The Effects of Fisheries Capture on the Physiology and Post-Release Fate of Adult Pacific Salmon

by

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Abstract

All animals encounter acute stressors during their lifetimes, and while the immediate response to those stressors is well understood and presumed to be adaptive, relatively few studies have linked those responses with subsequent fitness outcomes. This problem finds particular relevance in the fisheries realm, where there is interest in developing a) an understanding of what leads to mortality for caught-and-released animals, b) methods for reducing post-release mortality, and c) predictors of delayed mortality. Pacific salmon are a tractable model for studying post-release mortality because the migration success of individuals after release can be easily and effectively tracked, and migration failure means zero lifetime fitness. In this thesis I report on research in which I used physiological assessments and tracked Pacific salmon fitness outcomes in the wild to examine the response to and recovery from capture, and whether individual differences in responses could be linked to migration or spawning failure. A key finding that arose throughout was that reflex impairment is an effective indicator of the whole-animal response to capture stressors, is correlated with dermal injury, reflects underlying physiological processes, and can predict delayed mortality. I demonstrate that mortality rates currently used in management models are likely inaccurate, but use several lines of evidence to show that mortality could be reduced using different capture and handling techniques. Specifically, more proactive efforts to reduce handling time reduced physiological disturbance and reflex impairment. Revival using industry-standard revival totes and novel in-river recovery bags did not reduce delayed mortality, although the latter and forced-flow revival boxes appeared effective at expediting short-term revival. I found some evidence that sensitivity to capture stressors may change dynamically

throughout the spawning migration, with fish becoming particularly resilient once reaching spawning areas. Well-controlled experiments are required if the knowledge gaps arising from this thesis are to be addressed in future research: namely, how does the response to and recovery from acute stressors change over the course of the spawning migration, and when does facilitated revival benefit fish survival? Collectively, the work presented in this thesis provides a useful addition to our understanding of the effects of fisheries capture on the physiology and survival of fish.

Acknowledgements

I am very fortunate to have worked under the supervision of Dr. Steven Cooke and Dr. Scott Hinch, and am indebted to both. A late-night call from Steve in the final months of my undergraduate degree persuaded me to agree to join his lab for graduate school – a decision that has benefited me enormously. Working with Scott's dynamic research team in British Columbia was a remarkable experience that I'll never forget, and I realized early on that I wanted to continue working with him and Steve for an extended period, which is why this document is a doctoral dissertation rather than an M.Sc. thesis. Steve's energy, drive, and accomplishments have inspired and motivated me, and he was always positive and encouraging. I am grateful to both supervisors for the opportunities they afforded me throughout my graduate career, and for shaping my personal and professional development for the better.

I owe thanks to my advisory committee, who were supportive, encouraging, and helpful throughout the course of my studies: Dr. Michael Davis, Dr. Gabriel Blouin-Demers, and Dr. Bill Willmore. Each has served as a collaborator with unique contributions and has offered ideas, feedback, and challenged my thinking in constructive ways.

The work presented in this thesis would not have been possible without a remarkable amount of input from others. Mike Donaldson spent several weeks in the field with me and his mentorship ensured the success of my work and contributed significantly to my development as a biologist. Tim Clark was an important mentor who regularly provided useful advice and flew all the way from Australia to train me on respirometry and fish surgery. David Patterson is an exceptional resource on anything to do with

Preface

All research presented in this thesis was conducted in accordance with animal care protocols approved by the animal care committees of the University of British Columbia, Fisheries and Oceans Canada, and Carleton University, in accordance with guidelines set by the Canadian Council on Animal Care.

Thesis format

This thesis is written in a manuscript-based format. However, I modified some of the titles and content of the manuscripts I use as thesis chapters. Though some redundancy does occur, I made efforts to avoid repeating information on background and methods in each chapter. In addition, acknowledgments from each manuscript are combined at the start of the thesis, and references are compiled at the end of the thesis. The six data chapters (manuscripts) are ordered in a logical progression that will hopefully become clear from explanations in the first and final chapters of the thesis.

Co-authorship

This thesis contains results of my own empirical research, but I conducted the research with the support and guidance of numerous co-authors. Because chapters 2-7 are either published or under journal peer-review and were co-authored as such, they frequently contain "we" and "our" and I have left them in that form. Below I list the manuscripts used as thesis chapters and the contributions of authors on each paper.

Chapter 2: Mechanisms to explain purse seine bycatch mortality of coho salmon: interactions between injury, reflex impairment, and blood physiology

Raby, GD, SG Hinch, DA Patterson, JA Hills, LA Thompson, and SJ Cooke. Mechanisms to explain purse seine bycatch mortality of coho salmon. Under review in Ecological Applications (re-submitted 27-10-2014).

The study was designed by Raby and Cooke. Raby collected the data with help from Hills and Thompson, using equipment provided by Hinch, Patterson, and Cooke. Hills helped analyze blood samples. Raby analyzed the data and wrote the paper. All authors provided input on the manuscript.

Chapter 3: Validation of reflex indicators for measuring vitality and predicting delayed mortality in coho salmon

Raby, GD, MR Donaldson, SG Hinch, DA Patterson, AG Lotto, D Robichaud, KK English, WG Willmore, AP Farrell, MW Davis, and SJ Cooke. 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. Journal of Applied Ecology 49:90-98.

The study was designed by Raby, Cooke, and Donaldson. Data were collected by Raby, with significant contributions from Donaldson and Lotto, and using equipment provided by Hinch, English, and Cooke. Robichaud contributed significantly to data processing. Raby analyzed the data and wrote the paper. All authors provided input on the paper.

Chapter 4: Bycatch mortality of endangered coho salmon: impacts, solutions, and aboriginal perspectives

Raby, GD, MR Donaldson, VM Nguyen, MK Taylor, NM Sopinka, KV Cook, DA Patterson, D Robichaud, SG Hinch, and SJ Cooke. 2014. Bycatch mortality of endangered coho salmon: impacts, solutions, and aboriginal perspectives. Ecological Applications 24:1803-1819.

This study was designed by Raby, Cooke, Donaldson, and Nguyen. Raby and Nguyen collected the data with significant contributions from Donaldson, Taylor, Sopinka, and Cook. Hinch and Cooke provided equipment. Robichaud and Nguyen contributed significantly to data processing. Raby analyzed the data and wrote the paper. All co-authors provided input on the paper.

Chapter 5: Facing the river gauntlet: understanding the effects of fisheries capture and water temperature on the physiology of coho salmon

Raby, GD, TD Clark, AP Farrell, DA Patterson, NN Bett, SM Wilson, WG Willmore, CD Suski, SG Hinch, and SJ Cooke. Facing the river gauntlet: understanding the effects of fisheries capture and water temperature on the physiology of coho salmon. Under review in PLOS ONE (submitted 01-11-2014)

This experiment was designed by Raby, with input from Clark, Patterson, Hinch, and Cooke. Raby conducted the experiment with significant contributions from Clark, Bett,

and Cooke, using equipment provided by Clark, Farrell, Hinch, and Cooke. Wilson contributed significantly to laboratory analyses, which were conducted using equipment provided by Willmore and protocols developed by Suski. Raby analyzed the data and wrote the paper. All authors reviewed and provided input on the paper.

Chapter 6: A physiological comparison of three techniques for reviving sockeye salmon exposed to a severe capture stressor during upriver migration

Raby, GD, SM Wilson, DA Patterson, SG Hinch, TD Clark, AP Farrell, and SJ Cooke. A physiological comparison of three techniques for reviving sockeye salmon exposed to a severe capture stressor during upriver migration. Under review in Journal of Comparative Physiology B (submitted 05-10-2014).

Raby, Hinch, Clark, and Cooke designed the experiment. Raby conducted the experiment with significant contributions from Wilson, using equipment provided by Hinch and Cooke. Raby analyzed the data and wrote the paper. All authors provided input on the paper.

Chapter 7: Resilience of pink and chum salmon to simulated fisheries capture stress incurred upon arrival at spawning grounds

Raby, GD, SJ Cooke, KV Cook, SH McConnachie, MR Donaldson, SG Hinch, CK Whitney, SM Drenner, DA Patterson, TD Clark, and AP Farrell. 2013. Resilience of pink salmon and chum salmon to simulated capture stress incurred upon arrival at spawning grounds. Transactions of the American Fisheries Society 142:524-539.

This experiment was designed by Raby, Cooke, Donaldson, Hinch and Farrell. Raby conducted the experiment and collected the data with significant contributions from Cooke, Cook, McConnachie, Donaldson, Whitney, Drenner, and Clark. Raby analyzed the data and wrote the paper. All authors provided input on the paper.

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Chapter 1. General introduction

1.1   The stress response system and its link to fitness

All animals encounter challenges to their fitness during their lifetimes. Perception of those challenges (i.e., stressors) results in a suite of physiological and behavioural changes known as the stress response (Chrousos and Gold 1992, Wendelaar Bonga 1997). Behavioural reactions to a stressor can occur immediately upon its perception, vary depending on the type of stimulus (stressor), and ideally, serve to enhance the likelihood of survival and maintenance of homeostasis (Morilak et al. 2005). The general endocrine features of the stress response system are conserved across vertebrates (reviewed in Chrousos and Gold 1992, Wendelaar Bonga 1997, Sapolsky et al. 2000). Within seconds of perceiving an acute stressor (e.g., an immediate threat to survival), catecholamines (CAs; epinephrine and norepinephrine) are released from chromaffin cells, which occur in the adrenal medulla in tetrapods and in the head kidney in fishes. At the same time, the hypothalamo-pituitary-adrenal (HPA) axis is activated (in fish, the hypothalamo-pituitary-interrenal [HPI] axis). Activation of the HPA (or HPI) axis ultimately involves release of glucocorticoids (GCs), the second main class of stress hormones, into circulation – with a more gradual and prolonged increase than for CAs. In amphibians, reptiles, and birds the primary GC is corticosterone, while in teleost fishes and most mammals, including humans, it is cortisol (Sapolsky et al. 2000). Elevated circulating GCs stimulate the down-regulation of their precursors (corticotropic-releasing factor and adrenocorticotropic hormone), thus initiating a negative feedback loop that eventually returns GCs to low levels, assuming the stressor is overcome (or following habituation to the stressor; Schreck 1981).

The two primary classes of hormones (CAs and GCs) involved in the stress response system have interactions with other components of the endocrine system and secondary effects on physiology that are numerous and complex (see reviews by Chrousos and Gold 1992, Wendelaar Bonga 1997, Sapolsky et al. 2000). Perhaps the most well-known and fundamental effects of CAs are the release of glucose from the liver into circulation, and a large, rapid increase in oxygen delivery to tissues via increases in ventilation rate, cardiac output, and the oxygen carrying capacity of the blood (Wendelaar Bonga 1997). GC elevation lasts longer than that of CAs, and can persist for hours or days. GCs facilitate energy mobilization, affect immune and reproductive physiology, increase metabolic rate, and cause changes in hydromineral balance (Mommsen et al. 1999, Sapolsky et al. 2000, Barton 2002, Schreck 2010).

The way the stress response system is thought of has evolved somewhat since the concept was first crystalized as the "general adaptation syndrome" (Selye 1950). In actual fact, even today there is no universal agreement on the definition of stress (Schreck 2010), and McEwen and Wingfield (2003a) proposed virtual abandonment of the term in favour of allostasis – the concept of achieving stability (i.e., homeostasis) through change – but accepted that "most people will continue to use "stress" to mean many different things" (McEwen and Wingfield 2003b). The confusion arises partly because of the conceptual mismatch between the negative connotation of "stress" and the fact that elevation in "stress" hormones occurs during normal life challenges (e.g., reproduction, migration, social interactions), and sometimes in anticipation of those challenges (Sapolsky et al. 2000). It has long been recognized that sustained activation of the stress response system and the phenotypic changes that result can be thought of as an "allostatic

load" that changes the realized performance or behaviour of the animal, either temporarily or permanently (Schreck 1981, McEwan and Wingfield 2003a, Ellis and Del Guidice 2014). The standard view is that stress-induced changes to phenotype are usually adaptive in the short-term but in most cases become maladaptive if stress responses become chronic. For example, while elevation in GCs could assist an animal in escaping suboptimal environmental conditions (e.g., via increased cognitive functioning, oxygen transport, energy mobilization), elevation in GCs affects somatic growth, reproduction, and immune function such that if GC elevation is sustained without realizing clear benefits (e.g., escaping poor conditions, establishing social dominance, successfully mating) the net effect of the stress response on fitness will be a negative (i.e., allostatic load or overload; McEwen and Wingfield 2003a, Schreck 2010, Boonstra 2013).

In the study of ecology and evolution, and indeed human medicine (Ellis and Del Guidice 2014), most of the effort to understand the effects of stress responses has been in the framework of what is now called allostatic load (or overload), where studies examine the (usually negative) effects of chronic stress responses (Sapolsky et al. 2000, Romero 2004). Changes to behaviour, life history processes, and animal fitness – known collectively as the tertiary response to stress – have been of particular interest for ecologists. Fewer attempts in ecology have been made to link acute stress responses (i.e., "emergency-type responses"; Schreck 2010) to tertiary effects. Indeed, Breuner et al. (2008) highlighted that exceptionally few studies have linked acute stress responses to direct measures of fitness. The physiological and behavioural responses to acute stressors (e.g., a predator attack), sometimes referred to as activating an emergency life-history stage (Wingfield et al. 1998), are widely presumed to be adaptive. Nevertheless, there can

be substantial inter-individual variation in how animals respond to and recover from immediate (perceived) threats to survival (Breuner et al. 2008), responses can differ depending on context (Wingfield et al. 1998, Barton 2002), and the magnitude of the response could affect fitness-related outcomes (e.g., via immediate allostatic overload). The causes and consequences of that variation could play a role in evolutionary processes (Careau and Garland 2012) and represent an interesting intersection of ecology, physiology, behaviour, and life history (Calow and Forbes 1998, Dantzer et al. 2014).

1.2   Defining the problem – capture and release

In their seminal review on the effects of GCs, Sapolsky et al. (2000) describe the prototypical acute stressor as a predator attack that occurs without warning, elicits injury, and includes one hour of being stalked and chased – a perceived challenge to survival that constitutes psychological stress while also requiring extended exercise, heightened cognitive functioning, and later, the process of injury repair and physiological recovery. Fisheries capture can be as severe as the example described above, and for most animals it would likely be more severe than any acute stressor previously experienced in their lifetime. Aquatic animals belonging to all major vertebrate taxa including mammals, reptiles, birds, and fishes, are captured by fisheries. Regardless of the fishing gear used, fisheries capture elicits (to varying degrees) strenuous exercise, asphyxiation or exposure to hypoxia, injury, and a neuroendocrine stress response (see reviews by Chopin and Arimoto 1995, Davis 2002, Skomal 2007, Wilson et al. 2014) that, presumably, is initiated the moment the animal perceives that it is blocked or trapped by a net or feels the hook. The response of the animal (the emergency life-history stage, Wingfield et al.

1998) is meant to enable escape from the stressor, and indeed many animals are able to escape fishing gear (Gilman et al. 2013). However, the suite of responses elicited by fisheries capture can result in rapid allostatic overload (i.e., compromised vitality), from which a full recovery may not occur. Those that are landed and survive the initial stress will be released if they have no value for the fisher or if their release is required for conservation reasons (Hall 1996, Arlinghaus et al. 2007). The major theme of this thesis involves examining the response to and recovery from fisheries capture stressors of varying severity and the resulting consequences for fitness.

While it has some relevance to basic biology, the organism-level effects of fisheries capture have obvious applied relevance. Globally, billions of fish and other animals experience capture-and-release each year while an unknown number of others escape fishing gear (Cooke and Cowx 2004, Bartholomew and Bohnsack 2005, Kelleher 2005, Davies et al. 2009). In some commercial fisheries, bycatch (capture of non-target organisms) and discards (non-target catch released overboard) collectively constitute >50% of total catch (Davies et al. 2009), while many recreational fisheries have release rates >50% and some as high as 99% (Cooke and Cowx 2004, Arlinghaus et al. 2007). Despite the good intentions of releasing animals alive, cryptic post-release mortality (PRM) can occur hours or days after release (or escape) from fishing gear (Coggins et al. 2007). Rates of PRM can be quite high, which creates obvious sustainability concerns (Coggins et al. 2007, Gilman et al. 2013). Correspondingly, a considerable literature has developed over the past 20 years focused on estimating post-release survival in a variety of fisheries (Davis 2002, Donaldson et al. 2008), assessing sublethal effects of capture (Wilson et al. 2014), and developing fishing methods that reduce bycatch (Kennelly and

Broadhurst 2002) and PRM (e.g., Farrell et al. 2001a, Broadhurst et al. 2008, 2009, Donaldson et al. 2013).

1.3   The response to fisheries capture and its link to delayed mortality

The physiological response to fisheries capture has been well documented in fishes, and is thought to be similar to the experimental imposition of exhaustive exercise so frequently used in the study of fish physiology (Kieffer 2010). It involves rapid, massive release of CAs followed by a slower rise of cortisol that typically peaks 1-2 h after initiation of the stressor (Wood et al. 1983, Milligan 1996, Milligan et al. 2000). At least in fasted animals (Romero et al. 2009), there is an increase in circulating glucose (Wydoski et al. 1976). Because of the behavioural response of fish to attempt escape via burst swimming (i.e., using anaerobically-fueled white muscle), short-term energy stores in white muscle are depleted (e.g., glycogen, adenosine triphosphate [ATP], and phosphocreatine [PCr]; Farrell et al. 2001a,b). Lactate, a byproduct of anaerobic metabolism, increases rapidly in muscle cells but typically begins to fall significantly within an hour (Suski et al. 2006). Muscle lactate is partly leaked to blood plasma and typically continues to rise there for 1-2 h after capture (Wood et al. 1983, Farrell et al 2001a, Suski et al. 2006). Lactate's increase in plasma is mirrored by a decrease in pH, while there is an also an increase in arterial CO_2 partial pressure (Milligan and Wood 1986). The changing acid-base balance creates an osmotic shift of water to muscle cells and erythrocytes, which concentrates ions in plasma (Wood et al. 1983). The marked change in plasma ion concentrations is also affected by direct effects of CAs and cortisol

on osmoregulatory balance (Mommsen 1999), and can take hours to correct (Wood 1991).

A persistent challenge in both the ecology of stress and fisheries science has been that of linking the immediate response of individuals to acute stressors with subsequent fitness outcomes (Davis et al. 2001, Breuner et al. 2008, Cooke et al. 2013a). In fisheries science, this topic is of interest because vitality indices measured at the time of release that predict delayed mortality could allow for rapid estimates of PRM for different species and fishing gears, which would facilitate more accurate estimates of total fishing mortality (Davis 2007). Identification of such biomarkers could also help develop guidelines to reduce PRM and explain why it occurs. Blood biochemistry has been a popular approach to assessing the response to capture stressors, with such application dating back to the 1930s (Huntsman 1938). Blood measures can provide useful insight into the effects of capture (Cooke et al. 2013a), but often fail to predict delayed mortality (Davis et al. 2001, Davis and Schreck 2005, Davis and Ottmar 2006, Cooke et al. 2013a) – perhaps partly because the full effect of capture stressors is often not evident in standard measures of blood biochemistry until 30-60 min after capture. Injuries can play an important role in delayed mortality and represent a component of allostatic load and vitality impairment (Davis 2002, 2010); presumably they act as chronic stressors and can lead to mortality via infection and disease enhanced by the immunosuppressive effects of the cortisol response (Olsen et al. 2012), although this interaction is poorly understood (Lupes et al. 2006). Fisheries scientists have sometimes used macroscopic assessments of animal vitality that include the extent and severity of injuries and the lethargy/vigour of the animal (e.g., Kaimmer and Trumble 1998, Trumble et al. 2000, Farrell et al. 2001a,

Yergey et al. 2012). While those assessments are an intuitive inclusion to such research and have been useful for understanding mortality, they involve assigning fish to descriptive categories and rely on subjectivity.

Reflex Action Mortality Predictors (RAMP) is a recently introduced approach (Davis 2007, 2010) that aims to provide a simple and objective assessment of animal vitality following fisheries capture, and a means of predicting delayed mortality. RAMP assessments involve rapidly determining the presence or absence of a series of simple reflex responses to handler stimuli that, for vigorous animals in good condition, are always present, but that become progressively impaired (absent) as vitality deteriorates with more severe stressors. The argument in favour of using this approach instead of (for example) blood physiology is that reflexes can integrate whole-animal vitality and departure from homeostasis (or allostatic load, Davis 2010), whereas blood measures represent discrete components of animal function that exhibit large natural variation and whose influences are complex. Reflex assessments are also rapid and simple enough to be used by fishers and fisheries observers and integrated into fisheries management (Davis 2010). Much of the work I present in this thesis is focused on evaluating the utility of RAMP in a variety of contexts and assessing its physiological correlates.

The majority of research on post-release fate after exposure to capture stressors has been conducted using containment experiments. Holding animals is useful because their fate can be verified, but these studies fail to integrate the challenges of the wild environment, which could affect PRM estimates. This is a shortcoming that has been pointed out numerous times (e.g., Donaldson et al. 2008, Yergey et al. 2012, Rogers et al. 2014), and the use of biotelemetry has been proposed as the optimal alternative

(Donaldson et al. 2008). Biotelemetry involves remote monitoring of free-roaming animals that are equipped with transmitters, enabling the release of animals into the wild (Cooke et al. 2004a). However, use of biotelemetry in studies of PRM has its own challenges, including a) the sometimes poorly-understood effects of the transmitter of tagging procedure, b) the need to surgically-implant or externally-attach transmitters which could add further stress and injury and bias mortality rates, c) difficulties in assessing fate and survival based on movement data, and d) insufficient receiver coverage or detection efficiency issues. Moreover, even if detailed movement data are obtained, it can be difficult to discern whether an animal is still alive – in some cases the transmitter can be ingested by a predator along with the tagged animal, and such data might appear to indicate continued survival of the tagged individual (Yergey et al. 2012).

1.4   Pacific salmon as a model

The unique nature of the spawning migrations of Pacific salmon (*Oncorhynchus* spp.) makes them well-suited to the study of the effects of acute stress responses on fitness outcomes because biotelemetry can be used in ways that avoid most of its usual limitations. Salmon cease feeding during upriver migration and their stomachs atrophy, which makes gastric insertion of radio transmitters an option for tracking individuals to spawning areas (Ramstad and Woody 2003, Cooke et al. 2005). Gastric tagging is rapid, requires no anesthetic or dermal injury, and creates little or no additional hydrodynamic drag (unlike external transmitter attachments). Moreover, Pacific salmon are semelparous, which means that failure to reach their natal spawning area (identifiable using DNA from a small tissue biopsy; Beacham et al. 2011) has clear consequences for

fitness which makes interpretation of movement data straightforward. Radio and acoustic telemetry receiver stations can be set up at key checkpoints along the migration pathway, which allows for monitoring of migration success of individuals tagged with uniquely-coded transmitters (Eiler 1995, English et al. 2005).

Pacific salmon life histories are fascinating and diverse (described in detail in Groot and Margolis 1991, and Quinn 2005), and involve some of the most spectacular migrations in the animal kingdom. The life cycle of most Pacific salmon is broadly characterized by i) adults migrating from the ocean to freshwater where they spawn and bury their eggs in gravel, ii) overwinter incubation of eggs before spring emergence of fry, iii) downstream migration of juvenile salmon to the ocean following, in most cases, some period spent growing in lakes or streams, iv) migration to ocean feeding grounds where the vast majority of growth takes place. Generally-speaking, Pacific salmon return to their natal stream (or lake) on a fixed schedule, which creates genetically distinct populations, although fidelity to spawning locales differs among species. The concentrated and predictable nature of the adult migration makes the fish easy targets for predators (including humans), particularly when fish become concentrated while moving through rivers. The spawning migration, which is the focus of fisheries and this thesis, has been studied to the extent that we now know much about the energetics, ecology, life history, physiology, and behaviour of migrating adult salmon (Groot and Margolis 1991, Groot et al. 1995, Quinn 2005, Hinch et al. 2006). That extensive knowledge provides a helpful base on which to interpret data on the effects of fisheries capture.

This thesis includes work on four of the seven species of Pacific salmon that occur in Canada: sockeye (*O. nerka*), pink (*O. gorbuscha*), chum (*O. keta*), and coho

salmon (*O. kisutch*). Pink and chum salmon are distinguished from the other species by having offspring that migrate to the ocean immediately upon yolk-sac absorption, rather than rearing in freshwater – although some of the sockeye I used belong to a population that does the same (from the Harrison River, British Columbia). Pink salmon are the smallest and most abundant species in the genus *Oncorhynchus*, are distinguished by having a two-year life cycle (spawn-to-spawn), and exhibit relatively low fidelity to natal spawning sites (Heard 1991). Chum salmon are the second largest Pacific salmon (after Chinook salmon, *O. tshawytscha*), third-most abundant, and usually spawn quickly after migrating to freshwater (Quinn 2005). Sockeye salmon predominantly spawn in streams adjoining lakes (or in lakes themselves) and their offspring typically rear in lakes for 1-2 years before smoltification, migration to the ocean, 1.5 years of feeding at sea (two full summers), and a spawning migration that is notable for extremely high stream fidelity and some of the more arduous upstream migrations (Quinn 2005). Coho salmon, which are the subject of most of this thesis (chapters 2 through 5) for reasons described below, typically have a three-year life cycle (Decker and Irvine 2013) characterized by protracted adult migration that occurs later in the year than most other species, stream spawning, offspring that rear in streams for one year before ocean migration, and piscivorous ocean feeding that persists during the coastal approach to freshwater (Sandercock 1991).

Aside from logistical advantages and their unique life histories, there are several reasons to use Pacific salmon as models for the study of post-release fisheries mortality. Pacific salmon are iconic because of the remarkable scope and scale of their life cycle, but they are also integral to the economies, ecosystems, and cultures of the Pacific

Northwest (Scarnecchia 1988). They are particularly important to aboriginal peoples, and

have been targeted by their fisheries in North America for thousands of years (Campbell

and Butler 2010). Because of their importance, Pacific salmon receive more attention

from resource managers than do most fish populations. The declines of some salmon

stocks (populations) have been attributed to overexploitation (e.g., Bradford and Irvine

2000), and while fisheries managers can halt directed fishing for a certain species,

incidental catch will occur in fisheries targeting other species (Donaldson et al. 2011). In

British Columbia's salmon fisheries, there has been a policy shift towards the philosophy

of "selective fishing", defined as the goal of avoiding non-target animals and releasing

them "alive and unharmed" if encountered (DFO 2001). Thus, there is some appetite

among managers of Pacific salmon fisheries to gain further insight into post-release

mortality. Indeed, fisheries management in British Columbia (Fisheries and Oceans

Canada, referred to as DFO) take steps to minimize incidental mortality for stocks and

species facing conservation issues. For example, fisheries are curtailed at key times and

locations to avoid bycatch, or gears that elicit high bycatch mortality (i.e., gillnets) are

restricted in favour of those that are thought to enable higher post-release survival (i.e.,

seines). Moreover, in efforts to meet spawning escapement targets (numbers of fish that

are allowed to reach spawning areas), fisheries managers apply estimates of bycatch

mortality to non-target fish that are released (DFO 2011). If bycatch mortality causes

total allowable fishing mortality for a particular stock or species (e.g., Chinook salmon)

to exceed set limits (based on pre-season management plans), the fishery generating that

bycatch mortality can be prematurely terminated.

All seven species of Pacific salmon that spawn in the Fraser River basin are caught and released to varying extents. Of particular interest to managers are populations or species facing conservation problems. Most notable among these is the interior Fraser River population of coho salmon (hereafter, interior Fraser coho), which is the focus of much of this thesis. Interior Fraser coho are those that spawn in the upper watersheds of the Fraser River basin (upstream of Hell's Gate, Fig. 1.1). Their collapse in the 1990s (Bradford and Irvine 2000) and the ensuing motivation to expedite the population's recovery was partially responsible for the formation of the selective fishing policy (DFO 2001). The population collapse was attributed to overfishing, changing ocean conditions, and altered freshwater habitat (Bradford and Irvine 2000). The collapse led to an "endangered" listing by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) and the closure of all directed harvest of wild Fraser River coho salmon in BC waters. To this day, retention of wild coho salmon (distinguishable from hatchery-produced coho salmon whose adipose fins are clipped off prior to release) is restricted in British Columbia waters. This would appear to be a sensible policy based on the fact that the productivity of interior Fraser coho took a marked downturn starting in 1991 such that the population can sustain very little fishing mortality in most years (Decker and Irvine 2013). However, the mortality of coho salmon caught incidentally in fisheries targeting other species remains a source of uncertainty (Decker and Irvine 2013), and most of the bycatch mortality rates currently used in management models are not based on direct, scientifically defensible evidence.

1.5   Hypotheses and objectives

The overarching hypothesis of this thesis is that the magnitude of physiological disturbance resulting from an acute stressor (here, fisheries capture) is inversely proportional to the likelihood of the animal recovering from that stressor and avoiding negative tertiary effects (e.g., migration or spawning failure). Moreover, physiological disturbance (and the likelihood of mortality) is expected to be proportional to the intensity and duration of the capture stressor (Fig. 1.2). However, even if the capture stressor is kept perfectly consistent among individuals, there are *a priori* reasons to expect some variation in stress responses and physiological impairments among individuals (Fig. 1.2). Stress responses can be cumulative (Barton et al. 1986, Schreck 2007) such that recent escapes from predators or other fishing gears could have an effect on the response. Within species, there is also variation in thermal tolerance, exercise performance, hypoxia tolerance, and reactivity to stressors (also termed stress responsiveness; Pottinger and Carrick 2001) – these and other phenotypic variations (see Careau and Garland 2012) could affect responses to capture and subsequent survival. Therefore, it is important to measure the response of individuals to capture stressors (e.g., via reflex assessments) rather than merely measuring the intensity or duration of the stressor as a way of predicting mortality.

This work presented in this thesis addresses several objectives. The chapters are ordered to roughly coincide with the spawning migration of Pacific salmon (Fig. 1.1). The first two chapters (2 and 3) focused on comparing the response of coho salmon to fisheries capture with their post-release survival and migration success. In chapter 2 I telemetry-tagged salmon caught on a marine commercial fishing vessel and tracked their

success in completing the coastal migration to freshwater, and compared PRM data between telemetry-tagged fish and a concurrent short-term holding experiment. Chapter 2 also involved a first marine-based assessment of the RAMP method (in adult Pacific salmon), and I assessed interactions between reflex impairment, blood physiology, and external injury as mechanisms leading to delayed mortality. In chapter 3 I tagged fish caught in aboriginal fisheries in the lower Fraser River, tracked their success in reaching spawning areas, compared RAMP scores to survival, and compared RAMP scores with blood physiology as means of assessing the response to capture. Chapter 4 builds on chapters 2 and 3 by providing a robust mortality estimate for use in management models, assessing options for reducing PRM, and comparing those options with the views of the fishery participants, which were characterized using human dimensions surveys. In the latter three data chapters (5, 6, and 7) I used fish that had reached spawning areas and shifted to an experimental approach. Chapter 5 is a controlled evaluation of the effects of capture variables (stressor duration and water temperature) that could not be assessed in the earlier field-based telemetry studies (chapters 2-4). Instead of assessing mortality, I used physiological assessments to examine response and recovery patterns (the latter could not be assessed in field studies), with the hope that those data could help shed light on underlying causes of impairment and mortality observed in the field. For chapter 6 I also focused on physiological recovery profiles with the objective of comparing three methods of reviving salmon from capture stress – including one that was assessed for its survival benefit in chapter 4. The final data chapter (7) is a field-based experiment with fishing simulations aimed at teasing apart the effects of exhaustion-related stress and dermal injury on post-release fitness outcomes. That study took place at spawning areas

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such that I was able to determine spawning success of fish after release. As such, it represented an opportunity to examine resilience to capture stressors for fish that had completed their migration. In chapter 8, I synthesize findings from the six data chapters and discuss future directions for basic and applied research. Collectively, the work presented in this thesis represents a useful and novel contribution to the literature on the effects of acute stressors on the physiology and fitness of wild fish.

Figure 1.1 Map of the study area where research occurred for this thesis. The spawning migration pathway shown is a general representation for populations of Pacific salmon that spawn in the interior Fraser River watershed (i.e., upstream of Hell's Gate).
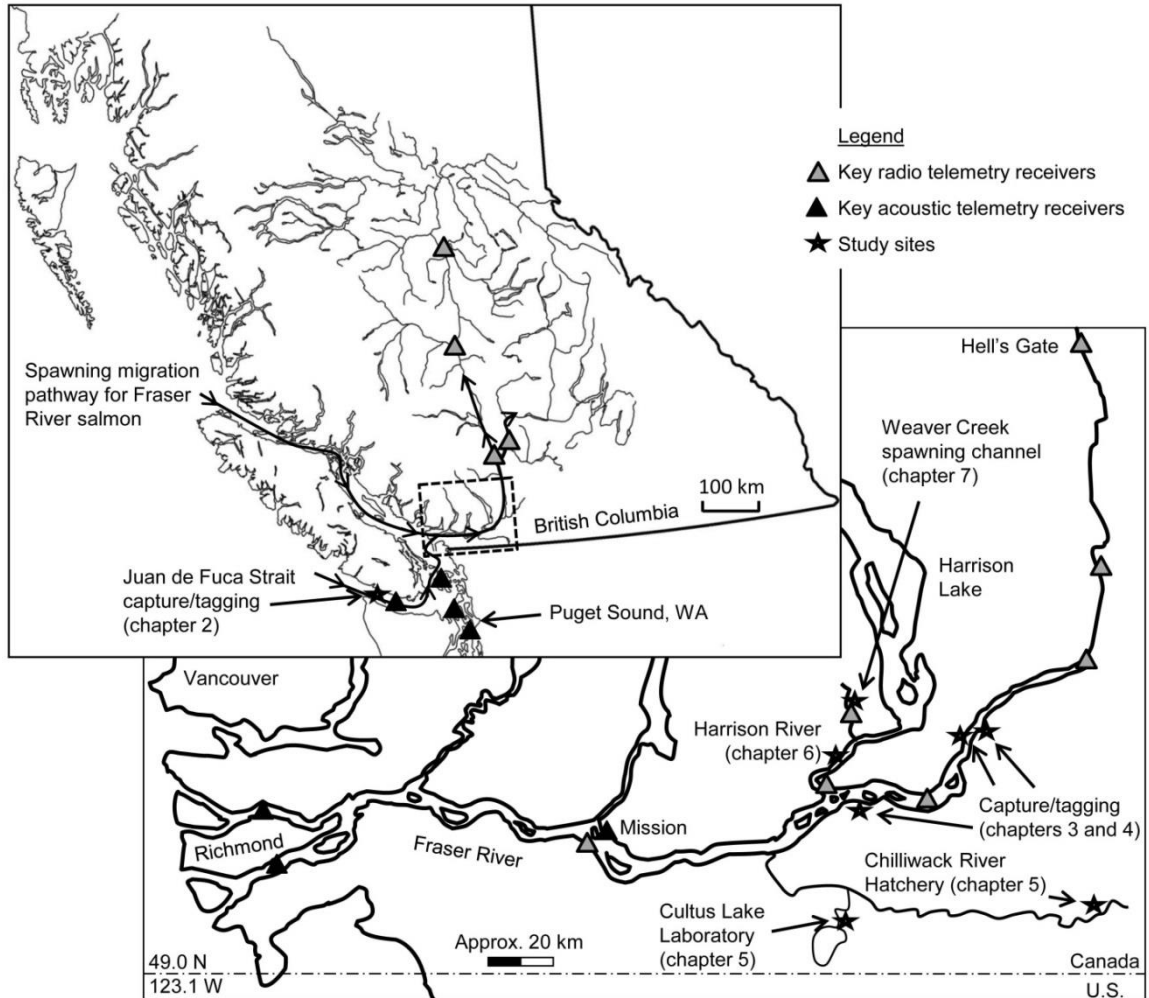
Figure 1.2 A visual representation of the overarching hypothesis of this thesis, based on longstanding notions that the severity (or duration) of acute stressors affects the resulting level of physiological disturbance and impairment of vitality at the animal level (i.e., the response, e.g., Davis and Schreck 1997, Davis 2002), that identical stressors will elicit some inter-individual variation in responses (i.e., the grey area connecting 'stressor' and 'response'), and that resulting vitality indices will predict the likelihood of loss of fitness for that individual (i.e., outcomes, such as survival, migration success, reproductive success; Anderson 2000, Davis 2010). "Stressor severity" on the left-most axis is meant to be inclusive of or interchangeable with stressor duration. For example, a 15 min stressor would be considered more severe than a 2 min version of the same stressor.
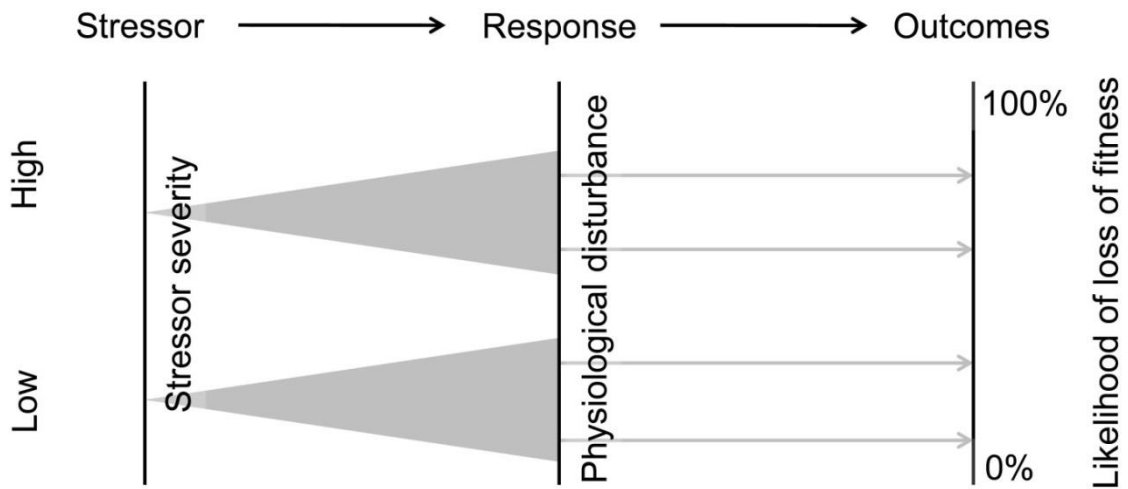
Chapter 2. Mechanisms to explain purse seine bycatch mortality of coho salmon: interactions between injury, reflex impairment, and blood physiology

2.1   Abstract

Research on fisheries bycatch and discards frequently involves the assessment of reflex impairment, injury, or blood physiology as means of quantifying vitality and predicting post-release mortality but exceptionally few studies have used all three metrics concurrently. We conducted an experimental purse seine fishery for Pacific salmon in the Juan de Fuca Strait, with a focus on understanding the relationships between different sublethal indicators and whether mortality could be predicted in coho salmon bycatch. We monitored mortality using a ~24 h net pen experiment (N = 118) and acoustic telemetry (N = 50), two approaches commonly used to assess bycatch mortality that have rarely been directly compared. Short-term mortality was 21% in the net pen experiment (24 h) and estimated at 20% for telemetry-tagged fish (~ 48-96 h). Mortality was predicted by injury and reflex impairment, but only in the net pen experiment. Higher reflex impairment was mirrored by perturbations to plasma ions and lactate, supporting the notion that reflex impairment can be used as a proxy for departure from physiological homeostasis. Reflex impairment also significantly correlated with injury scores, while injury scores were significantly correlated with plasma ion concentrations. The higher time-specific mortality rate in the net pen and the fact that reflexes and injury corresponded with mortality in that experiment but not in the telemetry-tagged fish released into the wild could be explained by confinement stress. While holding

experiments offer the potential to provide insights into the underlying causes of mortality, chronic confinement stress can complicate the interpretation of patterns and ultimately affect mortality rates. Collectively, these results help refine our understanding of the different sublethal metrics used to assess bycatch and the mechanisms that can lead to mortality.

2.2   Introduction

Bycatch mortality in marine commercial fisheries is a leading conservation issue (Kappel 2005) because it can contribute to population or species imperilment (Lewison et al. 2004a, Read et al. 2006) and affect the sustainability of fisheries (Milton 2001, Coggins et al. 2007, Davies et al. 2009). A large component of bycatch is typically discarded, often with the hope that it survives, whereas in reality post-release mortality rates can be substantial but are often unknown (Davis 2002). For management, post-release bycatch mortality is a particular problem in marine systems because it often represents an un-quantified source of fishing mortality that is viewed as wasteful and may negatively affect threatened species (Hall et al. 2000, Coggins et al. 2007).

In recent years a growing body of research has arisen on the use of tools for rapidly assessing delayed bycatch mortality, with several examples of indices of animal condition that have been shown to predict delayed mortality (e.g., Davis 2007, Campbell et al. 2010, Stoner 2012). Increasing in popularity is an approach where a suite of simple reflex responses is rapidly (< 15 seconds) tested for impairment in an animal to be released, and validated as a predictor of mortality (i.e., reflex action mortality predictors,

RAMP – Davis 2010). RAMP goes beyond the traditional approach of categorizing bycatch condition as 'good' or 'poor', because it involves checking for the presence or absence of simple responses using a conservative, consistent, and objective approach which then produces a condition score. Injury and blood physiology can also be used as predictors, given that injuries can strongly influence post-release mortality by compromising osmoregulatory function (Olsen et al. 2012), impairing animal locomotion (Ramsay and Kaiser 1998), and providing a pathway for infection (Udomkusonsri and Noga 2005). Blood plasma measures, such as lactate, provide a glimpse of underlying physiological disturbance at the time of release and can be predictive of mortality in some contexts (e.g., Moyes et al. 2006). Rapid and simple mortality predictors are powerful because of a) the cost and logistical challenges involved in monitoring mortality in wild animals, and b) the utility of having post-release mortality estimates for different contexts. The notion that a greater departure from homeostasis predicts a greater likelihood of subsequent mortality is intuitively appealing - this is the premise upon which RAMP (and other mortality predictors) is largely based. Needed, however, is evidence that reflex impairment reflects underlying physiological disturbance – evidence that could help fisheries managers decide whether implementing the use of RAMP assessments in fisheries observer programs is based on sound science. Moreover, physiological measurements are regarded as objective measures of fish welfare and health (Iwama 2007, Arlinghaus et al. 2009) and have the potential to reveal the cause-and-effect relationships needed to justify management action (Cooke et al. 2013b).

Pacific salmon are a useful model for studying discard fate because they are discarded from multi-sector fisheries (commercial, aboriginal, recreational) while

migrating on a known trajectory towards natal spawning grounds. Of particular concern in British Columbia (Canada) are coho salmon, which are caught incidentally in fisheries targeting other species of salmon (primarily pink salmon and sockeye salmon). Fisheries management (DFO) have strategically adjusted the timing and extent of harvest fisheries for other species to minimize fishing mortality of coho salmon via bycatch. Coho salmon bycatch mortality limits are set by DFO, both overall and for specific fishery openings. If those limits are reached, based on a product of the total bycatch and the mortality rate used, that fishery is typically closed for the season. Mortality rates used vary extensively by fishery and fishing zone (e.g., 5% for in-river beach seines, up to 70% for purse seines, 60-70% for gillnets; DFO 2013). Application of accurate bycatch mortality rates facilitates DFO meeting spawning escapement targets.

Virtually all bycatch mortality rates used by DFO are based on captivity studies, wherein fish are captured and held, usually in a net pen, for a short period of 1-3 days. Temporary captivity is also by far the most common method used to estimate mortality throughout the bycatch literature (Rogers et al. 2014). As an approach to monitoring mortality, short-term captivity has some disadvantages such as the elimination of post-release predation (Raby et al. 2014) and potential imposition of chronic confinement stress (Portz et al. 2006), the latter of which appears to be especially true for migrating adult salmon (Donaldson et al. 2011). Biotelemetry studies are the most common alternative to net pens, and offer the benefit of monitoring animals released back into the wild (Donaldson et al. 2008). The drawbacks of biotelemetry include costs and logistical constraints, difficulty in quantifying tagging effects, and the inability to physically verify the fate of individuals. Side-by-side comparisons of net pen captivity and biotelemetry

have been exceptionally rare in fisheries science and such work could help improve our understanding of the functional benefits and drawbacks to estimating mortality using both approaches – knowledge that would be of use both to fisheries managers and scientists.

This study used coho salmon caught by purse seine in Juan de Fuca Strait to address two primary objectives: 1) compare methods of estimating bycatch mortality, 2) evaluate the interaction of RAMP, injury, body size, and blood physiology in relation to predicting short-term bycatch mortality. To evaluate mortality, we conducted ~24 h net pen holding experiments and an acoustic telemetry tagging experiment using separate groups of fish. This study provides a rare comparison between net pen confinement and biotelemetry as means of monitoring mortality. It is also the first evaluation of RAMP as a vitality index and mortality predictor in salmon caught in the marine environment, after some success in freshwater fisheries (Donaldson et al. 2012, and see chapters 3 and 7) and in numerous other fish and crustaceans (Davis 2010, Stoner 2012). Moreover, this study takes the novel step of exploring relationships between different sublethal effects of fisheries capture: injury, reflex impairment, and blood physiology. Understanding relationships among sublethal metrics used to assess bycatch condition could lead to refined research and management practices in a range of fisheries.

2.3   Methods

2.3.1   Fish capture

This study took place from August 20-27, 2012, in the Juan de Fuca Strait near Port Renfrew, British Columbia (Canada; see Fig. 1.1). In total, 30 purse seine sets were carried out on the Canadian side of the strait, between Sombrio point (48°29'N,

124°17'W) to the east and adjacent to Bonilla Point on the western-most edge of the strait (48°36'N, 124°43'W), ranging in depths from 86 m (near shore) to 220 m (at the international boundary). A 549 m long x 55 m deep seine with 100 mm bunt mesh was deployed and towed by the *Franciscan No. 1* and her power skiff. The net was always deployed such that it was stretched from north to south and towed from east to west, against the general direction of movement of adult salmon homing to coastal spawning streams, as is standard for open water purse seine salmon fishing in this locale. Towing time was always 20 min and the distance covered varied depending on tidal flow (up to 1.5 km towing distance) before the net was closed and pursed. After 20 min, the two ends of the net were pulled together such that the fish were fully encircled (which required 6 min 26 s ± 3 min 25 s; mean ± standard deviation), at which point the net was drawn in and up (pursed) to the side of the boat such that the fish could be retrieved (which required a further 24 min 9 s ± 1 min 45 s). Water temperatures recorded by the boat's subsurface digital thermometer ranged from 8.5 – 9.8 °C (mean = 9.3 °C) during net sets. Weather conditions were consistent during the eight-day study: mostly sunny, little precipitation (6 mm total), with a mean daily air temperature of 14.3 °C (range: 12.8 – 15.5 °C), and a mean daily max temperature of 19.4 °C (18 – 22 °C). All fishing was conducted during daylight hours, with set landing times ranging from 0837 to 1619 h.

Fish were brought from the pursed seined net into the boat using an industry-standard brailer (a large dip net operated with the assistance of hydraulic winch, see Fig. 2.1), directly into a recovery tote (61 cm deep × 109 cm × 119 cm) that was continuously flushed with fresh seawater (Fig. 2.1). Braling resulted in ~ 20 s of air exposure and potential physical trauma due to crushing. In cases where the number of salmon brailed

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into the recovery tote resulted in severe crowding (e.g., > 20 fish in the tote), a portion of

the fish were rapidly transferred with dip nets to a second, smaller recovery tote (61×71

$\times 119$ cm). The two recovery totes used were industry standard "half totes" normally

used to revive bycatch before release. Catches were not brailed into the metal sorting bin

(Fig. 2.1C) prior to transfer to recovery totes, as in a standard fishery, because in each net

set virtually all of the catch was bycatch coho salmon. Catch rates were sufficiently small

that only one brailer load was required for each set (the largest catch was 82 fish). The

small catch sizes and direct brailing into recovery totes were the only two ways in which

our capture process differed markedly from standard commercial practice. Coho salmon

were in recovery totes for a mean duration of 27 min 41 s (range = 10 s – 1 h 47 min)

before they were processed by the research team. The crew was directed to conduct one

experimental set where fish were brailed into the sorting bin and then transferred to the

recovery totes, which added ~ 60 s of air exposure and the potential for further dermal

injury. Coho salmon from that set were used only to test for differences in plasma

measures, RAMP, and injury (see below) and not mortality assessment.

2.3.2   *Reflex, injury, and blood measurements*

Once fish were on board, individual coho salmon were randomly selected from the

recovery totes and dip-netted to an adjacent V-shaped foam-padded sampling trough that

was continuously flushed with seawater. First, fish were held supine for withdrawal of a

1-2 mL sample of blood via caudal puncture (only a subset of fish were blood sampled;

21 gauge needle, 3-mL vacutainer coated with lithium heparin; BD, Franklin Lakes, New

Jersey).

All fish were assessed for reflex impairment (i.e., RAMP; Davis 2010), which required < 15 s to complete and involved checking for the presence of five reflexes (tail grab, body flex, head complex, orientation, vestibular-ocular response) previously described and validated in adult Pacific salmon (see chapters 3 and 7, Donaldson et al. 2012, Nguyen et al. 2014) and other marine fishes (Brownscombe et al. 2013, Cooke et al. 2014). Each reflex was assessed categorically (0 = unimpaired, 1 = impaired) in a conservative manner – that is, if the handler had doubt as to whether the reflex was present it was recorded as being impaired. Presence of the tail grab response was assessed by the handler attempting to grab the tail of the fish with the fish submerged in water (in the sampling trough); a positive response was characterized by the fish attempting to burst-swim immediately upon contact. The body flex response was tested by holding the fish out of water using two hands wrapped around the middle of the body. The fish actively attempting to struggle free was scored as a positive response. Head complex was noted as positive if, when held out of water, the fish exhibited a regular pattern of ventilation (for ~5 s) observable by watching the opening and closing of the lower jaw. VOR was observed by turning the fish on its side (i.e., on a lengthwise axis) out of water. Positive VOR was characterized by the fish's eye rolling to maintain level pitch, tracking the handler. Finally, the orientation reflex was noted as impaired (absent) if the fish failed to right itself within 3 s when turned upside-down below the water's surface. The entire reflex assessment took ≤ 20 s to complete. If a fish was too vigorous to allow researcher handling and assessment of reflexes, it was assigned an unimpaired status for all reflexes. The reflex actions included in our protocol are thought to be sufficiently varied that they involve different neurological pathways and/or muscle groups such that there are no

redundancies. For example, some of the reflexes are part of the autonomous nervous system (head complex, i.e., respiration) while others are clearly not (tail grab, body flex). Moreover, using this RAMP protocol with Pacific salmon, no two reflexes in the suite of five are consistently present/absent together (see chapters 3 and 7). The reflex responses were used to calculate a RAMP score of 0-1 that represents the proportion of reflexes that were impaired. Thus, a high RAMP score corresponds to a fish in poor condition.

For each coho salmon, we also recorded observations about whether gillnet-like injuries were visible around the head (0/1), or the centre of the body (0/1). Gilling injuries were characterized by a combination of dark contusion lines and focused rings of scale loss. In addition, we noted whether overall scale loss was low ($< 5\%$ of total body), moderate (~ 5-30%), or high ($> 30\%$), which was scored as a 0, 1, or 2 respectively. Finally, we recorded whether any other injuries were present (0/1), such as old hooking or predator wounds. The injury scores were combined into an overall injury score (proportion) in a similar manner to RAMP scores, by dividing by the highest possible total (i.e., 5). Thus, a higher injury score represented a fish that experienced greater and/or more numerous injuries. For some analyses we also used a combined condition score which was a combined proportion of RAMP and injury scores.

After blood sampling (when applicable) and RAMP-injury assessments, fish were measured (fork length, FL, nearest cm), followed by removal of ~ 0.5 g piece of adipose fin tissue using a hole-punch (except for hatchery-origin whose adipose fins are removed prior to release to the wild as smolts) which was stored in 95% ethanol for later analysis of DNA. Finally, coho salmon were tagged for the net pen holding experiment (mix of

hatchery-origin and wild fish, see section 2.3.3) or telemetry tracking (wild fish only, see section 2.3.4).

Additional fish were assessed (as above) to increase our sample sizes for evaluation of interactions among capture variables, and those fish were released untagged following assessment (i.e., if the holding experiment was already underway or to conserve transmitters for other net sets or study days). Many of the coho salmon caught were not used in this study and were merely counted; some were lethally sampled for a separate study, while others were simply released because of time limitations (i.e., so that fishing could resume). We did not collect tissue for population-identification of hatchery-origin fish (e.g., from operculum clips). Hatchery-origin fish (identifiable by missing adipose fins) were likely to have consisted of a mix of origins in the United States and Canada but with the majority coming from tributaries in the Puget Sound (WA, U.S.A.) basin (based on high hatchery production there and the high proportion of Puget Sound fish among wild fish sampled, see *Results*). Hatchery-origin coho salmon were included to ensure adequate sample sizes (except for in the telemetry component) because they consisted of ~50% of the total catch of coho salmon. Although they have inherently different conservation value than wild fish, incidental mortality of hatchery coho salmon is not distinguished from that of wild-origin salmon for bycatch management purposes in marine commercial fisheries. Both sets of fish would have presumably migrated to sea from freshwater 1.5-2.5 years earlier after either leaving natural rearing areas (wild) or having being released from (often adjacent) hatchery facilities.

Adipose fin tissue samples collected from coho salmon for which mortality was assessed, was used for population-origin identification via analysis of variation of 17

microsatellite loci (Beacham et al. 2011). Analyses identified fish to individual spawning streams, but spawning stream fidelity within subpopulations can be low in some years if low flows necessitate finding an alternate spawning site (R. Bailey, DFO, *personal communication*). For that reason and for statistical power, fish were grouped into major population groupings for analyses (Beacham et al. 2011).

