphology, gross somatic energy and egg number, and migration behaviour likewise differ between populations depending on migration difficulty (Crossin et al. 2004). Survival and migration rates can also be population-specific for sockeye salmon (Hinch and Rand 2000), with certain populations being more vulnerable to predicted changes in climate (Martins et al. 2011) and fisheries effects (Donaldson et al. 2010a).

Previous work developed from comparative physiology studies on exercise stress has highlighted tat the type and duration of stressor has consequences on the level of stress incurred by fish (Wood 1991; Kieffer 2000) and the magnitude of the physiological disturbance shows a typical recovery profile, which if severe may lead to mortality (Black 1958, Wood 1983). For salmon, the magnitude of the physiological disturbance may be predictive of post-release migration behaviour and delayed mortality for certain populations of Fraser River sockeye salmon populations (i.e., early-entry Adams-Shuswap) but not others (normal-timed Chilko; Donaldson et al. 2010a). Donaldson et al. (2010a) found evidence of a carry-over effect, where an event that occurs in one life history phase (i.e., onset of freshwater migration) profoundly affects a subsequent life history phase (i.e., spawning). The concept of a carry-over effect is well illustrated by migrating taxa, particularly birds where one event can influence subsequent migratory and reproductive outcomes (Norris and Taylor 2006; Sorenson et al. 2009). However, no known studies have linked the effects of a controlled set of experimental stressors on migration survival among populations.

While chapter 2 identified that different fisheries capture methods can have different outcomes for sockeye salmon survival, it remains unclear whether or not the fisheries stressors can have population-specific outcomes for sockeye salmon. This chapter contrasts with several recent observational studies (Vander Haegen et al. 2004; Baker and Schindler 2009; Donaldson et al. 2010a) by adopting an experimental approach (Donaldson et al. 2008) to examine how simulated fishing gear encounters influence the survival of two populations of adult sockeye salmon that migrate through the same location at the same time, to limit variation in environmental variables. While both populations share a similar migration path and spawn within a few kilometres of one another, peak spawning of Harrison sockeye salmon occurs one month later than Weaver. I wished to test the hypothesis that different capture methods would have population-specific outcomes for stress and survival. I predicted that the protracted migration timing and longer freshwater residency of the Harrison population would result in higher migration failure relative to the earlier-timed spawning Weaver population following fisheries-related stress. I place these results in the context of population-specific carry-over effects and discuss the relevance of these findings for the conservation of co-migrating salmon populations.

3.2 Methods

3.2.1 Study site and experimental treatments

Two populations of Fraser River sockeye salmon, the Weaver Creek and Harrison River populations, were captured in the Harrison River, British Columbia, Canada. Fish capture and experimental procedures were conducted on the Harrison River on Chehalis First Nations land downstream of Harrison Lake on September 10th, 11th, 14th and 17th, 2009. These genetically distinct populations migrate through the lower Fraser River and enter the Harrison River, but

Weaver either spawns within Weaver Creek proper or in an artificial spawning channel after diversion by Fisheries and Oceans Canada. Harrison spawn within Harrison River itself (Figure 5). Although these populations migrate together and spawn within a few kilometres of one another, Harrison spawn approximately one month after Weaver. Individual population origin cannot be determined visually, so DNA analysis of adipose fin tissue biopsies was required (Beacham et al. 2005). Mean river temperature during experimental treatments was 17.2 °C, as determined by iButton thermal loggers distributed throughout the release area. Harrison River temperatures averaged 16.3 °C throughout September, 11.6 °C throughout October, and 9.4 °C throughout November.

Fish were captured by beach seine using a powerboat to lead the net into the middle of the river in a semi-circle pattern, then rapidly drawing the net closed to a vehicle tie-off on shore. The net was brought to shore and closed but remained submerged under water (~60 cm depth) while individuals were collected using dip nets. Several seine sets were conducted during each day of the study to enable sufficient sample sizes in each treatment group. While the beach seine methods were identical for each set, the number of fish captured ranged from zero to several hundred. Even still, the time from seine deployment to being closed and brought to shore was always ~5 min and having additional fish in the seine (i.e., fish not used for experimental treatments) mimics the reality of beach seine fisheries that can capture several hundred fish per set that typically need to be sorted or released. Netting individuals from the beach seine necessitated the possible disturbance of other individuals in the seine. Dip net capture generally took < 15 s per individual. All fish were either biopsied or tagged, as described below, following one of four experimental treatments.

Beach seine capture was a common treatment for all four experimental treatments. Following beach seine capture, the following additional interventions were also performed (1) Immediate release, which involved keeping the fish submerged and corralled in the beach seine while they were either tagged or biopsied within 3 min from the closure of the seine. (2) Prolonged beach seine capture, involved crowding fish in the fully submerged seine for a period of 10-15 min. (3) Tangle net and (4) gill net simulations were designed to simulate a selective fishery, where non-target species would be rapidly removed from a net and released. For both net simulation treatments, fish were rapidly placed in a landing net that was loosely strung with 13.3 cm or 8.9 cm monofilament mesh netting (representing standard mesh sizes for commercial gill nets and selective harvest tangle nets in the Fraser River, respectively) while underwater for a period of 3 min. The net and fish were then lifted from the water for an additional 1 min while the fish was untangled and released. The additional 1 min of air exposure was designed to simulate air exposure that occurs during sorting of commercial and native fisheries. In each case, the net soak times and air exposure times were intentionally rapid, to test methods for simulating fisheries-related stressors that could be employed in selective fisheries.

Individuals were randomly assigned to treatments. At the time of capture, populations could not be visually distinguished (i.e., DNA identification was required), ultimately resulting in higher sample sizes for the Harrison population relative to Weaver. For Harrison, the handling time between dip net capture and release did not differ significantly for gill net and tangle net treatments (23 min 3 s and 24 min 54 s, respectively), but was twice as long as than the prolonged beach seine (11 min 26 s) almost 8-times longer than the immediate release treatments (3 min 6 s). Similarly for Weaver, handling time did not differ significantly for gill net and tangle net (18 min 50 s and 22 min 49 s, respectively) and almost twice as long as the

prolonged beach seine (12 min 57 s) 6-times longer that the immediate release treatments (2 min 52 s).

3.2.2 Biopsy and tagging methods

Fish were either biopsied (blood sample) or tagged (gastric radio telemeter implant) in an alternative fashion. Established protocols were used for both procedures that have been validated previously for adult salmon (Cooke et al. 2005; Young et al. 2006). All fish were handled in the same manner, with the biopsy and tagging procedures completed in ≤ 2 min. Two teams of technicians worked in parallel for sampling and tagging, while additional technicians conducted the actual fisheries simulations. Fish were transferred by dip net from the enclosed seine to a cylindrical, coarse mesh open-ended Hypolon bag (length =100 cm, diameter = 20 cm), which was submerged in-river and constantly supplied with clean flowing river water. Biopsy involved a 2 mL blood sample collected using caudal venipuncture with a 3.8 cm, 21-gauge needle and a vacutainer (lithium heparin, 3 mL, Becton-Dickson, NJ) to assess plasma physiological indices.

Acoustic transmitters (V16-1H-R64K coded tags, Vemco Inc., Shad Bay, NS, Canada; 16 mm diameter, 54 mm length, and weighing < 2% of fish body mass) were inserted gastrically (Cooke et al. 2005). A unique coding system for the transmitters enabled the identification of individual fish as they were detected at receiver stations. For all fish, a scale sample and a 0.5 g adipose fin clip were taken for identification of population complexes, and fork length (FL) measurements were made. All fish handling procedures were approved by the Animal Care committees of University of British Columbia and Carleton University, in accordance with the Canadian Council of Animal Care.

3.2.3 Reflex and injury assessments

A reflex impairment index score, developed from the Reflex Action Mortality Predictor (RAMP) method (Davis 2005, 2007), was determined immediately prior to release of all tagged fish. A scale from 0 for unimpaired and 1 for impaired was assigned based on the following reflexes (Davis 2007): tail grab (i.e., fish successfully burst-swim away from the technician); body flex (i.e., the fish actively attempting to struggle free from the technician when held briefly out of water); head complex (i.e., the fish exhibited a regular pattern of ventilation by opening and closing of the lower jaw); vestibular-ocular response (i.e., the fish's eye rolling to maintain level pitch, tracking the handler); and orientation reflex (i.e., could the fish turned upside down on release and righted itself within 3 s). The entire reflex assessment took ≤ 10 s to complete. The reflex impairment score was the average of the 5 measured reflexes.

The severity of net injury by gill and tangle net was assigned as either 0 for minor scale loss or other signs of injury or 1 as severe, if they had considerable mucus, scale or blood loss. Severe scores were typically associated with fish that had wrapped the net either around the gills and head, resulting in damage to the gills and opercula, or around the middle of the body, resulting in scale and mucous loss and gill net marks along the body.

3.2.4 Biotelemetry methods and determination of survival

To monitor fish movement throughout the Harrison River, Weaver Creek and Harrison Lake following release and determine survival to the spawning area, a fixed array of 20 acoustic telemetry receivers (VR2 and VR2W, Vemco Inc., Shad Bay, NS, Canada) was strategically located both down-river and up-river of the release site at locations known to spatially cover the migration route of sockeye salmon from each population, following that used by Mathes et al.

(2010). While chapter 2 used radio telemetry to determine position as fish moved predictably through the Fraser watershed, this chapter used acoustic telemetry to provide a fine spatial scale with overlapping receiver detection fields to monitor fish position and movement as they hold and move in river and lake environments. Receivers were distributed to ensure that detection fields overlapped whenever possible to strengthen the likelihood of detection and determine if fish were no longer actively migrating. Mortality was assigned based on inactivity. Immediate survival represented fish that survived the treatments and migrated away from the release site, short-term survival represented detection of activity for up to 5 days post-release and long-term survival represents detection at spawning areas. DNA population identification enabled the determination of arrival at a potential spawning area for each individual. For Weaver fish, arrival at the spawning area was determined by detection with receivers positioned at Morris Lake, Weaver pool, Lower Weaver raceway, and Weaver Creek spawning channel. Detection at one or more of these locations was used to assign successfully reaching a potential spawning area. Similarly for Harrison fish, detections at receivers that were positioned where the majority of spawning activity is known to occur (Schaeffer et al. 1951) on or after October 20th, 2009 were considered to have successfully reached spawning areas (Figure 5). This date represents a time well before the mean spawning date and fish detected at this time and afterwards and confirmed to remain in this location can be considered potential spawners. If Harrison individuals had their last detection at this site prior to October 20th and were not determined to be actively moving in this region again, they were not considered to be survivors (i.e., these were fish that were present in the area only transiently, but ultimately were not detected in the area during the spawning period for this population). Manual tracking by foot and by boat occurred

throughout the study period to determine fish position and confirm mortalities or arrival at spawning areas.

3.2.5 Laboratory assays of physiological variables

Similar to chapter 2, plasma cortisol, ions (K^+, Cl^- and Na^+), glucose, lactate and osmolality were quantified from blood samples based on procedures described in Farrell et al. (2001), except osmolality analyses were conducted using an AI 3320 Freezing Point Osmometer (Advanced Instruments Inc, Norwood, MA) and K^+ and Na^+ were conducted using a Model 410 Single Channel Flame Photometer (Cole Parmer, Montreal, QC).

3.2.6 Statistical analyses

Normality was assessed using Shapiro-Wilk tests and homogeneity of variance was assessed using Levene's test and variables were log-transformed as necessary. Multiple Analysis of Variance (MANOVA) was used to test for population-specific differences in physiological response to treatments and one-way analysis of variance (ANOVA) was used to test for differences in length, longevity, and time to release for each treatment group and population. Where significant differences were found, Tukey-Kramer post-hoc tests were used (Zar 1999). Pearson chi-square analysis was used to test for differences in post-release survival between groups and populations. Logistic regression was used to relationships between reflex impairment and survival. Fisher's exact test was used to compare injury score between populations for each of the gill net and tangle net treatments. Additional statistical tests are summarized in the results section. All values presented here represent means ± S.E., unless otherwise noted. Statistical analyses were conducted using JMP v. 8.0.2 (SAS Institute Inc., Cary, N.C.).

3.3 Results

3.3.1 Survival

Independent of treatment groups, surprisingly few salmon (33 of 116 fish [28.5 %]) reached their spawning areas after a fisheries treatment (Table 2). Furthermore, almost twice as many Weaver (13 of 38 [34.2 %]) survived to reach spawning areas compared with Harrison (14 of 78 [17.8 %]). Weaver and Harrison had similar short-term survival (23 of 38 [60.5 %] and 50 of 78 [64.1 %], respectively).

3.3.2 Treatment effects on survival

Treatment significantly influenced survival of Harrison sockeye to reach spawning areas ($\chi^2 = 11.28$; d.f. = 3; $p = 0.010$). For Harrison, individuals in the immediate release (7 individuals, [33.3 %]) and beach seine (5 individuals [33.3 %]) groups were more likely to reach spawning areas relative to the gill net (0 individuals [0.0 %]) and tangle net (2 individuals [8.7 %]) simulations. Weaver had proportionately higher survival in the gill net and tangle net groups relative to the immediate and beach seine, but there was no statistically significant relationship between treatment and survival to reach spawning areas for this population ($\chi^2 = 2.26$; d.f. = 3; $p = 0.519$).

All but 2 Harrison individuals from each of the tangle net and gill net simulations survived the treatments, and the immediate release and beach seine groups resulted in 100 % survival. Weaver fish had 100 % immediate survival for all treatments. Treatment significantly influenced short-term survival for Harrison ($\chi^2 = 11.33$; d.f. = 3; $p = 0.010$) but not Weaver ($\chi^2 = 0.56$; d.f. = 3; $p = 0.905$). Longevity (i.e., the amount of time between an individual's first and

last detections at a receiver) was not influenced by treatment for either population (one-way ANOVAs; $p = 0.381$ and $p = 0.786$, respectively).

The FL of Harrison was significantly smaller than Weaver fish (mean $\pm$ SEM; 56.8 ± 0.4 cm; 63.1 ± 0.6 cm; respectively; t-test = 82.527; d.f. = 1; $p < 0.001$). Even so, FL did not differ among treatment groups for either Harrison ($F = 1.348$, d.f. = 3; $p = 0.265$) or Weaver ($F = 0.259$, d.f. = 3; $p = 0.855$). FL had no effect on short-term survival for either Harrison or Weaver, ($F = 3.886$; d.f. = 1; $p = 0.052$ and $F = 0.079$; d.f. = 1; $p = 0.780$, respectively) or survival to reach spawning areas ($F = 1.924$; d.f. = 1; $p = 0.170$ and $F = 0.121$; d.f. = 1; $p = 0.297$, respectively).

3.3.3 Migration behaviour

Seventeen Harrison and 19 Weaver sockeye fell back downriver and were detected at the furthest downriver receiver, located at the Harrison/Fraser River confluence. Treatment had no effect on the likelihood of falling back for either population; Harrison ($\chi^2 = 3.63$; d.f. = 3; $p = 0.304$) and Weaver ($\chi^2 = 2.03$; d.f. = 3; $p = 0.566$). However, Weaver sockeye that did not fall back were significantly more likely to reach spawning areas ($\chi^2 = 9.47$; d.f. = 1; $p = 0.002$; Table 3). This trend was not apparent for Harrison fish ($\chi^2 = 0.02$; d.f. = 1; $p = 0.879$).

Twelve fish (6 Harrison and 6 Weaver) were detected entering Harrison Lake. Treatment had no effect on the duration or likelihood of fish reaching Harrison Lake for either population ($p > 0.05$ in each case). Of these, only 1 Harrison and 2 Weaver sockeye ultimately reached spawning areas.

3.3.4 Physiological condition

Each treatment produced a physiological stress response but this response did not differ significantly between the two populations as evidenced by a significant treatment effect but no significant effect for population or their interaction (Two-way MANOVA whole model: $F_{50,207.5}$ = 2.059, p <0.001; treatment effect: $F_{21,115.47}$ = 3.336, p < 0.001; population effect: $F_{7,40}$ = 0.965, p = 0.469; interaction: $F_{21, 115.41}$ = 0.978, P= 0.496).

3.3.5 Treatment effects on physiological condition

The gill net and tangle net simulations induced a more severe physiological disturbance, including increases in plasma cortisol, lactate, glucose, osmolality and ions (Na^+ and Cl^-) compared with the immediate release group. Specifically, significant relationships were detected among treatment groups for plasma cortisol ($F_{3,50}$ = 4.771, p = 0.005; Tukey-Kramer HSD test, p < 0.05), glucose ($F_{3,50}$ = 11.262, p < 0.001; Tukey-Kramer HSD test, p < 0.05), lactate ($F_{3,50}$ = 10.496, p < 0.001; Tukey-Kramer HSD test, p < 0.05), osmolality ($F_{3,50}$ = 20.203, p < 0.001; Tukey-Kramer HSD test, p < 0.05), sodium ($F_{3,50}$ = 5.775, p = 0.002; Tukey-Kramer HSD test, p < 0.05), chloride ($F_{3,50}$ = 5.057, p = 0.004; Tukey-Kramer HSD test, p < 0.05), but not potassium ($F_{3,50}$ = 0.729, p = 0.539; Tukey-Kramer HSD test, p > 0.05; Figure 6).

Physiological condition is likely reflective of the time course of stress response rather than simply the treatment itself, since a significant relationship was found between treatment and the time between seine net closure and sampling for both Harrison ($F_{3,82}$ = 34.065, p < 0.001; Tukey-Kramer HSD test, p < 0.05) and Weaver ($F_{3,41}$ = 19.606, p < 0.001; Tukey-Kramer HSD test, p < 0.05). Thus, these measures should be interpreted as an indicator of physiological

condition at the time of release, rather than as an absolute measure of the stress response to the treatment itself.

3.3.6 Reflex impairment

For Harrison, but not Weaver, reflex impairment score was influenced by treatment ($F_{3,74}$ = 5.302; SS = 0.510; p = 0.002; Figure 7). Harrison mean reflex impairment score was higher for gill net (0.365) and tangle net (0.275) treatments relative to immediate release (0.133) and beach seine (0.175). Logistic regressions revealed that reflex impairment score was predictive of both short-term survival (r^2 = 0.117; χ^2 = 11.93; d.f. = 1; p < 0.001) and survival to reach spawning areas (r^2 = 0.153; χ^2 = 11.21; d.f. = 1; p < 0.001) for Harrison but not Weaver. Reflex impairment score had no relationship with fallbacks (p > 0.05 for both populations).

3.3.7 Injury score

All four (100 %) fish that did not survive the treatments had an injury score of 1, suggesting severe injury due to the net simulations. Treatment type (i.e., gill versus tangle net) did not influence injury score for Weaver (χ^2 = 0.53; d.f. = 1; p = 0.467) or Harrison (χ^2 = 4.33; d.f. = 1; p = 0.055), so both treatments were combined into a single variable for subsequent comparisons of injury score. Harrison fish (35.7 % of individuals with an injury score of 1) were significantly more likely to have an injury score of 1 relative to Weaver (5.6 % of individuals with an injury score of 1; χ^2 = 5.86; d.f. = 1; p = 0.016). Injury score had no relation with short-term survival for either population (Weaver χ^2 = 0.53; d.f. = 1; p = 0.467; Harrison χ^2 = 1.91; d.f. = 1; p = 0.167). Injury score did not influence the likelihood of Weaver fish to reach spawning areas (χ^2 = 1.32; d.f. = 1; p = 0.250). The two Harrison fish reaching spawning areas after either gill or tangle net simulation had injury scores of 0.

3.4 Discussion

While overall survival for all treatment groups combined appears low, the survival of Weaver fish to reach spawning areas in this study is similar to survival of telemetry-tagged sockeye salmon released from a recreational fishery in 2009 (i.e., ~36%) but lower than those released from a beach seine in the Fraser River (i.e., 52.2%; chapter 2). The survival observed here also falls within the ranged observed by Donaldson et al. (2010a), who found that survival ranged between 18 and 42 % for sockeye released from tangle nets in the Fraser River. The much lower survival of the Harrison fish is driven largely by the gill and tangle net simulations whereas survival from the beach seine treatments is comparable to that of the recreational fishery survival in chapter 2. For Weaver, the result that the immediate release group had the lowest survival was unexpected but treatment did not affect survival for Weaver fish even though it did affect Harrison fish survival.

The mechanism of delayed mortality from fisheries-related stress has been suggested to be linked to injury and physiological stress, but the proximate causes of delayed fisheries-related mortality are not always apparent (Wood et al. 1983, Davis 2002). I found that the net simulation treatments resulted in a significantly greater proportion of fish from the Harrison population exhibiting severe injury. The four immediate mortalities from the tangle and gill net treatments for Harrison were all related to the fact that gill net (and occasionally tangle net) fisheries function by entangling fish around the gills and preventing their escape. Immediate mortality, which was assigned an injury score of 1, occurred when the net mesh had bound the individual's opercula and gills, damaging the gill tissue and preventing normal ventilation throughout the duration of the treatment. I can only speculate the dermal injuries sustained during the treatments may have likewise influenced short-term survival and survival to reach spawning

areas. Individuals may have died either due to the injuries themselves or were more susceptible to secondary disease development, particularly when coupled with presumed stress-mediated immunosuppression (Lupes et al. 2006).

Injuries may have been more detrimental for Harrison fish since they had to continue holding in the Harrison River for up a long period of time post-release allowing more time for disease development, whereas Weaver fish had only three or four weeks remaining until peak spawning. Peak spawning for Weaver fish occurs about 1 month after the treatments (~October 20th) whereas Harrison spawn one months afterwards (~November 20th). This would allow more time for latent mortality to manifest in Harrison fish than in Weaver fish, and would be in line with recent data indicating Pacific salmon may be more resilient to fisheries encounters at later stages in their migration (Donaldson et al. 2010a; G. Raby *pers comm*). Since sockeye salmon reabsorb their scales during reproductive maturation, they may have been less likely to be affected by net abrasion and susceptibility to fungal development, which may have been the case for Weaver. If disease development is indeed a factor influencing latent mortality, Harrison Lake is a thermal refuge that is available to both populations that has been shown to improve survival for early entrants (Farrell et al. 2008). Neither Weaver nor Harrison populations need to enter Harrison Lake to reach spawning areas, however they may do so volitionally to mitigate thermal stress which has been shown to increase the likelihood of survival in warm temperature years (Mathes et al. 2010). Harrison River temperatures were not above average in 2009, and few individuals from either population entered Harrison Lake. Of the twelve fish that entered the lake, only three ultimately returned to spawning areas.

The predictive power of the reflex index was population-specific, where reflex impairment was influenced by the severity of the stressor for Harrison but not Weaver. Reflex

impairment score is an established measure of fish vitality (Davis et al. 2010) and has been used previously to monitor animal condition and to predict delayed mortality (Davis and Ottmar 2006, Davis 2007; 2010; Humborstad et al. 2009). Of the reflexes used to develop the reflex impairment score, the tail grab response (i.e., failure to engage in burst swimming when stimulated), orientation (i.e., inability to maintain equilibrium), and head complex (i.e., inability to maintain rhythmic ventilation) are all indices that may be suggestive of exhaustive exercise (Davis 2010). This assertion was corroborated by elevated plasma lactate and cortisol, two indices of exhaustive exercise stress (Wood et al. 1991; Milligan et al. 1996; Kieffer 2000), in the prolonged beach seine, gill net, and tangle net treatments. My finding that reflex score predicted mortality even several weeks after treatment suggests that a response to a stressor that is presumed acute can still have long-term consequences on survival.

Physiological condition did not differ between populations for any of the treatments. The net simulation treatments resulted in a greater stress response relative to the immediate release and beach seine groups, however this may simply reflect the fact that these variables were changing on a fixed time course, rather than the severity of the treatments themselves. Most of the plasma variables measured here typically continue to increase after the stressor until they peak and begin to recover (generally between 30 min and 2 h; Wood et al. 1983; Milligan 1996; Barton 2002). This response pattern has indeed been shown in adult migrating Pacific salmon in freshwater (e.g., coho salmon, see chapter 4). While the values obtained from my longer treatments (net simulations and prolonged beach seine treatments, and all treatments required initial beach seine capture) may reflect the duration between capture and sample collection, they provide an indication of physiological condition at the time of release and suggest a major stress response was being mounted at that time. The physiological stress response in fishes has

previously been linked with latent mortality due to bacterial or fungal disease development (Mazeaud et al. 1977; Schreck et al. 2000; Lupes et al. 2001). For the immediate release group, plasma values were similar to plasma collected from post-exercise (Hinch et al. 2006) or rapidly captured sockeye salmon in freshwater (i.e., dip net, Young et al. 2006; tangle net of identical mesh size, Donaldson et al. 2010a; angling, chapter 2).

