

**The physiological, behavioural, and survival consequences of two radio transmitter  
attachment techniques on migrating adult sockeye salmon**

By

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## **ABSTRACT**

Biotelemetry is widely used to study the behaviour and survival of migrating adult Pacific salmon, but little is known about if and how the tagging process and burden of the transmitter pose risks to the study animal. Minimizing the adverse impacts of tagging is important for reasons of animal welfare, but also to accurately derive representative data from tagged individuals. In Chapter 2, I compare the short-term physiological responses of adult sockeye salmon tagged either via gastric insertion or external attachment to untagged controls and report no differences in physiology between the treatment groups. In Chapter 3, I monitored the movement and status of gastrically and externally tagged individuals and reported that externally tagged fish were almost twice as successful in reaching spawning grounds than gastrically tagged individuals. These results reveal that the failure to detect immediate physiological disturbances and behavioural differences in tagged adult migrating Pacific salmon does not negate the possibility that long-term tag-specific adverse effects may occur.

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## **GLOSSARY**

ACTH: Adrenocorticotrophic hormone

ANOVA: Analysis of variance

ATP: Adenosine triphosphate

GSI: Gonadosomatic

HPI: Hypothalamic-pituitary-interrenal

MHz: megahertz

PIT: Passive integrated transponder

RAMP: Reflex action mortality predictors

Rkm(s): River kilometer(s)

SEM: Standard error of the mean

## **CO-AUTHORSHIP STATEMENT**

M.L. Dick held primary responsibility for designing the research project, establishing experimental protocols, collecting and analyzing data, and preparing manuscripts. Throughout the process, colleagues provided considerable logistical support, guidance, and feedback. The research presented herein was therefore the result of a collaborative effort and all co-authors contributed to its completion.

**CHAPTER 2:** The effects of different transmitter attachment methods on the physiology of adult sockeye salmon

M.L. Dick, E.J. Eliason, D.P. Patterson, and S.J. Cooke contributed to the study design. Fieldwork was completed by M.L. Dick, E.J. Eliason, D.A. Patterson, and K.A. Robinson. All writing was conducted by M.L. Dick. All co-authors provided comments and feedback on the manuscript. A version of this chapter will be submitted for publication in the journal *Transactions of the American Fisheries Society*.

**CHAPTER 3:** The effects of gastric and external tagging on behaviour and fate of adult migrating sockeye salmon in the wild

M.L. Dick, D.A. Patterson, K.A. Robinson, E.J. Eliason, and S.J. Cooke designed this study. Fieldwork was completed by M.L. Dick, D.A. Patterson, and K.A. Robinson. D.A. Patterson and K.A. Robinson assisted with data management and analysis. All writing was conducted by M.L. Dick. S.J. Cooke provided comments and feedback on the manuscript. A version of this chapter will be submitted for publication in the journal *Ecology of Freshwater Fish*.

## **CHAPTER 1: Introduction**

The movement of animals within and across landscapes is associated with important biological processes that are integral for survival; consequently, understanding these movements is critical for conservation (Nathan et al. 2008; Kays et al. 2015). Biotelemetry has provided tools for studying the movement behaviour, ecology, and ecophysiology of wild fish (Cooke et al. 2004a; Cooke et al. 2012). The earliest use of biotelemetry to study fish occurred when Trefethen et al. (1956) externally attached acoustic transmitters to adult salmon to monitor dam passage behaviour on the Columbia River in Washington. Since this pioneering study, important technological advances have occurred and biotelemetry is now a common tool in fisheries sciences (Lucas and Baras 2000; Cooke et al. 2004a; Block 2005; Nielsen et al. 2009). Recent technological advances have allowed transmitters to become smaller and lighter, for the incorporation of sensors that measure various environmental parameters, and for the ability to remotely monitor a tagged individual in real time via orbiting satellites (Block 2005). Given the complex logistical challenges associated with studying fish behaviour, physiology and survival in the wild, electronic tagging tools have provided remarkable insight into complex movements of wide-ranging species, such as those that undertake vast migrations that span shallow freshwater streams to the high seas.

A key assumption of telemetry studies is that the attachment and burden of the electronic transmitter does not adversely affect the behaviour and survival of the tagged individual; however, this assumption is rarely acknowledged and even less often tested (Murray and Fuller 2000). Indeed, the stress response elicited by a tagging event requires a period of metabolic recovery to regain physiological equilibrium and normal performance (Bridger and Booth 2003). The acute stress response caused by the capture event, associated handling, and attachment or

implantation of the tag can be exacerbated to chronic costs to the animal if latent effects remain (Broell et al. 2016). To investigate potential sublethal and lethal effects of tagging in salmon, this thesis compares two electronic transmitter attachment techniques to determine best practices for tracking migrating adult sockeye salmon (*Oncorhynchus nerka*) using endpoints related to physiology, behaviour, and survival.

### *Transmitter attachment methods*

A variety of transmitter attachment methods and tag types allows for application to taxa with different physical characteristics (i.e. size, morphology, locomotor processes, wound healing abilities, tag retention rates), in numerous life history stages, and living in various aquatic environments. Methods of applying electronic transmitters to fish include: 1) external attachment, 2) gastric insertion, and 3) intracoelomic implantation via surgery (Cooke et al. 2012). Transmitter attachment techniques are important to consider in the design of a telemetry study to ensure that the method of choice is least invasive to the study species and to minimize any bias created by differential tagging effects (Bridger and Booth 2003; Cooke et al. 2012). A general rule of thumb for safe tag weight is less than 2% of the body mass of the fish, but few experiments have explicitly investigated the validity of this rule (Cooke et al. 2012). In addition, duration of the experiment must be considered when examining appropriate transmitter attachment methods and tag types. Anesthesia is sometimes used prior to attaching a transmitter to the experimental fish to decrease muscle movement and facilitate handling (Bridger and Booth 2003). However, there are a number of situations in which use of an anesthetic is not appropriate, particularly for field-based projects, such as the dangers that chemical anesthesia pose to human health if the experimental fish is consumed, and logistical limitations to the use of electroanesthesia or CO<sub>2</sub> in the field (Cooke et al. 2013).

External attachment and gastric insertion are most commonly used in the relatively short-term investigations of migrating adult Pacific salmon (6-8 weeks; Brown et al. 2011). Gastric tags are inserted by guiding the transmitter through the esophagus and into the stomach of the fish using a smooth plunger (Cooke et al. 2012). Advantages of the gastric insertion method include rapidity of the procedure, minimal training required by the tagger, placement of the tag at the fish's center of gravity, protection from the outside environment, and limited drag force impacting swimming performance (Bridger and Booth 2003). Disadvantages include the potential for perforation or other injury to the stomach lining and abrasions to the corner of the mouth due to the protruding antenna (Bridger and Booth 2003). External transmitters are often attached with steel wires or strong thread through the dorsal musculature (Thorstad et al. 2013). This attachment method requires more skill by the tagger than gastric insertion and longer handling times, which may cause increased stress to the experimental fish (Jepsen et al. 2015). Disadvantages include possible interference of the tag with the streamlined body shape of the fish and increased drag (Thorstad et al. 2000), entanglement in aquatic vegetation and fishing gear (Rikardsen and Thorstad 2006; Mellas and Haynes 1985), increased vulnerability to predation (Beguér-Pon et al. 2012), and fouling of the immediate area around the tag (Thorstad et al. 2013; Jepsen et al. 2015).

#### *Assumptions in biotelemetry*

The assumption that tagged individuals are representative of their population is a central tenant of biotelemetry studies (Brown et al. 2011). However, the attachment of an electronic transmitter inherently poses a risk to the study animal. The capture event, associated handling, attachment or implantation of the tag, burden of the tag, as well as possible confinement and transportation before release, can contribute to acute and chronic stress responses. Minimizing

the adverse impacts of tagging is important for reasons of animal welfare, but also to ensure that the data collected accurately reflect the behaviour of the species being studied (Jewell 2013; Kays et al. 2015). There are examples of varying degrees of negative effects of different tag types on particular animals (Murray and Fuller 2000). For example, marking techniques have shown to significantly impact growth, survival, behaviour, physiology, reproduction, parasitism, disease, and predation for certain species of birds, mammals, reptiles, amphibians, and fish (reviewed in Murray and Fuller 2000). Another assumption central to tagging studies is that tags are retained for the duration of observation, rates of which vary between tag attachment method and study species. For salmonids, gastric tags are susceptible to regurgitation but retention rates are generally high (98% in Ramstad and Woody 2003; 96-98% in Keefer et al. 2004a; 90% in Corbett et al. 2012). The constriction of the esophagus creates a suction that aids in the retention of the gastric tag. External tags tend to have high retention rates as well (Gray and Haynes 1979) but have been shown to shed after time for tagged fish held in concrete environments (Corbett et al. 2012). Estimating tag retention is often difficult in the wild because differentiating between the detection patterns of a rejected tag and those of a dead fish is often impossible, and in studies where secondary tags are used, most fish are rarely recaptured to allow for inspection of tag retention (Keefer et al. 2004a). The key to any successful telemetry study is the power of the researcher to derive representative data from tagged animals (Bridger and Booth 2003). Ultimately, if tagging causes changes in fish behavior or health or if tags are not retained, data cannot be generalized to the broader population, which can lead to incorrect conclusions. Researchers must make informed decisions about the attachment method for their study in order to create an appropriate study design and to reliably interpret the tagging data.

Despite acknowledgement of the potential for tags to influence the welfare of study animals and that tag loss may occur for any telemetry study, sub-lethal effects and tag-related mortality are rarely estimated or even acknowledged (Murray and Fuller 2000; Drenner et al. 2012). In a survey of 238 ecological studies, Murray and Fuller (2000) indicated a tendency for studies that show significant negative effects to be published over those failing to show effects, which can cause a misrepresentation of the effect of tagging and inhibit dissemination of information about the best marking methods. Additionally, if significant tagging effects remain undetected or unaddressed, conservation and management decisions based on those results might not be appropriate (Murray and Fuller 2000).

#### *Physiological consequences associated with a tagging event*

Wild fish are exposed to natural and anthropogenic stressors. The recognition of a real or perceived threat by the central nervous system causes a stress response that elicits a suite of physiological and behavioural responses (Barton 2002). The response to stress is considered an adaptive mechanism that allows fish to mitigate the negative consequences of a stressor(s) in order to maintain homeostasis (Barton 2002). Primary physiological responses to stress include endocrine changes that can be characterized by increases of circulating catecholamines and corticosteroids (Barton 2002). Activation of the hypothalamic-pituitary-interrenal (HPI) axis stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary region (Schreck et al. 2001; Barton 2002). ACTH is a promoter hormone responsible for triggering the release of corticosteroids (i.e., cortisol) into circulation (Mommsen et al. 1999). Cortisol elicits the secondary physiological responses that include the catabolism of carbohydrates, proteins, lipids, and the release of ions into the blood stream (Barton 2002). Secondary responses include changes in metabolism, hydromineral balance, and cardiovascular,



respiratory, and immune functioning (Barton 2002). If the intensity of the stressor is severe or long lasting, the stress response may no longer be adaptive and become dysfunctional, leading to tertiary stress responses, which are whole-animal changes in performance such as in growth, disease resistance, behavior, which ultimately affect survivorship and fitness (Wendelaar Bonga 1997; Barton et al. 2002). If the intensity of the stressor is severe or chronic, physiological response mechanisms may become strained, which can negatively influence the probability of survival (Barton et al. 2002).

To quantify an individual fish's stress response, biochemical indicators can be measured in blood plasma to assess the physiological condition of a fish. A primary indicator is circulating cortisol, which is the principal corticosteroid in teleosts. Secondary indicators include plasma lactate, plasma glucose and ions (potassium, sodium, chloride) and plasma osmolality. The secondary physiological changes that occur tend to take longer to manifest themselves in circulation than primary responses, from minutes to hours, but often remain altered for more extended periods (Barton 2002). Indeed, many exercise-related changes observed in blood plasma are driven by changes within the musculature, and thus muscle tissue may provide a more direct and sensitive measure of anaerobic exercise in fish (Pon et al. 2012). The depletion of anaerobic substrates, such as glycogen, generates metabolic wastes, which can be measured from tissue samples (Milligan 1996; Kieffer 2000). For example, lactate, the metabolic end product of anaerobic glycolysis, initially accumulates in muscle tissue but can also leak into the circulatory system (Kieffer et al. 1994; Wang et al. 1994). Using both plasma and muscle samples to assess physiological parameters is common in lab-based studies (Black et al. 1962; Milligan and Wood 1986; Wang et al. 1994), but is more rarely applied to field-based studies. However, it has been successfully applied in a field study assessing passage efficiency of fishway structures for pink

salmon, and demonstrated that muscle tissue exhibits a greater sensitivity to exercise-related physiological changes than blood (Pon et al. 2012).

### *Study species*

Sockeye salmon are a semelparous and anadromous species of Pacific salmon that migrates from the ocean to natal freshwater streams and lakes for a single spawning event and dies soon thereafter (Scott and Crossman 1973). Sockeye salmon from the Fraser River watershed in British Columbia are an economically, ecologically, and culturally important species that has experienced abnormal migration timing and declines in productivity in recent decades (Lapointe et al. 2003). This phenomenon inspired a federal judicial inquiry and increased funding and interest in examining the potential causes of this decline.

Adult sockeye salmon cease feeding in the ocean prior to their freshwater migration, therefore endogenous energy stores fuel all upriver swimming and associated physiological changes (Mommsen et al. 1980; Burgner et al. 1991). Sockeye salmon undergo important physiological changes during this life history stage and are sensitive to adverse environmental conditions or anthropogenic interactions (Hinch et al. 2006). Their osmoregulatory systems, plasma sex hormone concentrations, lipid content, cortisol levels, and other physiological variables are constantly changing throughout the migration and while on spawning grounds. Location, timing, and method of capture, as well as tag types, tagging methods, the use of anesthetics, handling time, tag size and release technique (e.g. recovery period) can all alter behaviour, cause physiological imbalances, and alter the probability of pre-spawn mortality (Cooke et al. 2011; Drenner et al. 2012). The magnitude of the physiological response to a stressor can be quantified by measuring concentrations of biochemical indicators in the blood plasma and muscle tissue. However, stress responses typically revealed with blood physiology

are not always clear mortality predictors in migratory adult salmon. Part of the difficulty lies with obtaining good baseline measures and the additional problem that plasma cortisol increases progressively with maturation and independently of stress (Carruth et al. 2002; Cooke et al. 2012). Increased plasma cortisol is an adaptive response to a stressor and is often naturally upregulated in migratory adult salmon in response to this challenging life history stage (Carruth et al. 2002).

Today, telemetry is widely used in fisheries monitoring and research of Pacific salmon throughout the northeast Pacific. Telemetry has helped us learn more about the behavior and survival of juvenile salmon migrating past dams (Steig et al. 2005; Ploskey et al. 2008), as well as in estuary and near-shore ocean environments following post-smolt migration (Lacroix et al. 2005; Semmens 2008). Telemetry has also yielded novel insights into adult salmonid migration biology (Hinch et al. 2002; Cooke et al. 2004*b*), specifically spawning migration patterns including the timing of river entry, travel speeds, and mortality (Hinch and Bratty 2000; English et al. 2005). Mortality is a key parameter in population models and telemetry can identify natural mortality (Cooke et al. 2004*b*) and post-release mortality from commercial fisheries (Candy et al. 1996). Furthermore, telemetry has been used to quantify the consequences of angling, beach seining, and confinement of adult sockeye salmon during upriver migration (Donaldson et al. 2011), the influence of post-capture ventilation assistance following catch-and-release (Robinson et al. 2015), and dam and reservoir passage rates and survival in a hydrosystem (Keefer et al. 2004*b*). Telemetry is therefore entrenched as a key tool employed by researchers and fisheries managers in answering fundamental and management questions associated with migrating Pacific salmon. However, few efforts have been made to quantitatively assess and characterize the effects of tagging on adult Pacific salmon. In a recent synthesis on salmon tagging in marine

environments, it was found that most biotelemetry studies have focused on the juvenile portion of the salmon life cycle (Drenner et al. 2012). Of those studies, only 10.6% assessed tagging/handling effects, and an acknowledgement of potential tagging/handling effects was made in only 33.8% of publications (Drenner et al. 2012). More attention to the effects of tags and research on the subject is needed to understand the long-term influences of electronic transmitter attachment on adult Pacific salmon. Not only is this information crucial to researchers designing telemetry studies or interpreting tagging data but it will also directly inform fisheries management models that use telemetry tags and facilitate stakeholder adoption of studies that use electronic tags.

The Harrison River sockeye salmon population from the Fraser River watershed was studied in this thesis. This population has maintained high abundances in recent years in contrast to many other Fraser River sockeye salmon populations (Fig. 2.1; Peterman and Dorner 2012; Beamish et al. 2016). Several tagging studies have occurred on adult Harrison River sockeye salmon in the past, employing methods such as Petersen disc tagging, gastric tagging, and external tagging, using both acoustic and radio technologies (i.e. Department of Fisheries and Oceans Stock Assessment Program; English et al. 2005; Mathes et al. 2010; Donaldson et al. 2012; Robinson et al. 2015). Past tagging studies have provided information relevant to the relative survival of adult Harrison sockeye salmon (English et al. 2005), however no research has been specifically designed to quantify the tag-related effects. The lack of validation of different transmitter attachment methods may result in over- or underestimation of survival, migration timing, straying behaviours, and holding behaviours of salmon during migration. Given the ecological and socio-economic importance of Pacific salmon, understanding the potential

consequences of different tagging methods on fish behaviour, physiology and survival is important to inform study design and data interpretation.

### *Thesis rationale and objectives*

For anadromous salmonid species, studies have shown that the tagging process and the presence of an electronic transmitter can have adverse effects at different life history stages. In the smolt or juvenile stage, tag to body size ratio is particularly important to consider in order to maintain normal performance, growth, and survival (McCleave and Stred 1975; Adams et al. 1998a; Adams et al. 1998b; Chittenden et al. 2009). For adults, the attachment and presence of a telemetry tag has been shown to result in failed passage of hydroelectric dams (Gray and Haynes 1979), reduced swimming ability (Mellas and Haynes 1985), and differences in survival (Corbett et al. 2012). This is the first study to comprehensively investigate the influences of different tagging methods on adult migrating sockeye salmon using both short-term (physiological) and long-term (behavioural and survival) endpoints.

The goal of this thesis is to compare gastric and external tagging techniques on adult Harrison River sockeye salmon to assess the effects on behaviour, physiology, and fate in wild fish. Chapter 2 reports the findings from a riverside holding study that compares the short-term responses of blood plasma and muscle metabolite variables of adult sockeye salmon tagged either via gastric insertion or external attachment and untagged controls. The physiological response profiles 1 and 4 h post-tagging were compared to those of untagged controls to assess the magnitude of the stress response elicited by the two attachment procedures. In Chapter 3, a telemetry study was conducted to compare the behaviour and fate of sockeye salmon affixed with either a gastric or external radio tag. In both Chapters 2 and 3, the influence of sampling period on the physiology, behaviour, and survival of sockeye salmon post-tagging was assessed.

Furthermore, the transmitter attachment techniques were consistent in the methodology of both chapters, thus coupling physiological and telemetry perspectives to generate a comprehensive study of the short- and long-term consequences of tagging. Chapter 4 includes a synthesis of my results, implications of this research for fisheries management and researchers, and possible areas for future studies.