Blood plasma samples were stored in an ice slurry for <1 h before they were centrifuged at $7,000 \times g$ for 5 min, after which plasma was separated and stored in liquid nitrogen until later transfer to a -80 °C freezer. Plasma was then analyzed in the laboratory for cortisol (Neogen enzyme-linked immunosorbent assay with Molecular Devices Spectramax 240pc plate reader), chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Palmer, model 410 single-channel flame photometer), osmolality (Advanced Instruments 3320 freezing-point osmometer), and lactate and glucose (YSI 2300 Stat Plus analyser) using methods described previously (Farrell et al. 2001a).

2.3.3   Short-term holding experiment

One of the two large, below deck holds on the *Franciscan No. 1* that is normally used for packing target catch on ice was used as a holding tank for ~ 24 h after capture to monitor short-term mortality. Only the area of the tank immediately below the 1.8 m x 1.4 m hatch was used – this area was sectioned off by means of a net pen that could be easily drawn to the surface as needed to retrieve fish. The net pen was 1.8 m wide x 1.4 m long x 2 m deep and made from 70 mm diamond soft nylon mesh, within a tank that was approximately double the volume of the net pen. The tank was continuously flushed with fresh seawater using an on-board bilge pump and pure oxygen was bubbled through a

diffusion plate at the bottom of the net pen, as needed, to maintain saturation between 85 – 115 % through the 24 h period. The tank was kept covered and in darkness during the entire holding period. Fishing operations were able to continue while fish were held below deck. Seas were mostly calm during the eight day study, and periodic visual inspection confirmed that the water in the tank remained hydrodynamically neutral while the boat rolled over waves, with no apparent swaying of fish within the tank. Overnight, the boat was anchored inside Port San Juan across from Port Renfrew (48°33'N, 124°28'W). In that location, the water was visibly more turbid than in the Strait, ~ 2 °C warmer, and likely contained some mixture of freshwater. The following day while in Juan de Fuca Strait, 24 h after the most recently tagged fish entered the tank; the fish in the holding study were removed individually by dip net and processed in the sampling trough.

All fish placed in the holding tank for monitoring were tagged with a uniquely numbered spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) that was threaded through the dorsal musculature just posterior to the dorsal fin and tied with a double knot. Sixty (60) of 118 fish in the holding study also had 'dummy' transmitters (8 mm diameter, 20.5 mm length, made from high density plastic) threaded onto the spaghetti tags to test short-term retention of the transmitter to be used in the telemetry component (2.3.4). A subset of fish was blood sampled: 32 of 60 fish with dummy transmitters, and 33 of 58 with only spaghetti tags. Of the 118 fish that were used for the 24 h net pen experiment, 84 were wild fish (intact adipose fins), while the other 34 were hatchery-origin (adipose fin clipped). Post-24 h processing included a RAMP assessment, a blood sample for a subset of fish, removal of the spaghetti tag, and description of macroscopic

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condition, followed by release overboard after removal of any tags. The net pen

experiment was replicated four times (N = 36, 25, 32, and 25 salmon, respectively), each

iteration comprising fish caught in three, one, two, and one net set(s), respectively (Table

2.1). Fish were taken from multiple net sets in some cases in order to increase sample

sizes, which resulted in a range of durations of net pen confinement. The longest gap

from first to last entry into the net pen (from round one of the holding study) was 7.5 h,

such that the fish in that replicate group were held for 24-31.5 h depending on when they

were caught and tagged relative to when the experiment was terminated (details on each

replicate in Table 2.1). The overall mean duration of net pen monitoring (from tagging to

retrieval) was 26.80 h, but the duration was significantly longer for the first iteration of

the holding study than for the three other replicates, because fish were taken from three

net sets (based on Tukey HSD post-hoc comparisons after significant among-group

differences for analysis of variance [ANOVA]; $F_{3,113}$ = 36.4, $P < 0.001$; see Table 2.1).

2.3.4   Telemetry tracking

From August 21-27, 50 wild coho salmon were externally affixed with acoustic

transmitters and immediately released to resume their migrations. We used a 'backpack'-

style attachment method with 8 mm diameter acoustic tags that transmitted their unique

codes every 25-65 s (random time delay; V8-4X with pre-installed end caps, 30 mm

length, 2.95 g in air; VEMCO, Bedford, NS, Canada). The transmitters were threaded

onto a spaghetti tag posterior to the dorsal fin such that they laid flush against the back of

the fish, behind the dorsal fin (Fig. 2.2). This method of tagging required < 30 s to

complete, involved no anesthetic or suturing, and avoided the risk of regurgitation that

can be a concern when gastrically tagging adult salmon that may continue feeding during the coastal approach to freshwater. The 50 coho salmon we tagged were selected at random from 21 different net sets, representing a range of post-capture recovery times, RAMP and injury scores. Blood samples were not taken from telemetry-tagged fish to minimize handling and injury that could potentially affect post-release outcomes.

For this chapter, the acoustic telemetry data were used solely to assess survival. Fish were classed as short-term survivors if they were detected by the Juan de Fuca (JDF) acoustic receiver line, which consisted of thirty receivers arranged in a straight line from Sheringham Point on the Canadian shoreline (48°22'N, 123° 55'W) to Pillar Point on the American shoreline (48°13'N, 124°6'W). The JDF line was en route to spawning areas for all populations of coho salmon we encountered, except for two fish DNA-identified to a coastal Washington stream (Clearwater Creek). Those two fish were nevertheless detected on the JDF line and classified as short-term survivors. On average, the JDF line was 32 km from the release site to the first receiver at which fish were detected (range: 25-41 km), and thus represented an appropriate short-term migration checkpoint. It appeared to have 100% detection efficiency because all of the fish detected there were detected on multiple receivers on the line, often by two or more receivers simultaneously, and no fish detected at subsequent receivers was undetected there.

Long-term survival was assessed for 43 telemetry-tagged fish whose migration pathway towards natal tributaries (assessed via DNA analysis) had appropriately placed receiver infrastructure. For Puget Sound-origin coho salmon, long-term survival was based on success moving past a 13-receiver array that crossed Admiralty Inlet (between 48°4'N, 122° 40'W and 48°4'N, 122°37'W), the entrance to Puget Sound. Out of 14 coho

salmon that were detected on various receivers in Puget Sound beyond the Admiralty line, three were never detected on the Admiralty line (21.4%). Thus, detection efficiency for that line was 78.6%. That detection efficiency was used as a correction factor to estimate the total number of individuals that were successful in reaching the Admiralty receiver line (i.e., long term survival for Puget Sound stocks). In addition, two Puget Sound fish were detected on receivers in the San Juan Islands (48°41.5'N, 123°14.5'W and 48°47.2'N, 122°58.3'W) but not elsewhere beyond the JDF line – those fish were included as long-term survivors. For Fraser River stocks, long-term survival was assessed based on detection in the Fraser River. All fish detected in the Fraser River progressed upstream past all four receiver arrays there, with the upstream-most receiver array ~ 70 river km upstream of river entry, at Mission (49°7'N, 122°18'W). We assumed a 100% detection efficiency for the lower Fraser River lines because each fish was detected on all four lines. The few fish from other spawning populations (e.g., Vancouver Island, coastal Washington) were not included in analysis of long-term survival due to lack of appropriately placed receiver along their presumed migration pathway, beyond the Juan de Fuca line.

2.3.5   *Data analysis and statistics*

Principal components analysis (PCA) was used to distill the original variables into one or more synthetic variables (factors) for further analyses. The former was accomplished by running a PCA using all blood samples (at-capture and post-24 h, N = 231). We sought to use factors for further analyses that would represent and replace two or more of the original variables. From the initial PCA, variables were successively removed and the

analysis re-run if a) a variable had a Kaiser-Mayer-Olkin (KMO) measure of sampling

adequacy of < 0.5 (Field et al. 2012), or b) a plasma variable's only factor loading > |0.6|

was for a factor which had no > |0.6| factor loadings from other variables (and thus, that

factor would largely be replicating one of the original variables rather than synthesizing

two or more). Scores from the resulting factor (PC1, un-rotated) along with four original

plasma variables not included in the final PCA were compared between at-capture and

post-24 h using Welch's t-test. We similarly used t-tests and Kruskal-Wallis ANOVA to

explore whether there were effects of the following at-capture variables on post-24 h

physiology: loss of orientation (0/1), attachment of dummy transmitter (0/1), caudal

puncture blood biopsy (0/1), and injury score (0/0.2/0.4). Loss of orientation was used in

place of RAMP score for comparisons of 24 h blood data because it was shown to be

particularly influential in 24 h mortality whereas RAMP score was not (see *Results*).

Since we ran each of the above tests with five separate blood variables, α was set at 0.01.

The plasma variables we measured change along a predictable trajectory

following exhaustive exercise stress (e.g., Wood et al. 1983, Milligan et al. 2000,

Marcalo et al. 2006), and fish in this study were sampled after varying amounts of time in

the recovery tote after capture. Thus, there was a need to control for the effect of

sampling time on blood physiology (see Cooke et al. 2013a) to assess the influence of

other variables such as RAMP or injury scores, which did not change over time in the

recovery tote (see *Results*). To do so, each of the five plasma variables (PC1 scores,

plasma lactate, cortisol, potassium, and glucose) was regressed using linear regression

against time spent in the recovery tote prior to blood sampling (Fig. 2.3). For regressions

that were significant, the residuals were used for further analyses of at-capture blood

physiology in place of the raw data. To assess relationships between RAMP and injury scores and the five physiological responses (time-controlled) we used Kruskal-Wallis ANOVA. If Kruskal-Wallis ANOVA was significant ($\alpha = 0.01$), we assessed directional trends across groups using Spearman rank-order correlation and the Jonckheere-Terpstra test ($\alpha = 0.05$). The Jonckheere-Terpstra test is analogous to Kruskal-Wallis ANOVA except groups are ordinal such that the test evaluates whether the order of group medians is meaningful (Field et al. 2012). Because the combined condition score involved up to ten condition levels, only spearman rank-order correlations were used to assess its relationship with plasma variables.

Predictors of post-release mortality were assessed using forced entry logistic regression (Field et al. 2012). For the on-board holding experiment, we ran two models: one using only the five physiological variables (time controlled, as above) for the subset of fish that were blood sampled prior to the holding study ($N = 63$), and a second with seven non-blood variables ($N = 118$): whether a blood sample was taken at capture (0/1), whether a dummy transmitter was attached (0/1), fork length (cm), time spent in the recovery tote (s), injury score, RAMP score, and loss of orientation at capture (0/1). Two separate models were used for post-release mortality among telemetry-tagged fish: one for short-term mortality ($N = 50$) and one for long-term mortality ($N = 43$). Those models used the same predictor variables as the second model described above except that the effects blood sampling and dummy transmitters were not included (not applicable). All statistical procedures were completing using R (v. 3.0.2; R Core Team 2013).

Figure 2.1 Photo showing the process by which the purse seine catch was brought on board; by transferring fish using a brailer (A) into an industry-standard recovery tote (B). In a true fishery, catch would be brailed into the metal sorting bin (C) and then sorted into recovery totes (or onto ice, in the case of the harvest species).
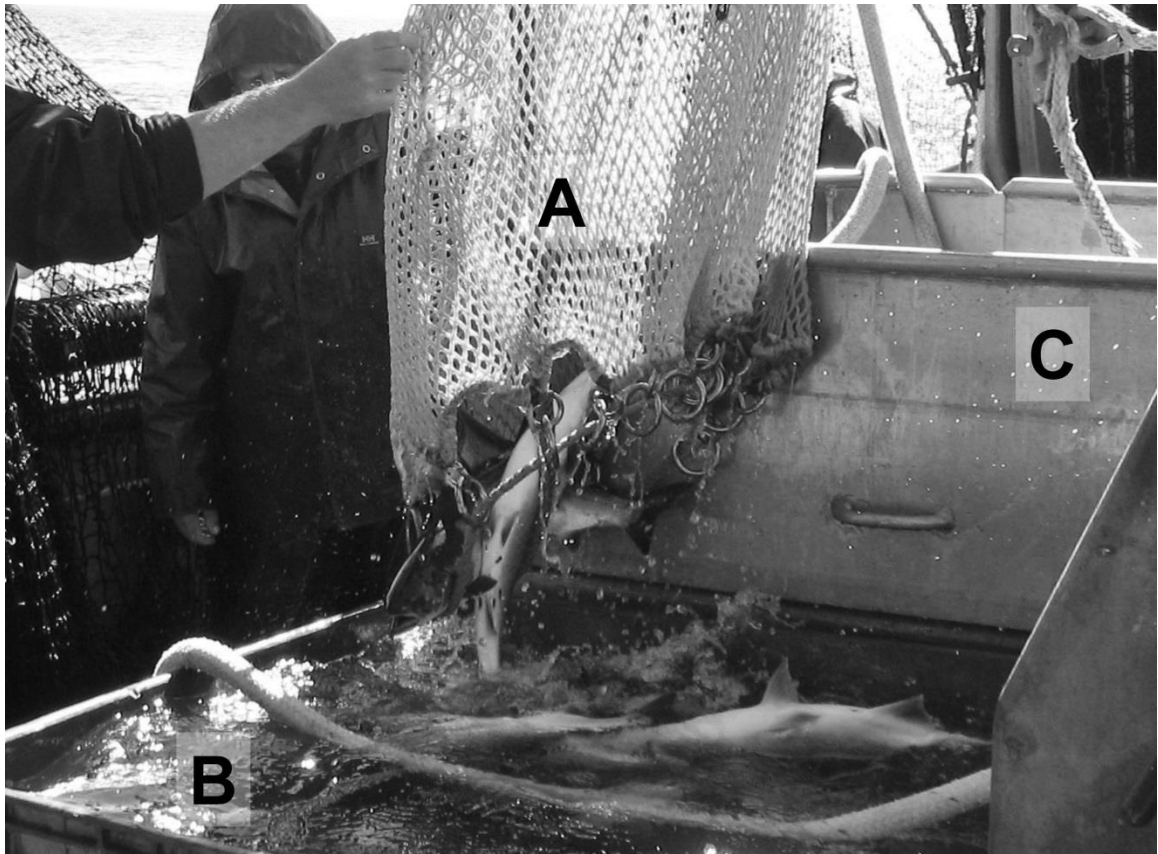
Figure 2.2 Photo depicting the external 'back-pack' acoustic transmitter attachment method used for this study. The spaghetti tags and dummy transmitters used in the net pen holding study were attached in the same way.

Table 2.1 Dates, capture variables, animal condition, and holding durations for each of the four replicates of the short-term holding experiment. The holding duration varied based on when a fish was captured, processed, and the exact time when the holding experiment was terminated. For survival rates, moribund fish were included as mortalities (which were 3 of 10, 0 of 3, 3 of 9, and 1 of 3 total mortalities counted for each of the four replicates, respectively). Differences among replicates were statistically significant for RAMP score such that fish in replicate 1 of the experiment had significantly higher RAMP scores than those replicates 3 and 4 (Kruskal-Wallis ANOVA $P < 0.001$, post-hoc differences assessed using multiple comparisons). Fish in replicate 1 were also held in the net pen for a longer duration, on average, than the other three replicates (based on Tukey HSD comparison, ANOVA $F_{3,113} = 36.4$, $P < 0.001$). Differences among replicates for injury scores (Kruskal-Wallis ANOVA) and time in recovery totes (ANOVA) were not significant ($P > 0.10$ in both cases). The number of mortalities was not significantly different among the four replicates of the holding study (Chi-square test, $\chi^2 = 0.82$, df $= 3$, $P = 0.84$).

Replicate	Dates (dd/mm/yy)	Total catch for sets used (number of fish)	Net set water temperatures (°C)	Mean mm:ss in recovery tote (range)	RAMP score (mean ± S.D.)	Injury score (mean ± S.D.)	Mean holding duration in hours (range)	Survival rate (number of fish)
1	20/08/12-21/08/12	58, 20, 28	9.6, 9.7, 9.2	36:20 (00:30-104:00)	0.47±0.19	0.14±0.16	28.97 (24.00-31.45)	72% (26 of 36)
2	22/08/12-23/08/12	46	9.4	35:57 (00:59-75:20)	0.35±0.12	0.23±0.18	25.30 (24.55-26.06)	88% (22 of 25)
3	24/08/12-25/08/12	15, 50	9.2, 8.7	32:17 (02:10-70:07)	0.30±0.20	0.20±0.22	26.26 (24.95-28.04)	72% (23 of 32)
4	26/08/12-27/08/12	66	9.5	33:15 (02:02-67:03)	0.32±0.14	0.24±0.19	25.78 (25.07-26.31)	88% (22 of 25)
	Weighted mean	48.13	9.32	34:29	0.36	0.20	26.80	79% (93 of 118)

2.4 Results

2.4.1 Catch characteristics

Total catch of salmon for individual purse seine sets was consistently low (mean = 37 adult salmon using the standard netting, range = 0 – 82 fish) but coho salmon did make up 83% of this total (~ ½ were hatchery origin). The remaining Pacific salmon were sockeye (10% of catch), Chinook (6%), chum (< 0.5%), and pink salmon (< 0.5%). Of the 137 wild coho salmon for whom we determined population-origin (using DNA), 67% belonged to populations in Puget Sound (Washington, USA) and only 8% were identified to the interior Fraser River watershed (10% to the Fraser River overall; Table 2.2). RAMP scores (Kruskal-Wallis ANOVA, $\chi^2 = 4.7$, df = 5, $P = 0.46$), injury scores ($\chi^2 = 4.8$, df = 5, $P = 0.44$), and fork length ($\chi^2 = 10$, df = 5, $P = 0.08$) were not significantly different among major population groupings (Table 2.2). Rapidly sorting bycatch in a dry sorting box prior to transfer to recovery totes did not result in significantly different blood physiology for the one set where it was used ($P > 0.05$) except for time-corrected plasma potassium, which was marginally lower for fish exposed to the sorting box ($t_{28} = -2.8$, $P = 0.009$). Use of the sorting box resulted in no significant differences in RAMP or injury scores (Wilcox rank-sum test, $P > 0.10$ for both).

2.4.2 Bycatch mortality estimates by method

The immediate bycatch mortality rate (i.e., died during capture or in the revival totes) for coho salmon was 2% (15 of 673 fish). The 24 h mortality rate for fish held in the on-board tank was 21% overall (25 of 118; Table 2.3), which included seven fish categorized as moribund. Moribund fish were characterized by fungus-infected eyes that caused loss

of the VOR reflex (and likely blindness; 6 of 7 cases) and some loss of other reflexes, or extreme lethargy characterized by complete loss of reflex actions. The logistic regression model predicting mortality in the holding experiment that included all fish and excluded plasma variables explained more of the variation in mortality (N = 118, χ^2 = 40.1, df = 7, $P < 0.001$, Nagelkerke R^2 = 0.45) than that which used only plasma variables (N = 63, χ^2 = 11.3, df = 4, P = 0.045, R^2 = 0.25). Injury score (P = 0.003) and loss of the orientation reflex ($P < 0.001$; but not RAMP score) were both positive predictors of mortality within the main model (Table 2.3). The amount of time spent in the on-board recovery tote did not significantly affect survival. Among plasma variables, only glucose was predictive of 24 h mortality; higher glucose at capture (controlling for sampling time) was associated with lower mortality (P = 0.02, Table 2.3). The number of mortalities was not significantly different among the four replicates of the holding study (Chi-square test, χ^2 = 0.82, df = 3, P = 0.84; Table 2.1).

Short-term post-release mortality of coho salmon (~ 48-96 h) based on telemetry tracking was 20% (10 of 50 fish were undetected on the JDF receiver line). The long-term mortality rate was 47% (20 of 43 failed to pass receiver lines in Puget Sound or the Fraser River). Among the five interior Fraser coho salmon that were telemetry-tagged, four (80%) successfully reached the Fraser River and progressed upstream past all receivers there. However, small sample sizes precluded statistical assessment of whether survival was different among populations. Logistic regression models predicting short-term (Nagelkerke R^2 = 0.25, P = 0.14) and long-term mortality (R^2 = 0.21, P = 0.20) for telemetry-tagged fish were not significant.

2.4.3   Blood physiology at capture and after 24 h confinement

PCA revealed very strong positive associations among plasma chloride, sodium, and osmolality within the overall dataset of seven plasma variables (Table 2.4). Low KMO statistics (< 0.5) and lack of sufficient shared variation within a factor among the other plasma variables meant that they were successively eliminated from the PCA until only chloride, sodium, and osmolality remained. Thus, the final PCA produced a single principal component (PC1) variable that was used in further analyses as an integrator of the strong shared variation (Table 2.4) between those three measures. The other four plasma variables were used separately for further analyses.

There were some notable changes in plasma physiology in coho salmon following a ~24 h on-board net pen confinement (shaded areas in Fig. 2.3). Cortisol was significantly elevated after ~24 h (541 ± 31 ng mL^{-1}) compared with the post-capture blood samples (331 ± 17 ng mL^{-1}; Welch's t-test using log-transformed data; $t_{142} = $ -6.6, $P < 0.001$; Fig. 2.3E). Circulating plasma lactate was significantly lower after ~24 h (2.3 ± 0.16 mmol L^{-1}) than in the minutes following capture (13.8 ± 0.42 mmol L^{-1}; $t_{134} = $ 25.8, $P < 0.001$; Fig. 2.3B). Log-transformed plasma glucose ($t_{134} = 25.8$, $P < 0.001$) and potassium ($t_{160} = $ -6.3, $P < 0.001$) were also significantly elevated at ~24 h relative to post-capture samples, while PC1 scores were not significantly different between capture and 24 h ($t_{160} = $ -1.1, $P = 0.30$; Fig. 2.3).

Post-24 h blood physiology was not significantly different between fish that did and did not have an attached dummy transmitter (Welch's t-test; all $P > 0.10$) and was not affected by whether the fish had been previously blood sampled (all $P > 0.20$). Log-transformed plasma lactate was significantly higher at 24 h for fish that had lost

orientation at capture than in fish that had not ($t_{16} = -5.3$, $P < 0.001$), but no other

significant differences in 24 h blood physiology were predicted by loss of orientation (all

$P > 0.20$). PC1 scores were significantly different among injury scores after 24 h

(Kruskal-Wallis ANOVA; $\chi^2 = 13$, $P = 0.002$) with post-hoc tests indicating significant

elevation for PC1 at 0.4 relative to fish with an injury score of 0 at capture. The other

physiological variables were not significantly different among injury scores (all $P >$

0.01).

Time from landing to blood sampling (i.e., time spent in the recovery tote) had a

significant positive effect on each of the five plasma variables (Fig. 2.3). However, the

relationship with potassium could not be transformed to meet both the assumptions of

heteroscedasticity and normality, so that regression was not used for further analyses. For

the other four plasma variables, the residuals of their regressions with sampling time (Fig.

2.3) were used for further analyses (section 2.4.4, below, and for the physiology-

mortality net pen model, section 2.4.2), as a means of controlling for the effect of

sampling time.

2.4.4 Relationships among sublethal measures

RAMP and injury scores were positively correlated (Spearman's rank-order correlation,

$r_s = 0.27$, $P < 0.001$). Catch size was positively correlated with RAMP score ($r_s = 0.36$, P

< 0.001), injury score ($r_s = 0.19$, $P = 0.02$), and the combined condition score ($r_s = 0.35$,

$P < 0.001$). Conversely, time spent in the recovery tote after capture did not correlate

significantly with RAMP score, injury score, or the combined condition score (all $P >$

0.20). Moreover, for individual reflexes there was no apparent change over time: the

duration of recovery in the revival totes did not differ significantly between fish with and without each of the reflexes (Welch's t-test, all $P > 0.25$). RAMP score had a significant positive relationship with the plasma PC1 response (i.e., controlling for sampling time – see Fig. 2.3), plasma lactate response, and a significant negative relationship with plasma cortisol response (Table 2.5, Fig. 2.4). Similarly, there was a significant positive trend in the PC1 response relative to injury scores (Table 2.5, Fig. 2.5). The combined condition score (RAMP + injury) was positively correlated with PC1 and lactate responses and negatively correlated with the cortisol response (Table 2.5, Fig. 2.6) but there was no pattern with the other blood variables measured.

Overall, fork length was negatively correlated with injury score (Spearman correlation, $r_s = -0.18$, $P = 0.03$) and RAMP score ($r_s = -0.29$, $P < 0.001$). There was no correlation between fork length and time-corrected cortisol ($r_s = 0.15$, $P = 0.06$), or potassium ($P = 0.15$) but significant negative correlations did occur between fork length and lactate ($r_s = -0.20$, $P = 0.01$), glucose ($r_s = -0.17$, $P = 0.03$), and the PC1 response ($r_s = -0.29$, $P < 0.001$).

Figure 2.3 Linear regressions between five plasma variables in purse seined coho salmon and the time elapsed from capture (and transfer to a recovery tote) to blood sampling (N = 157 for each regression). PC1 score is a principal component variable that represents chloride, sodium, and osmolality (all positively correlated with PC1; see Table 2.4). The shaded area represents mean (±S.E.) values from fish sampled after being held ~24 h in the below-deck holding tank (N = 59). The residuals of each of these regressions were used for subsequent statistical analyses to control for the effect of sampling time, except in the case of potassium where the assumption of heteroscedasticity could not be met. Each regression was significant ($P < 0.001$), and the lines were described by (with standard error of the slope): (A) $[K^+] = [4.9 \times 10^{-4} (1.1 \times 10^{-4}) \times$ Time (s)$] + 2.25$; (B) [lactate] $= [2.8 \times 10^{-3} (2.4 \times 10^{-4}) \times$ Time (s)$] + 1.03$; (C) [glucose] $= [4.3 \times 10^{-4} (1.1 \times 10^{-4}) \times$ Time (s)$] + 5.76$; (D) PC1 score $= [3.2 \times 10^{-4} (3.4 \times 10^{-5}) \times$ Time (s)$] + -0.63$; (E) $\log_{10}$Cortisol $= [0.24 (0.04) \times \log_{10}$Time (s)$] + 1.75$.
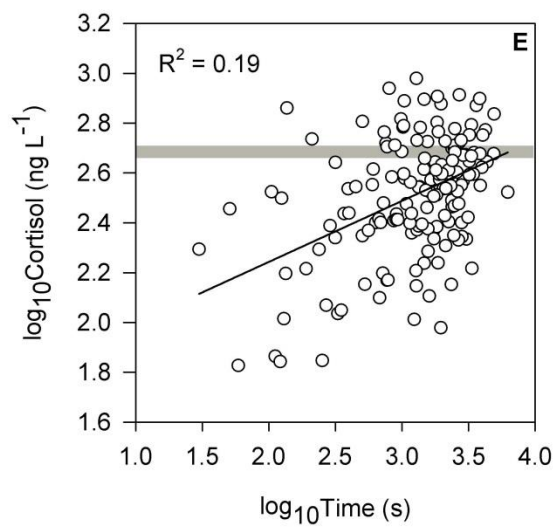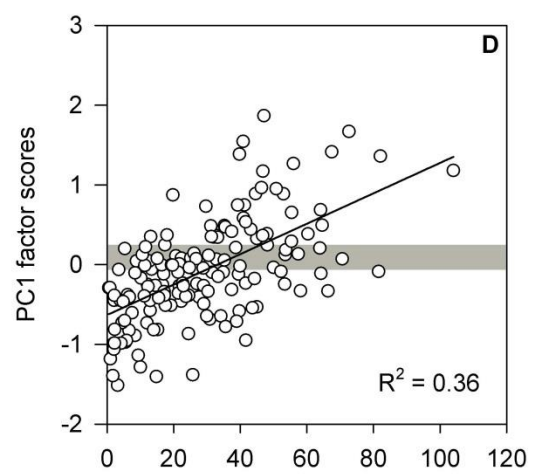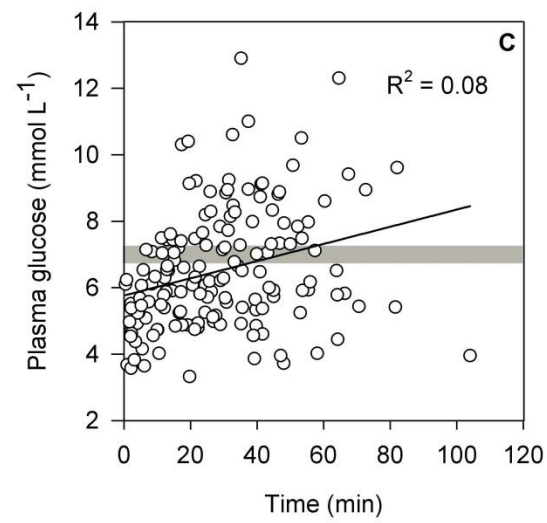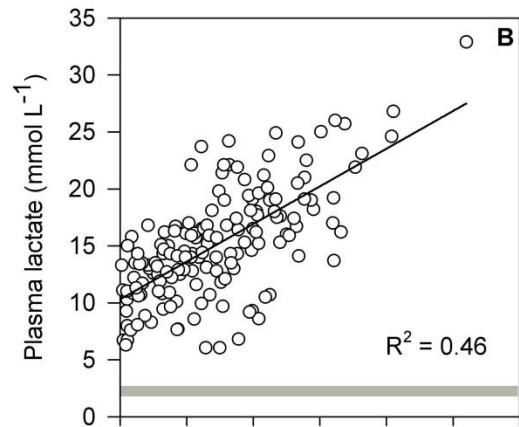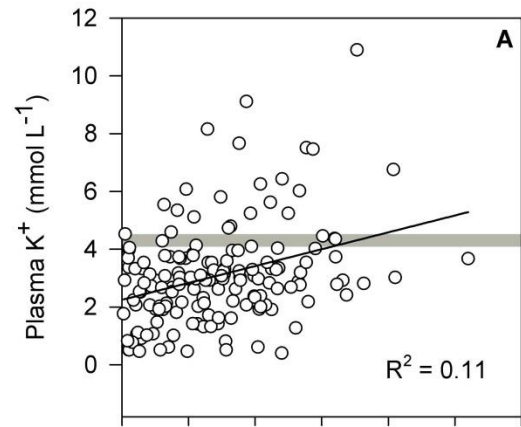
Figure 2.4 Differences in physiological responses among fish assigned different RAMP scores (see Table 2.5 for statistics). Y-axis values represent blood concentrations of lactate (A), cortisol (B), and a combination of sodium, chloride, and osmolality (C – the PC1 variable; Table 2.4) corrected for the time-point at which they were sampled after capture, using the residuals of a regression with sampling time (Fig. 2.3). Higher RAMP scores correspond to fish in poorer condition, and simply represent the proportion of reflexes that were impaired (see 2.3.2 in *Methods*).
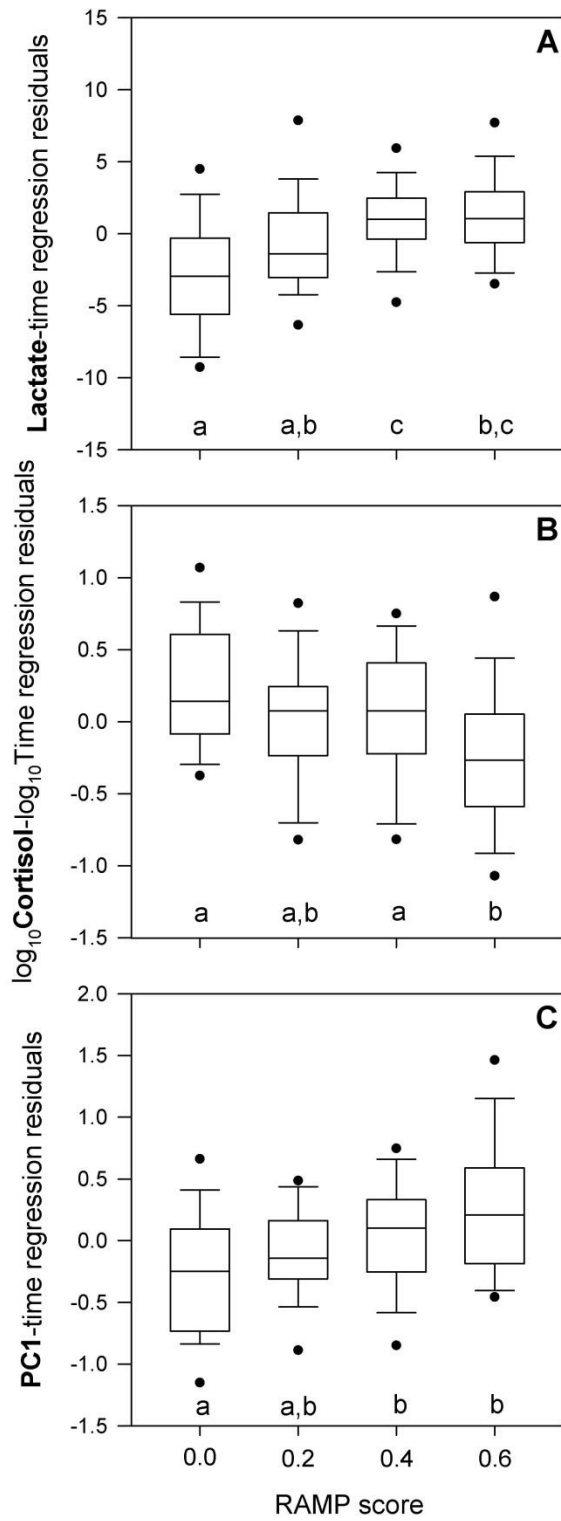
Figure 2.5 Differences in the PC1 (i.e., a synthetic variable that combined plasma sodium, chloride, and osmolality – Table 2.4) response of coho salmon assigned different injury scores (for statistics, see Table 2.5). Injury scores were based on the extent of scale loss and the number of different injury types that occurred, with higher values indicating a fish in poorer condition (see 2.3.2 in *Methods*).
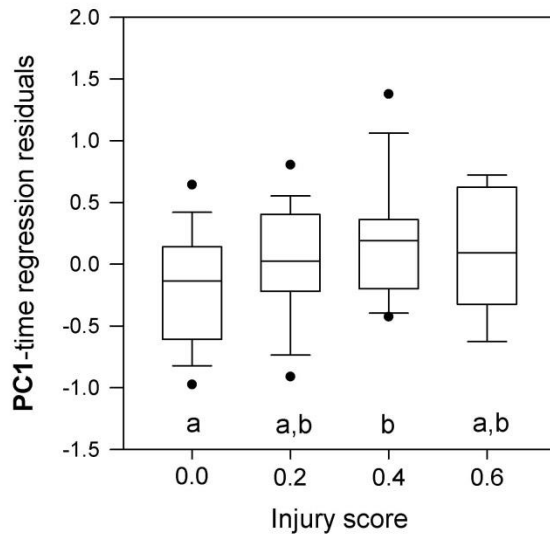
Figure 2.6 Differences in physiological responses across combined condition scores, which represent a simple combination of RAMP and injury scores (see Table 2.5 for statistics). Y-axis values represent blood concentrations of lactate (A), cortisol (B), and a combination of sodium, chloride, and osmolality (C – the PC1 variable; Table 2.4) corrected for the time-point at which they were sampled after capture, using the residuals of a regression with sampling time (Fig. 2.3).
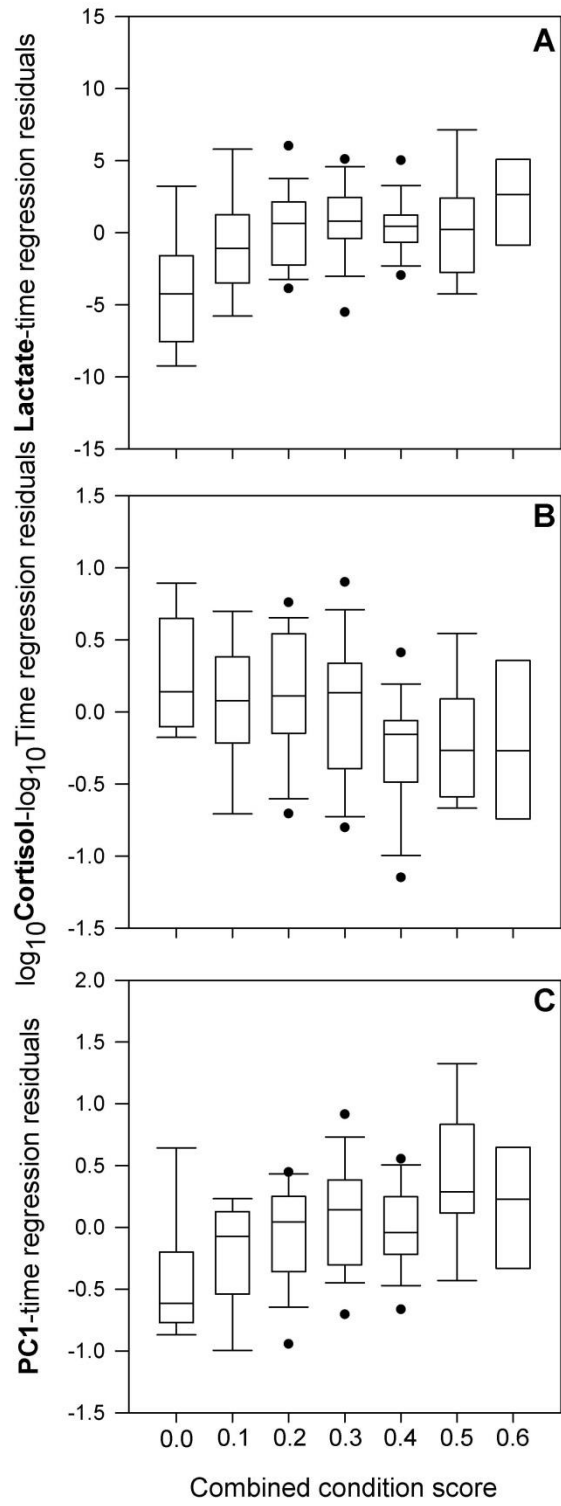
Figure 2.7 Mortality rates specific to fish assigned different injury scores in the telemetry study (A) and net pen experiment (C) and specific to different RAMP scores (B – telemetry, D – net pen). The numbers in each bar represent the total number of fish assigned that condition score. RAMP and injury scores represent proportions, whereby higher scores denote fish in poorer condition (i.e., more reflexes impaired or more injury; see 2.3.2 in *Methods*).
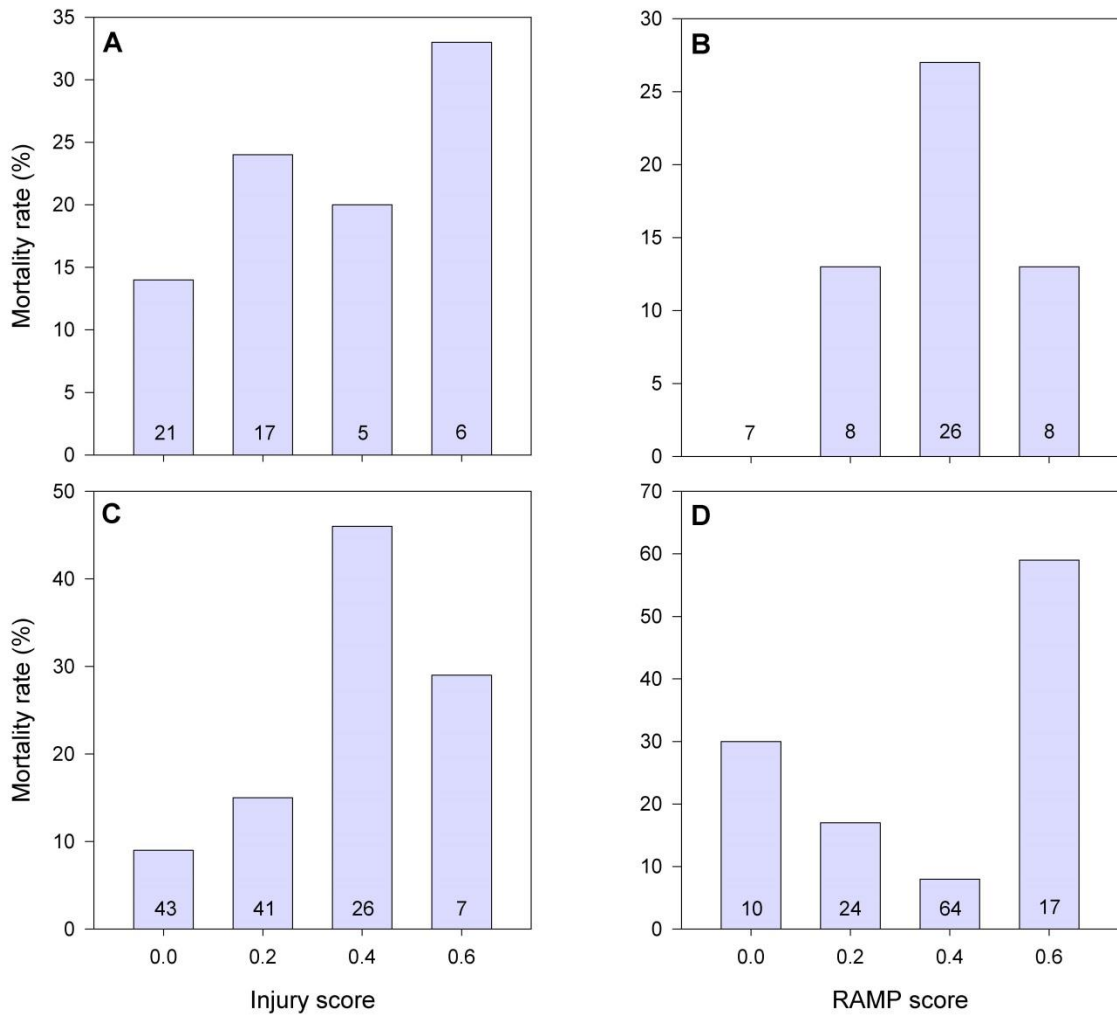
Table 2.2 Population-origin identification of coho salmon along with sizes and mortality rates for fish originating from population groups. Differences in survival among population groupings (for statistics, grouped as BC south coast, interior Fraser, Puget Sound Hood Canal, Puget Sound North, Puget Sound South/central, Vancouver Island) were not significant for either net pen mortality rates (Chi-square test: $\chi^2 = 0.32$, df = 5, $P = 0.99$) or for success in surviving to reach the JDF receiver line ($\chi^2 = 0.66$, df = 3, $P = 0.88$). Differences in fish length among population grouping were not significant (Kruskal-Wallis ANOVA; $\chi^2 = 10$, $P = 0.08$).

Region (% of overall)	FL (cm ± 95 CI)	24 h net pen mortality	Failed to reach JDF line	Population complex	Individual stream IDs (N)
Puget Sound N = 92 (67%)	54 ± 1.0	12 of 54 (22%)	8 of 37 (22%)	North	Skykomish River (44) Jones Creek (10) Stillaguam River, north fork (2) Marblemount Hatchery (2) Nooksack River (1) White River (25)
				South/central	Puyallup River (1) Dewatto River (6)
				Hood Canal	Quilcene Hatchery (1)
Vancouver Island N = 17 (12%)	54 ± 2.1	4 of 15 (27%)	1 of 1 (100%)	East coast	Puntledge River (5) Cowichan River (3) Shawnigan Lake (1)
				West coast	San Juan River (3) Robertson Creek (1) Sarita River (1)
				Northeast	Quatse River (2)
Fraser River N = 14 (10%)	57 ± 4.9	1 of 7 (14%)	0 of 6 (0%)	Interior, North Thompson	Mann Creek (3) Dunn Creek (2) Tumtum Creek (1)
				Interior, middle drainage	Bridge River (1) McKinley Creek (1)
				Interior, South Thompson	Harbour Creek (2)
				Interior, lower Thompson	Coldwater Creek (1)
				Birkenhead River	Birkenhead River (2)
				Lower Fraser	Alouette River (1)
BC South Coast N = 12 (8.8%)	55 ± 3.7	1 of 8 (13%)	1 of 4 (25%)	North	Homathko River (5) Phillips River (3)
				South	Seymour River (4)
Coastal Washington N = 2 (1.5%)	51	n/a	0 of 2 (0%)	Coastal WA	Clearwater River (2)

Table 2.3 Statistical output for logistic regression predicting mortality in the on-board net pen holding experiment using data for all fish held in the study (N = 118; top) and for a second model predicting 24 h survival using blood physiology responses (bottom, blood values corrected for sampling time-course by using the residuals of their relationships with time – see Fig. 2.3; N = 63). Both models were significant overall; top - $\chi^2 = 40.1$, df = 7, $P < 0.001$, Nagelkerke $R^2 = 0.45$; bottom - $\chi^2 = 11.3$, df = 4, $P = 0.045$, $R^2 = 0.25$.

Predictor variable	B (SE)	95% CI for odds ratio		
		Lower	Odds ratio	Upper
Predicting mortality using non-blood variables				
Constant	-6.96 (4.15)			
Recovery tote time (s)	2.6×10^{-4} (2.0×10^{-4})	1.00	1.00	1.00
Fish size (FL, cm)	0.07 (0.07)	0.94	1.07	1.23
Injury score	**4.68 (1.57)**	**5.77**	**108**	**3146**
RAMP score	-1.65 (2.12)	0.003	0.19	14.2
Orientation reflex	**3.33 (1.00)**	**4.22**	**27.9**	**225**
Caudal sampling	0.49 (0.60)	0.51	1.62	5.44
Dummy transmitter	-0.86 (0.61)	0.12	0.42	1.34
Predicting mortality only using blood variables				
Constant	-1.47 (0.71)			
PC1	1.06 (0.73)	0.74	2.91	13.9
Lactate	-0.06 (0.12)	0.74	0.94	1.18
Cortisol	-0.66 (0.78)	0.10	0.52	2.34
Potassium	1.1×10^{-4} (0.19)	0.67	1.00	1.43
Glucose	-0.67* (0.30)	0.27	0.51	0.87

Table 2.4 Output of the final PCA used to synthesize chloride, osmolality, and sodium into a synthetic (PC1) variable for further analyses of the effects of capture on physiology (N = 231). An initial PCA was successively run on all seven plasma variables with stepwise elimination of variables based on plasma variables having either a) a low KMO statistic, or b) not having a loading > |0.6| (shown in bold) for any factor which also had other > |0.6| loadings (i.e., not correlating strongly with other variables within a factor).

Final PCA used for subsequent analyses

	PC1 loadings	Communality (h^2)
Eigenvalue	2.66	
% variance explained	89%	
Chloride	0.92	0.84
Osmolality	0.96	0.91
Sodium	0.95	0.91

Table 2.5 Statistical relationships among sublethal measures: injury, reflex impairment, and blood physiology. Blood physiology measures tested were the residuals of regressions with sampling time to correct for the confounding effect of the sampling time-course. Note that this only involves using RAMP scores and injury scores from 0-0.6 (= 4 groups). Total N = 153.

Plasma variable	Direction of relationship (+ / - / none)	Statistical test		
		Kruskal-Wallis test	Jonckheere-Terpstra test	Spearman rank-order correlation
RAMP score				
PC1	+	$\chi^2 = 18, P < 0.001$	$P = 0.013$	$r_s = 0.34, P < 0.001$
Lactate	+	$\chi^2 = 31, P < 0.001$	$P = 0.013$	$r_s = 0.42, P < 0.001$
Cortisol	-	$\chi^2 = 15, P = 0.002$	$P = 0.013$	$r_s = -0.27, P < 0.001$
Glucose	none	$\chi^2 = 18, P < 0.001$	$P = 0.05$	$P = 0.06$
Potassium	none	$\chi^2 = 2, P = 0.57$	n/a	n/a
Injury score				
PC1	+	$\chi^2 = 11, P = 0.009$	$P = 0.027$	$r_s = 0.27, P < 0.001$
Lactate	none	$\chi^2 = 2, P = 0.58$	n/a	n/a
Cortisol	none	$\chi^2 = 6, P = 0.13$	n/a	n/a
Glucose	none	$\chi^2 = 1, P = 0.83$	n/a	n/a
Potassium	none	$\chi^2 = 3, P = 0.38$	n/a	n/a
Combined condition score				
PC1	+	n/a	n/a	$r_s = 0.38, P < 0.001$
Lactate	+	n/a	n/a	$r_s = 0.30, P < 0.001$
Cortisol	-	n/a	n/a	$r_s = -0.30, P < 0.001$
Glucose	none	n/a	n/a	$P = 0.38$
Potassium	none	n/a	n/a	$P = 0.38$

2.5   Discussion

The present study is among the first to directly compare the use of short-term captivity with biotelemetry as methods for estimating post-release bycatch mortality. The short-term mortality rates observed in the net pen experiment and acoustic telemetry component were similar: 21% and 20%, respectively. However, while fish were monitored for mortality in the net pen for an average of 26.8 h, the telemetry component involved monitoring survival over distance along the migration route, rather than time. The time from release to arrival at the first receiver line (JDF) was highly variable (mean = 125 h, median = 95 h, range = 14 - 587 h), and 32 of 40 fish required more than 48 h. Thus, although the two mortality estimates appear quite similar, the short-term mortality estimate for telemetry-tagged fish represented a considerably longer monitoring period in almost all cases. The long-term mortality rate for telemetry-tracked fish, which represented a period of 10-20 days in most cases, was 47%. That longer duration meant that there was increased potential for latent pathogenic mortality to occur via injury (although there was no link between injury and mortality) and that some natural mortality would likely have occurred. In the discussion below, we attempt to make connections between our novel physiological findings and the RAMP, injury, and mortality patterns we observed in the two mortality experiments.

2.5.1   Predictors of post-release mortality

Mortality could be predicted by injury and reflex impairment in the more controlled net pen experiment, whereas there were no statistically significant predictors of mortality in the telemetry experiment with a smaller sample size (Table 2.3, Fig. 2.7). In the net pen,

higher injury scores were positive predictors of mortality, consistent with our expectations and a number of past studies that demonstrate dermal injury is detrimental to fish (e.g., Kaimmer and Trumble 1998, Olsen et al. 2012). Reflex impairment was predictive of mortality but it was the orientation reflex on its own that was a strong predictor rather than the full suite of five reflexes included in RAMP scores. RAMP-mortality curves are often sigmoidal in shape whereby reaching a threshold score, often associated with the addition of a particular reflex (Fig. 2.7D), causes a large increase in mortality (Davis 2007). The fact that neither injury nor RAMP scores were predictive of mortality for telemetry-tagged fish was surprising, as it suggests that mortality was not influenced by either a) the extent of departure from homeostasis, or b) the extent of dermal injury. In light of a number of other studies, our results show that RAMP may not be an effective mortality predictor in some contexts and further work will be needed to understand why this is the case. However, we caution that because few fish in the telemetry component (8 of 50) experienced high RAMP scores (≥ 0.6), there was limited power to calibrate RAMP to post-release outcomes, especially given that mortality was strongly associated with RAMP scores ≥ 0.6 for fish in the net pen (Fig. 2.7) and for coho salmon bycatch in freshwater (see chapter 3). In a future study with a larger sample size and a greater range of stressor severity and RAMP scores (e.g., in a real fishery with thousands of fish caught rather than dozens) it is entirely possible a strong RAMP-mortality relationship would occur for telemetry-tagged fish. Regardless, this and other studies on RAMP in Pacific salmon show that it is an effective approach for rapidly assessing animal vitality (Davis 2007, Donaldson et al. 2012, Nguyen et al. 2014, chapters 3, 4, 6, 7).

2.5.2   Mechanisms of reflex impairment and mortality

RAMP scores in coho salmon bycatch were clearly mirrored by certain plasma variables, providing the strongest confirmation to date that a simple reflex assessment can be used to assess the extent of physiological departure from homeostasis in bycatch. In particular, RAMP score was positively correlated with plasma lactate and the PC1 response (i.e., plasma sodium, chloride, and osmolality). Significant elevations in the PC1 and lactate responses of fish were both likely the result of excessive anaerobic metabolism in the white muscle. Lactate produced in muscle cells is partly leaked into blood and the osmotic pressure created by intracellular metabolic acidosis causes water to move from plasma into white muscle cells, thereby increasing the concentration of ions in plasma (i.e., the PC1 response; Wood et al. 1983). In salmon, a RAMP score of 0.4 almost always represents the combined loss of the tail grab and body flex responses (this study and see chapters 3 and 7) – two responses that, when positive, involve use of white muscle to burst forward in water (tail grab) or struggle free of the handler in air (body flex). RAMP scores of 0.6 (or higher) virtually always reflect the additional impairment of the orientation response (present study and chapters 3 and 7). Increases in plasma lactate (chapters 7 and present study) and PC1 response (present study) appear to occur up to a RAMP score of 0.4 but not beyond (Fig. 2.4), suggesting the mechanism for loss of body flex and tail grab reflexes is metabolic acidosis and exhaustion of white muscle whereas orientation (RAMP score of 0.6) becomes impaired via another pathway and requires a more severe capture stressor. Loss of orientation may be analogous to fainting in humans (i.e., syncope), a loss of consciousness caused by insufficient oxygen delivery

to the brain (i.e., cerebral hypoxia or hypoperfusion, Low and Mathias 2011). Exhaustive exercise during net hauling and asphyxiation via entanglement or air exposure could, when combined, lead to a drop in the oxygen partial pressure of the blood sufficiently large that could in-turn cause loss of consciousness (i.e., loss of orientation).

Given that the orientation reflex was predictive of mortality in the net pen, we assessed whether it was associated with patterns in blood physiology after 24 h. Our data provide support for the connection between loss of orientation and mortality based on the novel finding that lactate was higher after 24 h for fish that had lost orientation at capture (but survived 24 h), representing a departure from homeostasis extreme enough that metabolic recovery was impaired. In addition, cortisol was very high for fish in the net pen (Fig. 2.3), and elevated cortisol has previously been associated with slowed clearance of lactate in exercised fish (Milligan et al. 2000). The 24 h elevation in lactate we observed for fish recovering from loss of orientation may not have occurred in fish that were telemetry-tagged and released because those fish did not experience confinement stress. This key difference between the net pen and immediately released fish could explain why orientation predicted mortality in one and not the other. However, as mentioned above, small numbers of fish at higher RAMP scores precluded a rigorous calibration between RAMP and mortality for telemetry-tagged fish.

