A Conservation Physiology Approach to Understanding the Migration Biology of an
Imperiled Stock of Summer-run Chinook salmon (*Oncorhynchus tshawytscha*) in a
Regulated River

By

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Dedication

nanos gigantium humeris insidentes

(Dwarfs standing on the shoulders of giants)

-Bernard of Chartres (*circa* 1120)

During my time as a doctoral candidate I lost two mentors whom I wish to dedicate this thesis to. Debbie Chambers was my high school biology teacher, and had it not been for her guidance, friendship, excitement for science, and mentoring I would have never pursued biology as a career path. Dr. George Niezgoda was a collaborator and mentor early in my graduate career. His confidence in me, and the opportunities he provided me, will never be forgotten.

Abstract

Understanding the interactions between migrating fishes and the environment is needed in order to properly manage fish populations in disturbed habitats. Firstly, this thesis reviewed a suite of physiological tools that could be used in an interdisciplinary way to understand the basic biological mechanisms that lead to conservation issues; and it was the aim of this thesis to explore the use of these physiological tools to understand the migration of a summer-run Chinook salmon through a hydropower-impacted river system (i.e., the Puntledge River on British Columbia's Vancouver Island). Osmoregulatory, nutritional, and metabolic conditions were compared to migratory behaviours of the stock and revealed few correlates to migration success/failure. Thermal histories of fish moving through the river suggested that fish had no opportunity to choose cool water refuge, and that failure to access the cooler Comox Lake may result in premature mortality. When activity levels of fish were compared to fish location, river discharge, and temperature it was found that substantial interindividual variation was present. Furthermore, activity was not found to change during prescribed pulse flows and these flows were not found to be effective at inducing upstream movements of salmon. Future research should aim to understand migration in stressed environments across spatial scales and across multiple taxa. Managers of the Puntledge River may consider using other techniques for inducing movement of fish upstream such as habitat enhancement based on fine scale flow dynamics, and could alleviate thermal stress by ensuring fish passage to Comox Lake.

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First and foremost I must acknowledge the outstanding supervision I received from my supervisor, Dr. Steven Cooke. His excitement for fish, his craving for research, and his ability to multi-task will hopefully one-day rub-off on me. I am also forever thankful to Dr. Kyle Hanson and Dr. (soon-to-be) Michael Donaldson. Their friendship throughout my career has been enjoyed immensely and I look forward to future drinks at random pubs, conversations of all sorts, and collaborations.

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Without all of you, I would be a very lonely person.

Co-Authorship

Chapter 2: Expanding the “toolbox” for studying the biological responses of individual fish to hydropower infrastructure and operating strategies

Hasler, C.T., Pon, L.B., Roscoe, D.W., Mossop, B., Patterson, D.A., Hinch, S.G., and Cooke, S.J. 2009. *Environmental Reviews* 17: 179-197

The paper was conceived by Hasler, Mossop, Patterson, Hinch, and Cooke. Hasler completed all research and wrote the paper with supervision by the other co-authors. Hasler, Mossop, Patterson, Hinch, and Cooke conceived, carried out the field work and analysis for case study 2; Pon, Roscoe, Patterson, Hinch, and Cooke conceived, carried out the field work and analysis for case study 2. The paper was also submitted to Fisheries and Oceans Canada and was supported by the Canadian Centre for Expertise in Hydropower Impacts on Fish and Fish Habitat (administered by Dr. J.-D. Dutil).

Chapter 3: The influence of osmoregulatory, metabolic, and nutritional condition on the migratory behavior and fate of an imperiled stock of summer-run male Chinook salmon in a regulated river.

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The paper was conceived by Hasler, Patterson, Mossop, Hinch and Cooke. Significant field data collection and analysis were performed by Hasler, Donaldson, Sunder, and Guimond. Hasler wrote the paper with assistance from all other authors.

Chapter 4: Thermal biology and bioenergetics of different upriver migration strategies in a stock of summer-run Chinook salmon

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All authors conceived of the paper. Hasler, Donaldson, and Guimond collected data. Data were analyzed by Hasler, Patterson, Hinch, Cooke and Donaldson. Paper was written by Hasler and reviewed by all authors.

Chapter 5: Influence of individual variation, river reach, water temperature, and discharge on relative activity of upstream migrating summer-run Chinook salmon in a regulated river

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Paper was conceived by Hasler, Mossop, Patterson, Hinch, and Cooke. Field work was completed by Hasler. Hasler wrote the paper and did all analysis. All authors reviewed the paper.

Chapter 6: Effectiveness of pulse flows in a regulated river to induce upstream movement of an imperiled stock of Pacific salmon.

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All authors conceived the paper. The study was completed by Hasler, Guimond, Hinch, and Cooke, and is the result of a BC Hydro (Mossop) funded three-year study to understand fish migration during pulse flows. All data analysis and writing was completed by Hasler and reviewed by all co-authors.

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Chapter 1: General Introduction

Migration

Humans the world over, and throughout history, have been fascinated by animal migrations. The annual arrival of song birds in North America, the gathering of Monarch butterflies in Mexico, and the large herds of American buffalo once roaming the plains of the American mid-west, all conger up visions of grandeur. Pre-historic humans followed the migrations of animals in order to survive, and these migration paths have influenced the locations of human settlements in many parts of the world. So captivating are animal migrations that many have been interlaced into human cultures and recorded for the ages in artwork, song, and dance. Even Aristotle himself pondered the movements of animals between feeding and breeding locations. Therefore, it is not surprising that the biological phenomenon of animal migrations has been well studied by modern-era scientists.

Animal migration is broadly described as the seasonal movements undertaken by organisms between breeding, feeding, and wintering areas. A review by Dingle and Drake (2007) suggested a more general definition of animal migration that consists of four different but overlapping concepts: (1) long range and directed locomotory activity; (2) large scale relocation of animals; (3) seasonal movements for feeding and breeding; and (4) movements that redistribute populations across large spatial scales. These movements occur in every major taxa of the animal kingdom and represent essential components of the life history and ecological niche of the organism. Migration is thought to be an adaptation driven by individual selection as a

result of changes in resources (both in space and abundance; Åkesson and Hedenström 2007; Dingle and Drake 2007; Ramenofsky and Wingfield 2007).

Holistically, Drake et al. (1995) describe migration in terms of a ‘migration system’ (Figure 1.1). This system comprises 1) a migration arena representing the environment; 2) a migration syndrome representing the suite of traits (behaviours, physiology, locomotion and responses to environmental cues) that result in migratory activity; 3) the genetic complex that causes migration; 4) a population trajectory that makes up the migratory route, and the life history milestones that occur during migration (e.g., spawning). The expression of genotypes leads to the migration syndrome, which in turn results in the population trajectory *via* migratory behaviours. The population trajectory shapes the genetic complex through the process of natural selection. The migration arena (i.e., the abiotic and biotic factors of the environment) influences the expression of genotypes, the migratory behaviours and natural selection, and the population trajectory influences the environment (e.g., resource abundance) (Figure 1.1). Broadly, this thesis focuses on how the variation among individuals may shape the population trajectory due to differences in migratory syndromes and influences from the migration arena. Specifically, this thesis attempts to validate the link between the migration arena and migratory behaviours.

Pacific Salmon

One iconic fish that undertakes annual migrations is the Pacific salmon (*Oncorhynchus* spp.). Pacific salmon are a genus within the family Salmonidae and consist of 13 species, 7 of which inhabit North America (Quinn 2005). The genus is

known for its life cycle which broadly is: *anadromous*, a life-history pattern that implies fish spawn in freshwater and migrate to the ocean to grow and mature; *semelparous*, meaning fish die after spawning once; and most salmon are capable of *homing* to their natal streams to spawn. Each species of Pacific salmon exhibits (to a varying degree) these life-history traits (Quinn 2005). Specifically, adults return to natal streams where spawning occurs and eggs are fertilized (Figure 1.2). Alevin are hatched and develop into parr. After varying lengths of time, parr develop into smolts, which are adapted for salt water. Migration to the ocean environment occurs at this time. Once in the open ocean, juvenile fish begin to feed and grow. This stage can encompass 3 to 5 years and ends once the fish has begun to reproductively mature. At this time, the adult fish returns to its natal stream where it will spawn and die (Quinn 2005). The migrations require an integration of both behaviour and physiology (Hinch et al. 2006). There are many natural challenges fish face during the migration, including variable water flows, predators, disease and the transition between saltwater and freshwater. Collectively, adult Pacific salmon embark on annual migrations from the North Pacific Ocean to the freshwater rivers and streams of northwestern North America.

Annually, millions of adult salmon migrate to their natal streams to spawn and subsequently die. This migration plays several key roles in ecosystem function along the migration routes, as it translates to millions of nutrient-rich carcasses being transported to nutrient-poor rivers and riparian zones (Helfield and Naiman 2001). Salmon carcasses are eaten by multiple food web vectors leading to mobilization of nutrients in water and soil and serve as a significant source of marine-derived carbon

and nitrogen for the riparian ecosystem (Naiman et al. 2002; Reimchan et al. 2003; Helfield and Naiman 2006). Humans directly benefit from the salmon migration too, as many First Nations, recreational, and commercial fisheries operate on many rivers. For example, estimates for fish returning to the Fraser River (British Columbia, Canada) fishery in 2004 ranged from 3,000,000 to 9,000,000 (Pacific Salmon Commission 2008) representing a significant exploitable natural resource.

Since the early 1990's, the number of Pacific salmon returning to the Pacific Northwest (North America) has been declining. The reasons for the dwindling return of salmon have been widely debated by academics, managers, and fishers (Lackey 2003). A combination of poorly understood factors including oceanic conditions, fisheries over-harvesting, farming and ranching practices, hydropower developments, flood control, water diversions, hatchery production, habitat degradation, predation, competition with exotic fish species, and diseases and parasites represent the plethora of possible interacting variables causing the decline of Pacific salmon returns to the Pacific Northwest (Lackey 2003). To properly mitigate these problems and to progress to sustainable management practices, each of the factors must be examined.

Hydropower

Of the above factors influencing the returns of Pacific salmon, hydropower development and regulated rivers represent a significant research focus for scientists, as numerous studies have been conducted that relate hydropower infrastructure and operational strategies to negative impacts on salmon populations and communities in general (Murchie et al. 2008). The use of hydropower to meet growing energy

demands is on the rise, and transcends continents (Yüksel 2007). Over 50 % of the world's freshwater habitats have been impacted by hydropower infrastructure and/or operations (Rosenburg et al. 2000). Hydropower facilities typically use a diversion method (e.g., containment dam, penstock, etc.) that diverts flowing water through power generating turbines. Operational strategies, such as 'hydropeaking' are often used to control water release to ensure water flow for energy production at peak energy use hours. Globally, the use of hydropower facilities to generate electricity is increasing because it is seen as a 'clean' and safe energy source, as there are no fossil fuel requirements, and no hazardous waste byproducts. Regardless, hydropower infrastructure and operations can influence fish communities, populations, and individuals, particularly for Pacific salmonids, which are at increased risk due to their migratory behaviours (Riede 2004).

To negate the impacts of hydropower developments on migratory fish, scientists must first understand how migratory fish, like salmon, interact with hydropower infrastructure and operations. Most studies have quantified community, population, and individual responses to either changes to the natural flow regime (Poff et al. 1997), or to obstructions and fishways (e.g., Gehrke et al. 1995; Anderson et al. 2006). However, suborganismal and organismal responses have largely been ignored (Murchie et al. 2008).

Conservation Physiology

Conservation physiology is a nascent discipline that combines physiology and conservation science. The premise of conservation physiology is that physiological

tools can be used to assess the suborganismal responses to conservation issues such as hydropower development (Wikelski and Cooke 2006). Wikelski and Cooke (2006) broadly define conservation physiology as “the study of physiological responses of organisms to human alteration of the environment that might cause or contribute to population declines”. The term ‘physiology’ in this context encompasses whole-organism functions such as metabolism, thermal relationships, nutrition, and changes to endocrine responses, and immune parameters caused by environmental change (Wikelski and Cooke 2006). The discipline is couched in the physiological and behavioural responses of organisms to environmental stress and uses the three concepts of ‘allostasis’ (the maintenance of homeostasis through change), ‘allostatic load’ (stress from normal activities that an organism can cope with up to a limit), and ‘allostatic overload’ (a state in which organisms can no longer deal with external stresses) described by McEwen and Wingfield (2003). Overall, conservation physiology provides integrated measures of suborganismal stress due to environmental change that conventional studies (studies that focus on population outcomes) may overlook. Furthermore, conservation physiology is relevant to managers as it may offer solutions for fisheries problems (Cooke and O’Connor 2010).

One effective tool for understanding the conservation physiology of animals is telemetry (Cooke et al. 2004a). Telemetry enables researchers to track the behaviour and survival of tagged animals in their natural habitat. Subsequently, telemetry can be coupled with numerous other techniques to study many interdisciplinary topics, including migration biology and physiology (Cooke et al. 2004a). Pacific salmon

researchers have long used telemetry and its associated methods to reveal the biology of migration, including coupling telemetry with conservation physiology techniques (Cooke et al. 2008a,b). In this respect, by combining river-wide fish movements with fish physiology at the time of freshwater migration, insights into the possible mechanisms that drive migration and migration success/failure can be addressed. Recent studies have focused on the physiological and energetic limitations that adult Pacific salmon experience during their spawning migration (Cooke et al. 2008b).

In terms of energetics, migrating Pacific salmon have three types energetic stores available for fueling activity, growth and maintenance, and reproduction (Idler and Clemens 1959; Brett 1995). The first fuel that is utilized at the start of migration is fat (Idler and Clemens 1959). Fats are burned during the early migration to fuel locomotory activity and when the spawning migration is short (< 800 km) will be burned until close to the spawning grounds (Idler and Bitners 1958). Protein (mostly white muscle; red muscle is conserved) will be burned as the fats are exhausted and will further fuel locomotory activity and mature gametes (Idler and Bitners 1958; Mommsen et al. 1980). Amino acids are for the most part are converted to glucose to fuel movement. Finally, once the fats and proteins are exhausted, glycogen is burned to sustain spawning behaviours and burst activity (Brett 1995). As each of the fuel stores are broken down, the blood is used to mobilize the ions, dietary minerals, lipids, amino acids, and proteins to the reproductive tissue and the red muscle. Thus, blood parameters are indicative of the undergoing processes and not the available endogenous stores.

To empirically understand how physiological processes affect migratory salmon, non-lethal blood samples are often used (e.g., Cooke et al. 2008a,b). Few studies exist that have outlined baseline data of this nature for Chinook salmon. In 1971, Snodgrass and Halver sampled tissues from numerous life stages of Chinook salmon and found adults in freshwater had mean sodium blood plasma concentrations of $152 (\pm 10, \text{SD}) \text{ m-equiv}\cdot\text{L}^{-1}$ which was significantly below levels of sodium found in Chinook salmon in seawater. Likewise, Snodgrass and Halver (1971) found that magnesium and calcium was decreased in adults in freshwater as well when compared to adults in seawater ($1.81 [\pm 0.14] \text{ m-equiv}\cdot\text{L}^{-1}$ and $5.77 [\pm 0.7] \text{ m-equiv}\cdot\text{L}^{-1}$, respectively). The same study found potassium was greatly increased in freshwater adults ($1.6 [\pm 0.7] \text{ m-equiv}\cdot\text{L}^{-1}$) when compared to adults in seawater. difference between circulatory and tissue bourne. Plasma phosphorous was not measured; however, in seawater and freshwater Chinook salmon it did not differ in all other tissues sampled (brain, heart, muscle, liver, kidney, and gills; Snodgrass and Halver [1971]). An early study by Robertson et al. (1961) found that glucose in migrating spring-run Chinook salmon was $11.65 \text{ mmol}\cdot\text{L}^{-1}$, cholesterol was $19.5 \text{ mmol}\cdot\text{L}^{-1}$, and total protein ranged from 4.31 to 5.8 gm/100 ml. All three values were significantly lower in spawning fish. The above two studies represent the most through papers on baseline blood parameters in migrating Chinook salmon. In general, the authors attribute much of relationships to osmotic changes due to the transition from seawater to freshwater (Parry 1961) and starvation (Prakash 1962).

The spawning migration for salmon is physiologically and energetically challenging because fish must transition from a saltwater environment to a freshwater environment, fish have ceased feeding and must rely solely on endogenous energy stores, and the rivers that fish are migrating through offer a variety of natural and anthropogenic obstacles (e.g., constricted waterways, poor water quality, unpredictable flows, shoreline developments, etc.; Brett 1995; Quinn 2005).

Coupling telemetry with physiological sampling can help to better understand the challenges that migrating adult salmon are exposed to (Cooke et al. 2005a). Recent work that linked watershed-wide fish movements to gene expression arrays has suggested that fish physiology at sea and at the spawning area may predict spawning success/failure in Fraser River sockeye salmon (Miller et al. 2011). Other work on sockeye salmon has found that energetic status (Young et al. 2006b), as well as energy use may also influence the spawning migration (Hinch and Rand 1998; Crossin et al. 2004a,b). Elevated blood-borne stress indicators such as cortisol, plasma glucose, and plasma lactate have been found in salmon that fail to migrate into the freshwater environment (Cooke et al. 2005b, 2006). Fish that die in the freshwater environment have had poor osmoregulatory capability (Cooke et al. 2006) and been characterized as having elevated plasma lactate (Young et al. 2006b). Reproductive hormones have been shown to also relate to spawning success (Young et al. 2006b). Increasing water temperature has also been implicated as a likely reason for declining salmon stocks and has an additive stress effect on the physiological ecology of migrating salmon (Farrell et al. 2008). Much of the work on the physiological ecology of migrating salmon has been undertaken in the Fraser

River on a number of Pacific salmon species (reviewed in Hinch et al. 2006 and Cooke et al. 2008b). Important to this thesis, the Fraser River is among one of the largest rivers in North American and has no hydropower developments along its main stem.

Studies on Pacific salmon migration in the hydropower-developed Seton River (a tributary of the Fraser River) have assessed the physiological ecology of migrating salmon near their spawning grounds. Biosampled and telemetered sockeye salmon were found to not use burst swimming during passage through a fish ladder and unsuccessful fish had lower plasma Na^+ levels (Pon et al. 2009a). In a similar study, physiological condition and energetic status of sockeye salmon did not differ during three different flow conditions at the Seton River hydropower facility (Pon et al. 2009b). Mortality upstream of the Seton hydropower facility was found to increase when fish were released below the dam, as opposed to above the dam (Roscoe et al. 2010a). Interestingly, though fish were biosampled in the study, no physiological variable was found to predict spawning success/failure. With the exclusion of the above-cited papers, no other studies have focused specifically on the physiology of salmon migration through hydropower-impacted rivers; however, work completed on the Columbia and Snake River in the USA (both large hydropower-impacted rivers) have greatly progressed our knowledge of salmon migration in human altered environments.

A number of studies completed on the Columbia and the Snake River have sought to understand the importance of the environment and hydropower facilities on the spawning migration of salmon. Major findings on these rivers are slow passage

rates at hydropower facilities (Keefer et al. 2004; Naughton et al. 2004; Goniea et al. 2006; Caudill et al. 2007), altered energy use near facilities (Brown et al. 2006), increased mortality during the freshwater spawning migration (Keefer et al. 2005, 2008a, 2010) changes in behaviours due to increasing temperature (Goniea et al. 2006; Keefer et al. 2008a,b) and altered flow regimes (Tiffan et al. 2010). Suggested reasons for these findings are premature depletion of energy stores, overall physiological condition and the cumulative effects of passing multiple hydropower facilities during the spawning migration (Naughton et al. 2004; Naughton et al. 2006; Caudill et al. 2007).

Goals

The purpose of this thesis is to gain insight into the basic biological responses of an imperiled stock of Pacific salmon known to have spawning migration failure possibly due to hydropower infrastructure and operations. This thesis stems from the need for hydropower utilities to effectively manage fish populations in impacted river systems. The Canadian federal government's department in charge of aquatic habitat and fish (i.e., Fisheries and Oceans Canada) mandates that utilities must make habitat improvements and/or support hatcheries to ensure the sustainability of Canada's aquatic ecosystems. Furthermore, Canada and many other developed/developing countries are increasing their reliance on hydropower to meet growing energy demands. It is for this reason that there is a need to better understand how this infrastructure may influence vulnerable fish populations. A stock of Chinook salmon that dwells in the a hydropower-impacted Puntledge River on Vancouver Island has

seen marked decreases in returning adults despite the utility and the government supporting several habitat enhancements and a hatchery on the river. It has been hypothesized that suborganismal physiology and variations among individual behaviours may be leading to the migration failure. As such, my co-authors, in collaboration with the utility and government and local scientists, and myself completed a three-year study to understand possible biological mechanisms for the decline in returning adults.

To address my objectives this thesis has been organized into 5 manuscript chapters: 1) a review of conservation physiological tools that could be used in hydropower-impacted settings; 2) three chapters focused around the biological responses of the migrating Chinook salmon in the Puntledge River; and 3) a chapter on the effectiveness of a management directive. To begin with, I have thoroughly reviewed the physiological tools that I have used in this thesis, as well as several other tools, to address the need for researchers and managers to incorporate suborganismal biology when addressing questions associated with hydropower infrastructure and operational strategies (Chapter 2). I then use the hydropower-impacted Puntledge River and its summer-run Chinook salmon to address several biological and hydropower related reasons for the decline of the stock (Chapters 3-5). The goal of Chapter 3 was to assess the migratory behaviours of the stock and to relate the behaviours to the physiological condition of fish near the time of their river entry. The goal of Chapter 4 was to understand the river temperatures that are experienced by fish migrating through the hydropower-impacted portion of the river and to model possible effects of different migratory strategies on energy stores. The

goal of Chapter 5 was to quantify activity patterns of tagged fish within the hydropower-impacted reach, and to relate variation in activity to habitat and environmental characteristics. Lastly, I assessed the outcome of a change in hydropower operations to influence the movement of returning adult fish (Chapter 6). In Chapter 7, I discuss the specific findings of my research as it relates to the physiological ecology and conservation of the Puntledge River summer-run Chinook salmon. Furthermore, I explain the overall contribution of this thesis to fisheries management with respect to hydropower research, and the contribution of my research to the understanding of the migration biology of Pacific salmon. In addition, I suggest how future developments could utilize the knowledge of biological responses to operate in a manner that promotes healthy Pacific salmon populations in regulated rivers.

Study Location

The Puntledge River is located near Courtenay, British Columbia, Canada on the east shore of Vancouver Island. The river begins at the northeast outlet of Comox Lake and drains part of the Comox Watershed into the Tsolum River, which flows into the Comox Estuary (Figure 1.3). The Puntledge River is known for great fly-fishing for returning Pacific salmon and trout, and during the spring run-off, the river has world-class kayak flows. The river has stocks of Chinook (*O. tshawytscha*), chum (*O. keta*), pink (*O. gorbuscha*), sockeye (*O. nerka*), and coho (*O. kisutch*) salmon and cutthroat (*O. clarkii*) and rainbow trout (*O. mykiss*). Other species that reside in the river

include coastrange sculpins (*Cottus aleuticus*), three-spined sticklebacks (*Gasterosteus aculeatus*) and Pacific lamprey (*Lampetra tridentate*). Recently, a freshwater diatom (*Didymosphenia* sp.) that forms mats on the river bottom has invaded the river.