## **CHAPTER 2:** The effects of different transmitter attachment methods on the physiology of adult sockeye salmon

### **ABSTRACT**

A premise of telemetry studies is that the tagging techniques do not compromise the welfare, behaviour, or fate of tagged fish. It is common to tag Pacific salmon to learn about their migration biology and to inform fisheries management, but it is unclear which tagging methods induce the least physiological disturbance. In this study, female adult Harrison River sockeye salmon *Oncorhynchus nerka* were radio-tagged by gastric insertion or external attachment, and their physiological response and recovery were measured and compared to that of control fish. Experiments were conducted during two distinct time periods representing an early season when fish were new arrivals to the freshwater system and less sexually mature, and a later season when fish had been staging in the freshwater environment for some time and were closer to spawning. Plasma levels of cortisol, glucose, lactate, sodium and potassium, as well as concentrations of white muscle lactate and glycogen were measured after fish were held for 1 or 4 h post-treatment in flow-through riverside pens. There were no differences in the physiological responses following gastric and external tag attachment, and tagged fish showed similar response profiles to those of untagged control fish. Concentrations of most physiological variables returned to baseline levels similar to those reported in other studies of the recovery of adult Pacific salmon following an acute stressor; however, plasma lactate and cortisol remained elevated, indicating the potential for negative consequences to metabolic recovery and the ability to burst-swim. Perforation of the intestinal wall was observed in 66% of gastrically tagged fish in the late sampling season in contrast to 0% in the early sampling season; however, stomach perforation

was not associated with any distinct physiological disturbances 1 or 4 h post-treatment. This study concludes that the capture and handling associated with a tagging event are stressful, while the addition of the transmitter, regardless of external or gastric tagging methods, is non-additive over the relatively short assessment period. The long-term consequences of gastric or external transmitter attachment methods on fish physiology, behaviour and survival must be understood in order to determine which technique is less invasive and best suited to a particular study design.



## INTRODUCTION

Advances in electronic tagging and tracking technology provide researchers with unprecedented opportunities to study wild fish in their natural environment (Cooke et al. 2013; Hussey et al. 2015). These tools have provided remarkable insight on complex fish behaviours and survival, and are now a fundamental part of fisheries monitoring and research toolboxes. A central tenant of tagging studies is the assumption that the behaviour of tagged individuals is representative of their population (Murray and Fuller 2000; Brown et al. 2011). Adverse effects on behaviour and survival caused by transmitters or the tagging process will result in a bias and limit the application to the broader population (Mellas and Haynes 1985). There are current doubts surrounding individual tagged fish being truly representative of non-tagged counterparts and, as a result, stakeholders and fisheries managers are wary in interpreting these data (Cooke et al. 2013; Young et al. 2013). Minimizing the adverse impacts of these tags is important for reasons of animal welfare, but also to ensure that the data collected accurately reflect the behaviour and survival of the species being studied (Kays et al. 2015). The impacts of tagging techniques on salmonids have been studied to supplement field studies or to determine tag limits for certain species; however, most of these studies have taken place in laboratory settings and have focused primarily on juvenile fish (Drenner et al. 2012).

Electronic tagging has yielded novel insights into the behaviour and survival of adult Pacific salmon on their homeward spawning migration, such as the energetics of migration (Hinch and Bratty 2000; Wilson et al. 2013), the consequences of capture and release fisheries (e.g., Candy et al. 1996; Donaldson et al. 2011; Raby et al. 2014) and hydropower interactions (e.g., Matter and Sandford 2003; Keefer et al. 2004*b*; Ferguson et al. 2006; Burnett et al. 2014). However, adverse effects associated with the presence of electronic transmitters on adult

salmonids have been reported and include failed dam passage (Chinook salmon: Gray and Haynes 1979), decreased survival (Chinook salmon: Corbett et al. 2012), and irregular swimming patterns (rainbow trout: Mellas and Haynes 1985). These behavioural observations warrant further investigations to identify the physiological mechanisms that underlie such negative consequences.

The transmitter attachment techniques primarily used to monitor the behaviour and survival of adult migrating Pacific salmon are gastric insertion and external attachment. Surgical implantation is a third option, but is rarely used in field settings due to longer handling times, logistical constraints associated with the use of anesthetics and extra tagging equipment, and the risk to human consumption if chemical residues remain in the tissue post-release (Cooke et al. 2013). Gastric implantation is a common tagging method for semelparous adult salmonids that have generally ceased feeding prior to freshwater entry. This tag attachment method is simple, rapid, and requires minimal fish handling (Bridger and Booth 2003) but has the potential for tag loss due to regurgitation (McCleave et al. 1978; Mellas and Haynes 1985; Smith et al. 1998; Keefer et al. 2004*a*). Additionally, during the spawning migration of Pacific salmon, tissues atrophy, particularly in the gastrointestinal tract, and overall condition declines with advancing sexual maturity and longer time spent in freshwater (Dickhoff 1989). Thus, the potential of stomach perforation for gastrically tagged fish increases (Corbett et al. 2012). An alternative to gastric tagging is external attachment. This method requires longer handling time and more skill from the tagger but may be more appropriate if tagging is taking place at an early stage in the migration when the fish may still be feeding in the marine environment or at a late stage when the degenerated gastrointestinal tissues could be damaged by gastric insertion. Evaluation of the

short-term physiological response of each tagging method is required to better interpret the potential influences on long-term fish behaviour and survival.

The attachment of an electronic transmitter to a fish is an acute stressor due to capture, handling, tagging, and potentially recovery from anesthetic. The energy demand associated with struggling during capture and handling is similar to that required in exhaustive exercise (Donaldson et al. 2011). Following such exhaustive exercise, a variety of physiological disturbances occur. For example, tissue energy stores (e.g. glycogen, ATP, and creatine phosphate) are rapidly depleted, muscle and blood lactate levels increase, ionic homeostasis is disturbed, and plasma catecholamines increase (Cameron and Cech 1990; Pagnotta and Milligan 1991). Excessive energy use during the tagging event could compound the environmental and anthropogenic stresses already encountered in the spawning migration. Prolonged recovery would presumably represent the primary mechanism underlying any tertiary effects associated with tagging (e.g., alterations in behaviour or survival). Indeed, such an accumulation of stress could be detrimental to migration and reproductive success (Hinch et al. 2006). Furthermore, the burden of the transmitter itself may cause chronic stress post-tagging. Identifying the short-term physiological response profiles to different tag attachment techniques may point to potential long-term causes of mortality.

The objective of this study was to compare the short-term physiological consequences of different transmitter attachment to untagged controls. Comparisons focused on adult female sockeye salmon *Oncorhynchus nerka* that were staging near spawning grounds and were either gastrically tagged, externally tagged or untagged (i.e., control fish). Because telemetry tags are applied to sockeye salmon at different stages of the spawning migration and thus over a range of physiological and environmental conditions, from in the ocean prior to freshwater entry (Cooke

et al. 2005; Wilson et al. 2014; Drenner et al. 2015) to mature fish on or near spawning grounds (Schubert and Scarborough 1996; Young et al. 2006; Pon et al. 2009; Pon et al. 2010; Roscoe et al. 2011; Burnett et al. 2014), we wanted to assess the effect of maturity on the tagging response profile. As such, we had two distinct sampling periods representing an earlier- and later-timed group with respect to maturation. We expected our results to provide information on which attachment techniques may be least invasive at this life stage and to determine if sampling period influenced outcomes. Additionally, these results will be useful in understanding the recovery profiles of fish that have undergone exhaustive exercise in the context of a realistic tagging event in the wild.

## **METHODS**

All research was conducted in accordance with Canadian Council of Animal Care guidelines and scientific collection permits were obtained from Fisheries and Oceans Canada. This study took place on the Harrison River, BC, Canada, one of the largest spawning tributaries for Pacific salmon of the Fraser River (Fig. 2.1). It is relatively wide (2-3 km in some sections), shallow (much of the river is <2 m deep with a thalweg ~7 m maximum depth) and short (16.5 km total length) (Mathes et al. 2010), and is approximately 100 km upriver from the Fraser River mouth. Harrison River sockeye salmon have a protracted river entry pattern, with individuals arriving from late July to mid-October. Peak spawning occurs in early- to mid-November (Gilhousen 1990).

The Harrison River sockeye salmon population has maintained high abundances in recent years in contrast to many other Fraser River sockeye salmon populations (Peterman and Dorner 2012; Beamish et al. 2016). Additionally, Harrison River sockeye salmon display a “sea type” life history in which juveniles remain in the river for several months after emergence from gravel and enter the ocean in spring, while typical “lake type” sockeye salmon will rear in a lake for about one year after emerging (Birtwell et al. 1987; Burgner et al. 1991). Furthermore, these adult sockeye salmon exhibit unique behaviour during their protracted freshwater waiting period prior to spawning in the middle of the river in a rapids area. After entry into the Harrison River system, sockeye salmon do not exhibit a linear migratory trajectory towards an upstream spawning site. Rather, they move upstream and downstream of the spawning site, sometimes milling together in deeper sections of the river or in the lake before spawning. Several tagging studies have occurred on adult Harrison River sockeye salmon in the past, employing methods such as Petersen disc tagging (common method used by Department of Fisheries and Oceans

Stock Assessment Program), as well as gastric and external telemetry tagging using both acoustic and radio technologies (English et al. 2005; Mathes et al. 2010; Donaldson et al. 2012; Robinson et al. 2015). Over recent years, considerable effort and resources have gone into tagging studies of adult Harrison sockeye salmon; however, no research has been specifically designed to determine the short-term physiological effects of different transmitter attachment methods.

### *Tagging and holding*

Capture, tagging, holding, and biopsy took place on the Harrison River approximately 9 km from the confluence of the Fraser and Harrison rivers (Fig. 2.1). Tagging took place over two periods in 2014, an early sampling period (18 and 25 September; water temperature range = 17.3 - 18.4°C) and a late sampling period (16 and 23 October; water temperature range = 12.8 - 13.0°C). This represents fish that are 7 to 8 weeks and 3 to 4 weeks away from peak spawning dates, respectively. Adult female sockeye salmon ( $n = 135$ ; mean body mass =  $2354.7 \pm 293.3$  g; mean fork length =  $59.0 \pm 2.3$  cm) were captured by beach seine. Some individuals were removed immediately once the seine net was bagged and rapidly sampled to assess physiological status (time 0). The period of time from when the seine net was deployed to when time 0 individuals were sampled ranged from 5 to 33 min, so the time 0 sample was actually taken following capture fatigue, which would include some anaerobic exercise and crowding (Wood 1991; Boutilier et al. 1993; Milligan 1996). A flow-through riverside net pen (dimensions: 2.4 m in length, 1.3 m in width, bisected to make two partitions 0.65 m wide, approximately 1 m in depth) was used to hold fish for 1 or 4 h, which is consistent with other studies assessing the responses of fish to exhaustion (e.g., Wood 1991). There were 3 treatment groups: (1) control, untagged fish, (2) gastrically tagged fish, and (3) externally tagged fish. We aimed to have groups of 10 fish per treatment, holding time, and sampling period. Control, untagged fish were

dip netted from the bagged beach seine and placed directly into the net pen. Fish that underwent a tagging treatment were placed in a tagging trough affixed with a pump that allowed fresh river water to continuously flow over the mouth, gills, and body throughout the entire procedure.

Either a gastric or external radio telemetry tag was affixed (procedure outlined in detail in Cooke et al. 2012) using established protocols for tagging adult salmon without anesthetic (Cooke et al. 2005). External tags (model TX-PSC-E-45 from Sigma Eight, 32 mm in length, 10 mm in width, 9.8 mm in height, and weighed 3.7 g in air) were affixed using two metal pins inserted through the dorsal musculature at the base of the dorsal fin and secured by twisting over the ends of the pins (Fig. 2.2). Gastric tags (model TX-PSC-I-1200 from Sigma Eight, 43 mm in length, 16 mm in width, 16 mm in height, and weighed 15.2 g in air) were inserted through the mouth into the stomach using a smooth plunger (Fig. 2.2).

The tagging procedure was designed to mimic a real study with basic tagging protocols. A team of three people assisted with the tagging process: the first person held the fish in the trough and reported fish condition and estimated sex, the second individual applied the transmitter, and the third person recorded all relevant information. Each fish was assessed for capture and release vigor, maturity, and injuries. Sex was estimated by observing secondary sexual characteristics and was later confirmed during autopsy. Fish were held in the seine net prior to tagging for 6 - 73 min (median 30 min), which is a typical period of time for tagging studies (Clark et al. 2010; Nguyen et al. 2014). Immediately following tagging, individuals were placed in net pens alongside the untagged control fish. Groups of 9 - 12 individuals were held in a net pen during a single holding period, and it took approximately 10 min to load up a net pen with fish from all 3 treatments.

### *Blood and tissue collection*

After a treatment group was held for the allotted time period, all fish were quickly removed from the pen and euthanized by cerebral concussion. A blood sample was taken immediately from the caudal vasculature using a 21-gauge needle and a 3 ml vacutainer syringe containing lithium heparin (B.D. Vacutainer, Franklin Lakes, NJ). The blood sample was temporarily stored in an ice-water slurry and then centrifuged at 7 000 g for 5 min (Clay Adams Compact II Centrifuge). Plasma was flash frozen in liquid nitrogen and stored at -80°C until processing. Immediately following extraction of the blood sample, a thin muscle sample (<0.5 cm thick) was excised halfway between the dorsal fin and the anal fin and across the lateral line using a scalpel, blotted to remove excess blood, freeze-clamped in liquid N<sub>2</sub> and stored in a -80°C freezer until processing. A sample of tissue from the adipose fin was removed using a hole-puncher for DNA analysis and stock determination, and a scale was removed for age information and stock composition. Morphometric information (e.g., body mass, organ masses, length) and gross somatic energy levels (Distell Fish Fat Meter, Distell, West Lothian, Scotland) were recorded for each fish and the stomach was assessed for perforation.

Plasma sodium and plasma potassium were analyzed using a Cole Parmer Model 2655-00 Single-channel Digital Flame Photometer. Plasma glucose and plasma lactate were measured using a YSI 2300 STAT Plus Glucose/Lactate Analyzer. Plasma cortisol was analyzed using an ELISA hormone kit (Neogen Corporation). Methods are described in Farrell et al. (2001). Muscle samples were ground to a fine powder under liquid nitrogen using a pre-cooled mortar and pestle. Muscle glycogen and lactate were isolated and assayed spectrophotometrically as described by Richards et al. (2002).



### *Data analysis and statistics*

All data are presented as mean  $\pm$  standard error of the mean (SEM), unless otherwise indicated. All analyses were conducted in JMP (version 12.0.1). A series of factorial analysis of variance (ANOVA) tests were used to examine for differences in plasma metabolites, plasma ions, and muscle metabolites. A 3-way ANOVA was used to test for differences in physiological variables between untagged, externally, and gastrically tagged fish held for 1 or 4 h over both sampling periods. When it was found that tag type had no effect, we removed it from the analysis and used 2-way ANOVA on each variable. Where statistical differences were detected post-hoc tests were performed. Tukey HSD post-hoc tests were used to determine the nature of the differences between the holding time groups. T-tests were used to determine the nature of the difference between the sampling periods. Significance levels were set at 0.05. A t-test was used to assess differences in physiological variables between fish in the late sampling period with a ruptured or intact stomach. To do so, physiological results from fish in the late sampling period with an intact stomach from untagged, external, and gastric tag treatments were pooled and compared to those from gastrically tagged fish with a ruptured stomach in the same sampling period. When assumptions of normality and homogenous variance were not met, the variables were transformed or non-parametric ranking tests (for the ANOVA and t-test analyses) were used.

## RESULTS

The tagging procedures took an average of 14 s (range = 5- 29 s) for the gastric procedure, which was significantly faster ( $t = 13.39$ ,  $df = 75.98$ ,  $P < 0.001$ ) than the 45 s (range = 25 - 101 s) required for external tagging (Fig. 2.3). Throughout the study, one control fish (4 h treatment group in the early sampling period) became moribund while in the net pen and was removed and euthanized. Gonadosomatic index (GSI) was significantly higher ( $t = 10.46$ ,  $df = 76.23$ ,  $P < 0.0001$ ) for fish from the later sampling period (range = 12.9 – 24.4%; mean 18.6%) than those in the earlier sampling period (range = 4.9 - 19.4%; mean 11.5%) (Fig. 2.4).

The 3-way ANOVA revealed that despite differences in tagging time, the plasma and muscle physiological variables did not significantly differ among control untagged, gastrically tagged, and externally tagged sockeye salmon (Tables 2.1, 2.2; for example, plasma lactate, Fig. 2.5). Furthermore, no significant interactions with tag type were observed (Table 2.2). As such, we dropped tag type from subsequent analyses. Doing so enabled us to incorporate a time 0 sampling period that we only had for untagged fish and thus could not be incorporated into the 3-way ANOVA. Control untagged, gastrically tagged and externally tagged fish were subsequently pooled to examine how the physiological variables varied with holding time (0, 1 and 4 h post treatment) and between periods (early and late) (Table 2.3).

The effect of holding time was significant for all response variables other than muscle glycogen, and sampling period was significant for plasma sodium, potassium, and glucose (Table 2.3). There was no interaction of holding time post-treatment and sampling period (2-way ANOVA; Table 2.3). Post-hoc tests indicated the nature of the differences between groups within holding time and sampling period (Figs. 2.6, 2.7). The effects of holding time and sampling period were analyzed separately because the effect of the interaction was not

significant (Table 2.3), indicating that the response variables responded similarly across holding times and sampling periods. Plasma sodium concentrations increased significantly from time 0 to 1 h and then decreased significantly at 4 h to values below time 0 by approximately 5% (Fig. 2.6a). Plasma sodium was significantly elevated in the late period by ~3% relative to the early period (Fig. 7a). Plasma potassium concentrations for time 0 and 1 h were similar, and significantly increased by about 52% in the 4 h group (Fig. 2.6b). A significant difference was also observed in levels of plasma potassium between sampling periods, with the mean concentration measuring 27% greater in the late period than in the early period (Fig. 2.7b). Cortisol (Fig. 6c) and plasma lactate (Fig. 2.6d) showed similar trends over holding times, both increasing after time 0 and remaining elevated. Plasma cortisol concentrations at 4 h were similar to the corresponding 1 h values (Fig. 2.6c), whereas a significant decrease toward time 0 values was observed for plasma lactate values at 4 h (Fig. 2.6d). Plasma glucose increased significantly from time 0 to 1 h and decreased to an intermediate level after 4 h (Fig. 2.6e). Sampling period had a strong effect on plasma glucose in which values in the early period were 38% greater than in the late period (Fig. 2.7c). Muscle lactate concentrations were elevated in the time 0 group and a significant decrease occurred over time (Fig. 2.6f).

We observed ruptured stomachs in 66% of gastrically tagged fish in the later sampling period (Fig. 2.8), but none in the early sampling period. In our assessments of the stomach quality of all fish, we observed thinning of the stomach wall in the later sampling period but only saw damaged stomachs in the gastrically tagged fish. However, stomach perforation had no significant impact on the physiological response profiles of female sockeye salmon in the late sampling period when compared to fish for which the stomach was not ruptured (Table 2.4).

During the late sampling period, there was one occurrence of a gastrically tagged female regurgitating the tag in the net pen. There was no loss of external tags during the experiment.