Perplexingly, plasma cortisol was lower for fish that lost orientation (i.e., RAMP score of 0.6; Fig. 2.4) than for vigorous fish (RAMP score of 0). This result is contrary to our expectation that the same processes that result in stress and the release of cortisol during fisheries capture would also cause reflex impairment. In reality, however, all fish in this study were clearly quite stressed and it is unclear whether a particularly elevated

cortisol response after capture would have been adaptive or maladaptive for the fish (but see Cook et al. 2014). In addition, a caveat with respect to plasma cortisol is that sex has a strong and significant influence on circulating cortisol in migrating adult Pacific salmon (Hinch et al. 2006, Baker and Vynne 2014) and sex was not identified for the fish in this study (i.e., they were morphologically indistinguishable and we did not measure sex steroids). Mature female Pacific salmon have higher baseline cortisol than males (Sandblom et al. 2009, Donaldson et al. 2010a, chapter 3) because of cortisol's role in mediating other sex hormones that drive reproductive maturation and egg production (Hinch et al. 2006, Baker et al. 2013). If the male coho salmon in our study were, because of a morphological or behavioural difference, more prone to severe net entanglement and associated exhaustive exercise, a greater number of males would have been assigned RAMP scores of 0.6, lowering the mean cortisol concentration for that RAMP score. Future studies on reproductively maturing fish should ensure that sex is identified (e.g., via sex steroid analysis) and controlled for when using cortisol as an indicator of capture stress.

In the present study, injury score was an index that incorporated both the extent of scale loss and the number of different injuries occurring for a single individual (i.e., gillnet-like marks on the head, the body, or other injuries). Injury score had a positive, medium-strength correlation with RAMP score, perhaps because fish exposed to injurious entanglement are likely to struggle more vigorously, become more exhausted, and thus suffer greater reflex impairment. Among blood physiology variables, injury score was only significantly associated with the PC1 response (Fig. 2.5) with a significantly elevated response at an injury score of 0.4 (e.g., moderate scale loss +

gilling marks on body) relative to a score of 0 (i.e., low or no scale loss, no notable injuries). The relationship between injury score and PC1 response was weaker and less linear than its RAMP equivalent, suggesting it is likely indirect and not causal. However, there is a physiological basis and evidence in the literature to expect that dermal injury could cause latent problems (Gadomski et al. 1994, Zydlewski et al. 2010, Olsen et al. 2012); including signs of osmoregulatory distress we observed in our study whereby PC1 scores remained elevated after 24 h for fish with higher injury scores. Similar evidence exists that scale loss causes increases in plasma ions over a period of days in Atlantic herring (*Clupea harengus*, Olsen et al. 2012), and outmigrating Atlantic salmon smolts (*Salmo salar*, Zydlewski et al. 2010) in seawater. The mechanism for this phenomenon is not clear but likely includes direct loss of body water via the compromised integument, although research on herring suggests water is lost directly from the gills because of a secondary stress response caused by scale loss (Olsen et al. 2012). Injury score predicted mortality in the net pen, and the 24 h plasma data suggest that failure to restore osmoregulatory homeostasis, as a result of dermal injury, may have contributed to that mortality. Adult Pacific salmon remodel their osmoregulatory physiology during their approach to freshwater (Cooperman et al. 2010). Any latent and sustained effects of scale loss on osmoregulation during this period could therefore be detrimental to migration success. Loss of osmoregulatory capacity is also a precursor to pre-spawn mortality for maturing sockeye salmon held in freshwater tanks, where large decreases in plasma chloride, sodium, and osmolality (i.e., PC1 in our study) precede death (Jeffries et al. 2011).

As with the orientation reflex, the fact that injury predicted mortality in the net pen but not in telemetry-tagged fish could be related to confinement stress. Plasma cortisol was remarkably high after 24 h of net pen holding (Fig. 2.3E) - higher even than in post-capture samples which were drawn from fish at a time point when cortisol typically peaks during the acute stress response in fish (i.e., within 2 h of initiation of the stressor, Mommsen et al. 1999). Cortisol has a role in regulating gill ATPase activity, and is considered a saltwater adapting hormone because cortisol treatment has been shown to promote saltwater tolerance (Clarke and Hirano 1995, Mommsen et al. 1999). Olsen et al. (2012) point out that sustained cortisol elevation caused by scale loss could therefore be explained by fish attempting to regain osmoregulatory control but if left unconstrained, such a response could initiate a lethal spiral via impaired immune function and accelerated depletion of glucose stores. However, in our study cortisol was not significantly higher after 24 h for fish with higher injury scores. On its own, cortisol elevation caused by crowding stress in aquaculture facilities can cause skin damage and infections in fish (Iger et al. 1995, Udomkusonsri and Noga 2005). It is possible that in our net pen experiment, a magnified and prolonged cortisol response (caused by confinement) worsened existing injuries in ways that did not occur for the fish we released overboard with transmitters. In the net pen, these factors would have both increased overall mortality and strengthened the association between injury and mortality.

2.5.3   Management relevance and future research

Few past studies have directly compared short-term captivity and biotelemetry as means

of estimating post-release mortality. The collective evidence from our study and past

research (Milligan et al. 2000, Donaldson et al. 2011, Olsen et al. 2012) highlight caveats

associated with the use of confinement as a means of assessing short-term mortality in

wild fish, particularly in fisheries where substantial dermal injury occurs. DFO assign

post-release mortality rates to bycatch in all major Pacific salmon fisheries as a means of

estimating total incidental fishing mortality each year, in efforts to ensure spawning

escapement targets are met (DFO 2013). Nearly all of the mortality rates currently used

by DFO are based on short term (~ 24-48 h) captivity studies (e.g., using a net pen). Wild

adult salmon clearly experience severe stress from short-term confinement (Farrell et al.

2001a, Donaldson et al. 2011, present study), and sustained cortisol elevation can

compound problems that lead to mortality (discussed above). As an alternative means of

assessing survival, biotelemetry poses a different set of issues such as the long-term

negative effects of the transmitters (largely unknown), lack of controls, and the inability

to physically verify the fate of the animals. However, we noted that survival (i.e.,

migration success) was quite high for telemetry tagged fish and that two individuals were

recaptured in spawning areas, meaning that the external tag application did not

necessarily prevent some fish from reaching freshwater and completing > 400 km

upstream migrations. Managers and scientists should carefully consider the benefits and

drawbacks of both net pens and biotelemetry when designing mortality experiments and

using resultant data in management models. Moreover, some weight should be given to

long-term survival (> 10 d), which can be effectively monitored using biotelemetry (Donaldson et al. 2008, 2011).

The mortality estimates provided by this study are not appropriate for direct application to management of purse seine fisheries that release coho salmon. The catch sizes were very small (largest catch = 82 fish) because our fishing charter occurred during a year of low sockeye salmon and pink salmon abundance, and catches were brailed directly into the recovery tote (although we saw no effect of the alternate dry box sorting on RAMP or injury scores). Actual seine fisheries often land thousands of salmon in single sets and such catches would necessitate longer entanglement times, higher crowding, and more brailer loads. In Atlantic herring and mackerel (*Scomber scombrus*) purse seine fisheries, longer crowding time and higher crowding densities are correlated with greater stress, behavioural impairment, and mortality (Marcalo et al. 2006, 2010, 2013, Huse and Vold 2010, Tenningen et al. 2012). Even within our small range of catch sizes there was a positive correlation with both RAMP and injury scores, leading to the prediction that larger sets of fish would likely cause higher post-release mortality than in our study. Thus, our short-term bycatch mortality estimate of 20-21% likely represents a 'best-case' scenario, while being lower than the 70% rate currently applied to the fishery. We suggest further research under a more representative fishing scenario to get a better understanding of the underlying mechanisms to explain this large discrepancy.

Some of our data reveal possible avenues for future work aimed at developing ways to reduce or better manage salmon bycatch mortality in purse seines. We noted that reflex impairment showed no improvement over time for fish placed into the on-board recovery tote (Fig. 2.1B); a revival technique currently used for bycatch in BC purse

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seine fisheries. Also, time spent in the recovery tote did not significantly affect survival. There may therefore be some value in investigating whether other revival techniques could promote survival, although options like the Fraser recovery box (Farrell et al. 2001a, and see chapter 6) are inappropriate for the simultaneous revival of dozens of adult salmon, since they can only revive two fish at a time. Fork length was significantly correlated with injury and RAMP scores, but was not directly predictive of mortality. While we did determine population-origin via DNA analyses, larger sample sizes are required to generate any real conclusions about differences among populations in body size, post-release mortality, or other metrics. Our physiological data help demonstrate that RAMP can be an effective tool for assessing the condition of bycatch relative to different capture, handling, and revival practices but more work is needed to establish whether it can be an effective mortality predictor in ocean-caught coho salmon. The integration of reflex and injury assessments with blood physiology in the net pen experiment provide key insights into the mechanisms of mortality and the limitations associated with the two mortality assessment methods.

Chapter 3. Validation of reflex indicators for measuring vitality and predicting delayed mortality in coho salmon

3.1   Abstract

Effective management of fish and wildlife populations benefits from an understanding of the effects of stressors on individuals in those populations. Such an understanding has conventionally been gleaned from measurements of stress-related blood constituents in animals, both in the laboratory and the field. However, while such physiological tools can help provide a mechanistic understanding of organismal responses, their applied utility is limited because they cannot easily be used by stakeholders. Reflex Action Mortality Predictors (RAMP) is a method that involves checking for the presence or absence of natural animal reflexes to generate a condition (RAMP) score in response to stressors and to predict fate. The method has previously been validated with fishes in artificial laboratory- and field-based holding studies as a responsive measure of fisheries capture stress and a predictor of delayed mortality. We used radio telemetry with fixed-station radio receivers to monitor migration success of 50 endangered coho salmon following incidental capture in the lower Fraser River (Canada). RAMP was used to measure the condition of fish at release and to predict migration success following capture. Biopsy of an additional 43 coho profiled physiological condition at time of release. Individuals with greater reflex impairment (higher RAMP scores) at release experienced significantly

higher rates of migration failure. RAMP scores were also significantly correlated with fishery handling time. Plasma variables showed that the fish had experienced physiological stress characteristic of exhaustive exercise and hypoxia, with significantly elevated cortisol and lactate values for individuals entangled longer in fishing gear. This is the first validation of Reflex Action Mortality Predictors in a wild setting. Based on our findings, fishers could use the method to make adjustments in fishing behaviour in real time to improve condition and reduce mortality of bycatch, and conservation practitioners could monitor animal condition and identify problems that deserve management attention. RAMP is an easy, rapid and inexpensive approach to predicting mortality and measuring vitality, and performed better than physiological tools which cannot easily be used by stakeholders.

3.2 Introduction

Management of animal populations can benefit from an understanding of stressors and their effects on individuals (Cooke and O'Connor 2010), particularly for exploited species, or animals incidentally harmed by human activity. Fisheries bycatch is a common example of human-caused acute stress in wild animals and is regarded as an important conservation issue worldwide in both marine (Hall 1996, Hall et al. 2000, Lewison et al. 2004a) and freshwater (Raby et al. 2011) systems. Releasing bycatch alive (i.e., discarding) is commonly employed to conserve populations of incidentally captured animals but relies on the assumption of survival, an assumption that may be false in certain contexts (e.g., Chopin and Arimoto 1995, Campbell et al. 2010). Currently, the

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unintended capture of turtles (Watson et al. 2005), seabirds (Gales et al. 1998), mammals (e.g., Read et al. 2006), and non-target fishes (Broadhurst et al. 2006) is occurring en masse in fisheries and can result in immediate or latent lethal and sub-lethal outcomes (Davis 2002) that can lead to population declines (Lewison et al. 2004b).

Most research in the bycatch realm has focused on bycatch rates, but significant bycatch sometimes remains even with the most selective fishing gear available (Davis 2002, Broadhurst et al. 2009). In such cases, researchers have focused on understanding and mitigating the mortality of bycatch (Davis 2002). Research that informs managers on how to reduce the mortality of discards is valuable (e.g., Broadhurst et al. 2009), but can be costly as it is commonly achieved by combining measurements of blood physiology with the monitoring of mortality in a contained environment (holding studies, e.g., Davis 2007). In holding studies, animals are exposed to a fishing gear encounter and monitored for mortality in an artificial enclosure over some time period (typically 2-3 days for field studies, weeks for laboratory studies; Gallinat et al. 1997, Davis 2007). The survival estimates derived by monitoring captive animals fail to integrate the conditions and challenges of a wild environment (e.g., predators, environmental heterogeneity and variability in food resources). Applying biotelemetry to bycatch research can provide data on behaviour and survival discards in the wild, and thus address the shortcomings of holding experiments. The use of biotelemetry in recreational fisheries (mostly freshwater) has been relatively common, whereas in bycatch research (mostly marine) it has been rare, due to technological/logistical limitations and prohibitive costs (Donaldson et al. 2008). Therefore, studying bycatch in freshwater using telemetry can serve as the basis

for advancing an understanding of the fate of discards and for developing predictors of mortality (Raby et al. 2011).

Simple measures of animal vitality made following capture may predict future survival and hence generate more rapid and inexpensive estimates of bycatch mortality (Davis 2010). Traditional physiological tools (e.g., measuring blood constituents) can be useful (Moyes et al. 2006) for predicting delayed mortality but are expensive and require expertise. RAMP (Reflex Action Mortality Predictors) is an easy-to-use and inexpensive field-based assessment tool that measures fish vitality before release and correlates with future survival (Davis 2010). Since its introduction, RAMP has successfully predicted post-release mortality for fish and shellfish in laboratory- and field-based holding experiments (e.g., Davis 2007, Stoner 2009, Campbell et al. 2010, chapter 2). RAMP assessments involve checking for the presence or absence of multiple reflexes identified to be consistently present in vigorous individuals. Assessments of reflexes is rapid (< 20 s) and the results are cumulated into a simple index. The appeal of reflexes is that they are intuitive to fishers and are whole-animal indicators of a compromised physiological state (Davis 2010, chapter 2). If validated, RAMP could be used to rapidly generate bycatch mortality estimates for different gears, seasons, and fishing techniques – data that can inform the management and reduction of bycatch mortality and support conservation initiatives.

Wild coho salmon released from aboriginal beach seine fisheries in the Fraser River, British Columbia (Canada) were used to provide a comparison of telemetry-based survival estimates with RAMP scores. The interior Fraser River coho salmon population is listed as endangered by the Committee on the Status of Endangered Wildlife in Canada

and therefore fishers in British Columbia are required to release all wild coho salmon. We assessed whether RAMP scores correlated with delayed mortality, and compared RAMP scores with plasma physiology to determine if RAMP is a reasonable approach to assessing stress and vitality

3.3   Methods

3.3.1   Study site and capture method

An aboriginal band operating on the lower Fraser River mainstem near Hope, British Columbia, Canada (49º 18' 32" N, 121º 40' 03" W; Fig. 3.1), allowed us to tag or biopsy the coho bycatch in their pink salmon beach seine fishery, from 21-23 September 2009. Fish were captured using a 90 m x 9 m x 5cm mesh beach seine that was anchored to shore, dragged away from shore, allowed to drift downstream, and pulled in using a power boat. Once the net was closed, it was pulled onto the beach by hand until fish were crowded into shallow water (< 0.5 m; see Fig. 3.2). Once the net was landed, the fishing crew sorted pink salmon into bins, released other species, and handed coho salmon to us as they were found. If we were not present, these fish would have been released directly into the river. If coho salmon were located in the net while we were occupied processing other fish, they were removed immediately and placed in black hypalon fish bags with mesh ends (1.0 m x 0.2 m) and oriented into flow for holding until they could be processed. Fish were typically exposed to air for 20 s between removal from the seine and placement in a fish bag. For each net set, we recorded the time required to close the net and to pull the net into the beach, as well as the number of fish of each species caught. For each coho salmon, we recorded the amount of time spent in the beached seine

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net (i.e., fishery handling time) and the duration between being pulled from the net and release by the research team (i.e., researcher handling time). Water temperature in the Fraser River during the tagging period was ~16 °C, as measured by a permanent temperature probe installed 20 km upstream at Hope (see Fig. 3.1).

3.3.2   Radio tagging

Radio telemetry transmitters were deployed in 50 bycaught coho salmon; biopsies were performed on 43 separate individuals. We used established, minimally invasive protocols for gastric tagging of salmon in the Fraser River (e.g., Cooke et al. 2005, Donaldson et al. 2010b). The method involved using a plastic plunger to push a radio telemetry transmitter down the throat of each fish until it reached the fish's stomach, leaving the antenna trailing out of the mouth and down the side of the body. This procedure was carried out while fish were manually held inside flow-through fish bags submerged in flowing river water. The entire tagging and handling procedure lasted < 2 min, and no anaesthesia was used. We did not use anaesthesia for a number of reasons, most of which are outlined in Cooke et al. (2005). Most importantly for our study, the pharmokinetics of analgesics are poorly understood in fish and any drugs we administer would potentially alter behaviour and physiology more than the treatment itself (fisheries capture), and could potentially interfere with physiological recovery and survival (e.g., via depressed ventilation rates). Immediately prior to release, reflex impairment (RAMP) was assessed using the same five reflexes described in chapter 2 (section 2.3.2).

We used coded transmitters (MCFT-3A-3V, Lotek Wireless Inc., Newmarket, ON) so that individual fish were identifiable at receiver stations. A thermal logger (data

not reported here) was glued to the end of each transmitter and waterproofed using Plasti Dip multi-purpose plastic coating (Plasti Dip International, Blaine, MN). The whole transmitter/thermal logger unit weighed 17 g in air, 7 g in freshwater and measured 56 mm long, 16 mm in diameter, with a 460 mm long antenna. The radio tags used three pulse intervals per frequency (4.5, 5.0, 5.5 s) to reduce signal collisions and transmitted at 150 MHz on six unique frequencies (320, 360, 440, 460, 600, and 800 kHz).

3.3.3 Physiological sampling

Blood physiology is commonly used in fisheries and conservation biology to measure stress and can illustrate organismal response to a capture experience (e.g., Farrell et al. 2001a, Wikelski and Cooke 2006). To minimize handling time for the radio telemetry study, a separate group of 43 coho salmon was biopsied and released using capture methods identical to the telemetry component. For blood sampling, individuals were removed from fish bags and processed in a padded V-shaped trough. The trough was continuously supplied with fresh river water by an electric pump that provided strong flow through the mouth and across the gills (we avoided using the plumbed holding trough for tagging to not bias survival estimates: the high rate of flow in the trough appeared to accelerate revival). Fish were biopsied for blood by caudal puncture and adipose fin clips were collected for DNA analyses (described in chapter 2, section 2.3.2). In addition, visible injuries were described, and fork length (FL, nearest cm) was measured. The entire biopsy procedure lasted < 3 min. Biopsied fish were released into the river following a reflex assessment (described above and in 2.3.2). After biopsy,

blood and adipose tissue samples were processed and stored, and subsequently analyzed in the laboratory, as described in chapter 2 (see section 2.3.2).

3.3.4   Radio tracking

Radio telemetry tagged coho salmon were tracked at fixed radio telemetry receiver stations (SRX400 or SRX400A, Lotek Wireless Inc., Newmarket, ON) using 3 or 4-element Yagi antennas (Maxrad Inc., Hanover Park, IL, or Grant Systems Engineering Inc., King City, ON). A total of eighteen receiver stations were strategically positioned throughout the Fraser River watershed to intercept radio tagged salmon (Fig. 3.1). The receiver stations nearest to the release site were 20 km upstream (Hope) and 16 km downstream (Rosedale), respectively.

Fish were characterized as en route mortalities if they were undetected at subsequent upstream receiver locations. There was no apparent en route mortality for radio-tagged coho beyond the Thompson River confluence with the Fraser River: all fish detected there were detected at subsequent upstream receivers en route to spawning areas. Therefore, we characterized fish as successful migrants if they were detected by the Thompson River receiver. Previous studies (e.g., Cooke et al. 2006) have similarly used arrival at natal sub-watersheds to indicate successful migration given the challenges of tracking fish directly to the many potential spawning areas. For statistical analyses, survival to Hope, Qualark, and Hell's Gate were also evaluated (Fig. 3.1). In general, the detection efficiencies of the receiver stations were quite high. For example, the Thompson sub-watershed receiver in 2009 detected 81.8% of tags in passing sockeye salmon (Robichaud et al. 2010). Sockeye salmon provide a good surrogate for measuring

the efficiency of the telemetry system given that over 500 sockeye were concurrently tagged in other studies using the same system in 2009, whereas we tagged relatively few coho. For the fifty coho salmon we did tag, detection efficiency was 100% at both the Hell's Gate and Thompson River receiving stations, the two most important receivers for this study.

3.3.5   *Data analysis and statistics*

To evaluate whether RAMP score was associated with delayed mortality (i.e., migration failure) we carried out two tests. First, radio-tracked fish were separated into two groups; successful migrants and unsuccessful migrants (based on criteria outlined above) and the RAMP scores measured at release were compared between these two groups using the non-parametric Mann-Whitney U Test. Second, we treated RAMP score (0, 0.2, 0.4, 0.6, or 0.8 – no 1.0 scores occurred) as a categorical predictor variable and used the non-parametric Kruskal-Wallis ANOVA to compare the survival rates of fish among those groups. We also wanted to test the power of RAMP as a response to stressor duration. Based on the prediction that fishery handling time (time entangled in the beached seine before removal) would have a negative effect on fish vitality, we evaluated whether RAMP score would indicate lower vitality (higher RAMP score) using a Spearman-rank correlation test comparing fishery handling time with RAMP score. We used the same test to evaluate the effect of researcher handling/holding time on RAMP score. Because we made four different comparisons here using RAMP scores, we used a Bonferroni-corrected significance threshold to account for type I errors ($\alpha = 0.0125$).

Entanglement time and handling time are relatively conventional, intuitive predictors of post-release mortality and therefore we evaluated whether these variables affected outcomes. ANOVA was used compare both fishery and researcher handling time among immediate mortalities, successfully migrants, and unsuccessful migrants. These tests were assessed as significant at $P \leq 0.05$.

We used seven plasma physiological measurements that were analyzed in the laboratory (glucose, lactate, chloride, sodium, potassium, osmolality, and log-transformed cortisol titres) to evaluate whether physiological state was reflective of fishery handling time and/or RAMP score. There was a large range in researcher holding times (between removals from the seine to blood sampling, see *Results*) and plasma variables typically continue to change in a predictable way for quite some time following the initiation of a stressor. Therefore, we included researcher handling time in our analysis of plasma titres. We used multiple linear regression with type III (orthogonal) sums of squares, including fishery handling time and researcher handling time (both log-transformed) as independent variables, and plasma measurements as the dependent variable. We grouped fish by RAMP scores and used one-way ANOVAs to compare mean concentrations of each plasma variable to evaluate whether there was concordance between blood parameters and reflex impairment. We used a Bonferroni-corrected α of 0.007 because of multiple comparisons with blood physiology data. All data are presented as means ± S.E.M unless otherwise noted.

Figure 3.1 Map of the Fraser River watershed, British Columbia, Canada, the release site, and radio receiver locations. The letter A indicates the point of river entry. The star symbol indicates the location of the study/release site. The remaining letter symbols represent receiver locations used for calculating survival and/or migration rate for radio tagged coho salmon: B (Hope), C (Qualark), D (Hell's Gate), E (Thompson River confluence), F (Nicola/Thompson confluence), G (Seton River confluence), H (Chilcotin River confluence). Plus symbols indicate the locations of additional receivers that operated as part of the array.
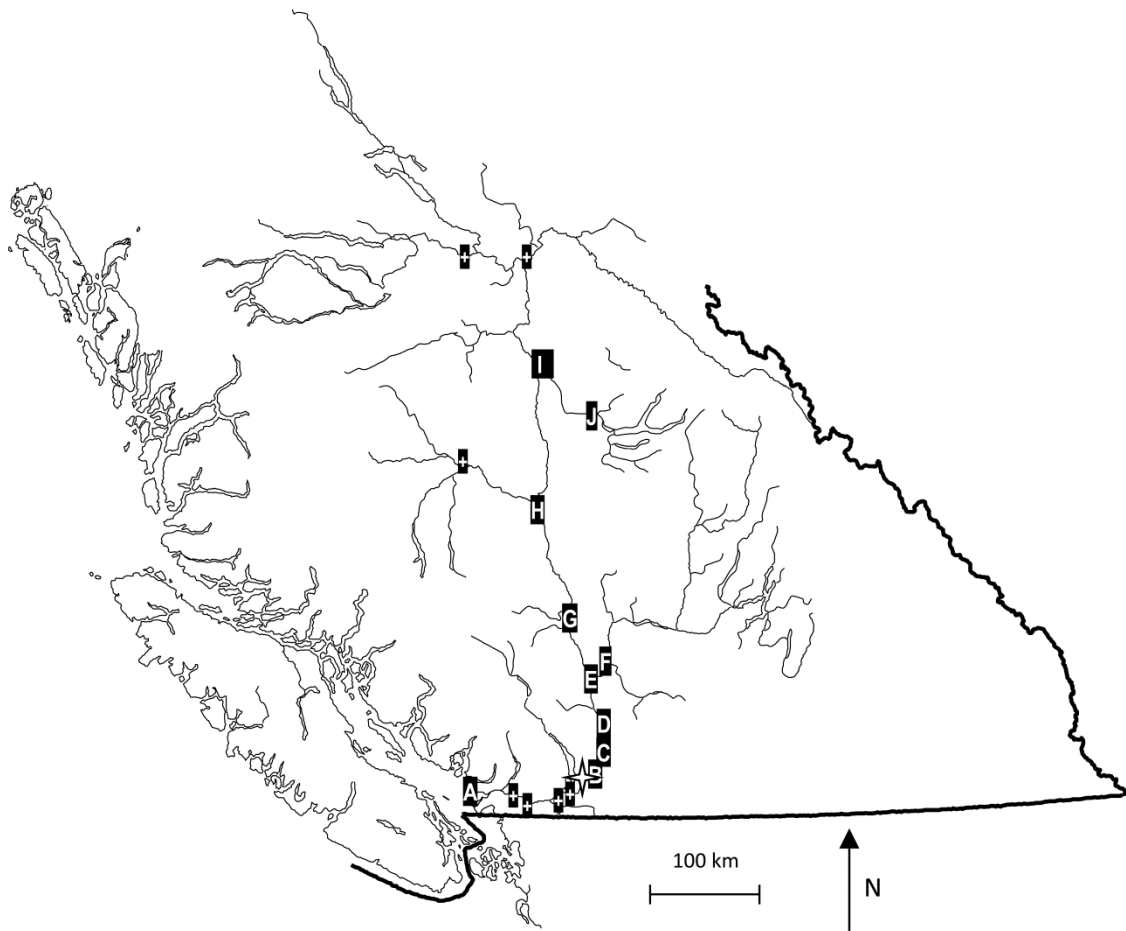
Figure 3.2 The beach seine being pulled into shore to crowd fish for sorting. Photo credit: Sarah McConnachie.

3.4 Results

3.4.1 Summary

The study encompassed 26 beach seine net sets in which 13,060 pink salmon were caught. The bycatch comprised 105 coho salmon, six sockeye salmon, one chinook salmon, and one white sturgeon *Acipenser transmontanus*. The total time required to deploy and pull in a beach seine averaged 7 min 28 s ± 29 s (Range: 3 min 55 s – 12 min 40 s). The net was always pulled into shallow water where fish reacted by thrashing vigorously for 1-2 minutes. Coho salmon used for telemetry and biopsy were in the seine (i.e., fishery handling time) for an average of 8 min 18 s ± 42 s (0 min 5 s - 41 min 0 s) before being removed. Telemetry tagged fish spent an additional 6 min 22 s ± 21 s (1 min 37 s – 11 min 38 s) in the possession of the research team (mostly holding time in hypalon fish bags while waiting to be processed). Biopsied coho salmon required an additional 9 min 41 s ± 75 s for holding and processing (1 min 0 s – 28 min 40 s; hereafter referred to as researcher handling time). There was a significant positive correlation between fishery handling time and reflex impairment at release (Fig. 3.3; Spearman-rank correlation, $r_s = 0.40$, $P < 0.01$). In contrast, researcher holding/handling time was negatively associated with reflex impairment ($r_s = $ -0.37, $P < 0.01$; Table 3.1). Of the 105 incidentally captured coho salmon, five died before removal from the net (i.e., immediate mortality; Table 3.2). Of the 50 radio-tagged fish we released, 37 (74%) migrated successfully and 13 (26%) died en route. Short-term mortality (i.e., died within 96 h of release) was evident in 12% of radio-tagged coho.

3.4.2   RAMP scores, handling time, and migration success

RAMP score exhibited a significant relationship with post-release survival whereas handling times were not significant predictors of survival. Coho salmon that failed to reach natal subwatersheds (mortalities) exhibited significantly higher reflex impairment upon release than did fish who migrated successfully (Mann-Whitney U Test: $P = 0.005$). However, neither log-transformed fishery handling time (One-way ANOVA; $F_{2,52} = 2.57$, $P = 0.09$) nor researcher handling time ($F_{2,52} = 0.04$, $P = 0.96$) differed significantly among successful migrants, unsuccessful migrants, and immediate mortalities (Table 3.2).

With successively increasing RAMP score from 0 to 0.6, the proportion of telemetry tagged fish successfully migrating to natal subwatersheds decreased (though these differences were not statistically significant; Kruskal-Wallis ANOVA, $H_{4, 45} = 9.0$, $P = 0.06$). For example, coho salmon with one reflex impaired (RAMP = 0.2) had 93% survival, whereas this figure was 68% for fish with 2 reflexes impaired, and 44% for fish with three reflexes impaired (Table 3.1). The pattern of individual reflex impairment showed that body flex was most commonly impaired, closely followed by tail grab: these were the reflexes impaired in nearly every fish with a RAMP score of 0.4 (Table 3.3). While body flex and tail grab were often impaired, orientation was virtually always the next reflex to become impaired with decreasing vitality. Impairment of the orientation reflex alone was a non-significant predictor of premature mortality: a lower proportion (0.54) of fish with impaired orientation at release successfully migrated to the Thompson than did fish with positive orientation (0.81; Mann-Whitney U test, $P = 0.06$). Head

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complex was rarely impaired, while impairment of VOR was not observed among live fish in this study (Table 3.3).

3.4.3   Plasma physiology

There was high inter-individual variation in blood plasma measures among the 43 coho salmon we sampled. Using multiple regression, both fishery handling time ($F_{1, 40} = 8.77$, $P = 0.005$, $\beta = 0.97$) and researcher handling time ($F_{1, 40} = 34.66$, $P < 0.001$, $\beta = 0.75$) were significantly ($\alpha = 0.007$) predictive of log-transformed cortisol titres (model $R^2 = 0.46$). Likewise, plasma lactate concentration in captured coho salmon was significantly affected by fishery ($F_{1, 40} = 20.80$, $P < 0.001$, $\beta = 13.14$) and researcher handling times ($F_{1, 40} = 22.39$, $P < 0.001$, $\beta = 5.25$; model $R^2 = 0.42$). Only researcher handling was a significant predictor of plasma glucose ($F_{1, 40} = 14.42$, $P = 0.005$, $\beta = 1.44$, $R^2 = 0.21$), and osmolality concentrations ($F_{1, 40} = 18.16$, $P < 0.001$, $\beta = 15.85$, $R^2 = 0.33$). Plasma chloride was not significantly affected by either fishery ($P = 0.23$) or researcher handling time ($P = 0.62$). Similarly, sodium was not significantly affected by fishery ($P = 0.57$) or researcher ($P = 0.03$) handling time. Likewise for potassium ($P = 0.46$, $P = 0.03$, respectively). Grouping coho by RAMP scores did not reveal any differences in plasma constituents (all $P > 0.05$). Females exhibited notably higher cortisol concentrations (not significant; $F_{1, 40} = 7.00$, $P = 0.01$) while glucose was significantly higher in males ($F_{1, 40} = 16.13$, $P < 0.001$; all others plasma variables $P > 0.10$; table 3.4).

Figure 3.3 Fishery handling times (time in landed seine net) and mean proportion of

reflexes impaired at release (RAMP score; spearman rank-order correlation, $P < 0.001$).

Error bars represent standard error.
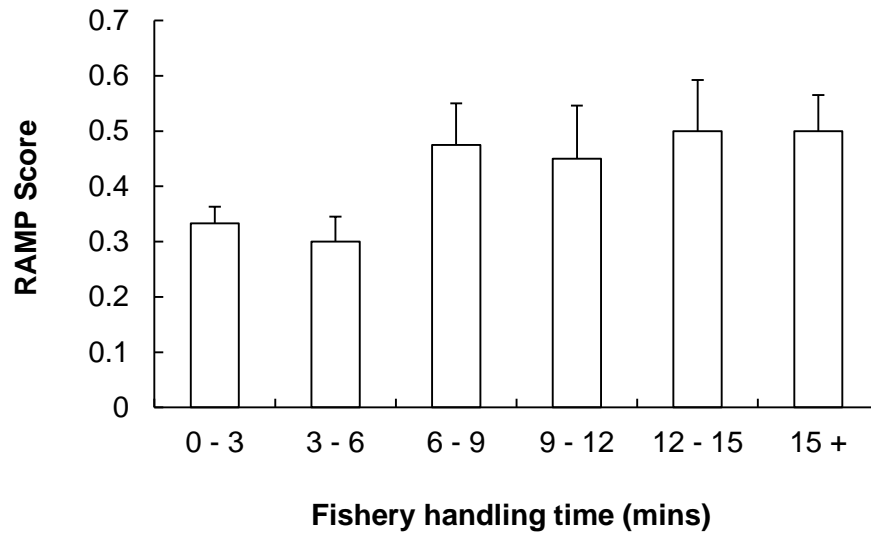
Table 3.1 Relationships between reflex impairment (RAMP score) and fishery handling time, researcher holding time, and post-release survival (migration success). Values are given in means ± standard error.

RAMP score	Fishery Handling time (mm:ss)	Researcher holding time (mm:ss)	Post-release survival (%)
	N = 12	N = 12	N = 4
0	3:12 ± 1:02	10:30 ± 1:32	1.00 ± 0
	N = 22	N = 22	N = 15
0.2	4:01 ± 0:57	8:14 ± 1:16	0.93 ± 0.07
	N = 39	N = 39	N = 19
0.4	3:27 ± 1:01	9:40 ± 1:09	0.68 ± 0.11
	N = 18	N = 18	N = 9
0.6	8:31 ± 1:42	6:14 ± 1:18	0.44 ± 0.18
	N = 5	N = 5	N = 2
0.8	8:45 ± 2:50	8:35 ± 3:17	0.5 ± 0.5

Table 3.2 Immediate and post-release survival of coho salmon captured in an aboriginal beach seine fishery. Handling time data are means given in minutes:seconds ± 1 S.E. RAMP scores represent the average proportion of reflexes impaired in individuals of that group.

	All fish pooled	**Telemetry tagged fish**			
		Died before Hope	Died before Hell's Gate	Died before Thompson	Reached Thompson
	Survived < 1 h	*Survived < 24 h*	*Survived < 96 h*	*Migration failure*	*Migration success*
Percentage of fish	5% (5 of 105)	6% (3 of 50)	12% (6 of 50)	26% (13 of 50)	74% (37 of 50)
Fishery handling time	17:10 ± 5:24	9:01 ± 4:14	6:33 ± 2:17	8:36 ± 1:46	6:45 ± 1:15
Researcher handling time	5:22 ± 4:36	5:18 ± 1:53	4:52 ± 1:01	5:08 ± 0:35	5:21 ± 0:25
Total handling time	22:32 ± 4:08	14:19 ± 5:16	11:25 ± 2:42	13:44 ± 1:43	12:06 ± 1:09
RAMP score	1.00	0.46 ± 0.07	0.47 ± 0.04	0.49 ± 0.04	0.31 ± 0.03

Table 3.3 Impairment of individual reflexes with increasing overall reflex impairment (RAMP score). Values represent the proportion of individuals with a particular reflex impaired within each group.

RAMP score	Tail grab	Body Flex	Orientation	Head Complex	VOR
N = 12					
0	0	0	0	0	0
N = 22					
0.2	0.36	0.59	0.05	0	0
N = 39					
0.4	1.00	1.00	0.03	0.03	0
N = 18					
0.6	1.00	1.00	1.00	0	0
N = 5					
0.8	1.00	1.00	1.00	1.00	0

Table 3.4 Physiological measures of stress compared between resting coho salmon (borrowed from Donaldson et al. 2010a) and coho salmon sampled following capture in a beach seine (current study). Both sexes are pooled where sex is not indicated. Values are means ± standard error.

Plasma variable		Baseline reference	Beach seined (current study)
Cortisol (ng mL^{-1})	Male	78.5 ± 19.8	159.7 ± 40.1
	Female	94.1 ± 21.9	328.5 ± 43.0
Glucose (mmol L^{-1})		5.6 ± 0.3	5.2 ± 0.2
Lactate (mmol L^{-1})		1.9 ± 0.1	12.3 ± 0.6
Chloride (mmol L^{-1})		134.5 ± 0.8	138 ± 0.6
Sodium (mmol L^{-1})		155.2 ± 0.9	163.5 ± 1.0
Potassium (mmol L^{-1})		1.9 ± 0.2	4.1 ± 0.14
Osmolality (mOsm kg^{-1})		321.4 ± 1.3	341.2 ± 1.8

3.5   Discussion

RAMP was developed as a tool for rapid, simple, and inexpensive evaluation of fish vitality and fisheries-induced mortality without the use of conventional research methods (i.e., holding pen experiments, telemetry, and blood physiology). This study demonstrates that RAMP scores can be used to predict post-release mortality in salmon captured as bycatch while migrating to spawning grounds. A higher proportion of reflexes were impaired at release in radio-tagged coho salmon that failed to reach their natal sub-watersheds than for individuals that migrated successfully. This result is particularly interesting given the lack of concordance between the duration of the stressor (here, fisheries handling time) and delayed mortality. In fact, RAMP score was the only measurement we made for radio-tagged fish that predicted post-release mortality. Further, RAMP integrated the organismal response to varying degrees of capture stress, showing a significant positive correlation with stressor duration (i.e., fishery handling time; Fig. 3.2, Table 3.1). Research elsewhere has shown similar correlations between capture stress severity, reflex impairment, and mortality in Atlantic cod *Gadus morhua*, walleye pollock *Theragra chalcogramma*, northern rock sole *Lepidopsetta polyxystra*, Pacific halibut *Hippoglossus stenolepis*, sablefish *Anoplopoma fimbria*, and coho salmon smolts (Davis 2005, Davis and Ottmar 2006, Davis 2007, Humborstad et al. 2009). However, in addition to being the first study to use RAMP in freshwater and on adult salmonids, our data are the first data to show a correlation between RAMP score and mortality for a fish released into the wild, thus validating this approach.

Physiological variables were indicative of both fishery and researcher handling durations but did not show concordance with RAMP scores – the former is not surprising

as most of the variables measured increased following initiation of a stressor for up to an hour (Barton 2002). Regardless, the biopsy data showed that the captured salmon had experienced substantial stress, as plasma values (e.g., lactate, cortisol) were clearly higher than those considered routine for non-stressed adult coho salmon (Table 3.4). Indeed, capture and crowding in the beach seine would constitute a combination of exhaustive exercise and hypoxia, two stressors known to result in severe physiological disturbance (e.g., Ferguson and Tufts 1992, Kieffer 2000). Longer time entangled in fishing gear was predictive of higher lactate accumulation in plasma (controlling for researcher handling time). Lactate values (i.e., ~12 mmol L^{-1}) revealed that fish were clearly using anaerobic metabolism which is characteristic of both exhaustive exercise and hypoxia. Physiological exhaustion was probably a driver of reflex impairment, although plasma lactate (our best measure of exhaustion here) was not significantly different among RAMP scores. Like lactate, cortisol titres were positively correlated to fishery handling times, but did not demonstrate a pattern with respect to RAMP score. This result was surprising, given our assumption that reflex impairment has a basis in physiological stress (Davis 2010). It is possible that alternative measures could better relate to RAMP scores (e.g., muscle physiology, plasma pH, arterial P_{O2}). More importantly, had every fish been blood sampled instantly upon removal from the seine we would probably have found a stronger relationship between plasma physiology, RAMP score, and fishery handling time. Although basic physiological analysis is not as widely accessible as RAMP, it can offer researchers an added understanding of capture stress. Future efforts to predict mortality in Pacific salmon could attempt to combine blood physiology and reflex measures (e.g., Campbell et al. 2010). Regardless, our study demonstrates that in an

applied context, RAMP appears to be more useful than traditional physiological tools in field studies of bycatch.

At this time, we can only speculate about the mechanistic links between reflex impairment, fish vitality, and the physiology of morbidity and death. Reflexes are neurological responses to external stimuli although in our assessment we included two responses (head complex - the pattern of regular ventilation, and VOR) that are functions of the autonomic nervous system. Neurological control of respiration in fish is complex and originates in the brain where neurons discharge rhythmically in time with respiration (De Graaf and Roberts 1991). The proximate motor nerve controlling the opening and closing of the lower jaw in fish (the pattern we observed to assess this reflex) is the trigeminal fifth (Taylor et al. 2006). VOR was not impaired in any fish in this study, although its impairment has been observed in sockeye and pink salmon air exposed for three or more minutes in other studies in the lower Fraser River (see chapters 6 and 7). Analogous to 'ocular counter-rolling' in humans (MacDougall et al. 1999), the VOR reflex in teleosts occurs when static signals in the otolith reach the extraocular motoneurons (Suwa et al.1999). When fish lose equilibrium, it may be caused by a combination of the breakdown of neural and muscle function broadly, but perhaps in particular at the fins involved in controlling balance (e.g., the pectoral fins), or via insufficient oxygen delivery to the brain (discussed in chapter 2). Body flex and tail grab both involve use of white myotomal musculature innervated by local motoneurons (Lauder 2006): these reflexes are likely to be impaired as a result of white muscle exhaustion (e.g., high lactate loading) rather than neurological dysfunction. Collectively, this suite of reflexes became progressively more impaired with increasingly severe

capture stress. Our supposition is that, as a whole-animal index of vitality, a higher

RAMP score predicts delayed mortality by indicating a state further from homeostasis

(i.e., higher allostatic load) from which a complete recovery is less probable (and

mortality is more likely). However, the proximate causes of delayed mortality following

exercise and air exposure stressors have yet to be identified (Wood et al. 1983, Davis

2002). Future research that uncovers the links between physiological disturbance, reflex

impairment, and mortality would be valuable from both an applied and fundamental

perspective.

Arguably the most important contribution of the present study is compelling

evidence that RAMP deserves further consideration as a tool for predicting delayed

mortality of fish released into the wild. Particularly for comparing vitality among fish,

assessing handling techniques, and determining post-release mortality, RAMP should be

considered for use in Pacific salmon management and research. In the case of fisheries in

the lower Fraser River, RAMP assessments could be conducted on coho prior to release

to determine if capture conditions are impairing fish condition to a point that increases

the likelihood of mortality. Fishers could change their behaviour in real time by

conducting shorter net sets and leaving fish in deeper water if fish condition is poor.

RAMP could also be used to determine the likelihood that a fish will survive if released,

facilitating decisions on whether or not the fish should be released, retained, or recovered

using recovery tools (e.g., Farrell et al. 2001a). Education programs could be used to

develop capacity for RAMP assessment among fishers and management agencies. Most

importantly, RAMP could be used to rapidly generate inexpensive mortality estimates for

different combinations of fishing conditions, handling techniques, and gear-types that

otherwise would be impossible given the prohibitive costs of conducting numerous telemetry (or confinement) studies. Paired with estimates of bycatch rates, discard mortality estimates facilitate a more accurate accounting of total fishing mortality in management models (Baker and Schindler 2009).

Although the data we have presented here represent a single case study, RAMP may be widely applicable in fisheries and potentially in other areas of wildlife management where animal welfare and mortality outcomes are monitored. A weight of evidence has already accumulated that RAMP works across taxa, systems, and in a variety of laboratory and field conditions. The most obvious value of RAMP lies in bycatch management and although mostly used with fish and shellfish thus far, all animals have reflexes that can become impaired. Thus, it is conceivable that conservation practitioners could use RAMP for managing the welfare and mortality of birds, turtles (see Stoot et al. 2013), and mammals caught as bycatch. There may even be potential for application of reflex measures of some form outside of fisheries, such as in monitoring the welfare and predicting the survival of translocated wildlife (e.g., Pinter-Wollman et al. 2009). The use of reflex measures has the potential to play an important role in assessing and managing fisheries-induced mortality and, in turn, contribute to conservation of Pacific salmon populations and possibly other species elsewhere.

Chapter 4. Bycatch mortality of endangered coho salmon: impacts, solutions, and aboriginal perspectives

4.1   Abstract

We used biotelemetry and human dimensions surveys to explore potential solutions to migration mortality of an endangered population of coho salmon caught as bycatch in an aboriginal beach seine fishery. From 2009 to 2011, 182 wild coho salmon caught as bycatch in the lower Fraser River (Canada) were radio-tagged and tracked as they attempted to complete their migrations to natal spawning areas over 300 km upstream. Failure to survive to reach terminal radio receiving stations averaged 39% over three years. This mortality estimate is low compared to those obtained from telemetry studies on other salmon fisheries in the Fraser River. However, this value is markedly higher than the mortality estimate currently used to manage the fishery's impact. It is also in contrast to the perceptions of the majority of aboriginal fishers, who do not think survival of coho salmon is affected by capture and release in their fishery. Increased probability of survival was associated with lower reflex impairment which is consistent with previous findings. Reflex impairment was positively correlated with entanglement time, suggesting that greater efforts by the fishers to release bycatch from their nets quickly would minimize post-release mortality. Survey responses by aboriginal fishers also suggested that they are receptive to employing new bycatch handling methods if they are shown to increase post-release survival. However, attempts to facilitate revival of a subset of captured fish using cylindrical in-river recovery bags did not improve migration success. Fisheries managers could use the new information from this study to better

quantify impacts and evaluate different harvest options. Since aboriginal fishers were receptive to using alternate handling methods, efforts to improve knowledge on minimizing reflex impairment through reductions in handling time could help increase bycatch survival. Such a direct integration of social science and applied ecology is a novel approach to understanding conservation issues that can better inform meaningful actions to promote species recovery.

4.2   Introduction

There are numerous examples in the literature of conservation scientists calling for an integration of the social sciences into biological research, and for the need to bridge the gap between science and conservation action (e.g., Campbell 2005, Fox et al. 2006, Lowe et al. 2009, Sutherland et al. 2009, Margles et al. 2010). Yet, research papers that include and integrate both sociological and biological data remain uncommon, despite their obvious potential to develop more "actionable" science (Cook et al. 2013). There are some examples. Irvine et al. (2009) interviewed managers of red deer *Cervus elaphus* populations, and combined their perspectives with scientific data to build habitat use models for the species. Interview approaches alone have been used to build models – such as for assessing threats to endangered sea turtles (Donlan et al. 2010). Donaldson et al. (2013) demonstrated that comparative physiology and radio telemetry could be combined with human dimensions surveys to address revival strategies for angled and released sockeye salmon. Given that application of the best science can be improved with stakeholder input, incorporating human dimensions data into conservation science is a natural fit.

Pacific salmon are among the most well studied wild animals. Their contribution to human culture, economies, and the functioning of ecosystems, coupled with the fact that many populations are in decline means that they receive enormous research attention from conservation scientists (Scarnecchia 1988, Gende et al. 2002). Their importance as a natural resource and the complexity of socio-political considerations in how they are managed (Scarnecchia 1988, Lackey 1999) make this a particularly fruitful area for integrative research. In the Fraser River, British Columbia, Canada, the fishery for Pacific salmon is complex, involving different user groups (aboriginal, recreational, and commercial) which target several species comprising hundreds of unique populations, many of which migrate upriver toward spawning grounds at the same time. Inherent in managing these fisheries is the objective that diversity be maintained, both within and among species (DFO 2005). Interior Fraser River coho salmon (that spawn in tributaries of the Fraser River upstream of Hell's Gate; Fig. 4.1, described in section 1.4) have received particular attention in recent years owing to their listing as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Directed harvest of wild coho salmon in British Columbia was closed in 1999 and the population has since stabilized at 20,000-30,000 returning adults, an incomplete recovery to pre-1990s abundance (R. Bailey, DFO, *personal communication*). To ensure that bycatch mortality is not limiting population recovery, in-river fisheries for other species have been significantly curtailed at the time of year that interior Fraser coho begin migrating through the river (IFCRT 2006). There have been some exceptions to this management strategy including permitting aboriginal groups to conduct beach seine fisheries in the lower Fraser River targeting pink, chum, or sockeye salmon. Beach seines are thought to

enable the live release of bycatch with higher subsequent survival than alternative gear such as gill nets. Regulations for the beach seine fishery state that fishers must release all wild coho salmon that are alive, with an inherent assumption that most of these fish will survive, continue their migration, and ultimately spawn. The agency responsible for managing this fishery (DFO) applies a 5% mortality rate to coho salmon bycatch for the purposes of accounting for fishing mortality and meeting spawning escapement targets (DFO 2011 - IFMP). However there is very little empirical evidence to inform estimates of beach seine bycatch mortality. Factors affecting mortality remain poorly understood, limiting the ability of fisheries to implement practices that minimize impacts.

When a fish is captured in a fishery, it experiences a suite of physiological disturbances (Farrell et al. 2001a, Davis 2002, Clark et al. 2012) and typically, some degree of injury (Chopin and Arimoto 1995). Although the proximate causes of post-release bycatch mortality are not well understood in fishes (Wood et al. 1983), certain components of the capture experience are likely more harmful than others. For example, crowding, hypoxia, air exposure, and exhaustive exercise are all thought to negatively affect fish (Ferguson and Tufts 1992, Davis 2002, Marcalo et al. 2006). The magnitude and duration of the capture stressor can influence physiological recovery time (Donaldson et al. 2010a), suggesting that minimizing capture stress is important. In addition, there are techniques that can facilitate metabolic recovery of fish and, in some cases, improve post-release survival (Milligan et al. 2000, Farrell et al. 2001a). For example, provision of a dark recovery environment that is free from predators and provides high flow across the gills can promote re-oxygenation of tissues, re-invigorating fish before release (Farrell et al. 2001a). Recent work in a sockeye salmon recreational

fishery has shown that specially-designed fish "recovery bags" (Donaldson et al. 2013) could be used to increase survival in some contexts.

This three-year study aimed to quantify capture experience for individual fish and use radio telemetry to assess survival. Combined with interviews of the aboriginal fishers taking part in the fishery, this study used an all-encompassing approach to provide results relevant to resource managers. There were three general objectives: 1) estimate immediate (i.e., at the time of capture) and post-release mortality for wild coho salmon caught in beach seines, 2) identify factors associated with bycatch mortality, and 3) assess potential ways to manage or reduce mortality. Given that a reflex impairment index can be used to predict delayed mortality in this fishery (chapter 3), we also explored correlates of reflex impairment. For objectives 2 and 3, we hoped to identify specific aspects of the capture experience that were most associated with mortality (e.g., crowding time) and evaluate whether cylindrical in-river fish "recovery bags" could be used to promote survival. Semi-structured interviews were used to understand fisher perspectives on issues relating to each of our objectives.

4.3   Methods

4.3.1   *Study area and fish capture*

This study took place in the Fraser River watershed (British Columbia, Canada). Data collection occurred over three years (2009-2011) during openings of the aboriginal beach seine fisheries. The fishery was targeting pink salmon in 2009 and 2011, and sockeye salmon in 2010. In each case, Fraser River coho salmon caught as bycatch were en route upstream towards their natal streams to spawn. We attended the fishery to collect data on

every day it was open in each year; Sept. 21-23 in 2009, Sept. 15 – 17 in 2010, and Sept. 17 – 19, 22, and 24 in 2011. The mean daily river temperature, measured at a temperature monitoring station upstream of Hope (Fig. 4.1), was 15.88 °C (range: 15.76-16.08 °C) on the fishing days in 2009, 15.12 °C in 2010 (15.07-15.18 °C), and 15.32 °C in 2011 (14.85-15.59 °C).

We worked with multiple fishing crews at five different locations (some fishing sites had two or more fishing crews operating adjacent to each other). Almost all of the data collection occurred at three sites: Peg Leg, Seabird, and Peters (Fig. 4.1), which were 108, 129, and 131 river kilometers (rkm), respectively, upstream of the river mouth. Those sites and crews were chosen based on accessibility, the number of crews, and the size of catches. The two other sites were Mountain Bar (just downstream of Peg Leg – 8 fish tagged) and a location halfway between Peg Leg and Seabird (2 fish tagged; Fig. 4.1). The fishing crews used beach seines that were 90 m long x 9 m deep x 5 cm diamond stretch mesh. Nets were pulled out from the riverbank using powerboats with one end anchored on shore and were allowed to drift downstream for ~ 30-60 s then pulled by the boat in an arc to a downstream point on shore (total time ~ 5 min), before being pulled in by hand or with a truck until fish were crowded into shallow water (< 0.5 m deep) for sorting (Fig. 4.2). Attempts at locating and releasing bycatch varied among crews, but in most cases crews spent 1 – 3 min searching for bycatch prior to collecting their target species. When coho salmon were found by the fishing crew they were given to us for tagging and biopsy (details below, 4.3.2) rather than released directly to the river. Entanglement time was calculated for each fish as the time between the net being pulled to shore and the fish being released from the net. For each set, we also obtained

the total number of salmon caught from a member of the fishing crew whose duty was to enumerate the catch. For some sets only the number of females was recorded (only females were being retained); for such sets we estimated the total catch by assuming a 50:50 sex ratio.

4.3.2   Fish sampling and tagging

Upon removal from the seine, coho salmon were immediately placed in individual fish holding bags for tagging, biopsy, and measurements (described in chapter 3, section 3.3.1). Radio transmitters were gastrically inserted; a rapid (< 10 s) tagging method with no anesthesia that has been validated and used extensively in Fraser River salmon (e.g., Cooke et al. 2005, English et al. 2005, Martins et al. 2011, Wilson et al. 2013). We used two models of individually-coded radio transmitters that were functionally identical with the same transmission frequencies (as in chapter 3), dimensions (16 mm diameter, 46 mm long, 460 mm long antenna), and weight (17 g in air, 7 g in water; Pisces 5, Sigma-Eight Inc., Newmarket, Ontario, Canada; and MCFT-3A-3 V, Lotek Wireless Inc., Newmarket, ON).