The treatment effect observed for Harrison survival to reach spawning areas contributes to a growing body of ecological research identifying latent mortality and carry-over effects. A carry-over effect refers to an event that occurs at a discrete time point and which influences subsequent migratory and reproductive outcomes (Sorenson et al. 2009). This phenomenon has been described previously for migratory birds (Norris and Taylor 2006) as well as non-migratory (O'Connor et al. 2010) and migratory (Donaldson et al. 2010a) fish. By traditional definition, carry-over effects typically have a 'seasonal' component, but growing evidence for Pacific salmon suggest that carry-over affects may emerge in discrete life history stages. For adult migrating Pacific salmon, latent mortality has been linked with a range of stressors, including navigating challenging migration barriers (Budy et al. 2002; Caudill et al. 2007), capture stress (Donaldson et al. 2010a), and confinement stress (chapter 2). Donaldson et al. (2010a) found that physiological condition at the time of capture was predictive of fate for the Adams-Shuswap population complex, but not Chilko. Comparable to the results of this chapter, Donaldson et al. (2010a) found that Adams-Shuswap had slower migration rates and were less likely to reach natal subwatersheds relative to the Chilko population. Interestingly, both the Chilko and Weaver populations migrate directly to spawning areas (i.e., within days), while the Adams-Shuswap and Harrison populations tend to spend weeks in freshwater prior to spawning. Remarkably similar to the findings of the 2-fold difference in survival for Weaver vs Harrison in this chapter, Chilko

had a nearly 2-fold higher survival to reach spawning areas relative to Adams-Shuswap. Understanding how Pacific salmon recover from capture stress may provide an indication of the mechanisms of post-release mortality (see chaptrs 4 and 5).

Regardless of the mechanisms of mortality, the population-specific treatment effects highlight the need to better understand population-specific mechanisms for migration mortality in sockeye salmon. My finding that even a short duration net simulation resulted in elevated mortality suggests that selective fisheries aimed at releasing non-target Pacific salmon could still encounter high mortality rates. Other methods, such as the immediate release beach seine which involved less handling and potentially less mucous and scale loss could be a more viable means of reducing mortality of released fish (which was also the case in chapter 2). My results indicate that, in addition to a diversity of behavioural and physiological traits (Lee et al. 2003; Farrell et al. 2008; Mathes et al. 2010; Eliason et al. 2011), Fraser River sockeye salmon populations show diversity in their resilience to fisheries encounters. The differences between populations suggest that mortality associated with fisheries-related stressors is context dependent, meaning that management regimes must be careful applying generalizations across co-migrating populations. This is particularly relevant to vulnerable populations of sockeye salmon (e.g., Cultus Lake sockeye salmon; Rand 2010). While the fishery is already managed on a population-specific basis, co-migrating populations are typically simultaneously targeted during fisheries openings. This suggests that even if fisheries were selective and non-target populations could be released, there may be reduced population-specific post-release survival. Given that even short durations of gear entanglement can result in delayed mortality, further research is required to test methods for promoting recovery and improving methods for handling and releasing fish to reduce post-release mortality (see chapter 6). This study is one of the first involving an experimental

approach using physiological measurements and telemetry in a fisheries bycatch context. The findings provide a cautionary note: researchers and managers should consider the potential role of inter-population variation when interpreting research findings and making management decisions related to fisheries.

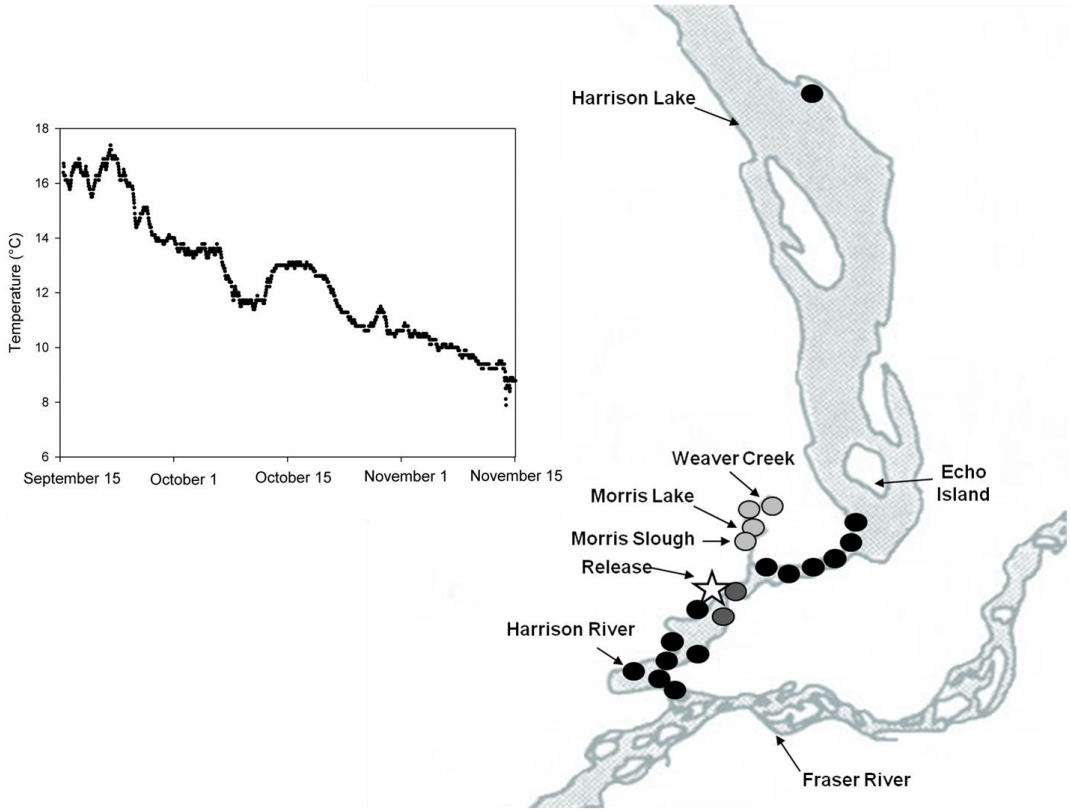

Figure 5. A map of the study area in Harrison River, British Columbia, Canada. The star represents the treatment and release site. Black circles indicate key VR2 receiver coverage. Light grey and dark grey circles indicate receivers at general spawning locations for Weaver Creek and Harrison River sockeye salmon, respectively. Inset shows water temperatures throughout the study period.

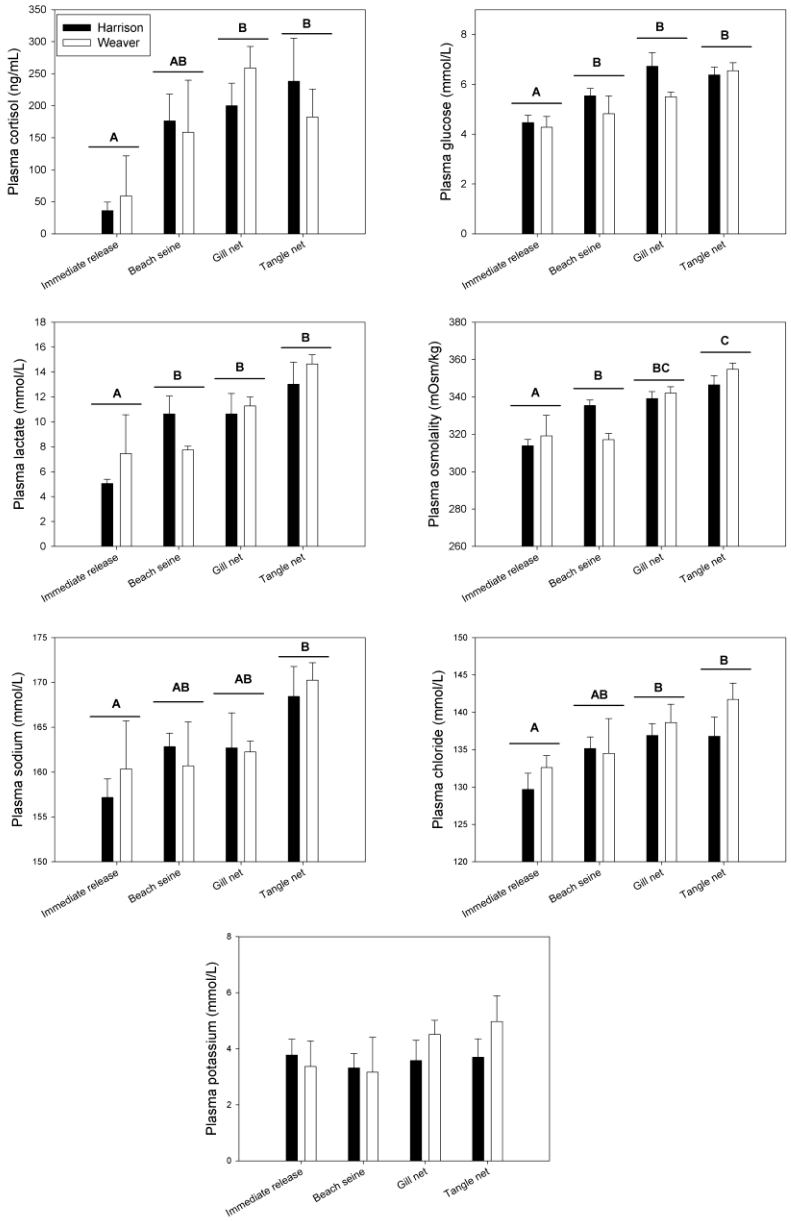

Figure 6. Plasma physiological variables for four experimental treatment groups for adult Harrison and Weaver sockeye salmon in the Harrison River, British Columbia, Canada. Populations did not differ significantly among treatments for all variables, and were pooled for analysis. Dissimilar letters represent significant differences between treatments.

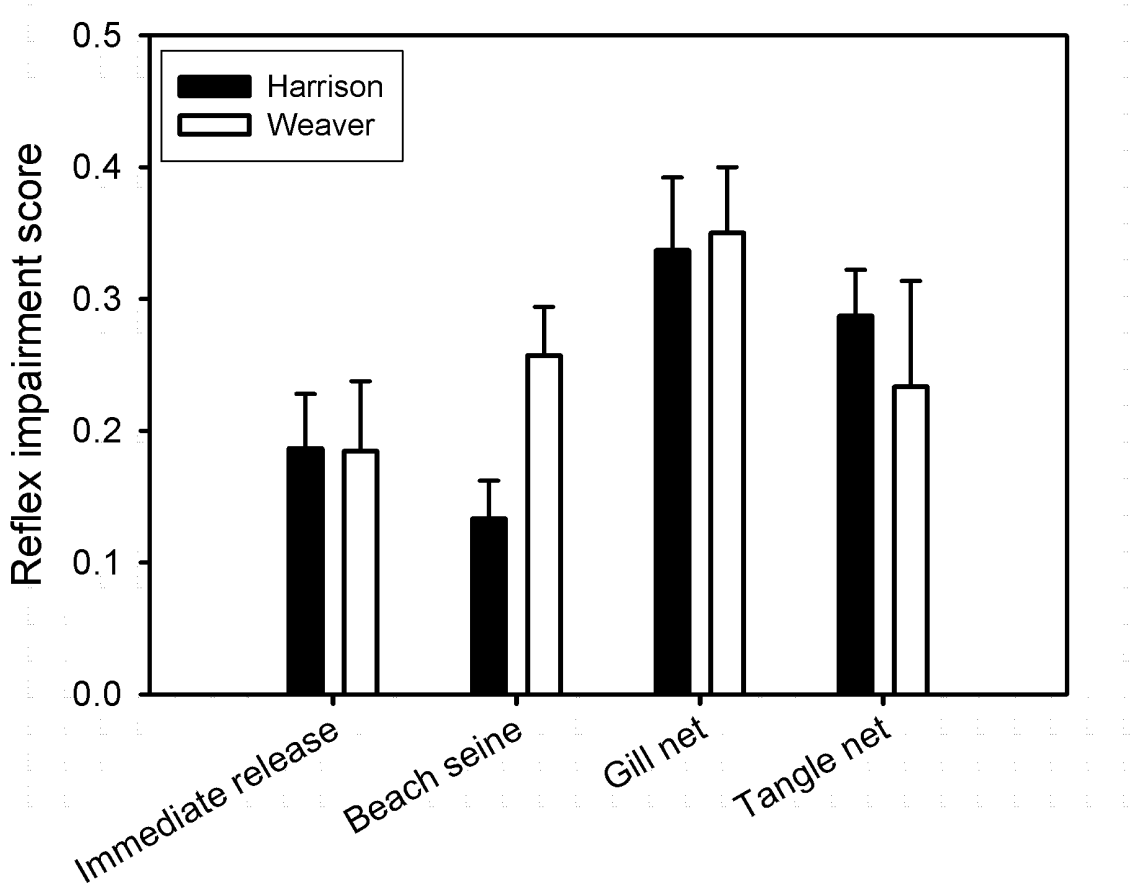

Figure 7. Reflex impairment score versus treatment for adult Harrison and Weaver sockeye salmon in the Harrison River, British Columbia, Canada.

Table 2. Percentage of fish that survived treatment (i.e., immediate survival), survived 5 days or survived to spawning areas for adult Harrison and Weaver sockeye salmon in the Harrison River, British Columbia, Canada.

Population	Treatment	N	Survived treatment (% [N])	Survived 5 days (% [N])	Survived to spawning area (% [N])
Harrison	Immediate release	21	100.0 (21)	85.7 (18)	33.3 (7)
	Beach seine	15	100.0 (15)	80.0 (12)	33.3 (5)
	Gill net simulation	19	89.5 (17)	42.1 (8)	0.0 (0)
	Tangle net simulation	23	91.3 (21)	52.2 (12)	8.7 (2)
	Total	**78**	**94.5 (74)**	**64.1 (50)**	**17.8 (14)**
Weaver	Immediate release	7	100.0 **(7)**	4 (57.1)	14.3 (1)
	Beach seine	13	100.0 **(13)**	7 (53.9)	30.8 (4)
	Gill net simulation	12	100.0 **(12)**	8 (66.7)	41.7 (5)
	Tangle net simulation	6	100.0 **(6)**	4 (66.7)	50.0 (3)
	Total	**38**	**100.0 (38)**	**60.5 (23)**	**34.2 (13)**
	Grand total	**116**	**96.6 (112)**	**62.9 (73)**	**28.5 (33)**

Table 3. Fall back and survival to reach spawning areas for adult Harrison and Weaver sockeye salmon in the Harrison River, British Columbia, Canada.

Population	Fell back to the Harrison/Fraser River confluence	N	Survived to spawning area (% [N])*
Harrison	Fell back	17	17.7 (3)
	Did not fall back	57	19.3 (11)
	Total	**74**	**18.9 (14)**
Weaver	Fell back	19	10.5 (2)
	Did not fall back	19	57.9 (11)
	Total	**38**	**34.2 (13)**
	Grand total	**112**	**23.3 (27)**

*excludes immediate mortalities

4 PHYSIOLOGICAL RESPONSES AND RECOVERY OF FREE-SWIMMING ADULT COHO SALMON TO SIMULATED FISHERIES ENCOUNTERS

4.1 Introduction

The ability of an animal to recover from a stressful encounter is fundamental to the persistence of animal populations (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Romero 2004) and is particularly important for adult Pacific salmonids during their spawning migrations that routinely encounter natural and anthropogenic stressors that presumably test their physiological limits. Throughout their coastal approach and in-river migration, Pacific salmon are targeted by predators (e.g., sharks, seals, orcas, bears) and fisheries (e.g., commercial, recreational and First Nations fisheries sectors). These encounters can kill fish directly or lead to sublethal physiological or behavioral impairments following either predation evasion (Lima and Dill 1990) or escape/release from fisheries gear (Chopin and Arimoto 1995). Chapters 2 and 3 identified that different capture methods can result in a dramatic stress response. However, the recovery process and the duration of recovery from fisheries-related stressors remains poorly understood. This chapter endeavors to determine how different durations and intensities of stressors influence the duration of recovery.

Fish generally respond to a startle stimulus by engaging in brief, burst swimming activity that is powered predominately by white muscle tissue and supported by anaerobic metabolism (Milligan 1996). Burst activity triggers acute bradycardia (Stevens et al. 1972; Priede 1974), which may function to prevent hypertension associated with constriction of peripheral blood vessels by skeletal muscle contractions (Farrell and Jones 1992). However, burst swimming cannot be sustained beyond a few minutes, after which avoidance behaviors rely on slower,

steady swimming activity supported by slow-twitch, aerobic red muscle (Webb 1984). Exhaustive exercise pushes fish to their physiological limits, resulting in maximal endocrine disturbances, acidosis and metabolite accumulation, responses that can temporarily impair physiological performance during recovery (Milligan and Wood 1986; Milligan 1996). In severe cases, delayed mortality is possible (Wood et al. 1983). During recovery, metabolic rate may increase to maximum levels (Reidy et al. 2000; Cheng and Farrell 2007), supported by increased cardiac output, heart rate (f_H) and cardiac stroke volume. Until routine cardiac output is restored, the circulatory capacity to respond to future encounters is likely compromised.

Technological limitations have made measuring cardiac output in free-swimming fish difficult, particularly for actively migrating species like Pacific salmonids. One study on Atlantic salmon (*Salmo salar* L.) using f_H telemetry found that f_H increased by no more than 30 % after fisheries capture (angling), and f_H returned to pre-capture levels after ~16 h (Anderson et al. 1998). However, implantation of the electrocardiogram (ECG) electrodes had breached the pericardium (Anderson et al. 1998), which is known to impair normal cardiac performance in rainbow trout and sharks (Farrell et al. 1998). Largemouth bass (*Micropterus salmoides*) equipped with large external acoustic f_H transmitters increased f_H by 1.7-fold in response to fisheries capture (angling), but recovery profiles could not be quantified due to substantial variations in f_H and small sample sizes (Cooke et al. 2004).

Implantable data loggers (Clark et al. 2008a; 2009a; 2010) can monitor f_H without disrupting the pericardium and therefore enable long-term, continuous assessment of the sublethal consequences of fisheries encounters on fishes. Chapters 2 and 3 identified a stress response in relation to capture that had tertiary outcomes, but the recovery process has not yet been resolved. This chapter tested the hypothesis that varying intensities of exercise stress

designed to simulate fisheries encounters would differentially affect the response and recovery of f_H and haematological indices for free-swimming, adult migratory coho salmon. I predicted that (1) the magnitude of the f_H response would depend on the intensity and duration of the stressful encounter; 2) the duration of recovery of f_H to pre-stress levels would depend on the intensity and duration of the stressful encounter; and (3) f_H responses would correlate strongly with haematological indicators of physiological stress. Through an examination of these predictions, I aimed to determine if f_H could act as a holistic indicator of the physiological response to and recovery from an ecologically relevant stressful encounter.

4.2 Methods

4.2.1 Study site and animals

Experiments were conducted on adult Chehalis River coho salmon at the Chehalis River Hatchery, a Fisheries and Oceans Canada facility near Agassiz, British Columbia, during October and November 2008. Some fish showed early signs of secondary sexual characteristics, but most were silver in color, which is the preferred stage for many predators as well as fisheries. Mean (± S.E.) fork length was 67.9 ± 0.9 cm and mean body mass was 3.6 ± 0.2 kg. Fish were individually removed from hatchery raceways by dip-net following their normal 140 km river migration from the ocean. Thirteen fish (6 males, 7 females) were implanted with data loggers and an additional 12 fish (7 males, 5 females) were surgery controls (subjected to identical handling treatments as the instrumented fish, but without surgery to implant a data logger).

4.2.2 Implantation of data loggers and blood sampling

Fish were individually anaesthetized in a bath containing 100 $mg \cdot L^{-1}$ tricaine methanesulfonate (MS-222; Sigma, St Louis, M.O.) buffered with 200 $mg \cdot L^{-1}$ $NaHCO_3$. Upon

loss of equilibrium (< 1.5 min), biopsies of blood, gill tissue and skeletal muscle tissue were taken. A 2.5 mL blood sample was taken by caudal puncture using a heparinised vacutainer, which was then stored in ice-chilled water for <1 h until subsequent processing. A 3 mm diameter lateral tissue biopsy and a small gill biopsy (1 mm from the tips of 5-8 gill filaments, ~0.03 g) were taken for another study. Very minor bleeding (<0.05 mL) was sometimes observed at the biopsy sites, but ceased after 10-20 s. Similar biopsy procedures have been shown to have no detrimental effects on the health or behavior of fish (Cooke et al. 2005; Clark et al. 2009).

Once the fish were fully anaesthetised (~5 min), body mass, length, girth and height were measured before placing the fish on a damp plastic-lined foam surgery bench, where the gills were continuously irrigated with a maintenance dose of buffered anaesthetic (70 $mg \cdot L^{-1}$ MS-222, 140 $mg \cdot L^{-1}$ $NaHCO_3$). Peterson disc identification tags were inserted into the dorsal tissue approximately 1 cm ventral and anterior of the dorsal fin. At this point, surgery control fish were recovered in one of two sections (L x W x D = 15 x 5 x 2 m) of a concrete, flow-through holding channel (9.0 ± 0.1^{o}C) with water depth ~60 cm. For the remaining fish, a data logger (iLogR, mass 23 g in air; B.D. Taylor, La Trobe University, Melbourne, Australia) was implanted with the fish supine, using previously described surgical methods (Clark et al. 2009; 2010). Briefly, a sterilized data logger was inserted through a 3-4 cm incision along the ventral midline of the fish, anterior of the ventral fins and associated cartilage. A pair of uterine forceps was used to position the electrocardiogram (ECG) leads ventral to the liver and close to the peritoneal membrane separating the pericardial and visceral cavities. The body of the data logger was loosely sutured to the peritoneal wall and associated ventral tissue, and the incision was closed with silk sutures. The data logger recorded the date, time, temperature and ECG output at 200 Hz for a 10 s period

at 10 min intervals. The entire ECG waveform was stored to memory for subsequent analysis of heartbeats (see Clark et al. 2010 for sample of raw ECG traces).

The entire surgical procedure lasted about 25 min, after which each fish recovered in a section of the holding channel detailed above. All fish resumed unassisted ventilation within several minutes and within 2 h exhibited behaviors indistinguishable from the surgery control fish and non-experimental coho salmon at the hatchery. All surgical procedures were completed over a two-day period.

4.2.3 Blood processing and analysis

Haemoglobin concentration ([Hb]) was assessed on whole, well-mixed blood using a hand-held haemoglobin analyser (HemoCue 201^{+}, Ängelholm, Sweden) calibrated for fish blood (Clark et al. 2008b). Haematocrit (Hct) was quantified using micro-capillary tubes centrifuged at 10,000 x *g* for 3 min. Mean corpuscular haemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). As in previous chapters, the remaining blood sample was centrifuged at 7,000 x *g* for 5 min and plasma was stored in liquid nitrogen prior to being frozen at –80°C until analysis. Plasma was subsequently analysed for cortisol (Neogen ELISA with Molecular Devices Spectramax 240pc plate reader), lactate, glucose (YSI 2300 stat plus analyser), osmolality (Advanced Instruments 3320 freezing point osmometer), chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Parmer, model 410 single channel flame photometer; Farrell et al. 2001).

4.2.4 Experimental protocols

For the corralling protocol, after anesthesia, fish recovered undisturbed for at least 24 h before being guided into a raceway (L x W x D = 6 x 1 x 1.5 m, water depth = 0.6 m, water

velocity ~0.2 m s^{-1}, water temperature 9.3 ± 0.1^{o}C) attached to each recovery holding channel. To initiate fish movement, a large corralling fence, which spanned the width of the holding channel, was used to gently and slowly coax the fish into a 2 x 5 m section of the holding channel without physically contacting the fish. The fish were spontaneously active in the 2 x 5 m section. The corralling duration was set at either 10-min (N=6 with data loggers, N=6 surgery controls) for the group in one raceway and 30-min for the group in the other raceway (N=7 with data loggers, N=6 surgery controls). Movement into the raceway was completed with a smaller corralling net, after which each raceway was closed with a plastic mesh gate and half of the water surface was covered with high-density foam to provide shelter. The intent of this corralling procedure was to simulate conspecifics being cornered in fisheries gear (e.g., crowding in a seine net) without direct physical contact for periods of 10 and 30 min. Thus, no biopsies were taken during the corralling procedure. Fish recovered undisturbed in the raceways for 4-6 d while data were logged continuously.