## DISCUSSION

In comparing the short-term physiological response of wild migrating female sockeye salmon radio tagged via either gastric insertion or external attachment to untagged females in a field setting, we found no differences in blood and muscle variables 1 or 4 h post-tagging, both during early and late periods in the spawning migration. Previous studies that compare the effects of tag attachment techniques on adult Pacific salmon and salmonids have focused on a number of indicators of fish well being, such as spawning migration timing (Chinook salmon: Gray and Haynes 1979), survival (sockeye salmon: Ramstad and Woody 2003; Chinook salmon: Corbett et al. 2012), swimming performance (Atlantic salmon: Thorstad 2000) and behaviour (rainbow trout: Mellas and Haynes 1985). To our knowledge, this is the first study to report and compare quantitative measures of the blood and muscle physiological response of adult Pacific salmon tagged with external and gastric radio transmitters to untagged controls in a natural environment, and represents an important benchmark towards understanding the physiological mechanisms underlying the impacts of the overall tagging event. Furthermore, conducting a streamside assessment with 4 h as an endpoint is a novel aspect in the exhaustive exercise literature.

The failure to identify differences in the acute physiological responses between tag types and controls suggests that the cumulative effect of capture, handling, and holding with the addition of tagging is antagonistic, meaning the combined effect is less than the sum of the individual stressors (Johnson et al. 2012). Allowing the fish to recover in the net pen for a period of time prior to tagging may have reduced the influence of capture on the stress response profile and help isolate the incremental effects of the tagging procedure itself. Disentangling the effects

of capture, handling, tagging, and holding on wild fish is an inherent problem in evaluations of tagging effects (Jepsen et al. 2015).

The temporal response profiles of the physiological variables in this study were similar to those reported in other field studies looking at the recovery of adult Pacific salmon following an acute stressor (Farrell et al. 2001; Raby et al. 2015; time points for blood sampling were 0, 1 h, 2 h, and 24 h, and 0, 15 min, 30 min, 60 min, and 120 min, respectively). The blood variables here exhibited similar patterns, with immediate increases in sodium, lactate, glucose and cortisol (Fig. 2.6; Table 1 in Farrell et al. 2001; Figs. 3 and 4 in Raby et al. 2015). The decrease of plasma sodium following the peak that occurred at 1 h was observed 2 h after capture by Raby et al. (2015) but the magnitude with which it decreased over time to below time 0 values was only observed when measured after 4 h (Fig. 2.6a) and 24 h of recovery (Farrell et al. 2001). Plasma potassium responded differently by immediately decreasing then increasing significantly after 4 h (Fig. 2.6b), the magnitude of which was not captured <2 h post-stressor (Farrell et al. 2001; Raby et al. 2015). This significant increase after 4 h was consistent with the response profile of exercised rainbow trout (Wood et al. 1983) in which plasma potassium continued to increase to approximately twice the resting values after 2 - 4 h, and resting levels were not restored until 12 h post-exercise. The pronounced and prolonged rise in plasma potassium is thought to reflect the loss of this ion from muscle cells due to exercise (Wood et al. 1983; Sejersted and Sjøgaard 2000).

It can be challenging to interpret cortisol concentrations following a stressor in migrating adult Pacific salmon because plasma cortisol increases progressively with maturation and independently of stress (Carruth et al. 2002). In this study, cortisol levels increased significantly from time 0 to about 4.5-fold and remained elevated 4 h post-stressor (Fig. 2.6c). Concentrations

of cortisol can stay elevated 6 h or more after a stressor, and increasing plasma cortisol after exercise can have negative consequences to the fish, in terms of metabolic recovery (Milligan 1996) and migration success (Cook et al. 2013). High cortisol levels may hinder muscle lactate and glycogen recovery, but knowing whether this was a long-term or chronic elevation could not be assessed in this study. Lactate is the metabolic end product of anaerobic glycolysis, which initially accumulates in muscle tissue but can also leak into the circulatory system (Kieffer et al. 1994; Wang et al. 1994). Plasma lactate followed a similar response profile over time as cortisol; however the significant reduction observed between 1 and 4 h values for plasma lactate was not present in the cortisol response (Fig. 2.6d). The mean concentration at 4 h was  $12.75 \pm 0.83$  mmol/L, which exceeded the maximum threshold of 10 mmol/L indicated by Farrell et al. (1998) for which sockeye salmon failed further repetitive swimming. Previous studies have also related delayed mortality with elevated plasma lactate levels (see Black 1958). 64% of fish had plasma lactate concentrations exceeding 10 mmol/L after 4 h and 14% had concentrations surpassing 20 mmol/L. This suggests that sockeye salmon in this study were still experiencing a high level of circulating lactate that may have severely limited burst swimming even 4 h post-treatment (Jain and Farrell 2003). However, Farrell et al. (2001) also observed plasma lactate levels that were  $>20$  mmol/L in wild coho salmon 1 and 2 h post-capture by commercial gillnet but reported low rates of mortality after 24 h. It can take up to 8 h for plasma lactate to return to pre-exercise values (Milligan 1996). Our results clearly show that  $>4$  h is required for concentrations of cortisol (Fig. 2.6c) and plasma lactate (Fig. 2.6d) to decrease to basal values.

We anticipated that muscle tissue would provide a more direct and sensitive measure of anaerobic exercise in fish (Pon et al. 2012), and hence perhaps indicate differences between gastric and external tagging insofar as external tagging required a longer handling time in the

trough and may have entailed higher levels of anaerobic exercise (Fig. 2.3). However, muscle lactate values were similar for control, external, and gastric tag attachments (Table 2.2). The response profile and range of muscle lactate concentrations were similar to that reported in other exercise physiology studies in both laboratory (Milligan 1996) and marine field settings (Farrell et al. 2001) but exceeded maximum muscle lactate concentrations following exhaustive exercise of adult rainbow trout and adult Atlantic salmon (30 - 41 and 25 - 45 mmol/kg, respectively: Kieffer 2000). We expected muscle glycogen to decrease following capture stress and then to slowly increase, however there was no effect of time on the response profile (Table 2.3). Milligan (1996) stated that following exhaustive exercise in rainbow trout, 80 - 85% of the total lactate produced is retained within the muscle and its clearance is coincident with glycogen replenishment. Milligan (1996) and Milligan and Wood (1986) illustrated the peak of replenishment for muscle glycogen in rainbow trout occurring 8 h post-exercise, with levels from 0 - 4 h appearing to be similar and ranging from 0 - 5 mmol/kg. In this study, muscle glycogen remained similar over holding times and sampling periods and ranged from 9 - 13.5 mmol/kg and replenishment was not observed likely due to the shorter timeframe of the experiment. These values were consistent with what was reported in commercial troll captured coho salmon (Farrell et al. 2001). It is also known that continued elevation of plasma cortisol limits the restoration of muscle glycogen post-exercise (Pagnotta and Milligan 1991; Eros and Milligan 1996).

Physiological influences due to sampling period were evident in the response profiles of plasma sodium, potassium, and glucose suggesting that the absolute values of recovery profiles from salmon will change with maturation states. The elevated potassium levels we measured in the late sampling period have been associated with exercise and also with periods of hypoxia, possibly indicative of cell damage or muscle depolarization (Sejersted & Sjogaard, 2000; Matey



et al. 2008). Additionally, low concentrations of major plasma ions have been reported in mature sockeye salmon after facing a severe stressor (Hruska et al. 2010). Plasma glucose concentrations were significantly different over sampling periods with levels in the early period being greater than those in the late period (Fig. 2.7c). It is known that glucose levels increase alongside rising cortisol levels (Kubokawa et al. 1999), however our results indicate that despite high levels of cortisol occurring in the 1 and 4 h holding times, glucose in the late sampling period did not reach the relatively elevated levels observed in the early sampling period (Fig. 2.7c). Moreover, plasma glucose values in this study were lower than those reported in adult sockeye salmon in freshwater after simulated capture-and-release treatments (Gale et al. 2011), prior to biosampling and surgical implantation (Clark et al. 2010), and following repeated swim challenges (Eliason et al. 2013). The reason for decreased levels of plasma glucose in the late sampling period may have been due to excessive use of this energy substrate during final maturation.

The high occurrence of stomach perforation in the late sampling period is similar to reports of injury of the gastrointestinal tract for gastrically tagged fish in other studies looking at tagging effects on adult migrating salmonids (Gray and Haynes 1979; Corbett et al. 2012). In all cases, it was unknown if damage occurred immediately upon tag insertion or some time after tagging. A tagging effects study by Corbett et al. (2012) found that gastric tagging of adult Chinook salmon in the late stages of spawning migration caused 90% mortality when held for 50 days in captivity, compared to 30% and 10% mortality of control and externally tagged fish, respectively. Furthermore, Corbett et al. (2012) reported that death of gastrically tagged fish only started to occur 16 days post-tagging (50% mortality after 22 days), suggesting that any immediate or acute trauma associated with the tagging event did not lead to immediate mortality.

Postmortem dissections revealed that the stomach tissue in all fish was degraded to various degrees and that individuals from all treatment groups exhibited accumulation of fluid in the peritoneal cavity and internal hemorrhaging (Corbett et al. 2012). Further, reports from field studies suggest that gastrically tagged sockeye salmon with ruptured stomachs appeared to be dying prematurely and were less successful spawners (i.e. higher levels of egg retention) compared to those that were found dead with an intact stomach (Schubert and Scarborough 1996).

Interestingly, we did not detect an immediate physiological disturbance caused by stomach perforation (Table 2.4). Results from Corbett et al. (2012) combined with our short-term equivocal physiological results among tagged, control, perforated, and non-perforated fish suggests that adverse physiological effects of gastric tagging may only become apparent days or weeks after the tagging event. This further supports our findings that acute physiology is not informative of long-term survival. Based on their survival study, Corbett et al. (2012) recommended that gastric tags should be implanted at dates and locations closest to freshwater entry, when fish are in a robust state (Hinch et al. 2006). They also offered the recommendation that if fish are only available at a later period in the spawning migration, external tags should be used. Disadvantages of externally attached transmitters should still be considered, such as the potential for biofouling around the tag site (Thorstad et al. 2001), increased susceptibility to entanglement in fishing gear or other elements of the environment (Rikardsen and Thorstad 2006; Mellas and Haynes 1985), increased predation (Beguér-Pon et al. 2012), and tag loss (Corbett et al. 2012). Gray and Haynes (1979) also concluded that external tags might prove better for telemetry studies with salmon under starvation conditions due to higher susceptibility of stomach rupture during tagging. If logistics permit, checking the quality of the stomach prior

to tagging could help in assessing the risk of potential perforation or deciding to use an alternative attachment technique, such as external attachment. The creation of an index of the progression of gastrointestinal tissue atrophy and risk of perforation based on river-entry timing would be beneficial for researchers when considering transmitter attachment options. Further research is needed to determine the mechanism underlying low survival of gastrically tagged fish (Corbett et al. 2012) and to understand if and how stomach perforation adversely affects fish health over a longer period of time.

Tagging fish of any taxa and life history stage requires minimization of handling time and burden on the fish carrying the tag; however, there will always be some degree of negative impact on the fish which may be indistinguishable from capture and handling stress when assessed immediately post-tagging. The initiation of anaerobic activity likely takes place both during capture and tagging without anesthesia and the subsequent fatigue can be serious and include depletion of energy reserves, physiological dysfunction, and even death (Wood et al. 1983; Wang et al. 1994; Milligan 1996). However, the temporal profile of the physiological response may only reach levels indicative of such adverse tertiary effects >4 h post-tagging. Such long-term consequences may be especially important for migrating adult sockeye salmon, which are already dealing with a myriad of environmental and anthropogenic pressures as well as physiological changes in preparation for spawning. We do not know which of these fish would have survived or perished over the long-term. We also do not know the extent to which capture, holding in the seine net pre-tagging, confinement in the net pen post-tagging, and dip netting may have influenced the various response profiles. For example, holding in the net pens may have compromised their recovery, as it has been shown that short-term confinement may result in significant physiological disturbance (Portz et al. 2006; Donaldson et al. 2011). Regardless, all

field tagging studies cannot avoid most of these capture and handling stressors and this limits the ability of the experimenter to tease apart the additional acute stress associated with tagging itself. The dominance effect of capture and handling on the overall stress response needs to be recognized in future efforts investigating experimenter effects.

Limitations in this study should be considered when interpreting the results. The difficulty in identifying effects may have been influenced by the high natural variation in a wild population and the sample sizes used. Furthermore, isolating the effects of transmitter attachment techniques from the impacts of capture and handling stress is challenging because each of these processes are inherently part of a realistic tagging event. Also, the physiological tools used may not have been the most appropriate to determine differences in short-term physiological responses. Another method to assess the physiological state of a fish and to predict delayed mortality that may have been helpful is reflex assessment such as RAMP (reflex action mortality predictors) (Raby et al. 2012). Lastly, our handling times with the fish in the tagging trough were realistic but were lower than in other tagging studies not using anesthesia (e.g., 2 min in Mathes et al. 2010; 3 min in Roscoe et al. 2011; 2.5 min in Corbett et al. 2012). Longer tagging times as well as the addition of biopsy during tagging may elicit more profound tagging effects although it has been shown that tag and biopsy procedures of 150 s or less do not cause significant deleterious effects on travel times and survival (Cooke et al. 2005). Similarly, our results showing no incremental effect on acute physiology are limited in application to gastric or external tagging procedures that take less than 100 sec.

In summary, we know that capture by beach seine and handling are stressful, while the addition of a tagging stressor, regardless of external or gastric tagging methods, does not influence the trajectory or magnitude of the acute stress response. We have addressed the call to

measure levels of stress indicators and muscle metabolites to assess the effects of tag attachment methods (as expressed in Corbett et al. 2012); using this “realistic” study design under field conditions, we failed to identify differences in physiological response. However, the reasonably high level of gastric tag perforation could be problematic despite the fact that our short-term assessment of the physiological responses to perforation did not indicate physiological dysfunction. Until the long-term consequences of stomach perforation are better understood, we recommend that researchers use the gastric insertion method with caution if tagging migrating Pacific salmon that are sexually maturing and have been in the freshwater environment for an extended period of time. The equivocal acute physiological results for tagging effects and stomach perforation were not consistent with the long-term differences in survival reported in other studies. This potential disconnect between acute stress and long term survival highlights the need for comparative field studies to understand the long-term consequences of different tagging methods on fish behaviour and survival.

**Table 2.1.** Comparison of biological variables for untagged control (tag type = C), gastrically tagged (tag type = G), and externally tagged (tag type = X) female sockeye salmon held for 0, 1, or 4 h over the early and late sampling periods.

Variable		Early							Late						
		0 h		1 h		4 h			0 h		1 h		4 h		
		C	C	G	X	C	G	X	C	C	G	X	C	G	X
Plasma Na <sup>+</sup> (mmol/L)	Mean	136.99	141.58	147.84	145.22	130.41	130.70	130.26	140.00	151.56	148.13	148.94	134.51	131.03	135.73
	SE	1.36	4.41	2.24	2.42	1.7	2.1	1.72	1.58	1.91	3.42	2.04	3.00	2.79	2.51
	<i>n</i>	8	9	10	10	10	10	8	10	9	11	10	10	9	11
Plasma K <sup>+</sup> (mmol/L)	Mean	1.53	2.29	2.17	1.88	3.09	5.71	4.74	3.74	2.49	2.75	3.10	4.47	4.89	4.91
	SE	0.42	0.6	0.5	0.45	0.49	1.38	0.67	0.31	0.37	0.39	0.34	0.60	0.58	0.28
	<i>n</i>	8	9	10	10	10	10	8	10	8	11	10	10	9	11
Plasma cortisol (ng/mL)	Mean	122.45	416.91	418.39	445.75	446.84	456.99	420.52	68.59	395.37	424.56	433.6	348.43	339.93	416.37
	SE	72.42	32.78	32.19	54.89	45.56	48.76	37.52	35.09	50.49	20.16	44.40	56.16	25.29	33.05
	<i>n</i>	8	9	10	10	10	10	8	10	7	11	10	10	10	10
Plasma lactate (mmol/L)	Mean	8.13	18.2	17.62	18.35	10.46	14.36	14.62	6.00	14.86	14.41	16.00	13.34	9.95	14.14
	SE	1.59	1.18	2.04	1.50	1.37	1.89	2.71	0.53	1.94	1.20	1.46	3.05	1.35	1.41
	<i>n</i>	8	9	10	10	10	9	8	10	8	11	10	10	10	11
Plasma glucose (mmol/L)	Mean	4.15	5.96	5.50	6.18	6.76	5.10	5.47	3.48	4.03	4.19	4.63	3.14	4.12	3.39
	SE	0.32	0.89	0.56	0.67	0.73	0.83	0.89	0.26	0.30	0.33	0.41	0.47	0.23	0.31
	<i>n</i>	8	9	10	10	10	10	8	10	9	11	10	10	10	11
Muscle lactate (mmol/kg)	Mean	54.63	43.34	43.25	46.66	36.72	33.54	28.18	52.01	43.98	42.95	44.12	30.11	28.24	30.75
	SE	5.02	5.06	4.23	2.82	3.10	4.64	4.07	3.12	4.24	1.73	1.03	2.64	2.39	2.35
	<i>n</i>	8	8	10	10	10	10	8	10	8	11	10	10	10	11
Muscle glycogen (mmol/kg)	Mean	11.08	10.86	12.20	10.86	12.77	12.00	14.01	13.25	8.38	8.65	10.08	12.17	11.79	9.17
	SE	2.98	1.26	2.22	1.13	1.47	1.20	1.92	2.35	1.57	1.11	1.41	1.40	1.37	1.08
	<i>n</i>	8	8	9	10	9	10	8	8	8	11	8	9	10	11

**Table 2.2.** Results of 3-way ANOVAs with tag type, time, month, and all interactions as effects, comparing females tagged with gastric or external tags or held as untagged controls for 1 h or 4 h in the early and late sampling periods.

Variable	Tag type			Time			Sampling period			Tag type × time			Tag type × sampling period			Time × sampling period			Tag type × time × sampling period		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Plasma concentrations																					
Na <sup>+</sup> (mmol/L)	0.06	2	0.940	95.42	1	<b>&lt;0.001</b>	6.63	1	<b>0.011</b>	0.43	2	0.650	1.63	2	0.201	0.19	1	0.659	0.55	2	0.581
K <sup>+</sup> (mmol/L)	1.38	2	0.256	42.13	1	<b>&lt;0.001</b>	4.19	1	<b>0.043</b>	0.98	2	0.379	0.13	2	0.877	0.01	1	0.920	1.11	2	0.334
Cortisol (ng/mL)	0.44	2	0.647	0.53	1	0.467	2.93	1	0.089	0.04	2	0.966	0.47	2	0.626	1.77	1	0.186	0.66	2	0.521
Lactate (mmol/L)	1.08	2	0.345	12.83	1	<b>&lt;0.001</b>	2.99	1	0.086	0.25	2	0.778	1.01	2	0.367	1.19	1	0.277	1.04	2	0.356
Glucose (mmol/L)	0.09	2	0.909	3.57	1	0.062	16.45	1	<b>&lt;0.001</b>	0.20	2	0.819	2.41	2	0.095	0.70	1	0.404	1.83	2	0.165
Muscle metabolites																					
Lactate (mmol/kg)	0.23	2	0.794	45.71	1	<b>&lt;0.001</b>	1.03	1	0.312	0.76	2	0.472	0.26	2	0.769	0.39	1	0.530	0.99	2	0.372
Glycogen (mmol/kg)	0.01	2	0.99	4.76	1	<b>0.032</b>	6.23	1	<b>0.014</b>	0.39	2	0.680	0.20	2	0.817	0.05	1	0.817	1.88	2	0.158

Notes: Significant values ( $P \leq 0.05$ ) are in boldface type.

**Table 2.3.** Results of 2-way ANOVAs with time, sampling period, and interactions as effects, comparing physiological responses of females held for 0, 1, or 4 h in the early and late sampling periods. Gastric, external, and control fish were pooled for this analysis.