Once tagged, each fish was measured for fork length (FL, nearest cm) and any apparent injuries were noted. A ~ 0.5 g piece of adipose fin tissue was removed from each fish using a hole punch, and stored in 95% ethanol for DNA analyses. Finally, we conducted a rapid (< 20 s) assessment of the presence or absence of five simple reflex responses (RAMP; described in chapters 2 and 3). Following tagging, measurements, and the reflex assessment, coho salmon were released to resume their migration towards spawning areas (except for fish exposed to the recovery bag treatment – see below).

Beginning in 2010, tagged coho salmon were chosen at random for an experimental treatment to evaluate whether a cylindrical recovery bag could be used to increase post-release survival. The bags that were used for facilitated recovery (Fig. 4.3) were narrower than those used for tagging (20 cm diameter x 1 m length) and had much larger mesh (4 cm diameter rigid diamond mesh) to maximize the flow of river water through the bag along its longitudinal axis. Once a fish was transferred into a recovery bag facing into the current, the lengthwise zipper along the top was closed and the bag was attached to a 1.5 m long reinforcing bar that had been driven into the riverbed (Fig. 4.3B). The duration of the treatment was 30 min, after which time the zipper was opened with the bag fully submerged and the fish encouraged to swim out of the top of the bag. The recovery bag treatment occurred only at three tagging sites (Peg Leg, Seabird, Peters), and we consistently used the same location at each site. The mean water speeds where the recovery bags were placed were ~ 0.5 m·s^{-1} at Peg Leg, ~ 0.2 m·s^{-1} at Seabird, and ~ 0.7 m·s^{-1} at Peters.

Cumulatively, we radio-tagged and tracked 182 coho salmon: 50 in 2009, 53 in 2010, and 79 in 2011. In each year, all coho salmon were tagged until our supply of radio transmitters was exhausted – in total we handled 295 coho salmon. Fish that did not receive transmitters were otherwise processed in the same way, with the primary goal being to collect additional RAMP and entanglement time data. Of the 182 that were tagged, 64 were exposed to the recovery bag treatment – roughly half of the 132 fish we tagged over 2010 and 2011 (no recovery bag treatment in 2009). Most of the data collection in 2010 and 2011 occurred at Peg Leg and thus most of the recovery bag treatments (43 of 64). In 2011, we collected additional data on bycatch condition (e.g.,

RAMP, FL, entanglement time) from 53 individuals that were not tagged. Also in 2011, we opportunistically used a handheld meter (CellOx 325, WTW Inc., Weilheim, Germany) to monitor dissolved oxygen (DO) depletion over time in beached seines. This was done to estimate the extent to which localized DO depletion can occur in crowded seines during sorting.

4.3.3 Population identification

Adipose fin clips were analyzed in the laboratory for population origin, and subsequently grouped into populations as described in chapter 2 (Beacham et al. 2011). Populations into which fish were grouped for analyses in this chapter were: Fraser canyon, Fraser middle drainage, lower Thompson River, North Thompson River, and South Thompson River (Fig. 4.1, Table 4.1). Coho salmon tagged in this study also belonged to three additional populations that were not from the interior Fraser River watershed: Birkenhead River, Chilliwack River, and the lower Fraser drainage (Table 4.1). Identification of population-origin facilitated accurate determination of whether individuals successfully migrated towards their natal subwatersheds, beyond terminal radio receivers (see below).

4.3.4 Telemetry tracking

Radio-tagged coho salmon were tracked using an array of radio receiver stations installed at strategic points in the watershed (Fig. 4.1); methods previously used to assess survival of Fraser River salmon (e.g., English et al. 2005, Cooke et al. 2006, Donaldson et al. 2011, Donaldson et al. 2013, Nguyen et al. 2014). The receiver array differed in each year but key receivers were present at the same locales each year (e.g., Hope, Hell's Gate,

Thompson-Fraser confluence). We considered coho salmon as migratory 'survivors' if they were detected at the upstream-most receiver stations en route to spawning areas. In eight instances (among all 182 fish) there was clear evidence of straying whereby fish migrated to terminal spawning areas to which they were not DNA-identified (e.g., into the South Thompson watershed rather than the North Thompson), in some cases undergoing lengthier upstream migrations than if they had migrated to their natal stream. Those fish were assessed as survivors. Distances from terminal receivers to actual spawning sites varied depending on spawning location (~ 5 – 300+ rkm; Fig. 4.1). We also quantified two day survival. Detection efficiency was variable among receivers and years (range = 24 – 100%), but was generally high, particularly at the key receivers (i.e., Thompson-Fraser rivers confluence) and at many of those used as "terminal" points for assigning survivorship. For example, detection efficiency at the Thompson-Fraser confluence was 100% in 2009, 96.7% in 2010, and 85.4 % in 2011. Detection efficiency was 100% at Hell's Gate in 2009 and 2010, and 56.8% in 2011. Detection efficiencies were assessed by diving the number of fish detected on a receiver by the total number known to have passed that receiver – confirmed either by detection at subsequent receivers, by mobile tracking efforts, or by tag recaptures that were reported. Detection efficiency estimates were therefore inherently less robust for the upstream-most receivers and in some cases, unknown for a given terminal receiver in a given year. In general, very little mortality was apparent in upper watersheds (beyond the Thompson-Fraser confluence) such that non-detection at terminal receivers was uncommon for fish that had reached the next most upstream receiver. Among all 182 fish, there were six individuals assigned as mortalities based on non-detection at a terminal receiver whose efficiency

was unknown in that year (two in 2010, four 2011), meaning there was some uncertainty associated with final migratory success of those fish. Nevertheless, receivers in upper watersheds typically have good detection efficiency because fish are generally moving through narrower and shallower waters as they approach spawning areas, thus reducing the likelihood of fish using deep water locations that attenuate the signals emitted by their transmitters.

4.3.5   Data analysis and statistics

To determine what factors affected post-release survival (0 = died, 1 = survived), we used a forced-entry binary logistic regression with six predictor variables: entanglement time (s), total catch size (number of fish), air temperature, FL, use of revival bag treatment (0 = not used, 1 = used), and RAMP score. Air temperature was used in place of river temperature because it varied widely among study days, possibly affecting water temperatures in shallow streamside areas where entanglement occurred, whereas deeper river temperatures exhibited minimal variation where temperature monitoring stations were located. Differences in survival among populations, years, and capture locales were assessed for significance using Pearson's chi-square test. To examine associations between RAMP scores, handling time, and total catch, we used Spearman rank-order correlations. Confidence intervals for mortality estimates were calculated to provide a measure of sampling error and took the form of binomial confidence intervals, based simply on the number of mortalities and the total number of fish (using the Clopper-Pearson exact method, similar to Stokesbury et al. 2011). All statistical tests were conducted using R (v. 3.0.0). Tests were assessed as significant at $\alpha = 0.05$.

4.3.6 Human dimensions surveys

In 2011, we conducted face-to-face interviews with aboriginal fishers and members

involved with the aboriginal fishing process, which included crew monitors, fish buyers,

and buying employees (N = 111). We included the latter groups (< 10 % of interviewees)

because they were also aboriginal and most had directly participated in this harvest

fishery in the past as fishing crew members or had extensive exposure to beach seining

through their role in the fishery. Moreover, buyers and monitors are highly involved with

the fishing process, are knowledgeable about fishing methods and locations, and can

potentially influence fisheries policy. Participants were chosen opportunistically due to

logistical limitations (i.e., access to fishing sites), and timing of when fishing crews were

on breaks from fishing. We aimed to interview at least 50% of the members of each crew,

including the crew chief, and to interview all crews present at a given site. We

interviewed members of ~ 30% of the fishing crews who participated in the beach seine

fishery in 2011 (K. Burnett, DFO, *personal communication*). Beach seine crews consisted

of 11 individuals on average (range = 8 – 13). Fewer than five percent of those

approached declined to take part in the interview. Seventy-two (72) of 111 interviews

were conducted streamside during the aboriginal economic opportunity beach seine

fishery. The remaining 39 interviews were opportunistically conducted during an

aboriginal economic opportunity gillnet fishery (targeting sockeye salmon) that occurred

a month earlier (on 24/08/11 and 25/08/11). Because questions #1-4 (see below) only

pertained to the beach seine fishery, respondents in the gillnet fishery were only asked

those questions if they stated that they also take part in the beach seine fishery, or have done so in the past (28 of 39).

The interviews were semi-structured, meaning that aboriginal fishing members could identify any additional topics, concerns, or ideas relating to coho bycatch during the interview. The interviews were used to address a number of research objectives, some of which are beyond the scope of this study (see Nguyen et al. 2012). We used two separate interviews: one shorter questionnaire and a long-form questionnaire. The short questionnaires were a subset of the long questionnaire, and included the same closed-ended questions (data not presented here) while open-ended questions alternated among the following themes: participant perspectives on coho bycatch, perspectives about biotelemetry science, and thoughts on the fish recovery bag. The short questionnaire was intended to be for individuals that only had 5-10 minutes to spare, while effort was made to ensure all crew leaders participated in the long questionnaire. For individual questions, the number of responses was somewhat lower than the total number of interviewees (111, see Fig. 4.5, 4.6, 4.7) because alternating versions of the shorter questionnaire contained only a subset of the questions, or because a question was not applicable to the interviewee (e.g., if they were a fish buyer). We asked the following questions:

1. Do you think that beach seine capture has any effect on a coho's chance of successful migration or spawning?

2. What do you think causes the greatest stress for coho released from beach seines?

3. What suggestions do you have to increase the survival of coho bycatch?

4. If you knew that leaving the seine in knee-high water for sorting would increase the chance of survival for released fish, would you voluntarily do it?

The interviewees were then shown a fish recovery bag (those tested in this study), accompanied by an explanation of its purpose and use. They were then asked the following questions:

5. What are your thoughts on the recovery bag?

6. If research data show the recovery bag improves post-release survival for salmon, how likely would you be to use one on a voluntary basis?

7. Do you think the recovery methods and gear should be mandatory for reviving coho bycatch from seine capture?

All survey responses were coded according to emergent themes following standard qualitative protocol (Strauss 1987, Creswell 2009). We assessed for consistencies among codes (similar meanings or pointing to a basic idea), and revealed themes and categories reflecting fisher responses to the particular questions (Fig. 4.5, 4.6, 4.7). In particular, responses to questions 1, 6, and 7 were coded into five themes: a) 'no', when respondents provided an assertive *no* to the question or scenario (e.g., coho are not affected by beach seine because we release them immediately, or no need for a recovery bag because revival can be made by holding fish in water); b) 'no conditional', when responses were no with conditions or dependent on certain situations (e.g., coho salmon are not affected by capture *if* they are handled carefully); c) 'neutral', when responses were neither yes or no (or both) or the respondents was uncertain; d) 'yes conditional', when responses were agreeable but with certain conditions, situations, or with some skepticism (e.g., coho are affected by capture depending on handling or release time, or fishers would use recovery bag if they did not have to pay for it); e) 'yes'; if respondents were fully in agreement with the question or scenario. The coding for 'no' in question 7 was extended to those

that were justified versus unjustified. Responses to question 5 were grouped into five themes as: a) 'negative', if respondents provided negative opinions and perspectives to the question; b) 'negative but legitimate', when respondents provided negative answers but with legitimate or justified reasons (e.g., recovery bags were not perceived to be necessary, bags could cause additional stress or damage to fish, number of coho salmon caught are too low to warrant recovery gear); c) 'alternative', when respondents provided an alternate response, solution, and/or idea; d) 'positive with condition', when responses were positive but dependent on certain conditions or situations (e.g., recovery bags are good idea but needs improvement on design, or as long as the bag did not stress the fish); e) 'fully positive', if respondents had completely positive thoughts about the recovery bag. Coded responses revealed 15 emergent themes for question 2, 12 categories question 3, and six for question 4 (see Fig. 4.5 and 4.6). We did not conduct any statistical tests on resultant survey responses, noting the paper by Drury et al. (2011) that recommends the use of qualitative approaches to using social science data in conservation research.

Figure 4.1 Fraser River watershed map with locations of radio receivers used in different years and the locations of the three main capture/release sites (Peg Leg, Seabird, and Peters). The spawning areas of the four main interior Fraser coho populations are indicated and circled by grey hashed lines (not circled: the Fraser canyon population, which comprises only one DNA-identified spawning tributary - the Nahatlatch River ~ 30 rkm upstream of Hell's Gate).
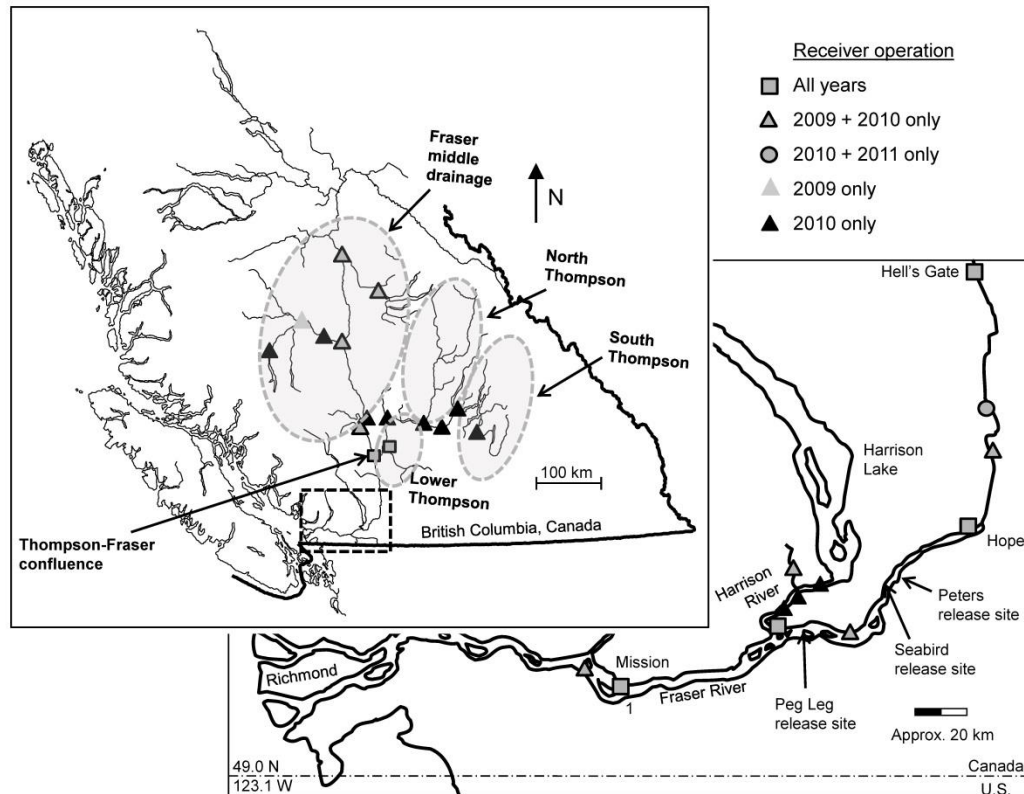
Figure 4.2 Photos showing different ways in which the aboriginal fishing crews pulled their nets into shore for sorting, during which "entanglement time" was recorded for individual coho salmon caught as bycatch. Photos show a typical set (A), a very large set with high crowding that required hours to sort through (B), and a smaller set that was pulled entirely onto shore for sorting (C).

Figure 4.3 Images showing the fish recovery bag open at the water's surface (A) prior to having a fish inserted for (B) a 30-min revival period in the river current with the bag attached to a reinforcing bar driven into the riverbed.
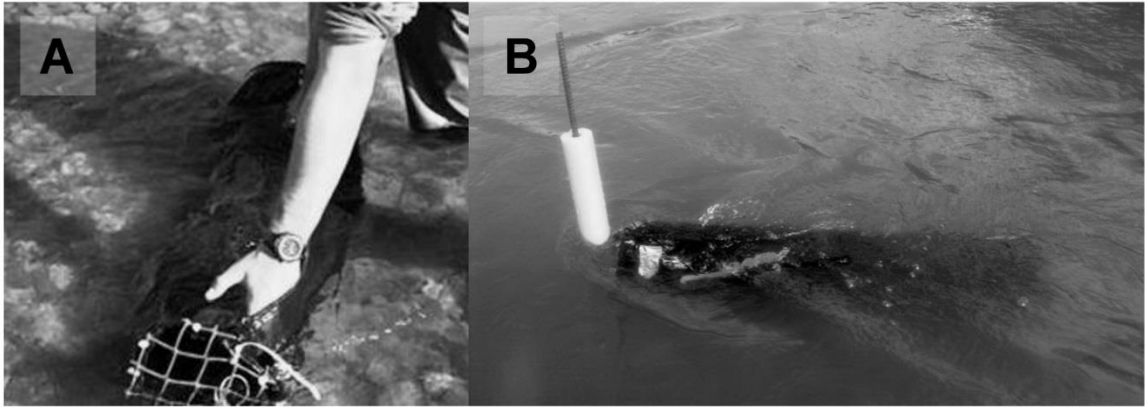
4.4 Results

Collectively, we handled 295 wild coho salmon, bycatch that was spread across 98 net sets and three study years. In those net sets, collectively, an estimated 54,790 adult salmon were caught, meaning that the coho salmon bycatch for those sets was ~ 0.5% of total catch. The real bycatch rate is somewhat lower, owing to the fact that we did not record any data for sets in which no bycatch occurred (~20-40% of all sets). The median total catch (number of adult salmon) for net sets from which coho salmon were tagged was estimated as 500 (range: 70 – 2000). All but eight of the coho salmon we tagged and tracked were DNA-identified as belonging to the interior Fraser River population complex (Table 4.1; note that no fish were excluded from the mortality estimate on the basis of population-origin).

Among all the coho salmon we handled from 2009-2011, the mean fish size (FL) was 61.6 cm (median: 62 cm, range: 37 – 82 cm). Median entanglement in the beached seine was 3 min 20 s (mean: 6 min 32 s, range: 5 s – 55 min 58 s). The most frequently observed RAMP score was 0.4, an impairment level that was characterized 97% of the time by loss of the tail grab and body flex reflexes. There was an apparent immediate effect of the recovery bag on fish vitality: after 30 min in the recovery bag treatment, all fish were highly vigorous (i.e., RAMP score of zero).

4.4.1   Bycatch mortality

Of the 182 coho salmon we tagged, 65 (36%) failed to migrate past terminal radio receivers en route to spawning areas, and were thus classified as mortalities (Table 4.2). Adjusting our sampling effort (N = 182) to a binomial distribution provides a 95% confidence interval (C.I.) for post-release mortality of 29-43% (Stokesbury et al. 2011). Nine (9) of the 295 coho salmon (3%) caught during the study were dead upon being pulled from the net (Table 4.2). Thus, the cumulative mortality rate was 37.3% (95% C.I. = 32 - 43%). Using the probability of mortality associated with each RAMP score to predict migration success of the non-tagged fish that were released alive (rather than applying a blanket post-release mortality rate to those fish whose fates were unknown), the total mortality estimate was 39.3% (Table 4.2; 95% C.I. = 34 - 45%). There were no significant differences in post-release mortality among the four main capture locations ($\chi^2_{3, 181}$ = 1.71, P = 0.63) or among years ($\chi^2_{2, 181}$ = 1.29, P = 0.53). Estimated population-specific differences in mortality were not significant (Table 4.1; Chi-square test: χ^2 = 1.39, df = 5, P = 0.99). Among all the variables we tested as predictors of mortality, none

were significant except for RAMP score, whereby fish with higher RAMP scores (more impaired) were less likely to be successful migrants (Table 4.3, Fig. 4.4). Use of a revival bag did not influence post-release mortality ($P > 0.05$, Table 4.3).

Since some in-river mortality is natural, there is a need to attempt to differentiate mortality caused by the capture itself. To do so, RAMP scores can be used whereby coho salmon released with little or no reflex impairment (vigorous) are assumed to experience no post-release bycatch mortality. Using that conservative assumption, the post-release mortality rate for those fish can then act as a baseline within the dataset. Additional mortality above that baseline that occurs at higher RAMP scores can then be assigned to the fishery (see Fig. 4.4). The 'baseline' post-release mortality rate at zero reflex impairment was 23% (Fig. 4.4). Applying a net mortality rate above that baseline for higher RAMP scores to the total number of fish at those RAMP scores yields an alternate estimated proportion of fish that died as a result of the capture stress − 13.6% overall of those released alive (Fig. 4.4). Combined with immediate mortality, this conservative approach accrues a bycatch mortality estimate of 16.6% (95% C.I. of 13 − 21%).

Entanglement time and catch size were not predictive of survival, but they were associated with reflex impairment (i.e., RAMP score - itself predictive of survival). There was a small but significant positive correlation between entanglement time and RAMP score ($r_s = 0.18$, $P = 0.03$). Total catch was not significantly correlated with RAMP score ($r_s = 0.15$, $P = 0.06$), but was strongly correlated with entanglement time (Pearson's $R = 0.64$, $P < 0.001$).

4.4.2   Human dimensions surveys

The majority of the participants of the beach seine fishery we surveyed stated that they thought beach seine capture does not or is not likely to affect a coho salmon's subsequent migration or spawning success (no effect – 41%, no effect under most conditions – 21%; Fig. 4.5A; total numbers of respondents provided in Figure). As such, only 12% of participants' responses were codified as a "yes", with a further 12% as a "conditional yes" (e.g., yes - stressful enough to reduce survival under certain scenarios). In a following question, many respondents identified crowding (23%), handling (19%), and air exposure (9%) as particularly stressful components of the capture experience (Fig. 4.5B). Fishers were then asked if they had any suggestions about how to increase the survival of coho salmon caught in their nets, and the most common response (26%) was to release fish quickly (Fig. 4.6A). Cumulatively, however, more respondents (35%) stated that nothing could be done (21%) or did not have an approach to propose (14%; Fig. 4.6A). When asked whether they would be likely to voluntarily leave the seine in knee-deep water for sorting (if it were shown to increase survival), 51% responded in the affirmative, while a further 26% stated that they already use this practice (Fig. 4.6B). Nine percent expressed concern that leaving the net in deeper water could compromise the safety of their crew.

       We received positive feedback to the concept of the fish recovery bag. Forty-seven (47) percent of respondents (44 of 94) gave fully positive responses when briefed about its design and purpose and asked for their thoughts, with a further 17% providing conditionally positive responses (e.g., it would have to be shown to improve survival; Fig. 4.7A). Many fishers thought the use of a revival bag should be mandatory, without

the qualifier that evidence would support its use (44%; Fig. 4.7B). However, responses were more positive to a proposal that recovery bag use be voluntary rather than mandatory, *given* scientific data to support use of recovery bags (Fig. 4.7C). A number of the conditionally positive responses arose from uncertainty about the monetary cost of the bags and whether they would be provided or subsidized by management.

Figure 4.4 Observed post-release mortality rates (whole bars) at each level of reflex impairment (RAMP score). The white sections of each bar represent a proposed 'baseline' mortality rate within the dataset, whereby only mortality that occurred above that level (black bars) was assigned to the fishery. Based on this calculation, the numbers at the bottom of each bar represent the number of fish assigned as survivors of the capture event at each RAMP score, while the numbers in the black bars are the numbers of fish assigned as mortalities at each RAMP score by applying a net mortality rate (total mortality minus 23%) to the total number of fish with each RAMP score. For example, the net mortality rate used for the fish with RAMP scores of 0.6 was 28% (51% - 23% baseline), which was then applied to the total number of fish tagged at that RAMP score (N = 37). The two numbers within each bar add to the actual total number of fish released with transmitters at that RAMP score, but note that the total N in this figure is 176 – although 182 coho salmon were tagged and tracked, RAMP scores were not recorded for 6 individuals. Thus, this alternate post-release mortality rate of 13.6% comes from dividing the cumulative fishery mortalities indicated (24) by a total of 176.
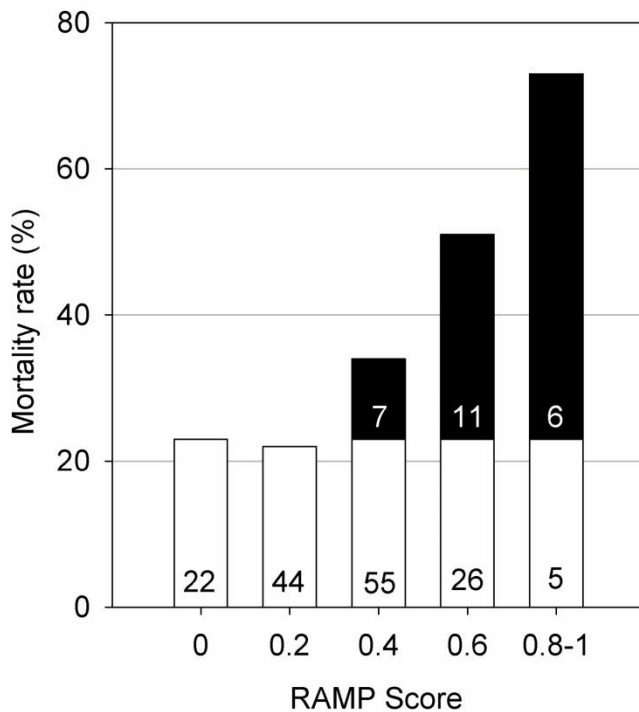
Figure 4.5 Responses of aboriginal fishers in our human dimensions surveys to questions relating to coho salmon capture stress and mortality. For A there were 58 responses and for B, N = 70. Responses of individual fishers were coded into the response categories shown in the figure and details described in 4.3.6.
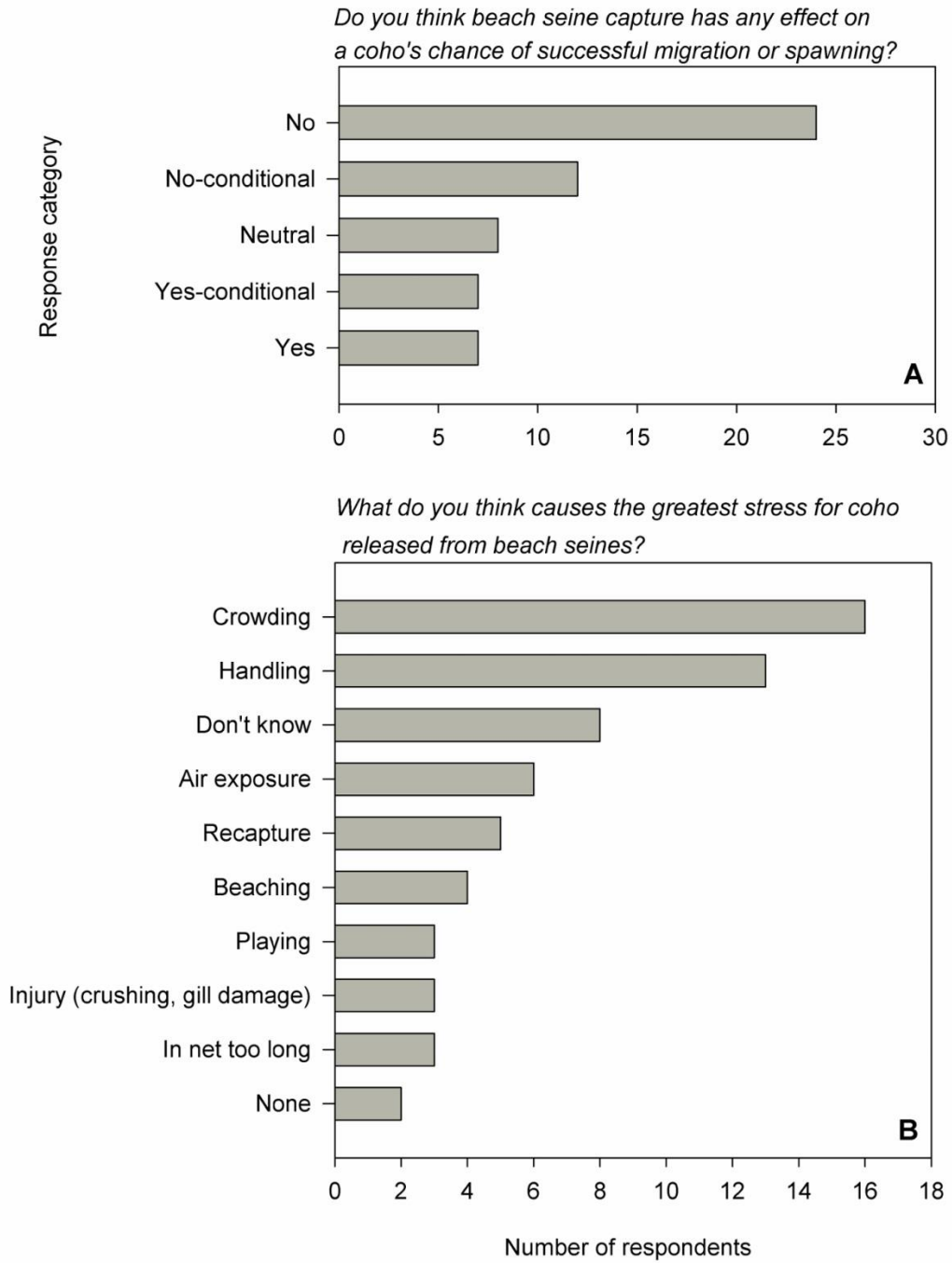
Figure 4.6 Responses of aboriginal fishers in our human dimensions surveys to questions relating to ways to improve the survival of coho salmon bycatch in the beach seine fishery. Responses of individual fishers for both questions were coded into the response categories shown in the figure, and details described in 4.3.6. There were 63 responses for question A. For B, N = 54.
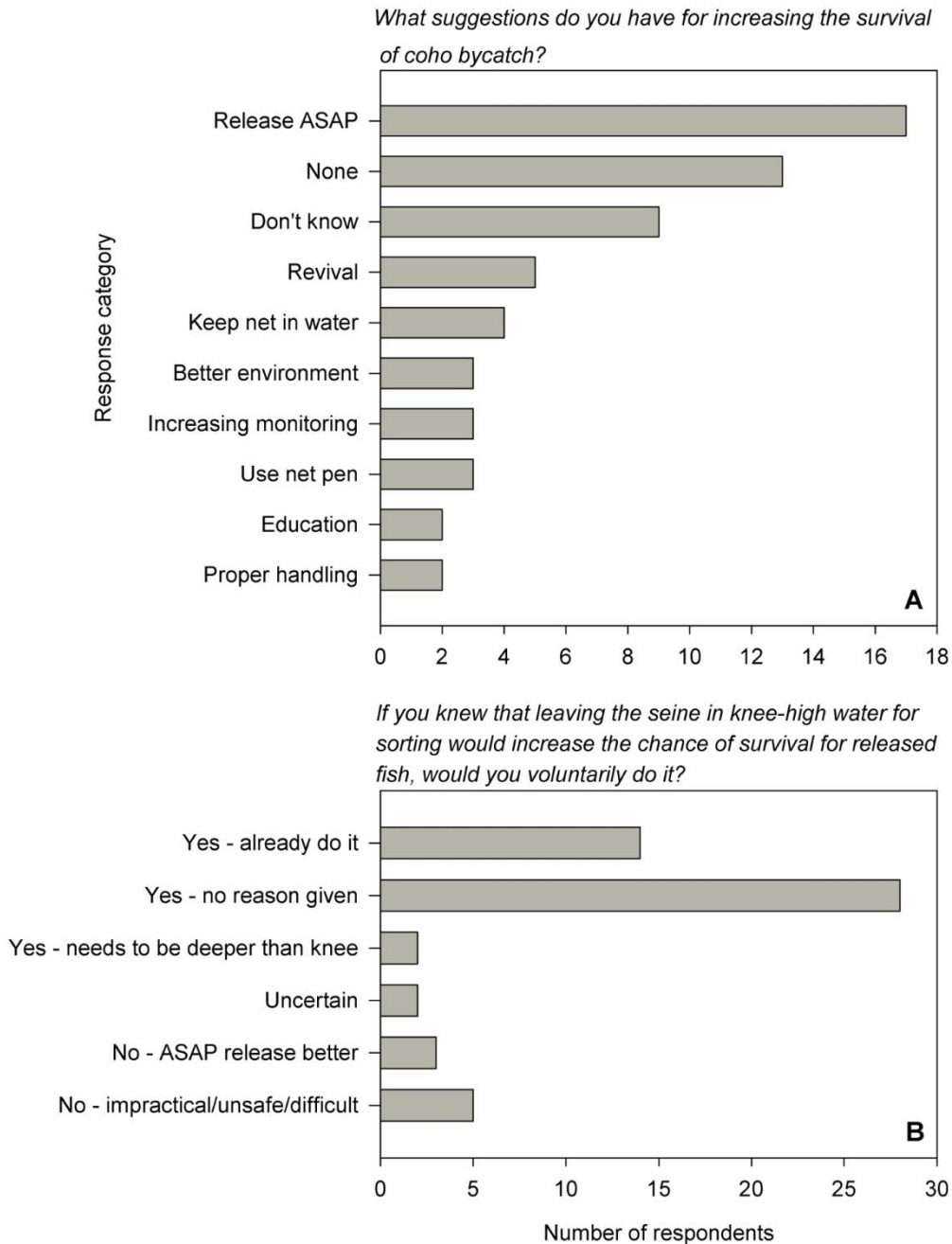
Figure 4.7 Responses of aboriginal fishers in our human dimensions surveys to questions relating to the use of fish recovery bags for coho salmon bycatch in the beach seine fishery. Prior to asking questions A (N = 94 responses), B (N = 96), and C (N = 86), the interviewees were shown a recovery bag and its purpose and use explained to them. Responses of individual fishers were coded into the response categories shown in the figure, and details described in 4.3.6.
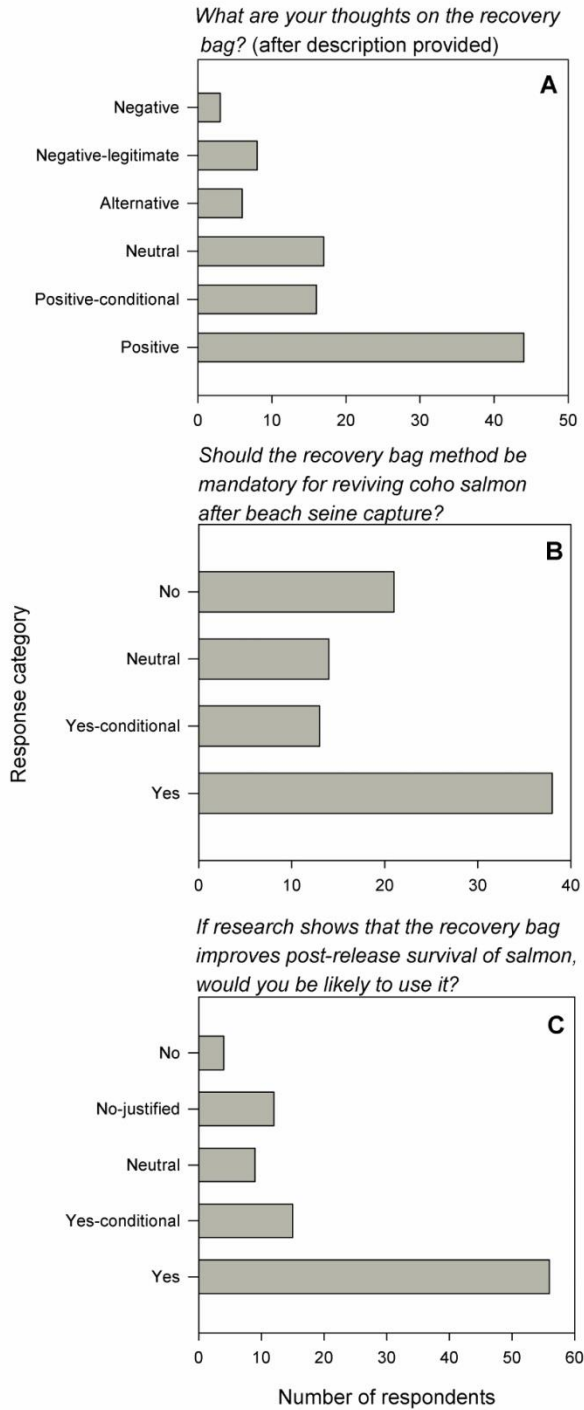
Table 4.1 Population (stock) proportions and individual stream IDs of coho salmon captured in the study from 2009-2011, determined via analysis of microsatellite variation in DNA (N = 223; see Beacham et al. 2011). These samples include fish tagged and released with transmitters and additional fish sampled for DNA (mostly blood-sampled fish from 2009; see chapter 3). All of these groups are part of the interior Fraser coho group except for the bottom three populations, which split off the Fraser River main stem downstream of Hell's Gate. "Actual percentage of overall population group" refers to the true percentage of the entire interior Fraser River coho salmon group represented by each population, based on DFO spawning ground stock assessment data for the years 2009-2011 (R. Bailey, DFO, *personal communication*). Post-release survival was not significantly different among populations ($\chi^2 = 1.39$, df = 5, $P = 0.99$).

Population (% of interior Fraser coho)	Actual percentage of overall population group	Mean Fork Length (± S.E.)	Post-release survival estimate (%)	Stream origin (N)
North Thompson River N = 86 (41%)	35%	61 ± 0.7 cm	67%	Pig Channel (37) Lemieux Creek (20) Louis Creek (8) Birch Island (7) Fennell River (6) Reg Christie Creek (5) Dunn Creek (2) Mann Creek (1)
Lower Thompson River N = 41 (20%)	23%	61 ± 0.9 cm	73.6%	Coldwater River (28) Spius Creek (12) Bonaparte River (1)
South Thompson River N = 38 (18%)	21%	62 ± 0.8 cm	58%	Eagle River (20) Harbour Creek (7) McMomee Creek (6) Bessette Creek (3) Salmon River (3) Wap Creek (2)
Fraser River middle drainage N = 37 (18%)	13%	61 ± 0.9 cm	52%	Bridge River (22) McKinley Creek (13) Gates Creek (2)
Fraser River canyon N = 7 (3%)	8%	67 ± 2.4 cm	71%	Nahatlatch River (7)
Birkenhead River N = 6		67 ± 3.8 cm	67%	Birkenhead River (6)
Fraser River lower drainage N = 4		71 ± 2.2 cm	75%	Chehalis River (4)
Chilliwack River N = 1		67 cm	0%	Chilliwack River (1)

Table 4.2 Observed mortality rates of coho salmon bycatch for each of the three study years and estimated cumulative mortality rates. Immediate mortality numbers were generated by direct observation of whether individual fish were alive when released from the net, whereas mortality estimates to 48 h and to natal sub-watersheds were made using radio telemetry. The overall estimates are weighted and cumulative such that the post-release mortality estimates were applied to fish that were released alive but not tracked. Differences in post-release mortality among years were not statistically significant (Pearson's chi-square test: $\chi^2 = 1.29$, df $= 2$, $P = 0.53$). The binomial probability 95% confidence interval for the final overall mortality rate (39.3%) was $34 - 45\%$. For 48 h mortality (18.6%) it was 14-24%.

		Post-release mortality	
Year (N tagged)	Immediate mortality	48 h	To upper watersheds
	5%	6%	26%
2009* (50)	(5 of 104)	(3 of 50)	(13 of 50)
	2%	15%	43%
2010 (53)	(1 of 55)	(8 of 53)	(23 of 53)
	2%	23%	37%
2011 (79)	(3 of 135)	(18 of 79)	(29 of 79)
Overall	**3%**	**18.6%**	**37.3%**
estimates	9 of 295	55 of 295	110 of 295
	Final mortality rate estimate if		
	RAMP scores used to predict fates	**39.3%**	
	of non-tagged fish	116 of 295	

*From chapter 3

Table 4.3 Regression coefficients (*B*, with standard error) and odds ratios (with 95% CIs) for each predictor variable included in the logistic regression model with post-release survival as the binary response variable (0 = unsuccessful migrant, 1 = successful migrant). Odds ratios of below 1 indicate a negative relationship with the response variable whereas the opposite is true of odds ratios above 1. RAMP score was the only significant predictor of survival (P < 0.001).

Predictor variable	*B (SE)*	Lower	95% CI for odds ratio Odds ratio	Upper
Constant	1.19 (2.60)			
RAMP score	**- 3.56 (0.93)**	**0.004**	**0.03**	**0.16**
Use of revival bag	-0.33 (0.42)	0.31	0.72	1.65
Entanglement time	4.5×10^{-4} (5.1×10^{-4})	1.00	1.00	1.00
Total catch	-6.8×10^{-4} (5.3×10^{-4})	1.00	1.00	1.00
Air temp.	0.05 (0.06)	0.94	1.05	1.17
Fork length (cm)	-1.1×10^{-3} (0.03)	0.94	1.00	1.07

Note: Overall model R^2 = 0.127 (Hosmer-Lemeshow), 0.154 (Cox-Snell), 0.21 (Nagelkerke). Model $\chi^2(6) = 24.45$, $P < 0.001$.

4.5 Discussion

We have provided an estimate of bycatch mortality for an endangered population of coho salmon captured in an aboriginal beach seine fishery, based on three years of radio-tracking the migration success of fish released from the fishery. In addition, we have evaluated whether altered capture and handling techniques (Fig. 4.2) or the use of a revival bag (Fig. 4.3) could reduce bycatch mortality. The inclusion of human dimensions data provides insight into the perspectives of the fishers on the impacts of, and solutions to, coho salmon bycatch in their fishery. This combined approach allows us to make recommendations to management that are informed by the resource users, who themselves can directly affect successful implementation of management strategies (e.g., rapid release of bycatch).

4.5.1   Bycatch mortality

The total mortality estimate of 39%, inclusive of natural mortality, is relatively low when compared against similar studies on post-release survival for Fraser River salmon. For example, sockeye salmon caught by anglers experienced ~65-70% total mortality from release in the lower river to reach terminal radio receivers, while those released in parallel from a beach seine had a mortality rate of ~43-48% (Donaldson et al. 2011, 2013). Gillnets typically cause higher post-release mortality – up to 70% within just 24-48 h based on net pen holding studies (in the marine environment; Buchanan et al. 2002); a 60% mortality rate is applied to gillnet bycatch by management to account for incidental mortality in attempts to meet spawning escapement targets (DFO 2011). Thus, there appears to be consistent evidence that beach seine capture results in the lowest post-

release mortality for Fraser River salmon, particularly when using the more holistic approach of examining long-term mortality.

Not all observed mortality (39%) can be attributed to beach seine capture as there is a component of natural en route mortality which we have no rigorous way of distinguishing. To account for impacts on numbers of salmon reaching spawning areas (termed escapement), DFO typically apply mortality rates to bycatch that are based on 24 or 48 h post-capture net pen holding studies. Net pen studies can under- or over-estimate mortality because of possible synergies between capture stress, confinement stress, and predation risk (Rogers et al. 2014 and see chapter 2), but do have the advantage that very little of any observed mortality is likely to be natural because of the small time window. Immediate and short-term (48 h) mortality (combined) was estimated to be 18.6% in the present study (95% C.I. of 14 - 24%). An alternate approach to estimating bycatch mortality that attempts to distinguish bycatch-induced mortality from natural migration failure, is to use RAMP scores and their mortality rates at each level of impairment, and assume negligible bycatch mortality for the fish that were least impacted (vigorous at release). That approach (Fig. 4.4) yields a bycatch mortality rate of 17% (rounded from 16.6%). The remaining mortality (39% total - 17% = 22%) could then be attributed to a combination of natural migration failure, unreported fisheries removals, and the unknown long-term effects of the transmitter. Although we have no true control for the mortality we observed, this approach (Fig. 4.4) uses low RAMP score fish within our dataset as comparative controls. The 'control' mortality rate within the dataset was 23%, from release to upper watersheds. For coho salmon, high uncertainty exists about natural in-river mortality, but available data for Thompson River sockeye salmon stocks with

similar migration timing show that natural mortality ranges from 8-20% from Mission (Fig. 4.1) to spawning areas (English et al. 2005, Martins et al. 2011).

True control mortality rates are often unknown in post-release mortality studies; every method of estimating mortality has limitations. Short-term captivity is the most common method (e.g., using a net pen, Rogers et al. 2014), but this method fails to expose fish to the challenges of upstream migration and imposes severe confinement stress (cortisol elevation) in wild Pacific salmon (chapter 2, Donaldson et al. 2011). The best alternative to captivity is to use biotelemetry (Donaldson et al. 2008), but most biotelemetry applications involve intracoelomic implantation via surgery or external attachment of a transmitter, with the latter typically requiring puncture of the dorsal musculature to attach a tag that creates external drag (e.g., 'backpack' attachment used in chapter 2). The gastric tagging method we used is among the most rapid and least injurious tagging methods available in fisheries science: it involved a $< 1\%$ tag burden (by weight), a rapid < 10 s procedure with no injury or anesthetic, and created minimal hydrodynamic drag. This tagging procedure has been widely used in Fraser River salmon (Cooke et al. 2008, Martins et al. 2011) and, to some extent, validated as benign (Cooke et al. 2005, English et al. 2005, Wilson et al. 2013). The other components of our handling procedure were similarly rapid – the length measurement, DNA biopsy, and RAMP assessment each required < 20 s to complete and instances of air exposure (e.g., for assessing certain reflexes) were very brief (~ 5 s). Although blood biopsy has been commonly included in past biotelemetry studies on the Fraser River as a means of assessing stress, exhaustion, and identifying sex (e.g., Cooke et al. 2005), we avoided using it here to allay concerns of managers that it could affect mortality, despite evidence

to the contrary (Cooke et al. 2005, chapter 2). Nevertheless, it would be near-impossible to empirically test the independent or synergistic effects of different components of researcher handling on delayed mortality in the context of a real fishery. Although the length of researcher handling time was no different between fish that died and those that migrated successfully (chapter 3), the potential for our handling and tagging to add to mortality remains an area of some uncertainty. It is for that reason that we offer a conservative approach for assigning mortality to the fishery for management purposes (Fig. 4.4).

As with any mortality estimate, uncontrolled factors undoubtedly affect its accuracy and it is near-impossible to quantify this error. Reporting rates for radio-tag recaptures in the Fraser River are quite good in the recreational angling community, but the aboriginal fishery has limited knowledge of the tag return-and-reward program in spite of its existence for ~ 10 years (Nguyen et al. 2012). In some cases, there were multiple beach seine crews directly upstream of where we were releasing tagged coho salmon, increasing the likelihood of recapture. Our interactions with many crews during the fishery will likely have increased awareness, but there may nevertheless have been some incidents of fish being recaptured and released after removal of the radio transmitter and this would have mainly had the effect of increasing our 48 h mortality estimate. Some fish may have died because of capture despite being vigorous at release (e.g., at RAMP scores of 0 or 0.2 – Fig. 4.4), resulting in an imperfect relationship between RAMP and mortality. Assuming that is the case, the baseline mortality rate we used (23%) in calculating our 'alternate' mortality rate of 17% would be an overestimate, with the true mortality caused by the fishery thus being higher than 17%. In addition, our

receiver array did not extend all the way to spawning grounds and it was not possible to verify spawning success. Latent capture-mediated disease-induced mortality likely occurs in fish (Lupes et al. 2006), though little is known about the potential for this to occur in any real fishery. In sockeye salmon, gillnet injuries can cause latent spawning failure for fish that reach spawning grounds because of their effect on reproductive physiology (Baker and Schindler 2009, Baker et al. 2013). Most importantly, observer effects, a well-known limitation to bycatch research (Benoit and Allard 2009), may have been a factor. All crews we worked with were clearly aware of our desire to radio-tag coho salmon, and spent a concerted 1-3 min attempting to locate bycatch upon landing of the net, prior to focusing on sorting their target catch. If such efforts are not typically made, entanglement times would increase, resulting in higher reflex impairment and thus higher mortality.

4.5.2   *Possible solutions to mortality*

Among all the variables we measured at the time of capture, the only one that was statistically linked to post-release mortality by our analyses was RAMP score (chapter 3). The finding that variables other than reflex impairment were not associated with survival was surprising. This discrepancy may be largely explained by some of the mortality occurring as a result of factors unrelated to capture (e.g., natural mortality or tagging effects). It also suggests that the severity of capture stress experienced by an individual fish was influenced by aspects of capture that were not measured. Interestingly, while entanglement time did not show a direct statistical association with post-release mortality, it was correlated with RAMP scores, which have previously been shown to predict survival in Fraser River coho salmon (chapter 3). Although this is not a strong statistical

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relationship, we can advise that any capture or handling techniques that result in lower

RAMP scores and more vigorous fish (e.g., reducing entanglement time) would be

beneficial to minimizing post-release mortality.

The full suite of factors that led to reflex impairment may have not been identified

by our study because a fish's RAMP score is likely to be reflective of its unique capture

experience, such as its location within a net or the techniques used by the crew that

catches it. We did not attempt to quantify such variables and it would be very difficult to

do so in an active fishery. We handled bycatch from 12 separate fishing crews during the

study, and there were variations in how their nets were pulled into shore, how fish were

crowded and sorted (Fig. 4.2), and slight gear differences (net material and mesh size).

Some of the differences likely depended on variation in the local riverbank morphology

(slope and substrate), the strength of the river current, the training and coordination of the

crew, whether the crew used a truck to pull in their net, and the size of the catch in a

given set. Very large hauls (> 1000 fish) would almost always necessitate leaving the net

in deeper water for sorting, thus suggesting that bycatch at least remained submerged and

supplied with oxygen until it was located and released (Fig. 4.2B). However,

overcrowding resulted in dissolved oxygen (DO) depletion to the extent that DO

saturation decreased to 56-60% within 10 minutes of the start of sorting in one large set

we measured (Fig. 4.2B). Approximately 30 minutes into the sorting process for that set,

DO had further decreased to 50% saturation at the upstream end of the net, and to 36-

40% at the downstream end. The effect of those changes in dissolved oxygen were

visibly evident: the target species (pink salmon) at the upstream end of the net were alive,

upright, and facing upstream, while at the downstream end all the catch appeared to be

dead or moribund. In smaller more typical net sets (Fig. 4.2A), the catch was brought into

a similar water depth but more easily spread out along the shoreline resulting in less

crowding, although the fish were often in water shallow enough that half their gills were

air exposed. In those sets it was easier to quickly identify and release coho salmon. In two

such sets we monitored, oxygen only descended to ~75-80% saturation during the < 20

min required to complete sorting, while in a third net set (e.g., Fig. 4.2A), DO saturation

decreased to 42% 25 min into the sorting process (by which time all bycatch had been

released). There were instances of crews pulling their entire catch completely onto the

beach for sorting, air exposing both the target catch and bycatch (Fig. 4.2C). Though

resulting in air exposure, this method always ensured coho salmon were identified and

released very quickly, typically after 1-2 min of air exposure. Pulling the entire net onto

shore was only physically possible with smaller sets of ~ 100-300 salmon, and often

necessitated by a steep riverbed and strong current that made leaving the net in deeper

water for sorting unsafe for the fishers (though this was not the case in Fig. 4.2C).

We found no evidence that the fish revival bag tested in this study benefitted post-

release survival. Coho salmon provided with a 30-min recovery period in the flow-

through fish bag (Fig. 4.3B) prior to release were no more likely to migrate successfully

than fish immediately released after tagging. While fish were clearly invigorated by

receiving 30-min of ram ventilation (i.e., forced water flow over the gills), the bag

seemed to offer no added survival benefit for fish beyond that provided by a free-

swimming recovery in the river. Physiological evidence has shown that a free-swimming

recovery is the optimal way for salmonids to return to homeostasis following exhaustive

exercise or fisheries capture (Milligan et al. 2000, Farrell et al. 2001b). Thus, we suspect

that facilitating revival using recovery bags only benefits those fish that are severely impaired (i.e., unable to swim), whereas it likely represents added chronic confinement stress for fish otherwise able to maintain equilibrium during the recovery period. Indeed, in the present study there was a small (non-significant) increase in survival associated with the recovery bag for fish with a negative orientation (equilibrium) reflex (47% survival with recovery bag treatment, 35% without, N = 17 fish per group). In general, facilitated revival techniques may be most useful in contexts where post-release predation occurs (Brownscombe et al. 2013, Raby et al. 2014); in the lower Fraser River where water turbidity is high there is little evidence that this is a major issue, as compared with the marine environment where revival techniques are currently in use for coho salmon (Farrell et al. 2001a). It is possible that a shorter revival duration would have been more beneficial (e.g., 10-15 min) by allowing fish enough time to regain basic vitality without extended confinement stress. Other research has shown that various means of facilitating post-capture revival (including recovery bags) can be effective but that attempts at revival can also be misguided in some contexts (Farrell et al. 2001a, Brownscombe et al. 2013, Donaldson et al. 2013, Robinson et al. 2013, Nguyen et al. 2014). Future research on facilitating revival of fish bycatch should take the form of well-controlled experiments that examine what recovery durations and techniques are optimal and at what level of impairment fish benefit from revival.

4.5.3   *Perspectives of the fishery participants*

The most notable pattern in the results of the survey was the general willingness among respondents to engage in strategies to mitigate bycatch mortality. For example, the

majority of aboriginal fishers were receptive to using the fish recovery bags (Fig. 4.7).

The recreational angling community in the lower Fraser River were asked similar

questions regarding fish recovery bags (Donaldson et al. 2013) and were less receptive (>

40% negative responses) when asked, "What do you think of the idea of a revival bag?".

Our biotelemetry data suggest that recovery bags may have limited potential for

improving survival in the beach seine fishery. Nonetheless, responses on the recovery bag

questions suggest there is a general likelihood that members of the fishery would

embrace strategies that benefit coho salmon they catch. Though a plurality of respondents

stated that beach seine capture does not affect survival of coho salmon, which conflicts

somewhat with our findings, many were able to identify causes of stress and suggest

ways to improve survival (Fig. 4.5 and 4.6). Even more encouragingly, there was a clear

willingness to alter handling practices to improve the condition of bycatch, such as by

leaving the net in deeper water for sorting to ensure adequate oxygen supply to bycatch

until it is found. Collectively, the human dimensions data painted a picture of a fishing

community that would be receptive to advice about how to best handle coho salmon to

maximize their survival if the advice is supported with scientific evidence.