In 1912, Canadian Collieries Ltd. installed a hydroelectric development that was later sold to British Columbia (BC) Power Commission (now known as BC Hydro) in 1953. The facility consists of infrastructure located at three main locations on the river. The northeast outlet of Comox Lake has a dam that is used to store water. Approximately 3.7 river (r) km downstream there is a dam that diverts water away from the natural course of the river and into a ‘penstock’ (i.e., a long tube). The penstock is released at the 6.8 rkm downstream through the turbine located at the power generation facility. The river reach between the diversion dam and the penstock outlet is known as Reach C and is 7.2 rkm in length (Figure 1.4).

Within Reach C there are a number of areas of difficult passage for returning Pacific salmon, specifically the summer-run Chinook salmon (Mazeaud et al. 1977; Taylor and Guimond 2004). Moving in the upstream direction, the first area of difficult passage is the turbine outlet (known as the ‘Powerhouse Pool’; Figure 1.4, 1.5). This area has a swirling deep pool that has been known to be suitable habitat for holding summer-run Chinook salmon (Taylor and Guimond 2004); however, fish may hold here for considerable time periods. The next area that has been known to cause delays in fish movement is a stretch of river that has a three-tiered waterfall known as Stotan Falls (Figure 1.4, 1.6). The area is roughly 300 m in length and has three blasted-rock fish stairs at each waterfall. These stairs were constructed in 1977

with the goal to reduce holding times at the falls areas. A study by private consultants found that the Stotan Falls complex may cause ~ 30 % of failure to migrate upstream (Taylor and Guimond 2006). The furthest upstream area of difficult passage in Reach C is another three-tiered waterfall complex known as Nib Falls (Figure 1.4, 1.7[upper panel]). This site is similar to the Stotan Falls complex, as it also has three blasted-rock fish stairs at the waterfalls (Figure 1.7 [lower panel]). The upstream end of Reach C is bound by the diversion dam and there is a fish ladder located there that is controlled by the Puntledge River Salmon Hatchery, Fisheries and Oceans Canada (Hirst 1991). Furthermore, in order to benefit upstream fish movement at the areas of difficult passage, BC Hydro has implemented controlled water releases at the diversion dam to increase water flow in Reach C (BC Hydro 2004).

Study Animal

Chinook salmon (also known as king salmon, “tyee” [fish > 13.6 kg], and spring salmon (Scott and Crossman 1973; Figure 1.8) were the species used in this thesis. The species name is *O. tshawytscha* (Walbaum) and is derived from the Russian common name. Chinook salmon are distributed throughout the Pacific Northwest in North America, and throughout rivers in Northeast Russia. The species is most closely related to coho salmon and are grouped with the other Pacific salmon (sockeye, chum, and pink; Utter et al. 1973); however, the coho-Chinook pair may be more closely related to the cutthroat-rainbow trout group (Utter and Allendorf 1994). The species is the largest of the Pacific salmon (839-915 mm; Scott and Crossman 1973) and is characterized by spots on the upper and lower lobe of the tail, on the

back, and by a black gum line (Quinn 2005). All Chinook spawn in the fall, but seasonal runs are typical (i.e., fall- and summer-runs). Fall-runs of fish migrate into the natal river during the fall season and spawn, whereas summer-runs return to the natal river in the late spring and summer before spawning in the fall (Quinn 2005).

The focus of this thesis is on the vulnerable Puntledge River summer-run of Chinook salmon. The stock has declined from 4500 individuals historically, to fewer than 1600 returning adults (Darcy Miller, Personal Communication, Fisheries and Oceans Canada). As a result, stakeholders have constructed a fish hatchery on the river to increase juvenile fish stock size and numerous habitat enhancements have been completed on the river to improve fish passage and spawning grounds. The power utility is also participating and experimenting with base water flows to improve river characteristics for returning adult Chinook salmon and outgoing juvenile fish (BC Hydro 2004).

Figure 1.1. The migration syndrome (adapted from Drake et al. 2005).

Figure 1.2. A depiction of the Pacific salmon lifecycle (Fraser Salmon and Watersheds Program, www.thinksalmon.com).

Figure 1.3. A map of the Puntledge Watershed with key locations and the study area labeled (borrowed with permission from Project Watershed).

Figure 1.4. A map of the study area. The area is known as Reach C in all reports by BC hydro

Figure 1.5. A photo of the outlet of the penstock at the Powerhouse (also known as the Powerhouse Pool; photo credit, C.T. Hasler)

Figure 1.6. An aerial photo of the Stotan Falls complex (upper panel; photo provided by E. Guimond) and a photo of the second blasted rock fish stair at Nib Falls (lower panel; photo credit, C. Dey).

Figure 1.7. An aerial photo of the Nib Falls complex (upper panel; photo provided by E. Guimond) and a photo of the second blasted rock fish stair at Nib Falls (lower panel; photo credit, C.T. Hasler).

Figure 1.8. A photo of a Puntledge River summer-run Chinook salmon being implanted with a transmitter (photo credit, C. T. Hasler)

Chapter 2: Expanding the “toolbox” for studying the biological responses of individual fish to hydropower infrastructure and operating strategies

Abstract

To date, few studies have evaluated suborganismal responses (e.g., physiological or energetic consequences) of individual fish to hydropower infrastructure (e.g., fishways, turbines) or operations (e.g., fluctuating flows, low flows). The field of “conservation physiology” (i.e., the use of physiological information to enhance conservation) is expanding rapidly and has great promise for hydropower research. However, there is a need to both expand the “toolbox” available to practitioners and to validate these tools for use in this context. This synthetic report details the behavioural, energetic, genomic, molecular, forensic, isotopic, and physiological tools available for studying suborganismal responses of fish to hydropower infrastructure and operating procedures with a critical assessment of their benefits and limitations. Furthermore, this paper provides two case studies where behavioural, energetic, and physiological tools have been used in hydropower settings. Progressive and interdisciplinary approaches to hydropower research are needed to advance the science of sustainable river regulation and hydropower development. The expanded “toolbox” could be used by practitioners to assess fishway performance, migration delays, and fish responses to fluctuating flows through a more mechanistic approach than can be offered by only focusing on population metrics or indices of community structure. These tools are also relevant for the

evaluation of other anthropogenic impacts such as water withdrawal for irrigation or drinking water, habitat alteration, and fisheries interactions.

Introduction

Globally, hydropower facilities contribute 17 % of the electricity needed by humans, and further developments are planned to meet the growing demand for clean energy (Yüksel 2007). In fact, over 50 % of the Earth's freshwater river systems have been altered for the generation of electricity (Rosenburg et al. 2000). Though hydropower is considered a clean and safe power source in comparison to fossil fuel and nuclear facilities, the infrastructure and operation of power generation facilities can have profound impacts on aquatic environments and the organisms that dwell in them. Given the importance of fish and fish habitat and the fact that freshwater fish represent some of the most threatened animals (Richter et al. 1997; Cowx 2002; Malmqvist and Rundle 2002; IUCN 2008; Jelks et al. 2008), there is a need to better understand how hydropower infrastructure and operations alter fish and fish habitat so that more appropriate guidelines can be developed to ensure the sustainability of the hydropower industry.

Hydropower infrastructure and the operational strategies used by power utilities have the potential to fundamentally change the local aquatic environment. These changes and their effects can be grouped into two main research topics: 1) *altered flow regime*, that encompasses numerous changes to river characteristics related to the amount of water present and its movement (Poff et al. 1997); and 2) *barriers and connectivity*, which include the hydropower infrastructure that prevents upstream and downstream

movement of fish and the constructed passage facilities for fish designed to make hydropower infrastructure as “invisible” as possible to fish. Because both of these topics have been reviewed elsewhere (e.g., Poff et al. 1997; Murchie et al. 2008; Roscoe and Hinch 2010), we will not provide an in depth review of these topics, but we will incorporate relevant research into our ‘tool’ summaries where appropriate. In addition, there are other associated processes that can lead to similar changes to the local aquatic environment and necessitate similar research needs, such as water withdrawal, flood control, and temperature regulations. However, for the purpose of this paper, we will focus solely on hydropower infrastructure and operations, but readers should note similar research needs and opportunities exist elsewhere.

Much of the research on hydropower impacts on fish has focused on evaluating changes in community structure or population size by sampling individual fish (Murchie et al. 2008). However, the response times can be lengthy, often extending beyond the monitoring periods required by regulatory agencies. In a recent synthesis, Murchie et al. (2008) revealed that few studies have evaluated suborganismal responses of fish to fluctuating flows associated with hydropower infrastructure, despite the fact that investigation into these effects can provide information on time scales that are consistent with most monitoring programs (Cooke and Suski 2008). In addition, it was apparent that few studies had included physiological or energetic endpoints (aside from several studies on the osmoregulatory consequences of dams on downstream migrants) or linked individual condition with behaviour and fate in a hydropower context. This is somewhat surprising as understanding organismal function and life history traits has been identified as an integral component of conservation and management initiatives (Young et al.

2006a). Also, the field of “conservation physiology” (i.e., the use of physiological information to enhance conservation [Wikelski and Cooke 2006]) is expanding rapidly and has great promise for research into the impacts of hydropower. Indeed, much of this work is possible because of the advent of tools that enable physiological research to take place outside of the laboratory. This sub-discipline is called “field physiology” and to date, has tended to focus on larger animals such as marine mammals and terrestrial vertebrates (see Costa and Sinervo 2004). However, there is a need to both expand the “toolbox” available to fisheries practitioners and to validate these tools for use in fish-hydropower research. In addition, for this toolbox to become practical in hydropower research, physiological endpoints need to facilitate regulatory action and/or decision-making by utilities and regulators.

The objective of this paper is not to present an overview of the impacts of hydropower on fish, but to summarize twelve types of tools that show promise for research on fish and hydropower interactions. First, we provide a brief summary of the current state of knowledge regarding the tool and its usefulness in fisheries science and environmental management. Second, we assess whether the tool is useful for studying individual and suborganismal responses to hydropower and discuss the benefits and limitations of using these techniques for the evaluation and monitoring of hydropower impacts on fish and fish habitat. Finally, we present two case studies to illustrate how the inclusion of individual-based information into hydropower research had helped to incrementally enhance the understanding of complex fish and hydropower issues.

Research tools for assessing possible individual level effects of hydropower infrastructure and operations

To assess the impacts of hydropower infrastructure and operations on individual fish, tools that measure suborganismal responses should be developed and validated to measure physiological changes that have the potential to be harmful to fish. Currently, tools developed to measure gene expression, heat shock protein expression, enzymatic activities, sensory physiology, and stable isotopic ratios have not been widely applied to research involving hydropower systems. Likewise, tools designed to quantify biomolecules like ions, lipids, hormones, and metabolites in fish tissue have been rarely applied to hydropower settings (with some exceptions, e.g., Wagner and Congleton 2004). Other tools such as physiological telemetry, forensic techniques and conventional measures of condition have also not been exploited to their full potential. Here, we outline several investigative tools to measure the individual and suborganismal responses to hydropower infrastructure and operations, as well we suggest how they could be contextually used (Table 2.1). Each tool is introduced by way of a general overview and is then discussed with respect to its actual or potential role for the study of individual and suborganismal responses of fish to hydropower impacts.

Genomics

Overview

Genomics is the study of whole genome patterns and regulation of genes and implies the use of high throughput DNA- or RNA-based methods, often involving costly microarrays, and bioinformatic approaches (Wenne et al. 2007). More simply put, this

tool uses computer analysis to determine the array of genes that are either up- or down-regulated in a given tissue sample. There are three main types of genomic studies: comparative, functional, and environmental. Comparative genomics refers to the study of whole genomes and their gene content. Functional genomics relates to the biochemical and physiological role of gene products. Environmental genomics encompasses the molecular variation in populations and across taxa, especially in response to environmental conditions. Whole genome sequences exist for multiple model species (e.g., zebrafish [*Danio rerio*], fugu [*Takifugu chrysops*], and stickleback [*Gasterosteus aculeatus*]). However, only partial sequences exist for species at a greater risk to be effected by hydropower (e.g., Atlantic salmon [*Salmo salar*], rainbow trout [*Oncorhynchus mykiss*]; Thorsen et al. 2005; Palti et al. 2004; Wenne et al. 2007). Genomic studies involving fisheries management have typically been undertaken to discriminate between different populations, assess immune response and disease susceptibility, and to investigate the effects of chemical contaminants and life history strategies on gene expression (e.g., Koskinen et al. 2004; Aubin-Horth et al. 2005a,b; Hook et al. 2006; Purcell et al. 2006; Roberge et al. 2006; Fisher and Oleksiak 2007). A recent study by Miller et al. (2007) found that genomics could be used to distinguish between successful and unsuccessful spawning salmon, both near the time of river entry and on the spawning grounds. To date, no studies have directly applied genomic techniques to fish exposed to a hydropower-related stressor.

Potential

Genomics could be used in a hydropower setting to develop biomarkers to predict if fish can successfully navigate through a hydropower impacted system, and to assess genome level responses (up- or down-regulation) to hydropower related stressors (for studies associated with the impact of hydropower on genetic variation please see Fagan 2002; Meldgaard et al. 2003; Alo and Turner 2005; Jager 2006). For instance, recent work by Miller et al. (2007) aims to develop predictive biomarkers for fate and entry times of Pacific salmon (*Oncorhynchus spp.*) by using cDNA microarrays from multiple tissues. If these techniques are to be used to understand gene expression of fish that successfully pass through hydropower infrastructure and deal with variable hydropower operations, it may be possible to understand, at a very basic level, the predictive biomarkers for successful navigation through a hydropower setting.

Other ways in which genomics could be used to assess the impact of hydropower infrastructure and operations on fish are to analyze the gene-level responses to abiotic and biotic stressors. Little is known about the genetic mechanisms present in fish to deal with acute changes to multiple environmental variables. Some recent studies have measured gene expression in relation to changes to habitat. For example, San Martín et al. (2007) quantified the expression of the prolactin gene receptor in response to seasonal changes to habitat in carp (*Cyprinus carpio*). In another study, Ju et al. (2007) found that a number of genes were up-regulated and down-regulated in Japanese medakas (*Oryzias latipes*) exposed to hypoxia. As hydropower infrastructure and operations can result in many changes to the surrounding habitat, genomic tools may be an effective way to assess the impact of these changes on fish. However, there is still a need to develop

species-specific tools, as well as link changes in gene expression to facility operations, before wide-use of genomic techniques in hydropower settings will be effective.

Heat Shock Proteins

Overview

Heat shock proteins, also known as stress proteins, are a unique group of highly conserved cellular proteins that are found in living cells of animals (see reviews by Iwama et al. 1998; 1999; and Basu et al. 2002). Briefly, there are two types that are primarily studied in fish, Hsp70 and Hsp80. Hsp70 is most often used in fisheries research, although more studies are needed to validate its use as a stress indicator, as it is not expressed in some cold water species (Barton et al. 2002; Iwama et al. 2004; Zakhartsev et al. 2005). Heat shock proteins are up-regulated in cells exposed to environmental (e.g., adverse levels of metals and contaminants, chemical toxins, physical and chemical water properties, and natural change), pathophysiological (e.g., crowding, confinement, etc.) and biological stressors (e.g., pathogens, parasites, etc.). Consequently, heat shock protein expression is a possible indicator of stress. However, heat shock proteins are still considered a developing research tool and, the methodology is fairly intensive (i.e., Western blotting or Enzyme-Linked ImmunoSorbent Assay). Heat shock protein expression has never been used in a hydropower setting, but represents a promising tool for quantifying the possible effects of hydropower infrastructure and operating strategies on the cellular stress of individual fish.

Potential

Studies aimed to understand the sublethal effects of crowding near fish passageways, the use of side-channel refuge habitat, and on individuals exposed to pulse flows may benefit greatly by using heat shock protein expression tools. Coupling heat shock protein expression techniques to local environmental monitoring and habitat assessment would allow researchers to bridge the gap between hydropower impacts and the stress response in fish. Certain attributes of heat shock proteins make them amenable to this type of research. To start, heat shock proteins are expressed in numerous tissues (blood, muscle, liver, fin). Another essential intrinsic characteristic of heat shock protein expression is that there are minimal effects on expression when fish are handled (Vijayan et al. 1997). Furthermore, quantification of heat shock protein expression permits the indication of only definitive stressors; as heat shock protein expression likely occurs in a threshold manner, rather than in a graded way (Currie and Tufts 1997; Vijayan et al. 1998).

Enzymatic Activity

Overview

Measuring the rates of enzymatic reactions involves the quantification of a particular rate limiting enzyme or reagent. With the development of commercial assay kits and spectrophotometers, enzyme activities can be measured with relative ease, though some can be costly. Some disadvantages of using enzymatic activities is that typically a variety of enzymatic activities need to be measured to fully understand the processes being measured, and results may be influenced by a number of endogenous and exogenous factors (McDonald and Milligan 1992; Wagner and Congleton 2004). Activities of a number of enzymes have been used to measure tissue damage (e.g., Wagner and

Congleton 2004; Morrissey et al. 2005), energetics (e.g., Garenc et al. 1999; Martínez et al. 2003; Kaufman et al. 2006), growth (Pelletier et al. 1995; Lamarre et al. 2004; Imsland et al. 2006), smoltification (e.g., Vanderkooi et al. 2000), and the effects of pollution (e.g., Ahmad et al. 2006; Tejeda-Vera et al. 2007).

Potential

Measuring enzymatic activities of fish in hydropower settings can be used for a number of applications, although perhaps the most relevant use is the measurement of tissue damage. When cells are damaged or die, the intracellular enzymes are released into the blood. By measuring the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) in the blood, inferences to the magnitude and type of tissue damage can be made (Grizzle et al. 1992; Wagner and Congleton 2004; Morrissey et al. 2005). In hydropower settings, tissue damage in fish can occur in passageways, turbines, or during high flow conditions.

Other ways in which the measurement of enzymatic activities would be useful in a hydropower setting would be to evaluate energetics, growth, osmoregulatory preparedness (i.e., smoltification for downstream migrating salmonids and preparedness for freshwater migration in upstream migrants), and pollution. By measuring LDH (an enzyme used in anaerobic metabolism to convert lactate to pyruvate and *vice versa*; and LDH is an indicator of burst swimming capacity [Garenc et al. 1999; Martínez et al. 2003]), Kaufman et al. (2006) were able to judge how active walleye (*Sander vitreus*) were in different habitats with varied food resources. It is possible that fish exposed to variable flows or in areas with fish ladders could have elevated levels of LDH. Growth is

another process that could be measured using enzyme activity (Pelletier et al. 1995; Lamarre et al. 2004). Imsland et al. (2006) found that growth positively correlated with pyruvate kinase (PK) and LDH (both indicators of glycolytic activity) in spotted wolffish (*Anarhichas minor*). Enzymes such as PK and LDH could potentially be used to compare growth rates between reference sites and areas affected by hydropower development. Smoltification is the process of adaptation to a marine environment (measured using Na^+/K^+ -ATPase; Zaugg and McLain 1972; Giles and Vanstone 1976). In fact, the measurement of Na^+/K^+ -ATPase activity has been used in several studies associated with hydropower impacts (Giorgi et al. 1988; Tiffan et al. 2000). Giorgi et al. (1988) found a relationship between gill Na^+/K^+ -ATPase activity and their susceptibility to guidance through gateways at dams located on the Snake-Columbia River system. Tiffan et al. (2000) found no link between travel time through a hydropower impacted stretch on the Columbia River and gill Na^+/K^+ -ATPase activity. In general, enzymatic activities can offer very direct measurements of the effects that hydropower infrastructure and operations have on fish.

That being said, there are numerous reasons why enzymatic activities may change near a hydropower facility. It will be important to validate field studies by using multiple tools from our proposed toolbox, researchers should be able to predict the causes of enzymatic activity change. For example, lactate (a metabolite indicative of LDH activity) may be elevated following severe exercise (Garenc et al. 1999; Martínez et al. 2003), in resting fish within close proximity of each other (Pickering and Pottinger 1987), or following tissue damage (Wagner and Congleton 2004). To distinguish the cause of the change in enzyme activity, biotelemetry could be used to understand if the change in

lactate is due to activity rates (see Case Study 1), and forensic techniques could be used to assess epithelial tissue damage (Colotelo et al. 2009).

Sensory Neurophysiology

Overview

Sensory neurophysiology concerns the study of how animals perceive and react to the ambient environment using their nervous system. However, little is known about the interactions between the structure of the sensory organs of fish, the environmental cues that fish are subjected to while moving, and the behavioural decisions fish adopt particularly when they encounter barriers. With reference to fish passage, sensory physiology can yield information on the roles of visual, auditory/mechanical (including the lateral line), and tactile (including temperature sensors) sensory systems in fish movement (Hara and Zielinski 2006). For example, scientists can understand lateral line mechanosensory ability by observing the neural response of superficial neuromasts to changing water velocity (Engelmann et al. 2000). Ablated superficial neuromasts have been shown to have a strong connection to rheotactic behaviour across several fish species (Montgomery et al. 1997) and it is likely that rheotactic behaviour has an important role in successful passage by fish through fishways.

Tools that researchers can use to measure sensory neurophysiology vary widely.

Manipulation studies can be completed by altering the sensory ability of fish. For example, Partridge and Pitcher (1980) blinded fish by creating temporary blindfolds using painted film, and altered lateral line function by cutting the lateral line.

Observation and measurements of superficial neuromasts can be done by fixing tissue for

view under scanning electron microscopes (e.g., Marshall 1996). While, Chagnaud et al. (2006) connected electrodes to the lateral line of gold fish and recorded afferents caused by stimulations made by a steel rod using an electrode amplifier connected to a digital audio tape recorder.

Potential

Understanding the sensory physiology associated with fish movement has great potential for the design of behavioural guidance technologies. By measuring lateral line sensory ability and fish swimming muscle activity, engineers may be able to construct fish passageways that guide fish through hazardous areas and making hydropower developments as invisible as possible to fish. By placing objects in the path of flowing water costly movement through fishways can be avoided, as fish may be able to sense and use water that decreases the cost of locomotion (Liao et al. 2003). Thorstad et al. (2003) located fish near hydropower water outflows and suggest that this may causes migration delays and could be energetically costly. By understanding how fish sense different flows, engineers may be able to prevent movement towards water outputs by developing guidance technologies that exploit lateral line mechanics or other sensory functions.