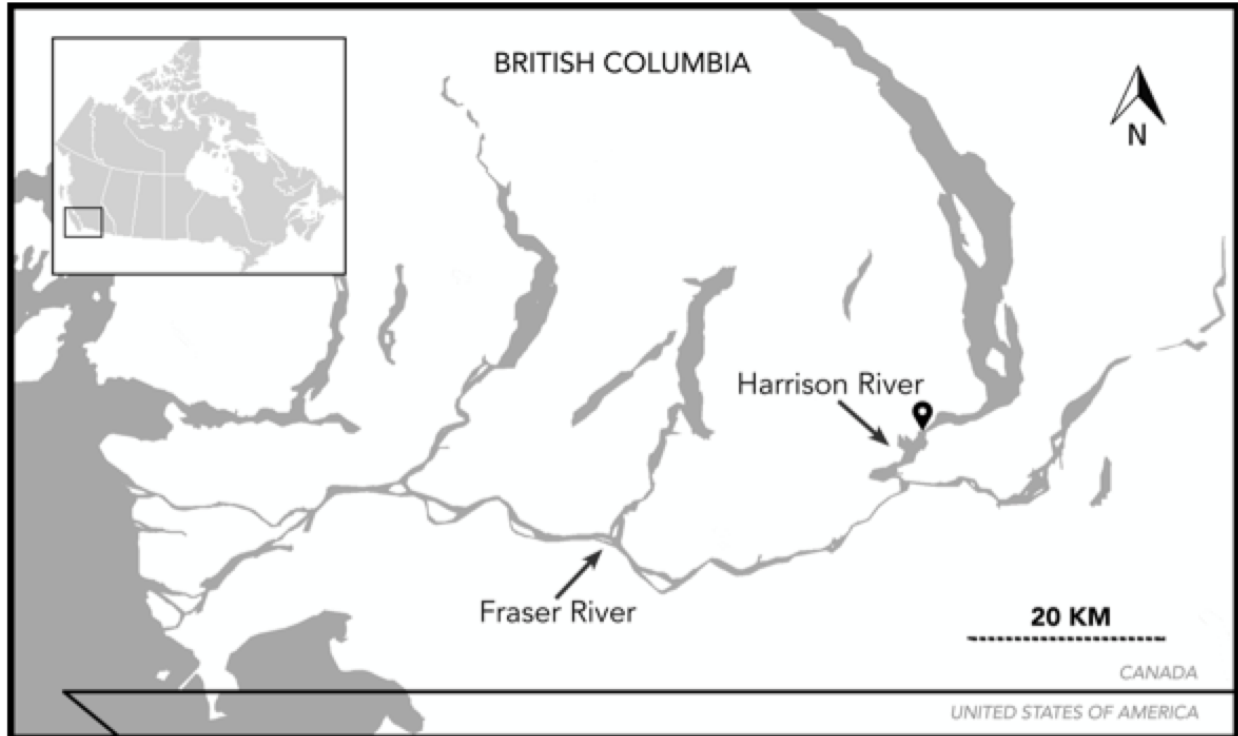
Variable	Time			Sampling period			Time × Sampling period		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Plasma concentrations									
Na <sup>+</sup> (mmol/L)	57.99	2	< <b>0.001</b>	5.89	1	<b>0.017</b>	0.029	2	0.971
K <sup>+</sup> (mmol/L)	23.59	2	< <b>0.001</b>	19.09	1	< <b>0.001</b>	2.200	2	0.115
Cortisol (ng/mL)	19.97	2	< <b>0.001</b>	1.907	1	0.169	1.112	2	0.332
Lactate (mmol/L)	27.11	2	< <b>0.001</b>	3.231	1	0.075	0.681	2	0.508
Glucose (mmol/L)	4.376	2	<b>0.015</b>	19.75	1	< <b>0.001</b>	1.304	2	0.275
Muscle metabolites									
Lactate (mmol/kg)	41.247	2	< <b>0.001</b>	1.087	1	0.299	0.527	2	0.592
Glycogen (mmol/kg)	2.665	2	0.074	0.083	1	0.774	2.392	2	0.096

*Notes:* Significant values ( $P \leq 0.05$ ) are in boldface type.

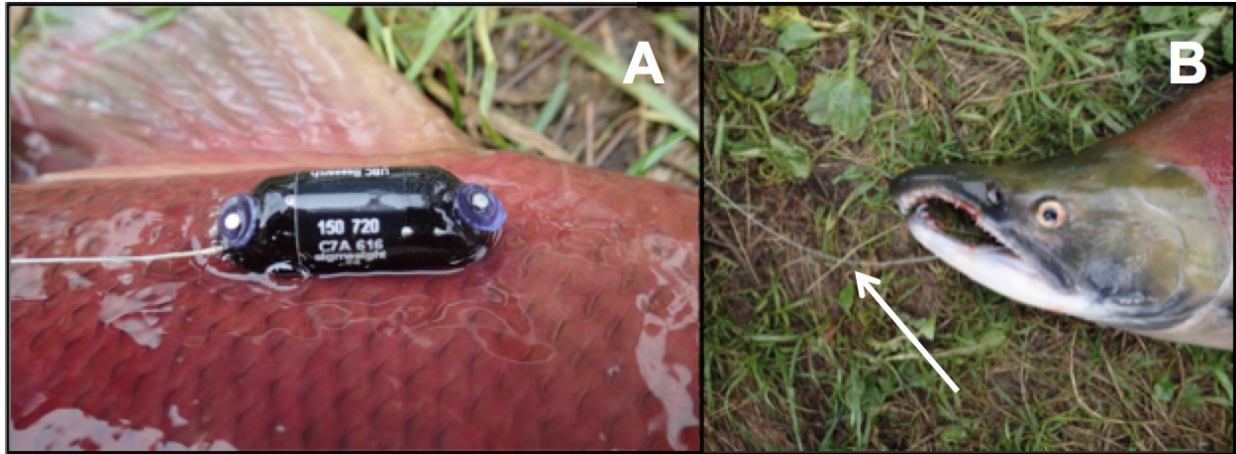


**Table 2.4.** Comparison of biological variables for females in the late sampling period with an intact or perforated stomach for the 1 h and 4 h holding times. The intact stomach group consists of untagged control and gastrically and externally tagged fish. The perforated stomach group consists only of gastrically tagged fish. T-test revealed that there were no significant differences in response variables between fish with an intact or perforated stomach over both holding times.

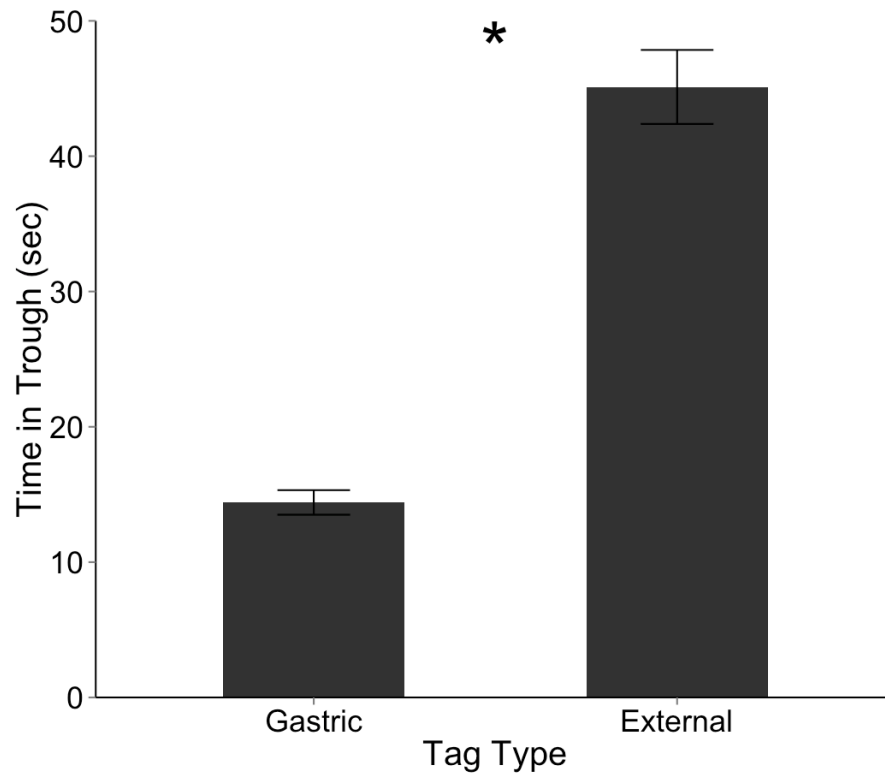
Variable	1 h				4 h			
	Intact		Perforated		Intact		Perforated	
	(n = 23)		(n = 6)		(n = 23)		(n = 8)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma Na <sup>+</sup> (mmol/L)	150.83	1.22	143.68	5.57	134.75	1.85	131.61	3.1
Plasma K <sup>+</sup> (mmol/L)	2.75	0.22	2.98	0.63	4.71	0.3	4.88	0.66
Plasma lactate (mmol/L)	15.52	0.97	13.42	1.62	13.54	1.46	9.62	1.64
Plasma glucose (mmol/L)	4.18	0.23	4.76	0.41	3.35	0.25	4.08	0.29
Cortisol (ng/ml)	414.31	25.89	443.15	27.04	375.15	30.04	349.25	31.08
Muscle glycogen (mmol/kg)	9.25	0.91	8.1	1.25	11.18	0.94	10.31	1.21
Muscle lactate (mmol/kg)	43.31	1.57	44.9	2.45	29.62	1.67	30.06	2.59



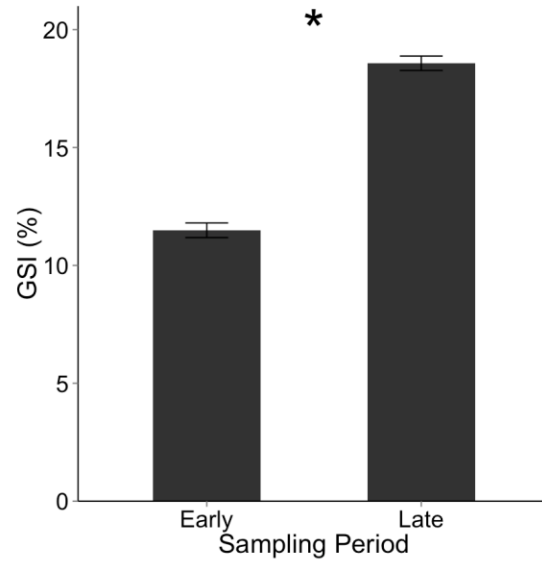
**Figure 2.1.** Map of the lower Fraser River and Harrison River in British Columbia, Canada. Pin indicates the study capture/tagging/holding site.



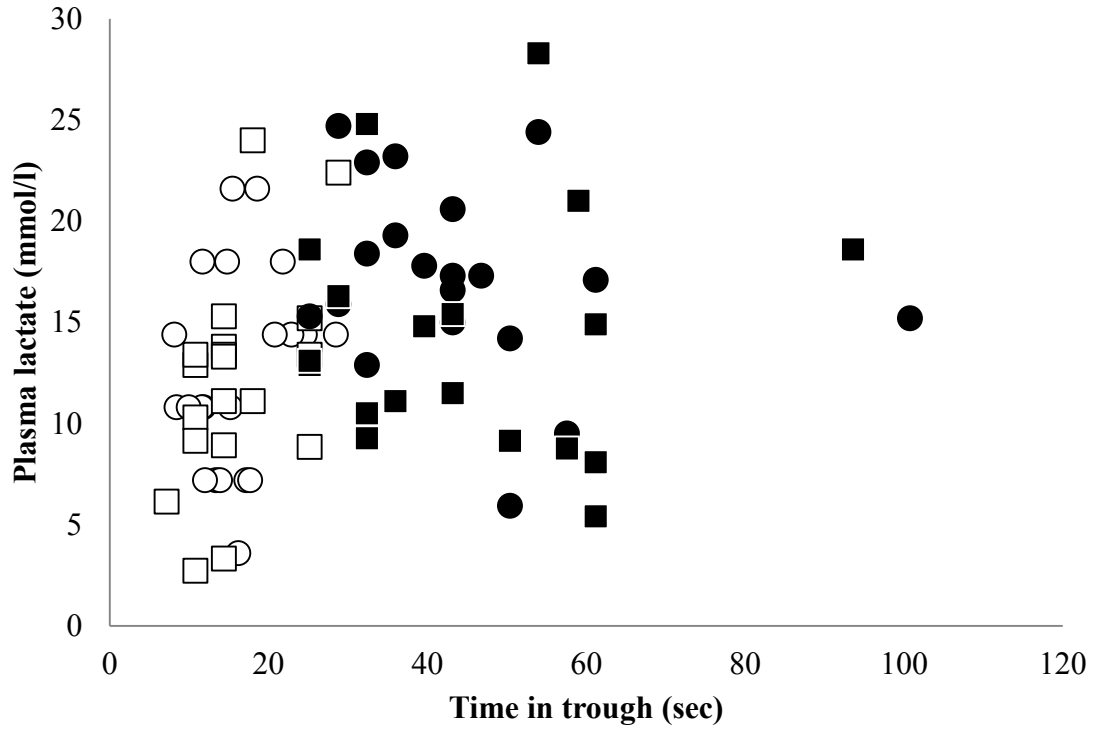
**Figure 2.2.** Tag attachment methods: (A) External tag affixed using metal pins inserted through the dorsal musculature and secured by twisting over ends of the pins; (B) Gastric tag inserted into the stomach through the mouth using a smooth plunger, antenna trails from mouth.



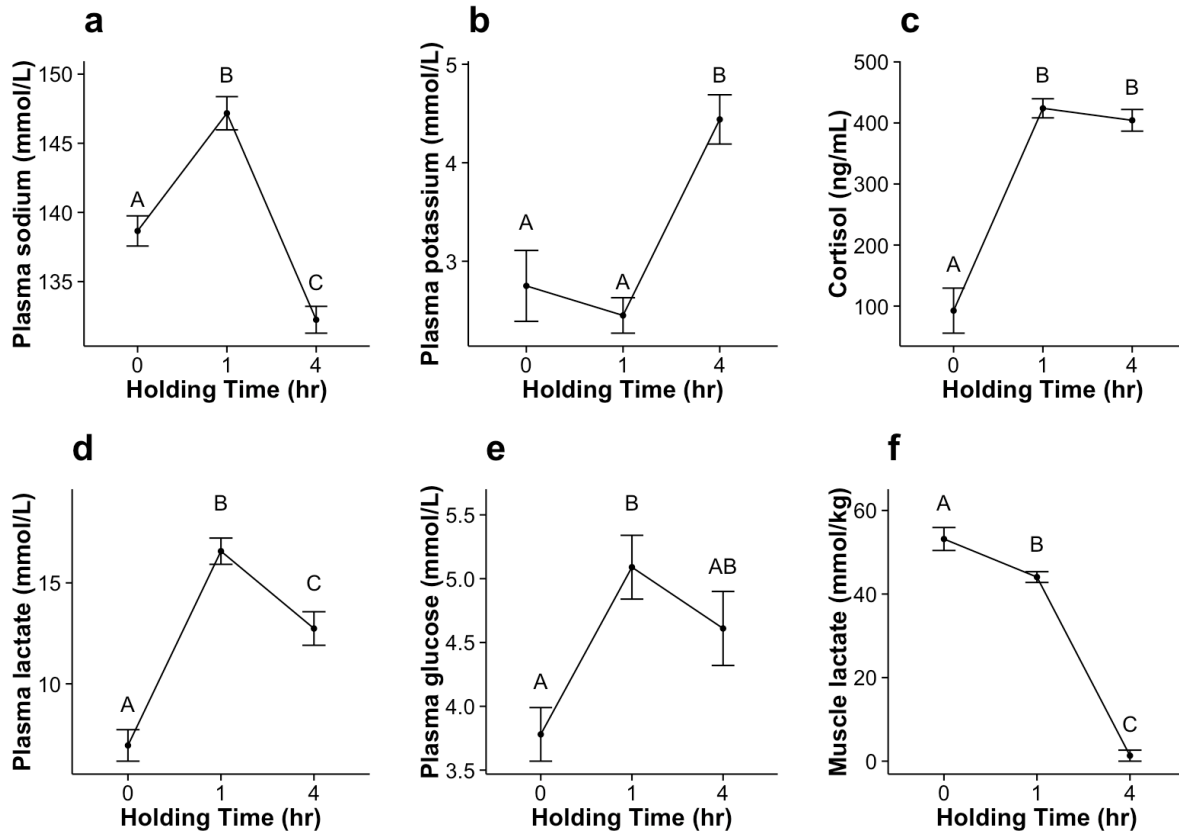
**Figure 2.3.** Time in trough per tag type (mean  $\pm$  SEM). Asterisk indicates significant difference in duration of time in the trough by tag type.



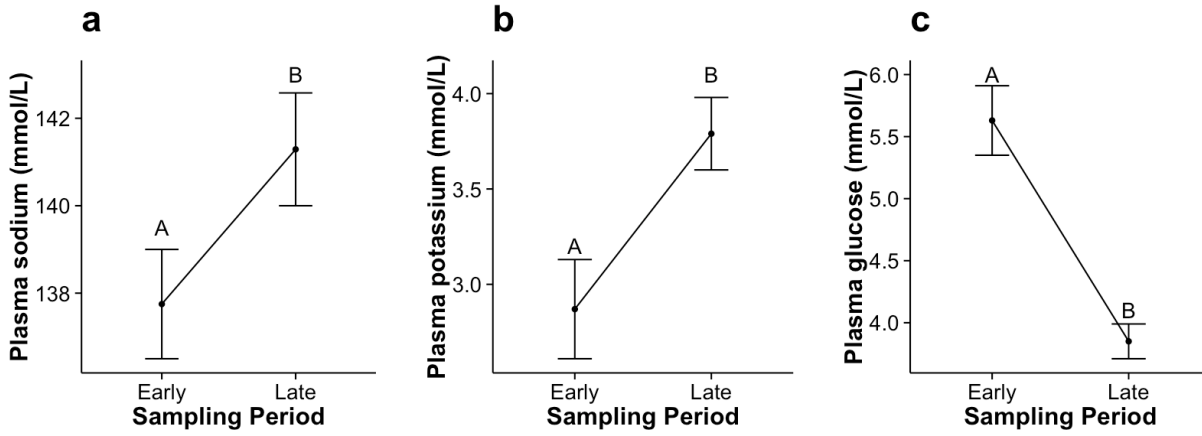
**Figure 2.4.** Mean ( $\pm$  SEM) gonadosomatic index ( $GSI = \text{gonad mass} \div \text{body mass}$ ) for females from the early and late sampling periods over the spawning season. Asterisk indicates significant difference between sampling periods.



**Figure 2.5.** Plasma lactate concentrations and time in trough in seconds. Fish tagged via gastric insertion are represented by white symbols and fish tagged via external tagging are represented by black symbols. Squares represent blood samples taken 1 h post-tagging and circles represent samples taken 4 h post-tagging.