The exhaustive exercise protocol was conducted on November 4th and 6th when water temperature was 8.1 ± 0.3°C and water velocity was ~0.2 m s^{-1}. Between 08:45 and 10:30, 6 fish were rapidly (< 3 s) removed from each raceway by dip-net and placed in pairs in one of 3 net pens (L x W x D = 1 x 1 x 1.5 m x 60 cm water depth) attached to each raceway. Fish recovered for 5-6 h before being randomly assigned to one of three protocols: (i) 3-min exhaustive exercise treatment, (ii) 3-min exhaustive exercise plus 1-min air exposure treatment, and (iii) untouched control. Exhaustive exercise involved individual fish being rapidly netted (< 5 s), placed in a donut-shaped exercise tank (diameter 150 cm, inner diameter 50 cm, water depth 40 cm) containing aerated water, and coaxed manually to burst swim for 3 min (Milligan 1996) by three experimenters positioned around the exercise tank. After 3 min of exercise with continuous

behavioral observations, the fish was either replaced directly into its respective net pen by dip-net, or temporarily held in air for 1 min in the dip-net. A 60-min post-exercise recovery period followed in the net pens [to anticipate the peak post-exhaustion plasma cortisol and lactate concentrations in other salmonids (Barton 2000), and as used in other studies with adult coho salmon (e.g., Farrell et al. 2000; 2001)]. At this time, biopsies were taken by individually removing all fish by dip-net and placing them supine in a water-filled V-shaped sampling trough. Within 2 min the fish was returned to its respective net pen to recover overnight for a further 16-18 h until a final biopsy was taken. Thus, the three biopsies were: (i) during initial anaesthesia, (ii) 60-min post-exhaustion, and (iii) 16-18 h post-exhaustion. Control fish were similarly biopsied but without the exhaustive exercise. Following the final biopsy, fish were released together into a central section of the large holding channel, where they were monitored for a further 2-3 d during which there was no delayed mortality. The intent of this exhaustive exercise procedure was to simulate responses of fish that might occur when wild fish are trapped but escape from a predator (e.g., a bear) or fishing gear (e.g., a gill net) while monitoring f_H and blood physiology.

4.2.5 Data logger removal, data handling, and statistical analyses

All fish were subsequently euthanized with a blow to the head, re-weighed and re-measured. The exact position of the ECG sensors within the body cavity was verified and the data logger was removed for subsequent download. The data file from each data logger was downloaded with a custom-built computer interface and the text file was imported into LabChart software (ADInstruments, Sydney, Australia) for analysis. A rate-meter function was applied to the ECG data to calculate f_H in beats min^{-1} and calculations were verified by manual examination of all data.

All continuous variables were assessed for univariate and multivariate model assumptions and in certain cases were subject to transformations as noted below. Student t-tests were used to test for relationships in the response of f_H to corralling and the time required for recovery to pre-treatment values. Multiple analysis of variance (MANOVA), with sex as a main effect, was used to compare all blood physiological variables between sexes at each time interval separately. MANOVA, with surgery control as an effect, was used at each time interval during recovery from the exercise treatment to compare all blood physiological variables between the surgery control fish and those fish that had been implanted with a data logger. Student t-tests were used to assess whether sex or exercise had an effect on f_H at each time interval following the exercise treatment. In cases where MANOVA whole models were significant, two-way ANOVAs were conducted on each variable to identify the variables driving the model. Two-way repeated measures analysis of variance (two-way RM ANOVA) with treatment and time and their interaction as effects was used to compare mean f_H and mean blood physiological indices at each time interval during recovery from the exercise treatment. Linear regressions were used to compare each of the blood physiological values (dependent variables) and f_H (independent variable) for all fish combined from each treatment at both the 1 h and 16 h sampling intervals. The level of significance (α) was assessed at 0.05 for the linear regressions and MANOVAs. In cases of multiple comparisons (i.e., two-way ANOVAs and two-way RM ANOVAs), α was Bonferroni-corrected to 0.006. All values are means ± S.E., unless otherwise noted. All statistical analyses were conducted using JMP v 7.0 (SAS Institute Inc., Cary, N.C.).

4.3 Results

4.3.1 Initial blood variables and heart rate

The initial blood variables were the same among surgery controls and instrumented fish (MANOVA; $F_{8,27}$= 0.376; p = 0.301) and therefore were pooled for further analysis. Sex-specific differences in blood variables (MANOVA; $F_{8,14}$= 3.889; p = 0.013) were limited to plasma cortisol (two-way ANOVAs; p < 0.001); cortisol was subsequently analysed by sex whereas all other variables were pooled (Table 4). Females randomly assigned to the subsequent exercise group had a significantly higher plasma cortisol compared with exercise controls at the time of instrumentation; however this was not significant following Bonferroni corrections.

Post-surgery, f_H reached a maximum of 58.7 ± 1.3 beats min^{-1} at 47 ± 8 min into recovery. Following an 18.4 ± 1.5 h recovery, f_H had stabilised at 35.1 ± 1.0 beats min^{-1} (8.6^oC). There were no significant differences in f_H between sexes.

4.3.2 Heart rate responses to corralling

Heart rate prior to corralling was 31.5 ± 1.2 beats min^{-1} (8.0^oC; N=13; Figure 8). Corralling induced short periods of burst swimming as fish attempted to avoid the net and experimenters. Even so, corralling was slow and fish could remain inactive for periods of ~1 min before swimming to a different region of the holding channel to evade the net. The individual level of exercise was considered uniform because fish swum as an aggregate, regardless of whether they were corralled for 10 min or 30 min.

Heart rate increased to about 60 beats min^{-1} during corralling independent of its duration (Figure 8; t-test, p > 0.05). However, the time taken for f_H to recover to pre-treatment values took 456 ± 56 min for the 10-min corral and 50% longer, 691 ± 65 min, for the 30-min corral (t-test, p

= 0.021). After 4-6 days of undisturbed recovery, minimum f_H was not significantly different among groups (25.1 ± 0.6 beats min^{-1}; 8.0^oC; N=13).

4.3.3 Physiological responses to exhaustive exercise and air exposure

Heart rate increased significantly after the rapid transfer (< 3 s) of fish by dip-net from a raceway to a net pen (Figure 9, Table 5). During manual chasing, burst swimming lasted for varying durations (range = 47-170 s; mean = 95 s), but no fish maintained burst swimming activity for the entire 3 min period. Other visual signs of exhaustion included slow righting response when fish were rolled supine, and slow body movements during air exposure. On return to the net pen, all fish regained equilibrium immediately, except one air-exposed fish which took 20 s.

Air exposure had no significant effect on the recovery of heart rate and blood variables measured at 1 h (heart rate: t-test; t_4= 0.329; p = 0.759; blood variables: MANOVA; $F_{8,7}$= 0.489; p = 0.871) and 16 h post-exhaustion (heart rate: t-test; t_4= -1.06; p = 0.348; blood variables: MANOVA; $F_{8,15}$= 0.435; p = 0.865). Thus, these two groups were pooled for subsequent analyses. There were no sex-specific differences in mean f_H at 1 h (t-test; t_8= -1.27; p = 0.238) and 16 h (t-test; t_8= 1.15; p = 0.283) following exhaustive exercise. Heart rate was significantly higher for exhaustive exercise (58.5 ± 1.8 beats min^{-1}) relative to controls (40.5 ± 3.2 beats min^{-1}) at 1 h post-treatment prior to biopsy. Biopsy similarly elevated f_H in control fish, which remained higher than exercised fish for 8 h following biopsy (Figure 9). Nevertheless, recovery of f_H in control fish had the same duration (926 ± 151 min) as exhaustively exercised fish (729 ± 68 min) (t-test; t_8= -1.34; p = 0.217).

Blood variables for surgery control fish and those implanted with data loggers did not differ at 1 h (MANOVA; $F_{8,14}$= 0.843; p = 0.251) and 16 h post-exhaustive exercise (MANOVA; $F_{8,3}$= 0.511; p = 0.973), and thus were pooled for subsequent analyses. Sex-specific differences in blood physiology at 1 h (MANOVA; $F_{8,27}$= 11.414; p < 0.001) and 16 h following exhaustive exercise (MANOVA; $F_{8,3}$= 13.594; p = 0.028) were again solely due to plasma cortisol (two-way ANOVAs ; p < 0.001). As expected, plasma lactate, glucose, sodium, osmolality and cortisol (males) concentrations were all significantly higher 1 h after exhaustive exercise compared with non-exercised controls, but were restored after 16 h.

4.3.4 Relationships between heart rate and blood variables during recovery

Significant relationships existed between f_H and several plasma variables at 1 h post-treatment for pooled data (exercise and non-exercised controls; Figure 10), but not at 16 h post-treatment (i.e., all p > 0.05). A positive linear relationship existed between f_H and plasma sodium (R^2 = 0.605; p = 0.008) as well as plasma chloride (R^2 = 0.596; p = 0.009), and a logarithmic relationship existed between f_H and plasma lactate (R^2 = 0.826; p < 0.001; Figure 10). A trend existed between f_H and plasma osmolality, but small sample sizes precluded making statistical inferences.

4.4 Discussion

4.4.1 Responses of fish to corralling with no physical handling

Routine f_H was stable and low (e.g., ~ 30 bpm) in comparison with previous studies on adult salmonids (Anderson et al. 1998; Gallaugher et al. 2001; Steinhausen et al. 2008), particularly those engaging in pre-spawning behaviors (Clark et al. 2009; Makiguchi et al. 2009). The immediate response to corralling (a near doubling in f_H) was uniform and independent of the

duration of the event. Even so, it is likely that the longer period of corralling was more stressful since f_H took 1.5 times longer to recover. Therefore, a three-fold increase in corralling duration increased the duration of recovery from the corralling treatment by 50% without affecting the maximum f_H response. Cooke et al. (2003) found that a 30 s presentation of avian predator models to largemouth bass without physical contact increased f_H by 30 – 50 % at ~24^{o}C, which is half the increase in f_H documented in this chapter. The bass recovered rapidly (20 – 40 min) from the short-duration stressor (Cooke et al. 2003) in comparison with the corralled coho salmon studied here.

4.4.2 Responses of fish to exhaustive exercise and handling

As with the corralling protocol, exhaustive exercise resulted in a rapid doubling of f_H, and additional air exposure did not further elevate f_H. Furthermore, the capture, handling, and biopsy procedure induced a similar doubling of f_H in the control fish at 1 h following the treatment protocols. These data confirm that the magnitude of the f_H response is independent of the magnitude of the stress, and thus my first prediction is rejected. That is, even minor stressful encounters initiate a maximal f_H response, possibly resulting from a rapid release of vagal tone (e.g., Sandblom et al. 2009). At similar temperatures, Atlantic salmon only increased f_H by 20% in response to brief angling (Anderson et al. 1998). Furthermore, the relative increases in f_H was unaffected by temperature (i.e., 30% at 16.5°C and 20% at 20°C; Anderson et al. 1998). Using similar methodology with largemouth bass in the laboratory, manual chasing doubled f_H at a range of temperatures from 13-25^{o}C (Cooke et al. 2004).

In accordance with my second prediction, recovery of f_H depended on the intensity of the stress, taking up to 16 h post-exhaustive exercise and either 7.6 h or 11.5 h depending on the duration of corralling. Following angling stress in the field, Atlantic salmon required up to 16 h

to recover f_H (Anderson et al. 1998). At 1 h post-exercise in this study, plasma cortisol, lactate, sodium and osmolality were elevated, which was to be expected given the time course for these variables (Milligan 1996; Barton 2000). Males and females, respectively, had a 3-fold and 1.5–fold increase in plasma cortisol, which is comparable to the 2.7-fold increase measured 30 min after coho salmon were exhaustively exercised in saltwater (Cech et al. 2004), and the 3.3-fold increase that occurred during the first hour following capture of coho salmon by a commercial gill net in saltwater (Farrell et al. 2001). Plasma cortisol was the only sex-specific plasma variable measured here and this difference may reflect physiological differences in reproductive development between sexes of Pacific salmon (Carruth et al. 2002; Clark et al. 2009; Sandblom et al. 2009).

In accordance with my third prediction, strong positive linear relationships existed for f_H and certain plasma variables (lactate, sodium and chloride) at 1 h post-treatment (pooled data for control and exercised fish). Plasma osmolality exhibited a similar pattern but insufficient sample sizes precluded statistical analysis. Lactate production during glycolysis is known to decrease muscle and blood pH (Wang et al. 1994), which can lead to a disruption of ion-osmoregulatory balance as water shifts from blood to muscle tissue. This leads to temporary increases in concentrations of some plasma ions in freshwater, followed by depressed ion concentrations over the longer term (Wood 1991). Temporary increases in plasma ions were observed at 1 h post-treatment for both plasma sodium and osmolality. However, plasma ion concentrations were significantly depressed relative to pre-treatment values when measured at 16 h post-treatment, consistent with the longer term decreases in ion concentrations following a stressful event. Similar physiological disturbances have been observed in the blood and muscle of salmonids captured by angling (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996; 1997) and

commercial fisheries gears (Parker et al. 1959; Farrell et al. 2000; Skomal 2007). Relationships between f_H and other variables may not have been detected due to the timing of blood sampling following the treatment. For example, blood glucose generally decreases and recovers rapidly (minutes) from exhaustive exercise, which may explain why I failed to detect relationships between f_H and plasma glucose when measured at 1 h post-exercise (Pagnotta et al. 1991; Milligan et al. 2000).

Some parameters had returned to pre-treatment levels at 16 h post-treatment (i.e., mean f_H, plasma lactate, sodium, osmolality, and cortisol [males]), yet mean MCHC, plasma glucose, chloride, potassium, and plasma cortisol (females) had not completely recovered. The time required to clear metabolites from the blood and restore muscle energy stores may limit performance since this recovery rate will determine the frequency of maximal performance (Milligan 1996). While prolonged swimming performance can be repeated with relatively short recovery times in adult Pacific salmon [e.g., 40 - 45 min (Farrell et al. 1998; Jain et al. 1998)], the duration of complete physiological recovery following the stressor may be prolonged (Tang and Boutilier 1991; Milligan et al. 2000; Farrell et al. 2001), potentially leaving the individual more susceptible to secondary predation or fisheries capture. Milligan et al. (2000) found that rainbow trout that recovered in flowing water and were able to swim at a constant low velocity (i.e., 0.9 body lengths•s^{-1}) following exhaustive exercise had a complete metabolic recovery in ~ 2 h. Similarly, plasma cortisol levels remained relatively low in exhausted individuals that swam at constant velocity compared with those held in static water. Given that the fish in this study recovered in a water velocity of only ~0.3 body lengths•s^{-1}, the differences in recovery times between studies may be partly a consequence of differences in water velocity (see chapter 6).

4.5 Conclusions

Together, the biopsy and f_H data provide a better understanding of the consequences of simulated fisheries encounters on free-swimming adult Pacific salmonids. Biopsy only provides a snapshot at various time intervals (e.g., chapters 2 and 3) and as my results show can induce a rapid increase in f_H, but the continuous f_H data provide a complete characterization of the recovery profile. It remains to be determined if recovery is species and sex-specific (see chapter 5). Clearly, while the level of elevation in f_H conveys little as to the magnitude of the stress, the intensity of the stress is reflected in the duration of recovery of f_H, an important finding that is relevant to exploring methods for facilitating recovery (see chapter 6). Even without physical contact, a fisheries encounter could maximally elevate f_H and result in a prolonged recovery period. The correlations observed between f_H and several plasma variables warrant further exploration under varying conditions of exercise and stress, but they suggest a potential for biologging or biotelemetry of f_H (Clark et al. 2009; Donaldson et al. 2008; Clark et al. 2010) to provide a general indication of the physiological responses of fish to stressful encounters.

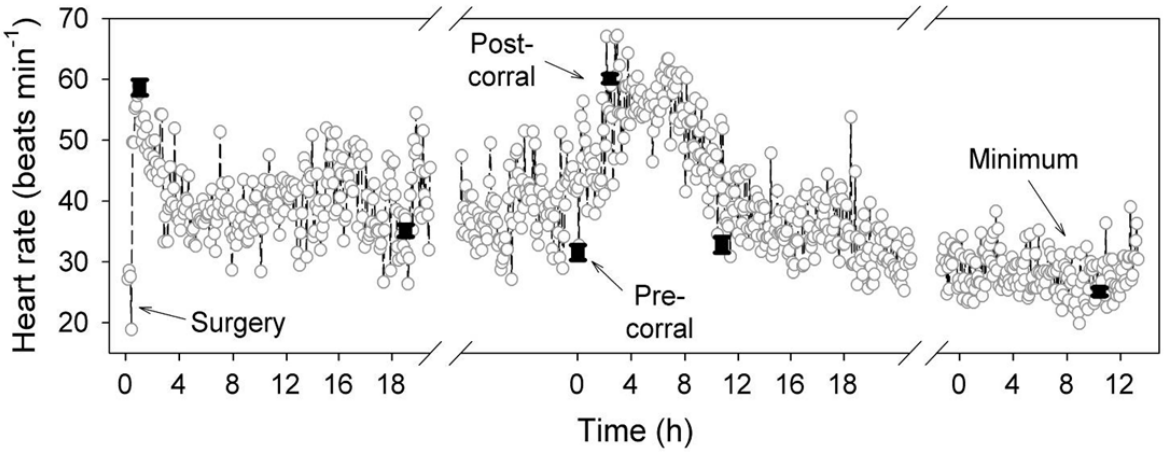

Figure 8. Heart rate (f_H)* traces during recovery from data logger implantation, for the short (10 min) corralling treatment, and for the period when f_H reached a minimum between the corralling treatment and the exercise treatment (~9^{o}C).

* Open grey circles represent raw data from an individual fish, while black squares represent mean (± S.E.M.) values from all fish that underwent the short corralling treatment (N=6). Fish had 24-48 h recovery from surgery prior to the corralling treatment. Heart rate profiles from the fish that underwent the long (30 min) corralling treatment were identical, except that *f*H took longer to reach pre-corral levels following the corralling treatment (see Results).

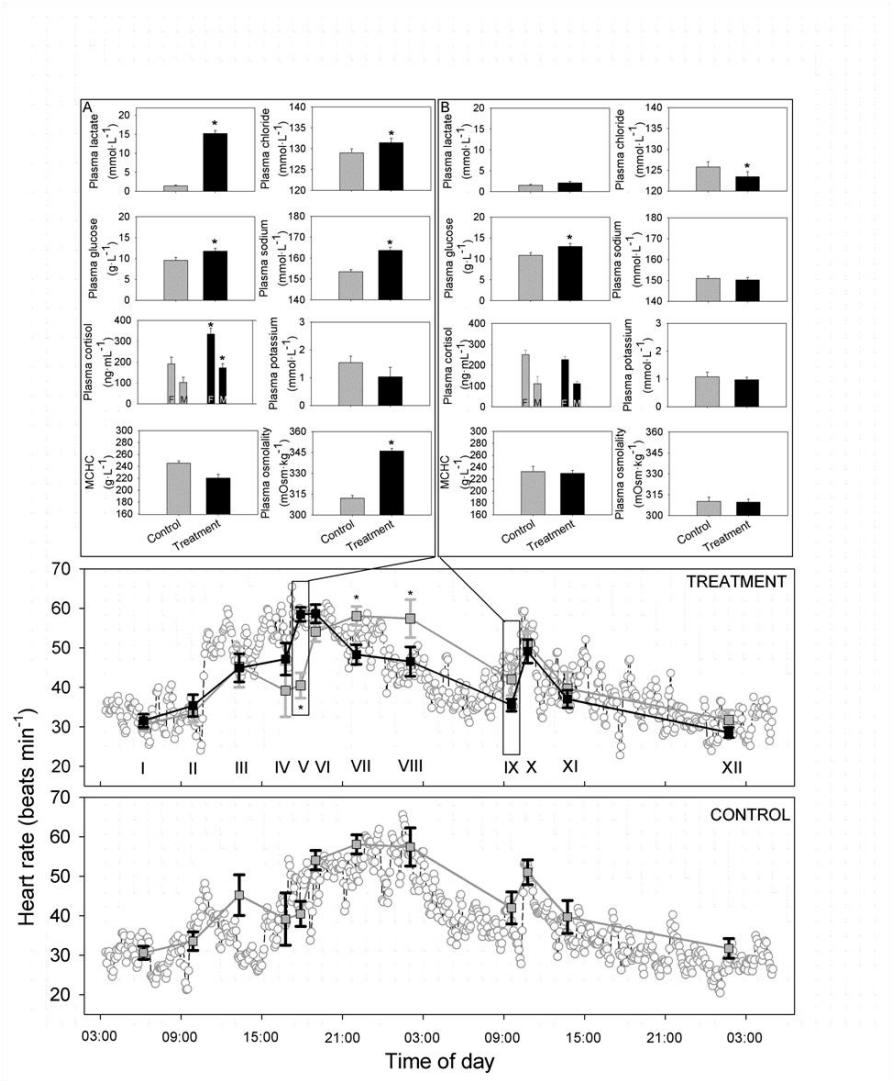

Figure 9. Heart rate (f_H)* traces for fish in the exercise treatment group and those in the exercise control group (~8^{o}C).

*Open grey circles represent raw data from an individual fish (one treatment fish and one control fish). Squares represent mean (± S.E.) values from all fish that underwent the exhaustive exercise treatment (N=6; black squares), or the control group (N=5; grey squares). The 'treatment' panel shows mean f_H for the exercise treatment group (black squares) and overlays mean heart rate for the control group (grey squares) for easy comparison. The top panels display the blood physiological data at the time intervals indicated by boxes on the bottom panel. Descriptions of the time periods marked with Roman numerals are given in Table 5.

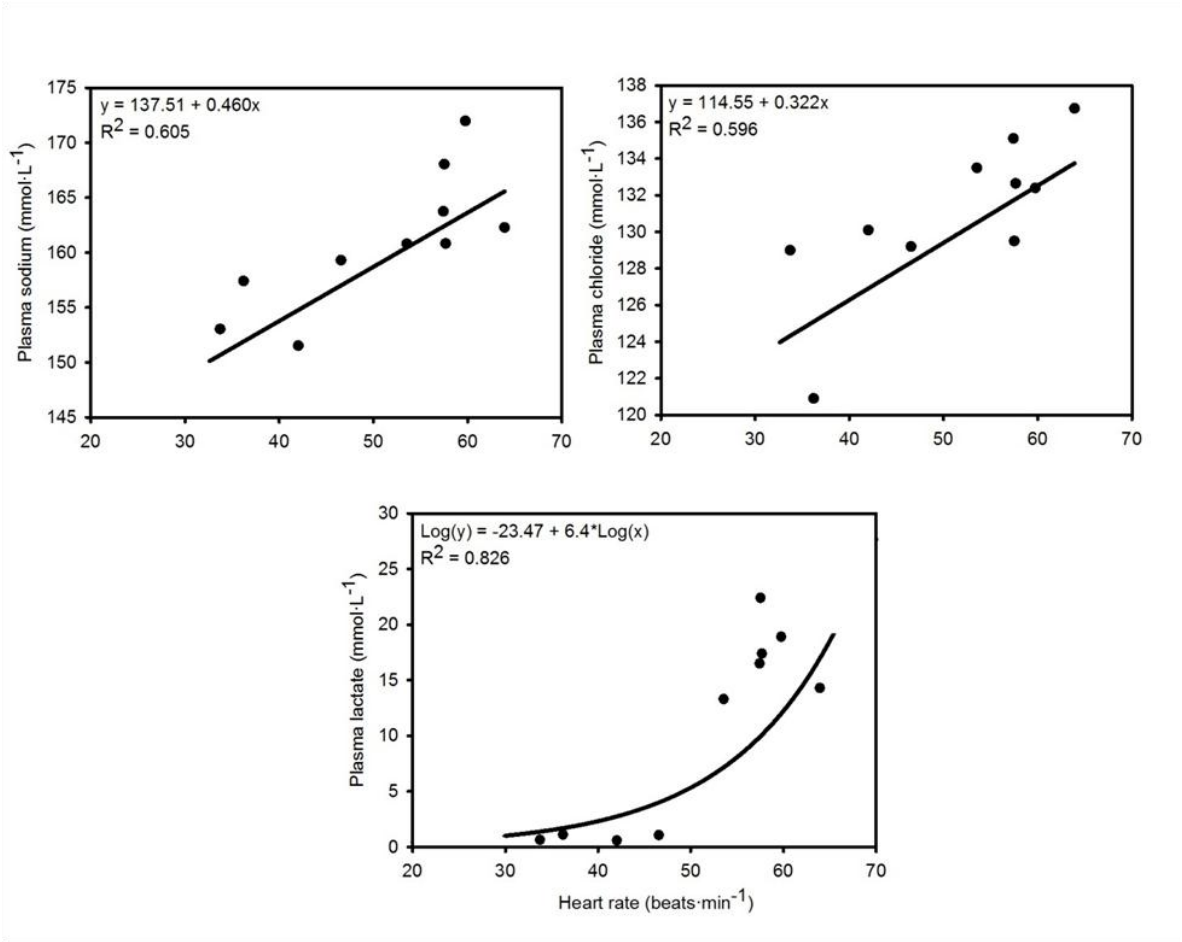

Figure 10. Linear regressions between plasma variables and heart rate (f_H) for pooled data (exhaustively exercised fish and non-exercised controls) from blood samples collected at 1 h post-treatment (~8^{o}C).