Ions and Dietary Minerals

Overview

Typically in studies aimed to quantify the magnitude or physiological consequences of stress in fish, the concentrations of specific ions (e.g., Na^+ , Cl^- , and K^+) or dietary

minerals (e.g., total calcium, total magnesium, total phosphorous) are measured to understand the secondary effects of stress (Mazeaud et al. 1977; Wendelaar Bonga 1997) and nutritional condition (Wagner and Congleton 2004); the primary response being the release of stress hormones. Plasma ions are often used to understand the ability of an organism to deal with stress responses, such as acidosis, a secondary response that is the result of lactic acid build-up caused by anaerobic metabolism (e.g., Wood 1988; Wood 1991). Following exhaustive exercise, ion concentrations may be disturbed over a period of 4 h – 24 h before returning to baseline levels (McDonald and Milligan 1997). In some cases, severe ion imbalances can lead to mortality (Wood et al. 1983; Wood 1988, 1991). Measures of plasma ion balance (plasma osmolality) have typically been used to measure the stress effects of catch-and-release angling (a practice that results in brief exhaustive exercise and subsequent release of the angled fish) on fate and physiology (e.g., Brobbel et al. 1996). Measuring dietary minerals (e.g., total calcium, total magnesium, total phosphorous) can also yield valuable information on the nutritional condition of fish (Wagner and Congleton 2004). Calcium is present in the plasma as both ionized and bound forms; however declines in plasma calcium are correlated to declines in plasma protein because the majority of plasma calcium is bound to proteins (Andreasen 1985). Magnesium may be associated with lipid metabolism (Wagner and Congleton 2004). Methods to determine ion and dietary mineral concentration are not difficult or expensive; some can even be done using handheld instruments. Measuring the secondary stress responses associated with ions and dietary minerals will give researchers a better understanding of the proximate causes of fish mortality associated with hydropower

impacts. Currently, few studies have applied measurements of plasma ion and mineral concentrations to assess fish dealing with hydropower infrastructure and operations.

Potential

In fish, periods of brief exhaustive exercise do occur in hydropower systems, as passage of fish through fish ladders is analogous to brief periods of exhaustive exercise (not always the case, for example, the Seton study; see Case Study 1) (Gowans et al. 2003). It is likely, that passage up a fish ladder results in changed levels of plasma ion concentrations, but after a recovery period, the levels of plasma ions would return to normal levels (Wood et al. 1983; Wood 1988, 1991). In many incidences, fish likely do not successfully ascend fish ladders on the first try, and must make multiple attempts to pass (Bunt et al. 1999; Moser et al. 2002; Castros-Santos 2004). Elevated ion concentrations are likely indicative of passage problems, as fish making several attempts to successfully pass likely incur a physiological cost. Managers may be able to use plasma ions and dietary minerals in a predictive model (once cause and effect are determined) to assess fish upon arrival as to whether they are ‘physiologically capable’ of successfully passing through hydropower infrastructure and dealing with hydropower operations (especially if passage is known to require multiple attempts). Dietary minerals reflective of nutritional condition could be used to evaluate habitat quality associated with restoration of altered habitats (Cooke and Suski 2008). Another possible use of ions relates to temperature, as ion concentrations tend to decrease in response to heat stress (Wood 1991) and this could be related to hydropower (stranding in side channels that get warm or warm water epilimnetic releases from dams).

Lipids

Overview

Lipids have widely varied structures and biological function. They are vital to the formation of biological membranes and are storage molecules for metabolic energy. Lipids are the primary store for energy and often are a major factor in proximate body composition. Proximate body composition is defined as a whole body energetic metric which measures the amount of lipids, proteins, organic ash, and water present in an animal and can indicate condition. Fatty acids are the simplest type of lipid and are aliphatic monocarboxylic acids that can be either saturated or unsaturated with hydrogen (further information is provided in the hormone section). In fish, triglycerides are the primary form of stored energy (Higgins and Talbot 1985) and are stored in the muscle, liver, subdermal tissue, and the mesenteries. In salmonids, triglycerides are primarily stored in the adipose tissues (Henderson and Tocher 1987). Cholesterol is another blood borne lipid that can easily be measured using non-lethal approaches and simple laboratory methods. Blood cholesterol levels indicate body energy stores. Energy stores and lipid content are important for fish health as they have been linked to overwinter survival; energy allocation strategies; reproductive performance; early life history strategies; and environmental stress response (Henderson and Tocher 1987; Jobling et al. 1998; Adams 1999). Lipid content in general can easily, and at a modest costs, be quantified using destructive extraction techniques that require relatively large pieces of tissue (usually requires lethal sampling; Bligh and Dyer 1959) or by various non-lethal electronic devices (e.g., handheld microwave energy meter, Crossin and Hinch 2005;

bioelectrical impedance analysis, Kushner 1992). Lipid analysis has only recently begun to be used in river systems altered by hydropower infrastructure and operations (Wagner and Congleton 2004; Cleary 2006).

Potential

Lipid analysis can likely be a robust tool to use in predictive models relating to fish response to hydropower infrastructure and operations because it has been shown to be indicative of numerous variables such as habitat quality, energy use, nutrition and overwinter survival. Assessments of habitat quality are imperative in understanding fish response to hydropower, and lipid analysis has revealed strong connections between habitat quality and lipid reserves in marine fish (Lloret et al. 2005). Energy use is also important in hydropower systems, as changes in flow rates can cause fish to compensate or seek refuge when faced with altered flow. It is likely that fish in an energetic demanding system would have less lipid reserves and researchers could exploit lipid analysis to judge the energetic requirements of a specific hydropower operational strategy. Lipid assessments may be especially useful in studies examining dam passage of anadromous Pacific salmon, as these species cease feeding prior to river entry and rely on energy stores to power migration, sexually mature, and spawn (Brett 1995; Magnoni et al. 2006).

Endocrine Measures

Overview

Stress hormones can be useful indicators of hydropower induced effects (Adams 1990; Wendelaar Bonga 1997). Stress hormones are endocrine response to perceived changes in the surrounding environment. For fish, stress responses occur for a number of reasons including elevated temperature, toxins, and handling (e.g., Mazeaud et al. 1977; Strange and Schreck 1978; Hontela et al. 1992; Fagerlund et al. 1995; Wendelaar Bonga 1997). Because of the stress response in fish being linked to environmental change, it is likely that hydropower impacts may be manifested in the response (see review by Fagerlund et al. 1995). Two main types of hormones are commonly used to measure stress are catecholamines and corticosteroids (Wendelaar Bonga 1997; Barton 2002). Plasma catecholamines are released rapidly in response to perceived ambient disturbance and are linked to tissue oxygen delivery and fuel mobilization for tissue metabolism. However, because catecholamines are very rapid acting, sampling fish in a manner that would be reflective of concentrations of catecholamines during the stressful event, and sampling control fish without inducing a catecholamine response would be difficult. Cortisol on the other hand may be a good indicator of the magnitude and duration of acute stress because there is a time lag between the stressor and the response (Barton 2002; Barton et al. 2002).

Reproductive hormones are typically fatty-acids secreted as thyroid hormones, growth hormones, or metabolic hormones and may also be relevant to hydropower applications. Specifically, some examples that have been studied in fish include: androgens testosterone, 11-ketotestosterone, $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one, 11-beta-OH-testosterone and 17-beta-estradiol (Brantley et al. 1993; Pankhurst 1995). Often reproductive hormones are used to link reproductive behaviours, reproductive

development, and the effects of endocrine disrupters on fish maturity (Pankhurst 1995; Arcand-Hoy and Benson 1998; McKinley et al. 1998). Quantifying reproductive hormones is well understood and can be done using a variety of laboratory techniques using small blood samples.

Potential

Cortisol and reproductive hormones are probably the most useful hormones to measure and quantify stress induced by hydropower infrastructure and operations. Cortisol has been used to link hydropower operations to suborganismal stress, as Flodmark et al. (2002) found that the amount of plasma cortisol changed due to a simulated hydro-peaking event. However, fish can become desensitized to cortisol and other variables like development stage, genetics, and environment could influence cortisol levels (Gamperl et al. 1994; Barton 2002). Other hormones that have been used to understand the impact of hydropower infrastructure are reproductive hormones such as testosterone and 17 β -estradiol (both fatty acids). McKinley et al. (1998) found that hydropower development caused delayed reproductive development because of impingement or entrainment. However, hormones can change naturally or as a result of non-hydropower related effects (Carruth et al. 2000), and so care should be used when interpreting these measures in making assessments of hydropower effects.

Metabolites and Tissue Energy Stores

Overview

With the onset of a hormonal response to stress, numerous secondary effects occur (Wendelaar Bonga 1997). Mainly, metabolites such as glucose, lactate, glycogen, and numerous other reactants and products of metabolism change to meet energetic demands to fuel the stress response (Mazeaud et al. 1977). In field studies, blood samples can be taken rapidly and non-lethally by caudal puncture (and/or gill puncture) and plasma glucose and lactate can be measured using inexpensive portable devices or simple laboratory methods. Increases in either glucose or lactate can indicate a metabolic response to stress. Plasma glucose is an indicator of mobilization of energy reserves, and plasma lactate a by-product of anaerobic metabolism. Other, more invasive techniques can be used to measure tissue glycogen or tissue lactate. Tissue glycogen is an indicator of the metabolic reserves stored in the liver and muscle and is a good indicator of muscular activity, but is highly dependent on temperature and when the fish last fed.

Potential

Fish experiencing acute changes in hydropower infrastructure and operations often exhibit a stress response (see Case Studies). It is likely that by measuring specific metabolites, indications of the magnitude and duration of stress can be found (Barton 2002). However, few studies have measured metabolite responses to hydropower infrastructure and operations (e.g., Flodmark et al. 2002). Fish swimming-up a fish ladder likely experience a rise in lactate, as exhaustive exercise typically leads to anaerobic metabolism (e.g., Schwalme and Mackay 1985; Wood 1988; Wood 1991). Plasma or tissue lactate could also change if fish are exposed to hypoxic water (e.g., if water is released from the hyperlimnion) (Morrissey et al. 2005). Glucose and glycogen

would also likely change in hydropower systems in relation to exhaustive exercise, however Flodmark et al. (2002) found no change to plasma glucose in fish exposed to simulated changes to water flow that could be attributed to the stressor. Metabolites would likely be a good indicator of short-term effects of hydropower systems, such as the energetic costs of pulse flows, fish ladder use, and water discharges. Metabolites should be used in studies investigating the impacts of hydropower infrastructure and operations, as the techniques can be informative, non-invasive and inexpensive.

Biotelemetry and Biologging

Overview

The term “telemetry” encompasses a variety of technological instruments that can be used to assess the physiology, behaviour, and energetic status of free-living animals (Cooke et al. 2004a). Conventional telemetry involves the use of transmitters and listening devices (i.e., radio-antennas, hydrophones) to locate the tagged animal in space and time (for simplicity, we also include the use of Passive Integrated Transponders [PIT tags] in this definition as they are often used to locate fish [Burke and Jepson 2006]). There are two principal types of telemetry: radio and acoustic. Radio telemetry uses antennas to receive waves of energy that are emitted from implanted/attached transmitters. By using arrays of antennas, or by using manual tracking approaches, animals can be tracked with a high degree of confidence. Acoustic telemetry involves the use of hydrophones to position implanted tags that transmit sound waves through water to convey fish location. As with radio telemetry, acoustic transmitter can be tracked manually or using a fixed hydrophone array. Both radio and acoustic platforms can be

used to transmit information such as temperature and pressure. In addition, there are a number of logging technologies (called “biologging” or archival loggers) that must be retrieved and downloaded to obtain data. The accuracy and confidence in telemetry and logging data depends on device choice, number of antennas/hydrophones/loggers being used, and the method used to interpolate the location of the transmitters (i.e., triangulation vs. successive gain reduction). Telemetry studies can be costly; however, they can provide large datasets and detailed information about fish movements, habitat selection, behaviour and physiology, data that are often unavailable from free-swimming fish using any other technique.

Remote physiological techniques (either radio-, acoustic-, or archival logger-based) can provide measurements of *in situ* physiological variables such as heart rate, opercular rate, tail-beat frequency and muscle activity (Cooke et al. 2004b). Electromyogram (EMG) transmitters are becoming a more commonly used tool to assess the volitional movements of fish (Cooke et al. 2004b). EMG equipped transmitters have electrodes that detect the bioelectrical voltage changes in the red muscle, which is proportional to the degree and duration of muscle tension (Sullivan et al. 1963) and is correlated with oxygen consumption (Weatherly et al. 1982; Weatherley and Gill 1987). EMG's can be calibrated to tail-beat rate to allow estimates of swimming speed, and/or oxygen consumption, and the metabolic costs of activity. EMG telemetry has been used to measure *in situ* metabolic rates at different temperatures and during seasonal and daily periods (Briggs and Post 1997a, 1997b), and has been shown to be useful in assessing energetic responses of fish to hydropower infrastructure and operating strategies (Gowans et al. 2003; Thorstad et al. 2003; Murchie and Smokorowski 2004; Brown et al. 2006;

Scruton et al. 2007a, 2007b). Forthcoming sensors including accelerometers may provide more opportunities for monitoring fish activity in the wild.

Potential

Conventional telemetry has been highly useful to track fish through hydropower systems (e.g., McKinley et al. 1998; Cooke et al. 2004a; Scruton et al. 2002, 2007a). For instance, by placing radio or acoustic receiving loggers throughout a hydropower impacted river system, detailed measurements of fish passage, fishway evaluation, turbine mortality, migration rates, survival, and habitat use have been made (Stier and Kynard 1986; Moser et al. 2002; Scruton et al. 2002; Behrmann-Godel and Eckmann 2003; Parsley et al. 2007). These methods are particularly important for assessing unanswered questions such as, how far fish will swim upstream when flows are high due to pulse flows, when temperatures vary because of water being released from below the thermocline, or to determine where fish spawn when hydropower systems have prevented passage to typical breeding grounds.

There are many applications of remote physiological devices (mostly telemetry) for assessing the response of fish to hydropower infrastructure and operations. Changing flow rates, fish passage, and habitat use can all be assessed in part by using physiological telemetry. Changing flow rates present an altered environment that can force fish to swim at high speeds for brief or extended periods of time. Physiological telemetry allows the researcher to track the location of individual fish, and collect data on swimming speeds and metabolic activity (see Case Study 2 for example; Murchie and Smokorowski 2004; Scruton et al. 2007a). Fish passage is also an important component of hydropower

infrastructure. Fish often physically exert themselves to successfully pass through a fishway; thus by using physiological telemetry, the costs of such movements can be quantified (See Case Study 1; Gowans et al. 2003; Scruton et al. 2007b). Assessing habitat use may also benefit from physiological telemetry. The physiological data could help researchers make links between habitat quality and individual behaviour and physiology (this aspect can have large impacts on fish habitat modeling and management; Cooke et al. 2004a). Overall, physiological telemetry can be used in a number of ways to address the impact of hydropower infrastructure and operations on fish physiology and behaviour.

Condition-Based Indicators

Overview

Condition-based indicators encompass length-weight relationships, organosomatic indices, and necropsy-based assessments. These assessments range from being relatively non-invasive to lethal. Length-weight relationships are typically very easy to measure, as fish only need to be handled briefly and they are appropriate indicators of general health. However, changes may not necessarily be due to stress, as seasons (Adams et al. 1982), stage of development and sexual maturation (Medford and Mackay 1978) can also influence length-weight relationships. Organosomatic indices involve the comparison of a particular organ to body weight ratio (e.g., hepatosomatic index [liver:body weight, HSI], gonadosomatic index [gonads:body weight, GSI], viscerosomatic index [entire viscera:body weight, VSI], and splenosomatic index [spleen:body weight, SSI], see Barton et al. 2002). These indices can be used to measure stress, as values that are lower

or higher than normal indicate that energy allotment to organ maintenance and growth is altered (Kebus et al. 1992). Another condition-based indicator is necropsy-based. This method involves autopsies of sacrificed fish and linking the condition of internal organs to stress based on published guidelines as to the condition of normal organs (Barton et al. 2002). It is difficult to relate condition-based assessments to stress, as results could be influenced by a variety of variables (i.e., disease, pollution, genetics, etc.), thus caution should be used.

Potential

Condition-based indicators may be most appropriate for assessments of the effects of hydropower infrastructure and operations on fish habitat and fish health downstream of the installation. A number of previous studies have used condition-based indicators to directly assess the impacts of hydropower (e.g., McKinley et al. 1998; McKinney et al. 2001; Paukert and Rogers 2004; Sato et al. 2005). McKinney et al. (2001) used a length-specific mean-weight equation to assess the condition of rainbow trout below the Glen Canyon dam and found that smaller fish were more strongly affected by dam operations than larger fish. Likewise, Sato et al. (2005) measured Fulton's condition factor and gonadosomatic index and determined that female curimatá (*Prochilodus argenteus*) were in better physiological and reproductive condition than male fish. Studies that measure condition-based indicators typically require control sites, and controlled laboratory experiments (possibly using underwater video) may be needed to fully understand the environmental conditions which lead to changes to condition-based indices.

Stable Isotope Analysis

Overview

In aquatic ecosystems stable isotopes are useful for understanding the sources of fish diets and the trophic positions held by a particular fish in a food web (Peterson and Fry 1987). By using the ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, diet source and trophic position, respectively, can be inferred (DeNiro and Estein 1978, 1981; Post 2002). Typically, researchers have lethally sampled the white muscle of fish (Pinnegar and Polunin 1999); but recent studies have promoted the use of fin clips and other tissues (e.g., blood, scales) to assess stable isotopes (Jardine et al. 2005) - mainly to sample species of conservation concern.

Potential

It may be possible to use stable isotope analysis in habitats downstream of hydropower facilities, as shifts in habitat caused by changes in flow and flooding events may alter food sources and trophic positions of fish. Recently, stable isotope analysis has been used to assess growth and feeding in yellow perch (*Perca flavescens*) to understand site-specific physical and chemical factors (Murchie and Power 2004), and to assess landscape-scale hydrological characteristics and carbon flow in river food webs (Hoeinghaus et al. 2007). In general, with the use of reference sites and/or archived specimens, stable isotope may be a unique tool to assess how individuals alter feeding behaviour in hydropower systems. However, caution should be taken when considering stable isotopes as a tool, as recent criticisms about the use of stable isotopes have been proclaimed (Hoeinghaus and Zeug 2008).

Forensic Techniques

Overview

Forensic techniques encompass tools that can be used to investigate external tissue damage, especially to the skin. These tools have recently been applied in fisheries to assess skin abrasion caused by nets. Researchers have used a combination of fluorescein bath and ultra-violet light exposure were used to assess tissue damage (Noga and Udomkunsri 2002), in a variety of species walleye pollock (*Theragra chalcogramma*), sablefish (*Anoplopoma fimbria*), northern rock sole (*Lepidopsetta polyxystra*), and Pacific halibut (*Hippoglossus stenolepis*) (Davis and Ottmar 2006). Other chemical enhancers including Hemastix®, Hemident™, Phenolphthalein and Bluestar® may offer alternatives to fluorescein for detecting fish skin abrasion (Colotelo et al. 2009). The use of chemical enhancers as a common tool in assessing tissue damage in fish is likely a few years away, as much work is still needed to determine any negative effects of the chemicals on the fish and aquatic environments, as well as to determine the extent to which false positives or false negatives may influence conclusions.

Potential

Using chemical enhancers to aid the detection of skin injury may be useful to assess the impact of hydropower infrastructure on fish. Numerous infrastructures including turbines and fishways either cause or likely cause tissue damage in both migratory and non-migratory fish. A quick, non-invasive method for determining and quantifying injuries would be useful for understanding how different mitigative options affect individuals.

Presumptive tests would also help researchers identify injury patterns which may lead to improvements in infrastructure and common practices. Davis and Ottmar (2006) have used presumptive tests, including forensic techniques to understand the effect of fish nets on multiple fish species. It is highly likely that similar methods could be used in a hydropower setting to determine external injury in fish passing through hydropower barriers and fishways.

Using Multiple Tools

All of the above ‘tools’ have the potential to provide useful information about the physiology of fish exposed to hydropower impacts. However, organismal physiology will be affected by other exogenous and endogenous factors. Many species that are affected by hydropower infrastructure are anadromous and semelparous (i.e., Pacific salmon). Depending on the species, stock, and location of the hydropower facility, these fish will have different maturation status and/or nutritional status and will be also influenced by other factors such as fishing and thermal stress. For example, lipid levels are related to glycogen stores or vitellogenesis; ions and enzyme levels (Na^+/K^+ ATPase) are inherently linked to both nutritional and maturation status. We suggest that studies should use multiple tools to clearly determine the impacts of hydropower on fish to ensure that other exogenous and endogenous factors are appropriately considered.

Case Studies

Here we present two brief case studies to illustrate how individual-based metrics can be used to understand fish responses to hydropower operations and infrastructure. Both case studies are based on data collected at hydropower facilities in British Columbia, Canada by an interdisciplinary research team consisting of academic, government, and industry partners.

Case Study 1: The Seton River

The Seton-Anderson watershed in the southern interior of British Columbia, Canada, is highly modified by hydropower development. Maturing sockeye salmon (*O. nerka*) returning to spawn in the watershed must pass a diversion dam via a vertical slot fishway (Figure 2.1) and two powerhouse tailraces before reaching spawning grounds. In 2005 and 2007, studies were conducted using physiological biopsy (Cooke et al. 2005a), and electromyogram (EMG) and conventional telemetry to evaluate physiological and behavioural responses to hydropower facilities in this system (Pon et al. 2006; Roscoe and Hinch 2008). Sockeye salmon were caught at the top of the Seton River fishway, blood sampled, and tracked by electromyogram, acoustic or radio-telemetry after release (fish were released downstream of the dam). Blood samples were analyzed for ion concentrations (i.e., Na⁺, K⁺, Cl⁻), metabolites (lactate and glucose) and cortisol to assess the degree of anaerobic exercise and physiological stress associated with hydro-system passage.

All stress indicators examined from fish captured at the top of the fishway were comparable or lower than previously reported for migrating adult sockeye (Crossin et al. 2008; Young et al. 2006b). Thus, the physiological variables measured suggest that

hydropower infrastructure passage was neither physically exhausting nor particularly stressful. However, when comparing successful and failed migrants it was found that fish that failed to ascend the fishway had lower plasma sodium ion concentrations ($P=0.020$, Pon et al. 2009a) and fish that failed to reach spawning grounds had higher levels of plasma lactate ($P=0.007$, Pon et al. 2006). Although lactate and ion levels in failed migrants were still within the range of values reported elsewhere for migrating sockeye, the results suggest that physiological stress may inhibit successful migration.

To examine the physical effort required to ascend the fishway, fish were tagged with EMG transmitters. Recordings of EMG signals converted into swim speed estimates (see Hinch and Rand 1998) revealed that fish generally did not invoke anaerobic activity for extended periods of time in the fishway (Pon et al. 2009a). Observations of low swimming intensity (i.e., aerobic activity), were consistent with low plasma lactate levels in fish caught at the top of the fishway and suggested that passage through the Seton River fishway is unlikely to be physically exhausting for migrant adult sockeye salmon. EMG telemetry also revealed that some fish used reverse flows to minimize swim efforts while others did not. As a consequence, these fish were able to ascend the fishway in a more energetically efficient manner than other fish.