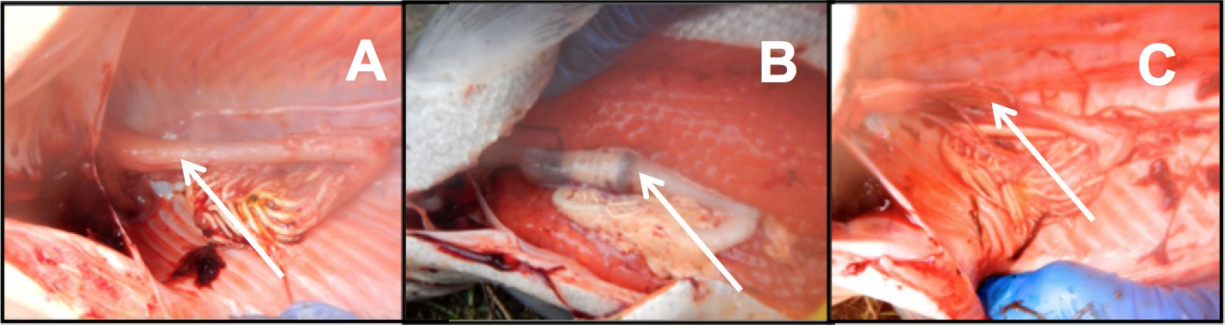


**Figure 2.6.** Mean  $\pm$  SEM of response variables assessed for the effect of holding time using Tukey's HSD, a) plasma sodium, b) plasma potassium, c) cortisol, d) plasma lactate, e) plasma glucose, and f) muscle lactate. Different letters indicate a significant difference between groups.



**Figure 2.7.** Mean  $\pm$  SEM of response variables assessed for the effect of sampling period using t-tests, a) plasma sodium, b) plasma potassium, and c) plasma glucose. Different letters indicate a significant difference between groups.





**Figure 2.8.** Degradation of stomach lining: (A) Intact stomach without a tag; (B) Positioning of the tag in an intact stomach; (C) Punctured stomach due to the insertion of a gastric tag.

### **CHAPTER 3: The effects of gastric and external tagging on behaviour and fate of adult migrating sockeye salmon in the wild**

#### **ABSTRACT**

Telemetry is a common tool for studying the behaviour and fate of migrating adult Pacific salmon with the assumption that the behaviour and survival of a tagged fish represents that of untagged conspecifics. Few studies focus on exploring this assumption, and those that do occur primarily in laboratory settings. In this study, adult Harrison River sockeye salmon in the Fraser River watershed of British Columbia were radio tagged by gastric insertion or external attachment in the dorsum. Similar numbers of the two tag types were deployed alternately, allowing for a paired comparison of behavioural patterns and survival. Tagging occurred over five days in September and October 2014 and encompassed fish in varying stages of maturity and freshwater residency, from early entrants to sexually mature individuals, to assess whether an interaction of tag type and sampling period affected the behavioural ecology of study fish. Tagged individuals were monitored over the spawning season using fixed receiver stations and mobile tracking. Regardless of tag type, study fish exhibited wide-ranging up- and downstream movement 35 h or less post-tagging, in which fish tagged in September tended to move more readily than those tagged in October. Tag type significantly influenced fate of Harrison sockeye salmon. Almost twice as many externally tagged fish (41.6% [42 of 101]) survived to reach spawning areas compared to gastrically tagged fish (22.4% [21 of 94]). Throughout the migration period, the number of active externally tagged fish in the Harrison River system was consistently greater than gastrically tagged fish released on the same date. More research is needed to elucidate the mechanism behind lower survival for gastrically tagged sockeye salmon. This is the first study to use adult sockeye salmon as a study species to evaluate and directly

compare the effects of gastric and external two tag attachment techniques in a field setting and is the first to describe intra-system movement of the Harrison River sockeye salmon population across the entire run timing period.

## INTRODUCTION

Telemetry is an important tool for studying wild fish in their natural environment (Cooke et al. 2013; Hussey et al. 2015). For species such as Pacific salmon that migrate vast distances from oceanic feeding grounds to natal freshwater streams to spawn (Groot and Margolis 1991), telemetry provides important insight into migratory behavior and survival (Drenner et al. 2012). Deciding what type of tag and attachment technique to use is dependent on a number of factors including morphology and life stage of the study species, the environment and habitat in which the study will take place, and duration of the study. Ultimately, selecting a tag type and attachment technique that is best suited to the individual is key to generating telemetry results that are unbiased and representative of the broader untagged population (Brown et al. 2011).

Tagging studies of migrating adult Pacific salmon are considered short in duration (6-8 weeks) given that the adults typically die after spawning (i.e., they are semelparous), compared to other studies that can track individual movement for many years. Tags are typically applied in the coastal approach, estuaries or in rivers/lakes partway along the spawning migration. It is necessary to expedite procedures so that animals can continue on their spawning journey with negligible delay. The main techniques for attaching electronic tags to fish are gastric insertion, external attachment, and intracoelomic surgical implantation (Cooke et al. 2012; Thorstad et al. 2013). Gastric insertion is commonly used to tag migrating adult Pacific salmon as they typically have ceased feeding upon leaving the marine environment (albeit species and population dependent) and it is a quick, less invasive method that requires little training on the part of the tagger (Ramstad and Woody 2003; Thorstad et al. 2013). External tagging is less common for adult Pacific salmon, but is a good alternative to gastric tagging given that it can be done rapidly and has been widely used on Atlantic salmon (Thorstad et al. 2000; Jepsen et al. 2015).

Intracoelomic surgical implantation is regarded as an approach used for long-term deployments (e.g., months to years) and is considered less favorable for telemetry studies on adult Pacific salmon en-route to spawning grounds due to additional logistic requirements such as the need for anesthetic and the more involved laparotomy procedure (Wagner et al. 2011).

Studies that assess tag effects are performed primarily under laboratory settings, with few occurring under field conditions (Cooke et al. 2011; Drenner et al. 2012; Wilson et al. 2016). There is an inherent challenge in conducting a tagging effects study in the wild due to the logistical impossibility of having an untouched “true” control group for comparison. One approach to getting around this challenge is the “staggered entry” approach in which the behaviour of newly released individuals is compared to previously-tagged conspecifics after a substantial time interval has elapsed (i.e. months or even one year) and assuming that negative behavioural alterations associated with tagging subside over time. This approach is appropriate for studying the effects of tag implantation on the behaviour of iteroparous fish that return to the same areas to spawn year after year (e.g., walleye; Wilson et al. 2016). However, the staggered entry approach is not applicable for migrating sockeye salmon, at least across multiple years, as they exhibit a semelparous reproductive strategy in which they perish after a single spawning event. Studies have compared different tag types (e.g. PIT vs. radio; Hockersmith et al. 2003), tag sizes (Matter and Sandford 2003), or tag attachment methods (Gray and Haynes 1979) on adult Pacific salmon in the field. To date, no studies have specifically used adult sockeye salmon as a study species to compare the long-term behavioural and survival consequences of gastric and external tags in the wild. Given the amount of effort focused on studying sockeye salmon in the northeast Pacific with telemetry, there is a need to identify optimal tagging methods to inform future research studies focused on adult behaviour and survival.

Abnormal behaviour of tagged adult Pacific salmon has been reported in previous telemetry studies, particularly immediately post-release. For example, tagged adult Chinook salmon demonstrated a tendency to pause or move downstream after release (gastric: Burger et al. 1985; Pahlke and Bernard 1996, gastric and external: Gray and Haynes 1979; external: Bernard et al. 1999). However, over time, abnormal behaviour appeared to cease in most cases. For example, despite initial fallback of their externally tagged group, Gray and Haynes (1979) found that rates of upstream movement of externally tagged and gastrically implanted adult Chinook salmon were similar. Other adverse behaviour reported in holding studies of other fish species affixed with tags include scouring by externally tagged Atlantic cod held in a large mesocosm (Broell et al. 2016) and substrate scraping by externally tagged rainbow trout (Mellas and Haynes 1985) and white sturgeon (Haynes et al. 1978), perhaps in an effort to dislodge the external tag. It is not uncommon for studies to disregard data from the first day or week post-tagging under the assumption that behaviour was altered but with no way of testing for this effect (Wilson et al. 2016; Murray and Fuller 2000). However, behaviour over this timeframe, even if it is considered ‘abnormal’, may be important to consider as it may reveal important tagging effects and could even influence the endpoints of a study such as delay in migration.

Differences in survival between gastrically and externally tagged Pacific salmon have also been reported. Corbett et al. (2012) observed low survival of gastrically tagged adult Chinook salmon compared to externally tagged and control fish. Adult hatchery Chinook salmon were collected during the spawning migration and transported to a holding tank and held for 12 h before being tagging under anesthetic (Corbett et al. 2012). In their 50 d holding study, gastrically tagged Chinook salmon began to die 16 d post-tagging, with 50% mortality occurring after ~27 days (Corbett et al. 2012). By the end of the study, 90% of gastrically tagged

individuals had perished while 70% of controls and 90% of fish with externally mounted tags had survived (Corbett et al. 2012). Corbett et al. (2012) posit that latent effects associated with stomach perforation of gastrically tagged Chinook salmon may have resulted in mortality but were unable to specifically determine why survival for this group was much lower than externally tagged and control conspecifics.

The objective of this study was to compare the behaviour and fate of sockeye salmon affixed with either a gastric or external radio transmitter. To do this, adult sockeye salmon were tagged and tracked in a freshwater river system throughout the spawning period to quantify the short-term (~35 h post-release) and long-term behaviour and fate. Research focused on the adult migrating Harrison River sockeye salmon in the Fraser River watershed in British Columbia. This population has been the focus of a number of telemetry studies over the past 15 years that have employed different tagging techniques (English et al. 2005; Mathes et al. 2010; Donaldson et al. 2012; Robinson et al. 2015). Harrison River sockeye salmon are considered part of the late run timing group and exhibit a unique migratory pattern. With early entrants arriving as early as August, Harrison River sockeye salmon exhibit a prolonged freshwater residency until peak spawning in mid-November (Gilhousen 1990). Harrison Lake is often used as a thermal refuge by migrating adult sockeye salmon seeking cooler waters (Mathes et al. 2010) and the spawning area is located in the middle reach of the river (Fig. 3.1; Schaeffer 1951). The prolonged residency of Harrison sockeye salmon in the river environment prior to spawning means that the movement of tagged fish does not adhere to a linear trajectory as is commonly seen in tagged fish from other Fraser River sockeye populations migrating to an upstream spawning site. In recent years, there have been inconsistencies between the projected spawning estimates generated by data from the hydroacoustic site in Mission, BC, and the actual spawning

escapements. Of late, there is much interest in generating prespawn survival estimates relative to different river-entry timing for this population, which necessitates an understanding of the relative effects of different tagging methods on fish behaviour and survival.



## **METHODS**

All protocols in this study were conducted in accordance with Canadian Council of Animal Care guidelines. Animal care protocols were approved by Carleton University and Fisheries and Oceans Canada. A scientific collection permit was also obtained from Fisheries and Oceans Canada.

### *Study area and species*

The study area was located on the Harrison River, ~18 km in length, which flows southwest from Harrison Lake to join the Fraser River, about 100km upriver from Vancouver (Fig. 3.1). The Harrison River is the natal stream of the Harrison Rapids sockeye salmon population and is a migratory waterway for Birkenhead River, Big Silver Creek, and Weaver Creek sockeye salmon populations. Both First Nations and recreational fisheries occur within the Harrison River. Fishing was planned to coincide with the appearance of adult migrating Harrison Rapids sockeye salmon and to encompass early entrants arriving in mid-August and early September through to fish arriving near peak spawning time in mid-November.

### *Transmitter attachment*

The basic components of the study were (1) tagging sockeye salmon with either a gastric or external radio telemetry transmitter to compare consequences relative to one another, (2) alternating tag attachment methods to ensure that both tag types were released into the system on each sampling day, and (3) planning for sampling days to occur across the run timing period to assess the consequences of migration timing on behaviour and survival. Sockeye salmon were captured in a narrow, fast-flowing section of the river traditionally used by the local Sts'ailes First Nations band and known as "the Park", located approximately 9 km upstream of the

Harrison-Fraser confluence (Fig. 3.1). This location is about 5-10 km from areas in which Harrison sockeye salmon are known to spawn (Schaeffer et al. 1951). A beach seine deployed from a jet boat encircled an area that typically holds staging sockeye salmon. The net was pulled in by hand to collect the catch in a manageable area on the riverside. Fish were held in the bagged seine net for 2 - 73 min (average 31 min) prior to tagging.

Study fish were transferred into a foam-lined V-shaped trough equipped with a continual flow of fresh river-water over the mouth, gills, and body of the fish. A team of three people assisted with the tagging process: the first person held the fish in the trough, the second individual applied the transmitter and collected biological samples, and the third person recorded all relevant information. Either a gastric or external radio telemetry tag was affixed (procedure outlined in detail in Cooke et al. 2012) using established protocols for tagging adult salmon without anesthetic (Cooke et al. 2005). Anesthetic is rarely used for tagging of adult Pacific salmon in the wild given the potential to disrupt migration and the fact that tagged fish could be captured and eaten in food fisheries. The two radio tag models used in this study were the externally attached TX-PSC-E-45 and the gastrically applied TX-PSC-I-1200 (Fig. 3.6), both made by Sigma Eight Inc. The external tag measured 32 mm in length, 10 mm in width, 9.8 mm in height, and weighed 3.7 g. Two holes at the extremities of the tag facilitated attachment using flexible nickel pins (77 mm in length), which were simultaneously pierced through the dorsal musculature of the fish at the base of the dorsal fin. Clear plastic discs (14 mm in diameter) were slid onto the exposed ends of the pins and, using pliers, the tag was secured against the body by bending and knotting the pin flat against the discs. Once attached, the tag profile was smooth and the antenna (305 mm) trailed from the posterior end. In comparison, the gastric tag measured 43 mm in length, 16 mm in width, 16 mm in height, and weighed 15.2 g. Gastric tags were inserted

through the mouth into the stomach of the supine fish using a smooth plunger to guide the transmitter down the esophagus (Ramstad and Woody 2003; Cooke et al. 2005). After insertion, the antenna (413 mm) trailed from the mouth of the fish. During the tagging procedure, fork length (cm), an estimate of sex based on secondary sexual characters, capture and release vigor, an estimate of maturity/freshwater residency time based on colour and scale quality, and duration in the tagging trough (mm:ss) was recorded. The adipose fin was clipped and a scale was collected for stock identification analysis (see Beacham et al. 2005). The tagging procedures took an average of 39 s (range = 22 - 76 s) for the gastric procedure, which was significantly faster ( $t = -9.6$ ,  $df = 182.9$ ,  $P < 0.001$ ) than the 64 s (range = 39 - 149 s) required for external tagging.

Overall, 195 sockeye salmon (mean fork length =  $61.5 \pm 3.5$  cm) were tagged and released in the study over 5 sampling days in 2014 (101 external tags, 94 gastric tags; on 11, 18, 25 September and 18, 23 October; Table 3.1). This approach of spreading tagging effort across the run timing period is consistent with previous studies given management interest in characterizing how time period influences success to spawning grounds. The electronic transmitters used in this study transmitted on the 150 MHz band and were set to one of eight radio frequencies: 600, 620, 640, 660, 680, 700, 720, and 740. Combining one of these frequencies with a unique transmission code allowed for each tag to be individually programmed. Transmitters were equipped with a motion sensor that was programmed to transmit at a different burst interval when a set movement threshold was attained to indicate a dead fish. When the number of movement events per second fell below 180 for a consecutive 24 h period, the coded transmission signal reversibly switched from the default 5 s burst rate to 7 s. At times, the burst rate from a tagged individual switched back and forth from the “live” 5 s to the “dead”

7 s interval burst rate. When this occurred, the fish was assumed to be alive up until the final 5 s burst interval, even if preceded by periods of 7 s burst intervals. Hence a fish was only deemed “dead” if its final detections were consistent 7 s burst intervals. Each tag was labeled with research contact information in case a radio tag was found or removed from a study fish (e.g. if a fish was harvested by a fisher). An ongoing tag-return reward program was in place to encourage the reporting of harvested individuals.

### *Tracking systems*

Radio-tagged sockeye salmon were monitored using fixed stations and mobile tracking. Five fixed receiver stations were installed on the Harrison River to track the activity of tagged fish (Fig. 3.1). An additional four fixed receiver stations were located in the Fraser River and were maintained in collaboration with concurrent radio telemetry studies to provide tracking information of tagged individuals that left the Harrison River system (Fig. 3.1). Each station was equipped with either an Orion radio receiver manufactured by Sigma Eight Inc. or a SRX400A manufactured by Lotek Wireless Inc. One fixed receiver station was located across the river from the tagging site in the Harrison River (Fig. 3.1), allowing for tracking to commence immediately upon release of tagged individuals. Radio receivers were powered by two 12V deep cycle marine batteries in parallel and housed in a waterproof metal enclosure. Energy from solar panels supplemented the power source at some sites. Each fixed-receiver station was equipped with at least one Yagi antenna with 3, 4, or 5 elements. Mobile tracking supplemented the data collected by the fixed-receiver stations by providing higher resolution information and allowing for monitoring of sections of the river that were not included in the detection range of the fixed stations. The general location of each tag detected by mobile tracking was determined using a mobile receiver and Yagi antenna. When coupled with GPS coordinates, we were able to

generate visual assessments of the distribution patterns over time. Mobile tracking occurred within ~35 h after a tagging event to determine short-term behaviour and survival post-release and was repeated on a weekly basis until the end of the peak spawning period. Data from fixed receivers were downloaded at regular intervals and stations were monitored for battery power levels and receiver performance.

### *Data analysis*

The raw data from the fixed-receiver sites were combined and archived. Analyses of the tagging data were conducted using custom functions created in RStudio (Version 0.99.467) with user-defined criteria. Filtering of the raw data was necessary to remove false detections caused by electronic noise. Criteria for valid records for detections from fixed receivers were set at power levels ranging from -155 to 0 for data collected by the Orion receiver and greater than 30 for the Lotek receiver, and a minimum of three detections within a time interval. Detections from mobile receivers were relatively much fewer and did not undergo such rigorous filtering as the fixed receiver detections. Once false positives were removed, a database of sequential detections for each fish was generated with both fixed and mobile detections. Each record included the fish identification code, fixed-receiver station, river kilometer in which it was detected, and detection power. The filtered database was used to generate spatiotemporal figures describing residence times at each station, detections between fixed sites from mobile tracking, and sites of last detection. Raw detections were analyzed to calculate the frequency of 5 s and 7 s burst rate intervals in each fish's tracking history, and to determine the burst rate interval of the final detections. Detections collected by mobile tracking were assigned positional data by matching corresponding GPS information.

### *Survival estimation*

Using the fixed-station and mobile tracking data, each radio-tagged fish was classified into one of two categories: (1) successful, and (2) unsuccessful. Successful fish were those that were detected in the spawning area (see Fig. 3.1) on or after 7 November 2014 (Fig. 3.2). This date was before peak spawning (10 – 20 November 2014, DFO, personal communication) and coincided with a mobile tracking event by boat that spanned the length of the Harrison River. The rest of the fish were deemed unsuccessful. Due to the configuration and large area covered by the spawning grounds, it was not possible to recover carcasses of tagged fish post-spawning, therefore it was not confirmed if a “successful” fish was actually able to spawn or not. Categorization of survival or success, in this case, was therefore assessed as whether the tagged fish was in the area of the river at a time that would have most likely allowed for successful spawning. Unsuccessful fish were further classified into one of four categories: (1) premature mortality, (2) left the system, (3) fisheries removal, and (4) unknown. Premature mortalities were those that were detected as being in the Harrison River and emitting 7 s burst frequencies consistently before 7 November (for example, see Fig. 3.3). Fish categorized as having left the system were last detected at either the Harrison/Fraser confluence (rkms 0 or 1, see Fig. 3.1) or the upper end of the Harrison River towards Harrison Lake (rkm 14, see Fig. 3.1), emitting 5 s burst frequencies, and not subsequently detected in the Harrison River system (for example, see Fig. 3.4). Fisheries removals were tagged individuals reported to us as having been captured by fishers. Unknown fish were those in the Harrison River emitting 5 s burst frequencies but final detections occurring prior to 7 November, or residing elsewhere than the spawning area for the remainder of their detections (for example, see Fig. 3.5). The reasoning behind including the “unknown” category was to avoid overestimating premature mortalities. Longevity was calculated as the proportion of the number of days between an individual’s first and last

detections and the number of days over the period of time during which it may have been detected.