Table 4. Heart rate and blood physiological variables for non-exercised control and exhaustively exercised treatment groups of adult coho salmon at instrumentation (Instr.; i.e., 8-9 d pre-treatment), and at 1 h and 16 h after the exercise treatment.

Variable	Group	Instr.	1 h post-treatment	16 h post-treatment	Two-way RM ANOVA		
					Treatment	Time	Interaction
Heart rate ($beats \cdot min^{-1}$)	Control	–	40.5 ± 3.2^{a}	42.0 ± 4.0^{a}	$F = 6.31$	$\mathbf{F = 27.73}$	$\mathbf{F = 33.63}$
	Treatment	–	58.5 ± 1.8^{b}	35.5 ± 1.5^{a}	$P = 0.036$*	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
Haematocrit (%)	Control	33.0 ± 1.0	36.0 ± 1.4	34.3 ± 1.5	-	-	-
	Treatment	33.4 ± 0.8	40.0 ± 1.5	34.8 ± 1.1			
Haemoglobin ($g \cdot L^{-1}$)	Control	99.4 ± 2.4	87.9 ± 2.9	78.8 ± 2.4	-	-	-
	Treatment	98.7 ± 1.4	87.1 ± 3.9	79.3 ± 2.2			
MCHC ($g \cdot L^{-1}$)	Control	302.9 ± 7.5^{x}	245.4 ± 3.6^{y}	232.4 ± 8.7^{y}	$F = 4.42$	$\mathbf{F = 63.70}$	$F = 2.01$
	Treatment	298.9 ± 7.4	216.2 ± 6.3	229.5 ± 4.9	$P = 0.041$*	$\mathbf{P < 0.001}$	$P = 0.141$
Lactate ($mmol \cdot L^{-1}$)	Control	1.9 ± 0.1^{by}	1.4 ± 0.2^{bx}	1.5 ± 0.2^{by}	$\mathbf{F = 94.25}$	$\mathbf{F = 109.94}$	$\mathbf{F = 122.10}$
	Treatment	2.1 ± 0.2^{b}	15.2 ± 0.8^{a}	2.1 ± 0.3^{b}	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
Glucose ($g \cdot L^{-1}$)	Control	5.6 ± 0.3^{dx}	9.6 ± 0.7^{cy}	10.8 ± 0.7^{bcz}	$F = 2.65$	$\mathbf{F = 102.89}$	$\mathbf{F = 5.63}$
	Treatment	4.9 ± 0.2^{d}	11.7 ± 0.7^{b}	12.9 ± 0.7^{ab}	$P = 0.113$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.005}$
Cortisol ($ng \cdot mL^{-1}$)	Control (F)	94.1 ± 21.9^{x}	191.6 ± 32.1^{y}	250.6 ± 22.7^{y}	$F = 5.95$	$\mathbf{F = 7.33}$	$F = 3.45$
	Treatment (F)	207.9 ± 33.3	327.9 ± 25.4	226.1 ± 34.0	$P = 0.03$*	$\mathbf{P = 0.002}$	$P = 0.04$*

Variable	Group	Instr.	1 h post-treatment	16 h post-treatment	Two-way RM ANOVA		
					Treatment	Time	Interaction
Cortisol ($ng \cdot mL^{-1}$)	Control (M)	78.5 ± 19.8^{bcx}	102.2 ± 27.9^{bcy}	111.4 ± 16.6^{bcy}	$F = 0.72$	$\mathbf{F = 13.07}$	$\mathbf{F = 6.05}$
	Treatment (M)	58.5 ± 8.5^{b}	173.1 ± 19.8^{a}	111.2 ± 11.5^{c}	$P = 0.410$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.005}$
Chloride ($mmol \cdot L^{-1}$)	Control	134.5 ± 0.8^{x}	129.0 ± 0.9^{y}	125.8 ± 1.2^{z}	$F = 0.05$	$\mathbf{F = 51.99}$	$F = 3.29$
	Treatment	133.7 ± 0.5	131.4 ± 1.1	123.4 ± 1.2	$P = 0.819$	$\mathbf{P < 0.001}$	$P = 0.04$*
Sodium ($mmol \cdot L^{-1}$)	Control	155.2 ± 0.9^{bx}	153.5 ± 1.1^{bcy}	150.9 ± 1.1^{bcz}	$F = 4.65$	$\mathbf{F = 25.87}$	$\mathbf{F = 14.29}$
	Treatment	155.2 ± 0.7^{b}	163.1 ± 1.5^{a}	150.2 ± 1.2^{c}	$P = 0.038$*	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
Potassium ($mmol \cdot L^{-1}$)	Control	1.9 ± 0.2^{x}	1.6 ± 0.2^{xy}	1.1 ± 0.2^{y}	$F = 0.02$	$\mathbf{F = 7.42}$	$F = 0.18$
	Treatment	1.9 ± 0.2	1.4 ± 0.3	0.9 ± 0.1	$P = 0.883$	$\mathbf{P = 0.001}$	$P = 0.835$
Osmolality ($mOsm \cdot kg^{-1}$)	Control	321.4 ± 1.3^{bcx}	312.1 ± 1.9^{cdy}	310.1 ± 3.0^{cdz}	$\mathbf{F = 36.73}$	$\mathbf{F = 30.91}$	$\mathbf{F = 44.00}$
	Treatment	322.6 ± 0.8^{b}	344.9 ± 1.9^{a}	309.5 ± 2.2^{d}	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$

MCHC = mean corpuscular haemoglobin concentration. Cortisol differed between males and females and thus was analysed independently for each sex. Values are means ± S.E.M. Results are given for two-way repeated measures ANOVAs conducted on each variable. Two-way repeated measures ANOVAs contained 'treatment' and 'time' as main effects, and their interaction. Bold values denote significance at Bonferroni-corrected $\alpha = 0.006$, * denotes significance at $\alpha = 0.05$. Superscript letters indicate the results of Tukey's post-hoc tests: dissimilar letters indicate differences between values, where ^{abc} denote differences with respect to the 'treatment' and 'time' interaction, and ^{xyz} denote differences with respect to the 'time' main effect across treatments.

Table 5. Description of the timing of treatment events that correspond with the responses observed in Figure 9.

Time	Description of event	Response
I	4 h before transfer to holding pen (fish housed with 11-12 conspecifics)	Resting heart rate ~30 beats min^{-1} for both the exercise treatment group and the exercise control group
II	30 min before transfer to holding pen (fish housed with 11-12 conspecifics)	Resting heart rate ~34 beats min^{-1} for both the exercise treatment group and the exercise control group
III	3 h after transfer to holding pen	Heart rate elevated above resting values in both the exercise group and the control group
IV	Immediately before exhaustive exercise treatment	Heart rate remained elevated and not significantly different between the exercise and control groups
V	Immediately before 1 h post-treatment biopsy	Heart rate significantly elevated in exercise group compared with control group
VI	1 h after 1 h post-treatment biopsy	Heart rate similarly elevated in both treatment and control groups in response to biopsy
VII	4 h after 1 h post-treatment biopsy	Heart rate significantly elevated in control group compared with exercise group
VIII	8 h after 1 h post-treatment biopsy	Heart rate remained significantly elevated in control group compared with exercise group

Time	Description of event	Response
IX	Immediately before 16 h post-treatment biopsy	Heart rate of both groups had returned to pre-exercise levels (i.e., IV)
X	1 h after 16 h post-treatment biopsy (fish housed with 24 conspecifics)	Heart rate similarly elevated in both treatment and control groups in response to biopsy
XI	4 h after 16 h post-treatment biopsy (fish housed with 24 conspecifics)	Heart rate of both groups had returned to values recorded immediately before the 16 h post-treatment biopsy (i.e., IX), but was still elevated above resting levels
XII	16 h after 16 h post-treatment biopsy (fish housed with 24 conspecifics)	Heart rate had returned to resting levels (i.e., I)

5 SPECIES-SPECIFIC RESPONSES AND RECOVERY OF WILD ADULT PACIFIC SALMON TO FISHERIES-RELATED EXERCISE STRESS

5.1 Introduction

The general stress response is well understood for many fish species (Wood 1991; Milligan 1996; Kieffer 2000) but less is known about how maturing wild Pacific salmon respond to stress and whether or not species-specific differences exist. Chapter 1 reviewed that, as a primary response to the stressor, catecholamines and corticosteroids are released which in turn initiate the secondary stress response at the tissue-level (Barton 2002). The secondary response includes osmoregulatory, ionic, metabolic and cellular responses to stress (Wendelaar Bonga 1997). The cellular suite of responses help to temporarily tolerate or counteract stress or remove damaged cells by apoptosis (Kültz 2005). Thus, changes in gene expression can be linked with various stressors, such as handling stress (Krasnov et al. 2005). For example, heat shock protein expression increases to maintain cellular homeostasis (Iwama 2004). Linkages between the acute stress response and gene expression are being elucidated, but the relationships between these responses and those that occur at other levels of biological organization still remain poorly understood (Kassahn et al. 2009). A complicating factor in understanding these relationships is the fact that studies conducted on fish to date are fragmented, focusing on different research questions, timecourses (Krasnov et al. 2005), species, tissues (Kassahn et al. 2009), and techniques (Prunet et al. 2008). While chapters 2 and 3 found that the type of fisheries stressor influenced physiological response and survival, recovery from fisheries-related stressors has rarely been explored. Chapter 4 filled this knowledge gap by elucidating the timecourse of

recovery following different magnitudes of stress and identified that the magnitude and duration of the stressor influences the duration of recovery. Despite the value of this information for understanding the timecourse of recovery, chapter 4 focused on a different Pacific salmon species than its predecessors. Chapter 4 also identified the role of sex in the magnitude of response. Therefore the focus of this chapter was to test for evidence of species- and sex-specific differences in recovery patterns for primary and secondary stress responses.

Pink salmon and sockeye salmon both undergo semelparous and anadromous reproductive migrations, but differ in their respective life history, performance, behavior, thermal tolerance, abundance, and conservation status. Sockeye salmon typically grow for one year in freshwater before migrating to sea, and generally return to spawn at age four. In contrast, pink salmon migrate directly to sea after emergence from redds, returning to spawn at two years of age. As a consequence, pink salmon are the smallest of the Pacific salmon species at maturity, which results in a lower absolute prolonged swimming performance relative to sockeye salmon, although length-adjusted swimming performance is not different than sockeye salmon (Williams and Brett 1987; MacNutt et al. 2006). Pink salmon also begin their spawning migrations with smaller relative somatic energy reserves compared to sockeye salmon (Crossin et al. 2003), but compensate behaviourally by reducing transport and activity costs compared to sockeye salmon (Standen et al. 2002) by seeking out optimal microhabitats of least migratory resistance. They also use more coastal rivers. In addition, the relatively narrow temperature range of sockeye salmon for optimal swimming performance and local adaption (Eliason et al. 2011) is not seen in the broader thermal performance window of pink salmon and less local adaptation to temperature conditions (Standen et al. 2002), perhaps as a consequence of a higher straying rate from natal

streams for pink salmon (Heard 1991). As a result, pink salmon have a remarkable cardiovascular performance and higher optimal temperatures for aerobic scope compared to other Pacific salmonids (Clark et al. 2011). Although pink salmon invest less energy into reproductive development than sockeye salmon, their unique swimming (Crossin et al. 2003) and cardiovascular performance (Clark et al. 2011) may account for their resilience. Given these species-specific differences in life history, migration strategies, and physiology, it could be assumed that differences in response and recovery to exercise stress may likewise differ, but this has not been examined to date.

Given that multiple Pacific salmon species migrate through the same location during overlapping time periods, non-target species are often caught as bycatch and released by fisheries. For released fish, the magnitude of response and timecourse of recovery are important, as these factors can be linked to mortality in extreme cases (Wood et al. 1983) and govern the frequency of maximal performance (Milligan 1996). Despite a growing understanding that post-release survival is influenced by capture method (e.g., chapters 2 and 3), population (e.g., chapter 3), and that the duration of recovery depends on the duration of the stressor (e.g., chapter 4), the sex- and species-specific nature of recovery have not been examined concurrently for wild, adult migrating Pacific salmon. To address this knowledge gap, this chapter compares the response and recovery of mature pink and sockeye salmon to a controlled, fisheries-related exercise stress treatment. This chapter tested the hypothesis that the recovery processes is sex- and species-specific by comparing the recovery patterns of one sockeye salmon population versus one pink salmon population using a series of plasma stress, osmoregulatory and reproductive variables as

well as examining the expression of genes active in cellular stress, cell maintenance, and apoptosis.

5.2 Methods

5.2.1 Study site and animals

Experiments on both species were conducted at the same location, Fisheries and Oceans Canada's Weaver Creek Spawning Channel, near Agassiz, British Columbia, Canada. Pink salmon experiments were conducted October 1st – 7th, 2009 and sockeye salmon experiments were conducted October 8th – 14th, 2009. In 2009, peak spawning was October 19-24th for pink salmon and October 15-19th for sockeye salmon.

Water temperatures throughout the course of the study were 11-12 ºC, measured using a permanent temperature probe operated by Fisheries and Oceans Canada staff. A total of 112 sockeye salmon and 88 pink salmon were used in the experiment. Equal proportions of male and female pink and sockeye salmon allowed sex-specific differences to be examined (e.g., plasma cortisol; Donaldson et al. 2010).

5.2.2 Study design

Fish were captured by dip net from a raceway downstream of the spawning channel entrance. Individuals were rapidly (1-2 s to minimize air exposure) transferred to a holding tote on a research vehicle, and transported ~300 m (2-3 min transport time) to the experimental area. Individuals in the exercise treatments were transferred into a donut shaped exercise tank (diameter 150 cm, inner diameter 50 cm, water depth 40 cm) supplied with fresh, flowing water pumped from the spawning channel. Fish were manually chased for 3 min, then collected by dip

net and exposed to air for 1 min as previously described (Donaldson et al. 2010). Briefly, fish placed in the tank were manually coaxed to continually burst swim (Milligan 1996) by three experimenters positioned around the exercise tank, which resulted in fish being visibly exhausted (unable to burst swim, unresponsive to tactile stimuli and some individuals unable to maintain equilibrium). The procedure was intended to simulate an exhausting wild fish capture and release event (e.g., angling and release or rapid release from a net fishery). The air exposure was intended to simulate air contact while being removed from a hook or net.

Fish were randomly assigned a sampling interval of 0, 0.5, 1, 4, 24 h. Control fish were neither exercised nor air exposed, but were transferred directly into holding boxes. They were sampled 24 h later to allow for full recovery from minor handling. Note that while this method could create a chronic confinement stress, control plasma glucose (~5-6 $mmol \cdot L^{-1}$) was identical to fish rapidly collected by dip net upon arrival at the spawning channel (5-6 $mmol \cdot L^{-1}$; McConnachie 2011). Individual fish were sampled once to collect gill tissue and a blood sample to avoid repeated handling and sampling. The rapid caudal puncture technique has been found in adult Pacific salmon to generally yield statistically similar plasma values compared to cannulation techniques (Clark et al. 2011).

Exercised and air exposed fish were immediately placed into individual, dark plastic holding boxes with secure lids (L x W x D = 93.7 x 54.0 x 47.3 cm). Each box received freshwater pumped at 0.63 L/s using an electric submersible pump placed in the spawning. The outflow was positioned at the back of the box. The inflow was centred in the lower third of the box to direct water at the fish's mouth. The boxes were large enough to enable fish to orient into the water flow with occasional tail beats to change or maintain position. However, when

collected for sampling, fish were often not observed oriented directly into the flow. I speculate that the sampling boxes provided an environment similar to fish in the wild holding in low flow riverine areas behind large rocks with lower direct velocity than methods designed to facilitate fish recovery using high velocity ram ventilation (e.g., Milligan et al. 2000; Farrell et al. 2001).

For tissue sampling, individuals were rapidly removed from their box and placed supine in a water-filled V-shaped foam-padded sampling trough (Cooke et al. 2005) for immediate biopsy and length measurements. The biopsy, which lasted < 2 min, collected a 2.5 mL blood sample by caudal puncture using a sterile 3.8 cm, 21-gauge needle and a heparinised vacutainer (lithium heparin, 3 mL, Becton-Dickson, NJ), which was then stored in ice-chilled water for < 1 h until subsequent processing. Also, ~3 mm gill filament tips from the first gill arch were collected using sharpened end-cutter pliers, sterilized with 95 % ethanol and rinsed with distilled water (Cooke et al. 2005). Gill samples were transferred using sterile forceps to cryovials, flash frozen in liquid nitrogen, and subsequently transferred to -80 C freezers for subsequent analyses.

5.2.3 Plasma assays

As in previous chapters, the chilled ~ 2.5 mL blood sample was centrifuged at 7,000 x g for 3 min and plasma was stored in liquid nitrogen prior to being frozen at –80 °C until analysis. Plasma was subsequently analysed for the following: cortisol, testosterone, and 17 B-estradiol using commercial ELISA kits (Neogen ELISA nos. 402710, 402110, 402510, with Molecular Devices Spectramax 240pc plate reader); lactate and glucose (YSI 2300 stat plus analyser); osmolality (Advanced Instruments 3320 freezing point osmometer); chloride (Haake Buchler digital chloridometer); and sodium and potassium (Cole-Parmer, model 410 single channel flame photometer; Farrell et al. 2001).

5.2.4 qPCR methods

Gill tissue was collected from non-lethal biopsies to quantify gene expression via RT-QPCR for genes involved in acute capture and handling stress including heat shock proteins (Krasnov et al. 2005), and cellular stress and maintenance (Momoda et al. 2007; Wiseman et al. 2007; Cairns et al. 2008). I examined four genes of interest and two housekeeping genes in gill tissue (Table 6). qPCR methods have been described previously by Jeffries et al. (*in press*), but briefly an iScript^{TM} cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to synthesize cDNA from 1 μg of total RNA. An ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) was used to perform Relative Quantification (RQ) assays. The cDNA template was diluted 1:2.5 and assays were conducted in 384-well plates using 20 μl reaction volumes [10 μl Kapa SYBR fast qPCR Master Mix (2x) (Kapa Biosystems, Inc., Woburn, MA, USA); 0.4 μl of a mixture of 0.2 μM forward and reverse primers; 2 μl of diluted cDNA; 7.6 μl of RNase/Dnase-free water]. The cycling conditions were 95°C for 3 min followed by 40 cycles of 95°C for 3 s and 60°C for 30 s, and a dissociation stage (95°C for 15 s, 60°C for 15 s, and 95 °C for 15 s) was added at each RQ run to ensure that there were no extra peaks in the dissociation curve. All samples were run in duplicate and with non-template controls included. Target gene expression was normalised to two housekeeping genes (78d16.1 and BMP4) that had been developed in house for sockeye salmon gill samples and that were not responsive to the experimental factors, as determined by the housekeeping gene association function of the DataAssist v 3.0 software suite (Applied Biosystems Inc., Foster City, CA).

5.2.5 Statistical methods

Relative expression of target genes was determined using the $^{-\Delta\Delta}$Ct method (Livak & Schmittgen 2001) using DataAssist v 3.0 (Applied Biosystems Inc., Foster City, CA). Statistical analyses were conducted on the expression of the genes of interest relative to two housekeeping genes. Fold change is presented in

Figure **12** to show the change in expression at each time period relative to controls. A principal component analysis (PCA) with a Varimax factor rotation was used, which conducts an orthogonal transformation of factors with eigenvalues ≥ 1.0, a technique that partitions correlated variables into factors for subsequent analyses (Wagner 2004). Variables with loading factors ≥ ± 0.5 were considered to contribute to the factor (marked in bold), but all variables are presented in Table 7. All response variables were $\log_{10}$-transoformed prior to PCA. Plasma estradiol was excluded from the PCA since it was only measured in females. MANOVAs and ANOVAs were conducted on the rotated factors to test for effects of time, sex, species, and their factorial interactions. Where noted, ANOVAs with Tukey's post-hoc tests were conducted and dissimilar letters represent statistical differences in figures and tables. All statistical analyses were conducted using JMP 9.0 (SAS Institute Inc., Cary, N.C.). For MANOVAs, significance was tested at $\alpha = 0.013$ (Bonferroni correction based on 4 rotated factors).

5.3 Results

No mortality was observed during the course of the study. The PCA resulted in four rotated factors (abbreviated RF; Figure 11). All four gill gene variables loaded on RF1. All osmoregulatory variables (i.e., plasma chloride, sodium, and osmolality) loaded on RF2. All

stress variables (i.e., plasma cortisol, lactate) loaded on RF3. All reproductive hormones (e.g., plasma cortisol and testosterone since cortisol is linked with reproductive maturity in Pacific salmon; Carruth et al. 2002) loaded on RF4.

A MANOVA of the rotated factors (RF1, RF2, RF3, and RF4) revealed a significant whole model (Wilk's lambda= 0.006; $F_{108,13.765}$= 13.765; $p < 0.001$), with significant effects for time (Wilk's lambda= 0.057; $F_{24,500.08}$= 26.517; $p < 0.001$), sex (Wilk's lambda= 2.215; $F_{4,143}$= 79.201; $p < 0.001$) and species (Wilk's lambda= 1.222; $F_{4,143}$= 43.673; $p < 0.001$), as well as the species * time interaction (Wilk's lambda= 0.462; $F_{24,500.08}$= 1.221; $p < 0.001$) and the species * sex interaction (Wilk's lambda= 0.101; $F_{4,143}$= 2.215; $p < 0.001$), but not the time * sex (Wilk's lambda= 0.816; $F_{24,500.08}$= 1.250; $p = 0.192$) or the species * time * sex interactions (Wilk's lambda= 0.849; $F_{24,500.08}$= 0.996; $p = 0.470$; Table 8). Only significant terms and interactions were included in subsequent ANOVAs testing the effects of time, sex, species, species * time interaction and species * sex interaction.

ANOVA testing found significant whole models for RF1 (SS= 59.065; F_{15} = 6.494, $p < 0.001$), RF2 (SS= 42.886; F_{15} = 24.221, $p < 0.001$), RF3 (SS= 121.348; F_{15} = 44.949, $p < 0.001$), and RF4 (SS= 100.874; F_{15} = 26.127, $p < 0.001$). RF1 was only influenced by time and species. RF2 was influenced by time, sex, species, and the species*time interaction. RF3 was influenced by time, sex, species, species*time and species*sex interactions. RF4 was influenced by time, sex, and their interaction.

While the general pattern of physiological response was similar for both species, pink salmon mounted a stress response of a greater magnitude for many of the plasma stress variables

that were measured (Table 9), whereas sockeye tended to have higher and more variable gene expression (Table 10). For both species, the plasma variables that responded to the stressor had recovered to control values by 24 h post-exercise (e.g., plasma lactate; Figure 12). Plasma lactate peaked between 0.5 and 1 h (pink females SS = 8.233; $F_6 = 62.666$; $p < 0.001$; pink males SS = 8.781; $F_6 = 20.360$; $p < 0.001$; sockeye females SS = 6.843; $F_6 = 28.949$; $p < 0.001$; sockeye males SS = 11.041; $F_6 = 64.836$; $p < 0.001$). Plasma cortisol peaked between 1 and 2 h for both species (pink females SS = 3.488; $F_6 = 22.469$; $p < 0.001$; pink males SS = 2.773; $F_6 = 9.315$; $p < 0.001$; sockeye females SS = 1.019; $F_6 = 11.958$; $p < 0.001$; sockeye males SS = 0.559; $F_6 = 5.007$; $p < 0.001$).