In using blood sampling to examine stress related to dam passage, there are several limitations highlighted by the Seton River dam study. For one, to obtain an accurate representation of fish condition, it is necessary to catch and sample fish in an expedient manner, so as to avoid biasing the blood sample with capture- and handling-related stress. At Seton River dam, fish could only be caught in such a manner by dip-

netting individuals from the top of the fishway, and thus it was not possible to capture fish downstream of the dam. It is also important to consider the effects of natural variability on measures obtained from blood samples, as common stress indicators such as cortisol and ions change over the course of migration (Carruth et al. 2000; Shrimpton et al. 2005). At Seton River dam, individual cortisol measures ranged from 32.5 to 927.6 ng mL⁻¹ in fish caught over the course of August and September of 2005, making it difficult to distinguish between stress effects and maturation-related changes.

EMG telemetry also has limitations, and should be used with care when making estimates of fishway passage efficiency. At the Seton River dam, a 40 % drop-back rate was observed in EMG tagged fish, while only 20 % of radio tagged fish failed to pass the fishway, a discrepancy that may be due in part to the additional stressors associated with EMG tag implantation (e.g., surgery). Also, as individual transmitters are relatively expensive, it may be cost prohibitive to tag a sample of fish sufficiently large enough to make statistical comparisons.

Case Study 2: The Puntledge River

The Puntledge River, is a regulated river which has a number of natural and artificial barriers to the upstream migration of adult Pacific salmonids (*Oncorhynchus* spp.), particularly in reach C (Figure 2.2). Throughout the reach there are a number of natural barriers, specifically Nymph Falls and Stotan Falls. These two areas have a number of fish ladders (steps carved out of bedrock) built into the natural terrain of the river to facilitate upstream migration of fish. Beginning in 2004, experimental release of water

from the diversion dam has occurred in an effort to mimic natural variation in river flow. These pulse flows lead to elevated water levels and increased flow rates, especially through the fish ladders (Figure 2.3). Research is currently being conducted to understand whether these pulse flows have an effect on upstream migration of summer Chinook salmon. Techniques being used include biosampling, thermal logging (biologgers), and telemetry.

In the summer of 2007 male summer-run Chinook salmon were measured for total length and blood and gill samples were taken (blood parameters measured included triglycerides, calcium, glucose, phosphorous, and AST [Aspartate transaminase; an indicator of tissue damage]; gill sample was collected for future genomic work). A microwave energy meter (Crossin and Hinch 2005) was used to estimate gross somatic energy density. Following these procedures, fish were either implanted with an EMG transmitter or a conventional radio transmitter. Both types of transmitters were equipped with a thermal logger (DS1921Z, iButton, Maxim Integrated Products and Dallas Semiconductor, Sunnyvale CA, USA) to record ambient body temperature every 20 min. Upon release, fish were tracked using multiple antenna telemetry arrays placed at the powerhouse, Stotan Falls, Nymph Falls, and numerous other strategic locations throughout the study period. The arrays were complimented with manual tracking whereby each fish was tracked for at least 10 min twice daily, increasing to 4 times daily during pulse flows, to ensure collection of EMG data for all fish (i.e., fish not tracked automatically by the multiple antenna arrays). River temperature and flow was also measured hourly at numerous locations.

Pulse flows increased the flow of water through reach C of the river. Some Chinook salmon (e.g., Chinook salmon #29) were found to move upstream during the periods of increased water flow (Figure 2.3). These movements are likely important, as migratory delays have been found to negatively affect spawning success (Quinn et al. 2000).

Fish were more likely to ascend Stotan Falls at lower flow rates. This finding will help managers select species-specific or community-specific (if multiple species are used) flow rates that increase the probability of successful ascent in difficult reaches. Also, in future studies we will investigate the thermal histories of tagged Chinook salmon using thermal loggers (e.g., Figure 2.4) and attempt to understand the importance of thermal refuge to spawning success (similar to studies by T.P. Quinn, e.g., Berman and Quinn 1991). Temperature has previously been shown to affect migration mortality and travel rates in sockeye with long migrations (Keefer et al. 2008a). If flow rates are combined with river temperature profiles, managers will be able to regulate rivers so that both flow and temperatures are optimal for upstream migration of Pacific salmonids (see Macdonald et al. 2007) and more closely resemble a natural flow.

Fish that were unable to move upstream during the pulses flows and were subsequently located downstream (though not necessarily as a result of the pulse flows) were found to have higher concentrations of plasma calcium and lower levels of triglycerides. These fish were sampled at the time of release, and thus, physiological condition at time of fallbacks was not known and can only be inferred. There were no significant impacts of time-of-release physiology and spawning success, but there may be multiple physiological variables related to maximum distance traveled, as calcium and

magnesium were strongly negatively correlated and gross somatic energy density was strongly positively correlated with maximum distance traveled.

Implications for Regulatory Agencies and Utilities

When results are obtained using suborganismal tools, it may be difficult for regulatory agencies and utilities to directly relate these data to management issues at the level of the population, community and ecosystem. However, we argue that suborganismal responses are just as important as population and community level responses for several reasons (as reviewed in Young et al. 2006a and Cooke and Suski 2008), and should be used by regulatory agencies and utilities when engaged in management decisions. The primary basis for this assertion is the notion that organismal condition, health and behaviour are influenced by both genetics and environments and that collectively these factors influence the fitness of an individual fish (Ricklefs and Wikelski 2002). In general, regulatory agencies responsible for hydropower operations should be making decisions based on a range of scientific data from the organismal level to the community level, ideally in an integrated manner.

Conclusions

Physiological indicators are sensitive and provide an integrative assessment of the condition of fish. Furthermore, energy is the currency of life and can be affected by a suite of factors including altered thermal environments, changes in food supply, delays in

migration, and variable water discharge. Ecological genomics will provide an even more mechanistic understanding of the biological systems regulating fish behaviour and survival. Heat shock proteins and stable isotope analysis could be used to assess fish response to changing abiotic and biotic environmental variables once further research is completed. Quantification of enzymatic activity (e.g., LDH and CK), ions (e.g., Na⁺ and K⁺), hormones (e.g., catecholamines and corticosteroids), and metabolites (e.g., glucose and lactate) likely will provide insight into tissue damage, energetics, and overall stress that fish endure when dealing with hydropower. Information on the sensory neurophysiology of fish can be used to help develop effective fish guidance and attraction technologies to facilitate safe passage past hydropower barriers. Lipids and condition-based indices may be useful for assessing habitat linked body condition. Physiological telemetry will continue to help researchers understand how fish respond to hydropower infrastructure and operations in real time. If researchers couple some or many of these tools, predictive models could be developed to assess how changes to fish habitat caused by hydropower infrastructure and operations will affect fish. The two case studies highlighted both the benefits and challenges of including individual-based metrics in studies of hydropower-fish interactions. Clearly, no single individual will possess the skills or expertise needed to mount a study that makes use of all of the tools identified in this review. Hence, implicit in expanding the “toolbox” is building more integrative and interdisciplinary teams to tackle complex hydropower questions. In summary, this paper should provide researchers with a comprehensive understanding of the tools available to understand individual and suborganismal responses to a variety of hydropower activities (but these tools may be useful during environmental assessment periods as well).

Furthermore, the expanded toolbox has the potential to provide the knowledge to move towards a more sustainable hydropower industry and provide regulators with additional tools for evaluating compliance and making more informed decisions.

Table 2.1. An assessment of possible research tools that could be used to measure individual level effects of hydropower infrastructure and operations on fish (see text for details)

Assessment Tool	Examples	Potential Use in a Hydropower Setting	Relative Applicability	Advantages	Disadvantages
Genomics	cDNA microarrays from multiple tissues, gene receptors	Predictive biomarkers, responses to abiotic and biotic stressors	Applicable	-Typically only requires a non lethal gill or muscle sample	-Expensive laboratory methods required -Still considered a novel research area and requires further validation -Species specific (or family specific) gene arrays required
Heat Shock Proteins (HSP)	Hsp70 expression in multiple tissues	Predictable fluctuations in abiotic environment (e.g., flows, water temperature)	Possibly applicable	-Found in most tissues -Validated for thermal applications	-Expensive laboratory methods required Still considered a reasonably novel research area
Enzymatic Activity	Na ⁺ K ⁺ ATPase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase	Tissue damage, energetics, growth, smoltification, effects of pollution	Very applicable	-Laboratory methods are relatively easy to do Can often be done with a non lethal biopsy	Other variables (both endogenous and exogenous) can influence enzyme rates -Some enzyme assays can be expensive
Sensory Neurophysiology	Neural stimulation, scanning electron micrographs of neuromasts	Developing fish guidance tools, attraction and avoidance studies	Very applicable	-Fundamental to understanding how fish locate and select migration paths around barriers	Rarely applied to hydropower issues -Challenges in conducting field studies on this topic
Ions and Dietary Minerals	Na ⁺ , Cl ⁻ , K ⁺ , Mg ²⁺ , Ca ²⁺ osmolality	Exhaustive exercise, nutritional condition	Applicable (fish ladder assessment and nutrition)	-Non lethal sampling (blood sample) -Easy to measure	- Can be influenced by any factors that alter homeostasis - Nutritional indicators can be influenced by feeding history

Lipids	Triglycerides, proximate body composition	Condition as it relates to habitat quality, energy expenditure, overwinter survival	Possibly applicable	-Laboratory methods are well-defined	-Some methods require lethal sampling
Endocrine Measures	Catecholamines, corticosteroids, reproductive status	Effects of hydro-peaking, abiotic and biotic stressors	Very applicable (cortisol and reproductive hormones)	-Very responsive -Time lag between stressor and response (cortisol only)	-Influenced by genetics, development and environment -Habituation or desensitization can occur
Metabolites and Tissue Energy Stores	Blood glucose, blood and muscle lactate, muscle glycogen, ATP, PCr	Indicate magnitude and duration of abiotic or biotic stress (e.g., fishway passage)	Very Applicable	-Can be done using simple methods and/or inexpensive meters -Reasons for responses well-defined	-Dependent on temperature
Biotelemetry and Biologging	Electromyogram transmitters (EMG), tail-beat transmitters, temperature loggers	Tracking fish through a hydropower system, fish way attraction, energetics, habitat use, thermal biology	Very Applicable	-Direct responses from organism -Can determine fate -Compare behavioural responses to physiological responses	-Expensive if using large sample sizes -Tracking is time intensive
Condition-Based Assessments	Length-weight relationships, hepatosomatic index, organ condition	Fish condition near facilities, growth rates	Possibly applicable	-Simple, inexpensive methods	-Gross indicator of organismal condition (may be influenced by many factors) -With the exception of length-weight relationships, involves lethal sampling
Stable Isotope Analysis	$^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$	Nutrient derivation, trophic position	Possibly applicable	-Can reveal food webs and diets associated with various flows or other system level changes -Non-lethal sampling	-Expensive laboratory methods required
Forensic Techniques	Fluorescein, Hemident	Tissue damage caused by turbines and fishways	Very Applicable	-Ease of use -Non-lethal sampling -Relatively inexpensive	-Not fully tested for effects on fish -Requires further

validation
-Not all false positives are
known

Figure 2.1. A photo of the vertical slot fishway at the Seton river hydropower facility used to facilitate upstream migration of salmonids (source, L.B. Pon).

Figure 2.3. The mean (whiskers represent standard error) EMG value for Chinook salmon number 29 before and after the first pulse flow (black line) and the water flow measured at gauge 6 (blue). The physical location of the fish along Reach C is also given (solid line indicates known location, dotted line indicates unobserved transition between known locations) (photo source, M.R. Donaldson).

Figure 2.4. Thermal history of a Chinook salmon (fish 28) in the Puntledge River with locations determined using conventional telemetry data.

Chapter 3: The influence of osmoregulatory, metabolic, and nutritional condition on the migratory behavior and fate of an imperiled stock of summer-run male Chinook salmon in a regulated river.

Abstract

We studied the migratory success of male summer-run Chinook salmon (*Oncorhynchus tshawytscha*) in the Puntledge River on Canada's Vancouver Island over a three-year period using biotelemetry and non-lethal physiological biopsy. Principal component analysis was used to group co-varying physiological variables prior to comparing fish with different migratory behaviors (e.g., migration rate, holding times) and fate (migration and spawning success). Fish with low levels of endogenous energy stores (total protein, cholesterol, and triglycerides) and dietary minerals (calcium, magnesium, and phosphorus) at the time of sampling were found to subsequently ascend the most upstream natural barrier (i.e., Nib Falls) significantly faster than fish with higher levels. Fate was weakly associated with several physiological characteristics - successful migrants had significantly higher hematocrit values and significantly lower plasma K⁺ relative to failed migrants suggesting that fish condition at river entry can influence subsequent behavior relative to hydropower-related barriers. Our results indicate that physiological and nutritional condition can influence adult migrating male summer-run Chinook salmon, but we did not find a physiological profile that could explain all behaviors and fates observed. This study represents one of the first to apply conservation physiology tools to study an imperiled river fish population.

Introduction

Hydropower infrastructure (e.g., dams, generating stations) and operations can interrupt a river's natural flow regime, fish habitat, and system connectivity, and thus has the potential to negatively influence the upstream migration of fish, particularly anadromous fish such as salmonids that must travel upriver to natal spawning grounds (Poff et al. 1997; Enders et al. 2009). Recent studies have demonstrated delays (Thorstad et al. 2003; Keefer et al. 2004; Keefer et al. 2008a; Pon et al. 2009a) or failure of mature adult salmon to reach spawning grounds (e.g., Gowans et al. 2003; Thorstad et al. 2003) and higher energetic costs for salmonids exposed to variable water flows (Murchie and Smokorowski 2004; Tiffan et al. 2010) in regulated rivers. Though numerous studies have measured fish behaviors in association with hydropower operations and infrastructure, only Pon et al. (2009a,b) and Roscoe et al. (2010a) have assessed the physiological status of free-swimming fish relative to their encountering a barrier, their ability to pass the structure, and post-passage survival. However, little is known about how physiological condition influences the behavior and survival of fish passing through multiple obstructions and barriers en route to spawning areas.

It is likely that the physiological status of maturing adults at the time of entry into a hydropower impacted river influences migratory behavior, as the freshwater migrations of spawning salmon, regardless of the degree to which the river is impacted by hydropower, are physiologically challenging and energetically demanding (Ueda and Yamauchi 1995; Hinch et al. 2006). Indeed, recent research on the Fraser River in British Columbia, Canada has determined that physiological condition of individual fish

during the coastal approach is correlated with behavior and fate at time of river entry (Cooke et al. 2008a; Crossin et al. 2009a) as is condition at time of capture in the river with behavior and survival in more upstream reaches (Young et al. 2006b; Mathes et al. 2010; Donaldson et al. 2010). These studies are possible through the use of a non-lethal biopsy approach where small blood and gill samples are obtained from fish that are subsequently affixed with a telemetry tag and released (Cooke et al. 2005a,b, 2008b; Miller et al. 2011). Although this correlative approach between physiological condition and subsequent behavior and fate does not reveal causal links (Cooke and O'Connor 2010), it can identify factors that may be contributing to migration failure or aberrant behaviors. Thus, understanding variation in fish condition and physiology can help us understand the impact of hydropower infrastructure and operations and better inform appropriate mitigation and compensation strategies (Caudill et al. 2007; Hasler et al. 2009 [Chapter 2]).

The aim of the present study was to understand the linkages between nutritional status, physiological stress and migratory behaviors and fates of adult salmon which must pass multiple obstructions *en route* to spawning areas. The study animals are male summer-run Chinook salmon (*Oncorhynchus tshawytscha*) which ascend the Puntledge River in British Columbia, Canada. Very little is known on the behavioral physiology of migration of SCS – furthermore this particular stock is imperiled (Hirst 1991) and recent management actions have focused on facilitating upstream migration. Specifically, the stock has decreased from 4500 individuals historically, to fewer than 1600 returning adults (Personal Communication, Darcy Miller, Fisheries and Oceans Canada). Thus our results have the potential to facilitate recovery and conservation. Previous studies of this

kind, although few, have tended to focus on larger, lower gradient systems with few obstructions (e.g., Fraser River, Cooke et al. 2008a,b, Crossin et al. 2009b; Thompson River, Young et al. 2006b) and have focused nearly exclusively on sockeye salmon as models. Radio-telemetry was used in tandem with blood and gill sampling obtained for physiological analysis (Cooke et al. 2005a) to test two broad hypotheses: (1) physiological status, energy use, and osmoregulatory status will differentiate migratory behaviors (i.e., time holding, time to ascend) of fish at ‘key barriers’ to upstream movement, and (2) summer-run Chinook salmon that successfully reach spawning grounds will be characterized by low levels of physiological stress, be in good nutritional condition, and be in a state of high osmoregulatory preparedness relative to fish that do not reach spawning grounds.

Methods

Summer-run Chinook Salmon and the Puntledge River

The Puntledge River summer-run Chinook salmon have been experiencing steep declines in returning adults (Hirst 1991; DFO Puntledge Hatchery, Unpublished Data). The decrease in returning adults is despite numerous restoration and enhancement efforts by government agencies and the hydropower utility, including: construction of fish ladders at Comox Dam and the diversion dam, modifications to natural obstructions, flow manipulations to facilitate upstream movement of returning salmon, stocking programs, and other habitat enhancement initiatives (i.e., addition of spawning gravel, side channels

for refuge use by juveniles) (Hirst 1991; Personal Communication, D. Miller, Fisheries and Oceans Canada).

The Puntledge River is 16.9 river (r)km and has been impacted by hydropower operations and infrastructure. The main impact is that there are multiple artificial and natural barriers to fish migration (Figure 3.1). At river km 13.3, a portion of the river's discharge river is diverted by a diversion dam into a penstock, and subsequently re-joins the river at river km 6.8 (Figure 3.1). At the output of the penstock there is a pool (Powerhouse Pool) that summer-run Chinook salmon must pass before entering the diversion reach (reach C in Figure 3.1). Reduced discharge in the diversion reach may affect the migration of summer-run Chinook salmon. In particular, discharge at two major natural barriers to fish migration (Stotan Falls and Nib Falls; Figure 3.1) seems to delay migration. Both of these barriers each have three sets of blasted-rock fish stairs. Once fish have migrated above Nib Falls, fish must navigate through a fishway (pool and weir type) at the diversion dam in order to access the headwaters and historic spawning gravel beds (river km 16.9 upstream of the river mouth). Summer-run Chinook salmon have been observed spawning from approximately river km 9.6 to river km 16.9 upstream and thus we classified fish as spawners once they passed the 9.6 rkm mark and exhibited further behaviors that indicated that they were alive. Fish were tagged and released at the lower hatchery (6.5 rkm) and were tracked throughout the study reach by walking the shoreline of the river and using fixed stations near three locations that are known physical or behavioral barriers: the Powerhouse Pool, Stotan Falls, and Nib Falls. During tagging days, river temperature and discharge in the diversion reach ranged from 15 to 17 °C, and 5.9 to 6.8 m³/s, respectively.

Study Animals, Tagging and Biosampling Procedures, Assays, and Tracking

Between June 25th and July 20th 2007 and 2008; and June 2nd and 26th in 2009, male adult summer-run Chinook salmon (females were not permitted to be used in the study because of the need to use them in the brood stock program) were diverted into raceways at the Lower Puntledge River Fish Hatchery (Fisheries and Oceans Canada; Figure 3.1) for physiological sampling and transmitter implantation (2007: 21 fish; TL = 691 ± 79 mm; 2008: 13 fish, TL = 663 ± 59 mm; 2009: 24 fish, TL = 645 ± 62 mm). Upon capture from the hatchery raceway using a ‘herding’ and dip net method, all fish were sampled for blood and gill tissue (see biopsy methods in Cooke et al. 2005a). Briefly, fish were placed supine in a v-shaped trough filled with water and 1.5 ml of blood was drawn from the caudal vasculature using 3 ml vacutainer (lithium heparin, Becton Dickson, NJ) and 1.5” 21 g needle. Less than 4 mm of tips from 6-8 gill filaments from the first gill arch were removed (McCormick 1993) and immediately frozen in liquid nitrogen. Blood was immediately placed in a water-ice slurry prior to centrifuging at 10,000 g (Compact II Centrifuge, Clay Adams, NY). Plasma was aliquoted into vials and frozen in liquid nitrogen prior to transferring samples to a -80 °C freezer.

Plasma was analyzed by IDEXX Laboratory (Markham ON) for total protein, aspartate transaminase (AST; an indicator of tissue damage), cholesterol, triglycerides, glucose, sodium, potassium, chloride, calcium, phosphorus, and magnesium using a Roche-Hitachi Analyzer (Wagner and Congleton 2004). Variables were chosen based on previous work (see Cooke et al. 2006; Pon et al. 2009a,b).

Once fish were sampled for blood and measured for total length (mm), each fish had a radio transmitter gastrically inserted with the antenna protruding from the oral cavity (MCFT-3A, Lotek Engineering, Inc., Newmarket, Ontario). Fish were held for up to 60 minutes in a cage (gated off portion [1.2 m x 2.0 m x 0.6 m] of the fishway entrance to the hatchery) to facilitate recovery and then released directly upstream of the Lower Hatchery Fence. Fish were manually tracked from shore twice a day using a telemetry receiver (SRX-600, Lotek Engineering, Inc., Newmarket, Ontario) and a 3-pronged Yagi antenna. Fish locations were determined using the successive gain reduction technique (Cooke et al. 2004a). The river is narrow and there are a minimum number of possible locations fish can inhabit, so transmitter locations are easily found with a high-degree of accuracy. Fish were tracked from June 30th to November 5th (in 2007), June 30th to November 7th (in 2008) and June 2nd to November 6th (in 2009). Tracking occurred twice a day up to August 5th, and then less frequent tracking (weekly) occurred up until the post-spawn die-off (early November).

Data Analysis

Five key metrics of migratory behavior in the Puntledge River were calculated by counting the number of days: 1) fish were located at the Powerhouse Pool, 2) to migrate between the Powerhouse Pool and Stotan Falls, 3) between arriving at and ascending over Stotan Falls, 4) to migrate between Stotan Falls and Nib Falls, and 5) between arriving at and ascending over Nib Falls. These milestones were used because they are key holding areas and sites of concern to the local stakeholders (Taylor and Guimond 2004). Furthermore, to avoid autocorrelation, relative times to ascend were used and were not

continuous from the previous behaviour metric. Yearly differences between each of the migratory behaviors were analyzed using Kruskal-Wallis non-parametric ANOVA and correlations between each of the migratory behaviors were done to assess the independence of each variable.