### *Statistical analysis*

Significance levels were set at 0.05. Statistical analyses were conducted using JMP, version 12.0. Pearson's chi-squared analysis was used to test for differences in fate between tag types, sex, and tagging date. Fisher's exact tests were used to assess differences in fate between external and gastric tag types. 3-way ANOVA was used to assess the effects of tag type, sex, and capture date on proportional longevity. Where statistical differences were detected, t-tests were performed to determine the nature of those differences. When assumptions of normality and homogenous variance were not met, the variables were transformed or non-parametric ranking tests (for the ANOVA and t-test analyses) were used.

## RESULTS

All but two sockeye salmon were confirmed to belong to the Harrison Rapids population. The tracking history of the two study fish with “unknown” population origin showed similar movement patterns to those of Harrison sockeye salmon. These fish were both assessed as “successful” with the assumption that they were from the Harrison population. Additionally, one gastrically tagged fish from September 11 was released prior to recording an estimate of sex, and is therefore referred to as “unrecorded” for sex estimation (Table 3.1).

Six tagged individuals were reported captured by fishers (5 externally tagged, 1 gastrically tagged; Table 3.2). Three tagged fish were not detected upon release or subsequently by fixed stations or manual tracking, two of which were later captured by fishers. The third tag was considered to be a tag malfunction and was removed from the dataset.

All Harrison River fixed receiver sites were actively scanning for detections 85 - 100% of the time except for site HR8 that experienced some technical difficulties and was operating for 71% of its operation period (Fig. 3.1). The four fixed receiver sites in the Fraser River performed 95 - 100% during their respective operation periods (Fig. 3.1). Results from a brief pilot study indicated that the average detection probability of both external and gastric tags were similar, in which gastric tags had slightly higher detection rates than external tags when in the air 90 – 180 m from the fixed receiver antenna (K. Dionne, Simon Fraser University, personal communication). Detection probability varied at depth when tested at different fixed receiver sites, with most detection probabilities estimated at 74% or higher at depths of 1 - 8 m, with some lower detection probabilities occurring such as 19% for gastric tags at 8 m depth at HR1 and 29% for external tags at 5 m depth at HR8 (see Fig. 3.1, K. Dionne, Simon Fraser University, personal communication). Detection efficiency of fixed receivers was estimated by



comparing the number of times a subset of fish (those that exhibited intra-system movement) passed a fixed receiver and the number of times it was detected by that receiver. Detection efficiencies were approximately 89% for HR1, 77% for HR4, 83% for HR6, 92% for HR8 and 100% for HR10 (see Fig. 3.1). The detection efficiency of HR10 may be overestimated due to the small number of study fish that appear to have moved to the upper reaches of the river.

### *Short-term behaviour*

On the tagging days in September, the majority of tagged individuals moved away from the release site within 35 h post-tagging (77.8% [42 of 54] on 11 September, 41.1% [23 of 56] on September 18, and 93.3% [42 of 45] on 25 September). Movement up- or downstream of the release site within 35 h post-tagging progressively declined on the tagging days in October (15.0% [3 of 20] on 16 October, and 5.0% [1 of 20] on 23 October). The likelihood of post-release movement was significantly influenced by capture date ( $\chi^2 = 81.263$ ,  $df = 4$ ,  $P < 0.001$ ). Tag type did not have an effect on the likelihood of immediate movement away from the release site ( $\chi^2 = 0.033$ ,  $df = 2$ ,  $P = 0.856$ ) or on the likelihood of up- or downstream movement within 35 h post-tagging ( $\chi^2 = 0.385$ ,  $df = 1$ ,  $P = 0.535$ ). Movement post-release primarily occurred downstream from the release site (63.1% [70 of 111]) and the magnitude of movement was primarily to the bottom reaches of the Harrison River (see Fig. 3.8). Some individuals did move upstream (36.9% [41 of 111]) and the limit of detection was limited to 4 rkm upstream of the release site (see Fig. 3.8). Upstream movement was most prevalent on the tagging day of 25 September, in which 82.2% [37 of 45] of tagged fish moved upstream immediately post-tagging.

Of the fish that moved up- or downstream of the release site within 35 h post-tagging, 21 did not return towards the release site and were classified as “unsuccessful: left the system”. This

group consisted of 10 externally and 11 gastrically tagged fish. Two fish (1 gastrically and 1 externally tagged) were last detected passing receiver station FR150 near Hope and 19 fish (11 gastrically and 8 externally tagged) were last detected passing receiver stations FR69 and FR70 near Mission (Fig. 3.1). Time between release and last detection before moving away from the release site was not significantly different between fish that left the system (range = 26 min to 31 h, median = 4.9 h, mean = 7.2 h) and those that eventually returned towards the release site (range = 4 min to 30 h, median = 3.3 h, mean = 6.7 h) ( $t = 0.47$ ,  $df = 37.97$ ,  $P = 0.642$ ). The amount of time that individuals spent up- or downstream after this initial movement and before returning toward the release site ranged from 14 min to 53 days (median = 2.1 days, mean = 10.6 days). Fish that left the system entirely and returned did so primarily at the Harrison/Fraser confluence (29 of 31) and only 2 exited temporarily via the upstream reaches towards Harrison Lake (2 of 31). Tagged sockeye salmon that were classified as “unsuccessful: premature mortality” ( $n = 10$ ) consisted of equal numbers of gastrically and externally tagged fish and included 7 females and 3 males. The proportion of days detected for this group ranged from 1.1 to 74.2 days and areas of highest frequency of mortalities were in rkms 1 ( $n = 2$ ) and 4 ( $n = 3$ ). 60% of study fish with fate “premature mortality” were tagged on 18 September.

Moving away from the release site increased the likelihood of survival to reach terminal spawning areas for fish tagged in September (Table 3.5). Additionally, of the fish that moved away from the release site, externally tagged fish had higher survival to the spawning area. The opposite was observed for the October tagging days in which fewer fish moved away in general, and those that did had lower survival to the spawning area (Table 3.5). Fish that did not move away from the release site in October had higher survival to spawning, with externally fish again

having higher survival to the spawning area. Statistically, fate was marginally non-significantly related to the occurrence of immediate movement post-tagging ( $\chi^2 = 3.244$ ,  $df = 1$ ,  $P = 0.071$ ).

### *Survival*

In total, 63 of the 195 sockeye salmon that were tagged and released were successful in reaching their spawning area by 7 November (Table 3.2). Almost twice as many externally tagged fish (41.6% [42 of 101]) survived to reach spawning areas compared to gastrically tagged fish (22.4% [21 of 94]) (Table 3.2). All but one externally tagged individual was detected as being active 35 h post-tagging (Table 3.3). Detections for five gastrically tagged fish terminated 29 hours or less post-tagging, so were deemed “unsuccessful”. A sharp decline in number of active fish occurred 15 d post-release, approximately halving the number of active fish from all tagging dates and for each tag type (Table 3.3; Fig. 3.7). The number of active externally tagged fish was consistently greater than gastrically tagged fish for all time points and tagging dates (Table 3.3; Fig. 3.7). The activity curve for 25 September exhibits the least difference in activity between tag types over time (Fig. 3.7c). The slopes of the activity curves for 16 and 23 October (Figs. 3.7d and 3.7e, respectively) are steeper than those from previous tagging days and reach or are close to 0% 40 and 50 d post-release due to temporal proximity to the peak spawning period and associated rapid senescence and death. Three-way ANOVA revealed that the only significant factor that influenced proportional longevity was tag type (Table 3.4). A t-test revealed that proportional longevity was significantly lower for gastrically tagged fish ( $P = 0.005$ ).

Tag type significantly influenced fate of Harrison sockeye salmon ( $\chi^2 = 8.244$ ,  $df = 1$ ,  $P = 0.004$ ). The likelihood of gastrically tagged fish being “unsuccessful” was significantly greater than that of externally tagged fish (Fisher’s exact test,  $P = 0.003$ ). Sex and tagging date

did not have an effect on fate ( $\chi^2 = 2.292$ ,  $df = 2$ ,  $P = 0.318$  and  $\chi^2 = 4.747$ ,  $df = 4$ ,  $P = 0.314$ , respectively).

## DISCUSSION

Deploying equal numbers of gastrically inserted and externally attached radio transmitters on wild adult Harrison sockeye salmon allowed for a comparison of post-tagging behaviour and survival of two tag attachment techniques commonly used in telemetry studies of Pacific salmon. This study revealed that the majority of tagged fish exhibited wide-ranging up- and downstream movement 35 h or less post-tagging, which was significantly influenced by capture date (i.e., fish tagged earlier tended to move more) but not by tag type. This study demonstrated that tag type significantly influenced survival to spawning areas, in which externally tagged sockeye salmon were nearly twice as likely to reach spawning areas as gastrically tagged fish. To our knowledge, this is the first study to use adult sockeye salmon as a study species to evaluate and directly compare the effects of these two tag attachment techniques in a field setting. Additionally, this study is the first to describe intra-system movement of the Harrison sockeye salmon population across the entire run timing period.

Assessing immediate behaviour post-tagging may reveal latent effects of the tagging event. Research on the physiological consequences of capture and/or tagging in the wild has reported high levels of circulating lactate and cortisol in the hours following the stressor (see Chapter 2; Raby et al. 2015; Farrell et al. 2001), which may result in decreased swimming performance (Driedzic and Kiceniuk 1976; Milligan 1996; Farrell et al. 1998). We therefore hypothesized that fish may pause for a period of time to recover after being tagged and either remain near the release site or move slowly downstream. However, the movements that occurred within 35 hours post-tagging varied in magnitude and suggested that some individuals left the release site in as little as 4 min after tagging and covered vast expanses of the Harrison River shortly thereafter. This pattern may indicate that some fish were not exhausted physiologically,

or that they were seeking out desirable (low to moderate) flows, or intentionally exercising in an attempt to facilitate metabolic recovery (Milligan et al. 2000). Greater numbers of study fish tagged in September exhibited immediate movement away from the release site than in October. Tagged individuals in September may have had sufficient energy stores to be able to seek environmental conditions to facilitate recovery. Overall, the decrease in the likelihood of movement from the release area from September to October may simply be a reflection of temporal proximity to spawning and thus fish stayed closer to the spawning grounds (Hruska et al. 2010; Mathes et al. 2010).

Movement within 35 h post-tagging occurred mainly downstream from the release site. Conversely, a pattern of immediate upstream movement post-tagging for gastrically tagged Harrison sockeye salmon was observed by Robinson et al. (2015) with tagging occurring in the same location as this study and over similar dates in September. Mathes et al. (2010) also reported upstream movement towards Harrison Lake, but primarily for fish tagged in late August and early September. We expected more study fish from earlier in our study to move upstream perhaps to seek thermal refuge in the cool waters of Harrison Lake (Mathes et al. 2010). However, the peak of upstream movement occurred for fish tagged on 25 September and there was little indication of prolonged residence in Harrison Lake. The two individuals that left the system temporarily via the upper reaches of the river appeared to have been gone for 2 and 6 days, respectively. The river temperatures were cooling slightly throughout the month of September, therefore decreasing the need to use Harrison Lake as cool water refugia and increasing residency in Harrison River. The prevailing direction of movement that occurred immediately post-tagging was downstream, which may be a function of passive fallback (Frank et al. 2009). Fallback post-tagging has been reported in other telemetry studies using Pacific

salmon. In their telemetry study in the Lower Snake River in Washington, Gray and Haynes (1979) reported consistent downstream movement of adult Chinook salmon post-tagging which was unique to their gastrically tagged treatment. In our study, downstream movement was not influenced by tag type but was influenced by capture date. Gray and Haynes (1979) implanted gastric tags to fish in both the spring and the fall and observed similar tag-specific movement patterns over both seasons, suggesting that capture period did not influence rates of fallback in their study. An important caveat is that the tagged Chinook salmon in Gray and Haynes' (1979) study were following a linear migration trajectory upstream whereas the Harrison sockeye salmon tagged in this experiment were already in vicinity of the spawning area and were likely exhibiting staging or milling behaviour. Therefore, downstream movement may be interpreted as migration delay in other telemetry studies of migrating adult Pacific salmon, but in our case it may simply be a reflection of in-river behaviour in the weeks prior to spawning.

The differences in numbers of gastrically and externally tagged fish that moved away from the release site within 35 h post-tagging and associated survival to spawning area suggests that immediate movement may be a beneficial migratory strategy for long-term survival (Table 3.5). Although the likelihood of immediate movement post-tagging did not statistically influence fate, there were consistent trends suggesting that, for tagging dates with frequent post-tagging movement, (1) externally tagged sockeye salmon moved away from the release site more readily than gastrically tagged individuals, and (2) fish that moved away were more successful in reaching spawning grounds (Table 3.5). We are not aware of any telemetry studies that have investigated correlations between immediate movement post-tagging and long-term survival.

Tagged individuals had an overall survival rate of 32.3%. This value is similar to studies evaluating survival after interactions with recreational fisheries (i.e. ~36%; Donaldson et al.

2011) and is within the range observed for sockeye salmon released from tangle nets in the Fraser River (18% to 42%; Donaldson et al. 2010). However, what is remarkable is the difference in survival between the two tag types. The mechanism behind this observation is unclear however it is presumably linked to physical placement of the tag in the stomach. Gastric insertion can lead to perforated stomachs (Chapter 2; Ramstad and Woody 2003; Corbett et al. 2012), particularly in fish that are well along in their migration and maturation. A holding study (see Chapter 2) revealed higher susceptibility of stomach perforation for gastrically tagged fish in the later sampling period (i.e. October). However, the short observation times did not identify physiological disturbances in gastrically tagged sockeye salmon with ruptured intestinal tract compared to those with an intact stomach. Presence of the antenna in the esophagus may also cause some leakage of water into the stomach that would otherwise not occur in externally tagged individuals. It is possible that the stomach has an important water balance and osmoregulatory role that we are not aware of and it cannot function when the tag is present. We speculated, alongside others (i.e. Corbett et al. 2012; Gray and Haynes 1979), that leakage or damage to the stomach could develop physiological imbalances and adverse whole-animal changes in performance over time. A higher frequency of gastrically tagged individuals was consistently assigned an “unsuccessful” fate category than externally tagged fish, except for those that were removed due to fisheries (Table 3.2). Activity curves suggest that fish tagged with either attachment method succumb to adverse effects >35 h but before 15 d post-tagging (Fig. 3.7), however the slope of decline for gastrically tagged fish is consistently steeper over this time period compared to that of externally tagged fish. The decline of activity for gastrically tagged individuals between 35 h and 15 d post-tagging is similar for all tagging dates, suggesting that even fish in a robust state tagged earlier in the sampling period via gastric insertion



experienced lower success, despite the lower likelihood of stomach perforation at this time. This suggests that gastrically tagged sockeye may be experiencing latent effects from the tagging event or are experiencing negative consequences associated with tag burden.

Areas of the river with highest frequency of final detections for fish with “unknown” fate were rkms 10 and 11 at receiver sites HR6 and HR8 (36.4% [20 of 55] and 21.8% [12 of 55], respectively; Fig. 3.1). These receiver sites, particularly HR6, were the most detection-intensive as they are located in proximity to the spawning areas where Harrison sockeye salmon stage for long periods of time. The area across from HR6 is where the capture, tagging, and releases took place for this study (Fig. 3.1) and is also a common boat launch site. It is possible that some of the “unknown” fish may actually be unreported fisheries removals with final detections occurring as they were harvested and removed from the system. Both tag types would be equally visible to any fishers given the presence of the long antenna such that there would be no a priori reason to expect difference in reporting rate. The external tags may be more likely to be ripped out in a gill net or seine net which would lead to more “unknown” fates or erroneous categorization as premature mortality but neither of those patterns were observed. The number of fish reported as fisheries harvest were primarily externally tagged individuals (5 of 6), which may speak to the risk of increased susceptibility of external tags to entanglement in fishing gear (Rikardsen and Thorstad 2006).

Understanding the technological limitations of each tag type is important to accurately interpret the results, such as differences in detection probabilities and performance under variable hydrological conditions. Determining the limitations of the mortality sensor would also aid in interpreting the data and potentially allow for finer resolution in confirming mortalities. One difference between the two tagging methods was the actual size of transmitter used. The

gastric transmitters were over 4 times heavier with 11 times larger volume than the external tags. The tag size selected for gastric placement was intentionally selected to be larger in an effort to reduce tag expulsion – the same tag size used for tagging thousands of sockeye in the Fraser watershed (English et al. 2005; Cooke et al. 2006). We selected a smaller external tag to reduce drag. We recognize that the difference in size/mass of the tag confounds the experiment but we also note that even the larger tag represents an incredibly small tag to body mass ratio (<1% calculated using mean body mass of study fish from Chapter 1). As such, we submit that it is unlikely that the difference in survival observed was a direct effect of the larger tag size. Alternate methods of assessing behavioural influences due to tag type include direct observation post-tagging or using accelerometers to measure aberrant swimming behaviours (as was done in Broell et al. 2016), however both these techniques may be more conducive to laboratory settings. Clearly there is more work needed on the long-term physiological consequences of stomach perforation and gastric tag placement on maturing sockeye salmon.

**Table 3.1.** The date and number of males and females tagged with either an external or gastric tag over the entire run time period. Fishing efforts occurred prior to 11 September but the quantity of fish captured was insufficient to allow deployment of both tag types in adequate numbers. The single individual with “unrecorded” sex on 11 September was released prior to sex estimation.

Date	External	Gastric	Total
11 September			
Female	14	11	25
Male	13	15	28
Unrecorded		1	1
			<hr/> 54
18 September			
Female	13	13	26
Male	17	13	30
			<hr/> 56
25 September			
Female	11	10	21
Male	13	11	24
			<hr/> 45
16 October			
Female	5	5	10
Male	5	5	10
			<hr/> 20
23 October			
Female	5	5	10
Male	5	5	10
			<hr/> 20
Grand Total	<hr/> 101	<hr/> 94	<hr/> 195

**Table 3.2.** Fate of study fish by tagging date. Successful fish were those that were detected in the spawning area on or after 7 November. Premature mortalities were those that were detected as being in the Harrison River and emitting 7 s burst frequencies consistently before 7 November. Fish categorized as having left the system were last detected at either extremities of the Harrison River, emitting 5 s burst frequencies, and not subsequently detected in the Harrison River system. Fisheries removals were tagged individuals reported to us as having been captured by fishers. Unknown fish were those in the Harrison River emitting 5 s burst frequencies but final detections occurring prior to 7 November, or residing elsewhere than the spawning area for the remainder of their detections.

	n	<i>Successful</i>	<i>Unsuccessful</i>			
			Premature mortality	Left the system	Fisheries removal	Unknown
<b>External</b>						
September 11	27	12	1	6	2	6
September 18	30	9	4	9	1	7
September 25	24	10	0	9	1	5
October 16	10	5	0	2	0	3
October 23	10	6	0	2	1	1
Total (%[n])	101	41.6 [42]	4.9[5]	26.7 [27]	4.9 [5]	21.8 [22]
<b>Gastric</b>						
September 11	27	5	1	8	1	12
September 18	26	4	2	10	0	10
September 25	21	9	0	10	0	2
October 16	10	1	1	3	0	5
October 23	10	2	1	3	0	4
Total (%[n])	94	22.4 [21]	5.3 [5]	36.2 [34]	1.1 [1]	35.1 [33]
<b>Grand Total (%[n])</b>	<b>195</b>	<b>32.3 [63]</b>	<b>5.1 [10]</b>	<b>31.3 [61]</b>	<b>3.1 [6]</b>	<b>28.2 [55]</b>

**Table 3.3.** Number of fish that were active 35 h, 15 d, 22 d, 30 d, 40 d, 50 d post-release. A tagged individual was considered active at a certain time point if its detections suggested movement in the Harrison River system or it was outside the system but returned at a later date. Data is visualized in Figure 3.7.