Indices of osmoregulatory status generally increased post-treatment. Plasma sodium increased over time and recovered by 24 h in both species (pink females SS = 0.015; $F_6 = 8.208$; $p < 0.001$; pink males SS = 0.0246; $F_6 = 9.444$; $p < 0.001$; sockeye females SS = 0.213; $F_6 = 8.008$; $p < 0.001$; sockeye males SS = 0.029; $F_6 = 4.301$; $p = 0.002$). Plasma potassium peaked at 2 or 4 h post-treatment for pink females (SS = 0.782; $F_6 = 3.323$; $p = 0.011$) and sockeye males (SS = 2.583; $F_6 = 12.490$; $p < 0.001$). Plasma osmolality increased over time and recovered by 24 h for pink salmon (pink females SS = 0.021; $F_6 = 32.527$; $p < 0.001$; pink males SS = 0.0167; $F_6 = 12.344$; $p < 0.001$) and was variable for sockeye salmon males (SS = 0.023; $F_6 = 3.246$; $p = 0.009$) and not significant for sockeye females. Plasma chloride decreased post-treatment for both sexes of pink salmon (pink females SS = 0.023; $F_6 = 30.948$; $p < 0.001$; pink males SS = 0.0183; $F_6 = 11.607$; $p < 0.001$), but remained variable for sockeye males (SS = 0.019; $F_6 = 2.785$; $p = 0.021$) and did not change for sockeye females.

In terms of reproductive hormones, plasma estradiol in females decreased immediately following stress in sockeye salmon (SS = 0.192; F_6 = 3.104; p = 0.018), but pink salmon (SS = 1.568; F_6 = 0.669; p = 0.675), while increasing slightly over time. Plasma testosterone showed high variability in both sexes and species and did not vary over time.

Gill Cyto C expression was significantly increased at 2 h, peaked at 4 h, and recovered to control values at 24 h post-treatment for pink salmon (SS = 0.163; F_6 = 4.179; p = 0.001). Gill Jun B peaked at 4 h and returned to control values at 24 h for both pink (SS = 0.201; F_6 = 5.789; p < 0.001) and sockeye salmon (SS = 0.116; F_6 = 2.521; p = 0.026; Fig 2). Gill HSP90 and NUPR1 expression remained unchanged over time for both species. Gene expression did not differ by sex for either species.

Although fork length varied between species and sexes (61.7 ± 0.4 cm and 66.4 ± 0.9 cm for females and male sockeye salmon, respectively, and 49.9 ± 0.4 cm and 51.4 ± 0.4 cm for females and male pink salmon, respectively), MANOVA did not reveal any significant influence of fork length on the physiological response variables for either pink (Wilk's lambda= 2.902; F_{18}= 0.806; p = 0.669) or sockeye salmon (Wilk's lambda= 0.614; F_{18}= 0.614; p = 0.845). Thus fork length could be excluded as a covariate in analyses.

5.4 Discussion

In accordance with the hypothesis of this chapter, the recovery processes following a fisheries-related capture treatment was found to be sex- and species-specific for many of the variables measured. These findings add new and valuable information to a field of research that began decades ago (Black 1955; overview in Kieffer et al. 2010) in that the magnitude of stress

response was found to be sex-specific for cellular stress, plasma stress, osmoregulatory, and reproductive variables as well as species-specific for these variables when reproductively mature individuals from two closely related species in the same spawning area and at the same temperature but with distinct life histories were compared. Species-specific responses to stress have been previously documented in fish for blood variables (Black 1955). Identical stressors can even elicit variable responses even within the same family (Pottinger et al. 2010) and so the sex-specific responses should not be too much of a surprise, but rather something that has not been previously investigated in detail. While species-specific variability was anticipated, the magnitude of the difference in plasma lactate and cortisol response, being dramatically higher for pink salmon, was unexpected given the similarity of the stress and environmental conditions. These species-specific differences may be the result of either inherent genetic differences in physiological responses to stress, or differences in the reproductive maturation state or body size between the two species. The results suggest that the later rather than the former explanation may be more important.

Pink salmon had comparatively higher plasma estradiol and testosterone levels relative to sockeye salmon, suggesting an earlier state of maturation. In Pacific salmon, reproductive hormones decrease rapidly prior to spawning following the progressive increases of these reproductive hormones during the coastal and river migrations (Williams et al. 1982). Indeed, pink salmon were sampled 12-23 d prior to peak spawning and sockeye salmon that were sampled only 1-11d prior to peak spawning. My analyses revealed estradiol was significantly higher for pink salmon females relative to sockeye salmon females, without species-specific differences for plasma testosterone. The enhanced stress response of less mature pink is

consistent with a pattern well-described for rainbow trout, where maturity and higher sex hormone concentrations moderate the stress response (Pottinger et al. 1995; Pottinger et al. 1996). The primary stress response of plasma cortisol has been linked with reproductive maturity in Pacific salmon (Carruth et al. 2002), which my analysis corroborates since testosterone loaded with cortisol in both PCAs. Thus, the species-specific responses in gene expression may be likewise influenced by reproductive maturity through the influence of cortisol in stimulating gene expression during the stress response (Kassahn et al. 2009).

Previous studies have had difficulty disentangling the influence of body size and reproductive maturity on species-specific responses to stress (Pottinger et al. 2010). Body size can influence the stress response and recovery of some species (Gingerich et al. in press), but plasma indices of stress were independent of body mass for mature adult Chinook salmon (Clark and Farrell 2011). Pink salmon are the smallest (in both length and weight) of the Pacific salmonids at maturity (Heard, 1991). However, my analyses did not reveal an effect of body size on plasma indices of stress. Therefore, I conclude that the observed species-specific differences were more likely reflective of sockeye salmon being more mature than pink salmon.

5.4.1 Plasma indices of stress

Plasma lactate values from control groups for both species (~ 1 $mmol \cdot L^{-1}$) are comparable to values expected for resting salmonids (Wood et al. 1983; Milligan 1996; Barton 2002) and are nearly identical to the control values obtained for adult coho salmon (chapter 4). Similarly, the peak responses to stress for plasma cortisol and lactate are consistent with this large body of literature which typically show a peak between 0.5 h and 2 h before recovering (Wood et al. 1983; Milligan 1996; Barton 2002). The immediate response upon capture (i.e., at time zero) of

plasma lactate but not cortisol was similar for sockeye salmon caught by either rapid angling or beach seine (~3 min) and sampled immediately (chapter 2). The higher plasma cortisol values observed here compared with chapter 2 and reflects either a higher level of stress or a different reproductive state (see above). Nevertheless, the stress used here was insufficient did not trigger a cellular heat shock protein response and plasma stress variables had recovered by 24 h post-stress, as expected from previous studies of exhaustive exercise (e.g., Wood 1991). Plasma glucose remained stable over time, similar to other studies that have considered the response and recovery of adult Pacific salmon to exercise and fisheries-related stress (Donaldson et al. 2010a; chapter 4).

5.4.2 Osmoregulatory indices

Here increased plasma ions generally paralleled the increase in plasma lactate, which is generated by glycolysis and is known to decrease muscle and blood pH (Wang et al. 1994), disrupting ion-osmoregulatory balance and shifting water from blood to muscle tissue. Thus, the temporary increases in plasma ions observed during the first 4 h of recovery are consistent with previous observations in freshwater fish, although in the longer term ion concentrations can become depressed (Wood 1991), something that I did not resolve with this time series. Chapter 4, however, observed depressed ion concentrations relative to pre-treatment values when measured 16 h after acute exercise stress in coho salmon. Plasma osmolality followed the increases in plasma solutes (lactate, sodium, and chloride), as observed previously (Gale et al. 2011), which resulted in each of these variables loading with osmolality on PCA RF2. For both species, potassium showed a decrease at 30 min post-stress, again similar to Gale et al. (2011), and possibly related with a temporary re-uptake of potassium ions from the extracellular space

(Nielsen, 1992). The general increase in plasma potassium over the longer term likely occurred as a result of potassium ions being gradually lost from muscle and accumulating in plasma (Sejersted and Sjøgaard 2002).

5.4.3 Reproductive hormones and maturity

My finding that sex was a significant factor for certain plasma variables is consistent with a very recent finding that sockeye salmon females have higher plasma cortisol, lactate, and glucose than males (Jeffries et al. 2012). Sex-specific differences for plasma cortisol are well known and have been observed in both resting and acute and chronically stressed sockeye salmon (Fagerlund, 1967). Male rainbow trout and Pacific salmon show a lower stress-response relative to females for cortisol (Pottinger et al. 1995; Pottinger et al. 1996; Sandblom et al. 2009; chapter 4). Furthermore, control female and male coho salmon had ~200 and ~100 $mmol \cdot L^{-1}$ plasma cortisol, respectively (chapter 4), values that compare with control values for pink and sockeye salmon obtained here.

Previously identified sex-specific differences in maturing Pacific salmon include higher routine heart rates, plasma cortisol, testosterone and estradiol in female sockeye salmon relative to males (Sandblom et al. 2009). Several studies on Pacific salmon have identified that females typically experience higher mortality compared with males (Patterson et al. 2004; Crossin et al. 2008; Jeffries et al. 2012) and it has been suggested that female salmon have less capacity to cope with environmental stressors (Clark et al. 2011). Reproductive hormones can become depressed by stress (Pickering et al. 1987; Schreck et al. 2001). This was certainly the case for estradiol female sockeye salmon here, which is relevant due to the essential role that

reproductive hormones play in the final stages of maturation and senescence for spawning Pacific salmon (Hruska and Hinch 2010; Jeffries et al. 2012).

5.4.4 Gene expression

Jun B provides an important link between the endocrine stress responses and downstream transcriptional processes (Vamvakopoulos and Chrousos 1994). JunB forms the transcription factor activator protein 1 transcription complex, which is linked to cellular proliferation, apoptosis and stress response (Piechaczyk and Farràs 2008). Jun B expression peaked at 2 and 4 h in pink salmon and between 0.5 and 4 h in sockeye salmon after the stressor, yet had recovered by 24 h much like the plasma stress variables. Similarly, Jun B expression in liver peaked at 3 h and recovery by 24 h when juvenile rainbow trout were stressed by a 0.5-h exposure to low water and a 30-s air exposure (Momoda et al. 2007). Similarly, red blood cell expression of this gene increased rainbow trout at 4 h and 24 h following acute heat stress (Lewis et al. 2010). In view of these broad responses, Jun B may be a robust indicator of both an initial cellular stress response and a prolonged cellular response, depending on the nature of the stress. Gene expression was not sex-specific, despite an earlier report to this effect for immature rainbow trout (Momoda et al. 2007). Cyto C expression was responsive to stress, with a peak at 4 h for pink salmon likely due to release from the mitochondria into the cytoplasm of cells undergoing apoptosis (Jiang 2004). Jeffries et al. (*in press*) found up-regulation of cytochrome C as well as Jun B in moribund sockeye salmon, suggesting that the ODC1 gene (the most up-regulated gene in that study) was the enzyme precursor involved in polyamine synthesis that lead to an increase in cytochrome C and Jun B for moribund fish. My finding that pink salmon showed increased expression of both Cyto C and Jun B over time, but that this expression returned to control

values by 24 h, suggests that while these genes may respond strongly to stress and can signal mortality, they may also recover following the stressor, indicating the role of these genes in cellular maintenance and recovery.

HSP90 remained largely unchanged over time, perhaps suggesting that the stressor used here may not have been of a magnitude sufficient to induce such a response (Iwama et al. 2004). NUPR1 which is a stress-responsive transcription factor found in several tissues that responds to a range of stressors (Chowdhury and Samant 2009; Cano and Hamidi 2011), including the glucocorticoid response for rainbow trout liver (Momoda et al. 2007) and mouse pancreas (Path et al. 2004), was not reflected in my PCAs.

5.4.5 Research applications

RT-qPCR enables researchers to focus on specific pathways to examine the expression of genes thought *a priori* to be relevant to specific research questions. Given that a non-lethal tissue biopsy (e.g., gill sample) can provide such valuable information, this approach is exceedingly informative for wild animal research, particularly when combined with other technologies such as telemetry to tackle fundamental (e.g., Miller et al. 2011) or applied research questions (e.g., catch-and-release fisheries, Donaldson et al. 2008; hydropower issues, Hasler et al. 2009). In terms of gene expression indices, Jun B emerged as the most informative stress indicator due to its rapid response and definitive recovery pattern for both species. Evidence now exists that Jun B responds to a range of stressors, including low-water and confinement stress (Momoda et al. 2007), acute temperature (Lewis et al. 2010), and chronic temperature and survival (Jeffries et al. *in press*). The time course of Jun B response, where peak expression can occur several hours after the stressor, points to this gene's role in the recovery process although

it is unclear whether that role is linked with arrested cell growth, apoptosis, or even cell proliferation (Piechaczyk and Farràs 2008). Investigating Cyto C, perhaps in conjunction with Jun B, may also be a valuable biomarker for exercise stress and recovery for pink salmon. Previous research suggests that NUPR1 is a sensitive indicator of handling stress (Momoda et al. 2007) and although no statistically significant changes over time were detected in this study, this gene did follow a similar general pattern to Jun B, and is worth investigating in future studies. Regardless, these genes should be measured in conjunction with well-studied variables in the plasma (e.g., cortisol), to not only lend more confidence to results, but also to identify correlations and a better understanding of the expression patterns of these genes.

5.5 Conclusions

The responses and recoveries of two Pacific salmon species were followed after exercise stress and air exposure, and were shown to conform to the generalized stress response of fishes. Many similarities existed between both species, but the magnitude of response differed, perhaps as a consequence of different reproductive maturity states. I suggest that the more rapid and stronger stress response of pink salmon, as indicated by the accentuated plasma cortisol and lactate, osmoregulatory disturbances, and higher expression of Jun B and Cyto C, is directly related to reproductively less advanced state compared with sockeye salmon, although an inherent species difference remains a possibility. The plasma stress response, but not the cellular response for both species, was generally higher for females compared with males. Recovery was completed in both species by 24 h, which provides important context for developing facilitated recovery methods (see chapter 6).

Table 6. Primer sequences for housekeeping genes and genes of interest used in RT-QPCR.

Gene type	Gene Name	Gene symbol	Primer sequence (‘5-‘3)
Housekeeping genes	Bone Morphogenetic Protein 4	BMP4	F-TTGCCCATAGTCAGTGTTAGCG R-GTGCCATCTCCATGCTCTACC
	Si:dkey-78d16.1 protein	78d16.1	F-AAAGGTCCCACGCTCCAAAC R-ACACACCCATCTGTCTCATCACC
Genes of interest	Cytochrome C	Cyto C	F-CGAGCGTGCAGATCTTATAGC R-CTTCTCCGCTGAACAGTTGATG
	Heat Shock Protein 90	Hsp90	F-TGGGCTACATGGCTGCCAAG R-TCCAAGGTGAACCCAGAGGAC
	Transcription Factor Jun B	Jun B	F-CTACACGCACAGCGATATTCG R-TCGTCGCTGCTCTGCATGT
	Nuclear Protein1	NUPR1	F-GACAAATCGGACGGCTAATCCT R-CTGCCTGCCCATTTGGTTTT

Table 7. Rotated factor loadings and final communalities for factor analysis of log10-transformed plasma and gill gene response variables for adult pink and sockeye salmon.

	Rotated Factor #				Final communality estimates
Response variable	**1**	**2**	**3**	**4**	
Eigenvalue	*3.198*	*2.688*	*1.485*	*1.291*	
Gill Cyto C	**0.918**	-0.039	0.150	-0.032	0.869
Gill Hsp90	**0.881**	-0.163	-0.018	-0.094	0.811
Gill Jun B	**0.635**	0.091	0.320	-0.071	0.519
Gill NUPR1	**0.584**	-0.141	-0.123	0.118	0.391
Plasma Glucose ($mmol \cdot L^{-1}$)	0.132	0.297	0.182	-0.195	0.177
Plasma Lactate ($mmol \cdot L^{-1}$)	0.088	0.319	**0.812**	-0.163	0.794
Plasma Chloride ($mmol \cdot L^{-1}$)	-0.182	**0.914**	-0.017	0.197	0.908
Plasma Sodium ($mmol \cdot L^{-1}$)	-0.220	**0.644**	0.155	-0.170	0.516
Plasma Potassium ($mmol \cdot L^{-1}$)	-0.193	-0.224	0.186	0.061	0.126
Plasma Osmolality ($mOsm \cdot kg^{-1}$)	-0.060	**0.955**	0.139	0.143	0.956
Plasma Cortisol ($ng \cdot mL^{-1}$)	0.147	-0.041	**0.734**	**0.662**	0.999
Plasma Testosterone ($ng \cdot mL^{-1}$)	-0.033	0.052	-0.042	**0.638**	0.412
Cumulative variance explained (%)	*26.647*	*49.045*	*61.417*	*72.174*	

Note: Variables with factor loadings ≥ ±0.5 are shown in bold. Response variables were log_{10} transformed prior to Principal Components Analysis.

Table 8. Effects tests from ANOVAs that were significant for the whole model comparing rotated factor loadings of log10-transformed plasma and gill gene response variables for adult pink and sockeye salmon.

Rotated Factor #	Source	D.F.	SS	F Ratio	*P*-value
	Time	**6**	**16.843**	**4.629**	**< 0.001**
	Sex	1	0.491	0.810	0.370
1	**Species**	**1**	**46.264**	**76.294**	**< 0.001**
	Species*Time	6	6.141	1.688	0.127
	Species*Sex	1	0.366	0.604	0.438
	Time	**6**	**26.650**	**37.628**	**< 0.001**
	Sex	**1**	**1.300**	**11.012**	**0.001**
2	**Species**	**1**	**6.069**	**51.412**	**<0.001**
	Species*Time	**6**	**6.067**	**8.567**	**<0.001**
	Species*Sex	1	0.366	3.102	0.080
	Time	**6**	**100.026**	**92.627**	**<0.001**
	Sex	**1**	**10.340**	**57.449**	**<0.001**
3	**Species**	**1**	**2.971**	**16.506**	**<0.001**
	Species*Time	**6**	**10.160**	**9.408**	**<0.001**
	Species*Sex	**1**	**1.658**	**9.215**	**0.003**
	Time	**6**	**5.885**	**3.811**	**0.001**
	Sex	**1**	**80.511**	**312.793**	**<0.001**
4	Species	1	0.281	1.093	0.298
	Species*Time	**6**	**7.688**	**4.978**	**<0.001**
	Species*Sex	1	0.018	0.069	0.793

Note: Bold values denote significant effects tests at $\alpha = 0.013$. Response variables were $\log_{10}$ transformed prior to Principal Components Analysis.

Table 9. Mean ± SE for adult pink and sockeye salmon plasma response variables following exercise stress.

Response variable	Sex	Pink							Sockeye						
		Control	0	0.5	1	2	4	24	Control	0	0.5	1	2	4	24
Plasma Glucose ($mmol \cdot L^{-1}$)	F	**5.74 ± 0.87^{AB}**	**5.74 ± 0.40^{AB}**	**8.15 ± 1.79^{A}**	**4.94 ± 0.37^{B}**	**5.39 ± 0.20^{AB}**	**5.10 ± 0.40^{B}**	**4.65 ± 0.32^{B}**	6.02 ± 0.84	5.38 ± 0.71	5.33 ± 0.59	5.52 ± 0.38	5.40 ± 0.15	6.44 ± 0.74	5.58 ± 0.21
	M	5.66 ± 0.34	5.58 ± 0.76	7.08 ± 0.20	6.17 ± 0.30	5.26 ± 0.36	6.47 ± 0.35	5.19 ± 1.07	5.41 ± 0.31	5.57 ± 0.71	6.22 ± 0.25	6.16 ± 0.28	6.26 ± 0.25	6.85 ± 0.51	5.18 ± 0.45
Plasma Lactate ($mmol \cdot L^{-1}$)	F	**1.12 ± 0.02^{A}**	**6.18 ± 0.58^{B}**	**18.68 ± 0.65^{C}**	**14.51 ± 1.12^{C}**	**10.68 ± 1.13^{BC}**	**6.94 ± 0.60^{B}**	**1.11 ± 0.34^{A}**	**1.60 ± 0.31^{Z}**	**4.67 ± 0.78^{Y}**	**8.62 ± 0.97^{X}**	**12.67 ± 0.97^{X}**	**7.21 ± 0.55^{YX}**	**4.64 ± 1.10^{Y}**	**1.31 ± 0.13^{Z}**
	M	**0.78 ± 0.09^{A}**	**5.99 ± 1.22^{B}**	**14.12 ± 1.04^{B}**	**15.30 ± 1.27^{B}**	**12.39 ± 1.71^{B}**	**9.55 ± 1.74^{B}**	**3.82 ± 2.96^{A}**	**0.75 ± 0.11^{Z}**	**2.68 ± 0.37^{Y}**	**9.00 ± 0.80^{X}**	**8.36 ± 0.76^{X}**	**7.19 ± 0.48^{X}**	**3.27 ± 0.64^{Y}**	**0.72 ± 0.10^{Z}**
Plasma Chloride ($mmol \cdot L^{-1}$)	F	**141.48 ± 3.98^{A}**	**137.01 ± 1.45^{A}**	**136.47 ± 0.56^{A}**	**132.74 ± 0.68^{AB}**	**121.10 ± 1.69^{C}**	**121.15 ± 1.16^{C}**	**130.34 ± 1.66^{B}**	116.97 ± 2.15	124.41 ± 2.16	120.05 ± 9.53	124.78 ± 2.05	125.15 ± 1.03	119.26 ± 1.81	122.00 ± 1.99
	M	**133.30 ± 3.24^{AB}**	**134.24 ± 1.27^{AB}**	**135.32 ± 1.01^{A}**	**131.26 ± 2.64^{AB}**	**121.78 ± 1.67^{C}**	**120.45 ± 2.07^{C}**	**126.22 ± 0.85^{BC}**	**118.59 ± 1.62^{ZY}**	**123.01 ± 1.25^{ZY}**	**126.80 ± 1.27^{Z}**	**125.13 ± 0.79^{ZY}**	**119.94 ± 1.00^{ZY}**	**114.94 ± 1.15^{ZY}**	**113.38 ± 6.37^{Y}**
Plasma Sodium ($mmol \cdot L^{-1}$)	F	**150.70 ± 3.23^{AB}**	**165.05 ± 1.83^{BC}**	**176.12 ± 3.25^{C}**	**163.71 ± 0.95^{AB}**	**153.39 ± 1.81^{A}**	**162.30 ± 2.75^{AB}**	**165.43 ± 3.71^{BC}**	**143.63 ± 1.73^{Z}**	**135.47 ± 2.79^{Z}**	**130.65 ± 10.46^{Z}**	**135.78 ± 2.65^{Z}**	**144.37 ± 4.00^{Z}**	**147.01 ± 3.29^{Z}**	**96.44 ± 7.54^{Y}**
	M	**149.83 ± 3.38^{A}**	**165.13 ± 2.68^{BC}**	**179.96 ± 4.19^{D}**	**165.63 ± 1.49^{BC}**	**154.63 ± 2.44^{AB}**	**168.38 ± 3.18^{CD}**	**165.92 ± 2.54^{BC}**	**146.54 ± 2.45^{ZY}**	**155.10 ± 3.41^{Z}**	**152.34 ± 2.40^{Z}**	**152.30 ± 1.14^{Z}**	**146.72 ± 2.25^{ZY}**	**141.17 ± 1.16^{ZY}**	**132.79 ± 7.02^{Y}**