To test for yearly differences between the blood variables, the mean of each blood variable was compared across years using Kruskal-Wallis non-parametric ANOVAs because the assumptions of a one-way ANOVA were not met. A principal component analysis (PCA) with a Varimax factor rotation (an orthogonal transformation of factors with eigenvalues ≥ 1 included) was used to simplify the predictor variables (blood variables) by partitioning autocorrelated variables together (Wagner and Congleton 2004). The PCA grouped the correlated blood variables together and created uncorrelated factors that were subsequently used for analyzes. A log transformation was used when blood variables were not normally distributed prior to the PCA to meet the assumption of normalcy. Within each factor, blood variables were considered to contribute to the factor if the rotated factor loading was ≥ 0.5 . This characterized the factors by only the blood variables that contributed the most to the variance explained for each factor. Rotated factors were then linearly regressed with each of the 5 metrics of migratory behavior (time spent at the Powerhouse Pool, time to move from the Powerhouse Pool to Stotan Falls, time to ascend Stotan Falls, time to move between Stotan Falls and Nib Falls, and time to ascend Nib Falls). In addition, rotated factors were logistically regressed with whether or not a fish held (minimum of one day) at the Powerhouse Pool, ascended Stotan Falls, ascended Nib Falls, and with fate (spawner [carcass found above Stotan Falls and movement occurred near time of spawning],

prespawn mortality [no movement prior to spawning period and/or tag found before spawning period], or fallback [fish moved downstream of the Lower Hatchery main fence]) (prespawn mortality and fallback were grouped in the logistical analysis). All statistical analysis was done using JMP 7.0.2 (SAS Institute Inc., Cary NC, USA) and significance was tested at $\alpha = 0.01$ (Bonferonni correction; 5 behaviors) for yearly differences in migratory behavior, at $\alpha = 0.004$ (Bonferonni correction; 12 blood variables) for yearly differences in blood variables, and at $\alpha = 0.013$ (Bonferonni correction; 4 rotated factors) for comparisons of blood variables and migratory behavior and fate.

Results

Migratory Behavior and Fates

There were few correlations (3 of 13) between the different migratory behavior variables used in the analysis (Days located at the Powerhouse Pool and Days to Stotan Falls, $r = 0.67$, $P < 0.001$; Days to ascend Stotan Falls and Days to Nib Falls, $r = 0.37$, $P = 0.017$; and Days to migrate between Stotan Falls and Nib Falls and Days to ascend Nib Falls, $r = 0.53$, $P = 0.001$).

Migratory behaviors of the tagged Puntledge River summer-run Chinook salmon did not vary between years (Table 3.1). Of the 58 tagged fish, 45 % held at the Powerhouse Pool (proportions do not include the fish that held for 0 days or spent 0 days traveling between sites; Table 3.1). The mean days held at the Powerhouse Pool was 4 days and ranged from 0-41, there was no significant year effect (Kruskal-Wallis; $\chi^2 =$

5.67; $P = 0.06$; Table 3.1). Ninety-one percent of tagged fish (53 of 58) moved upstream to Stotan Fall (8 days in 2007, 5 in 2008, and 15 in 2009); there was no significant year effect (Kruskal-Wallis; $\chi^2 = 8.12$; $P = 0.02$; Table 3.1). The migration failure rate (i.e., the number of fish that failed to pass Stotan Falls) was 26 % (14 of 53). Fish ascended Stotan Falls on average in 8 days (range = 1-47; Table 3.1). There was no significant year effect (Kruskal-Wallis; $\chi^2 = 3.76$; $P = 0.15$; Table 3.1). All of the fish that ascended Stotan Falls reached Nib Falls (39 of 39) and mean length of time to reach the barrier was 2 days (range 0-20 days), once the fish passed Stotan Falls and again, there was no significant year effect (Kruskal-Wallis; $\chi^2 = 4.80$; $P = 0.09$). The failure rate at Nib Falls was 15 %, as 33 of 39 fish ascended the falls in 3 days on average (range 0-11; Table 3.1) with no significant year effect (Kruskal-Wallis; $\chi^2 = 2.66$; $P = 0.26$).

In total, 38 fish were classified as being possible spawners (i.e., successfully ascended Stotan Falls and did not fallback or were not found dead prior to the spawning time); 8 as pre-spawn mortalities; and 12 as fallbacks (Table 3.2). The spawning fate of the tracked fish did not differ between years (Pearson; $\chi^2 = 3.767$; $P = 0.439$) and between dates tagged (Pearson; $\chi^2 = 10.725$; $P = 0.708$).

Blood Variables

Yearly means for blood variables were similar across years for all but one variable (sodium; Table 3.3). In 2008, sampled summer-run Chinook salmon had significantly higher values of sodium (171 [SD= 7] $\text{mmol}\cdot\text{L}^{-1}$) than in 2007 (164 [5] $\text{mmol}\cdot\text{L}^{-1}$) and 2009 (161 [7] $\text{mmol}\cdot\text{L}^{-1}$; Table 3.3).

A principal component analysis and subsequent orthogonal factor rotation of the loadings was found to explain 73.2 % of the variance in blood variables (Table 3.4). Rotated factor 1 represented endogenous energy stores and dietary minerals. It was characterized by high positive factor loadings for calcium, total protein, magnesium, cholesterol, triglycerides and phosphorous (Table 3.4), and accounted for 33.4 % of the variance. Rotated factor 2 was characterized by high positive factor loadings for chloride and sodium, and high negative factor loadings for AST and potassium (Table 3.4), mainly representing osmoregulatory preparedness (with the exception of AST) and accounting for 19.8 % of the variance. Rotated factor 3 was characterized by a high positive factor loading for glucose (Table 3.4), likely representing acute stress and explaining 10.7 % of the variance. Rotated factor 4 accounted for 9.4 % of the variance and had a high positive loading for hematocrit and a high negative loading for potassium (Table 3.4).

Blood Variables vs. Migratory Behaviors and Fates

The number of days to ascend Nib Falls was positively correlated with endogenous energy stores and dietary minerals (Rotated factor 1; $R^2 = 0.25$; Parameter estimate = 1.44, d.f. = 1, 26; $F = 8.49$; $P = 0.007$). Meaning that fish with higher positive values of calcium, total protein, magnesium, cholesterol, triglycerides, and phosphorous present in their blood at the time of sampling took longer to ascend Nib Falls than fish with high negative values of endogenous stores and dietary minerals (Figure 3.2). No significant relationships were found between any other migratory behavior and rotated factors.

Fate was positively predicted by blood condition (Rotated factor 4; $R^2 = 0.11$; $n = 53$; $\chi^2 = 10.60$; $P = 0.005$); meaning that fish with higher hematocrit and lower potassium

concentrations were more likely to be successful spawners than to be classified as prespawn mortality or fallback fish (Figure 3.3). No significant relationships were found between any other classified migratory behaviors and rotated factors.

Discussion

In an attempt to understand how a “snap shot” of physiological conditions may affect migratory behaviors and fate in a short regulated river, we compared blood variables to the migratory behaviors and fates of a migrating Pacific salmon. This study was the first of its kind to assess summer-run Chinook salmon migratory behavior and blood variables, unlike previous physiological assessments that have focused primarily on late-run sockeye salmon. Also, our study focused on a small, steep, and hydropower influenced river, whereas most previous studies have taken place on larger systems with comparatively low gradient (i.e., the Fraser and Thompson Rivers).

Blood Variables vs. Migratory Behaviors and Fate

We hypothesized that physiological status, energy use, and osmoregulatory preparedness would differentiate the variability observed in the migratory behaviors of fish at ‘key barriers’ to upstream movement. The length of time upstream migrating adult summer-run Chinook salmon took to ascend Nib Falls once they arrived at the base of Nib Falls was characterized by positively loading indicators of endogenous energetic stores and other dietary minerals. High-levels of energy reserves (i.e., lipids and proteins) in migrating salmon blood indicate that energy stores are being mobilized to fuel migration

(Brett 1995). Cooke et al. (2006) found that summer-run sockeye salmon (*O. nerka*) that failed to enter the Fraser River, British Columbia had higher gross somatic energy and suggest that fish with lower gross somatic energy may have committed to river entry and have already shifted energy reserves to fuel gonad maturation (Patterson et al. 2004). It may be that the Puntledge summer-run Chinook salmon that had higher endogenous energy stores and dietary minerals present in their blood at the time of tagging were not as reproductively advanced as fish that ascended Nib Falls faster.

We also hypothesized that fish that were able to reach spawning grounds would be characterized by low levels of physiological stress, be in good nutritional condition, and be in a state of high osmoregulatory preparedness relative to fish that did not reach spawning grounds. Fate was weakly related to the hematocrit/potassium factor. Specifically, fish that were classified as spawners were more likely to have higher hematocrit and lower potassium, while fish that were classified to have died prior to spawning had lower hematocrit and higher potassium, possibly indicating stress and poor condition (Barton et al. 2002). However, because potassium levels were indicative of healthy fish (Lall 2002), it is unclear how potassium contributed to fate.

It is important to note that for our significant relationships, the proportions of variability were not high (0.246 for factor one and relative days to ascend Nib Falls; 0.109 for factor four and fate). These low coefficients of determination mean that using these statistical relationships to assess future outcomes may lead to suspect conclusions. However, these significant relationships were found despite having a small sample size. For example, Wagner and Congleton (2004) used over 100 individuals in a PCA to assess blood chemistry correlates of condition, tissue damage, and stress in migrating juvenile

Chinook salmon; but, Hanson et al. (2007) used a PCA and found significant relationships between swimming performance and morphological correlates of 16 largemouth bass. The fact that we did find some significant relationships despite having a low sample size suggests our relationships may be biologically relevant and that had we had a greater sample size, we may have seen more stronger and significant physiological correlates to migratory behavior and fate.

Migratory Behaviors and Fate

The speed at which the Puntledge River male summer-run Chinook salmon took to reach spawning grounds (i.e., ascend Nib Falls, 11.9 km in ~22 days; Table 3.1) was less than in other systems. The Puntledge River summer-run Chinook salmon moved at a rate ~2 % slower than the rate of PIT (Passive Integrated Transponder)-tagged summer-run Chinook salmon that moved between the Bonneville Dam and the Lower Granite Dam in the Columbia River system (Matter and Sandford 2003). The fact that Puntledge River summer-run Chinook salmon moved at a slower rate may suggest that the river presents a number of challenges that delay or impede migration and/or the physiology of these fish is compromised such that they are unable to migrate at higher speeds. Alternatively, it is possible the Puntledge River stock has large variations in in-stream behaviors due to the long period from marine exit to spawning time. Furthermore, stocking, which does occur on the river, may have imprinted some fish to migrate in an alternative pattern (Power and McCleave 1980).

Fish spent considerable time at the Powerhouse Pool, Stotan Falls, and Nib Falls. These areas are likely ‘stop over’ sites used by upstream migrating fish. Hydropower

release sites and natural barriers often involve complex flow patterns and it has been suggested that this leads to difficulty in locating flow cues (Hinch and Rand 1998; Brown and Geist 2002). In the Puntledge River, fish held at the Powerhouse Pool between 0 and 41 days. Similarly, Scruton et al. (2007a) found that upstream migrating Atlantic salmon (*Salmo salar*) were attracted to a hydropower tailrace in a Newfoundland river for up to 12 days. Rivinoja et al. (2001) also reported that migrating Atlantic salmon were located by hydropower outlets in a Northern Swedish river. The stop-overs at Stotan Falls and Nib Falls tended to be longer than those experienced by fish at other fishways at man-made barriers (e.g., dams) in other river systems (Gowans et al. 2003; Scruton et al. 2007a; Pon et al. 2009a).

Twelve fish were classified as fallbacks which could have been related to tagging methods and handling stress (Bernard et al. 1999). However recent work has suggested that fallback is a part of typical movement behaviors in anadromous fish (Frank et al. 2009) and in the case of this study, fish that fell back were required to naturally re-ascend the hatchery fish ladder in order to resume their upstream migration. This happened for two fish and both were able to reach historic spawning areas (note that these fish were classified as spawners and not fallbacks).

Sex-differences have been shown to affect spawning migrations in Pacific salmon (Quinn 2005) and to influence physiological profiles (Brett 1995; Sandblom et al. 2009). In this study, only male fish were sampled because the female population was captured and used to produce hatchery brood stock. Males typically reach spawning grounds earlier than females (McKinstry 1993), thus the observed rates may have been slower if females were sampled. Furthermore, females may have held at preferable sites longer to

conserve energy and mature gametes as females allocate more energy into sexual maturation (Brett 1995). Females also have different physiological profiles (Sandblom et al. 2009), as female sockeye salmon were found to have higher levels of acute stress indicators (i.e., cortisol, glucose, and sodium) and reproductive hormones (i.e., 11-Ketotestosterone, testosterone, 17 β -Estradiol) when compared to males.

Blood Variables

Pacific salmon cease feeding before entering freshwater and do not eat during the upstream migration (Groot and Margolis 1991; Brett 1995). We found that factor 1 from the factor analysis represented a gradient among individuals in endogenous energy stores and dietary minerals (e.g., calcium, total protein, magnesium, cholesterol, triglycerides, and phosphorous). These variables reflect fasting and starvation in fish (e.g., Robertson et al. 1961; Congleton and Wagner 2006; Hanson and Cooke 2009). In general, the range of endogenous stores we found represent typical values for adult salmon at this point in their migration. Specifically, total protein indicates the amount of albumin and globulin molecules present in the blood and decreasing levels of total protein are found in starving fish and our total protein values reflected previous findings (Robertson et al. 1961; Congleton and Wagner 2006). Cholesterol is a lipid present in animal tissue and also is found to decrease in starving fish (Robertson et al. 1961; Congleton and Wagner 2006). Plasma cholesterol averaged $\sim 11.1 \text{ mmol}\cdot\text{L}^{-1}$, and this value is within the range reported by Robertson et al. (1961) for fish that were between ocean and spawning habitats. Triglycerides are the major storage form of fat, and like total protein and cholesterol, concentrations decrease in starving fish (e.g., Congleton and Wagner 2006; Hanson and

Cooke 2009) and fish undertaking spawning migrations (Ballantyne et al 1996; Kiessling et al. 2004; Magnoni et al. 2006). The Puntledge River summer-run Chinook salmon mean triglyceride value was $\sim 2.5 \text{ mmol}\cdot\text{L}^{-1}$, which is below what Magnoni et al. (2006) found in migrant sockeye salmon ($4.0 \text{ mmol}\cdot\text{L}^{-1}$).

Phosphorus and magnesium are abundant dietary ions. Magnesium typically is obtained from either the surrounding environment or dietary processes and is an essential cofactor in many enzymatic reactions. We found magnesium to range from 1.27 to $1.37 \text{ mmol}\cdot\text{L}^{-1}$ which is below what Snodgrass and Halver (1971) report for adult Chinook salmon in freshwater (i.e., 1.81 to $2.51 \text{ mmol}\cdot\text{L}^{-1}$). Phosphorous is mainly absorbed by fish *via* food sources and is an ion used for many metabolic and growth processes. We found yearly concentrations of phosphorous ions in the blood to range from 3.22 to $3.61 \text{ mmol}\cdot\text{L}^{-1}$, which is higher than those reported for fasting adult largemouth bass (Hanson and Cooke 2009). To our knowledge no baseline values for phosphorous in adult Chinook salmon have been reported in the literature.

Average plasma calcium values ranged from 2.80 to $3.00 \text{ mmol}\cdot\text{L}^{-1}$ which may indicate that the sampled fish are in a state of hypocalcemia, as previous work has found normal values to be above $5 \text{ mmol}\cdot\text{L}^{-1}$ (Snodgrass and Halver 1971). Low levels of calcium in Chinook salmon have been shown to reduce growth and result in poor feed conversion (Lall 2002). Sexual maturing Atlantic salmon utilize calcium for remodeling their body shape, so the hypocalcemia observed may impact the ability of the sampled Chinook salmon to form sexual characteristics (Kacem et al. 1998; Kacem et al. 2000; Witten and Hall 2003).

The osmoregulatory preparedness factor was composed of 4 variables. Sodium, chloride, and potassium represent a suite of ions that allowed us to investigate the effects of osmoregulatory preparedness. In general, our study fish had normal concentrations of osmoregulatory ions, but with a tendency to be more suited to 10 ‰ salinity water (Clarke and Hirano 1995), suggesting that these fish were still undergoing physiological changes associated with the transition into freshwater (Clarke and Hirano 1995; Hinch et al. 2006; Donaldson et al. 2010). In juvenile Chinook salmon invasive blood sampling techniques can elevate AST, plasma potassium, and decrease plasma sodium and chloride when compared to the least invasive technique, caudal vessel puncture (Congleton and LaVoie 2001). Overall, our fish were not undergoing osmoregulatory stress at the time of sampling.

The two remaining factors were a glucose factor and a hematological factor (Table 3.4). The glucose factor solely was comprised by glucose, which may indicate acute stress because plasma glucose is responsive to stressors (Wendelaar Bonga 1997); and not likely to be influenced by feeding because the sampled fish were fasting (Navarro and Guitiérrez 1995). Patterson et al. found similar plasma glucose concentrations in healthy migrating Chinook salmon (unpublished data). The blood factor was comprised of hematocrit and potassium. Hematocrit is the packed cell volume of blood and was found to be ~0.51 (Table 3.3). Hematocrit is not a particularly sensitive stress indicator (Barton et al. 2002) and, like glucose, can change rapidly in response to handling and blood collection methods (Sandblom et al. 2009). As discussed early, plasma potassium was within range of typical concentrations for Pacific salmon during the upriver portion of the spawning migration.

Conclusions

Our results indicate that while physiological and nutritional conditions can influence adult migrating male summer-run Chinook salmon, we did not find a physiological profile that could explain a large portion of the extensive variation in migratory behavior observed. This result suggests that variation in physiological and nutritional conditions among individual fish at the time near river entry is not an important influence on the migration behavior in this system. Our physiological profiles suggest that the Puntledge River summer-run Chinook salmon are in good condition with minimal physiological stress. Migratory behaviors were slower than in other larger systems, but fish were able to reach spawning grounds and presumably spawned. This study could serve as a baseline for assessing other populations and their physiological state and may be useful in identifying populations in need of conservation efforts. In addition, this study represents one of the first examples of using conservation physiology to understand problems with an imperiled population of river fish.

Table 3.1. A summary of the migratory behavior metrics in the upstream migration of the 58 radio-tracked summer-run Chinook salmon in the Puntledge River. There were no statistically significant differences between years and the 5 migratory behavior metrics.

Year	Salmon tagged	Number of Fish Held at the PHP	Mean Days Held at the PHP (min.-max.)	Salmon to SF	Days from PHP to SF	Salmon passing SF	Days to ascend SF	Salmon to NF	Days from SF to NF	Salmon passing NF	Days to ascend NF
2007	21	8	2 (0-17)	21	8 (2-22)	14	13 (2-47)	14	3 (0-20)	11	3 (1-11)
2008	13	4	5 (0-41)	13	5 (1-12)	7	7 (1-22)	7	1 (0-3)	5	4 (2-10)
2009	24	14	7 (0-25)	19	15 (2-29)	18	5 (1-19)	18	1 (0-5)	17	2 (0-5)
Sum:	58	26	Mean: 4 (0-41)	53	11 (1-29)	39	8 (1-47)	39	2 (0-20)	33	3 (0-11)

Note: Abbreviations: PHP (Powerhouse Pool); SF (Stotan Falls); and NF (Nib Falls).

Table 3.2. The fate of the 58 radio-tracked summer-run Chinook salmon in the Puntledge River and the percentage of total sample size. There were no statistical differences between year and fate.

Year	Number of possible spawners	Number of pre-spawn mortalities	Number of fallbacks
2007	14 (67 %)	4 (19 %)	3 (14 %)
2008	7 (54 %)	1 (8 %)	5 (38 %)
2009	17 (70 %)	3 (13 %)	4 (17 %)
Sum:	38 (66 %)	8 (14 %)	12 (20 %)

Table 3.3. Means ($\pm$ SD in parentheses) for blood physiology variables included in the datasets for summer-run Chinook salmon from the Puntledge River.

Variable	2007	2008	2009
Hematocrit	0.52 (0.08)	0.47 (0.07)	0.54 (0.07)
Total Protein (g/L)	46 (4)	47 (6)	47 (4)
AST (U/L)	1080 (277)	1106 (234)	1222 (389)
Cholesterol (mmol L ⁻¹)	10.4 (1.9)	11.1 (2.3)	11.8 (2.1)
Triglycerides (mmol L ⁻¹)	2.9 (1.1)	2.5 (1.4)	2.2 (0.6)
Glucose (mmol L ⁻¹)	5.4 (0.7)	5.3 (1.1)	6.2 (1.2)
Sodium (mmol L⁻¹)	164 (5)^a	171 (7)^b	161 (7)^a
Potassium (mmol L ⁻¹)	3.13 (3.17)	2.29 (1.35)	2.39 (1.36)
Chloride (mmol L ⁻¹)	136 (6)	140 (4)	129 (5)
Calcium (mmol L ⁻¹)	3.00 (0.24)	2.98 (0.32)	2.80 (0.26)
Phosphorous (mmol L ⁻¹)	3.22 (0.57)	3.36 (0.35)	3.61 (0.57)
Magnesium (mmol L ⁻¹)	1.37 (0.14)	1.35 (0.17)	1.27 (0.15)

Note: Rows in bold denote statistical differences between years (Sodium: Kruskal-

Wallis; $\chi^2 = 16.35$; $P < 0.001$; similar subscript letters indicate similarity between the years).

Table 3.4. Rotated factor loadings and final communalities for factor analysis of blood chemistry data for summer-run Chinook salmon in the Puntledge River (n = 58).

Plasma variable	Factor 1	Factor 2	Factor 3	Factor 4	Final communality estimates
<i>Eigenvalue</i>	<i>4.00</i>	<i>2.38</i>	<i>1.28</i>	<i>1.13</i>	
Calcium (mmol L ⁻¹) ¹	89	22	-10	3	0.842
Total Protein (g/L)	84	-12	18	15	0.783
Magnesium (mmol L ⁻¹) ¹	72	47	11	19	0.788
Cholesterol (mmol L ⁻¹)	68	-23	14	9	0.545
Triglycerides (mmol L ⁻¹) ¹	60	18	-18	-7	0.436
Phosphorous (mmol L ⁻¹) ¹	56	4	45	34	0.635
Chloride (mmol L ⁻¹)	0	85	-35	-8	0.858
Sodium (mmol L ⁻¹)	41	79	-13	8	0.819
AST (U/L) ¹	30	-62	-19	29	0.600
Potassium (mmol L ⁻¹) ¹	-20	-54	-35	-56	0.770
Glucose (mmol L ⁻¹)	0	-13	91	-7	0.847
Hematocrit (%)	5	-16	-9	91	0.866
<i>% variance explained</i>	<i>33.4</i>	<i>19.8</i>	<i>10.7</i>	<i>9.4</i>	

Note: Loadings are multiplied by 100 and rounded to the nearest integer. Variables with factor loadings $\geq \pm 50$ are shown in bold. ¹ indicates the variable has been log₁₀ transformed.

Figure 3.1. Map of the location of the study area in the Pacific Northwest and a map of the key areas in the study area with the distance from the estuary in parenthesis.