4.5.4   Integration – management recommendations

In the introductory text of DFO's selective fishing policy (DFO 2001), Thompson River

coho salmon were cited as the lead example of a fish population whose restoration was

being slowed because of bycatch in fisheries targeting other more abundant Pacific

salmon. Since the shift towards a selective fishing policy began in Canada's Pacific

fisheries (DFO 2001), managers have applied a 5% mortality rate to coho salmon caught

in the aboriginal beach seine fishery for the purpose of ensuring that overall bycatch mortality (all fisheries combined) is limited to a level that does not significantly compromise numbers of spawning adults. The bycatch mortality estimate in this study is higher than the 5% currently applied. Based on our data, we recommend fisheries management consider the following options for updating the bycatch mortality rate used to manage this fishery: a minimum of 17% bycatch mortality (Fig. 4.4); a value based on 48 h mortality (18.6%), or an estimate more reflective of total mortality (39%; Table 4.2). More work is needed to articulate the benefits and disadvantages of each approach. Unfortunately, comparable multi-year studies have not been done on all the other fisheries that bycatch interior Fraser coho salmon. Of the little work that has been done, beach seines have consistently resulted in the highest post-release survival relative to the other Fraser River fisheries that have been evaluated (i.e., higher than angling or gillnets; Buchanan et al. 2002, Donaldson et al. 2011, 2012, 2013, Nguyen et al. 2014), which suits the management shift towards a selective fishing policy (DFO 2001). In fact, management and stakeholders can use this information to contemplate the possibility of an even greater shift towards gear types that minimize release mortality.

Beyond simply documenting the extent of the problem, we have attempted to identify solutions to potential causes of mortality. At this time we are unable to recommend use of fish recovery bags in the beach seine fishery. Encouragingly though, many of the participants of the fishery were enthusiastic about the idea. Those responses suggest that fishers would similarly be enthusiastic about other means of improving survival of bycatch, perhaps including reductions in entanglement time. Alternatively, fish bags could be used on a voluntary basis with a precaution that they only be used with

severely impaired coho salmon and whereby the fish only remains in the bag until reflex actions are regained. The expanded validation of the RAMP approach in the present study provides confirmation that this simple technique is ready for use in this fishery if needed (chapter 3). The observers in the fishery could easily be taught how to conduct RAMP assessments to monitor the condition of bycatch in real time, provide advice to their crews on how to improve fish condition, and make decisions about whether individual fish should be revived using recovery bags.

The key variable that was significantly associated with reflex impairment was entanglement time. Entanglement time was also positively correlated with total catch, with bycatch often being difficult to locate in larger sets, resulting in a wider range of entanglement times for those sets. To minimize reflex impairment and maximize survival, the fishers should be encouraged to: a) make all efforts to release coho salmon to the river within 5 min of pulling the net into shore, and b) particularly if the catch is large (e.g., > 500 fish), minimize crowding by leaving the net in deeper water during sorting so that bycatch are adequately supplied with oxygen until they can be located. In our surveys, we learned that many of the fishers understand the benefits of such practices, are willing to use them, and that some already do so. However, there is clear potential for a) an increase in fisher awareness of best bycatch handling practices and b) reductions in reflex impairment. Most of the participants in the fishery have intuition based on a lifetime of experience capturing and handling salmon (i.e., traditional ecological knowledge; Huntington 2000) that could enable them to develop their own techniques that reduce reflex impairment. With observer-based collection of RAMP data, fisheries management would have the option to develop incentives that motivate the fishers to

minimize RAMP scores (and thus mortality; a general approach used with success in tuna fisheries; Hall et al. 2000).

4.5.5   Synthesis

This study demonstrates that fisheries science, biotelemetry, and human dimensions surveys can be combined to evaluate a conservation problem for an endangered population of salmon and inform resource managers and users. We consider this a model approach for conservation research, because it can help address the persistent challenge of generating science that "bridges the knowledge-action boundary" (Cook et al. 2013). A well-known barrier to transitioning from scientific knowledge to conservation action is the scientific structure that values publications and grant income but not engagement with stakeholders (Cook et al. 2013). As part of broader stakeholder meetings regularly held to present findings from several projects by our research group (Cooke et al. 2012), we disseminated the findings of this study throughout the course of data collection and received input for following phases of research. Collaboration with stakeholders was done to increase the likelihood that findings are subsequently adapted. In fact, this study was initiated by conversations with fisheries managers that followed a 2009 stakeholder meeting and was only possible with various forms of support from the resource users, government, and environmental NGOs (among others). Based on the experience with this study, adding a human dimensions component to conservation research represents good value considering the modest time investment required (e.g., conducting the surveys required ~ 8 days for two researchers). Previous research has shown the utility of such an approach for Fraser River sockeye salmon recreational fisheries (Donaldson et al. 2013).

Other papers have shown the value of combining sociology and natural sciences to address animal conservation issues (African elephants - *Loxodonta africana*, Guerbois et al. 2012; red deer - Irvine et al. 2009). In the conservation of tropical forests subject to logging, a conservation context somewhat analogous to harvest fisheries, this approach has been applied numerous times (Lele and Kurien 2011 and references therein). Fisheries bycatch is recognized widely as a leading global threat to biodiversity (Gray 1997, Kappel 2005, Davies et al. 2009), towards which a great deal of ecological knowledge and research have been applied (Hall et al. 2000, Soykan et al. 2008). Our effort is among the first integrative research studies in the realm of fisheries bycatch, and certainly the first for a freshwater bycatch issue (Raby et al. 2011). In addition to helping address a conservation issue for an endangered population of salmon, we hope this study can have value for future interdisciplinary research in aquatic conservation.

Chapter 5. Facing the river gauntlet: understanding the effects of fisheries capture and water temperature on the physiology of coho salmon

5.1   Abstract

This study examined the effects of water temperature and the duration of net entanglement on physiological disturbance and recovery in coho salmon after release from a simulated beach seine capture. Heart rate was monitored using implanted electrocardiogram biologgers that allowed fish to swim freely before and after release. A subset of fish was recovered in respirometers to monitor metabolic recovery, and separate groups of fish were sacrificed at different times to assess blood and white muscle biochemistry. One hour after release, fish had elevated lactate in muscle and blood plasma, depleted tissue energy stores (i.e., PCr, ATP), and altered osmoregulatory status, particularly in warmer (15 vs. 10°C) and longer (15 vs. 2 min) capture treatments. A significant effect of entanglement duration on blood and muscle metabolites remained after 4 h. Oxygen consumption rate recovered to baseline within 7-10 h. However, recovery of heart rate to routine levels was longer and more variable, with most fish taking over 10 h, and 33% of fish failing to recover within 24 h. There were no significant treatment effects on either oxygen consumption or heart rate recovery. We found evidence that recovery of plasma cortisol was impaired at the warmer temperature, especially for females, which may help to explain why exceptionally high post-release mortality has been documented in female salmon exposed to warm water and capture stressors. Our results indicate that fishers should minimize handling time for bycatch and

maximize oxygen supply during crowding, especially when temperatures are elevated.
Physiological data, such as presented here, can be used to understand mechanisms that
underlie bycatch impairment and mortality, and thus inform best practices that ensure the
welfare and conservation of affected species.

5.2   Introduction

Recent studies suggest climate warming affects the distributions and phenology of fishes
(Perry et al. 2005, Cheung et al. 2009), yet the relatively inflexible life history of some
species means that changes in distribution may be limited and thermal tolerance must
keep pace with the warming environment (Crozier et al. 2008). In Pacific salmon,
spawning stream fidelity and fixed reproductive schedules mean that fish have little or no
choice about what water temperatures or fisheries they will encounter during upstream
migration. High river temperatures can presumably act as a selective force because they
cause mortality via disrupted physiological homeostasis or acceleration of pathogen
development (Macdonald et al. 2000, Bradford et al. 2010, Martins et al. 2012, Hinch et
al. 2012, Miller et al. 2014). A variable portion of Pacific salmon intercepted by
commercial, aboriginal, and recreational fisheries will be released or escape (e.g., DFO
2001, Baker and Schindler 2009). The strategy of releasing certain species or populations
for conservation purposes hinges on post-release recovery and high survival after
exposure to an acute exercise stressor (i.e., capture and handling). The interaction
between water temperature and fisheries capture stressors is of increasing relevance to

management as salmon-bearing rivers are projected to continue to warm (Ferrari et al. 2007, Crozier et al. 2008, Hague et al. 2011).

A paradox of fisheries science is that while warmer water correlates positively with post- release mortality (Cooke and Suski 2005, Gale et al. 2013), it can also accelerate physiological recovery (Wilkie et al. 1997, Kieffer 2000, Schreer et al. 2001, Galloway and Kieffer 2003). Fisheries capture typically elicits exhaustive exercise, hypoxia, injury, and a neuroendocrine stress response (Ferguson and Tufts 1992, Chopin et al. 1996, Meka and McCormick 2005, Marcalo et al. 2006), which combine to cause rapid physiological changes from which the animal must recover. Physiological recovery profiles of exhaustively exercised and recreationally-angled fish have been well documented in the literature (Kieffer 2010, Cooke et al. 2013a), but few studies have done the same for commercial fishery scenarios (i.e., bycatch, but see Farrell et al. 2001a).

Physiological recovery profiles have traditionally focused on plasma and muscle tissue analyses (e.g., Wilkie et al. 1997, Farrell et al. 2001a, Suski et al. 2006), but some attention has been given to cardiorespiratory function (Cooke et al. 2004b, Donaldson et al. 2010a, Clark et al. 2012). A recent study found that heart rate (f_H) required an extended period (~16 h) to return to baseline following fisheries-related capture stressors in free-swimming coho salmon implanted with heart rate data loggers (Donaldson et al. 2010a). Though a small number of papers have described heart rate responses to recreational angling (e.g., Anderson et al. 1998, Cooke et al. 2004b), few have used cardiac measures to monitor recovery from fisheries capture because such measures normally require that the fish are tethered to recording equipment (e.g., to measure

electrocardiogram [ECG] or blood flow in the ventral aorta). Thus, sublethal fitness effects of fisheries capture are rarely considered (Wilson et al. 2014). Indeed, for adult migrating salmon, extended cardiorespiratory recovery could be a particular concern, potentially diverting a significant amount of their finite energy stores away from migration, gonad development, and spawning.

Of the seven Pacific salmon species found in British Columbia, Canada, one of the least abundant are coho salmon, which includes an endangered interior Fraser River population (interior Fraser coho) that are required by regulation to be released alive when caught incidentally (chapter 4). Field-based studies have examined factors influencing coho salmon survival in the marine (gillnets - Buchanan et al. 2000; troll fisheries - Farrell et al. 2001b; purse seines – chapter 2) and freshwater environments (chapter 4). Chapters 3 and 4 were aimed at understanding delayed mortality of interior Fraser coho released from the aboriginal beach seine fishery, which fish encounter shortly after initiating their upstream migration. In those field studies it was not possible to assess physiological recovery, how handling time (i.e., time entangled in the seine net) affected physiological impacts or recovery, and the extent to which water temperature can modulate such effects. It is not possible to study interior Fraser coho in an experimental setting due to conservation concerns, but other populations can act as surrogates.

Here, we use Chilliwack River coho salmon to examine physiological recovery profiles after release from a beach seine capture simulation. We conducted the simulation using two temperatures (10 and 15 °C) and two stressor durations (2 or 15 min seine net entanglement). We focused on monitoring the relative effects of different capture treatments on heart rate, oxygen consumption, and a suite of white muscle and blood

plasma indices of metabolic and osmoregulatory status. The involvement of the authors in recent research on bycatch in the beach seine fishery (chapter 4) enabled the use of a realistic fishing simulation and an experimental design that can help answer questions that could not be addressed in the field – specifically, the interactive effects of temperature and entanglement time on physiological disturbance and recovery. By unveiling mechanisms that underlie impairment and mortality, physiological data from controlled experiments can inform bycatch management and handling practices and assist with interpretation of trends observed in field studies.

5.3   Methods

5.3.1   Study site and animals

The fish used in this study were adult coho salmon from the Chilliwack River Hatchery (49°4'45" N, 121°42'21" W; Fig. 1.1). These fish had completed their 125 km upstream migration from the ocean to the hatchery where they had been reared and released ~2 years prior. Between 14-26 October 2011, fish were dip-netted from a concrete raceway at the hatchery and transported 22 km in aerated 8-10°C river water to Cultus Lake Laboratory (CLL; 49°4'44" N, 121°58'42" W; Fig. 1.1) for experimentation. At CLL, fish were held in the transport tank, within which oxygen was maintained between 85-120% saturation, and were dip-netted individually for surgery prior to transfer into either of two large, circular concrete ponds (5.3 m diameter). The concrete ponds, in which fish were held post-surgery (described below), were sectioned off by wooden frames lined with 5 cm diameter stretch beach seine mesh so that fish were kept in one half of the pond. Fresh cold water was continuously pumped into both ponds via an intake at 15 m depth in nearby Cultus Lake. Water in each pond was 60 cm deep, and each pond was serviced by

three large air stones that maintained air saturation > 90%. An additional submersible pump was used to create a circular flow within each pond (~ 10 cm s^{-1}).

5.3.2   Experimental protocol

The experiment, outlined in Fig. 5.1, was replicated three times for both the warm and cold test temperatures. Terminal sampling occurred at three time points (1, 4, and 24 h) after the fisheries simulation and involved three separate groups of fish that were separated into net pens within the pond at the end of the capture simulation. The 24 h group was surgically implanted with data loggers and tagged with spaghetti tags while the other two groups were only spaghetti-tagged.

a.   Fish surgery, transfer to experimental ponds, and temperature increase

Fish were dip netted from the transport tank and anesthetized with a knockout dose of 100 mg L^{-1} tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO) buffered with 200 mg L^{-1} NaHCO$_3$ in Cultus Lake water (8.5-10 °C). Fish remained in the anesthetic bath until they lost equilibrium and their opercular movement slowed (~5 min), at which point they were weighed and brought to the surgery bench where their gills were continuously irrigated with a well-aerated maintenance dose of anesthetic (70 mg L^{-1} MS-222 with 140 mg L^{-1} NaHCO$_3$). The fish were kept prone for insertion of a uniquely numbered spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) through the dorsal musculature, just anterior to the dorsal fin, tied with a simple double reef knot. Next, a data logger (23 g in air, coated in biocompatible silicon, University of Tasmania, Australia) was surgically implanted into the intraperitoneal cavity as previously described (Clark et al.

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2009). Briefly, the logger was inserted through a 3-4 cm incision and uterine forceps were used to guide the anterior-most electrode sensor as close as possible to the pericardial cavity, ventral to the liver. The logger (programmed to turn on and record ECG and temperature for 10 s every 6 min) was loosely sutured with one suture to the peritoneal wall to prevent it from moving post-surgery. The incision was closed using five or six sutures tied into square knots (size 0 monofilament PDS II absorbable sutures, 36 mm ½ circle reverse cutting needle; Ethicon, Somerville, NJ). After incision closure and post-surgery revival, fish were released into an experimental pond.

The remaining fish that had been transported from Chilliwack Hatchery were anesthetized (as above) before being briefly transferred to a water-filled (without maintenance anesthetic) and padded V-shaped sampling trough for spaghetti tag insertion (as above). Fish were then allowed to complete their revival from anesthesia in the experimental pond. Including data logger-implanted fish, ~35 fish were tagged and placed into each of the two ponds (~10-12 each for 1 h, 4 h, 24 h sampling groups) and allowed to recover over two nights (40-45 h, see Fig. 5.1) before the capture simulation.

Once all tagged fish were in the experimental ponds, the temperature in one of the two ponds was increased by supplementing the deep Cultus Lake water input with water that was run through a boiler system. Water inputs were adjusted to increase the warm treatment pond to ~15°C within 24 h after transfer, while the other pond was supplied only with deep Cultus Lake water (~10°C). Both temperatures are ecologically relevant in the context of coho salmon upriver migrations and the occurrence of harvest fisheries.

b. *Capture simulation*

A section of beach seine netting was used to gradually corral all fish to one corner of the pond. Once fish were corralled, the net was drawn under and around the fish such that they were pursed and could be pulled up onto a wooden platform that was dropped into the pond after corralling began. The platform was ~ 55 cm from the bottom of the pond, and 1×1.5 m across, resulting in high crowding and a water depth of ~5 cm on the platform – conditions comparable to those in real beach seine fisheries when the seine is pulled into the beach (see Fig. 4.2). Once fish were crowded on the platform for 2 min, approximately half of the fish were removed (fish identifiable by unique spaghetti tags) and rapidly transferred using knotless nylon dip nets to one of three net pens within the pond. The three net pens were used to hold fish to be euthanized in separate groups at three time points (Fig. 5.1). After 15 min of entanglement, the remaining fish were transferred into their respective net pens. Dissolved oxygen in the water on the crowded platform within the fishing net declined from ~90% air saturation to 50-60% saturation by the final minutes of the 15 min simulation. In real beach seine fisheries oxygen levels can decrease by 20-60% in beached seine nets during sorting (chapter 4). Thus, the capture stressor involved a stress response and exercise during corralling and netting, followed by confinement in shallow water with declining oxygen content.

c. *Terminal sampling*

Physiological sampling was carried out for each of the three pens at 1, 4, and 24 h after the initiation of the capture stressor (i.e., when the experimenters entered the pond to begin corralling fish; Fig. 5.1). All fish were rapidly dip netted from their net pen and

sacrificed by cerebral percussion within 30 s of the start of dip netting, at which point blood and white muscle sampling began. Blood samples (1-2 mL) were drawn within 3 min from the caudal vasculature of each fish using 21-gauge needles and heparinized vacutainers (3 mL with lithium heparin; BD, Franklin Lakes, New Jersey). Simultaneously, sections of white muscle were cut from the left side of each fish (within 5 min of being sacrificed), 1-2 cm above the lateral line anterior to the dorsal fin. Muscle samples were pressed firmly between a set of metal clamps that had been cooling in liquid nitrogen, and then stored in liquid nitrogen until later transfer to a -80 °C freezer. Blood samples separated for plasma and stored as described in chapter 2 (section 2.3.2). After physiological sampling was complete, fish were measured (fork length, FL, nearest cm) and sex was verified by examining gonads. In the case of 24 h fish, data loggers were removed, scrubbed clean in freshwater, and immersed in povidone-iodine for sterilization prior to re-use.

In addition to sampling fish exposed to the capture simulation, seven fish were dip-netted and sacrificed directly from the hatchery raceway (~7.5°C) and a further four fish were transported to CLL and held undisturbed at 15°C for 24 h in black cylindrical fish holding bags (Fig. 4.3). Data from these 11 fish were pooled to provide resting/routine values for illustrative (not statistical) purposes (light grey areas in Fig. 5.2).

d. *Respirometry*

A subset of data logger-implanted coho salmon exposed to the capture simulation (four fish in the 10°C group and five in the 15°C group) were rapidly transferred post-simulation to 138 L static intermittent flow-through respirometers for 20-26 h of oxygen

consumption rate ($\dot{M}o_2$) measurements using the same methods as Clark et al. (2011a). Briefly, the respirometers recorded oxygen saturation continuously (1 Hz) using electrodes (Loligo systems, Tjele, Denmark) placed within a re-circulation line that ensured each respirometer remained well mixed. The respirometers were flushed with fresh water for 45 min every hour and sealed for the other 15 min so that $\dot{M}o_2$ could be recorded (based on the slope of oxygen saturation vs. time). Though the small sample sizes precluded statistical comparisons, respirometry allowed us to characterize post-exercise $\dot{M}o_2$ and recovery patterns for the two temperatures.

5.3.3   Laboratory analyses

To extract metabolites from white muscle samples, they were first ground to a fine powder using a mortar and pestle kept partly immersed in liquid nitrogen (Farrell et al. 2001a). Approximately 0.5 g of powdered muscle was then briefly vortexed in a 15 mL falcon tube with exactly 4× the volume of perchloric acid solution (e.g., 0.5 g muscle = 2 mL solution of 8% PCA with 1 mM EDTA). The vortexed solution was incubated on ice for 10 min then centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was removed and balanced to a pH of 7-8 using a neutralizing solution (2 M KOH, 0.4 M KCl, 0.3 M Imidazole), before being centrifuged at $10,000 \times g$ for 3 min at 4°C. The resulting supernatant was removed and stored at -80°C for later analyses of lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP), which were measured in triplicate using enzymatic assays with a plate spectrophotometer (SpectraMax 340PC microplate reader with SoftMax Pro 4.8 data analysis software, Molecular Devices, Sunnyvale, CA) following details provided by Suski et al. (2006). Blood samples were

analyzed in the laboratory as described in chapter 2 (section 2.3.2) and Farrell et al. (2001a).

5.3.4   *Data analysis and statistics*

We used principal components analysis (PCA) to integrate responses of the seven plasma and three muscle variables we measured. An initial PCA with three factors was conducted using all 10 variables (N = 155 fish) but was successively re-run after stepwise elimination of variables that either had a) a low Kaiser-Olkin-Meyer (KMO) measure of sampling adequacy (<0.5, Field et al. 2012), or b) did not have a factor loading (eigenvector) $\geq |0.6|$ for a factor with loadings $\geq |0.6|$ for any other variables (as in chapter 2). That process led to a final PCA with five variables (see *Results*), from which factor scores were extracted for each fish and subsequently referred to as "metabolic PC scores". PCA was again used on the remaining five variables, and the same process was used to refine that PCA, from which factor scores were extracted (referred to as "plasma ion PC scores"; see Table 5.1 in *Results*). Plasma cortisol and glucose, which were ultimately excluded from both PCAs (criteria described above), were analyzed separately for further analyses.

To screen for the confounding effect of sex across treatment groups for muscle and plasma variables we used a two-way analysis of variance (ANOVA) with sex and group (groups shown in Fig. 5.2D) as main effects at each time point (non-significant interaction term removed). Where sex had a significant effect, further tests for capture variables were conducted using an ANOVA with three factors (sex, entanglement duration, temperature – non-significant interactions removed). For the other variables,

two-way ANOVAs were used. Data were visually examined to ensure they met parametric assumptions and tested for normality and heteroscedasticity using the Shapiro-Wilk and Levene's test, respectively. In one case (4 h metabolic PC scores) data could not be transformed to meet parametric assumptions so Wilcoxon rank sum tests were used to separately assess effects of temperature and entanglement duration. Fish size (FL) was not significantly different among treatment groups for any of the three time points (two-way ANOVA with entanglement duration and temperature as factors, all $P > 0.10$).

The data logger files were downloaded into LabChart (ADInstruments, Sydney, Australia) for processing with an ECG analysis tool that was used to calculate heart rate (f_H) in beats per minute. Some of the loggers failed to record data, resulting in a final N of 21; 5 each for 10°C/2 min and 10°C/15 min, 2 for 15°C/2 min, and 9 for the 15°C/15 min treatment (treatments shown in Fig. 5.1). All ECG data were manually examined to ensure correct beat counting. For each fish, we assessed its baseline (resting) f_H by averaging over a ~15 h period preceding the capture simulation. In some cases, certain sections were excluded or periods further than 15 h prior to capture were used to ensure baseline for each fish was calculated from a minimum of 10 h of low, stable f_H data. Time to recovery after the fisheries simulation was assessed as the time point when f_H returned to within the 99% confidence interval (CI) of that individual's baseline f_H (mean ± standard deviation CI = 1.39 ± 0.80 beats min^{-1}). We also calculated f_H elevation for each fish by subtracting its baseline f_H + 99% CI from its raw f_H at each time point, such that when f_H elevation decreased to ≤ 0 a fish was considered "recovered". Heart rate elevation was used to calculate excess post-stressor heart beats (EPHB) for each fish by

integrating the area under the recovery curve (until recovery was reached or until the fish was sacrificed if it did not recover within 24 h) using the simple trapezoidal method.

Effects of entanglement duration and temperature on post-capture peak f_H, f_H elevation, the factorial increase in f_H, recovery time, and EPHB were assessed using two-way ANOVAs using type III sums of squares with the interaction term removed because of unbalanced sample sizes (see Table 5.2). To assess whether capture variables resulted in relative elevations in f_H during an extended recovery, we used a linear mixed effects model with entanglement duration, temperature, and time as fixed effects, f_H elevation as the outcome variable, and fish ID as a random variable, focusing on f_H at 10, 15, and 20 h post-capture. Those time points were chosen to attempt to understand causes of extended f_H elevation, and because it was apparent that there was little variation in the initial response and recovery (e.g., up to 5-7 h). Statistics were conducted using RStudio (v. 0.98.953, RStudio, Inc., Boston, MA, USA; http://www.rstudio.com/). Tests were assessed as significant at $\alpha = 0.05$ and data are presented as mean ± standard error.

Figure 5.1 The experimental timeline that was repeated three times for each temperature. Letters correspond to sections in *Methods* (5.3).
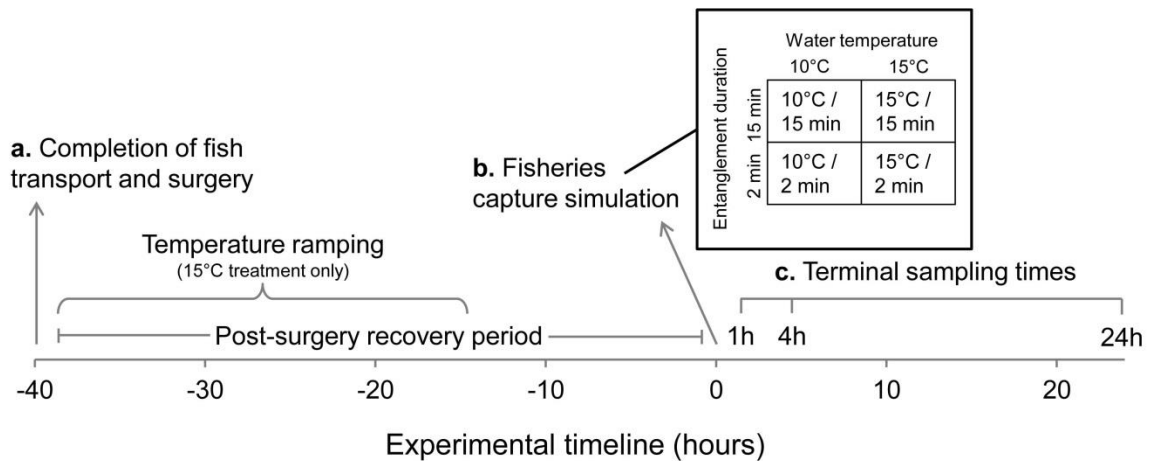
5.4 Results

5.4.1 Blood and white muscle physiology

The only fish to die in the experiment came from the most stressful group, 15-min entanglement treatment at 15°C, where 18% of fish died after release but prior to sampling (four in the 1 h group, three in the 4 h group, and one in the 24 h group; samples not included in analyses).

Fish exhibited an elevation in indices of exhaustion and stress 1 h after initiation of the stressor (Fig. 5.2). Lactate was elevated in plasma and white muscle relative to reference and 24 h values, as was plasma cortisol, while ATP and PCr were depressed. Metabolic indices were well-integrated by PCA (Table 5.1); the synthetic "metabolic PC scores" variable positively correlated with plasma and muscle lactate, and osmolality, and negatively correlated with muscle ATP and PCr. Overall, metabolic PC scores were

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elevated at 1 h but decreased to resting/routine (light grey areas in Fig. 5.2) values by 4 h

for most fish (Fig. 5.2D). At 1 h, there were significant and separate positive effects of

entanglement duration (Two-way ANOVA; $F_{1,44} = 9.5$, $P = 0.004$) and water temperature

($F_{1,44} = 10.3$, $P = 0.003$) on metabolic PC scores (Fig. 5.2D). At 4 h, metabolic PC scores

were significantly elevated across temperatures for fish exposed to the longer

entanglement duration (Wilcoxon rank sum test, $P = 0.034$), whereas there was no

apparent effect of temperature itself ($P = 0.29$). A Kruskal-Wallis ANOVA failed to

detect a significant difference among the four groups shown in Fig. 5.2D ($\chi^2 = 7.8$, df = 3,

$P = 0.051$). By 24 h, there were no significant differences among groups in metabolic PC

scores, with all groups apparently recovered (Two-way ANOVA, $P > 0.40$ for both

entanglement duration and temperature).

A second PCA on the remaining five variables strongly integrated the three

plasma ions (chloride, potassium, and sodium) into a synthetic variable referred to as

"plasma ion PC scores" (Table 5.1), which similarly showed signs of elevation at 1 h

followed by a decrease at 4 h (Fig. 5.2C). There were no significant effects of treatment

variables on recovering plasma ion PC scores (thus, all fish grouped in Fig. 5.2C).

Likewise, plasma glucose was not significantly affected by capture variables at the three

time points (all $P > 0.40$), but was significantly higher in males across groups at 1 h after

capture (Two-way ANOVA, sex $F_{1,43} = 7.7$, $P = 0.008$). Cortisol ($\log_{10}$-transformed) was

higher in females across time points ($P < 0.001$). Controlling for sex, plasma cortisol was

not affected by temperature or entanglement duration at 1 or 4 h, but after 24 h there was

a significant positive effect of temperature ($F_{1,40} = 8.74$, $P = 0.004$), in addition to a

separate effect of sex ($F_{1,40} = 13.43$, $P = 0.002$; using a three-way ANOVA, interactions removed due to non-significance; Fig. 5.2B).

5.4.2 Cardiorespiratory recovery

Confinement in a respirometer for 24 h of hourly $\dot{M}o_2$ measurements elicited periodic bouts of visually observable activity that were reflected in spikes in $\dot{M}o_2$ among the small number of fish placed in respirometers (N = 5 for 15°C, N = 4 for 10°C). Those data points were removed for a characterization of the respiratory recovery following our capture simulation (Fig. 5.3). In the first post-release measurements (mean 0.84 h after initiation of the stressor, ~ 0.54 h after release), $\dot{M}o_2$ reached 4.33 ± 0.45 mg kg^{-1} min^{-1} for fish in the 10°C treatment and 6.00 ± 0.45 mg kg^{-1} min^{-1} at 15°C; ~50% of $\dot{M}o_{2\,max}$ for both temperatures in this population of coho salmon (G.D. Raby, *unpublished data*; Fig. 5.3). $\dot{M}o_2$ returned to resting values quicker in the 15°C treatment (~5 h) than at 10°C (~8 h). Small sample sizes within time points precluded statistical analyses of $\dot{M}o_2$ data.

Heart rate (f_H) baseline and post-capture peak values reflected temperature differences but were not affected by entanglement duration (Fig. 5.4). Baseline f_H was 32.3 ± 1.5 beats min^{-1} at 10°C, which was significantly lower than at 15°C where it averaged 42.2 ± 1.4 beats min^{-1} (Welch's t-test, $t_{17.95} = -4.93$, P < 0.001). After capture, f_H peaked at 67.9 ± 1.3 (10°C) and 85.5 ± 1.7 beats min^{-1} (15°C) and the difference between temperatures was significant (Table 5.2). Net f_H elevation (Fig. 5.4B) was also significantly higher at 15°C than at 10°C, but there was no difference in the factorial increase, with heart rate approximately doubling at both temperatures (Table 5.2). Post-

capture peak f_H, peak f_H elevation above baseline, and factorial elevation were not significantly affected by entanglement duration (Fig. 5.4, Table 5.2). Likewise, after controlling for capture variables, sex had no effect on any of the above heart rate metrics (all $P > 0.08$).

Heart rate recovered to baseline levels notably slower than $\dot{M}o_2$ and other metrics measured in this study (Fig. 5.4B). Overall, 7 of 21 fish did not return to their pre-capture baseline f_H within the 24 h after release. Among the 14 fish that did recover before being sacrificed, recovery time ranged from 3.07 to 22.49 h. Effects of entanglement duration and temperature on EPHB, likelihood of recovery within 24 h, and recovery time were not significant (Table 5.2). A linear mixed-effects model examining heart rate elevation (Fig. 5.4B) across three time points (10, 15, and 20 h) with fish ID as a random variable revealed a significant negative effect of time (i.e., heart rate declined across the time points examined - evident in Fig. 5.4B; $\beta = -0.48 \pm 0.14$, $t_{39} = -3.44$, $P = 0.001$), but no significant effects of temperature ($t_{17} = 0.52$, $P = 0.61$) or entanglement duration ($t_{17} = 0.98$, $P = 0.34$).

Figure 5.2 Mean ± standard error (S.E.) plasma glucose (A), cortisol (B), plasma ion PC score (C), and metabolic PC score (D) in coho salmon 1, 4, and 24 h after initiation of a 2- or 15-min seine entanglement, at either 10°C or 15°C. The light grey shaded areas represent mean ± standard error control values based on seven fish sampled directly from the hatchery raceway (at ~7.5°C, no transport or entanglement stressor) and four fish allowed to recover from transport and handling undisturbed at CLL for 24 h (at 15°C). "Metabolic PC score" is a variable that was synthesized using principal components analysis from five original physiological variables relating to metabolic status, while plasma ion PC score was synthesized in a second PCA using three of the remaining five variables (see Table 5.1). For A and B, all fish were grouped because no significant differences occurred within any of the time points, except for plasma glucose at 1 h which was significantly higher in males across treatment groups (two-way ANOVA with group and sex as main effects using log-transformed data, $P = 0.008$). For B and D, significant effects within a time point are shown with * (effect of sex), † (temperature), or ‡ (entanglement duration). Separate and significant effects of temperature ($P = 0.003$) and entanglement duration ($P = 0.004$) for metabolic PC scores at 1 h were based on a two-way ANOVA (re-run after removal of a non-significant interaction term). The significant effect of entanglement duration (across both temperatures) at 4 h for metabolic PC scores ($P = 0.035$) was based on a Wilcoxon rank sum test − potential interactions with temperature could not be assessed because assumptions of parametric statistics could not be met. Using a Kruskal-Wallis ANOVA with fish grouped into their four groups (as in D) failed to find a significant effect ($P = 0.051$).
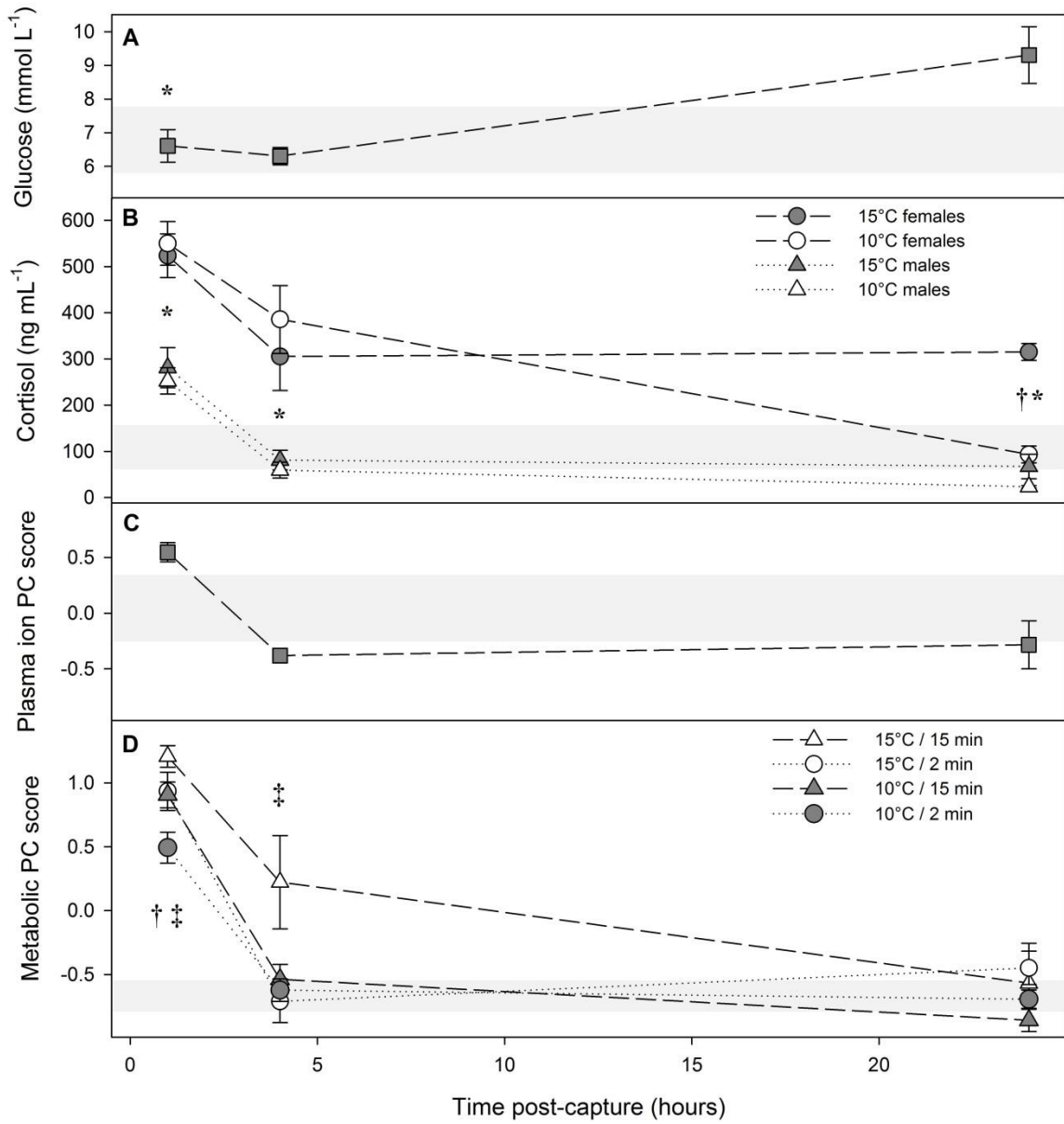
Figure 5.3 Mean ± S.E. oxygen consumption rate ($\dot{M}o_2$) for fish recovered in static respirometers after the capture simulation at 10°C (grey circles) and 15°C (white triangles). The total sample size was five individuals at 15°C (two following 2 min of entanglement, three following 15 min) and four at 10°C (all 15 min entanglement time) but the sample size was smaller for some time points where individuals exhibited spontaneous activity, reflected by sudden increases in $\dot{M}o_2$ (and confirmed by visual observation of respirometry chambers). Temperature-specific maximum ($\dot{M}o_{2\,max}$) and minimum ($\dot{M}o_{2\,min}$) aerobic metabolic rates for this population of coho salmon were measured in a separate experiment and are shown using dashed lines for illustrative purposes – maxima were obtained using a Brett-type swim tunnel respirometer (G.D. Raby, *unpublished data*)
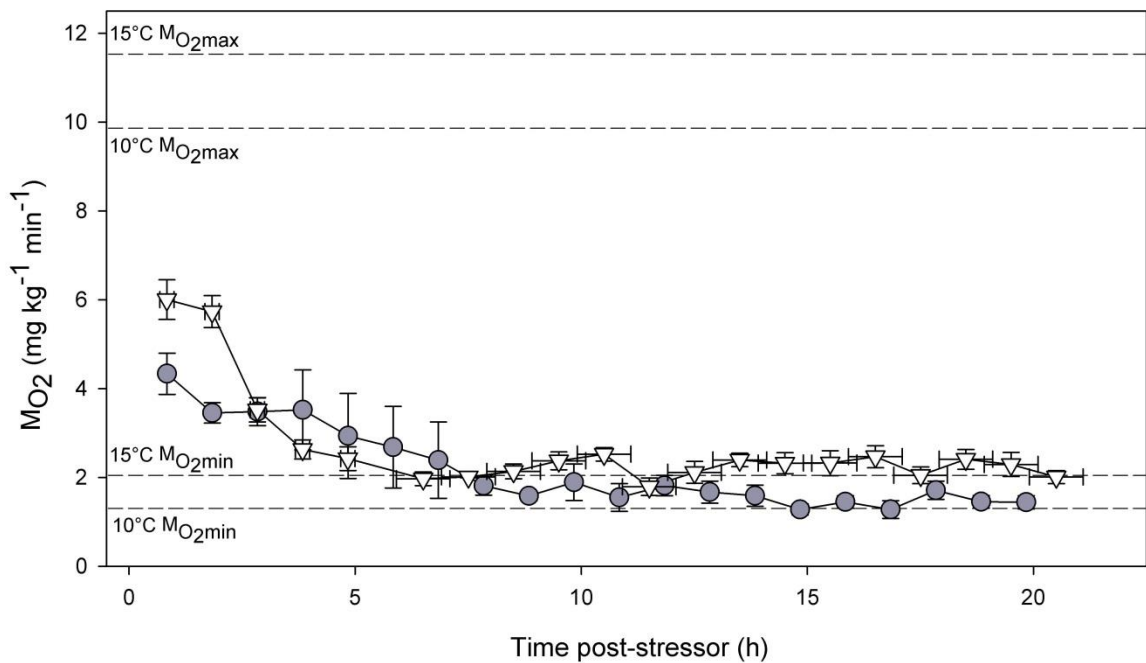
Figure 5.4 Mean heart rate (f_H) (A), heart rate elevation relative to baseline (B), and body temperature (T_b) (C) for coho salmon implanted with data loggers and subject to fisheries simulations at 10°C (grey circles) and 15°C (white triangles). Hourly means are based on data within ±30 min of each hour mark and error bars represent standard error (x-axis error bars present but too small to be visible in most cases; likewise for y-axis error for T_b in the 10°C group). Background data points in light grey are group means for each time point (i.e., every 6 min; triangles for 15°C, circles for 10°C). Heart rate relative to baseline (f_H elevation; B) was calculated for each individual by subtracting that individual's baseline from its actual heart rate at a given time point. Also shown in the top panels are sample electrocardiogram (ECG) traces (recorded at 200 Hz) used to assess heart rate from a logger-implanted salmon held at 15°C (i = baseline, ii = post-capture).

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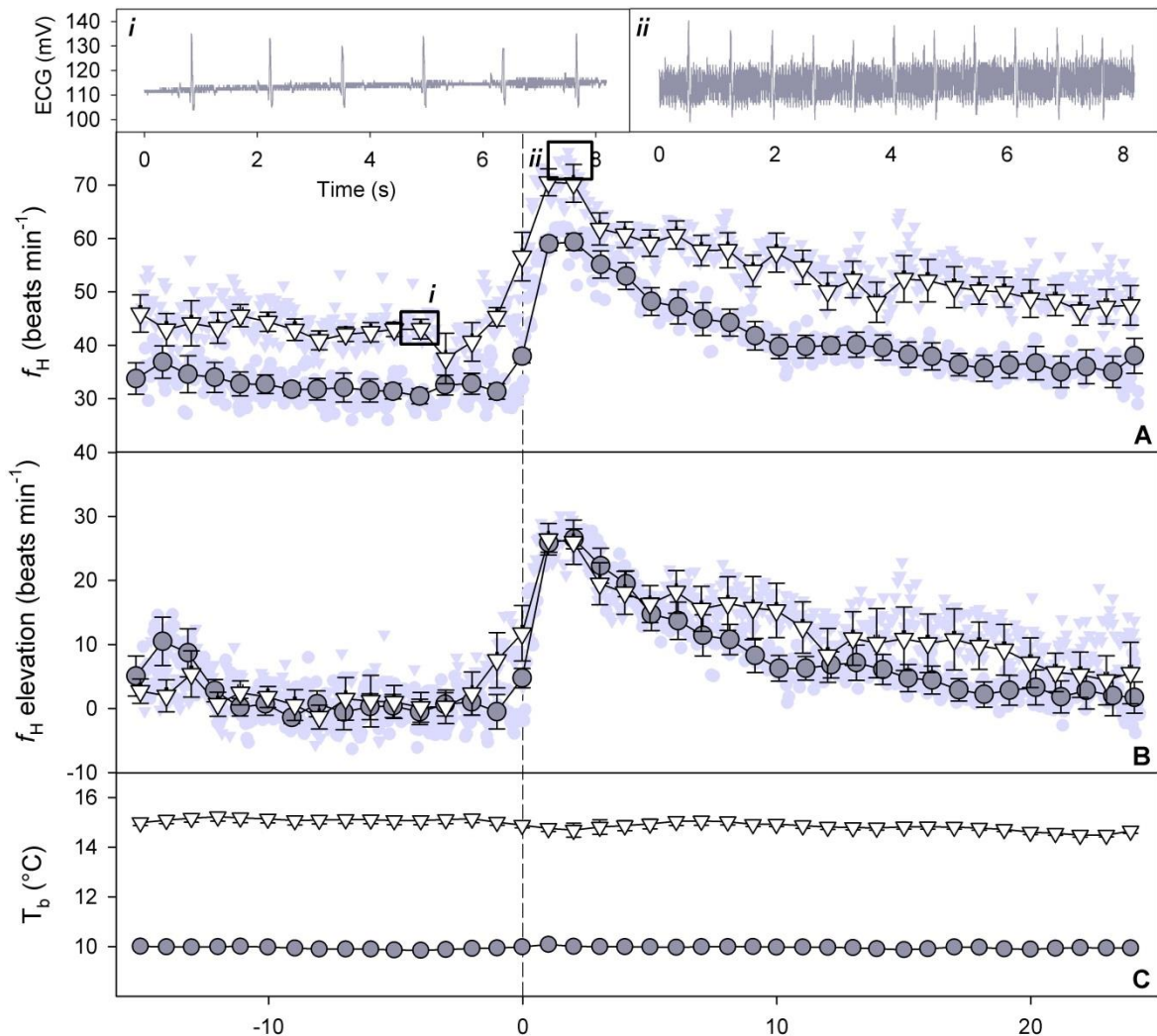
Table 5.1 Output of two separate principal components analyses (PCA; N = 155 individuals) whose resulting factor scores (PC1) were used for statistical analyses (see Fig. 5.2). The first PCA, whose resulting factor scores are referred to as metabolic PC (principal component) scores, resulted after an initial PCA with all ten original physiological metrics, from which variables were successively removed because of having either a) a low Kaiser-Mayer-Olkin (KMO) measure of sampling adequacy (see Field et al. 2012), or b) not having a loading ≥ |0.6| (shown in bold) for any factor which also had other ≥ |0.6| loadings (i.e., not agreeing strongly with other variables within a factor). PC loadings represent correlation coefficients (r) between the original variable and the new synthetic (e.g., PC1) variable. The second PCA (bottom) whose resulting factor scores are referred to as plasma ion PC scores, was initially run using the five remaining variables, and was simplified after the same iterative procedure used to refine the first PCA.

First PCA - metabolic PC score		
Tissue variable	**PC1** loading	Communality (h^2)
Eigenvalue	3.86	
Variance explained	77%	
Plasma lactate	**0.91**	0.83
Plasma osmolality	**0.89**	0.79
Muscle lactate	**0.94**	0.88
Muscle ATP	**-0.85**	0.73
Muscle PCr	**-0.80**	0.63
Second PCA - plasma ion PC score		
	PC1 loading	Communality (h^2)
Eigenvalue	2.23	
Variance explained	74%	
Plasma Cl$^-$	**0.91**	0.83
Plasma K$^+$	**-0.78**	0.61
Plasma Na$^+$	**0.89**	0.79

Table 5.2 Comparative summary of heart rate (f_H) responses and recovery among the four capture treatments. Values represent mean ± S.E.

Temperature, entanglement duration	N	Post-capture peak f_H (beats min-1)	Peak f_H elevation (relative to baseline)	Factorial post-capture f_H increase	Recovered to baseline f_H within 24 h	Recovery time (h) for fish that recovered	Excess post-capture heart beats
10°C							
2 min	5	67.9 ± 1.8	35.6 ± 2.3	2.2 ± 0.1	40% (2 of 5)	13.7 ± 0.3	18628 ± 2189
15 min	5	67.9 ± 2.1	33.1 ± 0.8	2.1 ± 0.1	100% (5 of 5)	8.5 ± 1.3	7421 ± 1348
15°C							
2 min	2	86.6 ± 3.3	39.1 ± 4.5	2.0 ± 0.2	100% (2 of 2)	5.9 ± 2.0	3785 ± 1418
15 min	9	85.3 ± 1.9	41.9 ± 2.3	2.1 ± 0.1	56% (5 of 9)	17.6 ± 2.2	23439 ± 4222
Statistics[1]							
Temperature		$F_{1,18} = 62.75$, $P < 0.001$	$F_{1,18} = 6.92$, $P = 0.02$	$F_{1,18} = 0.50$, $P = 0.49$	$Z = -1.19$, $P = 0.23$	$F_{1,11} = 0.70$, $P = 0.42$	$F_{1,18} = 1.50$, $P = 0.24$
Duration		$F_{1,18} = 0.05$, $P = 0.84$	$F_{1,18} = 0.02$, $P = 0.89$	$F_{1,18} = 0.03$, $P = 0.86$	$Z = -1.20$, $P = 0.23$	$F_{1,11} = 1.01$, $P = 0.34$	$F_{1,18} = 0.03$, $P = 0.86$

Notes: f_H elevation relative to baseline, factorial increase, and f_H recovery were assessed for each individual relative to that individual's unique baseline f_H. Factorial increase in f_H was calculated by dividing an individual's peak post-capture heart rate by its pre-capture baseline average. Total excess post-capture heart beats (EPHB) was estimated by integrating the total area under the recovery curve for each fish (to recovery or 24 h in the case of fish that did not return to baseline before being sacrificed at 24 h).

[1]Two-factor ANOVAs (no interaction term) used except for numbers of fish recovering to baseline within 24 h, for which a multiple logistic regression was used.

Table 5.3 Mean ± S.E. (range) blood plasma and white muscle measures for each treatment group (water temperature, capture stressor duration) at different times after initiation of the capture stressor. 24 h controls are fish that were transported to CLL and placed in black flow-through fish bags for 24 h before being rapidly sacrificed and sampled for tissue. 24 h control values were combined with those from the hatchery raceway to provide the control levels in Fig. 5.2 (grey shaded areas).

Post-capture time	Treatment		n	BLOOD PLASMA							WHITE MUSCLE		
				Glucose (mmol L^{-1})	Cortisol (ng mL^{-1})	Na$^+$ (mmol L^{-1})	K$^+$ (mmol L^{-1})	Cl$^-$ (mmol L^{-1})	Osmolality (mOsm kg^{-1})	Lactate (mmol L^{-1})	Lactate (mmol L^{-1})	PCr (µmol g^{-1})	ATP (µmol g^{-1})
1 h	10°C	15 min	14	6.3 ± 0.5 (4.0–9.0)	483 ± 64 (181–902)	153.0 ± 1.5 (143–163)	3.14 ± 0.25 (1.81–5.10)	132.4 ± 1.0 (125–138)	349.6 ± 2.9 (332–369)	15.4 ± 0.9 (10.5–20.0)	38.5 ± 1.5 (28.0-48.0)	11.2 ± 1.5 (4.1-22.2)	3.7 ± 0.4 (1.7-7.0)
		2 min	12	7.0 ± 0.7 (4.8–14.8)	430 ± 58 (135–759)	148.2 ± 2.7 (129–169)	3.28 ± 0.32 (1.41–4.98)	128.7 ± 2.0 (111–135)	334.1 ± 4.0 (316–357)	12.2 ± 0.8 (7.6–17.1)	33.2 ± 1.5 (23.8–40.0)	7.4 ± 1.2 (1.2–16.1)	5.1 ± 0.4 (3.0–7.8)
	15°C	15 min	12	6.8 ± 1.0 (3.4–14.4)	469 ± 79 (129–898)	154.3 ± 1.3 (145–162)	3.68 ± 0.56 (1.66–8.72)	126.9 ± 2.0 (113–139)	357.1 ± 3.0 (331–371)	20.8 ± 1.2 (13.3–28.2)	39.5 ± 1.1 (33.1–45.0)	9.4 ± 1.8 (0.9–16.7)	3.8 ± 0.4 (1.6–5.5)
		2 min	10	6.0 ± 0.5 (4.0–8.3)	347 ± 44 (166–616)	151.7 ± 2.8 (137–167)	4.38 ± 0.40 (1.71–6.64)	131.0 ± 1.3 (124–138)	345.6 ± 4.2 (315–361)	16.8 ± 1.3 (7.3 – 21.0)	37.2 ± 1.5 (25.1–42.5)	6.6 ± 1.1 (1.6–11.5)	4.6 ± 0.4 (3.3–6.7)
4 h	10°C	15 min	13	6.4 ± 0.5 (4.4–9.9)	329 ± 93 (21–1138)	137.7 ± 0.9 (133–143)	5.32 ± 0.57 (1.51–9.30)	116.5 ± 1.5 (103–124)	307.6 ± 1.9 (299–327)	7.5 ± 1.4 (1.9–18.7)	20.6 ± 1.3 (12.7–27.8)	16.1 ± 1.6 (3.9–25.2)	6.3 ± 0.7 (3.9–13.5)
		2 min	12	6.3 ± 0.5 (3.8–9.8)	202 ± 61 (16–703)	137.6 ± 1.7 (130–148)	4.71 ± 0.46 (2.31–7.80)	120.7 ± 1.9 (108–131)	308.3 ± 2.3 (297–319)	3.5 ± 0.6 (0.8–7.3)	23.0 ± 1.2 (15.9–30.3)	15.7 ± 1.0 (8.1–19.7)	6.8 ± 0.3 (4.6–8.4)
	15°C	15 min	10	5.8 ± 0.8 (1.3–9.1)	216 ± 61 (13–607)	143.3 ± 1.5 (136–152)	6.77 ± 0.70 (4.57–11.01)	118.5 ± 1.3 (113–125)	318.2 ± 4.6 (299–344)	14.2 ± 2.1 (6.6–26.3)	28.9 ± 4.7 (15.8–57.0)	13.0 ± 2.5 (0.7–23.5)	5.2 ± 0.9 (0.2–7.5)
		2 min	11	6.6 ± 0.4 (5.0–9.4)	212 ± 46 (47–517)	140.4 ± 1.7 (134–152)	5.21 ± 0.41 (3.52–7.57)	119.2 ± 1.3 (111–127)	310.8 ± 2.2 (297–321)	5.9 ± 1.1 (2.0–14.2)	20.3 ± 1.2 (15.7–27.3)	17.8 ± 1.8 (9.3–31.1)	7.3 ± 0.8 (4.4–14.6
24 h	10°C	15 min	4	7.7 ± 1.0 (5.8–9.5)	114 ± 42 (27–221)	139.1 ± 3.4 (130–146)	4.00 ± 0.62 (2.41 – 5.08)	123.0 ± 2.3 (120–130)	305.4 ± 2.5 (301–312)	1.7 ± 0.3 (1.2–2.4)	20.8 ± 2.3 (14.2–25.2)	15.5 ± 1.8 (13.0–20.6)	6.5 ± 0.2 (6.1–6.9)

(cont.)