Response variable	Sex	Pink							Sockeye						
		Control	0	0.5	1	2	4	24	Control	0	0.5	1	2	4	24
Plasma Potassium ($mmol \cdot L^{-1}$)	F	**2.47 ± 0.56^{AB}**	**3.53 ± 0.51 ^{AB}**	**2.13 ± 0.39^{B}**	**2.55 ± 0.35^{B}**	**5.18 ± 0.40^{A}**	**3.61 ± 0.26^{AB}**	**3.28 ± 1.09^{AB}**	2.60 ± 0.06	1.95 ± 0.39	2.45 ± 0.87	1.85 ± 0.14	2.38 ± 0.38	3.25 ± 0.12	2.42 ± 0.11
	M	2.22 ± 0.33	2.97 ± 0.64	2.20 ± 0.26	3.01 ± 0.21	5.07 ± 0.54	3.58 ± 0.71	2.61 ± 0.15	**2.06 ± 0.38^{ZY}**	**1.20 ± 0.10^{YX}**	**0.67 ± 0.11^{X}**	**1.92 ± 0.36^{ZY}**	**2.74 ± 0.32^{Z}**	**2.63 ± 0.34 ^{Z}**	**2.74 ± 0.27 ^{Z}**
Plasma Osmolality ($mOsm \cdot kg^{-1}$)	F	**309.88 ± 5.63^{AB}**	**324.33 ± 2.63^{B}**	**346.11 ± 4.15^{C}**	**322.29 ± 2.82^{B}**	**302.04 ± 2.46^{A}**	**299.47 ± 2.71 ^{A}**	**302.08 ± 2.28 ^{Z}**	**285.31 ± 2.64^{YZ}**	**307.81 ± 4.45 ^{Z}**	**295.72 ± 22.15 ^{Z}**	**313.34 ± 3.62 ^{Z}**	**303.03 ± 1.56 ^{YZ}**	**293.00 ± 2.93 ^{YZ}**	**292.78 ± 3.44 ^{Y}**
	M	**307.44 ± 5.33^{AB}**	**314.92 ± 5.13^{AB}**	**333.86 ± 2.61^{C}**	**319.43 ± 4.91^{BC}**	**297.00 ± 5.64^{A}**	**297.47 ± 3.65 ^{A}**	**299.79 ± 2.08 ^{A}**	292.34 ± 2.76	307.69 ± 2.53	312.06 ± 4.94	312.06 ± 1.42	305.56 ± 3.09	294.31 4.36	275.22 ± 15.44
Plasma Cortisol ($ng \cdot mL^{-1}$)	F	**84.52 ± 35.16^{A}**	**299.06 ± 63.58 ^{B}**	**369.97 ± 46.02^{BC}**	**646.60 ± 98.66^{C}**	**656.37 ± 82.79^{C}**	**298.75 ± 31.29^{B}**	**99.66 ± 12.36^{A}**	**207.46 ± 21.50^{Z}**	**351.96 ± 29.70 ^{Y}**	**387.37 ± 41.53 ^{Y}**	**384.67 ± 28.25 ^{Y}**	**354.40 ± 27.53 ^{Y}**	**398.79 ± 39.24 ^{Y}**	**178.23 ± 17.80 ^{Z}**
	M	**39.14 ± 2.50^{A}**	**133.23 ± 23.00^{BCD}**	**93.99 ± 11.78^{ABC}**	**283.67 ± 51.62^{D}**	**203.73 ± 34.61^{CD}**	**137.85 ± 41.81^{BCD}**	**53.92 ± 9.33^{ZY}**	**71.56 ± 5.26 ^{ZY}**	**118.43 ± 29.17^{YX}**	**104.65 ± 6.86 ^{YX}**	**111.54 ± 8.73 ^{YX}**	**107.37 ± 13.29 ^{YX}**	**119.97 ± 11.08^{Y}**	**62.39 ± 6.26^{Z}**
Plasma Testosterone ($ng \cdot mL^{-1}$)	F	193.90 ± 114.93	183.29 ± 32.39	162.91 ± 29.72	127.35 ± 24.04	89.85 ± 20.56	102.11 ± 24.86	212.01 ± 31.37	108.78 ± 12.81	71.04 ± 16.70	96.59 ± 13.57	97.74 ± 17.79	119.13 ± 8.63	112.11 ± 10.24	104.69 ± 15.05
	M	63.28 ± 17.60	12.76 ± 4.01	17.54 ± 4.63	9.22 ± 2.57	16.39 ± 10.23	12.96 ± 5.42	62.87 ± 11.44	22.74 ± 2.55	18.47 ± 2.79	15.86 ± 1.67	25.69 ± 3.82	24.31 ± 4.13	17.52 ± 3.68	22.81 ± 4.01
Plasma Estradiol ($ng \cdot mL^{-1}$)	F	3.74 ± 1.68	4.02 ± 1.73	7.00 ± 0.99	8.37 ± 2.40	7.23 ± 1.06	4.90 ± 1.26	**4.01 ± 1.95^{Z}**	**0.54 ± 0.04^{Z}**	**0.34 ± 0.03^{Y}**	**0.45 ± 0.04^{ZY}**	**0.42 ± 0.04^{ZY}**	**0.46 ± 0.03^{ZY}**	**0.46 ± 0.02^{ZY}**	**0.43 ± 0.05^{ZY}**

Table 10. Mean ± SE for adult pink and sockeye salmon gill gene expression following exercise stress.

Response variable	Pink							Sockeye						
	Control	**0**	**0.5**	**1**	**2**	**4**	**24**	**Control**	**0**	**0.5**	**1**	**2**	**4**	**24**
Gill Cyto C	**0.51 ± 0.15^{A}**	**0.80 ± 0.10^{B}**	**0.73 ± 0.09^{AB}**	**0.78 ± ± 0.13^{B}**	**0.93 ± 0.08^{BC}**	**1.17 ± 0.18^{B}**	**0.59 ± 0.05^{A}**	0.91 ± 0.06	1.04 ± 0.08	1.09 ± 0.08	1.07 ± 0.14	1.16 ± 0.08	1.11 ± 0.10	0.86 ± 0.09
Gill Hsp90	0.60 ± 0.22	1.16 ± 0.50	0.89 ± 0.17	0.78 ± 0.19	1.05 ± 0.23	0.97 ± 0.11	0.60 ± 0.07	1.32 ± 0.17	1.70 ± 0.22	1.93 ± 0.16	1.78 ± 0.44	1.66 ± 0.17	1.81 ± 0.29	1.51 ± 0.26
Gill Jun B	**0.74 ± 0.30^{AB}**	**0.92 ± 0.07^{B}**	**1.04 ± 0.08^{B}**	**1.23 ± 0.14^{BC}**	**0.99 ± 0.10^{B}**	**1.37 ± 0.23^{C}**	**0.61 ± 0.07^{A}**	**0.83 ± 0.09^{Z}**	**1.45 ± 0.24^{Y}**	**1.31 ± 0.13^{Y}**	**1.41 ± 0.20^{Y}**	**1.51 ± 0.15^{Y}**	**1.50 ± 0.22^{Z}**	**1.03 ± 0.14^{Z}**
Gill NUPR1	0.54 ± 0.17	0.62 ± 0.08	0.67 ± 0.13	0.70 ± 0.13	0.59 ± 0.05	0.92 ± 0.13	0.64 ± 0.05	1.03 ± 0.07	0.92 ± 0.09	1.14 ± 0.18	0.77 ± 0.09	0.81 ± 0.09	1.02 ± 0.08	0.94 ± 0.12

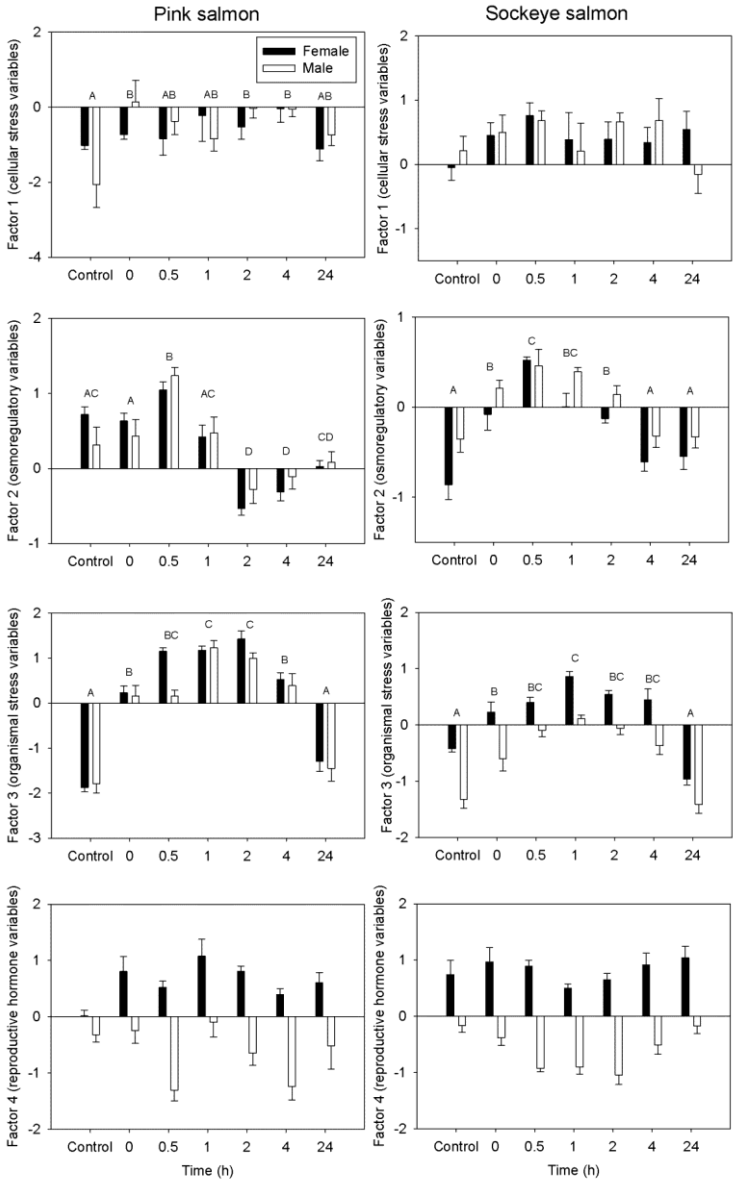

Figure 11. Means ± SE for rotated factor loadings of log10-transformed plasma and gill gene response variables for adult pink and sockeye salmon. PCAs based on plasma and gill samples. Dissimilar letters denote statistical differences from Tukey's post-hoc tests following ANOVAs.

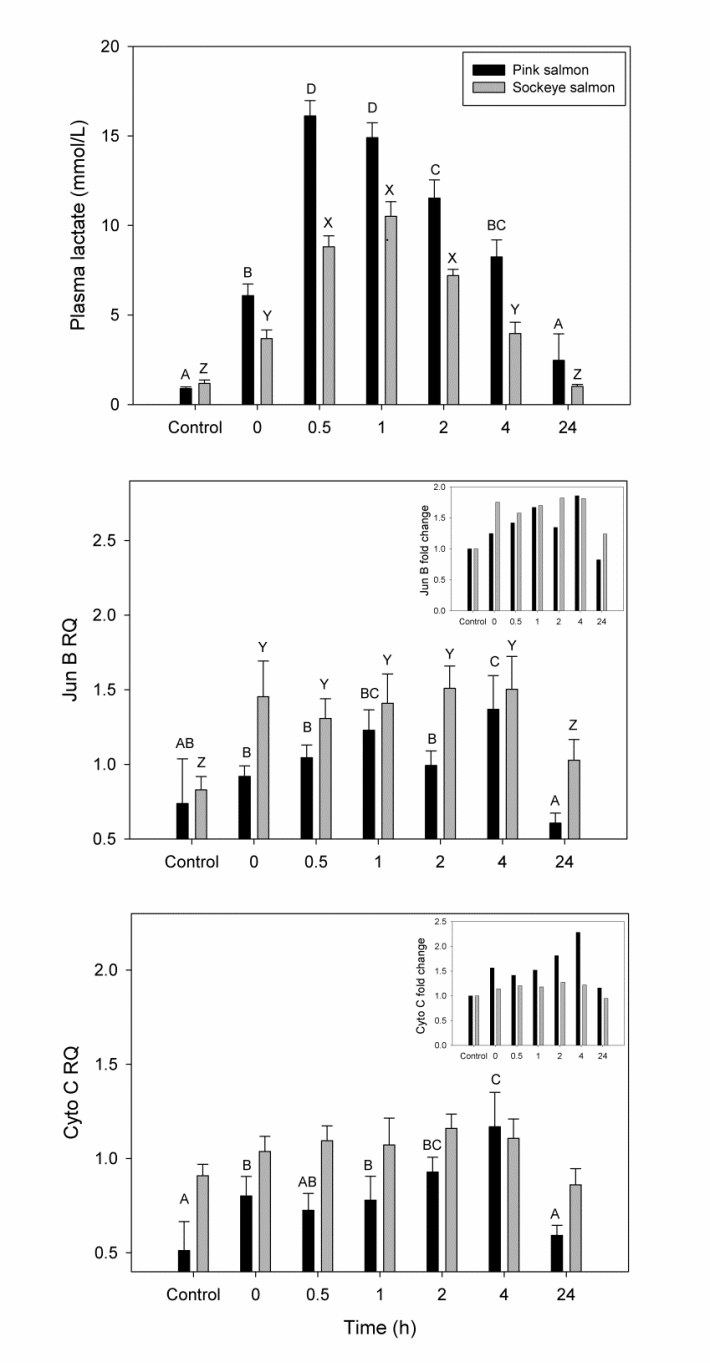

Figure 12. Means ± SE for plasma lactate, gill Jun B and gill Cyto C relative quantification and fold change (relative to control values) for adult pink and sockeye salmon. Sexes pooled. Dissimilar letters denote statistical differences from Tukey's post-hoc tests following ANOVAs.

6 EVALUATION OF A SIMPLE TECHNIQUE FOR RECOVERING FISH FROM CAPTURE STRESS: INTEGRATING PHYSIOLOGY, BIOTELEMETRY, AND HUMAN DIMENSIONS TO SOLVE A CONSERVATION PROBLEM

6.1 Introduction

Conservation physiology has emerged as a field that uses physiological tools and knowledge to inform conservation and management initiatives (Wikelski and Cooke 2006). One of the current limitations of conservation physiology is that physiological research is often disconnected from conservation practitioners and managers (Cooke and O'Connor 2010). In this chapter, I use a novel approach to bridge this gap by combining science and human dimensions research in the Pacific salmon recreational angling fishery, a highly relevant system given the precarious status of some Pacific salmon populations (Jonsson et al. 1999; Irvine et al. 2005; Gustafson et al. 2007). The physiological response of fish to capture stress is generally considered analogous to exercise stress and has been well characterized in the fish physiology literature (Milligan et al. 1996; Kieffer et al 2000). The time required to clear metabolites from the blood and restore muscle energy stores may limit subsequent performance since this recovery rate will determine the frequency of maximal performance (Milligan 1996). Stress and prolonged recovery may lead to tertiary consequences including delayed mortality (Black 1957; Wood et al. 1983; chapters 2 and 3). Prior to the work of Milligan (1996) and Milligan et al. (2000), the physiological time-course of recovery from exercise stress was thought to be long, for oxygen consumption to recover (Brett, 1964) and even longer for metabolites (Black, 1957; Turner et al. 1983; chapters 4 and 5). Milligan et al. (2000) found that when rainbow trout

recovered in water with a constant low velocity water current (i.e., 0.9 body lengths per second), complete metabolic recovery was much quicker (~ 2 h) relative to static water recovery.

The results of Milligan et al. (2000) have been adapted to facilitate physiological recovery and improve survival of coho salmon captured by various marine fisheries (Farrell et al. 2000; Farrell et al. 2001a,b). Farrell et al. (2001b) found that placing troll captured coho in a cage towed alongside the vessel promoted accelerated physiological recovery. A revival box (aka "Fraser Box") used on board a commercial gill net vessel which jetted seawater towards confined individual fish promoted rapid physiological recovery within 1-2 h, restored swimming ability and improved survival even for fish that appeared moribund at capture (Farrell et al. 2001a). The general physiological principle involved in these studies, is that recovery is facilitated by assisted gill ventilation by ramming high velocity water into the mouth and across the gills of recovering fish and/or the maintenance of steady swimming during the recovery process. Ram ventilation provides well-oxygenated water to pass over the gills, in turn resulting in greater oxygen reaching the tissues which is needed to pay back the oxygen debt incurred during the stressful fisheries encounter. These marine studies involved large vessel-based apparatus and survival was determined by observing fish in net pens for 24 h. There have been no investigations of this kind in freshwater environments or with recovery gear that is more portable and thus could be used by recreational salmon fishers, despite recent findings that angling can result in lower survival relative to fish released from other capture methods (chapters 2 and 3).

Human dimensions research and conservation social science (e.g., Mascia et al. 2003) are increasingly being used to inform policy and management actions, and in particular to help in understanding the factors that influence compliance with conservation regulations (Rudd et al.

2011a). While understanding socio-ecological dynamics has been suggested as an important part of ensuring sustainable recreational fisheries management (Post et al. 2008; Hunt et al. 2011), the viability of facilitated recovery methods has not been in examined interactively from the dual perspectives of social and natural sciences. In fact, management agencies throughout North America have typically not considered facilitated recovery methods in freshwater recreational fisheries (Pelletier et al. 2007), likely due to the limited data available on their effectiveness. If facilitated recovery were to be considered for implementation, an understanding of recreational angler perspectives on the issue of facilitated recovery gear would help fisheries managers determine whether such gear could be readily adopted by anglers.

The Fraser River sockeye salmon recreational fishery has grown in recent years (Kristianson and Strongitharm 2006) despite some small or unproductive populations being considered endangered due in part to mixed-population fishing (e.g., Cultus Lake sockeye salmon; Rand 2011). This fishery is traditionally catch-and-keep, but anglers may choose to voluntarily release fish if they are over their quota or if the fish is undesirable. The release to keep ratio can be as high as 1:2 for Fraser River sockeye salmon, which translates to high numbers when fish are migrating in high abundance (e.g., 100 000 released versus 200 000 harvested in 2010; Fisheries and Oceans Canada 2011) and post-release mortality can be relatively high compared to baseline estimates (chapters 2 and 3). While released fish may resume their migrations without delay, individuals showing signs of exhaustion at the time of release (e.g., loss of equilibrium, inability to swim against current, and/or physiologically compromised) may potentially drift downriver and be prone to capture by predators or secondary fisheries capture (e.g, subsequent capture by a gill net). In such cases, specially designed

recovery gear that enables high velocity water to pass over the gills could assist physiological recovery and allow fish to resume migration more quickly and effectively.

Recovery bags are structured, cylindrical hypolon bags with mesh ends that are submerged in high velocity water to enable flow through the bag and over the fish's mouth and gills resulting in ram ventilation akin to the Fraser box tested by Farrell et al. (2001a; Figure 13A). Recovery gear in general requires relatively high water velocities to be effective but recovery bags have the added benefit of being portable and not requiring an external power source, which is ideal for recreational anglers and shore-based net fisheries, such as native fisheries.

With chapters 2 and 3 finding that fisheries capture stressors influence the stress response and survival and chapters 4 and 5 identifying long durations required for recovery, the objective of this chapter is to test the hypothesis that facilitated recovery methods can influence the rate of physiological recovery and the likelihood of survival. This chapter explores the predictions that the recovery of primary and secondary stress indices can be expedited and that survival can be improved using facilitated recovery methods. I tested the utility of recovery bags for facilitating the recovery of Pacific salmon following capture in freshwater using three case studies: First, I assessed the effectiveness of recovery bags on the physiological response of adult pink salmon following a capture and release simulation. Second, I used biotelemetry to determine if recovery bags influenced the survival of sockeye salmon released by recreational anglers. Third, I surveyed salmon anglers to assess the potential for implementing recovery techniques in the Pacific salmon recreational fishery. These three case studies were integrated to discuss the

application of portable recovery gear in the management and conservation of Pacific salmon in the context of freshwater recreational salmon fisheries.

6.2 Methods

6.2.1 Case study 1: Physiology experiment

6.2.1.1 Study site and animals

Experiments were conducted at Fisheries and Oceans Canada's Weaver Creek artificial Spawning Channel (49º32°N, 121º88°W), British Columbia, Canada, draining into the Harrison River, a lower Fraser River tributary. Water temperatures during the study ranged between 10-12 ºC. Female pink salmon only were used in this experiment because sex-specific differences are known to occur in certain physiological variables (e.g., plasma cortisol, chapters 4 and 5). Fish were selected upon first arrival at the spawning channel. Fish were showing signs of secondary sexual characteristics but none were reproductively mature (i.e., "ripe").

6.2.1.2 Experimental design

Individuals were randomly assigned to controls or no, low, or high velocity treatments. Individuals were only biopsied (i.e., blood sampled) once. Controls (n = 8) were immediately placed into individual holding boxes (L x W x D = 93.7 x 54.0 x 47.3 cm) and held for 24 h before being biopsied. Each holding box was supplied with fresh water (0.63 L/s) pumped from the spawning channel. Fish from the no, low, or high velocity treatments were individually transferred into a donut shaped exercise tank (diameter 150 cm, water depth 40 cm) supplied with fresh water pumped from the spawning channel where they were manually chased for a

period of 3 min (coaxed by three experimenters positioned around the exercise tank to burst swim for 3 min [Black 1958, Wood 1991] to simulate an angling capture event), then given 1 min air exposure, simulating gear removal or photography. This treatment resulted in fish being visibly exhausted in that they had difficulty maintaining equilibrium and being unable to engage in further burst swimming.

The purpose of the recovery bags (Figure 13A) is to provide an environment where the exhausted fish can be oriented into the flow of water without risk of drifting down river and being susceptible to injury, predation, or fisheries capture. Recovery bags are particularly relevant where fish are exhausted to the point where they have lost equilibrium or are unable to swim to locations that are free from predators or locations of optimal flow conditions. In this chapter, fish were visibly exhausted following the exercise and air exposure treatments, typically unable to engage in burst swimming and/or having difficulty maintaining equilibrium. Fish assigned to the low (n = 75) or high velocity groups (n = 75) were transferred to one of three recovery bag types. The recovery bags were fine (1 cm mesh size) or coarse mesh (5 cm mesh size) made from hypalon and cylindrical in shape (L =100 cm, diameter = 20 cm), or simple draw-string mesh sacks (L x W = 80 x 40 cm). Bags were positioned in the natural water current of the spawning channel. The low velocity group was placed in a location of 0.19 $m \cdot s^{-1}$ water current and the high velocity group was placed in a location of 0.43 $m \cdot s^{-1}$. Velocities inside the fine and coarse mesh bags in the low velocity group were 0.15 and 0.17 $m \cdot s^{-1}$, respectively, and within the fine and coarse mesh bags in the high velocity group were 0.35 and 0.39 $m \cdot s^{-1}$, respectively (mean rates). For comparison, mean current velocity has been measured at 0.4 $m \cdot s^{-1}$ (Quinn 1999) and during this study, velocities measured in areas of the spawning channel where

fish typically maintained position averaged 0.18 $m \cdot s^{-1}$. Fish assigned to the no velocity group (n = 22) were transferred into individual holding boxes (as described above) and single biopsies were taken after 15, 30 or 60 min.

Single biopsies (i.e., individuals were sampled only once) were taken after 15, 30 or 60 min of recovery. For biopsy, individuals were collected from bags, placed supine in a water-filled V-shaped foam-padded sampling trough (Cooke et al. 2005) and biopsied immediately. The duration of the entire procedure was < 2 min. Fork length (FL) was also measured. Each biopsy collected 2.5 mL blood by caudal puncture using a 3.8 cm, 21-gauge needle and a heparinised vacutainer (lithium heparin, 3 mL, Becton-Dickson, NJ), which was then stored in ice-chilled water for ~ 1 h until subsequent processing.

6.2.1.3 Physiological assays

Methods are similar to those described in chapter 2. Briefly, the chilled ~ 2.5 mL blood sample was centrifuged at 7,000 x g for 3 min and plasma was stored in liquid nitrogen prior to being frozen at –80 °C until analysis. Plasma was subsequently analysed for cortisol (Neogen ELISA with Molecular Devices Spectramax 240pc plate reader), lactate, glucose (YSI 2300 stat plus analyser), osmolality (Advanced Instruments 3320 freezing point osmometer), chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Parmer, model 410 single channel flame photometer; Farrell et al. 2001b).

6.2.1.4 Statistical analysis

Normality was assessed using Shapiro-Wilk tests and homogeneity of variance was assessed using Levene's test, and variables were $\log_{10}$-transformed to reduce heteroscedasticity

where necessary, but all data are presented as non-transformed values. Three-way multivariate analysis of variance (MANOVA) was used to test for relationships among each of the physiological variables with recovery period (15, 30, 60 min), water velocity (no, low, high) and bag type (fine mesh, coarse mesh, mesh sack), as well as their interactions. Subsequent one-way ANOVAs were used to test for differences among groups at each recovery period and Bonferroni adjustments were made, resulting in $\alpha = 0.007$. Tukey's post-hoc tests were conducted on one-way ANOVA results ($\alpha = 0.05$). Statistical analyses were performed in JMP v 9.0 (SAS Institute Inc., Cary, N.C.).

6.2.2 Case study 2: Biotelemetry experiment

6.2.2.1 Study site and experimental design

Experimental procedures were conducted on male and female sockeye salmon at the Fraser River at Grassy Bar, near Chilliwack, British Columbia, Canada between August 9th and 26th, 2010 (Figure 13B). Treatment groups were established as follows: 1) angling, 2) angling + 1 min air exposure, 3) angling + recovery, 4) angling + 1 min air exposure + recovery, and 5) beach seine. Volunteer anglers captured sockeye salmon using standard bottom-bouncing gear from either shore or boats anchored near shore (as described in chapter 2). Capture durations ranged between 1 and 5 min. Once landed, hooks were removed and individuals were randomly assigned to a treatment. Fish in the angling only treatment were released immediately while those in the angling and air exposure treatment were given a 1 min air exposure by holding the fish by hand out of the water, and then released to resume their migrations.