Figure 3.2. Linear regression of rotated factor 1 (the only factor to elicit a statistical relationship between the factors and the migratory behaviors) and the relative number of days to ascend Nib Falls (the amount of days a fish took to ascend Nib Falls after ascending Stotan Falls).

Figure 3.3. Mean values and standard deviation of rotated factor 4 (the only factor to elicit a statistical relationship and the fates) of fish classified as either fallback, prespawn mortality, or spawner

Chapter 4: Thermal biology and bioenergetics of different upriver migration strategies in a stock of summer-run Chinook salmon

Abstract

By combining biotelemetry with animal-borne thermal loggers, we re-created the thermal histories of 21 summer-run Chinook salmon (*Oncorhynchus tshawytscha*) migrating in the Puntledge River, a hydropower impacted river system on Vancouver Island, British Columbia, Canada. Daily maximum water temperatures in the Puntledge River during the summer-run adult Chinook salmon migration and residency period frequently exceeded 21 °C, a value that has been observed to elicit behavioural thermoregulation in other Chinook salmon populations. We therefore compared river temperatures to body temperatures of 16 fish that migrated through the river to understand if cool-water refuge was available and being used by migrants. In addition, we used thermal histories from fish and thermal loggers distributed in the river to model the effect of thermal habitat on energy density using a bioenergetics model to predict energy depletion. In general, we found no evidence that cool-water refuge existed in the river, suggesting that there is no opportunity for fish to behaviourally thermoregulate in the river system during upriver migration through the regulated portion of the river. Of the thermal histories used in the bioenergetics model, fish that reached an upstream lake were able to access cooler, deeper waters which would have reduced energy consumption compared to fish that only spent time in the warmer river. We discuss the thermal refuge and potential fitness-level impacts of our bioenergetics findings on the summer-run Chinook salmon relative to

management and restoration opportunities for this imperiled population. We also emphasize the benefits of combining both biotelemetry and biologging techniques to study the thermal biology of wild animals.

Introduction

Understanding the thermal biology of fish has become increasingly important due to global climate change and the rise in water temperatures in some regions (Pörtner 2002; Pörtner and Farrell 2008, Farrell et al. 2008; Hofmann and Todgham 2010). Pacific salmon (*Oncorhynchus* spp.) are cold-water species and are at particular risk to increases in ambient water temperature throughout their life cycle (McDaniels et al. 2010), but especially during their spawning migration (Crozier et al. 2008; Farrell et al. 2008; Farrell et al. 2009). Pacific salmon have a thermal preference between 4 and 18 °C (Brett 1971) and many migratory routes, such as the Fraser River in Canada, routinely experience temperatures greater than 18 °C during time periods when salmon are migrating upstream (Patterson et al. 2007; Hague et al. 2010; Martins et al. 2010). Temperature is considered the “master” abiotic factor for fish (Fry 1968) and influences the behavioural ecology and most physiological processes of Pacific salmon (see reviews by Brett 1995 and Quinn 2005).

Fish can select their surrounding temperature by using behavioural thermoregulation to exploit thermal habitats at either a coarse- (e.g., lake vs. river) or fine-scale (e.g., groundwater, or cool water from a tributary). Migrating adult salmon can avoid warm temperatures by delaying or advancing up-river migration times (Berman and Quinn 1991; Newell and Quinn 2005; Keefer et al. 2008a, 2009) though most stocks have limited flexibility in migration timing because spawning times tend not to vary regardless of migration initiation dates (Cooke et al. 2004c). Pacific salmon can also thermoregulate when faced with water temperatures that exceed their tolerance by

seeking cool water in lakes, tributaries, or groundwater (e.g., Newell and Quinn 2005; Keefer et al. 2008a, 2009). In addition to aiding in survival, exploiting cooler water offers a trade-off between reproductive development and energy savings (Berman and Quinn, 1991). Cooler water will conserve energy, which is important as adult Pacific salmon have stopped feeding and rely on energy reserves to mature gametes and to complete migration and spawn; however, cooler water can slow gamete development which could impair spawning success.

Hydropower infrastructure and operations can affect the thermal regime of rivers (Angilletta et al. 2008) and thus, may impact the behavioural thermoregulation of salmonids. Controlled releases of cool hypolimnetic water, the presence of deep pools near dams, and water release sites that salmon can exploit as cool-water refuge (Nickel et al. 2004) may facilitate behavioural thermoregulation. However, dams, diversion structures, natural barriers, and inefficient fish passage ways may prevent fish from accessing thermal refuges, such as lakes and/or reservoirs located upriver. Furthermore, water drawdowns and low flows associated with hydropower operations can lead to elevated river temperatures (Angilletta et al. 2008), potentially reducing the availability of riverine thermal refuges.

The purpose of this study was to assess the thermal biology and associated bioenergetics of adult upriver migrating summer-run Chinook salmon (*Oncorhynchus tshawytscha*) in the Puntledge River, British Columbia. The stock typically arrives at the mouth of the river between late May and late July and has a spawning window between late September and late October (E. Guimond, private consultant, personal communication). Fish can either hold in the river or move through a number of fishways

to gain access to cool hypolimnetic water in Comox Lake, though migration through the river typically takes between 3 weeks and 4 months (Chapter 3). In addition, it is unknown whether cool-water refuge exists in the river, and because the stock can be present in the river for over 4 months during warm weather conditions (compared to other runs of salmon [i.e., fall-run Chinook salmon]), there is potential for the exposure to high temperatures to have negative consequences on fish energetics, health, condition and survival. The summer-run population of Puntledge Chinook salmon has declined over the last few decades as a result of several factors including overexploitation, habitat alteration and thermal conditions during up-river migration (Darcy Miller, DFO Restoration Ecologist, Personal Communication). Although the population is not officially listed, a recovery strategy exists and significant funds are spent annually on trying to enhance the stock. Elevated water temperature has been identified as a potential important factor influencing the recovery potential for this imperiled stock so this study has the opportunity to inform management and conservation efforts.

We used *in situ* biologgers and biotelemetry to understand whether fish were exposed to high temperatures and if fine-scale cool-water refuges were present. We then assessed the ability of the tagged fish to behaviourally thermoregulate and whether this varied by river location, water temperature, and river discharge. Lastly, we assessed how choice of thermal habitats could influence spawning success. Specifically, with a bioenergetics model, we estimated energy use of fish which were exposed to different thermal conditions during their migration (i.e., migration only in the river during hot and cool years, migration through the river and into the lake, and truck transport directly into the lake with no encounter of the river beyond the sampling point) and determined which

migratory conditions were most likely to result in fish surviving to the historical spawning dates.

Methods

Study Area and Sampling Locations

The Puntledge River is located on the east coast of Vancouver Island Canada (Figure 4.1). The river is approximately 16.9 km long and drains Comox Lake into the Comox Estuary. A hatchery located near the river supplements the natural populations of Pacific salmon in the river, but the summer-run of Chinook has experienced continuous declines in returning adults since the development of a hydroelectric facility, despite much effort from both Fisheries and Oceans Canada and the hydroelectric utility (Hirst 1991). The hydroelectric facility consists of a storage dam at the output of Comox Lake that releases water from near the surface of Comox Lake and has been outfitted with a vertical slot fishway. Approximately 3.0 km downstream, another dam diverts water through a penstock that funnels water through the turbine 7.2 km downstream. A fishway is also located at the diversion dam. The diversion results in the river having relatively lower flows between 6.8 river km to 14.0 rkm (rkm measured starting at the estuary). Within this reach there are two natural barriers, Stotan and Nib Falls. Both falls are three-tiered waterfalls with habitat enhancement to aid in the upstream movement of adult fish (i.e., concrete baffle fishways). The penstock output at the power station is located at 6.8 rkm and our capture and release point was located at 6.4 rkm. River temperature was measured at 4 locations throughout the river using Stowaway Tidbit Temperature

Loggers (Model TBI32-05+37; Onset Computer Corporation, MA, USA; Temperature accuracy = ± 0.20 °C, Temperature resolution = ± 0.16 °C) (Upper Hatchery, Highway Crossing, Upper Side Channel, and the Lower Hatchery; Figure 4.1; Figure 4.2). The thermologgers had a sampling rate of 15 min and were located in areas continuously wetted and exposed to typical river flow conditions.

Study Animals and Telemetry Methods

Between June 25 and July 20 2007 and 2008, and between June 2 and June 26 2009, 103 adult male summer-run Chinook salmon were diverted into raceways at the Lower Puntledge River Fish Hatchery (Fisheries and Oceans Canada; size distribution [2007, 38 fish; TL = 727 ± 14 mm [S.D.]; 2008, 26 fish, TL = 663 ± 11 mm; 2009, 39 fish, TL = 646 ± 56 mm]; Figure 4.1) for transmitter implantation. The conservation status of the stock meant that we were restricted from tagging female fish which is admittedly a limitation, but at this point we are unaware of studies that document differential behavioural thermoregulation or thermal tolerances in adult Pacific salmon. Of the 103 fish, thirty-seven fish (16 in 2007, 11 in 2008, and 10 in 2009) were implanted with coded electromyogram (CEMG) transmitters (CEMG2-R16-25 [2007, 2009] and CEMG2-R11-25 [2008], Lotek Engineering, Inc., Newmarket, Ontario; surgical methods outlined in Cooke et al., [2004b]), and sixty-six fish (22 in 2007, 15 in 2008, and 29 in 2009) had a conventional radio transmitter (MCFT-3A, Lotek Engineering, Inc., Newmarket, Ontario) gastrically inserted with the antenna protruding from the oral cavity. Beyond the 103-tagged fish, an additional 30 fish (2007, 10 fish; TL = 672 ± 5 mm [S.D.]; 2008, 10 fish, TL = 638 ± 4 mm; 2009, 10 fish, TL = 629 ± 4 mm) were

implanted with conventional radio transmitters (MCFT-3A, Lotek Engineering, Inc., Newmarket, Ontario) and transported *via* a tanker truck and released directly into Comox Lake. The entire confinement and transportation time was < 30 minutes and there was no more than 100 kg of fish in 1600 L of oxygenated freshwater. Temperature loggers (iButton DS1921Z; factory-stated resolution = ± 0.1 °C, accuracy = ± 1 °C; Maxim Integrated Products, Inc., Sunnyvale, California) were attached to each transmitter using white glue and Plasti Dip ® (Performix Brand, Blaine MN) and were programmed to record temperature at 2 h intervals (Donaldson et al. 2009). Similarly plasticized temperature loggers were tested in a laboratory setting to have an accuracy of 0.4 ± 0.3 °C and a temperature precision of 0.2 ± 0.3 °C (Donaldson et al. 2009).

Fish were manually tracked from shore at least twice a day to estimate location (time to determine location varied from 30 s to 15 min) between June and early October using a telemetry receiver (SRX-600 or SRX-400, Lotek Engineering, Inc., Newmarket, Ontario) and a 3-element Yagi antenna. Fish locations (approximate river km) were determined using zero point tracking, as the river is narrow and there are a minimum number of possible locations fish can inhabit, meaning individual fish could be readily found. Transmitter tracking is impossible at depths greater than 10 m; however, the river has no pools greater than this depth. The tracking period encompassed the time period that summer-run Chinook salmon enter the river and post-spawning die-off. River temperatures were assigned to each 'fish' temperature based on the closest river temperature sampled. Fish released directly into the lake occupied water depths in the main body of the lake where they could not be effectively tracked, and were only located

during the spawning period once they moved into the lake outlet and upper reach of the river (Reach 7, Figure 4.1).

Recovery of Transmitters and Thermal Loggers

Of the 133 deployed transmitters affixed with thermal loggers, 21 were recovered and contained data that were used in this study (Figure 4.3; low recovery rates were due to tags being washed out of the system during periods of high flow and tags being located in areas unsafe for tag retrieval). Transmitters and thermal loggers were recovered throughout the study period; however, most searching and collection occurred after the spawning period when fish were decomposing. Fish that were observed by radio-tracking to be moving upstream prior to the spawning period were classified as spawners ($n = 8$) and fish that were visually confirmed to have died prior to the spawning period (but spent greater than one month in the river) were classified as pre-spawn mortalities ($n = 8$). The remaining 5 fish had held in the lake and then spawned in the river below the lake (Reach 7). A further 23 transmitters with affixed thermal loggers were recovered but either contained no data, or were from fish observed to have been dead (dates of death unknown) after only a few weeks in the river.

Behavioural Thermoregulation

Mean body temperature was calculated for each fish (using all thermal records) and a Wilcoxon's sign-rank test was used to assess whether or not body temperature predicted spawning fate. The study area was divided into study segments to understand if fish location influenced body temperature and ΔT (body temperature – river temperature). In

order to assess the body temperature versus the river temperature, body temperature and river temperature were compared within each study segment using a Wilcoxon's sign-rank test. Telemetry data were used to determine positions of the fish. ΔT was compared among study segments using a Kruskal-Wallis non-parametric ANOVA. The slope of the relationships between each fish in each segment was calculated using a linear regression and then compared to a hypothetical slope of 1 using a Wilcoxon's sign-rank test which enabled us to test the hypothesis that there may be an influence of increased river temperatures on body temperatures if behavioural thermoregulation were occurring (Donaldson et al. 2009). A significant difference between the slopes would indicate temperature-dependent selection by the individuals. Linear regressions were also used to estimate if river discharge, total length, or distance from the river temperature sensor had significant effects on ΔT . All statistical analyses were done using JMP 7.0.2 (SAS Institute Inc., Cary NC, USA) and significance was tested at $\alpha = 0.05$.

Energy Density and Thermal Habitat

In order to predict energy density based on thermal habitat use, we developed a model using the software Fish Bioenergetics 3.0 (Hanson et al. 1997) and the physiological parameters found in Stewart and Iberra (1991) for Chinook salmon. In 2007 we used a handheld microwave energy meter (Crossin and Hinch 2005) to estimate somatic energy concentration of the 38-tagged summer-run Chinook salmon ($TL = 727 \pm 14$ mm [S.D.]). Using a species-specific length-weight relationship, we transformed total length of each fish into weight (Jasper and Evenson 2006), and then converted the energy meter values into somatic energy concentrations using the equation for sockeye salmon presented in

Crossin and Hinch (2005). The estimate of somatic energy density used in the bioenergetics model was 9.00 ± 0.57 (S.D.) $\text{MJ}\cdot\text{kg}^{-1}$. The bioenergetics model allowed for the calculation of routine daily energy use (in $\text{MJ}\cdot\text{kg}^{-1}$).

In the model we varied temperature using four different hypothetical thermal regimes based on the experiences of the Puntledge River summer-run Chinook salmon as recorded by the temperature loggers attached to the transmitters. Thermal regimes were based on temperatures experienced by fish in the river during 2007 (River Cool), fish in the river during 2009 (River Hot), fish that were transported to the Lake in 2009 (Lake), and fish that used both the river and the lake in 2009 (River and Lake [note that this is the River Hot year but fish were tagged earlier and the fish used in this scenario had migrated through the river before temperatures became warm]; Figure 4.4). These thermal regimes represent all the possible outcomes for the migrating salmon once they reached the tagging site.

Historically, it is believed that fish would migrate through the river and enter the lake and hold in the cool waters; thus the River and Lake thermal regime represents the historical normal, the River (Hot and Cold) regimes represent annual variation during post-hydropower development, and the Lake regime represents a possible management directive. Post-spawned salmon die when energy density are $< 4.0 \text{ MJ}\cdot\text{kg}^{-1}$ (Hendry and Berg 1999, Geist et al. 2000; Crossin et al. 2004b) therefore, we used this energy density value as a benchmark to assess spawning success – fish for which our modeling simulations indicate would be reduced to this energy level prior their historical spawning dates would be classified as dying unspawned. The simulation day start date was set at July 11 (the mean date of energy density sampling). Because all of our sampled fish

were males, and males allocate only a small fraction of total energy into gonads (e.g., 31-45 % of total energy and remains relatively unchanged (0-2 %) between river entry and spawning grounds; Crossin et al. 2004b) we did not account for energy partitioning to gonads in our modeling.

Results

Behavioural Thermoregulation

Mean body temperature for the 8 fish that spawned was 17.7 °C (individual fish means ranged from 16.9 to 18.3 °C; Figure 4.5a) and did not differ from the mean body temperature for the 8 fish that did not spawn (17.8 °C; individual fish means ranged from 17.0 to 18.6 °C; Figure 4.5b; Wilcoxon's sign-rank test, $Z = 0.053$, $P = 0.958$). Mean ΔT did not differ between spawning fates (Wilcoxon's sign-rank test, $Z = 0.473$, $P = 0.637$).

In total, 2145 of 10988 body temperature observations were warmer than river temperatures by ≥ 1.0 °C and 419 of 10988 body temperature observations were cooler than river temperature by < 1.0 °C. Over the three-year study period, the mean body temperature across all fish was 16.7 ± 1.5 °C in the Power House segment, 16.3 ± 1.4 °C in the Island Pocket segment, 17.7 ± 2.0 °C in the Upper Side-Channel segment, 18.1 ± 1.4 °C at Stotan Falls, 18.0 ± 1.1 °C in the Highway Crossing segment, 18.1 ± 1.3 °C at Nib Falls, and 17.6 ± 2.1 °C in the Upper Hatchery Site segment. Within each study segment, mean body temperature did not differ from the mean river temperature (Wilcoxon's sign-rank tests, all $P < 0.56$) and mean ΔT did not vary among study segments (Kruskal-Wallis test, $\chi^2 = 0.544$, $DF = 6$, $P = 0.997$; Figure 4.6). The mean

regression coefficients of body temperature and river temperature did not differ from a hypothetical slope of 1 in any of the study segments (Table 4.1).

River discharge did not affect the mean ΔT (Table 4.2) nor did total length have an effect on mean ΔT (linear regression; $R^2 = 0.003$; Sum of Squares = 3.540; $df = 15$; $P = 0.836$). In addition, the mean distances between the river sampling location and the location of the fish (body temperature) was -802 m (upper 95% = -773; lower 95% = 860). Mean ΔT was not related to the mean river distance from the river temperature sensors that the body temperature was assigned to (linear regression; $R^2 = 0.045$; Sum of Squares = 3.54, $DF = 1, 14$, $P = 0.484$).

Bioenergetics Modeling

The mean temperature for the River Cool group was 16.4 °C (13.5-18.8 °C min-max), River Hot was 17.6 °C (15.2-21.6 °C min-max), Lake was 14.6 °C (13.7-15.2 °C min-max), and River and Lake was 14.7 °C (12.9-18.8 °C min-max) (Figure 4.4). When the thermal regimes were entered into the bioenergetics model to assess the effect of temperature on energy density, it was found that by late September energy had depleted to $< 4.0 \text{ MJ}\cdot\text{kg}^{-1}$ for the River (Hot) and River (Cold) thermal regimes (Figure 4.7). The model outcome varied by 11 days for River (Hot), 15 days (for River (Cold), 20 days for Lake, and 25 days for River and Lake depending on the initial energy value (varied $\pm$ standard deviation[$0.57 \text{ MJ}\cdot\text{kg}^{-1}$]).

Discussion

Cool-water Refuge

Multiple studies have shown that migrating adult Pacific salmon will exploit cool water refuge to avoid warmer water temperatures during the freshwater migration, likely to conserve energy and to develop healthy gametes (Berman and Quinn 1991; Newell and Quinn 2005; Hyatt et al. 2003; Goniea et al. 2006; Keefer et al. 2009; Mathes et al. 2010). In our study, we found no evidence that the Puntledge River summer-run Chinook salmon were able to find and use cool water refugia which may indicate that the river is generally well mixed. Interestingly, fish were at times exposed to higher temperatures than recorded by river temperature loggers. This may suggest that in-river characteristics or flow-dynamics may be causing areas used by the fish to be warmer, possibly representing tradeoffs between the various habitat features that collectively influence habitat choice (e.g., flow characteristics, proximity to cover; Caissie 2006).

The lack of cool-water refuge resulted in no behavioural thermoregulation being observed. The study site was only 10.5 km long which is relatively small compared to rivers that have been used in the past to study behavioural thermoregulation (i.e., study segments in previous studies, Yakima River [~ 344 km; Berman and Quinn 1991], Columbia River [~ 404 km; Goniea et al. 2006], the Lower Fraser River [~ 238 km; Donaldson et al. 2009]). The Puntledge River is lake headed and regulated for power production in a manner typical of most hydropower operations, and results in short-term stable water temperatures during the upstream migration period. There are also no tributaries on the river that appear to provide water that is cooler than the mainstem Puntledge River. It is important to note that there was no relationship between river discharge and the difference between body and river temperatures. These characteristics

of the river may indicate that there are few, if any, cold-water refuge sites available for the summer-run Chinook salmon to use when migrating upriver to spawn. Conversely, in the Columbia River Keefer et al. (2009) found that adult steelhead (*O. mykiss*) exploited cooler tributaries for 3 to 4 weeks to avoid warmer mainstem temperatures and then resumed migration.

Historically, Comox Lake was likely less difficult for summer-run Chinook salmon to access (i.e., higher river discharge at time of migration such that natural barriers were easier to pass, no fishways to maneuver through, no recreational use of the river by humans, etc.). Comox Lake is a glacier fed, deep-water lake (max. depth = 109 m), and provides ample cool water for sexually maturing summer-run Chinook salmon to use. In fact, fish that used the Lake used cooler water during the warmer spawning migration periods (in comparison to fish using the river during the same period). This phenomenon has been observed in other river-lake systems (e.g., Newell and Quinn 2005; Mathes et al. 2010) and has direct benefits to reproductive and energetic status of maturing adult salmon (Roscoe et al. 2010b). Since the Puntledge River has only recently (~100 yrs) been impacted by anthropogenic factors, the summer-run stock may not yet have adapted to the habitat conditions of the river, and may be adapted to use behavioural thermoregulation in the lake. Waples et al. (2008) suggest that if anthropogenic changes to the environment occur at spatial and temporal scales that more closely mimic environmental changes that have occurred during the evolutionary history of Pacific salmon, stocks will be more “well-equipped” to deal with such changes. Evolutionary change and/or extirpation have been shown in large big dam river systems,

as thermal regime changes caused by hydropower has been shown to decrease the mean fitness of Chinook salmon (Angilletta et al. 2008).