Post-capture time	Treatment		n	BLOOD PLASMA							WHITE MUSCLE		
				Glucose (mmol L⁻¹)	Cortisol (ng mL⁻¹)	Na⁺ (mmol L⁻¹)	K⁺ (mmol L⁻¹)	Cl⁻ (mmol L⁻¹)	Osmolality (mOsm kg⁻¹)	Lactate (mmol L⁻¹)	Lactate (mmol L⁻¹)	PCr (μmol g⁻¹)	ATP (μmol g⁻¹)
24 h		2 min	10	10.1 ± 1.3 (6.0–19.2)	79 ± 24 (13–216)	137.5 ± 1.6 (128–145)	3.80 ± 0.30 (2.56–5.30)	122.2 ± 2.3 (112–132)	306.0 ± 1.4 (300–316)	1.9 ± 0.5 (0.6–5.8)	20.7 ± 1.5 (15.4–28.3)	15.7 ± 0.8 (11.4–19.8)	6.2 ± 0.4 (4.2–8.0)
	15°C	15 min	14	11.2 ± 2.4 (0.4 – 32)	312 ± 88 (17–1126)	131.2 ± 4.6 (75–146)	8.90 ± 4.36 (2.60–65.37)	115.9 ± 4.7 (61–130)	311.9 ± 7.8 (293–412)	4.7 ± 2.7 (1.1–39.5)	21.1 ± 2.3 (14.8–46.3)	17.1 ± 1.7 (0.1–25.3)	6.3 ± 0.5 (0.1–7.8)
		2 min	13	7.5 ± 1.0 (4.3–16.9)	133 ± 41 (13–464)	141.6 ± 1.8 (126–152)	4.36 ± 0.43 (1.91–7.47)	129.5 ± 1.5 (121–136)	314.1 ± 1.3 (305–321)	2.1 ± 0.3 (0.5–4.5)	23.8 ± 1.4 (15.9–31.4)	13.7 ± 1.7 (1.3–21.9)	6.7 ± 0.4 (4.6–8.3)
24 h controls	15°C		4	6.0 ± 0.6 (4.3–6.8)	47 ± 28 (16–131)	144.1 ± 1.5 (142–149)	4.39 ± 0.85 (2.73–6.76)	128.9 ± 1.2 (126–132)	310.1 ± 2.2 (306–314)	1.6 ± 0.5 (0.7–2.6)	25.1 ± 3.4 (19.1–30.9)	12.4 ± 3.1 (7.4–18.1)	5.7 ± 0.6 (4.9–6.8)
Hatchery raceway controls	7.5°C		7	7.3 ± 1.5 (3.9–14.3)	144 ± 60 (13–4.6)	140.8 ± 3.0 (124–150)	3.07 ± 0.22 (2.42–4.00)	124.6 ± 4.9 (95.4–134.9)	305.2 ± 4.9 (273–316)	1.6 ± 0.7 (0.3–6.1)	18.7 ± 2.0 (13.5–27.5)	16.7 ± 1.5 (12.0–23.8)	6.3 ± 0.5 (4.7–7.6)

5.5 Discussion

5.5.1 *Physiological responses*

Both warmer water and longer net entanglement result in greater physiological disturbance in salmon and, for some variables, an extended recovery after simulated fisheries capture, based on the data presented here. White muscle and plasma variables provided the strongest evidence of treatment effects (Fig. 5.2). Overall, we found that most physiological variables had recovered or approached routine levels within 4 h but that many individuals took much longer to return to pre-stressor f_H.

In a closely related population of coho salmon held at ~8°C, Donaldson et al. (2010a) found that f_H took up to 16 h to recover from an exhaustive exercise stressor, while Anderson et al. (1998) similarly found Atlantic salmon required 15 h to recover f_H after angling (at 8 and 16.5 °C). In contrast are centrarchids (family Centrarchidae), which return to routine f_H within 2-4 h of exercise and angling stressors (Cooke et al. 2001, Schreer et al. 2001, Cooke et al. 2003). Our data support previous findings that the relative increase in f_H from fisheries-related stressors is not affected by the nature of the stressor or water temperature, with an approximate doubling of f_H in all cases (Cooke et al. 2001, Donaldson et al. 2010a). However, whereas the duration of air exposure has a strong effect on f_H recovery time in rock bass (*Ambloplites rupestris*; Cooke et al. 2001), we found the duration of net entanglement had no such effect in salmon, though our ability to detect such effects was somewhat limited by low statistical power. More notable in our study was that several fish did not return to resting f_H within 24 h, and that recovery time varied widely even within treatments. As such, our data confirm that capture-related stressors can cause very prolonged f_H elevation in free-swimming salmon.

Accordingly, we suggest that f_H may provide the best indication of whole-organism

recovery from a stressor but that its immediate response is not indicative of the severity

of the stressor. Future research could explore why salmon exposed to seemingly identical

acute stressors can vary so widely in their recovery profiles – knowledge that could help

explain why delayed mortality occurs. Such differences could potentially be explained by

inter-individual variation in spontaneous activity levels, stress responsiveness (e.g.,

Romero and Wikelski 2001, Cook et al. 2014), physiological or behavioural syndromes

(Careau and Garland 2012), prior experiences (e.g., "training", Jain and Farrell 2003, or

"carry-over effects", O'Connor et al. 2014), or pre-existing pathogen loads (Miller et al.

2014).

The biological importance of an extended elevation in f_H (e.g., 15+ h) following

capture remains unclear, particularly in light of the fact that $\dot{M}o_2$ appeared to return to

baseline relatively quickly (Fig. 5.3), meaning the direct energetic cost of recovery was

modest. Based on the mean recovery profiles in $\dot{M}o_2$ in Fig. 5.3, excess post-exercise

oxygen consumption was 783 ± 284 and 501 ± 98 mg O_2 kg^{-1} at 10 and 15 °C,

respectively. Those values translate to 2358 ± 920 and 1623 ± 317 calories of excess

energy used during recovery from capture; energy that could otherwise be used to

achieve 1.4 ± 0.2 km (15°C) to 2.2 ± 0.8 km (10°C) of upstream migration, or 1.1 ± 0.2 h

(15°C) to 1.8 ± 0.6 h (10°C) of spawning activity (based on migration energetics data in

Brett 1995). Perhaps a more important energetic consideration was the mismatch between

oxygen demand and availability during entanglement. Both in the actual fishery (chapter

4) and our simulation, dissolved oxygen declined from 9 - 10 mg L^{-1} to ~ 5 - 7 mg L^{-1} in

the crowded seine within 10 - 15 min, while oxygen demand ranged from ~ 8 - 24 mg

min^{-1} per fish (depending on body size and water temperature), likely necessitating a significant shift towards anaerobic metabolism.

Reliance on anaerobic metabolism can explain why the fishing simulation caused changes in blood and muscle metrics that were modulated by temperature and the duration of net entanglement. The initial corralling and entanglement elicited ~ 1 min of exercise, followed by 2-15 min of crowding in very shallow water with declining oxygen content (e.g., 60% air saturation within 10 min). The protocol was more typical of a true fisheries net capture than those applied by exhaustive exercise studies (e.g., 5 min of manual chasing; Kieffer 2000). Nevertheless, the rich physiological literature that exists on the latter (Kieffer 2010) is relevant to understanding our results. Anaerobic exercise relies initially on using white muscle stores of PCr and ATP (which remain unchanged during aerobic swimming; Wood 1991, Milligan 1996), and thereafter shifts to greater consumption of glycogen (glycogenolysis), resulting in production of lactate and a drop in pH (Wood 1991). Some lactate leaks out of muscle cells to blood plasma (Milligan 1996), while decreased muscle pH creates an osmotic pull of water from plasma to muscle cells, effectively concentrating plasma ions (i.e., heightened osmolality and plasma ion PC scores at 1 h; Table 5.3, Fig. 5.2). ATP and PCr in muscle typically recover to resting levels within 1-2 h of exhaustion (Milligan 1996, Suski et al. 2006), while muscle lactate conversion back to glycogen (glycogenesis; Milligan 1996), the primary fate of muscle lactate, typically occurs more slowly (e.g., 6-8 h), as does the restoration of osmotic balance (Wood et al. 1983). These processes were effectively integrated into a synthetic variable (metabolic PC score) for our experiment, which can be thought of as a robust measure of departure from metabolic homeostasis, where higher

scores represent more exhausted fish and low scores represent a rested state (Table 5.1,

Fig. 5.2). Although we did not measure muscle glycogen, we expect that it would have

inversely tracked lactate (Milligan 1996). It was notable that although fish likely did not

exercise maximally, evidenced by only reaching 50% of maximum attainable $\dot{M}o_2$, lactate

reached maximal levels comparable to Atlantic salmon and rainbow trout in exhaustive

exercise experiments (i.e., ~ 40 and ~20 mmol L^{-1} in muscle and blood plasma,

respectively; Wilkie et al. 1997, Kieffer 2000), but only in the 15 min duration

entanglement (Fig. 5.2, Table 5.3). Entanglement time had a significant effect on

metabolic disturbance, which helps explain why it was negatively correlated with reflex

impairment in the field (chapter 4), and is supported by the catch-and-release literature

where longer angling or air exposure have both been shown to cause greater lactate

accumulation and longer recovery of cardiac variables (e.g., Cooke et al. 2001, Schreer et

al. 2001, Killen et al. 2006, Suski et al. 2007a).

Temperature had a significant effect on metabolic PC scores at 1 h, likely

resulting from a combination of higher metabolic rate at 15°C and to a lesser extent the

lower dissolved oxygen content of warmer water. Although past studies on the effects of

temperature on physiological responses to exhaustive exercise have found minimal

differences in resulting lactate loads (Wilkie et al. 1997, Kieffer 2000), the present study

exposed fish to hypoxia which only followed brief burst swimming during netting.

Therefore, exhaustion was likely to be a function of both entanglement time and

temperature, given the role of the latter in determining metabolic rate. In fact, it appears

fish were not fully exhausted in the 2-min duration treatments based on their lower

muscle lactate loads (and metabolic PC scores) at 1 h (Fig. 5.2, Table 5.3). After

exhaustive exercise, largemouth bass (*Micropterus salmoides*) accumulate more lactate and have more depressed white muscle energy stores if recovered in hypoxic or warmer water (Suski et al. 2006). Similar trends have been observed in bonefish (*Albula vulpes*, Shultz et al. 2011). Importantly, resting levels of ATP, PCr, and glycogen are relatively independent of acclimation temperature in fish (Kieffer 2000), such that exposure to hypoxia should deplete energy stores more quickly at a higher temperature (Fig. 5.3, Suski et al. 2006). In addition, the crowded fish would have depleted the oxygen content of the water more quickly, which is consistent with mortality occurring only in the 15°C/15 min treatment. The fish that survived the 15°C/15 min treatment exhibited the highest mean metabolic PC scores at 1 and 4 h, the longest heart rate recovery, and the highest number of excess post-stressor heartbeats (Table 5.2), although statistically significant differences did not occur in the latter two cases. Collectively, our data show that physiological disturbance in coho salmon is increased by longer entanglement time, particularly in warmer water.

A prediction from the literature is that physiological recovery from exhaustion in fish is more rapid in warmer water (Wilkie et al. 1997). The data from our experiment generally did not support that prediction, with the exception of our small $\dot{M}o_2$ dataset. However, the focus of our sampling design on a small number of time points, in the case of blood and muscle variables, likely precluded our ability to detect such effects. Most interestingly, there was an effect of temperature on plasma cortisol recovery, with cortisol remaining particularly high in females in the 15°C treatment 24 h after release (~ 300 ng mL^{-1}; Fig. 5.2B). Though we have little pre-capture control data (plasma cortisol was 15.9 and 131.2 ng mL^{-1} in two 15°C female controls [among four 15°C controls]; Table

5.3), the data suggest either a) female salmon may have impaired recovery of cortisol after capture in warm water, or b) cortisol is maintained at higher routine levels in warmer water, though this is not the case in sockeye or pink salmon (Jeffries et al. 2012). It has recently been established that mortality is exceptionally high in upriver migrating female sockeye salmon exposed to warm water and capture stressors (Gale et al. 2011, Jeffries et al. 2012, Martins et al. 2012, Robinson et al. 2013), and our cortisol data here may help explain why those trends occur. Integrating knowledge about sex-specific consequences of fisheries capture is particularly relevant to salmon populations in light of warming river temperatures because females are usually the limiting sex to spawning ground productivity (Peterman et al. 2010). Further experiments are required to establish whether impaired cortisol recovery is a mechanism for delayed sex-specific mortality of salmon caught-and-released in warm water.

5.5.2   Relevance to conservation and management

Our data are directly relevant to understanding bycatch of endangered coho salmon in Fraser River beach seine fisheries, which typically encounter coho salmon in mid-September (targeting pink and sockeye salmon) when water temperatures are 14-16°C (chapter 4). The fishery also sometimes re-opens in late October when water temperatures are 8-10°C to target chum salmon. It has been proposed that upriver migrating salmon are adapted to the modal water temperatures they historically experience at the time of river entry (Farrell et al. 2008) and in our case we used a population of salmon for which 15°C would represent the upper limit of their lifetime experience, with 10°C being closer to their modal upriver migration temperature (water

was 8-10 °C in the Chilliwack River at the time of the experiment). In the fishery, where entanglement times range from 5 s to 56 min (median = 3.3 min; chapters 3 and 4) plasma lactate averaged 12.3mmol L^{-1}, ~8-10 min after release from the net (chapter 3). This is only slightly lower than our 1 h samples, which were taken closer to when plasma lactate peaks during recovery (Farrell et al. 2001a, Donaldson et al. 2014). Thus, although in an experimental setting and using a surrogate population, our data are relevant to informing best handling practices for the beach seine fishery, and help highlight the importance of releasing bycatch from nets as rapidly as possible (see chapter 4), particularly at elevated temperatures.

In a human dimensions survey of fishery participants (chapter 4), rapid release of coho salmon was the means of reducing bycatch mortality most commonly suggested by respondents. Many fishers (35%), however, presented no ideas for reducing mortality, suggesting there is potential to increase awareness of the importance of rapid release (chapter 4). Our experiment illustrates that the mismatch between oxygen demand and supply during crowding results in additional and substantial physiological disturbances (Fig. 5.2); clear evidence of why, if rapid release is not possible, fishers should be urged to maximize oxygen availability by leaving the net in deeper water for sorting. Most fishers that participated in the survey (chapter 4) indicated a willingness to leave their nets in knee-deep water, although the depth required to ensure crowding does not deplete dissolved oxygen would likely depend on a variety of factors, such as catch size and local water flow.

In addition to informing bycatch management for an endangered population of salmon, the trends relating to handling time and temperature in our experiment are likely

applicable to other fisheries. For example, similar physiological data and recommendations exist in rainbow trout recreational fisheries (Meka and McCormick 2005) and fyke net fisheries that bycatch northern pike (Colotelo et al. 2013). Our maximal and recovered muscle lactate data are comparable to those that occur in coho salmon captured in marine gillnet and troll fisheries (Farrell et al. 2001a,b), further emphasizing that our data are likely replicable and relevant in real fisheries.

Estimates of global marine fisheries bycatch range from 6.8 to 38.5 million tonnes (Kelleher 2005, Davies et al. 2009)—a conservation problem that has caused population declines (e.g., Lewison et al. 2004b, Piovano et al. 2010) and drawn considerable research effort in the last 20 years (Raby et al. 2011). In Canada's Pacific fisheries, a policy to move towards "selective fishing" has been in place for more than ten years, which states that non-target fish should be released "unharmed" if bycatch cannot be avoided (DFO 2001). Our experiment is relevant in this context, given that physiological data are objective measures of fish welfare (Diggles et al. 2011). To date, there is sparse use of terms relating to stress, welfare, or the sublethal effects of bycatch in IUCN documents on imperiled species known to be captured in commercial fisheries (Wilson et al. 2014). Nevertheless, well-controlled experiments with physiological assessments can help provide mechanisms needed to facilitate evidence-based implementation of best practices (Cooke and O'Connor 2010), especially when complemented by field and human dimensions data (chapters 2-4, and see Donaldson et al. 2013). We hope the present study provides a helpful addition to a growing physiological literature (see Davis 2002, Cooke et al. 2013a, Wilson et al. 2014 for reviews) that can be used by

conservation practitioners to understand trends in fish impairment and mortality while

moving towards methods of live release that benefit the welfare and survival of bycatch.

Chapter 6. A physiological comparison of three techniques for reviving sockeye salmon exposed to a severe capture stressor during upriver migration

6.1   Abstract

Capture of fish in commercial and recreational fisheries causes disruption to their physiological homeostasis and can result in delayed mortality for fish that are released. For fish that are severely impaired, it may be desirable to attempt revival prior to release to reduce the likelihood of post-release mortality. In this study, male sockeye salmon undergoing their upriver migration were used to examine short-term physiological recovery in three revival treatments after beach seine capture and air exposure: a pump-powered recovery box that provided ram ventilation at one of two water flow rates, and a cylindrical, in-river recovery bag which ensured that fish were oriented into the river flow. A 3-min air exposure resulted in severe impairment of reflexes such that fish could not maintain positive orientation or properly ventilate. All three treatments resulted in significant reductions in reflex impairment within 15 min, with full recovery of reflex responses observed within 60-120 min. For most variables measured, including plasma lactate, cortisol, and osmolality, there were no significant differences among revival treatments. There was some evidence for impaired recovery in the low-flow recovery box, in the form of higher hematocrit and plasma sodium. These data mirror published recovery profiles for a recovery box study in the marine environment where a survival benefit occurred, suggesting the methods tested here are viable options for reviving salmon caught in freshwater. Importantly, with most of the benefit to animal vitality

accrued in the first 15 min, prolonging recovery when fish become vigorous may not

provide added benefit because the confinement likely serves as a stressor itself.

6.2   Introduction

The fate of fish released from recreational and commercial fisheries is a concern in many

systems (Davis 2002, Cooke and Schramm 2007) because post-release mortality is

cryptic and can impede conservation and management efforts (Coggins et al. 2007, Baker

and Schindler 2009, Gilman et al. 2013). Fisheries capture stressors result in a disruption

to homeostasis that has been well characterized (e.g., Wells et al. 1986, Davis et al. 2001,

Marcalo et al. 2006). Depending on the intensity of the stressor, physiological

disturbance can be significant enough to cause immediate mortality (Chopin et al. 1996),

or fish that fail to regain homeostasis can suffer delayed mortality hours or days after

release (Parker et al. 1959, Wood et al. 1983, Davis 2002, Skomal 2007).

Efforts to revive visibly lethargic fish prior to release are sometimes used in

fisheries as means of reducing post-release mortality. For example, some recreational

anglers will manually move fish back-and-forth or in a "figure eight" pattern to promote

flow across the gills (ram ventilation), techniques that are recommended by some

management agencies despite a lack of experimental support (Pelletier et al. 2007,

Robinson et al. 2013). Some commercial fisheries employ "recovery totes" that provide a

safe on-board recovery environment for bycatch before it is discarded (Farrell et al.

2001a). In catch-and-release recreational fisheries, flow-through recovery bags can be

used to reduce impairment of fish before release (Brownscombe et al. 2013), which may

be particularly useful where there is a threat of post-release predation (Cooke and Philipp 2004, Raby et al. 2014).

Facilitated revival techniques may be relevant in upriver-migrating Pacific salmon, where fisheries usually target a single species that co-migrates with others, resulting in capture-and-release of non-target salmon species and some portion of the target species (Gale et al. 2011). If a capture stressor is severe (e.g., via long angling times, air exposure, crowding or asphyxiation in nets) the resulting level of impairment can be high enough that fish exhibit negative orientation (drift downstream) and irregular ventilation patterns (chapter 3, Brownscombe et al. 2013), and can show a delay in upstream migration (Makinen et al. 2000, G.D. Raby, *unpublished data*). In the Fraser River (British Columbia, Canada), post-release migration failure has been shown to reach 20–40 % (Donaldson et al. 2013, chapter 4) despite a fisheries policy objective to release non-target fish in an "unharmed" state (DFO 2001). In the marine environment, a revival device known as the Fraser Box was validated, and can now be used as a way of promoting physiological recovery and short-term survival in coho salmon bycatch (Farrell et al. 2001a). The Fraser Box offers the advantage of a strong water current directed at the head of the fish (ram ventilation), which allows for effective revival of fish that have ceased to ventilate by themselves, something not offered by the "recovery totes" (chapter 2) that have been used in commercial salmon fisheries (Farrell et al. 2001a).

In freshwater, comparisons of the Fraser Box, a comparatively portable recovery bag, and traditional manual techniques (i.e., holding fish facing into the flow upon release) suggest the benefits of facilitated revival techniques are highly context-

dependent (Donaldson et al. 2013, Robinson et al. 2013, Nguyen et al. 2014, chapter 4).

Active swimming has been reported to promote rapid recovery and suppress the cortisol

response in exhaustively exercised hatchery rainbow trout *O. mykiss* (Milligan et al.

2000) and in troll-caught coho salmon (Farrell et al. 2001b), though it provided no benefit

to the recovery of angled largemouth bass *Micropterus salmoides* (Suski et al. 2007b).

However, if a salmon is unable to swim, maintain orientation, or ventilate after a severe

capture stressor, immediate release would risk short-term mortality via respiratory failure

(Farrell et al. 2001a, Skomal 2007) and make them vulnerable to predators (Quinn and

Buck 2001, Forrest et al. 2009, Raby et al. 2014). Thus, facilitated revival may only

reduce mortality when the severity of the capture stressor and resulting level of

impairment are high (Farrell et al. 2001a, Donaldson et al. 2013, discussed in chapter 4).

The purpose of the present study was to compare short-term physiological

recovery profiles among three revival treatments following seine capture of sockeye

salmon and three minutes of air exposure. The study was motivated by a need to assess

different options for reviving salmon from severe capture stress during upriver migration.

We exclusively used male fish to avoid data being confounded by sex because circulating

cortisol is higher and more variable in females (Baker and Vynne 2014). In addition to

assessing physiological status via blood measures, we used RAMP (Reflex Action

Mortality Predictors; see chapter 3) to assess whole-animal vitality both before and

during the recovery. One revival treatment was a lightweight, inexpensive and portable

in-river flow-through fish bag (identical to that used in chapter 4). The two other revival

treatments employed a specially-designed recovery box (which requires a powered pump

to generate flow) but used two different water flow rates (i.e., 0.2 L s^{-1} and 0.9 L s^{-1}).

6.3 Methods

6.3.1 Study site and capture treatment

Capture of sockeye salmon occurred from 20/09/2010 through 23/09/2010 on the

Harrison River (49°17'5.8" N, 121°54'27.4" W), a large tributary of the Fraser River

(British Columbia, Canada; see Fig. 1.1). Water temperature was ~14–15°C for the

duration of the experiment. The salmon had already migrated 115 river km upstream

from ocean entry and were likely a mix of Harrison River and Weaver Creek populations

(population-origin was not identified), both of which spawn within 5 km of each other.

Peak spawning activity occurs in mid-October for Weaver Creek sockeye salmon and

mid-November for the Harrison River population. Therefore, fish were about mid-way

through their development of secondary sexual characteristics and sex was externally

identifiable. Fish were caught using a beach seine that was pulled to shore but left in

sufficient water depth (~ 60 cm) that crowding was minimized (unlike in chapters 3, 4,

and 5) to allow fish to swim around in the enclosed net. Male sockeye salmon were

visually identified and dip netted for transfer into wetted black fish bags (cylindrical, 20

cm diameter, 1 m length) made of Hypalon (thick synthetic rubber). The fish bags had

lengthwise zippers that allowed fish to be enclosed, as well as 4 cm diamond mesh on

each end to allow water flow through the bags when they were submerged (see Fig. 4.3 in

chapter 4). The bags were first pulled onto the riverbank to expose fish to air for three

minutes, a duration chosen to maximize reflex impairment, i.e., fish would drift

downstream upside-down if released without a revival treatment. At the completion of air

exposure, the fish bags containing salmon were submerged in the river for the initial RAMP assessment (see below) and prior to transfer to revival treatments.

6.3.2   Reflex assessments, revival treatments, and physiological sampling

The fish bags were gently pulled to the surface and opened to perform the initial RAMP assessment (detailed in chapter 2, section 2.3.2). After the initial RAMP assessment, fish were placed in one of three revival treatments. The first treatment involved placing individual fish back into the fish bags and submerging the bags 10 m from the riverbank where water depth was 1 m. The bags were affixed to a metal rod that had been driven into the riverbed and the flow of water at the site of bag attachment was ~ 10 cm s^{-1}. Attenuation of water velocity within the bag was ~10% (measurement reported in Donaldson et al. 2013), meaning that the water velocity inside of the bag was approximately 9 cm s^{-1}, which converts to ~2.8 L s^{-1} based on the bag's diameter (20 cm).

The other two revival treatments involved the use of Fraser Boxes. Four Fraser Boxes were used (built to match Farrell et al. 2001a) which each had two fish compartments (each 20 cm wide $\times$ 40 cm deep $\times$ 90 cm long) with a 2.54 cm diameter inflow valve that could be adjusted to provide the two treatment water flows of 0.2 and 0.9 L s^{-1}. The outflow valve at the opposite end was the same in all the boxes. The Fraser Boxes were set up along the riverbank and continuously supplied with fresh river water via a gasoline-powered pump. Fish were placed in the boxes facing into the flow and the lids were replaced and secured to prevent fish being able to jump out if they were revived.

Fish remained in the revival treatments for 15, 30, 60, or 120 min (N = 7-11 fish per sampling time and revival treatment; average N = 9) before being reassessed for RAMP, sacrificed by cerebral percussion, and sampled for blood by caudal puncture using a 21-gauge needle and a heparinized vacutainer (3 mL with lithium heparin; BD, Franklin Lakes, NJ, USA). Fish were also sampled for several other tissues for separate physiological analyses (data not presented here). Whenever revival resulted in fish that were too vigorous to allow reflex assessment, fish were simply assigned an unimpaired status (RAMP score = 0; as in chapters 2, 3, and 4).

An additional 10 salmon were sampled as above within 1 min of landing of the seine without air exposure or RAMP assessment (which results in minor air exposure) and nine more salmon were sampled immediately after the 3-min air exposure (i.e., time zero).

Blood samples were placed immediately into an ice-water slurry and analyzed within 15 min for hematocrit (Hct, as a percentage using hematocrit tubes centrifuged at $10,000 \times g$ for 5 min) and for hemoglobin (Hb, g L^{-1} using a handheld meter calibrated for fish blood; HemoCue Hb 201^{+}; HemoCue, Ängelholm, Sweden; Clark et al. 2008). Mean corpuscular hemoglobin content (MCHC) was calculated for each fish as [Hb]/(Hct/100). Remaining whole blood was centrifuged to separate plasma, which was stored and analyzed in the laboratory for cortisol, sodium, chloride, potassium, lactate, glucose, and osmolality as in chapters 2, 3, and 5 (see section 2.3.2). The metrics chosen allowed us to assess the response and recovery of osmoregulatory status (osmolality, chloride, potassium, sodium), indicators of stress (cortisol, glucose, lactate), and oxygen transport capacity of the blood (hematocrit, hemoglobin, MCHC).

6.3.3   Data analysis and statistics

The main objective of our analysis was to assess whether recovery profiles differed among the three revival treatments. Also of interest was whether or not the rate of recovery differed. Tests were conducted on each physiological variable using two-factor analysis of variance (ANOVA using type III sums of squares) with revival treatment and time as fixed effects and with interactions removed if non-significant ($\alpha = 0.05$). Tukey HSD post-hoc comparisons followed ANOVAs where needed (family-wise α of 0.05). Plasma sodium and RAMP scores did not meet parametric assumptions (after attempts at transformations in the case of the former) so Kruskal-Wallis ANOVAs were used to separately test effects of time and treatment, with Kruskal-Wallis post-hoc multiple comparisons tests used where appropriate (family-wise α of 0.05). All statistical tests were conducted using RStudio (v. 0.98.953, RStudio, Inc., Boston, MA, USA; http://www.rstudio.com/). Data are presented as means ± standard error.

6.4   Results

After beach seine capture all fish were responsive, exhibited positive orientation, and were ventilating regularly (i.e., RAMP scores of ~ 0.2, Fig. 6.1). A three minute air exposure resulted in most fish becoming unresponsive: 46% exhibited complete loss of reflexes (RAMP score = 1.0), and a further 45% lost four of five reflexes (0.8). The former group would be characterized as either moribund or dead in a normal commercial fishery setting (Farrell et al. 2001a), while the one reflex retained in the latter group was vestibular-ocular response (VOR).

Revival significantly decreased RAMP scores, irrespective of either the duration of revival (dissimilar letters in Fig. 6.1; Kruskal-Wallis ANOVA, effect of time: $\chi^2 = 178.1$, df = 4, $P < 0.001$) or the revival treatment (treatment: $\chi^2 = 1.4$, df = 2, $P = 0.48$). Reflex impairment was significantly lower after 120 min (at which time no reflex impairment was observed) than in the 15 and 30 min groups (post-hoc differences shown by dissimilar symbols in Fig. 6.1; $\chi^2 = 27.1$, df = 3, $P < 0.001$). Therefore, independent of the treatment type, most of the benefit to fish vitality accrued in the first 15 min of recovery. Indeed, of the 28 fish revived for 15 min, 71% could be described as vigorous (RAMP score = 0-0.2) and 93% were self-ventilating.

Of the 121 immobile and poorly-ventilating fish that were air exposed, 97.5% were revived by the recovery treatments. The three fish that died exhibited negative orientation at the outset of revival treatment (two in 120 min group of 0.9 L s^{-1} Fraser Box, one in 30 min group of 0.9 L s^{-1} Fraser Box) and faced into the front corners of the chamber rather than directly into the inflow valve. Other fish were regularly observed making vigorous attempts to burst free of the Fraser Boxes prior to their pre-determined sampling times.

Most blood variables exhibited significant changes across time points but there were few differences among revival treatments, the latter being contrary to expectations. Air exposure significantly increased lactate, which then further increased during revival and remained elevated across revival durations, exhibiting few further changes (post-hoc differences shown in Fig. 6.2C; overall effect of time $F_{5,123} = 67.02$, $P < 0.001$). The main effect of treatment was not significant for lactate ($F_{2,102} = 0.08$, $P = 0.91$). Plasma cortisol and glucose exhibited similar patterns, with significant overall effects of time

(cortisol, $F_{5,121} = 8.65$, $P < 0.001$; glucose, $F_{5,123} = 4.78$, $P < 0.001$) but not treatment

(both $P > 0.10$), and a tendency for an increase throughout revival, especially for cortisol

(post-hoc differences among time points shown in Fig. 6.2A and B). During revival,

plasma potassium decreased sharply from time zero to 15 min (Fig. 6.3A; overall effect

of time, $F_{5,123} = 16.86$, $P < 0.001$) and then increased towards time-zero levels with

increasing durations of revival, but the type of revival treatment used had no effect ($F_{2,102}$

$= 0.33$, $P = 0.72$). Plasma chloride and osmolality exhibited opposite patterns to that of

potassium, with an increase from control levels by 15 min, followed by a decrease with

increasing time (overall effect of time for chloride, $F_{5,123} = 2.76$, $P = 0.02$; for osmolality,

$F_{5,123} = 19.78$, $P < 0.001$), and no differences among treatments ($P > 0.60$ for both

variables; Fig. 3). There was a significant overall effect of revival time on MCHC ($F_{5,121}$

$= 2.38$, $P = 0.04$) but post-hoc comparisons among time points were not significant (Fig.

6.4C). There were no statistically significant effects on hemoglobin with respect to time

or treatment (both $P > 0.25$; Fig. 4B).

Whereas there were no differences among revival treatments for most variables, a

notable difference among groups occurred for plasma sodium (Fig. 6.3B), with higher

concentrations across the four revival durations in the low-flow (0.2 L s^{-1}) Fraser Box

treatment (overall effect of treatment: $\chi^2 = 38.4$, df $= 2$, $P < 0.001$). Post-hoc comparisons

with revival durations pooled confirmed that plasma sodium was significantly higher in

the 0.2 L s^{-1} Fraser Box treatment than in the other two treatments during recovery ($P <$

0.05 in both cases). Kruskal-Wallis ANOVA multiple comparisons with fish grouped by

time and treatment (i.e., all groups shown in Fig. 6.3B) indicated that the 0.2 L s^{-1} Fraser

Box group was the only treatment to significantly increase plasma sodium above control

levels (dissimilar letters in Fig. 6.3B; overall Kruskal-Wallis ANOVA $\chi^2 = 64.94$, df = 13, $P < 0.001$). Fish in the 0.2 L s^{-1} Fraser Box treatment also had the highest hematocrit whereby there was a significant effect of treatment across the four revival time points (Fig. 6.4A, effect of time $F_{3,103} = 0.73$, $P = 0.67$; treatment $F_{2,103} = 5.63$, $P = 0.003$). With time removed as a factor and controls added as a fourth treatment, there were significant differences ($F_{3,125} = 9.32$, $P < 0.001$) and post-hoc comparisons revealed that a) mean hematocrit for the 0.2 L s^{-1} Fraser-box was higher than the control group and 0.9 L s^{-1} fish, b) hematocrit for recovery bag fish was significantly higher than controls but not different from the other two revival treatments, and c) 0.9 L s^{-1} Fraser Box fish were not different from controls.

Figure 6.1 Mean reflex action mortality predictors (RAMP) scores (± S.E.) for sockeye salmon upon beach seine capture (white triangle, data from Donaldson et al. 2012), after the addition of 3 min of air exposure (grey triangle), and after different durations of recovery in three revival treatments. All data points represent separate groups of fish (i.e., no repeat sampling), and higher scores represent more impaired fish (see section 2.3.2 for full details on the reflex assessment). There were no significant differences among revival treatments (revival time points pooled, Kruskal-Wallis ANOVA, $\chi^2 = 1.44$, df = 2, $P = 0.49$), but grouping fish by time point (including time 0, i.e., immediately post-air exposure) revealed a significant effect of time ($\chi^2 = 178.2$, df = 4, $P < 0.001$) with dissimilar letters indicating significant post-hoc differences (Kruskal-Wallis post-hoc multiple comparisons). Focusing on a comparison among revival durations there was a significant effect of time ($\chi^2 = 27.0$, df = 3, $P < 0.001$) and significant post-hoc differences that are shown by dissimilar symbols. Standard error bars are not present at 120 min because all fish had RAMP scores of 0 (likewise for the 0.9 L s^{-1} treatment at 60 min).
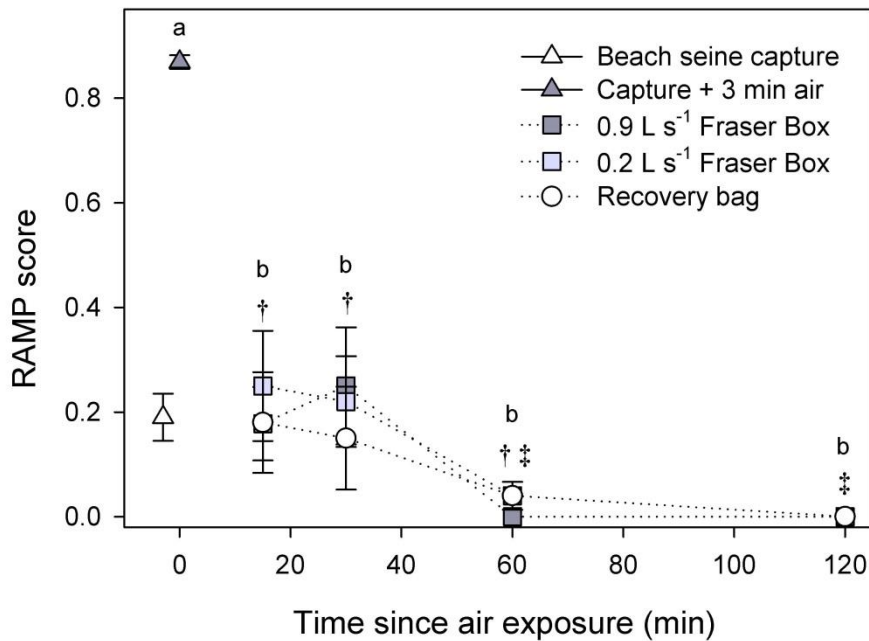
Figure 6.2 Mean ± S.E. plasma cortisol (A), glucose (B), and lactate (C) in sockeye salmon prior to and after 3 min of air exposure, and after different durations in three revival treatments that followed. Significant overall main effects of time occurred for cortisol, glucose, and lactate (see *Results*, section 6.4), and dissimilar letters indicate significant post-hoc differences among time points using Tukey HSD).
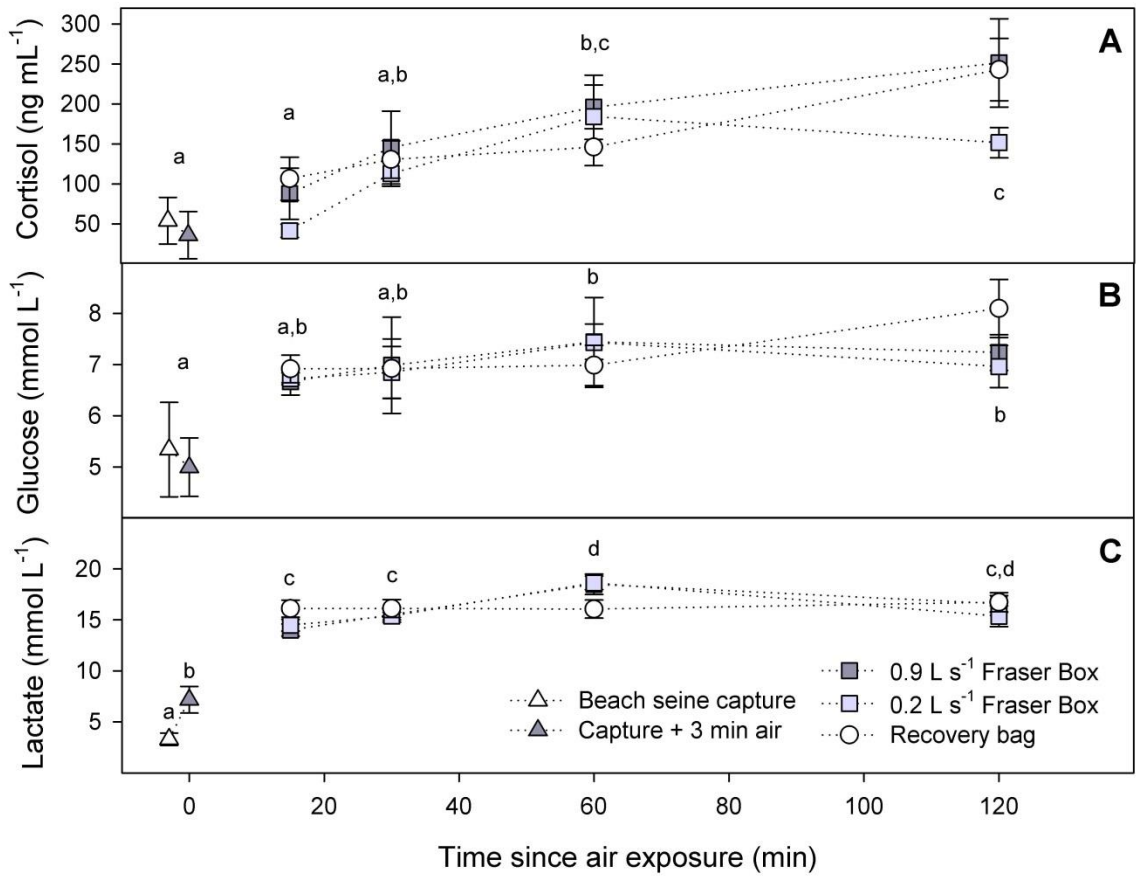
Figure 6.3 Mean ± S.E. plasma potassium (A), sodium (B), osmolality (C), and chloride (D) for fish captured by beach seine then air exposed for 3 min, followed by different durations in one of three revival treatments. Significant overall effects of time occurred for time for each variable (see *Results*; shown by dissimilar letters), and for sodium (B) a significant effect of treatment, with the 0.2 L s^{-1} treatment having significantly higher values than the other two treatments when comparing only within those four time points (see *Results* for statistics). Dissimilar letters in B show the data points that diverged significantly from control (pre- and post-air exposure) values, based on pairwise post-hoc comparisons among all data points shown in B (Kruskal-Wallis post-hoc multiple comparisons tests).

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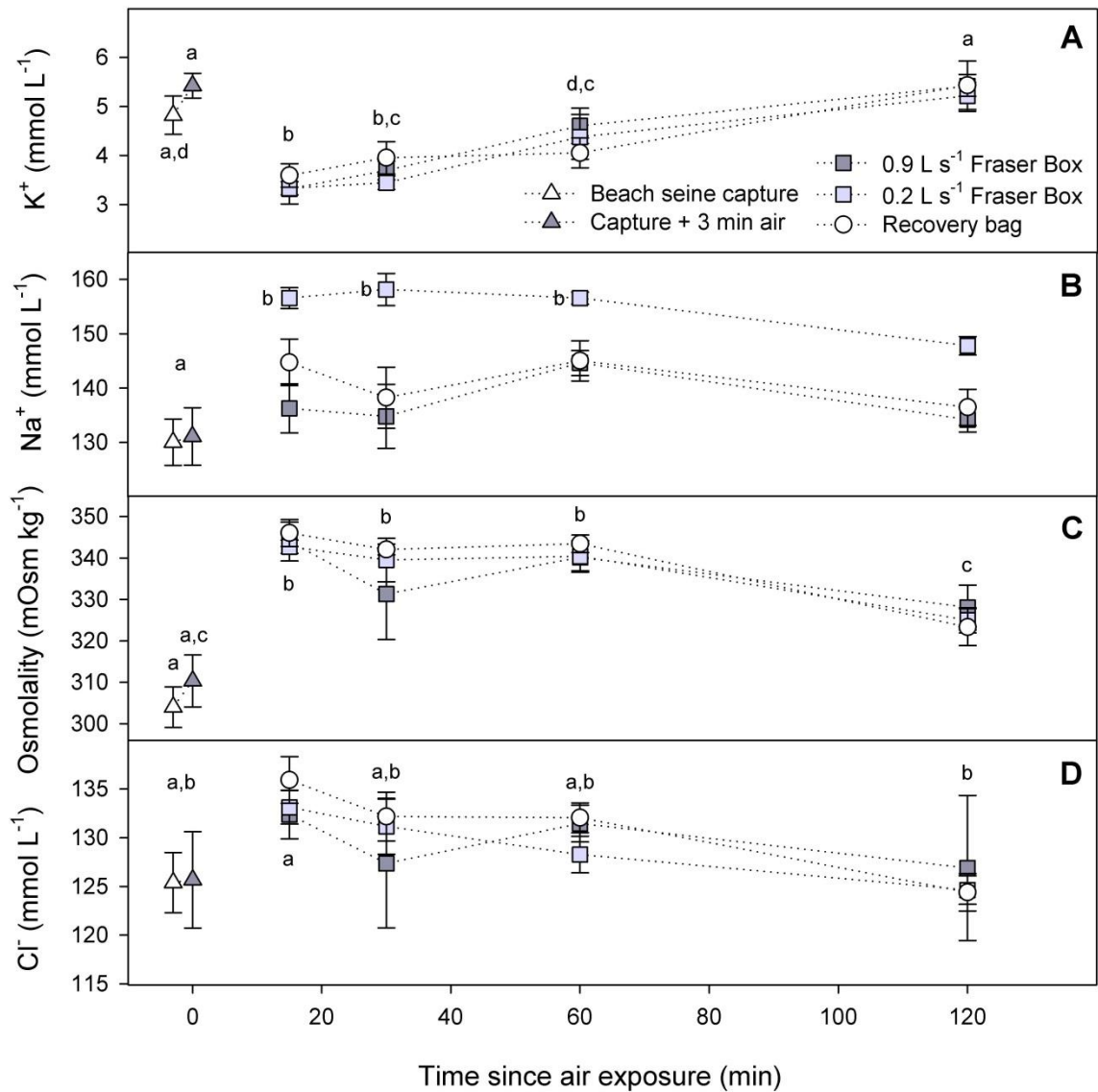
Figure 6.4 Mean ± S.E. hematocrit (Hct), hemoglobin (Hb), and mean corpuscular hemoglobin content (MCHC) measured from whole blood in the field in fish sampled upon capture, with the addition of 3 min of air exposure, and followed by different durations of recovery in three revival treatments. There was a significant overall effect of revival treatment on hematocrit (but not of time; post-hoc differences explained in text of section 6.4). MCHC was calculated from [Hb]/(Hct/100).
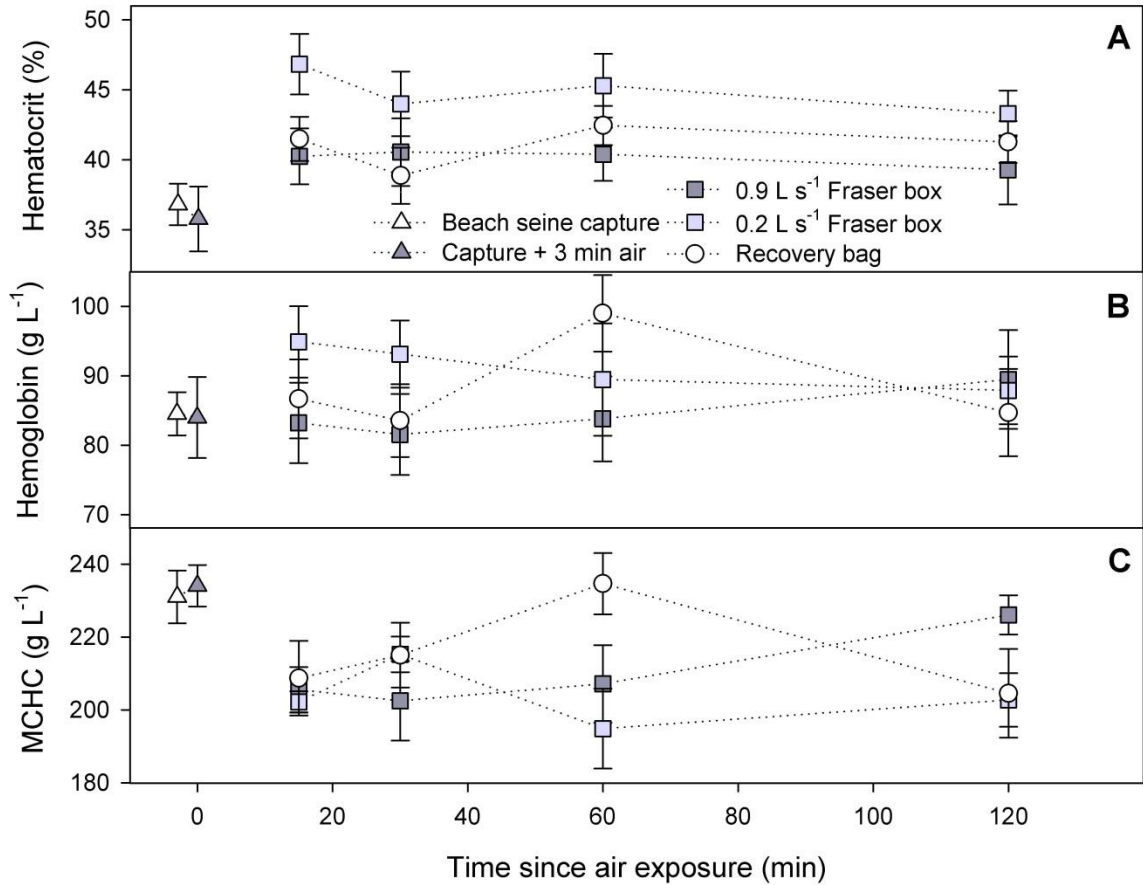
6.5 Discussion

This study confirms that the Fraser Box and recovery bag can both be used to achieve short-term revival of Pacific salmon after a capture stressor with extended air exposure incurred during upriver migration. Our experiment lacked true controls in the form of physiological data from fish sampled after being released to the river, and such data would be virtually impossible to obtain (see Cooke et al. 2013a for candid discussion of challenges with physiological sampling), but there are existing data against which ours can be compared (see below). Moreover, we caution that although immediate revival was achieved (97.5% of cases), we did not evaluate whether the revival treatments affected post-release survival or spawning success. Nevertheless, all three treatments showed evidence of being effective methods for reviving fish that, after the capture stressor we imposed, were so impaired that many of them would have been characterized as dead, asphyxiated, or moribund in a fishery setting (i.e., RAMP scores of 0.8-1.0; Farrell et al. 2001a). The RAMP scores recorded after 3 min of air exposure (0.8-1.0) were at or above the upper limit of those recorded in coho salmon caught in Fraser River beach seine fisheries, at which subsequent post-release mortality was very high (>70%) relative to fish with low RAMP scores (~20%; Fig. 4.4 in chapter 4). In such cases there may be the potential to increase post-release survival using revival techniques, but that possibility remains to be evaluated. The vestibular-ocular response (VOR) is always the final reflex to become impaired with increasing stressor intensity (see chapters 3 and 7); salmon without this reflex (46% of those in this study) ostensibly exhibit no signs of life. Thus, in some instances there may be opportunities to revive and release fish that otherwise would be retained because of a presumption that mortality has already occurred.

An important discovery was that after just 15 min in a revival treatment, 71% of fish were vigorous, ventilating, and were capable of some burst exercise (i.e., tail grab or body flex reflexes). In contrast, coho salmon caught in purse seines and revived in industry-standard recovery totes showed no significant improvement in RAMP scores irrespective of revival duration (chapter 2), indicating that not all revival methods are effective. While the present study did not assess post-release survival, the same revival methods have demonstrably improved survival in other contexts. The original validation of the Fraser Box in the marine environment noted a reduction in 24 h mortality from 57% to 6.5% for "asphyxiated" gillnet-caught fish (i.e., RAMP scores of 1.0) in a comparison with a traditional recovery tote (Farrell et al. 2001a, same as that used in chapter 2). The short-term mortality rate observed in the present study (2.5% overall) was similar to that in Farrell et al. (2001a). For sockeye salmon that were angled and air exposed for 1 min in the lower Fraser River, 15 min of recovery bag revival almost doubled post-release survival (from 28.6% to 50.0%; Donaldson et al. 2013). Conversely, substantial data have now accumulated that suggest these revival treatments do not benefit post-release survival of salmon after mild or moderate capture stressors, i.e., fish that are able to maintain orientation following their capture stressor (RAMP scores <0.6, Donaldson et al. 2013, Robinson et al. 2013, Nguyen et al. 2014, chapter 4). Manually holding salmon facing into flow for 1 min after moderate capture stressors may even reduce post-release survival in lab experiments (Robinson et al. 2013) and field studies (Robinson 2013). Those findings are important as they imply that the utility of revival techniques is context dependent. We concur with the earlier suggestion for commercially caught coho salmon that a vigorous fish should be released immediately to prevent

additional stress (Farrell et al. 2001a), such as that observed here with fish struggling to escape during prolonged recovery periods. Thus, revival treatments that are most likely to promote physiological recovery and minimize additional stress may be those in which fish are revived via ram ventilation until the moment they regain positive orientation and regular ventilation, and thereafter released to continue their recovery in the river (Donaldson et al. 2013, chapter 4). Such a treatment could be possible with recovery gears modified with a viewing window so that the handler is able to visually assess the condition of the animal without repeatedly subjecting it to physical assessment (i.e., to determine RAMP scores). However, in contexts where the risk of post-release predation is high, the locomotory benefit of a more extended revival treatment (e.g., 60-120 versus 15-30 min; Fig. 6.1) would perhaps outweigh the drawback of added confinement stress (Brownscombe et al. 2013, Cooke et al. 2014).

The present recovery data for physiological variables can be compared with those of Farrell et al. (2001a) where salmon were in similarly poor condition prior to a Fraser Box revival in saltwater. The blood variables here exhibited similar patterns, with immediate increases in osmolality, sodium, chloride, lactate, glucose and cortisol (Fig. 6.2, Fig. 6.3; Table 1 in Farrell et al. 2001a). There were important quantitative differences for the variables between the two studies. For example, plasma lactate was higher in the previous study (Table 1 in Farrell et al. 2001a) after 1 h in Fraser Boxes for gillnet-caught fish (24.2 versus 17.7 mmol L^{-1}), as were osmolality (379.5 versus 341.3 mOsm kg^{-1}) and hematocrit (50.1 versus 42.7 %), while plasma glucose was similar (7.7 and 7.3 mmol L^{-1}). These differences may reflect a variety of factors, including species differences (coho versus sockeye salmon), sex differences (mixed versus males only),

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salinity differences, maturation states (silver-bodied versus approaching spawning), the time elapsed between initiation of the capture stressor and when blood was drawn, or the nature of the capture stressor (Cooke et al. 2013a, Baker and Vynne et al. 2014, Donaldson et al. 2014).

As in the previous study on coho salmon (Farrell et al. 2001a), it is unclear whether the persistent physiological changes during recovery were a result of a natural time course or a result of recurrent stress as revived fish struggled to escape from the recovery treatment. Plasma lactate, glucose, and cortisol continued to rise or remained elevated across revival durations in all three treatments – evidence that, at first glance, would suggest physiological recovery was not taking place. However, those trends are in line with previous studies that show that these variables peak 1-2 h post-stressor (Milligan 1996, Clark et al. 2012), including during revival treatments that ultimately benefit survival (Farrell et al. 2001a, Donaldson et al. 2013). Nevertheless, there is good evidence that wild fish, particularly migrating adult salmon, are stressed by short-term confinement (Farrell et al. 2001a, Portz et al. 2006, Donaldson et al. 2011, chapter 2), and we expect that was the case in the present study. Moreover, a confinement-induced elevation in cortisol may slow the clearance of plasma lactate after exercise and capture stressors (Milligan et al. 2000, Farrell et al. 2001b), suggesting the sustained elevation of plasma lactate in the present study may have partly been an artefact of confinement.