Tagging date, treatment group	n	35 h	15 d	22 d	30 d	40 d	50 d
September 11							
External	27	26	18	17	17	16	15
Gastric	<u>27</u>	27	10	10	10	8	7
Total	<u>54</u>	53	28	27	27	24	22
September 18							
External	30	30	18	15	14	14	13
Gastric	<u>26</u>	21	11	8	7	4	4
Total	<u>56</u>	51	29	23	21	18	17
September 25							
External	24	24	17	15	14	12	10
Gastric	<u>21</u>	21	13	11	11	11	9
Total	<u>45</u>	45	30	26	25	23	19
October 16							
External	10	10	5	5	3	3	0
Gastric	<u>10</u>	10	4	1	1	0	0
Total	<u>20</u>	20	9	6	4	3	0
October 23							
External	10	10	7	5	4	1	1
Gastric	<u>10</u>	10	3	3	1	0	0
Total	<u>20</u>	20	10	8	5	1	1
Grand Total (%[n])	<u>195</u>	96.9 [189]	54.4 [106]	46.2 [90]	42.1 [82]	35.4 [69]	30.3 [59]

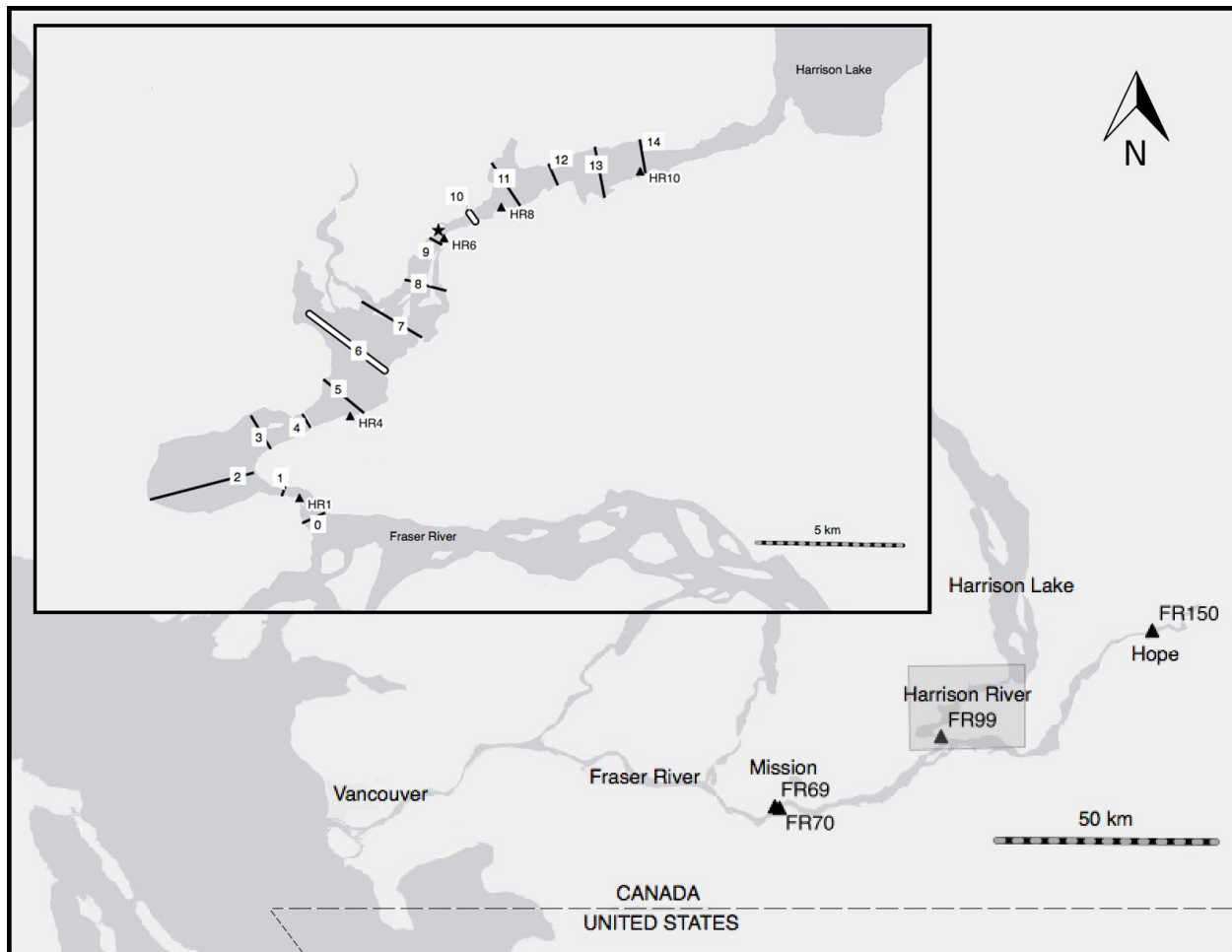
**Table 3.4.** Results of 3-way ANOVA with response variable proportional longevity and tag type, sex, capture date, and interactions as effects. Proportional longevity was calculated as the proportion of the number of days between an individual's first and last detections and the number of days over the period of time during which it may have been detected.

Variable	Tag type			Sex			Capture date			Tag type × Sex			Tag type × Capture date			Sex × Capture date			Tag type × sex × capture date		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Proportional longevity	8.27	1	<b>0.004</b>	0.85	1	0.357	1.69	4	0.155	0.03	1	0.86	0.70	4	0.59	1.06	4	0.377	1.10	4	0.357

*Note:* Significant values ( $P \leq 0.05$ ) are in boldface type.

**Table 3.5.** The number of study fish that moved away from the release site within 35 h post-tagging and associated survival to reach spawning areas.

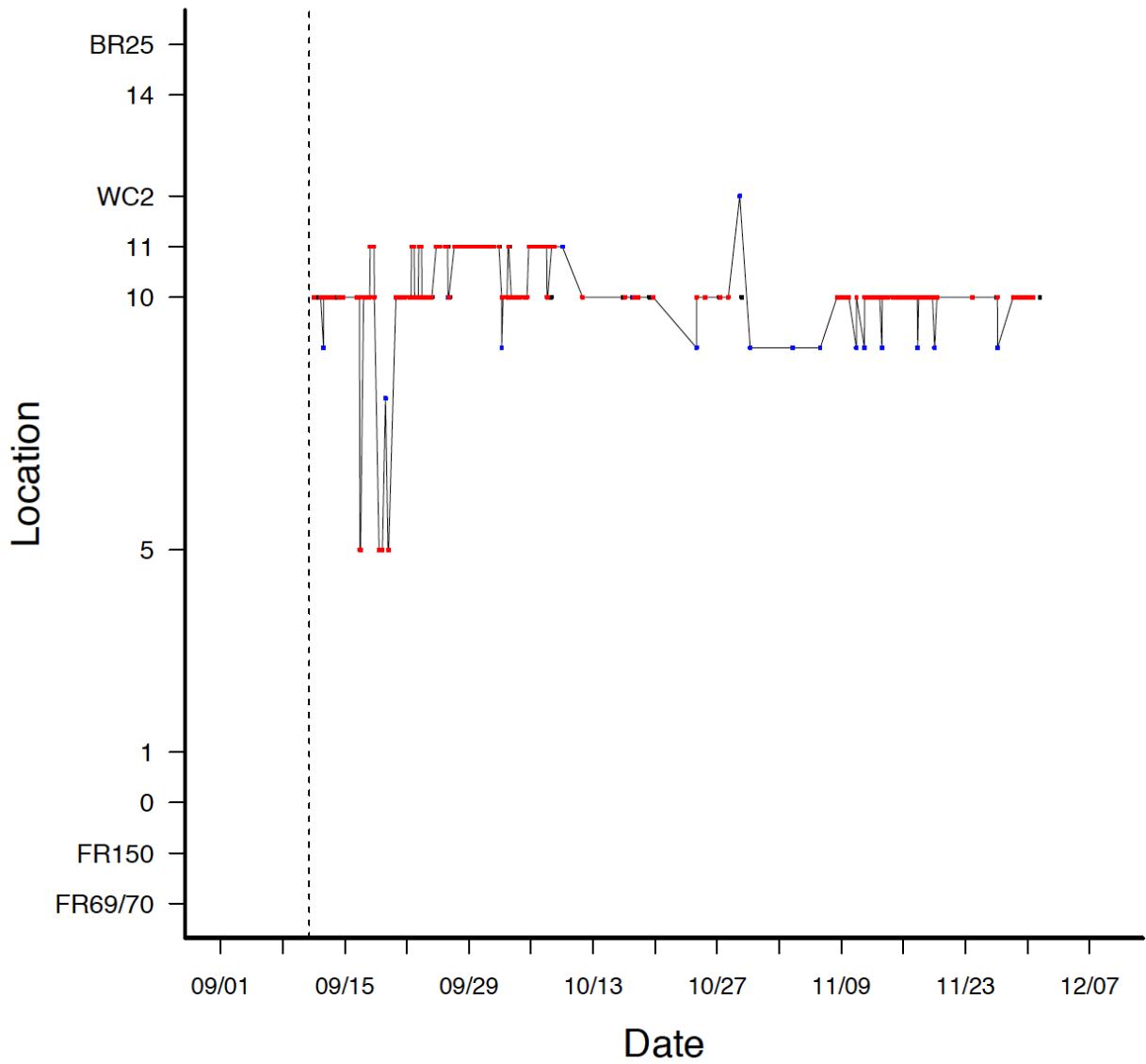
Tagging date, treatment group	n	Survived to spawning area (% [n])
September 11		
External		
Moved away	17	64.7 [11]
Did not move away	10	10.0 [1]
Gastric		
Moved away	15	26.7 [4]
Did not move away	12	8.3 [1]
September 18		
External		
Moved away	11	45.5 [5]
Did not move away	19	21.1 [4]
Gastric		
Moved away	8	25.0 [2]
Did not move away	18	11.1 [2]
September 25		
External		
Moved away	23	43.5 [10]
Did not move away	1	0.0 [0]
Gastric		
Moved away	17	52.9 [9]
Did not move away	4	0.0 [0]
October 16		
External		
Moved away	2	20.0 [1]
Did not move away	8	50.0 [4]
Gastric		
Moved away	1	0.0 [0]
Did not move away	9	11.1 [1]
October 23		
External		
Moved away	0	0.0 [0]
Did not move away	10	60.0 [6]
Gastric		
Moved away	0	0.0 [0]
Did not move away	10	20.0 [2]



**Figure 3.1.** Map of the lower Fraser River, British Columbia, Canada. with inlay map of the Harrison River. The triangles represent locations of fixed receiver stations. Fixed receiver stations labeled by FR are located along the Fraser River and HR that they are located along the Harrison River. River kilometer segments are delineated by numbered lines (0 – 14) perpendicular to the river thalweg and are 1 km apart. The upstream and downstream boundaries of the spawning area (rkms 10 and 6, respectively) are delineated by white lines bordered in black. The star indicates the study capture/tagging/release site.

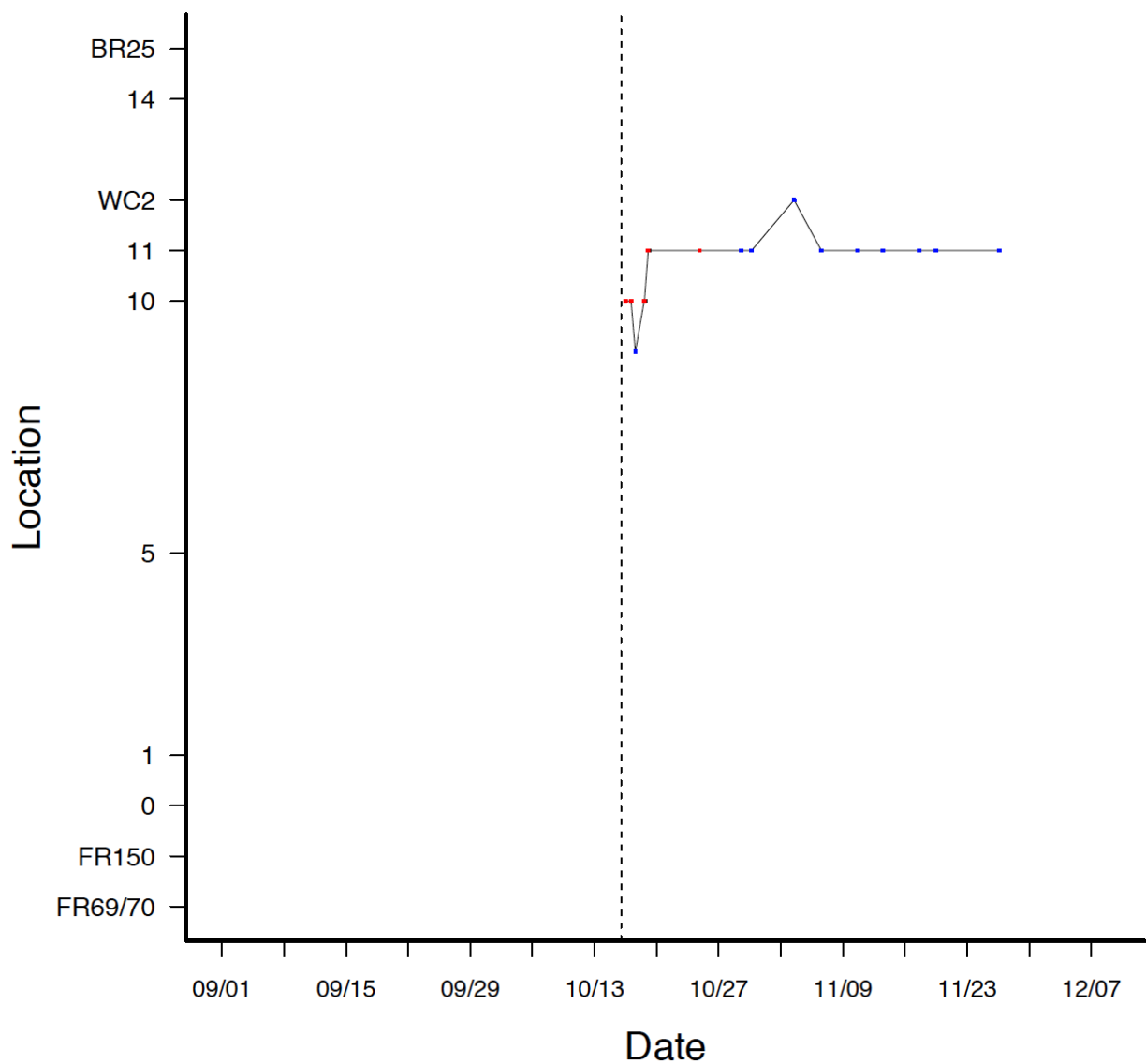


ID: 150.72-94 Tag: i  
Sex: m Stock: harrison Age: 41 FL: 64.8  
Capt.Date: 2014-09-11 Capt.Loc.: park Capt.Gear: beachseine



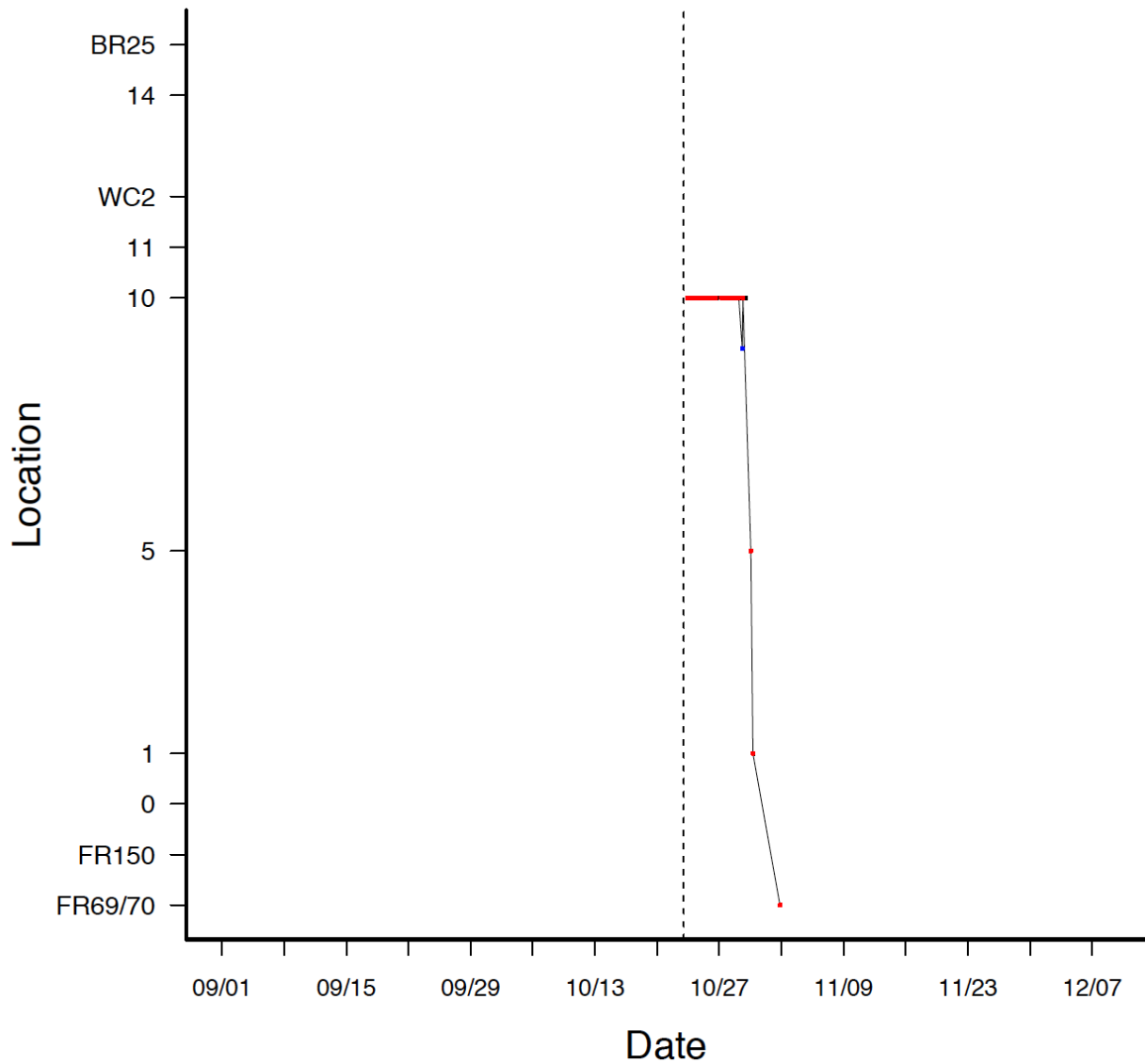
**Figure 3.2.** Spatiotemporal representation of detections from a tagged individual that was deemed “successful”. The individual was in the spawning area (between Location rkms 6 and 10) on or after 7 November.