For the angling recovery groups, fish were likewise either assigned to no air exposure or air exposure. Individuals were then placed in mesh-ended hypalon bags for a 15 min recovery period. The mean velocity in this area was 0.11 $m \cdot s^{-1}$, which is closer to my 'low velocity' treatment from first case study rather than the 'high velocity' treatment. However, this was the highest and most consistent water velocity available at the study site. Bags were positioned to ensure the velocity was directed through the bag and fish were oriented in the bag anteriorly to direct the flow of river water over the fish's mouth and gills. Following the recovery period, individuals were guided out of the bag, with minimal physical touching by technicians, and back into the river to resume their migrations. For the beach seine capture group, fish were captured using a 64 m x 7.5 m x 5 cm mesh beach seine net. Technicians continually monitored the catches from both capture methods and recorded qualitative information about angling durations, air exposure durations, injuries (e.g., hooking location and degree of bleeding), and general condition descriptions.

6.2.2.2 Telemetry methods and determination of survival

A total of 173 sockeye salmon were radio-tagged for this study. Individuals from each treatment were radio-tagged and released in equal proportions during the study period, as in chapter 2. Established protocols for the gastric tagging of sockeye salmon were used, where tags were inserted through the mouth and into the stomach of each individual since they do not feed during their migrations and their stomachs close around and secure the tag following placement (Cooke et al. 2005). Coded radio transmitters (MCFT-3A-3V, Lotek Wireless Inc., Newmarket, ON or Pisces 5, Sigma-Eight Inc., Newmarket, ON) were used. Coded transmitters enabled the identification of individual fish as they were detected at receiver stations. For all fish, a scale

sample and a 0.5 g adipose fin clip were taken for identification of population complexes, and FL measurements were made and a numbered cinch marker tag (Floy Tag and Mfg., Inc., Seattle, WA, USA) was attached through the dorsal musculature. Procedures were always completed in ≤ 2.5 min.

Twenty eight radio-telemetry receiver stations (SRX400 or SRX400A, Lotek Wireless Inc., NewMarket, ON) with 3-element or 4-element Yagi antennas (Maxrad Inc., Hanover Park, IL, or Grant Systems Engineering Inc., King City, ON) were strategically positioned throughout the Fraser River watershed (Donaldson et al. 2010a; chapter 2). Due to their high fidelity to natal spawning areas, DNA stock identification enabled us to determine the natal sub-watershed that each individual was migrating to. Arrival at natal sub-watershed was determined by detection with fixed station telemetry receivers located in tributaries en-route to spawning grounds. Failure of an individual to reach a subsequent receiver location was termed en-route mortality (Donaldson et al. 2010a; chapter 2). Individuals that were reported as fisheries harvest were excluded from this study.

6.2.2.3 Statistical analyses

One-way ANOVA was used to test for differences in FL among treatment groups. Pearson chi-square analysis was used to test for differences in post-release survivorship among treatment groups. Because sex could not be determined visually (i.e., fish were not showing secondary sexual characteristics), sex could not be included as a factor in analysis. All values presented here represent means ± S.E., unless otherwise noted. Statistical analyses were performed in JMP v 9.0 (SAS Institute Inc., Cary, N.C.).

6.2.3 Case study 3: Angler survey

6.2.3.1 Experimental design

A face-to-face human dimensions survey was conducted to gain insight into angler's opinions on the use of recovery bags in a recreational fishery. The objective was to see if underlying patterns of pro- or anti-recovery gear sentiment could be identified among the anglers in the sample with themes coded from semi-structured interview data. Such patterns of sentiment could signal the need to take segment-specific approaches to fisheries management and recovery gear education programs. A mixed-method research approach (i.e., both quantitative ratings data and open-end responses were collected) used semi-structured interviews lasting 10 to 50 min (Creswell 2009) in which key questions prompted structured conversations between interviewer and interviewee. The angler survey was exploratory in nature, testing a variety of questions relevant to anglers' willingness to adopt the use of recovery gear. Questions were designed to seek opinions on using recovery bags to help released salmon, whether or not the use of recovery bags is necessary, and whether or not the interviewee would use the bags either voluntarily or by mandate (Table 11).

Anglers were approached at random at fishing sites and boat launches along the lower Fraser River between July 30th and August 27th, 2010. Sixty-seven anglers (aged 18+) participated in the survey. With respondents' consent, responses were noted and audio-recorded and subsequently transcribed by the interviewer. Responses were coded and used as indicator variables for latent class cluster analysis modeling. During the interview period, anglers were targeting sockeye salmon, but the survey was designed to ask questions in general about facilitated recovery for Pacific salmon and did not focus on one specific species.

6.2.3.2 Statistical analysis

Latent-class (LC) cluster analysis (Hagenaars and McCutcheon 2002; Magidson and Vermunt 2004) can be used to statistically identify latent class membership using information from a set of observed variables (indicators) that imperfectly measure underlying true class membership (e.g., Morey et al. 2006; Ward et al. 2008; Rudd et al. 2011b). In the LC models, indicator variables, based on categorization of qualitative responses to four sets of questions about recovery bag use, were used to estimate a latent variable, 'supporters of recovery bag program implementation'. The LC models based on qualitative attitudinal data allowed us to characterize LC clusters whose members have statistically homogeneous beliefs or preferences regarding recovery bag use within clusters, but maximal difference between clusters. I used the Akaike Information Criterion (AIC) to identify the most parsimonious LC model by choosing a final LC model with the number of clusters than minimized AIC. Local independence between indicators was tested using bivariate residual (BVR) statistics (e.g., Rudd et al. 2011b). Significant BVRs ($\square^2 > 3.84$, 1 d.f., $p < 0.05$) signify local dependence between variables (Hagenaars 1988) and functionally mean that 2 or more indicators provide redundant information for the clustering process. Latent GOLD 4.0 (Vermunt and Magidson 2005) statistical software was used for all survey analyses.

6.3 Results

6.3.1 Case study 1: Physiology experiment

MANOVA revealed a significant whole model for $\log_{10}$-transformed plasma physiological variables and recovery period, velocity, and bag type (Wilk's lambda= 0.124;

$F_{119,785.87} = 2.497$; $p < 0.001$). Significant effects were found for recovery period (Wilk's lambda= 0.581; $F_{14,238}= 5.308$; $p < 0.001$) and velocity ($F_{7, 119}= 11.163$; $p < 0.001$) but not bag type (Wilk's lambda= 0.861; $F_{14,238}= 1.323$; $p = 0.194$) or its interactions. Mean FL was 50.2 ± cm and was similar among treatments (Two-way ANOVA with recovery period and water velocity as effects; whole model $F_{8, 209} = 1.45$; $p = 0.179$).

High and low water velocity with a recovery bag was more effective than no water velocity in mitigating simulated capture stress and was influenced by recovery period. For the 15-min recovery period, one-way ANOVA testing for differences among velocity groups revealed significant differences for plasma lactate ($F_{3,63}= 242.600$; $p < 0.001$), sodium ($F_{3,63}= 6.309$; $p < 0.001$), chloride ($F_{3,63}= 7.173$; $p < 0.001$), potassium ($F_{3,63}= 16.304$; $p < 0.001$), osmolality ($F_{3,63}= 62.144$; $p < 0.001$) and cortisol ($F_{3,63}= 26.925$; $p < 0.001$), but not glucose ($p > 0.05$; Figure 14). Similarly for 30-min recovery period, there was a significant effect of velocity on plasma lactate ($F_{3,61}= 253.972$; $p < 0.001$), sodium ($F_{3,61}= 5.785$; $p < 0.001$), potassium ($F_{3,61}= 19.298$; $p < 0.001$), osmolality ($F_{3,61}= 35.759$; $p < 0.001$) and cortisol ($F_{3,61}= 22.514$; $p < 0.001$), but not for either glucose or chloride (both $p > 0.05$). For the 60-min recovery period, there were significant differences among no, low, and high velocity types in plasma lactate ($F_{3,62}= 258.412$; $p < 0.001$), potassium ($F_{3,62}= 6.944$; $p < 0.001$), osmolality ($F_{3,62}= 17.105$; $p < 0.001$) and cortisol ($F_{3,62}= 27.385$; $p < 0.001$), but not for either glucose, sodium, and chloride (all $p > 0.05$). Thus, high velocity recovery emerged as the most effective treatment, with reduced plasma cortisol concentrations relative to the low velocity group at 15 and 60 min post-capture, and similar plasma sodium and chloride concentrations as control values at all recovery periods. For most

recovery periods measured, plasma glucose and potassium concentrations did not differ from control values.

6.3.2 Case study 2: Biotelemetry experiment

Significant differences were found for survival to natal subwatersheds among treatment groups (χ^2= 15.69; d.f. = 4; p = 0.004) but when the beach seine group was removed and only the four angling groups compared, differences were not observed (χ^2= 6.66; d.f. = 3; p = 0.084), suggesting that the beach seine group was driving differences (Figure 15). The beach seine group had the highest survival (57.5 %), followed by angling + air exposure + recovery (50.0 %), angling + no recovery (30.8 %), angling + air exposure + no recovery (28.6 %), and angling + recovery (16.7 %). FL did not differ among treatment groups ($F_{4, 168}$ = 0.514; p = 0.726).

6.3.3 Case study 3: Angler survey

Responses to the question (What do you think about the idea of a revival bag to help incidental [salmon] catches?) are shown in Figure 16A. I found 39.7 % provided *negative but legitimate* responses: they were unsupportive of recovery bags as a catch-and-release tool but for potentially legitimate reasons (e.g., they questioned their effectiveness or thought they were unnecessary when fish were handled properly). Interestingly, 29.4 % were conditionally supportive of the concept (e.g., recovery bags might be used if mandated, if they were shown to be useful, for beginners only). I found 23.5 % were *fully supportive* of the use of recovery bags as a tool to help reduce catch-and-release mortality of salmon. When asked explicitly, 66.7 % of respondents did not think there was a need for recovery bags. A total of 66.2% responded ‘yes’, that they would use a recovery bag voluntarily. When asked (Suppose using a recovery bag was

mandatory for reviving fish before releasing it – what are your thoughts on that?), responses were split among negative protest responses (10.8%), negative but legitimate responses (7.7%), supportive due to compliance (with mandatory use regulations) responses (35.4%), conditionally supportive responses (10.8%), and fully supportive responses due to the benefits for released salmon (30.8%; Figure 16B).

A two-class model of indicator questions minimized AIC and there were no significant BVRs, suggesting that all anglers cleaved into two clusters with internally homogeneous perspectives regarding recovery bag use (Table 11). Wald tests indicated that the coefficients for only two indicator questions were jointly significantly different than zero: 'angler thoughts on idea of recovery bag' (Wald=10.14, p<=0.01), and 'voluntary use of bag', (Wald=6.07, p<=0.05). The first cluster, which comprised 53.8% of the sample, included respondents who demonstrated positive attitudes towards recovery bags and greater support for mandatory implementation and use of bags. The remaining 46.2% did not see a need for a recovery bag, though almost half of respondents would use it on a voluntary basis if shown to increase salmon survival.

6.4 Discussion

6.4.1 Case study 1: Facilitated physiological recovery

The capture simulation resulted in pink salmon mounting a major stress response, typical of exercise stress (Milligan, 1996; chapter 5). The combined exercise and air exposure treatment resulted in fish generally showing signs of fatigue, including equilibrium loss and inability to burst swim. As a consequence many fish were unable to engage in normal swimming post-

treatment and likely would have drifted down-stream of the study area if they were not placed immediately in recovery bags. At 15 min, the high velocity resulted in reduced plasma cortisol concentrations relative to the non-recovery group, a result consistent with previous rainbow trout (Milligan 2000) and coho salmon (Farrell et al. 2001a;b) studies. Recovery using high water velocity was the only treatment where sodium and chloride consistently remained unchanged, further supporting the superiority of this recovery method. This is an important result for anadromous fish that had recently undergone a shift in osmoregulatory physiology upon transition from marine to freshwater environments. Impaired osmoregulatory function has been linked with the initiation of rapid senescence and premature death in sockeye salmon (Hruska et al. 2010), particularly for plasma chloride, which can correlate strongly with longevity for this species (Jeffries et al. 2011). Plasma potassium did not differ between control and recovery treatments for the 15- and 30-min recovery periods. Exercise can increase plasma potassium as this ion is lost from muscle (Sejersted and Sjogaard 2000), but I found that plasma potassium was unchanged. Likewise, plasma glucose was the same among groups and during recovery, and fell within the range measured in adult coho salmon captured by dip net from hatchery raceways (5-6 $mmol \cdot L^{-1}$, chapter 4) and sockeye salmon captured by hook-and-line or net (6-7 $mmol \cdot L^{-1}$, chapter 2), both in freshwater. These results suggest that facilitated recovery was generally effective at reducing the cortisol response and maintaining metabolic state and ion-osmoregulatory balance.

Despite a positive effect of recovery bags for some parameters measured, recovery was incomplete to the extent observed for rainbow trout in the lab by Milligan et al. (2000). Likewise, Farrell et al. (2001b) found that adult coho salmon placed in Fraser recovery boxes for

1 or 2 h had a partial recovery of muscle metabolites, but plasma metabolites and indices of stress and ion/osmoregulatory balance did not fully recover. Increased plasma osmolality and lactate as observed in my study are typical of post-exercise, due to a decrease in muscle and blood pH caused by lactic acid dissociation (Wang et al. 1994), which in turn disrupts ion-osmoregulatory balance as water shifts from blood to muscle (Wood, 1991). Plasma cortisol, even in my most effective high velocity treatment, was still 2.5-fold higher than controls, but still much lower than my no velocity group and is lower than for coho salmon following a 1 or 2 h recovery in a Fraser box (i.e., 380 – 1270 $ng \cdot mL^{-1}$ (Farrell et al. 2001b). Another challenge of working with a wild, senescing species is that plasma cortisol tends to be higher in the final stages of reproductive maturation and can vary greatly by sex and maturation, with females typically having higher circulating values (Sandblom et al. 2009; chapters 4 and 5).

6.4.2 Case study 2: Facilitated recovery and survival to reach spawning areas

Aside from the beach seine only treatment, which was included for reference, the air exposed recovery treatment represents the highest survival of the angling groups. However, this group was not significantly different from the other angling groups once the beach seine group was removed from the analysis. Nevertheless, the recovery bag treatment resulted in an over 20 % higher survival relative to immediately released fish for the air exposure group, suggesting that this method may benefit fish that have been previously exposed to air. The nearly 2-fold decrease in survival for the not air exposed recovery bag treatment suggests that care must be exercised when determining which fish require facilitated recovery and which instead benefit from immediate release. This finding lends further support to the recommendations of Farrell et al. (2001b) for the immediate release of individuals in vigorous condition (e.g., capable of

maintaining equilibrium and burst swimming) or perhaps individuals that underwent less stressful handling (i.e., short air exposure duration). The high mortality of fish 'recovered' in a net pen for 24 h in chapter 2 provides further evidence to support the idea that holding fish and facilitated recovery may not help and can in fact be deleterious to migrating salmon. In this study, following the air exposure treatment in particular, fish were incapable of burst swimming and many had difficulty maintaining equilibrium, suggesting that these individuals may have had short-term difficulty accessing locations of suitable velocity for recovery on their own volition. The increased survival for the air exposed recovery group relative to the air exposed immediate release group likely results from expedited physiological recovery and reduced metabolic costs associated with the stressor, as observed by Farrell et al. (2001b).

To put these survival proportions in context, Martins et al. (2011) found that sockeye salmon captured in freshwater environments by either fishwheel or tangle net had survival to spawning areas ranging between 30 and 50 % for similar populations as those examined here. However, sockeye salmon captured and released by purse seine in the marine environment had > 70 % survival from their first detection in the lower Fraser River to spawning areas (Martins et al. 2011). These marine tagged individuals may represent the best available telemetry survival data that approach true baseline survivorship values for the freshwater migration since tracking them from river entry (i.e., excluding mortalities that occur in the marine environment) enables the exclusion of capture and handling effects that occur in freshwater environments. If this 70% value is assumed to be baseline survival for similar sockeye salmon populations, the beach seine capture and release group had 12.5 % decreased survival relative to baseline, the recovery bag

following angling and air exposure resulted in 23.5 % decreased survival, and the other treatments resulted in at least 40 % decreased survival.

6.4.3 Case study 3: Potential for recovery bag use by anglers

Conflict between recreational angling communities and managers has been described previously (e.g., Danylchuk and Cooke 2011), providing rationale for seeking angler attitudes towards recovery bags prior to considering their implementation. My survey suggests that an equal proportion of anglers have generally positive attitudes towards recovery bags and support their implementation compared with the group that does not believe there is a need for recovery. Yet with only one quarter of survey respondents being fully supportive of using recovery bags when asked directly, implementation in this fishery could be challenging. Given the promising early evidence for recovery bags presented here, some attitudes may begin to change. In fact, supporters show overwhelming support for recovery bag use if such evidence were to be presented to them. While non-supporters may still not agree unanimously with using recovery bags, that group was more positive towards their use if they were presented with evidence of their effectiveness.

6.4.5 Synthesis and conclusions

How recovery bags are used by anglers will undoubtedly influence their effectiveness for recovery. My physiology results suggest that the type of bag is less important than water velocity itself, since several bag designs resulted in a reduced physiological disturbance relative to no velocity, particularly for the 15-min high water velocity group. Therefore, future work might focus on determining optimal velocities or a range of optimal velocities and exploring bag

designs that improve water velocity within the bag. Use of a light-weight, collapsible bag remains beneficial for this purpose since it can be easily transported. Fish in vigorous condition may benefit from immediate release, whereas those in poor condition (i.e., unable to burst swim or maintain equilibrium) are more likely to benefit from a short-duration facilitated recovery, provided that suitable water velocities can be located. For my telemetry study, bags were placed in the highest available velocity at the site of the study area. While there is realism to this approach, these velocities were still lower than the optimal 'high velocity' treatment from the physiology study. The observed benefit of recovery bags to air-exposed fish survival suggests that velocities of ~0.1 $m \cdot s^{-1}$ could still improve survival for fish in poor condition. While angler response was not unanimous, many respondents remain open to the possibility of recovery bag use if there is evidence of their effectiveness. Case studies 1 and 2 provide evidence that recovery bags have potential for promoting physiological recovery and survival, but the results here are not definitive, meaning that additional studies are required to further optimize recovery techniques. Additionally, with chapter 5 noting sex- and species-specific differences, future studies may benefit from focusing on one species and sex to reduce variation.

With the profound physiological responses to fisheries stress (chapters 2-5), prolonged recovery (chapters 4 and 5) and tertiary outcomes (chapters 2 and 3), exploring facilitated recovery methods is warranted. My case studies suggest that recovery bags hold promise for facilitating physiological recovery and promoting survival of Pacific salmon captured in freshwater. Given that recovery bags can be a simple, inexpensive, and portable means of facilitating recovery, they could be conducive for use in the recreational fishery and other small-scale inland fisheries that operate from shore. My results provide an important step forward in

identifying methods for promoting recovery from fisheries capture stress, which has consequences for increasing the sustainability of freshwater catch-and-release. This work provides a unique example where conservation physiology and human dimensions can be integrated to address a management concern.

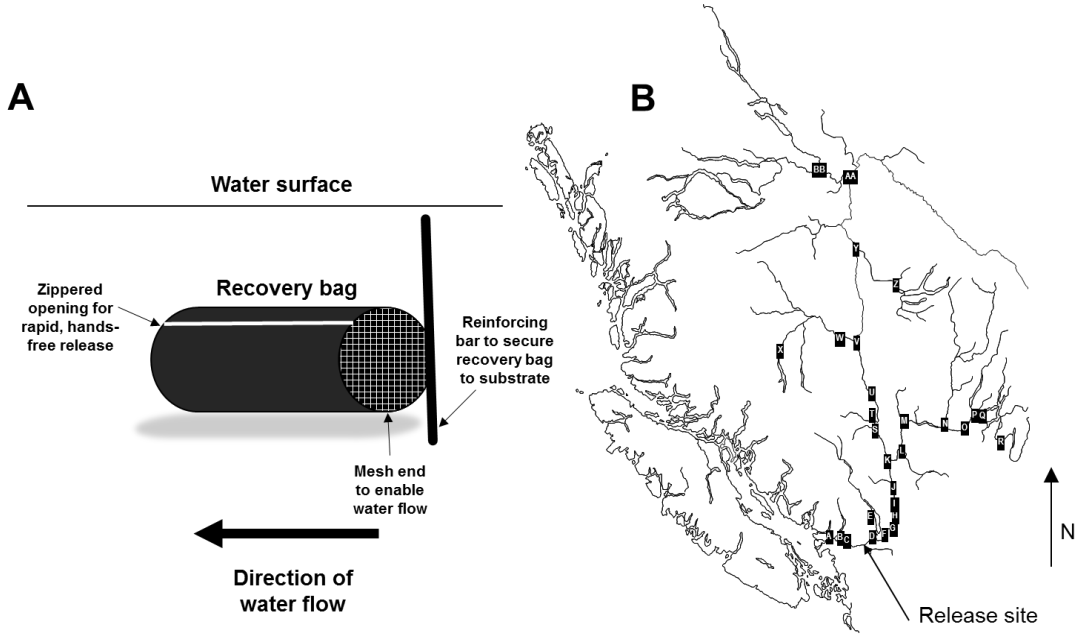

Figure 13. A: A schematic of a facilitated recovery bag. B: A map of the Fraser River, British Columbia, Canada showing locations of fixed station radio telemetry receivers. Receiver locations are denoted as follows: A Crescent Island, B Mission North, C Mission South, D Harrison Confluence, E Weaver, F Rosedale, G Hope, H Qualark, I Sawmill, J Hells Gate, K Thompson Confluence, L Spences Bridge M Ashcroft, N North Thompson O Timbers house, P Little River, Q Adams River, R Lower Shushwap, S Seton Confluence, T Bridge River, U Kelly Creek, V Chilcotin Confluence, W Farwell Canyon, X Chilko, Y Quesnel Confluence, Z Likely, AA Nechako Confluence, BB Stuart Confluence.

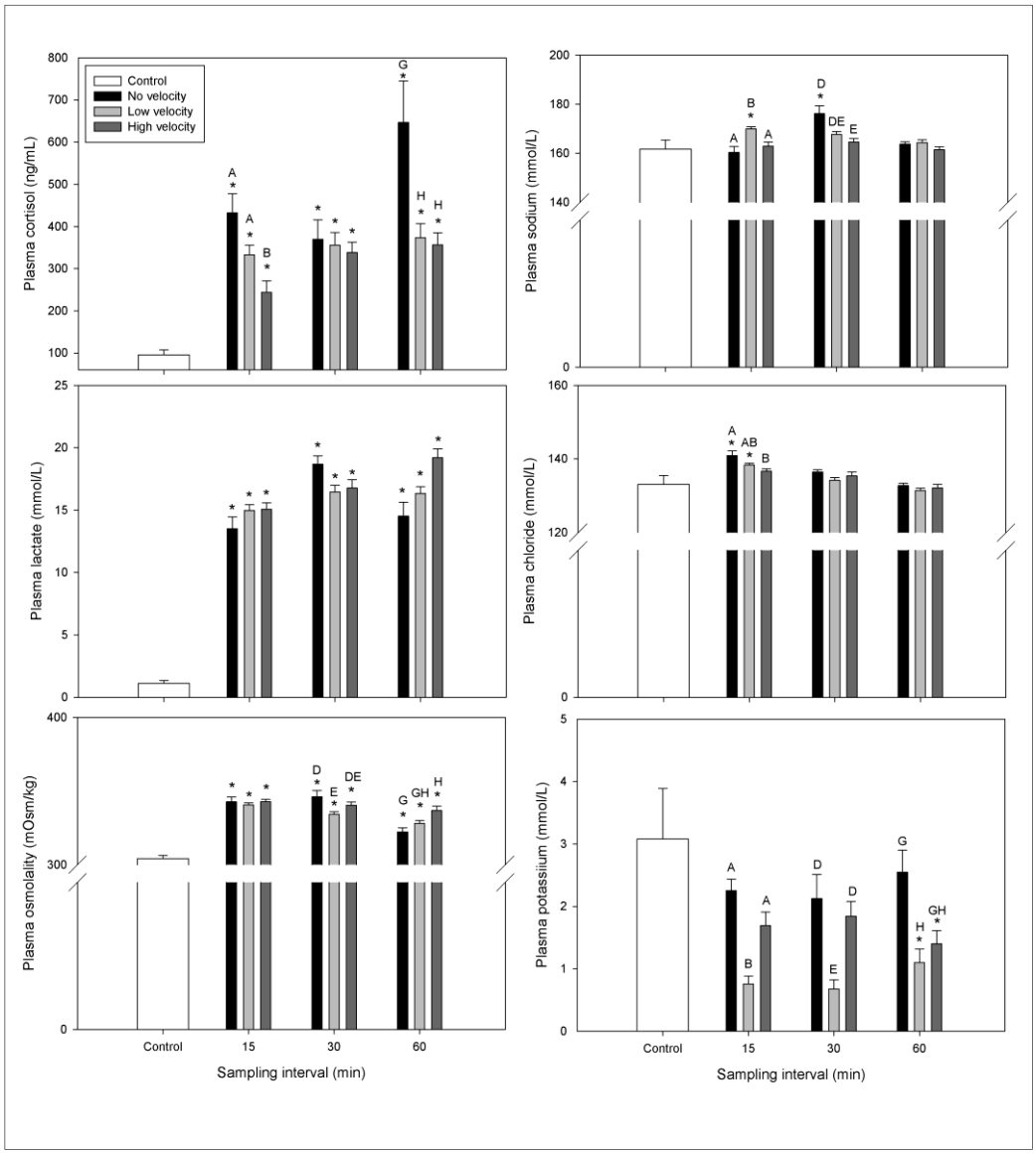

Figure 14. Plasma variables measured in adult pink salmon (*Oncorhynchus gorbuscha*) following an exercise treatment and a variable recovery period (15, 30, or 60 min) in portable recovery gears under no, low or high water velocity and controls. Different recovery bag types were pooled for analyses as bag type did not emerge as a significant effect in whole model MANOVAs. * denotes the group differs significantly from control values. Dissimilar letters denote differences among groups at each recovery period.