Differences in recovery profiles for the three revival treatments were absent for most variables we measured. The differences that did occur suggest that the low-flow Fraser Box treatment resulted in somewhat greater physiological disturbance or impeded physiological recovery. Patterns in hematocrit, and especially plasma sodium, suggested

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a possible limitation of the lower flow Fraser Box treatment. Hematocrit increases from resting values of 20-25% (Milligan and Wood 1986, Sandblom et al. 2009) almost instantaneously upon initiation of a stressor because of a massive release of catecholamines (CAs; adrenaline and noradrenaline), which trigger a splenic contraction that increases the number of circulating erythrocytes (Wendelaar Bonga 1997). Indeed, it was quite clear that upon landing the beach seine hematocrit had already been elevated (to ~ 37%). Further elevations in hematocrit appear to have occurred primarily through erythrocyte swelling rather than by an increase in number, given that MCHC tended to decrease and hemoglobin exhibited no clear patterns with respect to time or treatment (Fig. 6.4). During hypoxia or exhaustive exercise, an increase of arterial CO_2 partial pressure (Pa_{CO_2}) beyond a threshold causes the release of CAs, which bind to β-adrenoreceptors on erythrocyte membranes (Reid et al. 1991), activating an Na^+/H^+ antiporter that shifts protons to the extracellular fluid (plasma) in exchange for sodium ions (Fievet et al. 1988, Perry and Gilmour 2006). That ion exchange allows erythrocytes to maintain an internal pH favourable to the oxygen-binding affinity of hemoglobin, but causes erythrocyte swelling because water enters the cell along with sodium (Borgese et al. 1987, Motais et al. 1992). If efflux of CO_2 and influx of O_2 at the gills was impaired in fish in the low-flow treatment because of a lack of strong ram ventilation after collapse of gill lamellae, they may have experienced added or prolonged elevation in Pa_{CO_2}. Carbon dioxide is mainly carried in the blood as HCO_3^- with a dissociated proton (H^+), causing increased pH, referred to as a respiratory acidosis (Wood 1991). If a larger respiratory acidosis or CA response was present in fish in the low-flow treatment, those differences may have elicited greater erythrocyte swelling, and higher hematocrit.

The most consistent treatment difference was the elevated plasma Na^+ for the low-flow Fraser Box treatment, but it is unclear what caused this as there are few comparable results elsewhere in the literature. Plasma ions normally increase after hypoxia or exercise as a result of hemoconcentration when water content is drawn away from the blood by intracellular acidosis in muscle cells (Milligan 1996, Kieffer 2000). However, the few studies that have compared physiological recovery among different recovery environments have observed similar patterns in Na^+ and Cl^- post-stressor (e.g., Farrell et al. 2001b, Donaldson et al. 2013), whereas in the present study the Na^+/Cl^- ratio was significantly higher in the low-flow Fraser Box treatment. Sodium is important for regulation of acid-base balance because it is readily exchanged for protons via membrane exchangers that operate on red blood cells, muscle, and at the gills (Heisler 1989, Perry and Gilmour 2006). To satisfy electroneutrality with an elevated Na^+/Cl^- ratio, some other (unmeasured) anion(s) must have increased in concentration, perhaps partly HCO_3^-. A larger or more sustained respiratory acidosis in blood plasma for the low-flow treatment may have triggered a larger increase in plasma sodium, perhaps via transfer of Na^+ from a white muscle compartment that experienced relatively less intracellular acidosis than in other studies where fish were swum fully to exhaustion. Fish in the present study did "exercise" during netting but remained vigorous until removed from the net for air exposure, indicating they had not exercised maximally (Fig. 6.1). This suggests that the subsequent acidosis in plasma was likely more respiratory than metabolic in origin relative to previous exhaustive exercise studies. Indeed, plasma lactate, which is extruded from exhausted white muscle and is the primary source of metabolic protons (Wood 1991), did not reach maximal levels in this study (20-25 mmol L^{-1}; Farrell et al. 2001a,

chapter 5). Interestingly, van Raaij et al. (1996) found that rainbow trout exposed to severe hypoxia that subsequently died exhibited a significantly higher Na^+/Cl^- ratio in plasma during the post-hypoxia period in the lead-up to death. As in the present study, the authors were unable to determine the source of the elevated Na^+/Cl^- ratio given the complex nature of ion regulation, but noted that dramatic changes in ionic concentrations are maladaptive from a stress coping standpoint (van Raaij et al. 1996, Wendelaar Bonga 1997). Perturbations in plasma ions have elsewhere been linked to mortality in salmonids (Wood et al. 1983, Jeffries et al. 2011), and large changes in ion concentrations can affect the structure and function of macromolecules and directly damage tissues via osmotic swelling (Moyes and Schulte 2008).

In summary, from the perspective of small-scale freshwater fisheries, a key finding here is that lightweight and inexpensive recovery bags generate the same physiological recovery profiles as the previously validated Fraser box (Farrell et al. 2001a), which is a heavy, expensive, and non-portable device better suited to commercial fishing boats. Much is known about ways to minimize capture stress (e.g., Davis 2002, Cooke and Suski 2005), but in cases where angling times are long, if fish are air exposed for de-hooking or photography, or become asphyxiated in small-scale gillnet fisheries, recovery bags may represent a useful tool for fishers interested in reviving fish prior to release. Future experiments should clarify thresholds at which revival treatments can benefit physiological recovery and survival. The capacity of fish to recover from acute stressors has clear relevance to fitness, and as such represents a fascinating intersection of comparative physiology and ecology (Ricklefs and Wikelski 2002, Donaldson et al. 2010a). Further experiments comparing the effects of different recovery environments on

physiological responses can help generate knowledge that is of fundamental interest and

equally relevant to fisheries management and conservation.

Chapter 7.   Resilience of pink and chum salmon to simulated fisheries capture stress incurred upon arrival at spawning grounds

7.1   Abstract

We compared exhaustion-related physiological stress and physical injury as contributors to fish condition, longevity, and egg retention in two Pacific salmon species after their arrival at spawning areas. Adult female pink salmon (N = 174) and chum salmon (N = 120) were exposed to six experimental capture treatments that represented different levels of exhaustive exercise, air exposure, and injury. After evaluating reflex impairment and obtaining a blood sample, each fish was released into its natal spawning channel with an external tag and later retrieved post-mortem to evaluate spawning success via examining egg retention. Reflex impairment, plasma lactate, chloride, potassium, and osmolality varied among treatments with differences generally driven by longer exposure to capture stress, which included exhaustive exercise and air exposure. However, overall pre-spawn mortality was negligible (~ 5%) and consistent across treatments for both species. We hypothesize that pink and chum salmon are resilient to capture-related exhaustion upon reaching spawning areas because of a combination of low water temperature (~12 ℃ in this study) and a physiological shift towards increased use of anaerobic pathways during their final weeks of life. The capture and release of fish arriving at the spawning ground does not appear to influence survival, in contradiction to other studies that focus on earlier components of Pacific salmon spawning migrations. Fisheries adjacent to spawning sites represent the end of the continuum of salmon fisheries that begin with the

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high seas fishery and extend through the coastal and riverine environments. Mortality rates in this study should be interpreted cautiously by management until research efforts are broadened to provide a better understanding of how post-release outcomes at different life stages compare in natural systems and conditions more representative of real fisheries.

7.2   Introduction

Fisheries capture involves two general biological effects at the organism level: physiological stress and injury. In the context of fish capture, physiological stress has two parts: 1) a glucocorticoid-based stress response that elicits a cascade of physiological adjustments (Wendelaar-Bonga 1997, Barton 2002), and 2) metabolic exhaustion caused by anaerobic exercise and (in many cases) air exposure (Ferguson and Tufts 1992, Kieffer 2000). Physiological stress alone can lead to immediate or delayed mortality (Wood et al. 1983, Ferguson and Tufts 1992, Davis 2007, Gingrich et al. 2007).

Physical injury is the second general organismal effect of fish capture. Injuries could lead to immediate or delayed mortality (Broadhurst et al. 2005), behavioural impairments (Brouwer et al. 2006, Gravel and Cooke 2008), or secondary infections (Baker and Schindler 2009, Butcher et al. 2010). There are also interactions between stress and injury with injury tending to exacerbate the level of stress (chapter 2). Moreover, the stress response can result in reduced immune function (Barton 2002), which could act in synergy with dermal injuries to produce latent (and potentially fatal) infections. Few studies have compared the relative effects of (or interactions between)

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exhaustion-related stress and physical injury on post-release outcomes (but see chapter 2, Nguyen et al. 2014). Developing a better understanding of the stress-injury interaction could inform conservation initiatives. For example, if a particular bycatch-affected species proves adept at overcoming fisheries capture stress but suffers high mortality in cases where dermal injuries occur, mortality reduction efforts could focus on reducing injury (e.g., through gear or handling modifications) rather than reducing stress per se (e.g., through reducing entanglement times or air exposure).

Very little data exist on fisheries interactions in river headwaters where salmon are approaching and arriving at terminal spawning areas. Nearly all published studies on salmon capture and release have focused on marine fisheries, and none have investigated post-release impacts for fish captured upon arrival at spawning areas. Historically, the bulk of harvest fisheries for Pacific salmon did not occur close to spawning areas, with the exception of some small scale aboriginal fishing. However, in some cases there has been an increasing desire for such fisheries, motivated by attempts to reduce bycatch, to satisfy aboriginal treaty obligations, and by an increasing demand for roe. Though the large majority of fishing for Pacific salmon does occur in the marine environment and shortly after river entry, some capture and release occurs close to and within spawning areas. For example, in the Harrison River, one of the largest spawning tributaries of the Fraser River (and the subwatershed where this study took place), there are multiple First Nations fisheries that use gillnets and beach seines: "Economic Opportunity" fisheries (targeting all species), Food Social and Ceremonial (all species), in addition to ESSR harvest (Excess Salmon for Spawning Requirements – chum, coho, and pink salmon). Recreational fisheries in the Harrison River targeting mainly coho, Chinook, and sockeye

salmon also capture and release thousands of pink and chum salmon. In fact, during 2001 more than 45,000 pink salmon and 5000 chum salmon were released by anglers on or near spawning areas of the Harrison system. Catch-and-release angling also occurs throughout the upper watershed in a similar manner to that in the Harrison River (for data, see http://www.pac.dfo-mpo.gc.ca/fm-gp/fraser/index-eng.htm - accessed 07/09/2012). In addition, capture-mark-recapture is one of the most commonly used methods for stock assessment and research in spawning areas, especially in British Columbia (Schwarz et al. 1993, Rajwani and Schwarz 1997, Schubert 2000) but also in the United States and Japan (Miyakoshi and Kudo 1999, Naughton et al. 2009, Parsons and Skalski 2010). Those tagging programs rely on an assumption that the capture and tagging process itself does not affect spawning success.

The primary objective of this study was to elucidate the relative importance of physiological exhaustion and dermal injury as contributors to survival and spawning success in pink salmon and chum salmon released from fishing gears upon arrival at spawning grounds. A further objective was to evaluate RAMP for the first time in these species while comparing RAMP scores to plasma variables (see section 1.3 and chapter 3 for an introduction to the RAMP method). We sought to impose a range of experimental levels of stress and injury on pink and chum salmon arriving at spawning grounds, quantified RAMP for each individual, and subsequently monitored their fate after release into a large, artificial spawning channel. To our knowledge, this represents the first work on capture-and-release of pink or chum salmon in any context, two species commonly released by multi-sector fisheries that harvest sockeye, coho, and Chinook salmon.

7.3   Methods

7.3.1   Study site

The study was conducted at Weaver Creek Spawning Channel, a 2,930 m long, 6.1 m

wide and 0.25 m deep artificial spawning channel built in 1965 as part of British

Columbia's Salmonid Enhancement Program (Quinn 1999). The spawning channel is

part of the Fraser River watershed and is approximately 117 km (river kilometers)

upstream of river entry (Fig. 1.1, Fig. 7.1). Each year in October, Pacific salmon (sockeye

and chum salmon every year, pink salmon every odd year) enter the spawning channel

via a concrete raceway (1.5 m deep, 2.5 m wide, 100 m long). Individuals are

preferentially admitted into the channel through a fence based on pre-season spawning

targets for each species. Fish not permitted into the spawning channel are either released

into the natural creek adjacent the spawning channel or are treated as surplus and

harvested by an aboriginal commercial fishery. The channel contains gravel substrate

ideal for spawning (1.2 – 7.6 cm diameter), has consistent water flow (~ 0.4 m s^{-1}), and

fish densities are controlled. Fish in the raceway are often crowded and have fully

developed secondary sexual characteristics, making it easy to target individuals of a

particular sex and species using a dip net. Female salmon were used throughout all

experiments to facilitate an accurate assessment of spawning success (see below).

7.3.2   Experimental treatments, biopsy, and tagging

Female pink (N = 174; October 3-4, 2009, mean water temp. = 13.2 ºC) and chum

salmon (N = 120; October 7-12, 11.8 ºC) were dip netted from the spawning channel

raceway and randomly assigned to one of six experimental fishing simulation treatment

groups: (1 – 'high stress, high injury') 3 min gillnet entanglement and 1 min air exposure, (2 – 'high stress, minor injury') 3 min angling with a hook and line and 1 min air exposure, (3 – 'high stress, no injury') 3 min manual chase by hand and 1 min air exposure, (4 – 'low stress, high injury') 10 s gillnet entanglement without air exposure, (5 – 'low stress, minor injury')) 10 s angling without air exposure, and (6 – 'low stress, no injury') a control not subjected to a capture simulation. For all treatments but the control group fish were immediately transferred from the raceway to a small circular tank (2 m diameter, 0.4 m depth) continuously supplied with creek water where the capture simulation was undertaken. For the gillnet simulation treatments (groups 1 and 4), fish were placed in the tank and forced to swim into a dip net strung loosely with monofilament gillnet mesh netting (13.3 cm mesh; standard size for targeting sockeye salmon in the Fraser River). For the long duration gillnet treatment with air exposure (group 1; hereafter referred to as 'high stress'), the fish were disentangled from the net in air and kept in air for a total of 1 min following a 3 min entanglement. For the 'low stress' gillnet treatment (group 4), the fish were kept submerged for disentanglement which began after only 10 s in the net. The 'high stress' and 'low stress' angling treatments (groups 2 and 5, respectively) mirrored the gillnet treatments except that in place of a gillnet, a size 1 barbless J-hook attached to a line and rod was inserted through the upper jaw of the fish using pliers and angling was simulated in the circular tank, thus eliciting a hook injury. The chase treatment (group 3) was designed to provide the exhaustive exercise (3 min) and air exposure (1 min) of a capture event without any dermal injury, and involved three people leaning over the edge of the tank and stimulating the fish to burst swim by either touching its caudal fin or splashing beside it

(Gale et al. 2011). Air exposure involved fish being held in a knotless nylon dip net above ground adjacent to the circular tank for 1 min immediately following cessation of the tank treatment (i.e., 3 min chase, net entanglement, or angling). If gillnet disentanglement for the 'high stress' treatment was not completed within 1 min, disentanglement was completed with the fish submerged in the circular tank. These treatments were intended to be sufficiently varied so that we could make inferences about the relative effects of the injury and exhaustion-related stress components of capture. For example, were higher pre-spawn mortality to occur in treatments with more severe injuries, irrespective of the level of stress (i.e., exercise and air exposure) imposed, it could be inferred that injury was most influential. Conversely, were the highest mortality to occur in the 'high stress' groups with no differentiation resulting from injuries, one would conclude the exhaustion levels were most influential. Finally, were mortality rates far higher in group 1 ('high stress', high injury) than all others, it could be concluded that the interaction between stress and injury is most important.

Upon completion of the capture simulation, each individual was brought to a padded V-shaped sampling trough (described by Cooke et al. 2005), continuously supplied with flowing fresh water directed over the gills, where it was restrained by hand for biopsy and tagging. The control group (group 6) was dip netted from the raceway and brought directly to the sampling trough for processing (within 10 s of capture). Firstly, each fish was held supine for withdrawal of ~ 2 mL of blood using caudal puncture (described in section 2.3.2). Following phlebotomy, an individually-numbered cinch tag was inserted through the dorsal musculature anterior to the dorsal fin. Each fish was measured (fork length to the nearest cm) and any apparent injuries were described. Fish

were finally exposed to a RAMP assessment and released into the spawning channel

(RAMP assessment described in section 2.3.2). Plasma was separated from blood

samples using a centrifuge and stored as in previous chapters (2, 3, 5, and 6), and

analyzed in the laboratory for plasma glucose, lactate, osmolality, and ions (Cl^-, K^+, and

Na^+; see chapter 2, section 2.3.2).

7.3.3   *Post-release fate*

Subsequent to release of tagged salmon into the spawning channel, the entire length (~ 3

km) of the channel was searched twice daily for dead tagged fish. Fish were generally

recovered underwater and away from most gull scavenging. For each recovered

individual, we calculated longevity as the number of days from tagging to recapture. As a

proxy for reproductive success, we used egg retention (following Quinn et al. 2007,

Hruska et al. 2010, 2011, Cook et al. 2011). For egg retention, we calculated the

percentage of eggs remaining in the body cavity relative to the length-specific expected

number of total eggs based on length-to-egg-mass relationships we constructed for both

pink and chum salmon with separately sampled individuals (Cook et al. 2011).

Individuals were then categorized as successful spawners if they had released > 90% of

their eggs, while individuals retaining 50% or more of their eggs were considered

unsuccessful spawners (pre-spawn mortalities). Fish that had released 51% - 89% of their

eggs (pink salmon N = 10, chum salmon N = 7) were excluded from the analysis because

small numbers of eggs were sometimes lost from carcasses during transport from the

channel to the dissection bench (Cook et al. 2011). Carcasses were carefully inspected for

any evidence that they had been stripped of their eggs by gulls. Fish that were not successfully recovered (pink salmon N = 20, chum salmon N = 38) and fish pecked by gulls (pink salmon N = 14, chum salmon N = 4) were not included in analyses of egg retention (thus, assessments of egg retention were based on N = 130 pink salmon N = 71 chum salmon).

7.3.4   *Calibration of RAMP method*

We conducted an *a posteriori* experiment using female pink salmon to determine the limits of resistance to capture stress and to calibrate a RAMP-mortality curve. Our stress treatment involved aerial confinement in a wetted black hypalon fish-holding bag. We used air exposure durations in the range of those used by Gingerich et al. (2007) and Thompson et al. (2008): 0, 60, 120, 240, 480, and 960 s. Pink salmon females were dip netted from the spawning channel raceway and randomly assigned to one of the six treatment groups (read: durations; N ~ 20 per group). Following the aerial confinement stressor, each fish was measured and cinch tagged in a water-filled sampling trough as detailed above (except blood samples were not taken). RAMP assessments were conducted as described above and the fish were released into the spawning channel in the same location as for the earlier experiment (Fig. 7.1). In addition to the six treatment groups, there was a 'RAMP control' group of fish that was rapidly dip-netted and RAMP-assessed, without the air exposure treatment or the handling associated with tagging (total experiment N = 132). Tagging and release occurred over three days (October 19-21, 2009) during which the mean water temperature in the channel was 11.9

ºC. Following release, carcasses of tagged individuals were recovered and processed as detailed above (7.3.3).

7.3.5   *Data analysis and statistics*

To evaluate the status of fish at the time of release, we compared physiological measures from blood plasma and RAMP scores among treatment groups. The plasma variables we analyzed begin changing on a predictable trajectory that is initiated when a fish is first contacted. We therefore used analysis of covariance (ANCOVA) with treatment group as the main effect and handling time (from initial capture to release) as the covariate to compare plasma parameters among treatments (Bonferroni-corrected α of 0.007). Similarly, to compare RAMP scores to plasma variables, we used ANCOVA with RAMP score treated as the categorical main effect and handling time as the covariate ($\alpha = 0.008$). When ANCOVAs were significant, post-hoc differences were identified using the Tukey HSD test. RAMP scores themselves were compared among treatments (in both experiments) using Kruskal-Wallis analysis of variance (ANOVA) ($\alpha = 0.025$), with multiple comparisons of z values as a post-hoc test where necessary. We compared longevity among treatment groups using Kruskal-Wallis ANOVA because the data could not be normalized. In order to compare the proportion of fish categorized as successful spawners among treatment groups, we used a Pearson's chi square test. To evaluate the predictive power of RAMP score on the fate of released fish, mean longevity was compared among RAMP scores using Kruskal-Wallis ANOVA. Finally, fish were grouped as either spawners or non-spawners and the RAMP scores at release for those groups were compared using a Mann-Whitney U test. All analyses were conducted using

Statistica 8.0 (StatSoft, Tulsa, OK, USA). Data were normalized using $\log_{10}$

transformation for statistical analyses where necessary following assessment with

Shapiro-Wilk's test. All values in text are reported as means ± standard error of the mean.

Figure 7.1 Map of the location of Weaver Creek Spawning Channel within British Columbia, Canada (A), the lower Fraser River watershed (B), and of the layout of the spawning channel itself (C). Numbers denote 1) the natural Weaver Creek from which fish enter the spawning channel, 2) the fish ladder fish swim through to enter the raceway, 3) the sorting shed through which fish are normally allowed to enter the channel or diverted for surplus, 4) the holding area of the raceway from which fish were dip netted, and 5) the location in the downstream-most stretch of the spawning channel into which tagged fish were released following processing. Figure extracted from Hruska et al. (2011) and Cook et al. (2011) with permission.
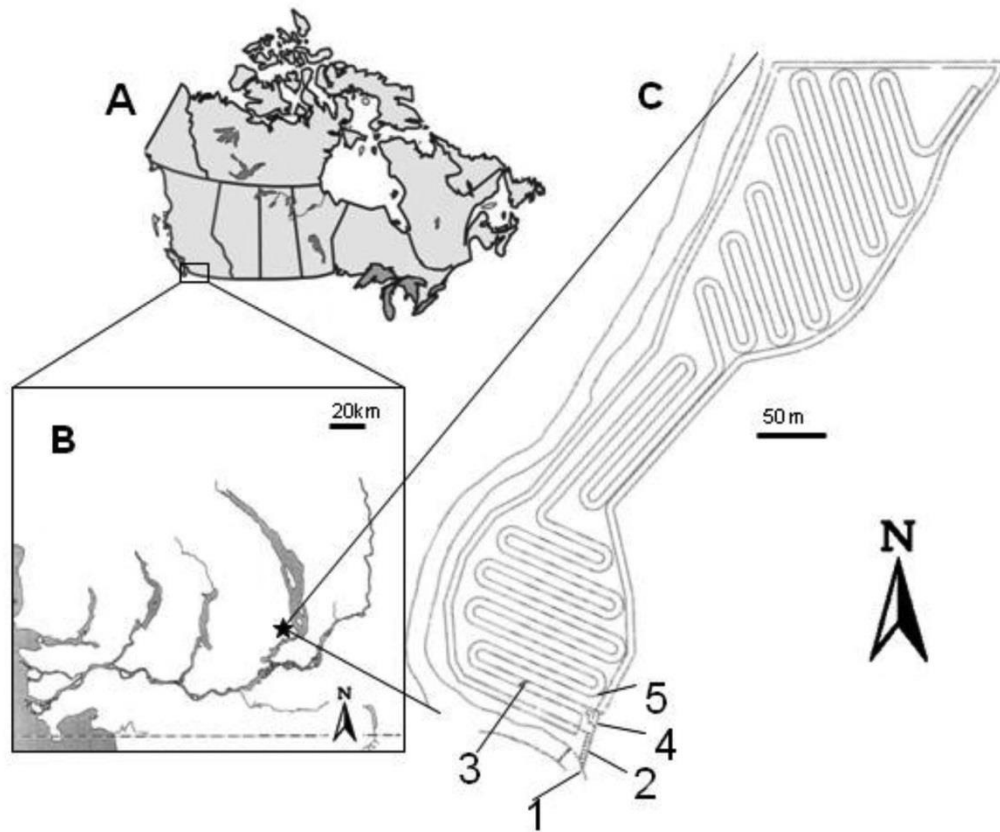
7.4 Results

7.4.1 *Qualitative observation of behaviour and injuries*

Both pink and chum salmon struggled vigorously once they were dip netted from the raceway into the experimental tank and forced to become entangled in gillnet mesh. Fish from the 10 s ('low stress') entanglement treatment remained vigorous as they were being disentangled. Fish from the 3 min ('high stress') treatment continued to struggle vigorously for the first ~ 30 s of entanglement and then attempted to burst free only periodically for the remaining ~ 150 s. A small number of the pink salmon were slender enough to struggle through the gillnet mesh sized used, and so had to be re-entangled multiple times during the 3 min treatment. Chum were typically too large for the mesh size used, and therefore the netting functioned as a tangle net with most tangling occurring around the head and on the teeth. Both species occasionally exhibited minor bleeding from the gills as a result of abrasion from the netting, which generally ceased after 1-2 min in the sampling trough post-treatment. In general, the gillnet entanglement caused few obvious injuries or scale loss, largely because all fish had reached the point in their lifecycle where their scales had fused into their skin in readiness for the spawning period. The most severe injuries consisted of minor bruising lines from net mesh and localized mucus loss - quite minimal relative to what can happen in gillnet fisheries further downstream (see photos in Baker and Schindler 2009). Salmon captured in most fisheries would suffer damage from the loss of highly perfused scales, plus a range of injury severity depending on fish size, duration of entanglement, and how fish are handled if they are pulled from the gillnet by hand and released (rather than escape the net before landing; Baker and Schindler 2009). We therefore caution that the post-release

effects of gillnet entanglement in a true fishery operating at spawning areas would likely differ from what we observed following our gillnet simulation. It remains a possibility that the external physical changes in matured Pacific salmon render them more resilient to gillnet injury than fish at earlier stages, though that possibility has not been sufficiently evaluated here.

The angling treatment was effective in that hooked fish typically struggled vigorously for > 2 min, similar to what would be observed in a true angling event (Donaldson et al. 2011). During de-hooking or dis-entangling, whether in water ('low stress') or in air ('high stress'), individuals of both species typically struggled and presumably increased their level of exhaustion. Injuries were consistent for the angling treatment because an identical hook was pulled through the same part of the upper jaw for each individual, leaving a small hole through the tissue and usually minor bleeding. Again, the injuries resulting from this treatment were considered to be minor, and comparable to those obtained during an actual river angling event.

The extent of the exercise resulting from the manual chase was similar to what was observed in the 'high stress' angling treatment, where fish would burst vigorously for the first 90 – 120 s of the treatment before visibly beginning to tire, and typically stopped responding to stimuli by the end of 3 min. However, we did observe that chum salmon typically ceased responding to researcher stimuli 30 – 60 s earlier than pink salmon in the manual chase treatment.

7.4.2   Interactive effects, RAMP, blood physiology, and fate

Pink and chum salmon responded similarly to the treatments, with RAMP scores and plasma lactate providing the clearest evidence of a treatment effect (Fig. 7.2). After controlling for handling time, the three 'high stress' treatments grouped together in that both pink and chum salmon had significantly higher plasma lactate levels upon release relative to the 'low stress' injury treatments and controls (ANCOVA with handling time as a covariate: pink salmon, $F_{5, 163} = 8.72$, $P < 0.001$; chum salmon, $F_{5, 110} = 6.48$, $P < 0.001$; Fig. 7.2, Table 7.1). Similarly, the longer capture simulations that included air exposure resulted in significantly more reflex impairment for both species (Kruskal-Wallis ANOVA: pink salmon, $H_{5, 173} = 95.53$, $P < 0.001$; chum salmon, $H_{5, 173} = 59.45$, $P < 0.001$; Fig. 7.2, Table 7.1). There was a significant difference in plasma potassium in pink salmon among groups ($F_{5, 163} = 8.72$, $P < 0.001$), but not in chum salmon ($F_{5, 110} = 3.10$, $P = 0.012$; Table 7.1). Specifically, a post-hoc test revealed that pink salmon in the control group had significantly lower levels of plasma potassium than all other groups, while the chase and high stress hook treatments resulted in the highest plasma K^+ (Fig. 7.2, Table 7.1). Treatment also significantly affected plasma chloride ($F_{5, 164} = 3.82$, $P = 0.003$) and osmolality ($F_{5, 164} = 3.74$, $P = 0.003$) in pink salmon after controlling for handling time, while osmolality was significantly different among groups in chum salmon ($F_{5, 109} = 4.67$, $P < 0.001$) whereas chloride was not ($F_{5, 110} = 2.01$, $P = 0.083$; Fig. 7.2, Table 7.1). There were no significant effects of the main effect (treatment) or the covariate (handling time) on plasma glucose or sodium in either pink or chum salmon (Table 7.1).

The pattern of impairment of individual reflexes with successively increasing levels of overall reflex impairment (RAMP scores) was largely consistent. Tail grab and body flex were by far the two most easily impaired reflexes (Table 2, Table 3). However, in chum salmon, tail grab impairment was more frequent than body flex impairment at low RAMP scores (0.2, 0.4) when compared with pink salmon. Orientation was typically the third reflex to become impaired for both species when overall RAMP score increased beyond 0.4 (Table 2, Table 3). VOR impairment was not commonly observed, and was almost always the last reflex impaired.

RAMP score also demonstrated some concordance with physiological variables in blood. In particular, lactate accumulation in plasma was significantly related to RAMP score for both pink ($F_{5, 163} = 3.73$, $P = 0.003$) and chum salmon ($F_{4, 111} = 4.75$, $P = 0.001$), with the higher lactate values occurring at higher RAMP scores (Fig. 7.3). Other patterns that were apparent between RAMP scores and plasma constituents (Fig. 7.3) were not statistically significant (Table 7.1).

Though physiological disturbances were significantly varied among the capture simulations, there were no resulting treatment differences in the post-release outcomes we measured (Fig. 7.4). Mean post-release longevity on spawning grounds for female pink salmon (tagged Oct. 3-4) in this study was 14.42 ± 0.33 d (range: $1 - 23$ d) and did not differ significantly among the six treatment groups (Kruskal-Wallis ANOVA: $H_{5, 154} = 4.81$, $P = 0.44$). Mean post-release longevity on spawning grounds for chum salmon was 7.54 ± 0.19 d (range: $3 - 12$ d) and there was no significant effect of treatment ($H_{5, 71} = 2.53$, $P = 0.77$). In general, spawning success was high based on egg retention: 92% and 96% of the released pink and chum salmon, respectively, were categorized as

successful spawners. The proportion of fish classed as successful spawners was not significantly different among the six treatment groups for either pink (Pearson's chi square: $\chi^2 = 0.24$, $P = 1.00$) or chum salmon ($\chi^2 = 0.11$, $P = 1.00$). With negligible variation in post-release outcomes, it is not surprising that RAMP score was not a significant predictor of longevity in pink (Kruskal-Wallis ANOVA: $H_{5, 139} = 9.56$, $P = 0.09$) or chum salmon ($H_{4, 78} = 5.50$, $P = 0.24$), nor of spawning success (Mann-Whitney U test, $P = 0.87$ and $P = 0.60$ for pink and chum salmon, respectively).

An objective of this study was to assess the utility of RAMP as a post-release mortality predictor for these species. However, given that we observed virtually no post-release (pre-spawn) mortality to compare with RAMP scores, we conducted an *a posteriori* experiment (7.3.4) using pink salmon that involved a range of exhaustion stress beyond what was included in the main experiment. With each increase in duration of the aerial confinement stressor, we observed an increase in reflex impairment (Fig. 7.5), with RAMP scores significantly different among durations (Kruskal-Wallis ANOVA: $H_{6, 132} = 108.79$, $P < 0.001$). There was an immediate mortality rate (i.e., fish did not leave the release site) of 25% for the 16 min period (5 of 20 individuals). However, excluding those five individuals, mean longevity (6.22 ± 0.14 d overall) did not differ significantly among durations ($H_{5, 75} = 7.43$, $P = 0.19$) and every single individual that was recaptured was categorized as a successful spawner based on low egg retention, including all eight fish recaptured from the 16 min group.

Figure 7.2 Mean RAMP score, as well as plasma lactate, potassium, osmolality, chloride and sodium values measured in female pink salmon (white bars) and chum salmon (grey bars) for each of the six capture treatments. RAMP score represents to the proportion of reflexes assessed as impaired for an individual among the five reflexes assessed (see 2.3.2). "Hook low" (treatment 5) and "gill low" (treatment 4) refer to injury treatments with brief exposure to fishing angling and gill netting respectively without air exposure (i.e., "low" exhaustion stress), whereas "hook high" (treatment 2) and "gill high" (treatment 1) refer to 3 min exposures to the same gears with the addition of 1 min of air exposure. "Chase" fish (treatment 3) were chased in the circular tank for 3 min followed by 1 min of air exposure, while "control" fish (treatment 6) were brought rapidly to the sampling trough for sampling and tagging. See *Methods* (7.3.2) and *Results* (7.4.1) for full details on capture simulations. Among-group differences were assessed for significance with ANCOVA using researcher handling time as the covariate; post-hoc differences among groups indicated by dissimilar letters (see *Results* and Table 7.1 for statistics). Error bars denote 95% confidence intervals.

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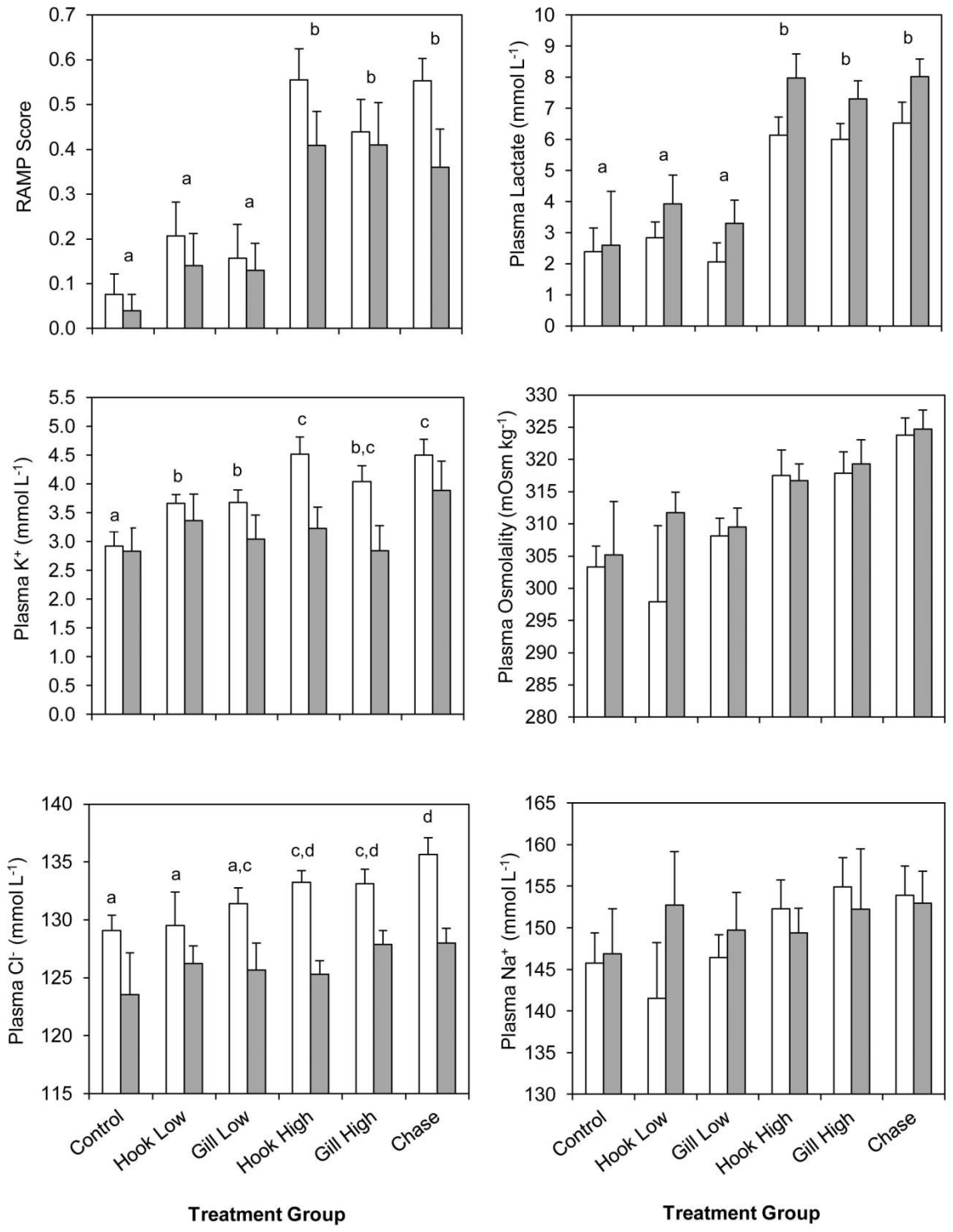
Figure 7.3 Mean plasma lactate, potassium, osmolality and chloride values for female pink salmon (white bars) and chum salmon (grey bars) assessed at five different levels (scores) of overall reflex impairment, with higher scores (on the x-axis) indicating a greater proportion of impaired reflexes (see 2.3.2). Among-group statistical differences were assessed using ANCOVA with handling time as the covariate and RAMP score as the main effect (categorical) variable; post-hoc among-group differences are denoted by dissimilar letters above bars (see *Results* and Table 7.1 for statistics). Error bars denote 95% confidence intervals.
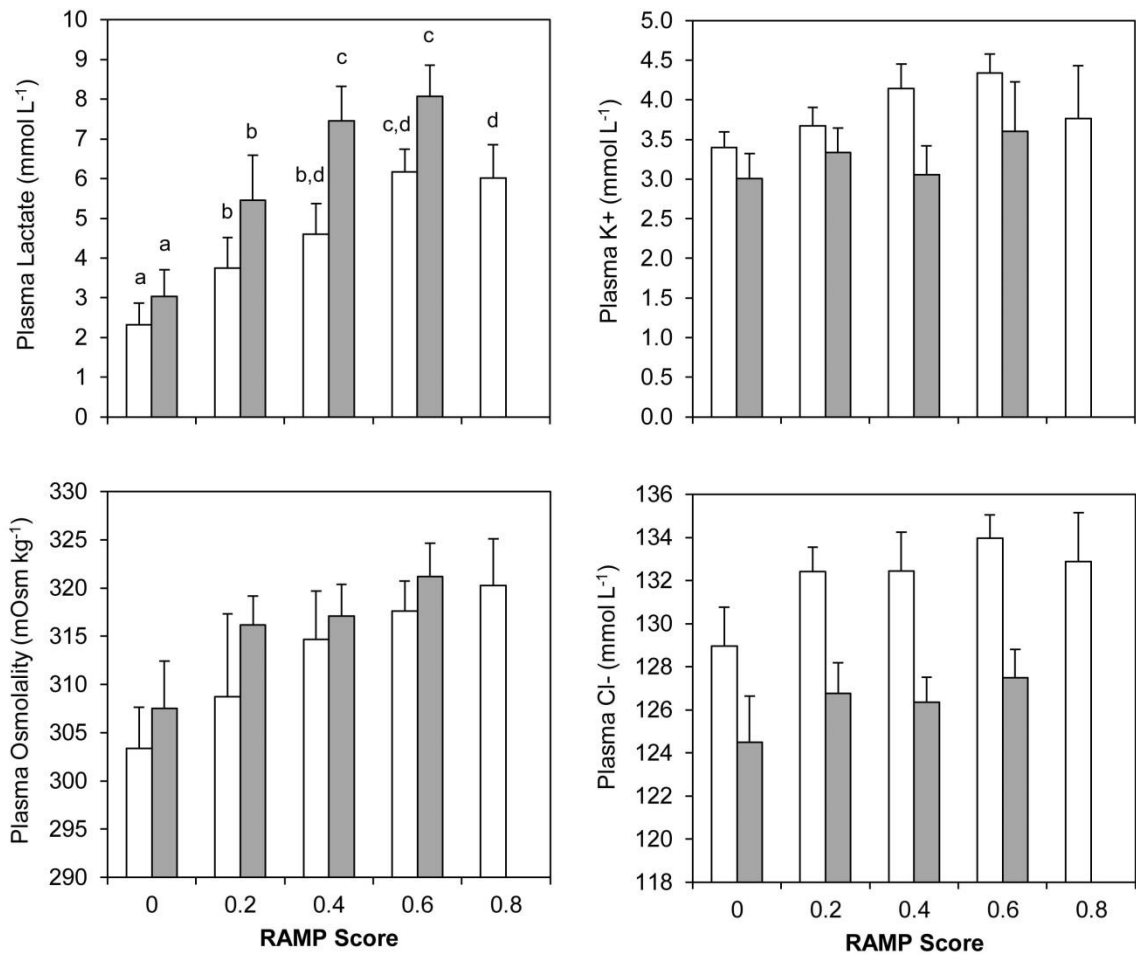
Figure 7.4 Mean post-release longevity and the proportion of successful spawners for each of the six treatment groups (pink salmon = white bars; chum = grey bars). Longevity was defined as the number of days from capture and released to post-mortem retrieval of the carcass. Individuals were categorized as successful spawners if they had retained ≤ 10% of their eggs following their time on the spawning channel (see *Methods* for more details). "Hook low" (treatment 5) and "gill low" (treatment 4) refer to injury treatments with brief exposure to fishing angling and gill netting respectively without air exposure (i.e., "low" exhaustion stress), whereas "hook high" (treatment 2) and "gill high" (treatment 1) refer to 3 min exposures to the same gears with the addition of 1 min of air exposure. "Chase" fish (treatment 3) were chased in the circular tank for 3 min followed by 1 min of air exposure, while "control" fish (treatment 6) were brought rapidly to the sampling trough for sampling and tagging. See *Methods* (7.3.2) and *Results* (7.4.1) for full details on capture simulations. Error bars denote 95% confidence intervals.
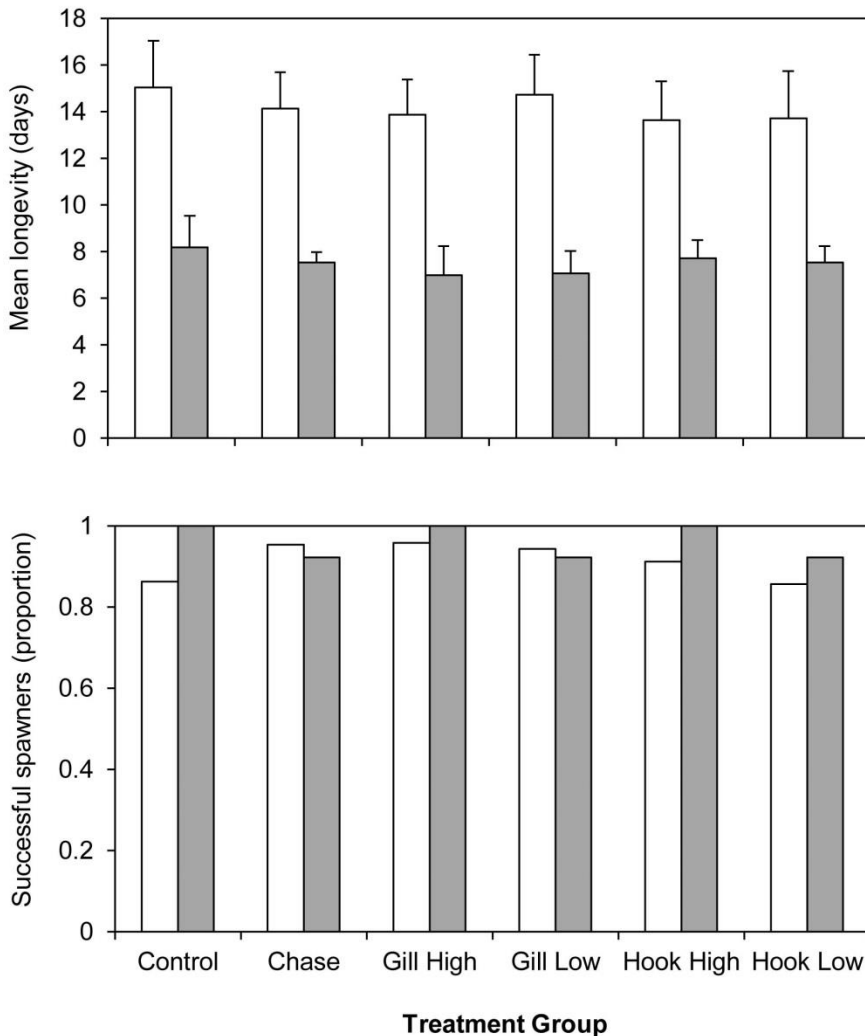
Figure 7.5 RAMP scores in female pink salmon following exposure to different durations of an aerial confinement stressor and cinch-tagging before release into the spawning channel (see 7.3.4). RAMP score represents to the proportion of reflexes assessed as impaired for an individual among the five reflexes assessed (detailed in 2.3.2). "Control" indicates fish that were rapidly dip-netted from the channel raceway entrance (Fig. 7.1) and immediately RAMP-assessed without tagging. Statistical differences among groups were assessed using Kruskal-Wallis ANOVA and post-hoc differences are indicated by dissimilar letters. Error bars denote 95% confidence intervals.
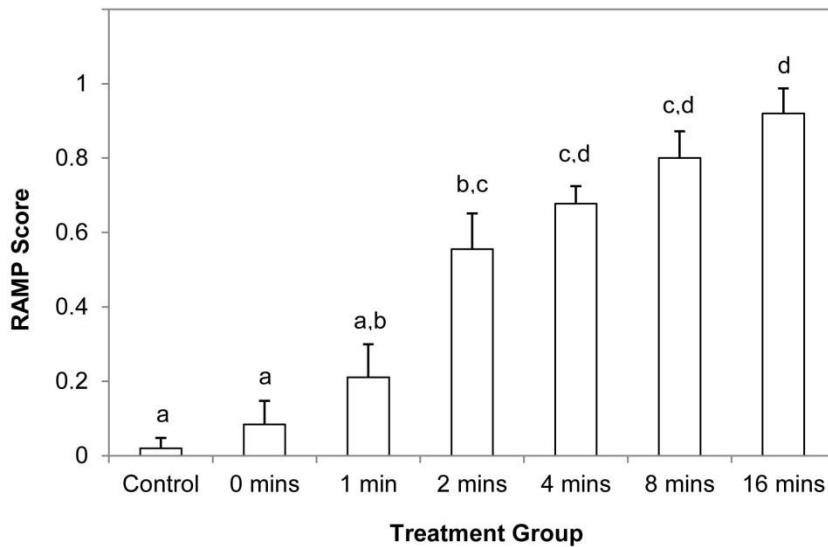
Table 7.1 Statistical output (*P*-values) of ANCOVAs comparing physiological differences among experimental capture treatments and among levels of reflex impairment (RAMP scores; all treatments pooled), with treatment/RAMP score as the main effect and total researcher handling time as the covariate for both. RAMP score represents to the proportion of reflexes assessed as impaired for an individual among the five reflexes assessed. RAMP score differences among capture treatments were assessed with Kruskal-Wallis ANOVA rather than ANCOVA. For treatment effects, $\alpha = 0.007$, for RAMP score effects, $\alpha = 0.008$. *P*-values in bold are significant at Bonferonni adjusted significance thresholds. Post-hoc differences are shown for treatment effects in Fig. 7.2, and for RAMP scores in Fig. 7.3. For pink salmon, N = 163; for chum salmon, N = 110.

		Treatment	Covariate	RAMP score	Covariate
Physiological variable		*P*	*P*	*P*	*P*
Plasma lactate					
	Pink	**<0.001**	0.005	**0.003**	**<0.001**
	Chum	**<0.001**	0.232	**0.001**	**<0.001**
Plasma glucose					
	Pink	0.796	0.363	0.913	0.573
	Chum	0.673	0.722	0.748	0.623
Plasma osmolality					
	Pink	**0.003**	0.053	0.492	**<0.001**
	Chum	**<0.001**	0.739	0.086	0.012
Plasma Cl$^-$					
	Pink	**0.003**	0.666	0.038	0.012
	Chum	0.083	0.908	0.436	0.585
Plasma K$^+$					
	Pink	**<0.001**	0.219	0.030	**<0.001**
	Chum	0.012	0.657	0.216	0.451
Plasma Na$^+$					
	Pink	0.078	0.126	0.406	**0.001**
	Chum	0.454	0.733	0.092	0.632
RAMP score					
	Pink	**<0.001**	n/a	n/a	n/a
	Chum	**<0.001**	n/a	n/a	n/a

Table 7.2 Impairment patterns of individual reflexes with increasing overall reflex impairment (RAMP score - the proportion of reflexes assessed as impaired for an individual among the five reflexes assessed – see 2.3.2) in pink salmon exposed to capture simulation treatments (all pinks in current study, N = 305). Values represent the proportion of individuals with each reflex impaired within that overall level of reflex impairment.

RAMP score	Tail grab	Body flex	Orientation	Head complex	VOR
N = 83 **0**	0	0	0	0	0
N = 50 **0.2**	0.60	0.36	0.02	0.02	0
N = 45 **0.4**	0.84	0.71	0.42	0.02	0
N = 76 **0.6**	0.99	0.93	0.96	0.12	0
N = 30 **0.8**	1.00	0.90	1.00	0.80	0.27
N = 21 **1.0**	1.00	1.00	1.00	1.00	1.00

Table 7.3 Impairment patterns of individual reflexes with increasing overall reflex impairment (RAMP score - the proportion of reflexes assessed as impaired for an individual among the five reflexes assessed – see 2.3.2) in chum salmon exposed to capture simulation treatments ($N = 120$). Values represent the proportion of individuals with each reflex impaired within that overall level of reflex impairment.

RAMP score	Tail grab	Body flex	Orientation	Head complex	VOR
N = 36 **0**	0	0	0	0	0
N = 39 **0.2**	0.87	0.08	0	0.05	0
N = 26 **0.4**	1.00	0.73	0.27	0	0
N = 17 **0.6**	1.00	0.94	0.88	0.18	0
N = 2 **0.8**	1.00	1.00	1.00	1.00	0

7.5 Discussion

The results of the present study provide evidence that, after reaching spawning areas, both pink and chum salmon may be resilient to certain forms of capture-related exhaustion stress imposed on them. Short of producing immediate mortality through extended anoxia, pink and chum salmon are apparently able to recover from substantial capture-induced physiological disturbance and ultimately spawn. A primary objective of this study was to tease apart the effects of exhaustion-related stress and dermal injury on post-release outcomes (here, pre-spawn mortality). However, given that we observed little pre-spawn mortality and no variation in post-release outcomes among treatments, that objective could not be realized. Natural pre-spawn mortality rates for pink and chum salmon in the channel during the study year were 6.5% and 3.2%, respectively (R. Stitt, DFO, *personal communication*); values nearly identical to fish subjected to our capture and tagging procedures (Fig. 7.3). It is important to note that our gillnet simulations did not generate severe injuries one would typically observe in a fishery more seaward of the spawning grounds areas (Baker and Schindler 2009), and thus we did not adequately evaluate the effects of the injury component of capture. This is partly a shortcoming of the simulation used, but likely also because such injuries may not be possible given the re-absorption of scales and thickened skin in spawning salmon. Future work aimed at disentangling the effects of stress and injury should focus on salmon in an earlier state of maturation, before secondary sexual characteristics are fully developed. The remainder of this chapter focuses on discussing apparent resilience to the exhaustion-related stress component of capture rather than injury, given that the injuries generated by our simulations were very minor.

The apparent lack of additive mortality documented here contrasts with previous

data on post-release mortality of Pacific salmon. As noted, some qualifications must be

made, including the lack of severe injuries that can occur in real gillnet fisheries. Indeed,

analyses of actual exposure to fisheries have often documented extensive delayed

mortality for adult Pacific salmon. However, the present study is the first to assess

mortality following a capture stressor incurred upon arrival at spawning areas. Pacific

salmon captured and released in the ocean have been shown to experience much higher

post-capture and release mortality rates: 33-55% for purse-seine caught sockeye salmon

(Cooke et al. 2006), 47% mortality for coho salmon bycatch in purse seines (chapter 2),

44% for troll-caught coho salmon (Parker et al. 1959), and up to 75% for coho salmon

captured by gillnet (Buchanan et al. 2002). During upriver migration, most studies of

capture mortality have focused on recreational angling, with mortality estimates ranging

from 1 – 69% depending on species, study location, duration of post-release monitoring,

and research techniques (Bendock and Alexandersdottir 1993, Vincent-Lang et al. 1993,

Vander Haegen et al. 2004, Nelson et al. 2005, Cowan et al. 2007, chapters 3 and 4).

Recent biotelemetry research revealed that sockeye salmon angled-and-released in the

lower Fraser River were 20-35% less likely to reach spawning areas than individuals

tagged in the ocean and subsequently tracked in freshwater (Donaldson et al. 2011,

2013). Most relevant to the present study was a biotelemetry study conducted at a nearby

study site on the Harrison River where sockeye salmon (mix of Harrison River and

Weaver Creek stocks, same capture locale as in chapter 6) were subjected to similar

capture simulations approximately two weeks earlier in their maturation status

(September 10-17, 2009). In that study, among Weaver Creek sockeye salmon subjected

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to the same gillnet treatment as the present study (3 min entanglement, 1 min air exposure); only 41.7% successfully reached the spawning channel (< 5 km away from tagging site; Donaldson et al. 2012). Among the other three treatment groups (all thought to be less severe than the gillnet treatment), the overall success of fish reaching spawning areas was 32%.

Clearly, survival differences among capture and release studies are wide enough to conclude that mortality rates are highly context-dependent. Nonetheless, we have documented exceptionally low mortality rates for pink and chum salmon, and did not observe significant variation in post-release outcomes in spite of varied and, at times, rather severe imposition of physiological stress. Herein, we propose hypotheses for our unexpected mortality results, discuss our physiological findings, and provide commentary on research and management relevance.