ID: 150.62-210 Tag: i  
Sex: f Stock: harrison Age: 41 FL: 57.2  
Capt.Date: 2014-10-16 Capt.Loc.: park Capt.Gear: beachseine



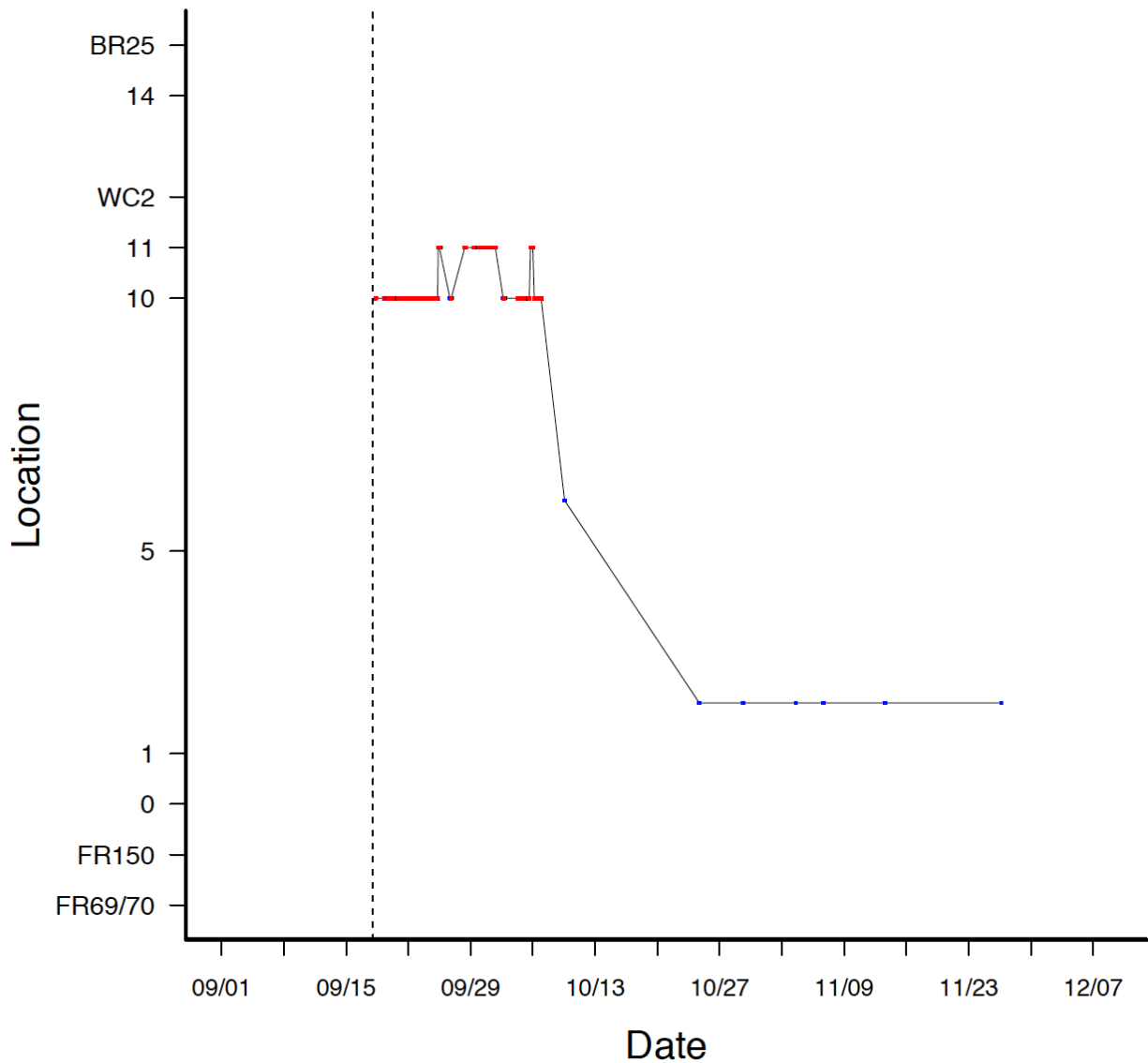
**Figure 3.3.** Spatiotemporal representation of detections from a tagged individual that was deemed “unsuccessful: premature mortality”. The mortality sensor is triggered by 29 October in rkm 11 and subsequent detections were consistently transmitted at the 7 s burst rate, indicating a dead fish.

ID: 150.72-84 Tag: i  
Sex: f Stock: harrison Age: na FL: 59.2  
Capt.Date: 2014-10-23 Capt.Loc.: park Capt.Gear: beachseine



**Figure 3.4.** Spatiotemporal representation of detections from a tagged individual that was deemed “unsuccessful: left the system”. The detection pattern showed that the fish remained near the release site for approximately a week post-tagging, then fell downstream and exited the system at the Harrison-Fraser confluence. The individual was detected passing by the receiver station further downstream in the Fraser River in Mission, BC (Location FR69/70).

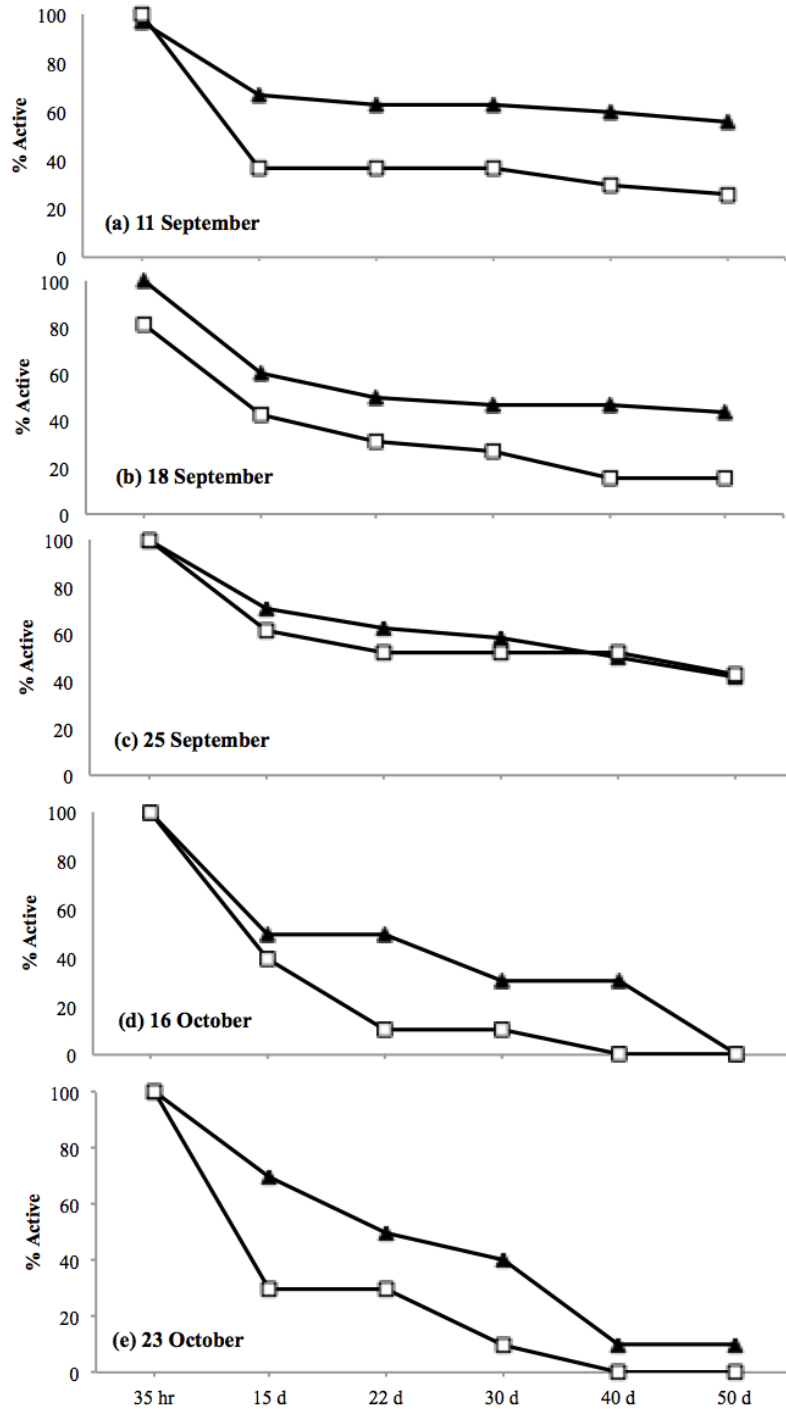
ID: 150.68-9 Tag: e  
Sex: m Stock: harrison Age: 41 FL: 64.2  
Capt.Date: 2014-09-18 Capt.Loc.: park Capt.Gear: beachseine



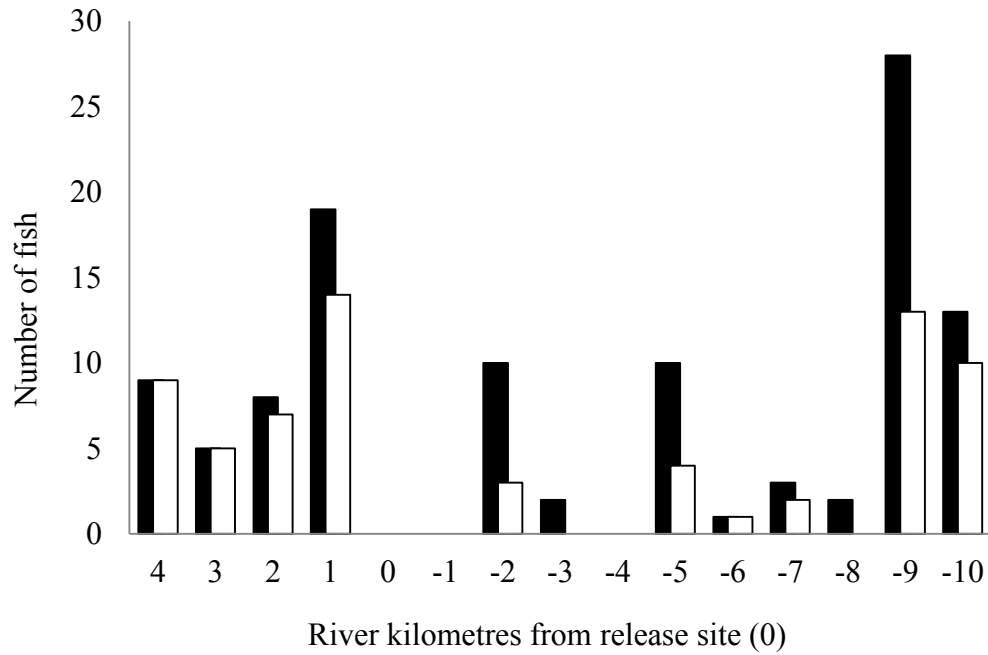
**Figure 3.5.** Spatiotemporal representation of detections from a tagged individual that was deemed “unsuccessful: unknown”. The detection pattern showed that the fish remained near the release site for approximately 20 days post-tagging then fell downstream and did not return towards the spawning grounds. The burst rate of the final detections could not be reliably determined due to low number of detections.



**Figure 3.6.** Tag attachment methods. (A) The gastric insertion method uses a smooth plunger to guide the tag through the esophagus and into the stomach; (B) The external mounting method uses two pins to pierce the musculature below the dorsal fin. Pliers are used to secure the tag by twisting over the ends of the pins flat against the buffer discs.



**Figure 3.7.** Percentage of active study fish over time. Triangles represent externally tagged fish and squares represent gastrically tagged fish. A study fish was considered active if its detections suggested movement in the Harrison River system or it was outside the system but returned at a later date.



**Figure 3.8.** Number of individual fish moving up- or downstream within 35 h post-release. The release site is represented by rkm 0. Black bars show number of individual fish that moved up- or downstream to that section of the river within 35 h post-tagging. White bars show proportion of those fish that eventually returned towards the release site.

## **CHAPTER 4: General Discussion**

This thesis aimed to explore a key assumption underpinning all telemetry studies; that the attachment and burden of the transmitter does not influence the behaviour and/or survival of tagged individuals. Adult sockeye salmon from the Harrison Rapids population in the Fraser River watershed of British Columbia were tagged with two models of radio transmitters that required either external mounting or gastric insertion. Study fish were in the midst of their spawning migration, and tagging occurred throughout the entire run timing period to assess whether sampling period, i.e. maturation state, influenced outcomes. In Chapter 2, tagged and control individuals were held in riverside net pens and short-term physiological responses to tagging were measured and compared. In Chapter 3, the movement and survival of externally and gastrically tagged adult sockeye salmon were monitored throughout their migration window and spawning periods to determine relative differences between the two tag types. I discuss how these findings advance our understanding of tag-related effects on migrating adult Pacific salmon, and inform future research activities. This study is the first to directly compare the consequences of gastric and external tags on the physiology, behaviour, and survival of migrating adult sockeye salmon throughout the entire run timing and spawning period.

### **Findings and implications**

Pacific salmon are an economically, culturally, and ecologically significant group of animals. They are the focus of many tagging studies at all life history stages in efforts to understand their complex migration biology and to inform fisheries management (Drenner et al. 2012). A number of studies on juvenile Pacific salmon are dedicated to determining tag size



limitations and the effects of tagging on a number of behavioural and health indices (e.g. Adams et al. 1998*a*; Adams et al. 1998*b*; Martinelli et al. 1998; Chittenden et al. 2009; Jepsen et al. 2011; Collins et al. 2013), but there is a paucity of reports designed to identify potential effects on adults. In order for telemetry studies to generate unbiased data and to influence appropriate conservation and management actions, tagging effects must be addressed and quantified.

In Chapter 2, I found no differences in the immediate physiological responses of adult female sockeye salmon tagged via gastric insertion or external attachment. Furthermore, tagged fish showed similar responses to those of untagged control fish. Plasma lactate and cortisol concentrations remained elevated 4 h post-tagging indicating that the fish had not fully recovered from capture, handling, and tagging. The persistent metabolic disturbances may have influenced the ability of fish to burst-swim (Farrell et al. 1998; Jain and Farrell 2003). I determined that the capture and handling associated with a tagging event were stressful, while the addition of the transmitter, regardless of tagging method, was non-additive over the relatively short assessment period. An important observation was the high frequency of perforation of the intestinal wall in more mature gastrically tagged fish. The occurrence of stomach perforation has been reported in other telemetry studies of migrating adult Pacific salmon (Gray and Haynes 1979; Schubert and Scarborough 1996; Corbett et al. 2012), and has been hypothesized to be a cause of premature mortality in gastrically tagged individuals compared to externally tagged or control conspecifics. We did not identify any distinct physiological disturbances due to stomach perforation 1 h or 4 h post-tagging, suggesting that any potential impacts on survival must manifest days or weeks following the tagging event, if at all, or involved physiological metrics that we did not quantify here.

The study design of Chapter 3 was planned to expand on the findings of Chapter 2 and to encompass long-term behavioural and survival outcomes. Consistent with reports of immediate movement post-tagging from other telemetry studies on migrating Pacific salmon (Gray and Haynes 1979), I reported high frequencies of movement away from the release area within 35 h for fish tagged with either a gastric or external tag. Some of these movements were downstream which could be interpreted as fallback. However, fish in the Harrison River mill around before spawning, and are known to spawn upstream and downstream of the tagging site. Fish tagged earlier in the spawning season were more likely to move immediately post-tagging. Tag type significantly influenced fate of adult Harrison sockeye salmon, in which almost twice as many externally tagged fish were successful in reaching spawning areas compared to gastrically tagged fish. These findings were consistent with results from a survival study by Corbett et al. (2012), which reported significantly lower survival of gastrically tagged adult Chinook salmon than externally tagged and control fish. Regardless of species or population origin, adult semelparous Pacific salmon in the late stages of spawning migration experience a suite of degenerative physical and physiological changes, which includes atrophy of the digestive organs (Morbey et al. 2005). Managers and researchers using telemetry to study adult Pacific salmon during advanced stages of the freshwater spawning migration must acknowledge that the gastric implantation method significantly increases the likelihood of tag effects (mortality, downstream fallback, decreased presence on or near spawning areas).

These results reveal that the failure to detect immediate physiological disturbances specific to gastric and external tag attachment techniques on adult migrating sockeye salmon does not negate the possibility that tag-specific long-term adverse effects on behaviour and survival may occur.

## **Future directions**

Understanding the limitations of different tag types is crucial to selecting the transmitter attachment technique that is best suited to generate unbiased data for the species in question. Telemetry will continue to be an important tool in studying the migration of adult Pacific salmon, but in order to have confidence in the reliability of the information generated, future efforts must take a step back to validate its use and to recognize potential impacts on tagged individuals, especially when attempting to quantify mortality and behaviour.

When exploring the question of the immediate stress response caused by different tagging methods, it is challenging to isolate the physiological disturbances caused by the tagging activity from those elicited by the capture and handling processes. This makes it difficult to directly compare different tag attachment methods in terms of adverse effects (Jepsen et al. 2015). Future research seeking to quantify the physiological response specific to different tagging techniques may consider allowing fish to recover for a period of time after the capture event and before the tagging and biosampling procedures. This may allow the effects of capture to subside and for a potentially more accurate measurement of tagging-induced stress. However, it is well-known that migrating adult Pacific salmon do not respond well to confinement (Portz et al. 2006) therefore creating a holding environment in the wild in which recovery from capture is maximized and holding-induced stress is minimized may be challenging. Moreover, capture of adult Pacific salmon will almost always immediately precede tagging such that the approach I used here is reflective of real tagging scenarios. To ensure that the stress response provoked when tagging an adult Pacific salmon in the wild remains below lethal levels, a review of capture and handling methods is necessary. Incorporating thresholds of holding time, crowding, risks of injury, air

exposure, and handling time that ensure maximum survival would aid in developing best practices for tagging adult Pacific salmon. However, the circumstances surrounding adult Pacific salmon tagging are often context-specific (freshwater or saltwater environments, cool or warm water temperatures, less mature or more mature individuals, a wide range of capture gears, biopsy or no biopsy, anesthetic or no anesthetic, etc.; see Raby et al. 2015 for review) and this complexity reduces the ability to make broad-scale suggestions to standardize capture, handling, and tagging techniques.

The high frequency of perforated stomachs in sockeye salmon tagged via gastric insertion in the late sampling period in Chapter 2 and the remarkably lower success of gastrically tagged individuals in reaching spawning areas in Chapter 3 support an existing call for future studies to assess the impacts of stomach perforation (Corbett et al. 2012, Gray and Haynes 1979). Studies describing the function of the gastrointestinal tract of migrating adult Pacific salmon are rare (but see Grosell et al. 2010 for a synthesis of gut functions in other fish) and it is widely accepted that there is little to no use of the gut at this stage because feeding has ceased prior to freshwater entry. This reasoning is also commonly referenced in support for the use of gastric tags for Pacific salmon during this life history stage. However, my findings suggest that physical placement of the tag in the stomach or injury to the stomach lining due to the gastric tag may influence premature mortality. Testing different methods or materials associated with the gastric insertion technique may help identify modifications beneficial to minimizing tagging impacts, such as using a lubricant on the tag upon insertion (Keefer et al. 2004a) and revising the physical properties, such as size, shape, and materials used, of the plunger, the gastric transmitter, and the antenna. Future studies that can successfully describe the physiological mechanisms associated with a perforated stomach will also help identify when the use of gastric insertion is more likely

to increase the likelihood of adverse tag effects. Quantifying the rate of weakening of the stomach lining and increased susceptibility to perforation as functions of freshwater residency would be beneficial for researchers who are considering transmitter attachment options for adult Pacific salmon in the late stages of maturation.

Finally, researchers leading telemetry studies on fish should acknowledge the potential for tagging effects and ensure that appropriate validation studies are cited or conducted. Increasing the reporting of unsuccessful telemetry studies or studies that failed to detect tagging effects is required to increase our understanding of acceptable tagging techniques (Murray and Fuller 2000; Thiem et al. 2011).

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