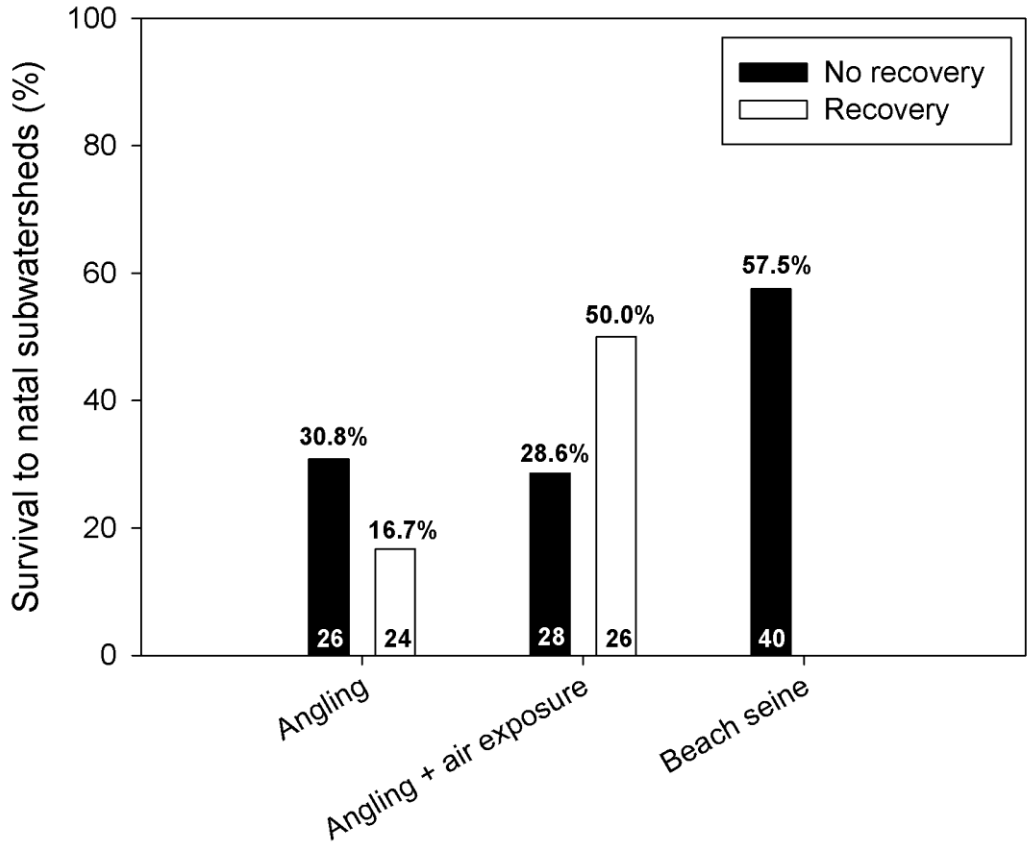

Figure 15. Survival to reach natal subwatersheds for adult sockeye salmon (*Oncorhynchus nerka*) captured and released by recreational anglers in the lower Fraser River, British Columbia, Canada. Angled fish were either immediately released, placed in a recovery bag for 15 min prior to release, air exposed and immediately released, or air exposed and placed in a recovery bag for 15 min prior to release. Beach seine survival is included for comparison and all individuals from this treatment were immediately released (i.e., none were given a recovery treatment). Sample sizes for each treatment appear within the vertical bars.

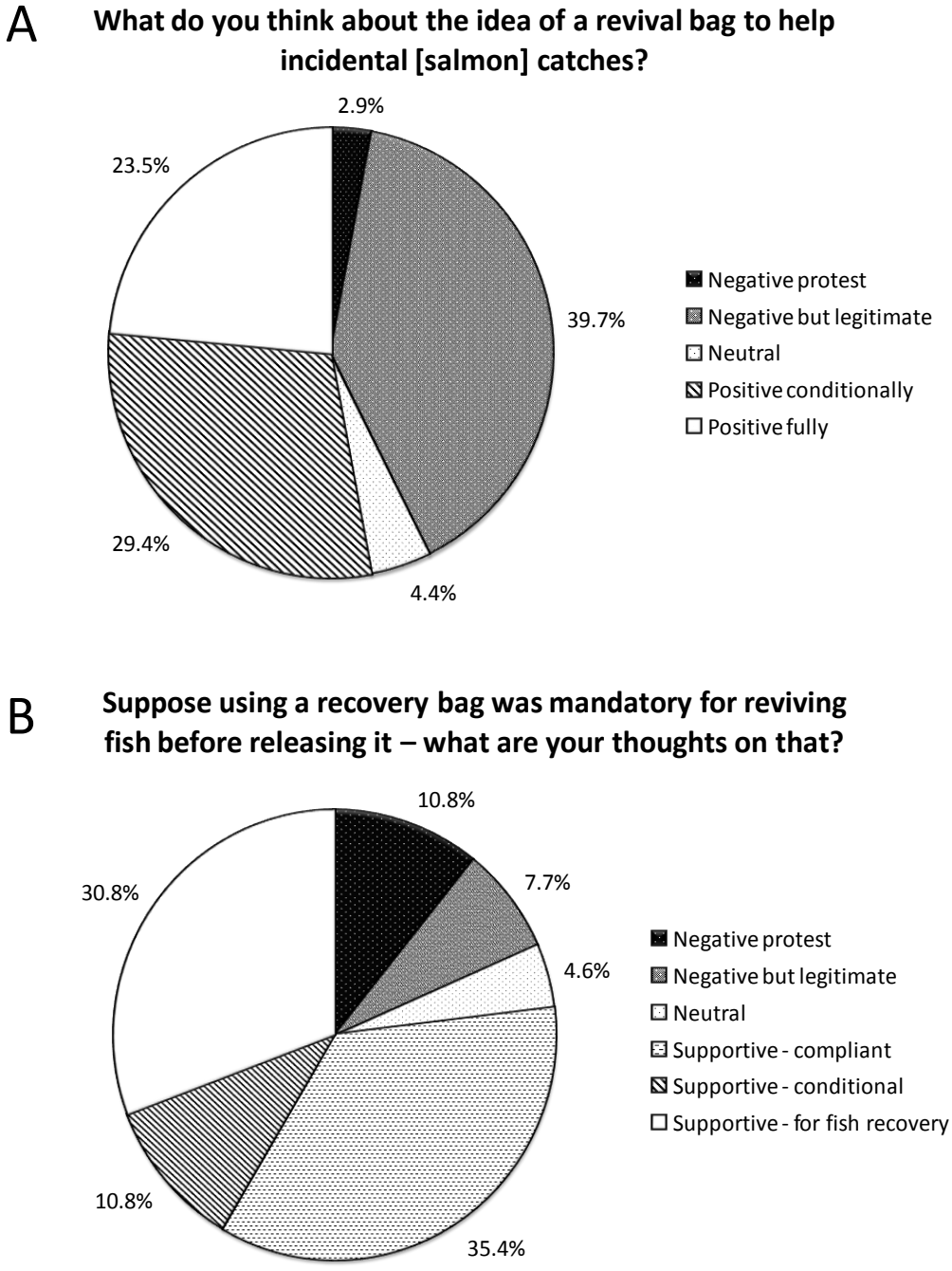

Figure 16. Fraser River salmon angler responses to human dimensions surveys on the support for using facilitated recovery gear to promote recovery of caught-and-released salmon.

Table 11. Latent class membership profile for survey questions on support for recovery bag use.

LC Cluster	Supporters (%)	Non-supporters (%)
Overall cluster size	53.8	46.2
Indicator variables		
1. What do you think about the idea of a revival bag to help incidental [salmon] catches?		
a) Negative (protest, legitimate)	13.1	78.6
b) Neutral	0.1	10.7
c) Positive (conditional, fully)	86.8	0.3
2. Is there a need for a recovery bag to revive incidentally caught salmon?		
a) No	39.8	98.0
b) Neutral	9.1	0.3
c) Yes	51.1	1.8
3. If the bag was shown to improve survival of released salmon, would you use it on a voluntary basis?		
a) No	15.5	54.1
b) Yes	84.5	45.9
4. Suppose using a recovery bag was mandatory for reviving fish before releasing it. What are your thoughts on that?		
a) Negative (protest, legitimate, conditional)	0.6	35.4
b) Other/Neutral	0.1	7.2
c) Supportive (compliant, conditional, fully)	96.3	57.4

7 CONCLUSIONS

My overarching hypothesis was that fisheries-related stressors would displace fish from homeostasis, resulting in primary and secondary stress responses that would have tertiary outcomes, which could be countered in part by facilitated recovery techniques. In support of this hypothesis, chapters 2 and 3 bridged the primary and secondary responses with tertiary outcomes and determined that a range of fisheries-related stressors resulted in physiological disturbances that reflected survival. Chapter 2 found that survival was lower for sockeye salmon released following angling compared to those released following beach seine capture. Chapter 3 identified that post-release survival was population-specific, and higher magnitude stressors resulted in higher mortality. With the results of chapters 2 and 3 finding profound effects of fisheries capture and release on survival, chapters 4 and 5 took a laboratory approach to investigate how Pacific salmon recover from fisheries-related stressors under controlled conditions. Chapter 4 showed that the duration of recovery was reflective of the magnitude of the stressor for heart rate, requiring several hours. Chapter 5 identified sex- and species-specific recovery using a series of primary and secondary stress indicators. Chapter 6 showed promising early results for facilitated recovery, but highlighted improvements needed before such techniques could be applied to freshwater release fisheries for Pacific salmon.

In the following sub-sections I revisit the four most salient and novel scientific findings from this thesis. In the first paragraph of each sub-section I discuss the relevance of each finding from a fundamental perspective, and then conclude each sub-section with a discussion from the applied perspective. I conclude the thesis with general and specific directions for future research.

7.1 Delayed mortality associated with fisheries capture and release

Chapters 2, 3, and 6 revealed that while short-term survival (i.e., within the first 3-4 days post-release) was quite high, latent mortality is a tertiary stress response that can occur several days post-release. While the mechanisms of mortality are not easily elucidated, reduced survival could be linked with stress, injury, disease, or a combination thereof. The fact that the most stressed treatment group in chapter 2 were also the least likely to survive (i.e., net pen group), further highlights the link between stress and latent mortality, although this finding certainly does not suggest causation, merely correlation. Chapter 3 provides some insight into a mechanism, where the stress of capture combined with injury and scale loss may have had a greater negative effect on survival for the less mature Harrison population that had to persist in the Harrison River or Harrison Lake for approximately 1 month longer than the more mature Weaver fish. I am left to speculate that greater stress and injury (or scale loss) from the more stressful treatments resulted in a higher susceptibility to disease (e.g., Picking and Pottinger 1989), contributing to the higher mortality of the Harrison fish that needed to persist for a longer duration before spawning.

From a management perspective, these results suggest that considerable delayed mortality in addition to natural mortality may go unaccounted for in management models. To reduce unnecessary mortality, this information should be considered when determining fisheries openings that overlap with abundant non-target species migrations. These findings challenge the assumption that catch-and-release survival is always high for Pacific salmon, which stems from short-term net pen holding studies (e.g., J.O. Thomas and Associates. 2009b) and that latent mortality can indeed occur and be profound. Although telemetry techniques do have some

limitations, such as additional handling and tag burden effects, receiver detection efficiencies, and small sample sizes, the value of being able to track fish to their terminal spawning areas enables long-term assessments of survival following a fisheries capture event, which could not be determined using holding pen studies alone.

7.2 The type of stressor affects the magnitude of response and duration of recovery

The duration and magnitude of the stressor influenced the duration of recovery and tertiary outcomes. Even brief fisheries-related stressors resulted in a dramatic stress response in all chapters (chapters 2-5). Fisheries capture events also contributed to injury and reflex impairment (chapter 3). Chapter 4 in particular highlights that the duration of recovery reflects the magnitude and duration of the stressor for heart rate and chapter 5 highlights that the response and recovery can be sex- and species-specific for may indices of primary and secondary stress. Recovery duration can be very long even following brief stressors, potentially limiting performance in the meantime. That the type of stressor also influences mortality (chapters 2 and 3) highlights the importance of using best practices when handling and releasing fish.

From an applied perspective, these cumulative results suggest that capture and handling methods should be optimized to ensure the rapid release of non-target species. For recreational hook-and-line fisheries, this would mean that angling durations should be kept short, air exposure and handling minimized, and release made rapidly. For commercial net fisheries, shorter net lengths for beach seines could enable faster net retrieval and sorting and in turn, less time between capture and release. Reduced net soak times for gill nets could be considered for mandate and advocacy of a “hot-picking” (i.e., rapid retrieval of the net upon contact with fish) strategy for removing non-target species rapidly to expedite release (e.g., chapter 3).

7.3 Fisheries-related capture and handling has context-, population-, sex- and species-specific outcomes

This thesis identifies context/gear-specific (chapters 2, 3 and 4), population-specific (chapter 3), sex-specific (chapters 4 and 5) and species-specific (chapter 5) consequences of fisheries-related stress. Chapter 5 identified, for the first time in maturing Pacific salmon, a timecourse of recovery following a stressor that was both sex- and species-specific. While species-specific responses to stress have been identified previously (e.g., Pottinger et al. 2010), the finding that two closely related species differ in their response and recovery patterns is novel. Cumulatively these results provide challenges for making generalizations on the effects of fisheries across species.

While generalizing management strategies across sectors (Cooke and Cowx 2006) and species (Cooke and Suski 2005) are often considered, these results suggest that making appropriate generalizations can be difficult and should be done cautiously. Given that Pacific salmon species co-migrate, and populations and sexes clearly overlap during migrations, it is impossible to completely avoid non-target catches. The best options to minimize these effects are to completely halt fisheries during periods where vulnerable populations are migrating, which already happens when certain populations are known to be migrating concurrently. As chapter 3 suggests, even if fisheries were selective and non-target populations could be released, there may still be reduced population-specific post-release survival. Thus if fisheries openings do occur when vulnerable populations are migrating, steps should be taken to ensure best handling practices and optimized fisheries-selective gear (e.g., chapter 2) are used to reduce stress and increase the likelihood of survival. However, as chapters 2-5 show, fisheries capture

is inherently stressful, and the likelihood of tertiary outcomes appears to be elevated for higher magnitude stressors, reaffirming the value of exploring facilitated recovery methods in freshwater (chapter 6).

7.4 Facilitated recovery has the potential to expedite recovery and improve survival

While facilitated recovery could help mitigate tertiary consequences and mortality (e.g., Farrell et al. 2001), chapter 6 highlights the fact that such methods have yet to be fully realized in freshwater for portable facilitated recovery methods. Facilitated recovery resulted in a reduced physiological response for certain variables, and a pattern of higher survival for air exposed fish, which was not statistically significant (chapter 6). Although anglers would be generally supportive of these techniques, more research is required to optimize recovery tools before such techniques could be used as a conservation tool in freshwater fisheries.

If more effective facilitated recovery methods cannot be found, given the high mortality from release fisheries (chapters 2, 3 and 6), managers must ask the difficult question: should catch-and-release even be an option for Pacific salmon fisheries or should these fisheries be mandated as strictly catch-and-keep? Exclusive catch-and-keep is controversial, particularly given the economic relevance of Pacific salmon fisheries (i.e., on the order of billions of dollars in subsidiary industries), but this philosophy has been adopted by specialized European recreational fisheries (e.g., Arlinghaus 2007). Given the high number of sockeye salmon released from recreational fisheries alone (a 1 in 3 release ratio with 100 000 released in 2010; DFO 2010) this is an issue worthy of attention from fisheries managers, particularly during periods of high temperatures.

7.5 Directions for future research

7.5.1 General directions for future research

While this thesis tackled questions specific to fisheries, the conceptual framework presented here and the general experimental approaches that were used provide a simple, integrated blueprint to help focus future research on the general stress response. The following two paragraphs suggest ideas for using a conceptual framework similar to the one presented here to help focus research questions and present an overview of how the field and laboratory methods used in this thesis could be adapted to explore general stress-related research questions.

The conceptual framework presented in this thesis represents a basic, generalized foundation for linking the stress response and recovery with life history consequences including reduced survival. The strength of this approach is that it enables an integration of the physiological mechanisms that may contribute to life history outcomes if the organism cannot adequately recover from the stressor. Upon assessing the primary, secondary, and tertiary responses to stress, more specific questions can be addressed, such as understanding the context-, species-, population-, and sex-specific nature of stressors as exemplified in chapters 2-5. Although the framework is geared towards fisheries-related stressors, it could be easily adapted to other stressors, ranging from environmental conditions to predator-prey interactions. The highly conserved nature of the general stress response would enable this approach to be expanded to other taxa as well.

The novel insights provided by this thesis were made possible by the effective use of new technologies and research methods that could be useful for future research on stress. While

telemetry is not new to fisheries science, the experimental (i.e., establishment of comparative treatment groups) and the integrative (i.e., combination of physiological biopsy with telemetry) studies in chapter 2 and 3 highlight new ways to tackle research questions that would be difficult to address with other approaches. The experimental telemetry studies provide excellent examples of how simple comparative approaches can help tackle difficult, mechanistic research questions. Heart rate biologgers were exceedingly informative here and are applicable to a number of ecological research questions. The experimental design used in chapter 4 required that fish were studied in a laboratory environment, but there may be opportunities to expand this type of work into the field, provided that the loggers could be recovered or integrated into transmitter technology. Given the importance of the heart in biological function and the relationship of heart rate with stress indices (chapter 4), heart rate loggers could be used on free-swimming fish to explore research questions related to predator-prey interactions, locomotion, performance, and reproduction. The biomarkers for stress identified in chapter 5 using qPCR are applicable beyond exercise and handling stress and may represent useful biomarkers for understanding acute and chronic stress in a number of contexts, including temperature stress (see below). While facilitated recovery gear is discussed in a fisheries-context in this thesis, the principles of facilitated recovery could be applied to studies on the recovery of exhausted fish attempting to navigate difficult barriers to movement (e.g., hydroelectric dams), following hatchery and aquaculture handling procedures (Portz et al. 2007) or even following some research methods (Wood et al. 1991). Integrating field and laboratory studies can be informative in this regard to enable both realism and to focus on mechanistic questions, respectively.

7.5.2 Specific directions for future research

I speculate that the consequences of fisheries-related stressors identified in this thesis may represent best-case scenarios, where more profound effects of fisheries stressors would be observed during periods of extreme water temperatures, which are increasingly frequent in the Fraser River, B.C. (Ferrari et al. 2007). Chapters 2, 3, and 6 involved releasing fish at ambient temperatures, which were moderate but stable during the study periods. In each case, temperature was not found to influence survival, likely because in each study fish were released over a relatively short time period and temperatures remained relatively constant. However, a rich body of literature has shown that high water temperatures can result in high mortality for released fish from multiple species in freshwater (Gale et al. 2012). Laboratory holding studies on adult sockeye salmon revealed profound mortality at 19 °C, particularly for fish stressed by a fisheries capture simulation and air exposure (Gale et al. 2011). Combining an experimental telemetry approach (i.e., similar to chapters 2 and 3) with biomarkers that show a sensitive response and definitive timecourse of recovery (chapter 5) may be useful for future temperature and fisheries interactions studies since some of these biomarkers are also temperature responsive in adult sockeye salmon (e.g., Jun B, Cyto C, NUPR1; M.R. Donaldson *unpublished data*).

Given the role of fisheries gear type and the magnitude of the stressors affecting survival, there is clearly a need to collect data from actual fisheries (e.g., chapters 2 and 3) rather than relying on simulations and laboratory studies alone. Much of our knowledge on fisheries-related stressors comes from laboratory-based studies and fisheries simulations. While some of the work conducted to date involves simulated fisheries encounters which has enabled a thorough understanding of fisheries-related stress in controlled settings, the data sets collected so far

would be further enhanced by working with actual fisheries in the field, as I have already done in this thesis. Given the prevalence of fisheries operating along the coastal migration route, studies in the marine environment would complement the freshwater research presented in this thesis.

My findings that the effects of capture on Pacific salmon can be sex-, population-, species-, and context-specific combined with our understanding of the role of temperature stress being sex- (e.g., Martins et al. 2012), population- (e.g., Eliason et al. 2011), and species-specific (e.g., Clark et al. 2011), points to an integrative series of studies to link fisheries capture stress in a broader context. With landmark studies identifying the roles of temperature tolerance (Eliason et al. 2011) and disease (Miller et al. 2011) in Pacific salmon migration mortality, there remains a need to integrate these factors in a fisheries-release context to understand how they potentially interact and contribute to delayed mortality. I believe that modelling approaches would be valuable in this regard. Given the impressive data sets already collected and sufficient data collection over the next several years, eventually thresholds could be identified and specific prediction models could be made each year on the combined roles of temperature, disease and fisheries capture on the survival of Pacific salmon at the species, population, and sex levels.

Facilitated recovery has the potential to increase post-release survival (Farrell et al. 2001), which has great relevance to freshwater fisheries. Chapter 6 showed promising albeit not perfect results, and it is clear that the optimal approach for facilitated recovery has yet to be identified in freshwater studies even when using Fraser boxes (V. Nguyen, unpublished data) and attempts to do so may actually result in lower survival (K. Robinson, unpublished data). A comparative study that tests different water temperatures, velocities, timing, and recovery gear type is warranted. Combining laboratory-based (e.g., under different temperature conditions)

and field-based (e.g., telemetry) study designs would be beneficial. While the rationale for using recovery bags is to have a light-weight, collapsible, and portable recovery option, modifying Fraser Boxes (Farrell et al. 2001) for portability could be useful for certain fisheries that are easily accessed by roads and have a high density of anglers or on-shore net fisheries. Using light-weight construction materials and portable pumps (e.g., battery or solar operated) could provide sufficient flow even in low-flow areas. Given the species-specific nature of the stress response, many research opportunities exist to determine the most effective methods of facilitating recovery depending on the physiological needs of different species. For example, low velocity swimming appears to not enhance recovery from exhaustive exercise in centrarchids in the way it does for salmonids (Suski et al. 2007).

The fundamental concern arising from my thesis is that even a brief stressor can have profound, latent outcomes on survival. Future work must investigate best handling practices to determine if there are simple techniques that fishers could use to minimize the effects of capture on released fish. To assess fish condition, a simple, integrated metric could combine a measurement of ventilation rate and the maintenance of equilibrium (Gale 2011) with reflex impairment metrics (i.e., RAMP; Raby et al. 2011; chapter 3). These simple techniques could inform decisions on whether or not to release or keep fish, or use facilitated recovery techniques. Many of the tools used in this thesis (e.g., biologging, biotelemetry, biomarkers) could be used to enhance experimental field and laboratory studies designed to test methods for the improvement of fisheries capture and handling, which could in turn further enhance the sustainability of Pacific salmon release fisheries.

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