7.5.1 *Resilience to capture stress*

The moderate water temperature in the present study (~ 12 ℃) likely contributed to the lack of pre-spawn mortality we observed; higher mortality would certainly be expected if water was warmer than the optimum temperature of the salmon (Farrell et al. 2008, Gale et al. 2013, and see chapter 5), which was not the case here. Temperature is an important consideration for any study of fisheries-induced mortality. Seventy percent of published capture and release studies with a temperature component have shown a positive relationship between water temperature, indices of stress, and mortality (reviewed by Gale et al. 2013). The bulk of Fraser River salmon fishing occurs in August and September in the lower river, where temperatures typically range from 14-18 ℃ (Farrell et al. 2008). Indeed, it should be expected that salmon experience cooler temperatures at

spawning grounds than during most of upriver migration. Sensitivity to capture is likely

not only temperature-dependent, but stock- and species- dependent as well. Recent work

has shown that Fraser River salmon stocks vary in their physiological capabilities at

higher temperatures (Lee et al. 2003, Clark et al. 2011a, Eliason et al. 2011, Martins et al.

2011, Eliason et al. 2013) and such inter- and intra-specific differences can drive

differences in mortality rates during upriver migration (Farrell et al. 2008, Tucker et al.

2009, Donaldson et al. 2010b, Mathes et al. 2010, Martins et al. 2011). It follows that

physiological differences among species and stocks could also translate into differences

in resilience to fisheries capture stress.

Disease may be a latent cause of mortality resulting from fisheries capture and

release, given the effects that the stress response can have on immune function (Lupes et

al. 2006). Chronic exposure to high temperatures during upriver migration can increase

the likelihood of natural pre-spawn mortality by increasing susceptibility to pathogens

(MacDonald et al. 2000, Crossin et al. 2008, Miller et al. 2014). Had the fish in our study

experienced temperature stress during migration, it is possible that the added stress and

energetic expenditure associated with capture would have resulted in spawning failure via

further acceleration of pathogen development. However, in many cases there may simply

not have been enough time for capture-induced pathogenic pre-spawn mortality to

become manifest, particularly when combined with the low temperatures. Irrespective of

whether fish in our study were able to spawn before development of capture-induced

disease, longevity did not differ among treatments. Given that cortisol is naturally

elevated (Hruska et al. 2010) and immune function is already collapsing in spawning

salmon (Jeffries et al. 2011), the additive effects of a capture stressor may be relatively

insignificant in this respect for spawning salmon. In general, links between fisheries capture and pathogenic mortality remain conceptual and speculative given that virtually no published research exists on the topic (but see Lupes et al. 2006). Future research could greatly advance the science of delayed bycatch mortality by developing an understanding of effects on immune function and pathogen proliferation.

An alternate hypothesis that could explain the lack of observed pre-spawn mortality in this study is the following: as Pacific salmon migrate towards spawning areas they become progressively more resilient to capture stressors because of a physiological shift that equips them to endure challenging environmental conditions, predators, and spawning interactions on spawning grounds. As salmon approach spawning areas, they undergo a broad-based metabolic shift towards a more frequent use of anaerobic pathways and an increased reliance on protein catabolism (Brett 1995, Miller et al. 2009). Therefore, once salmon have reached spawning grounds they may be particularly adept at enduring and recovering from acute stressors involving heavy use of anaerobic metabolism, and experience no tertiary stress response after release (i.e., life history consequences). It is logical that spawning salmon must already be physiologically configured to cope with repeated acute stressors and anaerobic activity associated with general crowding, fighting conspecifics, courtship behaviours, predator evasion, and the air exposure that can occur during migration through shallow streams (Quinn and Buck 2001, Hruska et al. 2010). For example, as part of the maturation process, circulating cortisol is already at very high levels during spawning activity, particularly for females (Hinch et al. 2006, Hruska et al. 2010, Baker and Vynne 2014). Parallel to the present study, Cook et al. (2011) exposed Weaver Creek female pink salmon to a rapid blood

sample, a 2 min air exposure, and 25 min holding time in fish bags followed by a second blood sampling; and documented a similar spawning failure rate (~ 11%) as the present study. However, fish with particularly large cortisol responses tended to experience spawning failure (Cook et al. 2011); suggesting intra-specific variation in the response to capture (Fig. 1.2) could drive post-release outcomes rather than the intensity of the stressor itself. Indeed, in our study we observed some spawning failure but no differences among treatments that differed in stressor intensity.

Finally, some consideration should be given to the potential confounding effects of the artificial nature of the spawning channel used for this study. Weaver Creek spawning channel provides uniformly high quality spawning habitat. In addition, fish are let into the channel at rates such that overcrowding (and density-dependent mortality) does not occur, theoretically reducing the intensity of competitive interactions relative to a natural system. In natural spawning areas, habitat is non-uniform meaning that there is competition for areas most suitable for redds (spawning sites where fertilized eggs are buried for incubation), and overcrowding can occur whereby many fish may not spawn successfully if they are unable to win spawning sites, or may spawn in low-quality sites resulting in low embryonic survival. Thus, had the study been replicated using a naturally varied and more crowded spawning area, there may have been differentiation of egg retention among capture treatments. Baker and Schindler (2009) demonstrated that sockeye salmon arriving at spawning areas with gillnet injuries are less likely to enter spawning sites than those without injuries, and exhibit reduced longevity. That finding was partly a result of a retarded maturation process whereby fish carrying old gillnet wounds did not fully develop secondary sexual characteristics (Baker and Schindler

2009, Baker et al. 2013). The injuries in that study were a legacy of gillnet interactions that occurred during the marine approach (i.e., Bristol Bay), where the bulk of Pacific salmon harvest occurs in most systems. For fish captured near spawning grounds whose secondary sexual characteristics (including scales being resorbed into skin) are developed, the consequences of injury for maturation, spawning behaviour, and egg retention are likely not as pronounced. This may be particularly true if water temperatures are low, slowing the rate of wound infection.

7.5.2   *Physiological findings*

The fishing simulations used in this study were sufficiently varied in their severity that physiological disturbances differed in magnitude among treatments. By rapidly dip netting control fish and sampling blood within 1 min of initial contact, we anticipated that the blood sample would be uninfluenced by the capture event (Clark et al. 2011b). Therefore, control values (Fig. 7.2) were likely representative of the fish's physiological state prior to capture in the channel raceway. Treatments differentiated as we had expected, with the three 'high stress' simulations being associated with RAMP scores, plasma lactate, osmolality, chloride and potassium values significantly diverging from those of the control fish. Although we did not analyze white muscle biochemistry (as in chapter 5), the increase in both plasma potassium and chloride associated with 'high stress' treatments was likely a result of extracellular water in plasma shifting to the intracellular compartment, instigated by muscle acidosis and resulting in a higher concentration of ions in plasma (Wood et al. 1983, McDonald and Milligan 1992). Further evidence that these fish were undergoing heavy anaerobic metabolism was

provided by an accumulation of plasma lactate in the 'high stress' groups (Fig. 7.2) – not surprising given that these treatments involved more burst exercise and an added 1 min of air exposure. Collectively, our physiological analyses leave little doubt that the stressors imposed physiological disturbance comparable to what can be expected in some fisheries for Pacific salmon, where post-release mortality can follow (Farrell et al. 2000, Farrell et al. 2001a, Donaldson et al. 2010b, 2011, 2013, chapters 2 and 3).

The potential for RAMP to predict delayed mortality was precluded by the lack of pre-spawn mortality in this study. Reflex impairment has been previously demonstrated as a predictor of delayed bycatch mortality in migrating Fraser River coho salmon (chapter 3) and in a number of marine species held in captivity (e.g., Davis and Ottmar 2006, Davis 2007, Humborstad et al. 2009). Aside from predicting mortality, RAMP can be used as an integrative whole-body measure of vitality in response to stressors (Davis 2010); indeed, it apparently performed that role in the present study, responding to varying degrees of capture stress (Fig. 7.2, Fig. 7.5). Novel in the present study is a demonstration of concordance between RAMP scores and plasma constituents (Fig. 7.3; and see chapter 2, McArley and Herbert 2014). RAMP score showed general agreement with plasma osmolality, chloride, and potassium (though not significant) but was most strongly (and significantly) associated with plasma lactate (Fig. 7.3) – not surprising, as plasma lactate is a by-product of anaerobic exercise (McDonald and Milligan 1992). Along with those in chapter 2 and a recent study by McArley and Herbert (2014), the data here are the first to provide some confirmation that reflex impairment indicates a compromised physiological state. We would suggest however, that plasma lactate is not itself a driver of reflex impairment but rather a by-product of the physiological processes

directly responsible for impairment of (at least some of) the reflexes used here. In this and other studies using RAMP with Pacific salmon, we have repeatedly observed that exhausted salmon can recover their reflex actions after a short recovery ($15 - 30$ min; chapter 6) whereas plasma lactate typically does not peak until $1 - 2$ h after capture and often does not return to baseline levels for 10 h (e.g., Parker et al. 1959, Wood et al. 1983, Ferguson and Tufts 1992, Farrell et al. 2001a, Cech et al. 2004). Moreover, RAMP scores were not associated with plasma lactate in blood sampled and RAMP-assessed coho salmon bycatch that had recovered for a range of durations < 30 min (chapter 3); presumably because lactate continued to accumulate in plasma while reflex impairment began to decrease during recovery. Other physiological measurements indicative of exhaustion that recover more quickly would therefore likely reveal more about what drives reflex impairment (as discussed in chapter 2, e.g., lactate, ATP, and PCr in white muscle; Farrell et al. 2001a, Suski et al. 2007a), although different reflex impairments may be associated with different types of physiological disturbances (e.g., pathway to VOR impairment may differ from that of tail grab – discussed in chapters 2 and 3). This study serves as a first "proof-of-concept" for RAMP with pink and chum salmon. We expect that further research in a fisheries context where mortality occurs would likely reveal a RAMP-mortality correlation, as has been observed for adult coho salmon (chapter 3) and sockeye salmon (Gale et al. 2011).

7.5.3   *Management implications and future research*

Our finding that pink and chum salmon may be resilient to capture stress at the final stage of maturation as they reach spawning areas should be of interest to managers and

scientists concerned with Pacific salmon. However, firstly, it should be made abundantly clear that the pre-spawn mortality rate documented in this study (near-zero) for salmon should not be considered a 'usable' estimate for migrating salmon that experience a comparable capture stressor, nor was it our objective to generate such an estimate. The capture stressors in this study were simulated and, in the case of the gillnet simulation, likely quite mild compared to a real fishery, particularly with respect to the injuries generated. In addition, most capture and release of Pacific salmon occurs much earlier in the migration than upon arrival at spawning areas. Nonetheless, there are fisheries near terminal spawning grounds in the Fraser basin, largely conducted by aboriginal groups using beach seines, dipnets, and gillnets but also by recreational anglers. Our results are relevant to the release of non-target fish caught by these groups on or adjacent to spawning areas. Such gears enable selective harvest so release of fish certainly occurs.

As discussed, the fish in this study may have been physiologically equipped to better endure acute stressors than earlier in their migration. As far as we are aware, this is the first study on capture and release of pink or chum salmon in any context, so it remains possible that these two species are more resilient to capture than sockeye, coho, and Chinook salmon. The capture-mark-recapture programs conducted on spawning grounds for stock assessment of Fraser River salmon assume negligible mortality from seine capture and external tagging with Petersen discs (similar tags to those used in this study), and the data presented here support that assumption. Our findings also bring light to the difficult issue that post-release mortality is dependent on biological context. For example, a mortality estimate for coho salmon released from a gillnet shortly after river entry may not be transferrable to the same fishery taking place 120 km upstream. Temporal

sequence could be a factor. In a separate study (mentioned above) where Weaver Creek

sockeye salmon were captured and released 5 km away in the Harrison River, ~ 2 weeks

earlier in their maturation status, mortality was nearly 10× higher (Donaldson et al.

2012). Even in the present study there was evidence for an effect of maturation status;

later arriving fish that were captured and tagged closer to senescence had higher rates of

spawning success. Stock and species differences further complicate management

decisions on assigning mortality rates to fisheries bycatch to meet escapement targets

(Baker and Schindler 2009).

Evidently, developing a better understanding of how interspecific, intraspecific,

and inter-population differences govern sensitivity to capture could greatly improve the

efficacy of Pacific salmon management. Validating rapid and inexpensive mortality

predictors like RAMP may be the most efficient avenue for researchers and managers,

given that such tools could quickly generate mortality estimates across different contexts

(chapter 3). Finally, it should remain a priority in bycatch research to better understand

the relative importance of (and interaction between) physiological exhaustion and dermal

injury. Studies that use an experimental approach and unveil mechanisms can serve as the

basis for solutions to unobserved fishing mortality.

Chapter 8. Synthesis and future research directions

My overarching hypothesis for this thesis was (Fig. 1.2) that i) the severity of capture stressors influences the magnitude of the response (e.g., RAMP impairment, changes in blood biochemistry), and ii) that the response itself would predict post-release fitness outcomes (e.g., migration and spawning success). The first part of my hypothesis (i) was supported by each chapter of the thesis. In chapter 2, the level of crowding (i.e., catch size) and occurrence of gillnet-like entanglement each affected reflex impairment and physiological measures of stress in blood. In chapters 3 and 4, longer net entanglement duration led to higher physiological disturbance and reflex impairment, while chapter 5 showed that physiological disturbances caused by crowding are further amplified by elevated water temperature. Chapter 6 involved a more severe capture stressor than in previous chapters, which correspondingly resulted in the highest RAMP scores (i.e., higher disturbance), along with blood parameters that approached what are thought to indicate maximal exhaustion (e.g., ~ 20 mmol L^{-1} plasma lactate; Jain and Farrell 2003, Crossin et al. 2009). In chapter 7, the largest range of capture stressor severity was imposed on pink and chum salmon, and the responses we measured matched well to stressor severity, including RAMP scores and to a lesser extent, blood plasma variables. These findings can hardly be considered novel or surprising, but were important nonetheless because they provided a thorough proof-of-concept of RAMP as an indicator of the immediate response to capture stressors in Pacific salmon. Taken together with a recent study on silver seabream (*Pagrus auratus*; McArley and Herbert 2014), results on coho salmon (chapter 2), pink salmon (chapter 7), and chum salmon (chapter 7) provide good evidence that reflex impairment likely occurs via the same physiological processes

that lead to lactate accumulation, changes in plasma ion concentrations, and decreased muscle pH (McArley and Herbert 2014). However, data in chapters 5 and 6 make it clear that the suite of physiological measures examined to date (blood and muscle biochemistry) are not directly causative of reflex impairment, as RAMP scores can recover quickly (i.e., 15 min; chapter 6) while the other physiological variables we measured remained elevated/depressed. As mentioned in chapters 2, 3, and 7, future investigations into the physiological basis of reflex impairment should compare recovery profiles between RAMP scores and physiological measures known to be corrected rapidly after cessation of exhaustive exercise (e.g., Pa_{CO_2}). That future work will be important from a conceptual standpoint. Meanwhile, the utility of RAMP as a useful measure of the response to capture stressors has now been established in Pacific salmon (this thesis, Davis 2007, Donaldson et al. 2012, Nguyen et al. 2014) and several other species (Davis and Ottmar 2006, Humborstad et al. 2009, Campbell et al. 2010, Barkley and Cadrin 2012, Stoner 2012, Brownscombe et al. 2013, McArley and Herbert 2014).

The second component of my hypothesis (ii – responses to capture predict fitness outcomes; Fig. 1.2) was generally supported where it could be properly evaluated (chapters 3 and 4) but evidence was equivocal in other cases (chapters 2 and 7). RAMP scores were effective predictors of post-release fate in chapters 3 and 4. Blood samples were not drawn from fish used in telemetry experiments, and did not correspond well with short-term mortality in chapter 2, or in chapter 7 where virtually no variation in fitness outcomes occurred. In chapter 5 there was indirect evidence of a link between blood parameters and immediate mortality because the most severe treatment group where all the mortality occurred also exhibited the highest physiological disturbance

among fish that survived. RAMP scores were not significantly predictive of delayed mortality among 50 ocean-caught and telemetry-tracked coho salmon (chapter 2), but only eight fish exhibited RAMP scores of 0.6 or higher (i.e., loss of equilibrium), meaning that there may not have been enough variation in RAMP scores for it to be predictive. Taken together with past research (e.g., Danylchuk et al. 2007, Gingerich et al. 2007, Gale et al. 2014), this thesis helps establish that equilibrium loss (here, termed the orientation reflex) is an important predictor of post-release mortality (chapters 2-4), and it can correspond with disturbances in blood physiology that last 24 h (chapter 2). Thus, without sufficient numbers of fish in a sample experiencing loss of orientation (i.e., chapter 2 telemetry component, Donaldson et al. 2012, Nguyen et al. 2014), RAMP scores will likely have limited power to predict fitness outcomes in Pacific salmon using the suite of five reflexes included here. Indeed, in a follow-up study to chapter 2 conducted on the same vessel in 2013 (K.V. Cook, *unpublished data*) with a larger range of stressor severity, there was a broader distribution of RAMP scores, and RAMP was a significant predictor of survival among 220 fish released with acoustic transmitters.

More work is needed to understand why RAMP scores, particularly loss of equilibrium, are predictive of delayed mortality. In physiological experiments, equilibrium loss is often used as a pre-lethal endpoint for predicting lethal thresholds (e.g., for assessing critical thermal maxima; Beitinger et al. 2000). Gale et al. (2014) found that both ventilation rates and equilibrium loss were predictive of mortality in sockeye salmon – fish with lower ventilation rates after capture stressors had a higher likelihood of delayed mortality. It is possible that depression of ventilatory operculum movements is caused by the same processes as loss of equilibrium (both are functions of

the autonomic nervous system). Slowed ventilation could lead to mortality because of impaired oxygen delivery to tissues (i.e., respiratory failure, see Farrell et al. 2001a) during what is likely a critical moment of recovery, immediately after cessation of exercise and air exposure. Future experiments could more closely examine individual variation in post-stressor oxygen consumption, ventilation rate, equilibrium loss, and their links to subsequent mortality.

Wood et al. (1983) suggested that the mortality that can be observed in some fish after exhaustive exercise may be caused by a failure to constrain a drop in intracellular pH (i.e., in erythrocytes or muscle cells) but that hypothesis remains to be properly investigated. That RAMP scores have recently been shown to strongly reflect muscle pH would seem to provide indirect support for that hypothesis (McArley and Herbert 2014). Past work (discussed in chapter 6) has identified loss of osmoregulatory control as a precursor to mortality (van Raaij et al. 1996, Jeffries et al. 2011) – perhaps a failure to constrain secondary stress responses and regain osmoregulatory homeostasis after a severe capture stressor (and high RAMP score) can lead to delayed mortality. Interactions between capture stressors and pre-existing pathogen loads represent virtually a complete unknown, although there is some evidence that capture stressors do impair immune function (Lupes et al. 2006). In humans, strenuous exercise or crushing injuries can lead to rhabdomyolysis, a condition where skeletal muscle breakdown leads to high circulating levels of myoglobin, which can damage the kidneys, lead to acute renal failure and (in some instances) death (Bosch et al. 2009). Whether such a mechanism could lead to mortality in fishes after exhaustive exercise, fisheries capture, or other acute stressors has not been investigated (Harper and Wolf 2009). In sockeye salmon traversing

challenging hydrological flows, some individuals (particularly females) exert themselves more than others, resulting in higher use of aerobic burst swimming (Burnett et al. 2014). 'Over-exertion' in those instances has been linked to delayed mortality, and the mechanism may be similar to that which causes mortality in caught-and-released fish. Whatever the reasons for delayed mortality, they are likely to be complex and varied. Future research in this area should focus on using wild fish fully acclimated to laboratory conditions (e.g., Olla et al. 1998) that are subject to simulated capture and closely monitored so that extensive tissue sampling can occur in fish in the moments leading up to death (as in Jeffries et al. 2011 in senescing Pacific salmon).

Even in cases where animal responses were predictive of fitness outcomes, models were only able to predict ~21-45% of the variation in survival (chapters 2 and 4). That gap in explanatory power leads to the prediction that there are important factors external to the nature of the acute stressor that affect tertiary outcomes (i.e., the link between "response" and "outcomes" in Fig. 1.2). As mentioned above, our understanding of the proximate mechanisms that can lead to delayed mortality is poor. Beyond that, there has been very little exploration of the role of phenotype (i.e., inter-individual differences) in the ability of animals to resist and recover from acute stressors. This knowledge gap should be of fundamental interest, and would also be of interest to fisheries managers even though it would not necessarily have obvious application to policy. Physiological analyses suggest smallmouth bass (*Micropterus dolomieu*) are less tolerant of acute hypoxia than largemouth bass, which has implications for air exposure thresholds during catch-and-release angling (Furimsky et al. 2003). Within species, there can be variation in tolerance of acute hypoxia (Faust et al. 2004). It follows that if two

coho salmon are exposed to crowding in low-oxygen water and one is physiologically-tuned to better endure and recover from those conditions (perhaps owing to different early-life experiences), that individual would be less likely to exceed thresholds leading to mortality. Individual differences in the perception of and reaction to stressors (e.g., Breuner et al. 2008) are also likely to be important features, a notion supported by recent work on Fraser River salmon. In sockeye salmon migrating through the lower Fraser River and rapidly captured using a fish wheel, the rise in cortisol from baseline to 30-min post-capture (i.e., stress responsiveness) was predictive of migration failure; high-responding individuals were less likely to reach spawning areas after being released with radio transmitters (Cook et al. 2014). On spawning areas, the same trend was observed in pink salmon with respect to spawning success (measured via egg retention as in chapter 7; Cook et al. 2011). Other than in a holding experiment in chapter 2, my work did not evaluate the link between cortisol responses and fitness – in most cases I avoided drawing blood because of management concerns that caudal puncture could affect mortality rates (though I provided evidence to the contrary in chapter 2). Repeat-sampling fish (e.g., after 30 min) to measure cortisol responses is not practical if the focus is on assessing post-release mortality for management purposes because it adds a confinement stressor and a second acute handling stressor that could compound the effects of the capture stressor.

The ecological and evolutionary relevance of repeatable individual differences in behaviour (i.e., animal personality) has gained traction in the literature over the past 10 years (Dingemanse and Reale 2005, Biro and Stamps 2008, Careau et al. 2008, Stamps and Groothuis 2010, Sih et al. 2012). It is now recognized that inter-individual

differences in behaviour can predict catchability (Uusi-Heikkilä et al. 2008), but animal

personality has not been investigated as a factor related to the response, recovery, and

survival of fish in a catch-and-release context. Within and among species, the extent of

struggling and exertion exhibited during capture and handling can vary, and "over-

exertion" has been recognized as a cause of capture-induced mortality since a 1938

Science article on the topic (Huntsman 1938). A future study could investigate whether

individual differences in behavioural types, assessed using standard metrics (e.g., bold-

shy, aggressiveness, activity/exploration rates, sociability; Sih et al. 2012), are correlated

to levels of exertion when faced with acute handling or capture stressors (e.g., a manual

chase) and resulting survival. In chapter 7, we noted that chum salmon ceased responding

to experimenter stimuli during manual chasing earlier than did pink salmon, and

correspondingly had lower RAMP scores, so such differences apparently may exist

between closely related species. In coral trout (*Plectropomis leopardus*), an important

fisheries species of the Great Barrier Reef, there is substantial inter-individual variation

in exertion during manual chases in the laboratory, and those differences can influence

mortality at higher temperatures (T.D. Clark, *unpublished data*).

Chapter 7 included the finding that pink and chum salmon were remarkably

resilient to capture stressors incurred at spawning areas, which ran contrary to my

expectation that some of the fisheries simulations would lead to pre-spawn mortality.

Taken with a parallel study (Donaldson et al. 2012), that finding highlighted the

importance of context when attempting to understanding post-release mortality. While

inter-individual variation could play an important role (discussed above) in immediate

responses, factors external to the animal's response or the severity of the stressor can also

shape outcomes (Fig. 8.1). The most well-known such factor is water temperature, which can have strong effects on post-release survival (reviewed by Gale et al. 2013). At this stage, it remains unknown if resilience to capture stressors changes in a systematic way across the spawning migration of Pacific salmon. As noted in chapter 7, water temperature could play a role, although water temperatures are low in marine fisheries (e.g., 9-10°C, chapter 2) where substantial post-release mortality can occur (e.g., nearly 50% delayed mortality occurred in chapter 2). One simple explanation in the case of Pacific salmon might be that a minimum amount of time before spawning is required for delayed mortality to manifest, yet longevity was 8 days for chum salmon and ~15 days for pink salmon in our study – a time frame in which substantial delayed mortality can occur in fish released with transmitters (e.g., chapters 2-4, Donaldson et al. 2011, 2013). An aspect of context internal to the individual is pre-existing pathogen load, and the interaction between capture-induced cortisol elevation, dermal injury, and subsequent proliferation of disease. Follow-up studies currently underway are attempting to shed light on that dynamic, as it remains poorly explored in any system and could play a particularly important role in Pacific salmon, where disease is apparently a major contributor to natural migration failure and pre-spawn mortality (Miller et al. 2011, Miller et al. 2014).

Further research on the role of migration stage will need to involve well-controlled field telemetry experiments and lab-based physiological investigations. Cortisol elevation normally serves to suppress reproductive functioning in vertebrates as part of the activation of the "emergency life history stage", in response to an acute stressor (Wingfield et al. 1998, Sapolsky et al. 2000). That functionality would be

maladaptive in Pacific salmon during spawning migrations because they are semelparous (Wingfield and Sapolsky 2003) and presumably have regularly encountered acute stressors during their evolutionary history, although most of those will have been predator attacks or other natural challenges rather than entanglement and air exposure in fishing nets. In birds, where multiple opportunities for breeding exist, stress responsiveness is modulated by seasonal effects, and can be suppressed during reproductive events (Wingfield et al. 1998). Cortisol naturally increases throughout the spawning migration of Pacific salmon (Carruth et al. 2000, Hinch et al. 2006, Baker and Vynne 2014) – clear evidence that it does not directly inhibit reproduction. Further acute stressor-induced cortisol elevations are apparently constrained enough in most instances (~51% elevation from baseline in spawning pink salmon) that reproduction is not impaired (Cook et al. 2011). As discussed in chapter 7, it is logical to expect spawning salmon to be physiologically-configured to endure repeated acute stressors with no ill-effects on reproductive success (reviewed by Wingfield and Sapolsky 2003). Spawning involves repeated 'stressful' agonistic interactions with other salmon, sometimes challenging environmental conditions, and a potentially heightened threat of predation because of conspicuous secondary sexual characteristics (e.g., dorsal hump, bright colouration) coupled with utilization of shallow streams (Quinn and Buck 2001). The mechanisms by which spawning salmon resist the usual effects of stress (i.e., cortisol elevation) on reproductive behaviour and physiology remain unclear, although it seems likely that the HPI or HPG (hypothalamic-pituitary-gonadal) axes should be re-configured in some way during this life stage (e.g., via changes to glucocorticoid receptors, Wingfield and Sapolsky 2003). As discussed above (and see chapter 7), stress-

induced disease development is a candidate mechanism for post-release mortality, but in spawning salmon immune function is already collapsing. Additional brief elevations in cortisol (e.g., 1-4 h, see Donaldson et al. 2014) may therefore be relatively inconsequential for immune function at this stage.

A further factor that could contribute to the context-dependency of the link between animal responses and post-release outcomes (Fig. 8.1) is the role of recovery environment. A sub-objective of this thesis was to assess the utility of post-capture facilitated revival as a means of expediting physiological recovery (chapter 6) and improving post-release survival (chapter 4). From my data, it seems clear that not all coho salmon benefit from a 30 min period in flow-through recovery bags prior to release (chapter 4). Based on the notion that only severely exhausted salmon would be likely to benefit from revival, I conducted a comparison among three revival techniques as a basis for informing future tests of the benefits of revival. That experiment did not reveal clear differentiation among treatments, although there was subtle evidence that (as expected) the low-flow Fraser Box may have failed to assist revival to the same extent as the other two treatments. Based on my data and that of others (Farrell et al. 2001b, Brownscombe et al. 2013, Donaldson et al. 2013, Robinson et al. 2013, Nguyen et al. 2014), facilitated revival appears to have inconsistent benefits. The yet-to-be-tested hypothesis that arises out of my work and that of others (Donaldson et al. 2013, Robinson et al. 2013, Nguyen et al. 2014) is that confining fish for revival purposes is probably a stressor that should be avoided unless the fish is severely impaired (i.e., as in chapter 6), which is where a potential benefit is thought to occur (Fig. 8.2). That relationship between fish impairment and the benefit of revival is likely mediated by predation risk (Fig. 8.2), which, for Fraser

River salmon, is almost certainly higher in the marine environment than in most parts of the freshwater migration – at least in the Fraser River main-stem where turbidity is high. These hypotheses relating to context-specific benefits of facilitated revival are not new (see Farrell et al. 2001a), but Fig. 8.2 provides a diagrammatic representation that should be tested with controlled experiments if further research on facilitated revival is conducted.

The knowledge gaps outlined above could be addressed using many of the same tools I employed in this thesis. The use of physiological assessments, biotelemetry, and human dimensions surveys in a combined field and lab approach is well-suited to address wildlife conservation problems. The significance of animal movement and survival data can be easily appreciated by resource managers, and physiological data can help identify cause-and-effect relationships behind the trends observed in field studies. Further innovations in study design and communication of findings (i.e., knowledge transfer) could help improve the uptake and use of physiological data by resource managers. I hope that the body of work included in this thesis can serve as a model for future research on capture and release, while providing new knowledge that can have use in the sustainable management of Pacific salmon fisheries.

Figure 8.1 An update to the hypothesis provided in chapter 1 (Fig. 1.2) that adds emphasis to the context-dependency of the link between immediate reactions to stress (e.g., reflex impairment, cortisol response) and post-stressor outcomes for individuals. Some context-specific variables that could affect whether capture and stress responses result in loss of fitness (e.g., migration failure, reduced spawning output) in Pacific salmon are: pre-capture pathogen load, water temperature (Olla et al. 1998, Gale et al. 2013), migration or life history stage, inter-individual variation in physiological phenotypes relating to the capacity to withstand and recover from stressors, predation risk (Raby et al. 2014), and whether facilitated revival is employed. For example, an individual with a high pre-capture pathogen burden could be exposed to a very mild capture stressor in cold water yet suffer delayed mortality, depending on its cortisol response. Conversely, a salmon exposed to a severe capture stressor could experience no loss of fitness because of internal and external factors leading to resilience (see chapter 7).
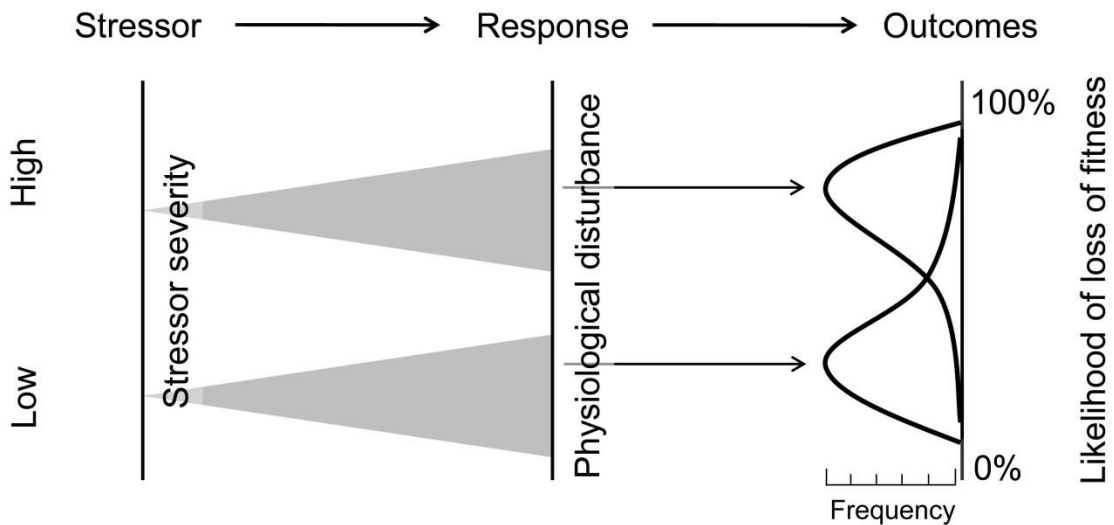
Figure 8.2 A hypothesis on how predation risk and the extent of post-capture fish impairment modulate the benefits of facilitated revival for physiological recovery and survival. This hypothesis predicts that holding fish to facilitate revival prior to release acts as a confinement stressor that compounds the negative effects of capture stress if fish are vigorous. In severely impaired fish (i.e., very high RAMP scores and physiological disturbance) the provision of ram ventilation accelerates physiological recovery and improves the likelihood of survival. For moderately impaired fish, the benefits of ram ventilation are counter-balanced by the negative effects of confinement. Across the range of fish vitality, high predation risk is predicted to increase the benefits to post-release survival by allowing fish to recover cognitive and locomotory functions prior to release (Brownscombe et al. 2013, Cooke et al. 2014), enabling predator evasion.
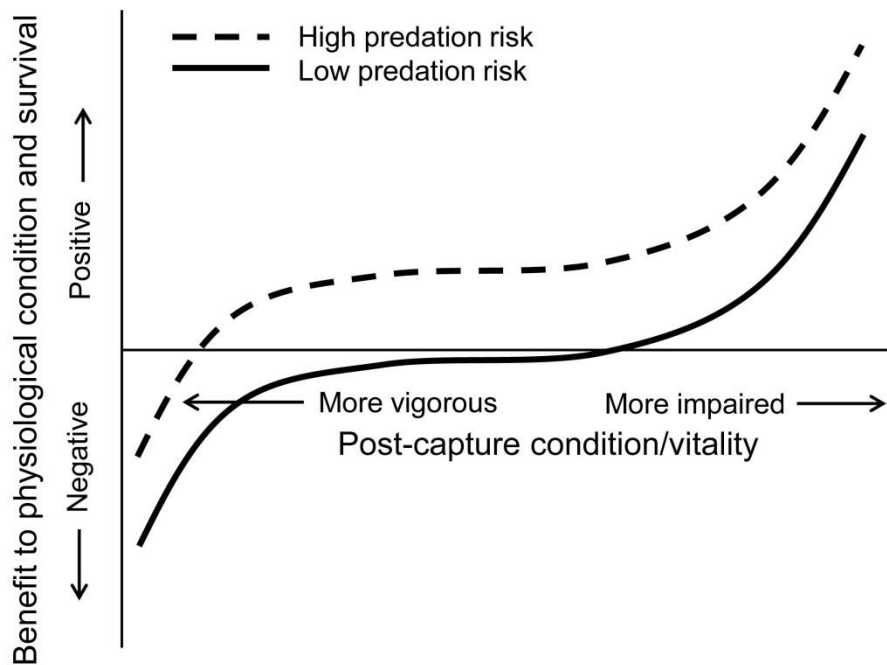
Appendices

Appendix A Abstracts of publications resulting from graduate courses

1. Wilson, SM, **GD Raby**, NJ Burnett, SG Hinch, and SJ Cooke. 2014. Looking beyond the mortality of bycatch: sublethal effects of incidental capture on marine animals. Biological Conservation 171:61-72.

There is a widely recognized need to understand and reduce the incidental effects of marine fishing on non-target animals. Previous research on marine bycatch has largely focused on simply quantifying mortality. However, much less is known about the organism-level sublethal effects, including the potential for behavioural alterations, physiological and energetic costs, and associated reductions in feeding, growth, or reproduction (i.e., fitness) which can occur undetected following escape or release from fishing gear. We reviewed the literature and found 133 marine bycatch papers that included sublethal endpoints such as physiological disturbance, behavioural impairment, injury, reflex impairment, and effects on reproduction, feeding, and growth for animals that survived a fisheries interaction. Of the 133 identified articles, 22 documented sublethal effects of capture using metrics directly related to fitness, life history, or population-level processes. Sublethal effects were classified as either short-term (e.g., acute stress response), which could lead to long-term or delayed sublethal outcomes (e.g., growth, reproduction), which are directly fitness-relevant and could have had population-level effects. We recommend further investigation into the effects of injury on fitness, and the effects of capture stress on reproduction. It is completely unknown whether sublethal effects can have significant consequences at the population or ecosystem-level. To date, the potential for discards to suffer from sublethal fitness effects has been almost

entirely ignored, and added knowledge on the topic could benefit both conservation and management.

2. Nagrodski, A, **GD Raby**, CT Hasler, MK Taylor, and SJ Cooke. 2012. Fish stranding in freshwater systems: sources, consequences, and mitigation. Journal of Environmental Management 103:133-141.

Fish can become stranded when water levels decrease, often rapidly, as a result of anthropogenic (e.g., canal drawdown, hydropeaking, vessel wakes) and natural (e.g., floods, drought, winter ice dynamics) events. We summarize existing research on stranding of fish in freshwater, discuss the sources, consequences, and mitigation options for stranding, and report current knowledge gaps. Our literature review revealed that ~65.5% of relevant peer-reviewed articles were found to focus on stranding associated with hydropower operations and irrigation projects. In fact, anthropogenic sources of fish stranding represented 81.8% of available literature compared to only 19.9% attributed to natural fish stranding events. While fish mortality as a result of stranding is well documented, our analysis revealed that little is known about the sublethal and long-term consequences of stranding on growth and population dynamics. Furthermore, the contribution of stranding to annual mortality rates is poorly understood as are the potential ecosystem-scale impacts. Mitigation strategies available to deal with stranding include fish salvage, ramping rate limitations, and physical habitat works (e.g., to contour substrate to minimize stranding). However, a greater knowledge of the factors that cause fish stranding would promote the development and refinement of mitigation strategies that are economically and ecologically sustainable.

3. **Raby, GD**, AH Colotelo, G Blouin-Demers, and SJ Cooke. 2011. Freshwater commercial bycatch: an understated conservation problem. Bioscience 61:271-280.

Bycatch from marine commercial fisheries has been regarded as a global conservation concern for decades. Fortunately, some headway has been made in mitigating bycatch problems in marine fisheries. Freshwater commercial fisheries, however, have been relatively understudied. Although freshwater yields comprise 11% of the global commercial catch, bycatch research focusing on freshwater commercial fisheries represents only about 3% of the total bycatch literature. This paucity of research is particularly alarming given that so many of the world's threatened species live in freshwater. The limited literature that does exist includes examples of population declines attributed to commercial bycatch (e.g., the Yangtze River dolphin) and illustrates that bycatch is substantial in some systems (e.g., lake trout in Laurentian Great Lakes fisheries). Encouraging results from the marine realm can serve as models for bycatch research and development in freshwater and can lead to measurable gains in the conservation of freshwater ecosystems. We summarize existing work on inland bycatch in an effort to draw attention to this understated and understudied conservation problem.

Appendix B Abstracts of non-thesis publications arising during doctoral studies

1. **Raby, GD**, JR Packer, AJ Danylchuk, and SJ Cooke. 2014. The underappreciated and understudied role of predators in the mortality of animals released from fishing gears. Fish and Fisheries 15:489-505.

The assumption that animals released from fishing gears survive has frequently been scrutinized by researchers in recent years. Mortality estimates from these research efforts can be incorporated into management models to ensure the sustainability of fisheries and the conservation of threatened species. Post-release mortality estimates are typically made by holding the catch in a tank, pen, or cage for short-term monitoring (e.g., 48 h). These estimates may be inaccurate in some cases because they fail to integrate the challenges of the wild environment. Most obvious among these challenges is predator evasion. Stress and injury from a capture experience can temporarily impair physiological capacity and alter behaviour in released animals, a period during which predation risk is likely elevated. In large scale commercial fisheries, predators have adapted their behaviour to capitalize on impaired fishes being discarded, while in recreational catch-and-release fisheries, exercise and air exposure can similarly impede the capacity for released fish to evade opportunistic predators. Owing to the indirect and often cryptic nature of this source of mortality very few studies have attempted to document it. A survey of the literature demonstrated that < 2% of the literature on incidental fisheries mortality has directly addressed or considered post-release predation. Future research should combine field telemetry and laboratory studies using both natural and simulated predation encounters and incorporate physiological and behavioural endpoints. Quite simply, predation is an understudied and underappreciated contributor to the mortality of animals released from fishing gears.

2. Nguyen, VM, EG Martins, D Robichaud, **GD Raby**, MR Donaldson, AG Lotto, WG Willmore, DA Patterson, AP Farrell, SG Hinch, and SJ Cooke. 2014.

Disentangling the roles of air exposure, gillnet injury, and facilitated recovery on the post-capture and release mortality and behavior of adult migratory sockeye salmon (*Oncorhynchus nerka*) in freshwater. Physiological and Biochemical Zoology 87:125-135.

We sought to improve the understanding of delayed mortality in migrating sockeye salmon (*Oncorhynchus nerka*) captured and released in freshwater fisheries. Using biotelemetry, blood physiology, and reflex assessments, we evaluated the relative roles of gill net injury and air exposure and investigated whether using a recovery box improved survival. Fish (*n*p238), captured by beach seine, were allocated to four treatment groups: captured only, air exposed, injured, and injured and air exposed. Only half of the fish in each group were provided with a 15-min facilitated recovery. After treatment, fish were radio taggedand released to resume their migration. Blood status was assessed in 36 additional untagged fish sampled after the four treatments. Compared with fish sampled immediately on capture, all treatments resulted in elevated plasma lactate and cortisol concentrations. After air exposure, plasma osmolality was elevated and reflexes were significantly impaired relative to the control and injured treatments. Injured fish exhibited reduced short-term migration speed by 3.2 km/d and had a 14.5% reduced survival to subnatal watersheds compared to controls. The 15-min facilitated recovery improved reflex assessment relative to fish released immediately but did not affect survival. We suggest that in sockeye salmon migrating in cool water temperatures ($\sim$13–16 °C), delayed mortality can result from injury and air exposure, perhaps through sublethal stress, and that injury created additive delayed mortality likely via secondary infections.

3. Cooke, SJ, NWR Lapointe, EG Martins, JD Thiem, **GD Raby**, MK Taylor, TD Beard Jr., and IG Cowx. 2013. Failure to engage the public in issues relating to inland fishes and fisheries: strategies for building public and political will to promote meaningful conservation. Journal of Fish Biology 83:997-1018.

Generating awareness of environmental conservation issues among the public is essential if there is an expectation of them to alter their behaviour, facilitate informed decisions and engage governments or regulatory authorities to take action. There are, however, exceedingly few public engagement success stories related to inland fishes and fisheries policy and resource allocation decisions. Inland aquatic resources and their associated fisheries provide employment, recreation, culture and, in developing regions, a considerable proportion of human nutrition and food security. Freshwater fishes are incredibly diverse but are among the most endangered organisms globally. Many threats to inland fisheries are driven largely by externalities to inland fisheries. The purpose of this paper is to draw attention to the role and plight of inland fishes and fisheries, and the need to generate the public and political will necessary to promote meaningful conservation. With this paper, the extent to which the scientific and environmental management communities have failed to engage the public in issues related to inland fishes and fisheries is characterized. Next, the barriers or factors that serve as the basis for the problem with public engagement are identified. The paper concludes by identifying strategies, including those focused on environmental education initiatives, for building the public and political will necessary to promote meaningful conservation of inland fishes and fisheries in developed and developing countries. Scientists, environmental managers, non-governmental organizations, politicians, regulatory authorities and the media all have important roles to play in overcoming challenges to inland fisheries. Failure to engage the public in freshwater conservation and management issues will impede efforts to stem the loss of freshwater habitats, fisheries and

aquatic biodiversity. Thankfully, there are opportunities to learn from success stories related to other environmental issues and initiatives that have been successful in marine fish conservation.

4. Donaldson, MR, **GD Raby**, VM Nguyen, SG Hinch, DA Patterson, AP Farrell, M Rudd, LA Thompson, CM O'Connor, AH Colotelo, SH McConnachie, KV Cook, D Robichaud, KK English, and SJ Cooke. 2013. Evaluation of a simple technique for recovering Pacific salmon from capture stress: integrating comparative physiology, biotelemetry, and social science to solve a conservation problem. Canadian Journal of Fisheries and Aquatic Sciences 70:90-100.

We evaluate the utility of an inexpensive, portable recovery bag designed to facilitate recovery of fish from capture stress by combining physiological assays, biotelemetry, and social science surveys. Adult migrating Pacific salmon (*Oncorhynchus* spp.) were used as a model, since some of their populations are threatened. While catch-and-release is common, there is a need to ensure that it is sustainable. A social science survey revealed that anglers generally have positive attitudes towards recovery bag use, particularly if research identifies that such techniques could be effective. Physiological assays on pink salmon (*Oncorhynchus gorbuscha*) revealed benefits of both high- and low-velocity recovery, but high velocity was most effective with reduced plasma cortisol concentrations and similar plasma sodium and chloride concentrations as those found in controls at all recovery durations. A biotelemetry study on sockeye salmon (*Oncorhynchus nerka*) captured by anglers and stressed by air exposure then placed in recovery bags had 20% higher, but not significantly different, survival than no-recovery salmon. The integration of natural science and social science provides an important step forward in developing methods for promoting recovery of fish from capture.

5. Cooke, SJ, MR Donaldson, CM O'Connor, **GD Raby**, R Arlinghaus, AJ Danylchuk, KC Hanson, SG Hinch, TD Clark, DA Patterson, and CD Suski. *2013*. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation, and relevance to stakeholders. Fisheries Management and Ecology 20:268-287.

Over the past 20 years, there has been a dramatic increase in the use of physiological tools and experimental approaches for the study of the biological consequences of catch-and-release angling practices for fishes. Beyond simply documenting problems, physiological data are also being used to test and refine different strategies for handling fish such that stress is minimised and survival probability maximised, and in some cases, even for assessing and facilitating recovery post-release. The inherent sensitivity of physiological processes means that nearly every study conducted has found some level of – unavoidable – physiological disturbance arising from recreational capture and subsequent release. An underlying tenet of catch-and-release studies that incorporate physiological tools is that a link exists between physiological status and fitness. In reality, finding such relationships has been elusive, with further extensions of individual-level impacts to fish populations even more dubious. A focus of this article is to describe some of the challenges related to experimental design and interpretation that arise when using physiological tools for the study of the biological consequences of catch-and-release angling. Means of overcoming these challenges and the extrapolation of physiological data from individuals to the population level are discussed. The argument is presented that even if it is difficult to demonstrate strong links to mortality or other fitness measures, let alone population-level impacts of catch-and-release, there remains merit in using physiological tools as objective indicators of fish welfare, which is an increasing concern in recreational fisheries. The overarching objective of this paper is to provide a balanced critique of the use of physiological approaches in catch-and-release science and of their role in providing meaningful information for anglers and managers.

6. Colotelo, AC, **GD Raby**, CT Hasler, TJ Haxton, KE Smokorowski, G Blouin-Demers, and SJ Cooke. 2013. Northern pike bycatch in an inland commercial hoop net fishery: effects of water temperature and net tending frequency on injury, physiology, and survival. Fisheries Research 137:41-49.

In lakes and rivers of eastern Ontario (Canada) commercial fishers use hoop nets to target a variety of fishes, but incidentally capture non-target (i.e., bycatch) gamefish species such as northern pike (*Esox lucius*). Little is known about the consequences of bycatch in inland commercial fisheries, making it difficult to identify regulatory options. Regulations that limit fishing during warmer periods and that require frequent net tending have been proposed as possible strategies to reduce bycatch mortality. Using northern pike as a model, we conducted experiments during two thermal periods (mid-April: 14.45 ± 0.32 °C, and late May: 17.17 ± 0.08 °C) where fish were retained in nets for 2 d and 6 d. A '0 d' control group consisted of northern pike that were angled, immediately sampled and released. We evaluated injury, physiological status and mortality after the prescribed net retention period and for the surviving fish used radio telemetry with manual tracking to monitor delayed post-release mortality. Our experiments revealed that injury levels, in-net mortality, and post-release mortality tended to increase with net set duration and at higher temperatures. Pike exhibited signs of chronic stress and starvation following retention, particularly at higher temperatures. Total mortality rates were negligible for the 2 d holding period at 14 °C, 14% for 6 d holding at 14 °C, 21% for 2 d holding at 17 °C, and 58% for 6 d holding at 17 °C. No mortality was observed in control fish. Collectively, these data reveal that frequent net tending, particularly at warmer temperatures, may be useful for conserving gamefish populations captured as bycatch in inland hoop net fisheries.

7. Nguyen, VM, **GD Raby**, SG Hinch, and SJ Cooke. 2012. Aboriginal fisher perspectives on use of telemetry technology to study adult Pacific salmon. Knowledge and Management of Aquatic Ecosystems 406:08.

Biotelemetry has become a popular tool accepted by the scientific community as a reliable approach for studying wild fish. However, stakeholder perspectives on scientific techniques and the information they generate are not uniformly positive. Aboriginal groups in particular may have opposition or apprehension to telemetry as a research tool. To that end, we conducted a river-bank survey of 111 aboriginal First Nations fishers that target adult Pacific salmon in the lower Fraser River, British Columbia, Canada. The majority of respondents had heard of telemetry, but few had knowledge of its function. Most responses regarding the use of telemetry in fisheries science were positive. The few negative perspectives were primarily concerned about the effects of tagging procedures whereas positive perspectives arose because telemetry was perceived to generate information on migration patterns and survival. Over half of the respondents would trust data arising from telemetry studies, but some had conditions related to the group conducting the research and their experience with fish handling. Several respondents noted the need for additional consultation and outreach with aboriginal communities (especially fishers) to better inform them of study questions and techniques which, in the case of telemetry studies, could promote better participation in tag return programs and uptake of knowledge emanating from use of telemetry.

8. Donaldson, MR, SG Hinch, **GD Raby**, DA Patterson, AP Farrell, and SJ Cooke. 2012. Population-specific consequences of fisheries-related stressors on adult sockeye salmon. Physiological and Biochemical Zoology 85:729-739.

The objective of this study was to determine whether fisheries-related stressors differently influence two populations of adult sockeye salmon (*Oncorhynchus nerka*) with shared migration timing and location but where one population (i.e., Harrison) spawns 1 mo after the other (i.e., Weaver). Four stressor treatments were used following beach seine capture: (1) immediate release, (2) release after 10–15 min in the beach seine, (3) an additional 3-min gill net entanglement and 1-min air exposure, and (4) an

additional 3-min tangle net simulation and 1-min air exposure. A comprehensive acoustic telemetry array and manual tracking revealed that survival was low overall, with moreWeaver fish (34.2% of 38 tagged) reaching spawning areas compared to Harrison fish (17.8% of 78 tagged). For the Harrison population but not the Weaver, the gill net treatment influenced immediate (i.e., survived treatment) and short-term (i.e., 5-d postrelease) survival as well as survival to reach spawning areas. Harrison fish were more likely to be injured by the treatment, and reflex impairment predicted their short-term and long-term survival. Physiological condition did not differ between populations at the time of release, although both populations showed signs of severe physiological disturbances from the gill and tangle net simulations. These results suggest that even short durations of gill or tangle net entanglement can result in profound population-specific physiological disturbances and mortality. The notion that there can be population-specific variation in response to fisheries encounters adds complexity to management and provides further evidence for intraspecific differences in migration success.

9. Cooke, SJ, SG Hinch, MR Donaldson, TD Clark, EJ Eliason, GT Crossin, **GD Raby**, KM Jeffries, M Lapointe, K Miller, DA Patterson, and AP Farrell. 2012. Conservation physiology in practice: How physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. *Philosophical Transactions of the Royal Society B* 367:1757-1769.

Despite growing interest in conservation physiology, practical examples of how physiology has helped to understand or to solve conservation problems remain scarce. Over the past decade, an interdisciplinary research team has used a conservation physiology approach to address topical conservation concerns for Pacific salmon. Here, we review how novel applications of tools such as physiological telemetry, functional genomics and laboratory experiments on cardiorespiratory physiology have shed light on

the effect of fisheries capture and release, disease and individual condition, and stock-specific consequences of warming river temperatures, respectively, and discuss how these findings have or have not benefited Pacific salmon management. Overall, physiological tools have provided remarkable insights into the effects of fisheries capture and have helped to enhance techniques for facilitating recovery from fisheries capture. Stock-specific cardiorespiratory thresholds for thermal tolerances have been identified for sockeye salmon and can be used by managers to better predict migration success, representing a rare example that links a physiological scope to fitness in the wild population. Functional genomics approaches have identified physiological signatures predictive of individual migration mortality. Although fisheries managers are primarily concerned with population-level processes, understanding the causes of en route mortality provides a mechanistic explanation and can be used to refine management models. We discuss the challenges that we have overcome, as well as those that we continue to face, in making conservation physiology relevant to managers of Pacific salmon.

10. Donaldson, MR, SG Hinch, DA Patterson, J Hills, JO Thomas, SJ Cooke, **GD Raby**, LA Thompson, D Robichaud, KK English, and AP Farrell. 2011. The consequences of angling and beach seine capture on the physiology, post-release behaviour and survival of adult sockeye salmon during upriver migration. Fisheries Research 108:133-141.

Few studies have examined the effects of fisheries capture on wild fish, particularly in the context of evaluating the sustainability of capture and release methods for Pacific salmon (*Oncorhynchus* spp.) during upriver migration. This study examined the physiological condition, post-release behaviour and survival of adult migrating sockeye salmon (*O. nerka*) in the Fraser River, British Columbia, Canada. Fish were captured by either beach seine or angling and released immediately, or were captured by angling and released following a 24-h recovery period in a net pen. Before release, all salmon were biopsied or

tagged with radio telemetry transmitters. Capture by either angling or beach seine with immediate release resulted in >95% survival 24 h after release, whereas net pen recovery after angling resulted in ∼80% survival. This differential in survival was similarly expressed in the percentage of released fish reaching natal sub-watersheds, with 52.2% and 36.3% of fish immediately released by beach seine and angling reaching natal sub-watersheds, respectively, compared with 2.9% of fish released after angling and net pen recovery. Blood plasma stress indices reflected the 10-fold difference in survival, with a ∼4-fold higher plasma cortisol, a ∼2-fold higher plasma glucose and significantly depressed plasma ions and osmolality relative to fish sampled upon capture. Plasma lactate did not differ among groups. Collectively, these results suggest that a 24 h recovery in net pen following angling failed to promote post-release survival experienced with immediate release after angling or beach seining.

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