There are several potential consequences to adult migrating salmon if they do not use cool-water refuge during their upstream freshwater migration. Exposure to high river temperatures can collapse aerobic scope (Farrell et al. 2008; Mathes et al. 2010), increase infection rate of parasites and disease (Wagner et al. 2005; Crossin et al. 2008; Bradford et al. 2010), slow travel rates (Crossin et al. 2008), and cause energy to be used at an elevated rate (this study), all of which can lead to high levels of migration mortality (Goniaea et al. 2006; Caudill et al. 2007; Crossin et al. 2008; Keefer et al. 2008a). However, we found limited evidence that the fish in the study segment were being exposed for prolonged periods of time (> 12 hours) to temperatures above which migration is halted (21°C , McCullough 1999). It is also unlikely that aerobic scope collapsed as summer migrating salmon tend to have critical temperatures $> 21^{\circ}\text{C}$ (MacNutt et al. 2006; Farrell et al. 2008) however we know relatively little about swimming performance of adult Puntledge River Chinook salmon and stocks can differ greatly in this regard (Farrell et al. 2008). In some populations of Pacific salmon, accumulated thermal units can be a reasonable predictor of migration success. In late-run Fraser River sockeye salmon, fish which migrate early and therefore encounter warm river temperatures and accumulate > 500 degree days are unlikely to successfully spawn whereas later migrants accumulate much fewer degree days and are more successful at spawning (Wagner et al. 2005; Crossin et al. 2008). In those studies, thermal-mediated diseases were likely responsible for the mortality. In our study, fish entered the study area at various times (i.e., those that perished were not the earliest migrants) but because

spawners lived longer than those which perished early, their cumulative thermal experience was much higher than that of the fish which perished before spawning. Future work should focus on identifying the stock-specific mechanisms that drive possible temperature caused pre-spawn mortality.

By only recovering 16 % of the thermal loggers, the thermal history data may be biased. Because variation among the 16 % thermal loggers was consistent between fish tagged in similar years, we do not believe this bias was significant and it is likely that our conclusions can be inferred for the entire population.

Energy Density and Thermal Habitat

The bioenergetics model predicts that fish behaviour with respect to thermal experience clearly influences energy available at the time of spawning. At the time of spawning fish must be reproductively mature and have enough somatic energy for spawning rituals (i.e., staging, competition, coitus, and minimal brood defense by females) during a window of opportunity when both sexes are present on the spawning grounds (Quinn 2005). Fish experiencing warmer thermal regimes will have less energy available resulting in a number of fitness-level implications: 1) fish will have lower amounts of energy to develop secondary sexual characteristics (Hendry and Berg 1999); and 2) males will have fewer energy stores to fight for access to spawning females (Quinn et al. 1996).

Summer-run Chinook salmon had higher energy densities further into the model simulation when they were exposed to the cooler waters in Comox Lake. The bioenergetics model predicts that only fish that use Comox Lake and fish that use Comox

Lake and experience early season river temperatures will have a sufficient amount of somatic energy available to potentially use to spawn during the typical spawning period. This finding supports the argument that thermal refuge is beneficial to the spawning success of migrating salmon. Because Pacific salmon do not feed during their upriver migration, energy use must be optimized to ensure reproductive status is sufficient for spawning during the historic spawning period (Brett 1995). Mathes et al. (2010) found that of the early-timed Weaver Creek sockeye salmon tagged in the study, only fish that held in Harrison Lake survived to spawn. The other fish held in the warmer Harrison River. Furthermore, Newell and Quinn (2005) found sockeye salmon hold in the cool water of Lake Washington during warm summer months prior to spawning. Likewise, other studies have shown reduced mortality in fish that use coldwater tributaries (Gonia et al. 2006; Keefer et al. 2008a). Direct links to energy levels have been found in female sockeye salmon (Roscoe et al. 2010b). These studies support our model prediction and further highlight the importance of ensuring migrating salmon have access to thermal refugia during the spawning migration.

The predictive model has limitations. Firstly, we presented only hypothetical thermal regimes of fish based on field obtained temperatures from a few fish (internal temperatures between fish varied little), all of which were male, and in-lake thermal loggers. Secondly, only routine activity level was used in the bioenergetics model (Stewart and Ibarra 1991). The activity level of River fish and Lake fish likely differs, as fish using the river may be exposed to more variable flow conditions which may require increased swimming activity (this would only accentuate the difference between them). Presumably lake fish do not have to expend the same level of activity swimming,

although it is worth noting that in the ocean salmon swim up to 40km/day so it is also possible that swimming activity was not appreciably different between those habitats. There is no research available that provides fine-scale measures of swimming activity of Pacific salmon in lake systems, though Young and Woody (2007) and Roscoe et al. (2010b) have measured travel rates in separate lake systems. If activity level were varied in the model (based on the assumption that River fish are more active than Lake fish), it would result in energy density depleting at a faster rate (Brett 1995). In addition, activity in warmer waters would reduce somatic energy stores faster than at cooler temperatures (Lee et al. 2003). Overall, our model represents a “best case” scenario based on temperature and by manipulating other variables, like activity, energy depletion will occur at a faster rate. However, if fish were to have greater somatic energy density at the time of river entry (though current research suggest that energy density at time of river entry is dependent on ocean conditions and may be declining [Crossin et al. 2004a]), energy density would persist to later in the year. Finally, the initial energy values were calculated using a lipid probe calibrated for sockeye salmon because we were unable to sacrifice fish to perform a Puntledge River Chinook salmon calibration. However, both Hendry and Beall (2004) and Crossin and Hinch (2005) show positive relationships with high R^2 values between lipid probe measured energy density and laboratory measured energy density in Atlantic salmon (*Salmo salar*) and sockeye salmon, respectively. Furthermore, the standard deviation of our calculated energy density values was low and resulted in deviations in the model predictions that did not change the conclusions.

Conclusions

We tagged individual fish with both radio transmitters and thermal loggers to link the spatial ecology of individuals with thermal characteristics of the river. We found no evidence that the Puntledge River summer-run Chinook salmon had opportunities to locate cool-water refuge in the regulated reach of the river, and thus fish were unable to use behavioural thermoregulation in the river. However, our bioenergetics model revealed that using cooler water refuge in Comox Lake (if fish are transported to the lake or are able to migrate through the regulated section to reach the lake) will lead to a reduction in the rate at which energy density declines. This finding suggests that there are potential fitness benefits if fish can access cool-water refuge in the lake, and highlights the importance of management strategies that consider lake access *via* modification to fish passage ways to enhance upstream movement, or by capture and trucking methods, particularly if current global climate change models are correct. Other strategies that take into account the necessity of cooler water, such as the installation of a cold-water release structure, or the use of a cold-water holding facility, could also benefit the population. Future studies should focus on successful fish passage through the regulated portion of the river and into Comox Lake (a behaviour that has been observed). In addition, stock conservation and increased adult returns may be promoted if studies highlighting the in-lake ecology of the stock and their pre-spawning movements are assessed. This study has the potential to reduce uncertainty regarding management options and provides a tool to evaluate thermal consequences of different management scenarios.

Table 4.1. Results of a Wilcoxon's sign-rank test (critical $\alpha = 0.007$) between the regression slope of river temperature versus body temperature and a hypothetical slope of 1 for each study segment for upriver migrating summer-run Chinook salmon.

Study Segment	<i>n</i>	Difference (mean $\pm$ SD) from hypothetical slope of 1	Test Statistic	<i>P</i>
Power House	11	-0.03 $\pm$ 0.33	-8.0	0.505
Island Pocket	9	-0.09 $\pm$ 0.22	-7.5	0.406
Upper Side-Channel	10	-0.23 $\pm$ 0.34	-18.0	0.068
Stotan Falls	15	-0.05 $\pm$ 0.21	-7.5	0.659
Highway Crossing	10	-0.03 $\pm$ 0.33	7.5	0.475
Nib Falls	13	0.07 $\pm$ 0.19	16.0	0.233
Upper Hatchery Site	8	0.10 $\pm$ 0.10	10.5	0.03

Table 4.2. Results of the mean difference of body temperature and river temperatures versus log-transformed river discharge ($\log RDischarge$; $m^3 \cdot s$; $\alpha = 0.007$) for each study segment for summer-run Chinook salmon

Study Segment	Regression equation	R^2	df	Sum of squares	P
Power House	$-1.504 + 1.608 \log RDischarge$	0.372	10	2.320	0.047
Island Pocket	$1.001 - 1.021 \log RDischarge$	0.119	8	4.380	0.364
Upper Side-Channel	$0.665 - 0.657 \log RDischarge$	0.049	10	1.518	0.512
Stotan Falls	$-0.041 + 1.608 \log RDischarge$	0.003	14	1.664	0.851
Highway Crossing	$-0.536 + 0.690 \log RDischarge$	0.077	9	0.995	0.4391
Nib Falls	$-0.042 + 0.150 \log RDischarge$	0.002	12	2.679	0.871
Upper Hatchery Site	$6.522 - 7.305 \log RDischarge$	0.387	7	4.177	0.100

Figure 4.1. The location of the study area in the Pacific Northwest (grey star) and a schematic of the study area with each study segment labeled, 1) Powerhouse, (2) Island Pocket, (3) Upper Side-Channel, (4) Stotan Falls, (5) Highway Crossing, (6) Nib Falls, (7) Upper Hatchery Site. The white circles represent river temperature sampling locations (1, Lower Hatchery Site, 2, Upper Side-Channel; 3, Highway Crossing; 4, Upper Hatchery Site).

Figure 4.2. River temperatures ($^{\circ}\text{C}$) recorded at multiple sites along the Puntledge River during 2007, 2008, and 2009.

Figure 4.3. An example of a thermal history recorded by the thermal logger attached to the radio-transmitter. The location of the tagged-fish is indicated.

Figure 4.4. The thermal regime scenarios used in the bioenergetics model to understand the effect of temperature on energy density depletion.

Figure 4.5. Whisker plots of the body temperatures of each fish classified as a spawner (A) and as a pre-spawn mortality (B).

A

B

Figure 4.6. Mean ΔT ($T_B - T_R$; and standard deviation) at each study segment, (1)

Powerhouse, (2) Island Pocket, (3) Upper Side-Channel, (4) Stotan Falls, (5)

Highway Crossing, (6) Nib Falls, (7) Upper Hatchery Site.

Figure 4.7. The bioenergetics output ($\text{MJ}\cdot\text{kg}^{-1}$) of the 4 thermal regime scenarios used to assess energy density depletion. The horizontal dashed line represents $y = 4.0$ $\text{MJ}\cdot\text{kg}^{-1}$. Spawning window is indicated by the hatched box on the x-axis.

Chapter 5: Influence of individual variation, river reach, water temperature, and discharge on relative activity of upstream migrating summer-run Chinook salmon in a regulated river

Abstract

Adult Pacific salmon (*Oncorhynchus* spp.) have a fixed amount of energy available to fuel their freshwater spawning migration. As such, they must optimize activity in a manner that conserves energy to ensure survival to, and success on, spawning grounds. The altered flow and thermal regime of the Puntledge River in British Columbia has the potential to impact activity patterns of the adult summer-run Chinook salmon (*O. tshawytscha*). The purpose of this study was to assess the relative changes to fine-scale locomotory activity in these Chinook salmon at varying discharges, temperatures, and reaches. Electromyogram (EMG) radio transmitters were used to evaluate aerobic swimming-muscle activity of free-swimming fish. Because transmitter calibration was not possible, a standardized EMG (SEMG) value was used to correlate relative activity to different environmental parameters. A random forest algorithmic modeling analysis was used to account for the non-independence of activity values repeatedly recorded on the same fish. The model explained 57.6 % of the variance in SEMG, and variation among individuals was the most important predictor variable, followed by reach, temperature, and discharge. These findings may indicate that individual variability in swimming strategies, migratory behaviours, and microhabitat-use influence activity to a greater degree than river conditions. If such findings are confirmed in other regulated systems, it

would have important implications for how alterations in flow and the biological consequences are evaluated.

Introduction

Pacific salmon (*Oncorhynchus* spp.) spend most of their lives in the ocean feeding and growing. However, once salmon begin migrating upstream, feeding ceases and energy above that required for routine maintenance is used for three things: to fuel locomotory processes, to mature gonads, and to spawn (reviewed in Brett 1995). Pacific salmon may utilize up to 50 % of total energy during migration (reviewed by Brett 1995; Hinch et al. 2006) and heightened activity may cause premature mortality (Rand and Hinch 1998; Macdonald 2000). It is for this reason that fish should select habitats and utilize behaviours that reduce energy consumption (i.e., low flow, low disturbance, temperature within thermal preference range) and minimize swimming energy expenditure. By doing so, fish can conserve energy for gonad maturation and spawning behaviours (Brett 1995). Understanding habitat-specific activity is important from a fisheries management perspective because it may offer explanations for stock- and habitat-specific difficulties in salmon migrations (Ney 1993; Hinch et al. 1996; Hinch and Rand 1998; Rand and Hinch 1998; Cooke et al. 2004a; Richter and Kolmes 2005). Given that en route and premature mortality is common in Pacific salmon (e.g., Cooke et al. 2004c), such knowledge has the potential to clarify mechanisms associated with mortality and reduce management uncertainty. Field-based measurements are an appropriate metric for determining the habitat-specific energetic costs of upstream migration in adult Pacific salmon (Cooke et al. 2004a,b).

A field-based method that has been used to assess habitat-specific energy-use is physiological telemetry, and more specifically, electromyogram (EMG) sensing transmitters (reviewed by Cooke et al. 2004a). EMG transmitters measure changes to

bioelectric voltages that are proportional to the degree and duration of muscle tension (Sullivan et al. 1963) and allow researchers to quantify energy expenditure and/or activity at biologically appropriate temporal and spatial scales. Several studies have used EMG transmitters to remotely assess habitat-specific activity levels in salmonids. Specifically, activity was found to relate to location of the tagged fish (Hinch et al. 1996; Hinch and Rand 1998; Hinch and Bratty 2000 [sockeye salmon, *O. nerka*], Hinch et al. 2002 [sockeye and pink salmon [*O. gorbuscha*]]; temperature (Briggs and Post 1997a,b [rainbow trout, *O. mykiss*]) and flow/hydrological conditions (Standen et al. 2002 [pink and sockeye salmon]; Brown et al. 2006 [Chinook salmon, *O. tshawytscha*]). Furthermore, variation in individuals, sex, and yearly differences have been found to be relatively strong predictors of swim speeds, while only reach characteristics have accounted for variation in migration rates (Hinch and Rand 1998; Hanson et al. 2008).

Hydropower infrastructure is pervasive globally and has been shown to significantly impact the environmental characteristics of rivers (e.g., physical habitat, flow regime, temperature; Poff et al. 2007; Nilsson et al. 2005; Arthington et al. 2010). The majority of studies that use EMG transmitters to assess activity in hydropower impacted rivers focus on fish passage at fish ladders (Cooke et al. 2004a; Hasler et al. 2009 [Chapter 2]). EMG-derived muscle activity increased as Atlantic salmon (*Salmo salmo*) ascended fish ways (Booth et al. 1997; Gowans et al. 2003). Sockeye salmon were found to exhibit burst swimming (an energetically costly behaviour) when they failed to ascend a fish ladder (Hinch and Bratty 2000). Holding near tailraces of hydropower dams has also been shown to increase EMG-derived activity (Brown et al. 2006; Scruton et al. 2007a). Changes to historic activity patterns may be detrimental to spawning

success (Bernatchez and Dodson 1987) and quantifying habitat-specific energy-use in hydropower-impacted rivers may offer managers insights into failed salmon migrations.

The Puntledge River on Vancouver Island, British Columbia, Canada is an example of a hydropower-impacted river. A stock of summer-run Chinook salmon that has had low returns of adults migrates through the river en route to spawning grounds at the head waters of the river (Hirst 1991). The facility consists of a power-generating turbine located on the river that uses diverted river water. This diversion results in a 7.2 km portion of the river having reduced flow and a swirling pool where the water is released back into the river. In addition, the reduced flows have resulted in the exposure of several natural structures (i.e., water falls, now equipped with blasted rock fish stairs) that may delay upstream migration of the adults. In particular, increased activity through the hydropower associated sections of the river (the turbine release pool and the water falls) and other environmental variables (e.g., river discharge and temperature) may be contributing to the failure to migrate to spawning grounds. To quantify the influence of individual variation, river reach, discharge, temperature and individual variability on the EMG telemetry-derived activity of the migrating salmon we used an algorithmic modeling analysis (Breiman 2001) to understand the importance of each variable on relative changes to EMG values.

Methods

Study Site

The Puntledge River is 16.9 river (r) km long and has a number of artificial and natural barriers to fish migration (Figure 5.1). At river km 14.0, a portion of the river's discharge is diverted by a diversion dam into a penstock, and subsequently re-joins the river at river km 6.8 (Figure 5.1). At the output of the penstock there is a pool (Powerhouse Pool [PHP]) that fish must pass before entering the diversion reach (Reach C in Figure 5.1). Reduced discharge at two major natural barriers may affect the migration of summer-run Chinook (Stotan Falls [SF] and Nib Falls [NF]; Figure 5.1) by delaying migration. Each of the two barriers have three sets of blasted rock fish stairs. Summer-run Chinook salmon have been observed spawning from approximately river km 9.6 to river km 16.9. Hourly discharge (m^3/s) and temperature ($^{\circ}\text{C}$) were measured for the Puntledge River. Discharge was recorded by the power utility at ~ 12.5 rkm using a Valcom Veda II data logger (Valcom Manufacturing Group, Guelph, ON, CAN). Temperature was measured at the Lower Hatchery site using Stowaway Tidbit Temperature Loggers (Model TBI32-05+37; Onset Computer Corporation, MA, USA; Temperature accuracy = ± 0.20 $^{\circ}\text{C}$, Temperature resolution = ± 0.16 $^{\circ}\text{C}$).

River Reaches

For the purpose of this study the river was divided into several zones (reaches) based on habitat characteristics, known holding areas for tagged-fish, and location in the river. The first zone was the lower hatchery (zone 1, Figure 5.1; LH). The LH is the location of the tagged-fish release site and is bound at the downstream end by a fence that crosses the river. The reach has mostly shallow cobblestone habitat with a pool of water near the north shore. The second zone, the PHP (zone 2, Figure 5.1), is a deep swirling pool

created by outflow of the penstock. The third reach of river is known as the Island Pocket (ISP) and it is characterized as having shallow riffles and small pools (zone 3, Figure 5.1). The fourth reach was the 500 m stretch of river that has the three-tiered waterfall known as Stotan Falls (SF - reach 4, Figure 5.1). The next area was called the Highway zone (zone 5, Figure 5.1; HW). This site is characterized as having mostly shallow riffles and small pools. The sixth area was the other tree-tiered waterfall, Nib Falls (NF - zone 6, Figure 5.1), and lastly the furthest upstream reach in the study was Barber's Pool (zone 7, Figure 5.1; BP). BP is characterized as having large deep pools.

Study Animals

Between June 25 and July 20 2007 and 2008, 27 adult male summer-run Chinook salmon were diverted into raceways at the Lower Puntledge River Fish Hatchery (Fisheries and Oceans Canada; Size distribution [2007, 16 fish; TL = 759 ± 81 mm [S.D.]; 2008, 11 fish, TL = 647 ± 60 mm]; Water temperature was between 14-17°C; Figure 5.1) for transmitter implantation. The serious conservation status of the stock meant that we were restricted from tagging female fish. However, it should be noted that male Pacific salmon are typically less efficient with respect to energy use than females (Hinch and Rand 1998) and length of the river decreases the differences between sexes in energy use (Bernatchez and Dodson 1987). Fish were captured using dip nets and implanted with coded electromyogram (CEMG) transmitters (CEMG2-R16-25 [2007] and CEMG2-R11-25 [2008], Lotek Engineering, Inc., Newmarket, Ontario; technology and surgical methods outlined in Cooke et al., [2004a]). Briefly, transmitters were cleaned with betadine and rinsed with distilled water prior to implantation. Fish were anesthetized

with a knock-out solution of 40 ppm clove oil and a maintenance bath of 30 ppm clove oil (clove oil was emulsified in 95 % ethanol). A 50 mm incision to the right side of the ventral midline, posterior to the pelvic girdle was then made and the transmitter was placed in the body cavity. Gold tips of the electrodes (14 mm in length) were then inserted into the axial musculature of the fish and the tips were positioned 10 mm apart. A 16-gauge needle was used to puncture a small hole through the body wall to allow for the antenna to exit. The incision was then closed by five simple interrupted, PDS-II absorbable sutures (3/0, sterile; Ethicon, Somerville, New Jersey). Fish were then placed upright and held in the river (a manufactured flow through cage at the lower Hatchery barrier) to recover for at least 30 min prior to release.

Fish were manually tracked from shore at least twice a day to estimate location (time varied from 30 s to 15 min) between June and early October using a telemetry receiver (SRX-600 or SRX-400, Lotek Engineering, Inc., Newmarket, Ontario) and a 3-element Yagi antenna. Fish locations (approximate river km) were determined using zero point tracking, as the river is narrow and there are a minimum number of possible locations fish can inhabit, meaning individual fish could be readily found. Transmitter tracking is impossible at depths greater than 10 m; however, the river has no pools greater than this depth. The tracking period encompassed the time period that summer-run Chinook salmon enter the river and post-spawning die-off. CEMG monitoring only occurred until the first week of August.

EMG Data Collection

To obtain CEMG values three fixed stations were installed along the river. Stations were installed overlooking the PHP and the ISP, at SF and at NF (Figure 5.1). Each station was equipped with a telemetry receiver (SRX-600, Lotek Engineering, Inc., Newmarket, Ontario), 2 5-element Yagi antennas (one pointing upstream at a 45° angle, the other pointing downstream at a 45° angle), 1 3-element Yagi antenna (pointing orthogonal to the shoreline), a multi-antenna switching port (ASP_8, Lotek Engineering, Inc., Newmarket, Ontario), and a 12 V battery. The equipment (with the exception of the antennas) was secured in a lock box to avoid tampering and theft. Another telemetry receiver and a single 3-element Yagi antenna were used as a mobile fixed station that was deployed at sites frequented by fish, but out of range of the other fixed stations. Receivers were downloaded regularly to laptop computers.

CEMG values (obtained from the fixed stations and manual tracking sessions) were summarized by calculating the hourly mode for each fish. A temporal scale of 1 h was used because of the receiver scanning settings. The receiver scans through transmitter frequency, transmitter IDs, and antennas, and this results in non-continuous records of individuals. Modes were used because the data were non-parametric and in theory, other calculations of central tendency may result in ‘false’ values (e.g., if a fish had CEMG values of only 10 and 20 during a particular hour, the mean would be 15- a CEMG value never measured by the sensor tag). Furthermore, because we were unable to calibrate the tags (Brown et al. 2007) due to limitations imposed on us by the conservation status of the stock and the long-term stock enhancement goals, standard EMG (SEMG) values were calculated to allow grouping of the data. SEMG was determined by dividing the CEMG hourly modes of each fish by the corresponding 90th

percentile (all fish were bound by 0 minimum). This resulted in each CEMG mode being converted to a SEMG value between 0 and 1. SEMG values greater than one, which were rare (< 10 %), were considered outliers and omitted from analysis. Hours with less than 10 CEMG records per fish were also omitted. This method resulted in each fish being measured on a similar scale and allowed us to group the SEMG values for each fish in order to understand relative differences in SEMG response to predictor variables.

Data Analysis

A large dataset of correlated data (i.e., values were repeatedly-measured in a short time interval on the same individuals) was collected and as such, the assumption of independence was violated. Our main focus was to quantify the possible relationships between the predictor variables (individual, discharge, river reach, and temperature) and SEMG values. A Random Forests (RF) regression analysis was used to explore associations between the untransformed predictor variables and the response variable (Breiman 2001). Individual variation in CEMGs due to differences in tag performance (see Brown et al. 2007) should have been greatly reduced by our standardization procedure assuming that all fish exhibited the same maximum and minimum range of muscle activity. However, we cannot assume that all of the variance in SEMGs explained by individual variation was due to differences in swimming performance and not due to tag performance (Brown et al. 2007). As a result, we focused on the relative contributions of the other variables (discharge, temperature and river reach) in our discussion as these are the factors that have been discussed in other studies related to activity and hydropower-impacted rivers.

RF provide accurate predictions that do not over fit the data by using bootstrapped samples to construct multiple trees (Breiman 2001; Prasad et al. 2006). The trees are grown with a randomized subset of predictors (Breiman 2001; Prasad et al. 2006). The main model outputs are the percent of variance explained and estimates of predictor variable importance. The RF analysis was done using the randomForest package in R (Liaw and Wiener 2002; R Development Team 2010). The default setting was used for m_{try} , the number of predictor variables available for selection at each node ($m_{\text{try}} = 2$; the square root of the total number of predictor variables) and 1000 trees were generated. Partial dependence plots based on RF results were created to visually describe the relationship between the predictor variables and the SEMG hourly modes, independent of other predictor variables. Partial dependence plot gives a graphical depiction of the marginal effect of a variable on the response. The Y-axes of the partial dependence plots are not meaningful for comparing different variables. The original RF analysis calculates biased estimates of importance for each predictor variable (Hothorn et al. 2006; Strobl et al. 2008; Strobl et al. 2007). Thus, estimates of predictor variable importance were calculated using the RF of conditional inference trees (cRF) that was developed in the party package in R. These estimates represent the mean decrease in accuracy and are calculated by permutating each predictor variable separately and calculating the increase in misclassification rate. The most important variable is the one, when omitted, degrades the model fit the most.

Results

Tagged-Fish

CEMG values were collected for all 27-tagged fish. On average, tagged fish were recorded for 250 h (141 h [interquartile range; IQR]) (Table 5.1). Ninetieth percentiles ranged from 20 to 50 (Table 5.1). Site-specific EMG output revealed variation in time spent in each reach (Table 5.1). EMG output was recorded for the most fish ($n = 26$) at the ISP, but only 4 fish spent the majority of time there. Almost half of the fish (13) spent most of the time at SF and in total, 24 fish were recorded near the area. Five fish spent the majority of time at the PHP and 15 fish had EMG output recorded at this site. Seventeen fish were recorded at NF; however, only 4 fish spent considerable time there. No fish spent the majority of time at the LH and at BP, and 11 and 12 total fish had EMG output associated with those reaches, respectively. In terms of total hours of EMG output records, irrespective of fish, most EMG output records were associated with SF, the PHP, and NF (Figure 5.2).

Environmental Variables

The Puntledge River discharge recorded at ~ 12.5 rkm during the study period ranged from 4 to $> 16 \text{ m}^3/\text{s}$ (Figure 5.3). The distribution of discharge was binomial, with two peaks, between 6 and $8 \text{ m}^3/\text{s}$ and between 12 and $14 \text{ m}^3/\text{s}$ (Figure 5.3a). Mean temperature of the river was 17.4°C ($\pm 1.4^\circ\text{C}$ S.D.; Figure 5.3b).

Random Forest Model

Using a RF analysis, 57.6 % of the variance in SEMG was explained by the predictors. A further conditional RF completed to assess the unbiased importance of each variable

found that individual fish (ID) was the most important predictor, followed by river reach, temperature, and discharge. The importance values were 0.045, 0.014, 0.009, and 0.007, respectively, meaning that fish ID had highest degradation in variance explained when omitted from the model. The relationships shown in the partial plots indicate that the marginal SEMG response due to fish ID varies by individual, with three fish (i.e., 1, 4, and 11) showing lower SEMG than the other fish (Figure 5.4d). Another fish (i.e., 21) had the highest SEMG values. The PHP and ISP reaches (2 and 3 in the plot; Figure 5.4c) had lower SEMG values than the other reaches, and all other reaches yielded similar SEMG values. The relationship between temperature and SEMG was positive and lower discharge resulted in lower SEMG values (Figure 5.4b,a, respectively).

Discussion

A limitation in this study was that we were unable to calibrate the CEMG transmitters once implanted into the fish. Brown et al. (2007) evaluated 20 CEMG transmitters implanted in rainbow trout and found that there was a strong positive relationship between CEMG output and swimming speed, but the relationship weakened when data were grouped. This suggested that the transmitters behaved differently as a result of small differences in electronic components so the authors suggested that each tag be calibrated to swimming speed separately to ensure accurate muscle activity estimates. Calibration of the transmitter typically involves prolonged handling and confinement of tagged fish, as well as forced swimming trials in either a swimming tunnel, or a circular swim flume. This process elevates the stress response and may induce pre-spawn

mortality (Geist et al. 2002; Standen et al. 2002; Cooke et al. 2004a). The Puntledge River summer-run Chinook salmon are of conservation concern, and the federal government has supported a hatchery on the river for over 35 years in an effort to increase the number of returning adults (D. Miller, Watershed manager, Puntledge River Hatchery, personal communication). Because of this effort, many fish are captured upon arrival at the hatchery and kept for enhancement purposes. The invasive tagging procedures required to use CEMG transmitters (i.e., level 5 anesthesia, and internal placement of electrodes and transmitters) is more stressful to the fish than conventional gastric tagging procedures (Cooke et al. 2004a), thus we were unable to risk further handling, confinement, and exhaustion; as such, our tags were uncalibrated. Nonetheless, calibrated EMG tags have shown that CEMG output increases with increasing swimming speed (i.e., linear relationship between EMG and swimming or metabolic metrics; Brown et al. 2007). Therefore, relative increases in CEMG are most definitely a proxy for an increase in swimming speed and associated energy cost. Although we limit our discussion to focus on relative changes in activity (not absolute energetic costs), the value of this work is that we have determined the relative contribution of the variables most associated with hydropower management (controlled flow releases, temperature and man-made migration barriers) in explaining the variation in swimming muscle activity. Furthermore, fine-scale field-obtained activity in free-ranging fish throughout a large area could not be collected in any other way. This approach represents the best means of obtaining fine-scale activity on free-swimming fish in a riverine system, given the serious conservation constraints imposed.

The central importance of fish ID in explaining SEMG activity levels, was determined by applying a random forest analysis (RF explained > 50 % of the total variance in SEMG). This type of analysis has not been done on a similar dataset, making it novel but difficult to interpret the absolute values; however, the calculated importance variables are similar to a paper currently in review; Lapointe and Light, unpublished data). While, the greater importance of fish ID could have been due to the use of uncalibrated transmitters, a number of studies using similar calibrated EMG transmitters have found significant variation among individuals (Hinch and Bratty 2000; Hinch et al. 2002; Scruton et al. 2007a; Pon et al. 2009a). Possible reasons for the variation may be individuals choosing different migration paths and consequently being exposed to different environmental conditions (Hinch and Bratty 2000). Indeed, this would be in accordance with a growing body of literature that demonstrates that individual variation in fishes swimming ability is repeatable and statistically valid (e.g., Kolok, 1999 and more, see below). In fact in this study, particular fish spent little time in most reaches, choosing only to hold in a single reach. In a similar study involving Fraser River sockeye, individual fish explained significant amounts of variation in swimming speed behaviours (i.e., time spent burst swimming and sustained swimming speeds) and the authors suggest that this may be due to difficulty in locating migration cues as they could find no evidence that individual traits such as sex and size had an effect (Hinch et al. 2002). Furthermore, a study by Hanson et al. (2008) revealed that the individual swimming speeds of upriver migrating sockeye salmon was highly repeatable which emphasizes the importance of individual variation. Hydropower infrastructure may also contribute to the individual variation observed, as EMGs obtained near facilities have

been found to vary widely (Pon et al. 2009a). Overall, this study further supports the contribution of individuality to estimates of activity in free-ranging fishes.

Location (i.e., reach) of the tagged fish was the second most important variable in the random forest analysis. Physical characteristics of fish locations have been shown to cause variability in EMG obtained activity estimates for salmonids (Standen et al. 2002; Scruton et al. 2007a). For example, reach characteristics was the primary factor influencing activity levels in adult pink and sockeye salmon migrating through the Fraser River Canyon (Standen et al. 2002). Increased activity levels were found when fish were migrating through reaches that were constricted by islands and river bars. The partial dependence plots in the present study suggest that higher SEMGs occurred in the constricted falls locations, SF and NF, when compared with less constricted reaches of the river pools, PHP and ISP. However, similar values to SF and NF were also measured at the LH, the HW, and BP (all less constricted reaches). Furthermore, a study on Atlantic salmon near a hydropower facility in Newfoundland and a separate study on Chinook salmon in the Columbia River suggest that EMG activity is heightened near tailraces associated with hydropower facilities (Brown et al. 2006; Scruton et al. 2007a). The present study did not demonstrate higher activity at the PHP, a reach with similar physical characteristics to the tailrace reach in the former studies.

In a relative sense, environmental conditions (i.e., temperature and river discharge) did not contribute much variable importance to the analysis. For temperature this is particularly surprising, as it is the “master factor” in fish physiological processes and numerous studies have shown heightened activity in Pacific salmon at warmer temperatures. Indeed, the partial dependence plot of temperature shows increased SEMG

values at greater temperatures, though the importance of temperature was low. In this study, river temperature ranged from ~14 °C to over 21 °C with a mean temperature of ~17 °C. The available thermal habitat was at the higher end of the species-specific thermal preference (Brett 1995), but differences among stocks do exist (Farrell et al. 2008). Likewise, because fish inhabit fluvial environments, river discharge may influence activity patterns in fish. The partial dependence plot of river discharge revealed a sharp increase in SEMG between low and moderate flows; however, the importance of river discharge was the smallest of the four variables examined. It is likely that in this study, the river discharge recorded at one location on the river (near the diversion dam) did not fully capture the variation in river flow that is present for fish in the river; greatly reducing the explanatory power of river discharge. A possible solution would be to design flow sensors that could be attached to a fish body and record the flow that fish are experiencing.

Prior to the study it was hypothesized that the CEMG transmitters would detect increased activity caused by fish ascending the fish stairs at SF and NF, as a previous study had found Chinook salmon frequently exceed sustained swimming speeds while swimming over water falls (Brown et al. 2002). However, the current study did not observe heightened SEMG at the water falls. Detecting burst swimming activity is difficult using CEMG transmitters because of the electrode placement being in the red muscle, and not the white muscle, which is utilized during anaerobic swimming (Cooke et al. 2004a). Furthermore, the temporal scale used in this study may have been too coarse to detect increased SEMGs during waterfall ascent. In other words, short waterfall ascents may have happened very quickly as, by definition, burst swimming occurs for

very short time periods (< 20 s). This suggestion is corroborated by a study by Pon et al. (2009a), which found few incidences of burst swimming during fish passage at a fish ladder.

Mortality likely occurred in the study. Three fish had few hours (< 100 h) of recorded CEMG and this may have been a result of the fish dying due to the tagging procedure. No mortalities were directly found despite conducting snorkeling observations on these fish. Gowans et al. (2003) suggest that fungal infections can cause fish mortality in EMG studies, and may be especially relevant when fish inhabit water at the top of their preferred thermal range. Anecdotally, the experienced fish surgeons used in this study commented that the tagged fish took longer to recover than previously tagged fish (Hasler and Cooke, personal communications). Data were processed in a manner that excluded CEMG values once fish mortality was suspected, thus it is unlikely that CEMG values from morbid fish biased the outcome of the analysis.

In conclusion, a recent review has highlighted the importance of developing quantitative relationships between river flow and ecological responses (Poff and Zimmerman 2010). Our study attempted to relate activity to environmental and habitat characteristics while also accounting for “subject effect”. The analysis also accounted for the non-independence of the sampling method and this type of method is encouraged where the common approach has been to compare grouped means. Our study did account for environmental and habitat characteristics in the energetic responses of fish in a hydropower river, but the observed relationships were moderate. Locomotor activity is likely influenced by numerous biotic and abiotic factors in hydropower systems (Taylor

et al. In Review). Our study highlights the importance of using interindividual variation in future analysis of fish activity.

Table 5.1. Information on the EMG-tagged fish used in the study. Fish 1 to 11 were tagged in 2008, fish 12-27 were tagged in 2007. Percentages of time at each location are provided along with the total number of hours that each fish had EMG recordings for.

Fish ID	90 th percentile of EMG output	Number of hour periods	% of time @ LH	% of time @ PHP	% of time @ ISP	% of time @ SF	% of time @ HW	% of time @ NF	% of time @ BP
1	21	413	3.1	0.0	5.8	41.2	0.5	49.4	0.0
2	23	377	0.0	0.0	2.7	8.2	2.7	54.4	32.0
3	49	220	5.5	85.9	8.6	0.0	0.0	0.0	0.0
4	20	338	3.6	0.0	6.8	10.7	1.8	71.5	5.6
5	28	264	2.3	67.0	3.0	12.9	0.0	7.2	7.6
6	25	319	0.0	0.0	16.3	27.0	0.6	56.1	0.0
7	24	264	1.1	7.6	40.9	50.4	0.0	0.0	0.0
8	24	356	2.8	5.6	2.8	25.6	25.8	24.4	13.0
9	50	291	12.4	61.8	0.0	25.8	0.0	0.0	0.0
10	25	247	0.0	17.0	5.3	35.2	0.4	32.8	9.3
11	31	241	4.6	64.6	1.7	9.1	2.1	7.9	10.0
12	41	314	0.0	0.0	37.3	62.7	0.0	0.0	0.0
13	25	308	0.0	0.0	3.9	81.5	1.9	3.9	8.8
14	23	263	0.0	0.0	4.6	77.1	0.8	11.8	5.7
15	24	70	0.0	0.0	94.3	5.7	0.0	0.0	0.0
16	34	183	2.2	1.1	7.7	89.0	0.0	0.0	0.0
17	23	267	0.0	28.5	6.7	55.4	4.9	4.5	0.0
18	33	178	0.0	12.4	15.2	55.0	10.7	6.7	0.0
19	29	319	0.0	0.0	15.7	75.2	4.4	1.6	3.1
20	35	185	0.0	17.3	54.1	28.6	0.0	0.0	0.0
21	22	174	0.0	7.5	7.5	60.3	1.7	7.5	15.5
22	24	501	0.0	0.0	1.2	97.6	0.4	0.8	0.0
23	50	81	33.3	40.7	25.9	0.0	0.0	0.0	0.0
24	40	344	0.0	23.3	2.9	56.1	0.6	9.3	7.8
25	26	105	0.0	0.0	98.1	1.9	0.0	0.0	0.0
26	36	21	0.0	0.0	100	0.0	0.0	0.0	0.0
27	20	102	10.8	11.8	15.7	46.1	0.0	2.0	13.7

Figure 5.1. The location of the Puntledge River in British Columbia, Canada (gray star)

and a drawing of the entire study site. Reaches used in the study are shown as numbers: 1 (LH), 2 (PHP), 3 (ISP), 4 (SF), 5 (HW), 6 (NF), 7 (BP). Reach C is defined as the stretch of the river from the diversion dam downstream to the penstock release at the PHP. Fixed antenna arrays are depicted as double crossed tees.

Figure 5.2. Box plot of the number of hours fish spent at each of the river reaches used in the study: 1 (LH), 2 (PHP), 3 (ISP), 4 (SF), 5 (HW), 6 (NF), 7 (BP). Each data point is the length of time in hours a tagged fish spent at the location.

Figure 5.3. Frequency histograms of river discharge (A; m³/s) and river temperature (B; °C) during the 2007 and 2008 study period.

A

B

Figure 5.4. Partial dependence plots depicting the marginal effect of each predictor variable on SEMG (A: river discharge, B: temperature, C: river reach, and D: fish ID).

The y-axis does not represent absolutes and are unitless.

Chapter 6: Effectiveness of pulse flows in a regulated river to induce upstream movement of an imperiled stock of Pacific salmon.

Abstract

A three-year study was carried out to assess the effectiveness of pulse flows on the upstream migration of an imperiled summer-run Chinook salmon (*Oncorhynchus tshawytscha*) population in the Puntledge River, British Columbia. During the months of July and August, radio-telemetered fish were released into the hydropower-impacted reach of the Puntledge River. The goal was to assess whether or not fish moved upstream, ascended natural barriers, moved away from the turbine outlet pool, and had gross scale changes in activity during pulse flows. Findings indicated that some pulse flows stimulated upstream movement although that was not always the case. Passage of natural barriers was only higher during an abnormal pulse flow where flows reached twice that of the prescribed flow. Some fish moved away from the turbine outlet pool during pulse flows and activity was unchanged. Though it is unclear as to the effectiveness of the pulse flows on the Puntledge River summer-run Chinook salmon population, no negative impacts were observed.

Introduction

River flow is an important factor for the upstream movement of salmonids (Banks 1969). Water levels that are both too high and too low can increase energy use, delay migrations

and in extreme cases lead to migration failure. Thus, the management of river flows by hydropower utilities may influence the effectiveness of salmonids to migrate to natal spawning grounds (e.g., Thorstad et al. 1998; Gowans et al. 2003; Thorstad et al. 2003; Keefer et al. 2004; Thorstad et al. 2005; Tiffan et al. 2010). Upstream movements by salmonids are often impeded by low river flow, exposure to artificial and natural barriers, attraction to artificial river currents (i.e., turbine out takes), and/or the presence of non-optimal environmental variables (i.e., cold/warm river temperature, supersaturation of dissolved oxygen). In addition, many hydropower developments are located in rivers that provide spawning habitat for migratory species like Pacific salmon and in some incidences, declines in population sizes have been noted (Nehlsen et al. 1991). Though loss of connectivity and habitat alteration are often cited as the primary reason for changes to population size in hydropower impacted rivers (e.g., Sheer and Steel 2006), there are also the associated changes to a fish's activity that may occur due to changes to water flow and local environmental characteristics (Murchie and Smokorowski 2004; Cocherell et al. 2011). Because of the economic importance associated with hydropower facilities and the need to conserve healthy salmon stocks, managing the flow of rivers with fish in mind is of particular concern to utilities and regulators.

Pulse flows, or artificial freshets, may offer hydropower utilities a method for ensuring that migrating fishes, such as salmon, can move upstream. A pulse flow consists of diverting an increased load of water through the hydropower impacted river stretch from the turbine generating pathway in order to simulate a "natural" runoff event that would typically correspond with the upstream migration of a given species. Pulse flows have been used to entice fish holding in estuaries to move into the river main stem

(Huntsman 1948) or to aid upstream movements through fishways (Thorstad et al. 1998; 2003); however, clear relationships between pulse flows and movement were not observed in all cases (Thorstad et al. 1998; 2003). Further studies, in particular species- and river-specific studies, are needed for water managers to better understand the potential for pulse flow releases to be used for conservation purposes.

The size of the Puntledge River stock of summer-run Chinook salmon on Vancouver Island, British Columbia has been identified as being in decline (Hirst 1991). One possible threat to the stock is the operations and infrastructure of a hydropower facility located on the river (Hirst 1991). The federal government and the power utility have attempted to increase returns of adult fish to the river by annually releasing thousands of smolts from a hatchery located on the river and by providing spawning habitat enhancement. However, to date, adult returns are not rebounding to pre-development numbers (D. Miller, Fisheries and Oceans Canada Puntledge River Watershed Manager, Personal Communication). The main issues for the returning adult salmon are the exposure (due to decreased water flow) of two natural barriers (waterfalls) and turbine release pool (Komori Wong Environmental and Bixby 2003). The reduction in river flow has meant that fish fail to successfully ascend the barriers, and/or they hold in the turbine release pool and not migrate to appropriate spawning grounds (Komori Wong Environmental and Bixby 2003; Chapter 3). In order to provide a greater amount of water at the natural barriers and to rheostatically stimulate fish to move away from the turbine release pool, the hydropower utility has mandated the release of pulse flows to mimic historical variation in river flow. Specifically, the goal of the pulse flows was to 1) facilitate upstream movement, 2) support fish ascending the two natural barriers, and

3) to reduce fish residency at the turbine release pool (Powerhouse Pool). In order to assess the potential of using the pulse flows as a permanent measure to assist in the conservation of the summer-run Chinook Puntledge River stock, we completed a three-year radio-telemetry study that measured migration rates, passage success, Powerhouse Pool attraction and changes to gross scale activity during pulse flows. This study offers a model for the effectiveness of pulse flows for summer-run Chinook salmon in small hydropower impacted rivers and may be used to support future adaptive management initiatives.

Methods

Study Site

The Puntledge River is located on the east coast of Vancouver Island, Canada (Figure 6.1). The river is approximately 16.9 km long and drains Comox Lake into the Comox Estuary. The hydropower facility was constructed in 1912 and consists of a dam at the outlet of Comox Lake, as well as a diversion dam approximately 3.7 river (r) km downstream that diverts water through a penstock that funnels the water through the turbine (Figure 6.1). The diversion results in the river having less river flow between 6.8 rkm to 14.0 rkm. Within this reach there are two natural barriers that have been identified as holding sites for summer-run Chinook salmon: Stotan and Nib Falls (Komori Wong Environmental and Bixby 2003). Both falls are three-tiered with habitat enhancement to aid in the upstream movement of adult fish (i.e., concrete baffle

fishways). Another area of concern is the turbine output (Powerhouse Pool) at the power station located at 6.8 rkm (Komori Wong Environmental and Bixby 2003).

Study Animals and Telemetry Methods

Between June 25th and July 20th 2007 and 2008, and in 2009 between June 2nd and June 26th, adult summer-run Chinook salmon were diverted into raceways at the Lower Puntledge River Fish Hatchery (Fisheries and Oceans Canada; Figure 1) for transmitter implantation (2007: 38 fish; TL = 727 ± 14 mm [S.D.]; 2008: 27 fish, TL = 663 ± 11 mm; 2009: 35 fish, TL = 646 ± 56 mm). Twenty-seven male fish (16 in 2007, and 11 in 2008) were implanted with coded electromyogram (EMG) transmitters (CEMG2-R16-25 [2007] and CEMG2-R11-25 [2008], Lotek Engineering, Inc., Newmarket, Ontario; surgical methods outlined in Cooke et al. (2004a); and 66 fish (22 in 2007, 15 in 2008, and 29 in 2009) had a conventional radio transmitter (MCFT-3A, Lotek Engineering, Inc., Newmarket, Ontario) gastrically inserted with the antenna protruding from the oral cavity, an approach that has high rates of retention in migratory adult Pacific salmon (Ramstad and Woodey 2003). A note about the coded EMG transmitters: Brown et al. (2007) have shown that the outputs of the transmitters requires calibration to each tagged fish in order to be used to assess energy use and to allow for grouping of coded EMG values from different fish. Due to the conservation status of the Puntledge River summer-run Chinook salmon and the added stress that calibration requires (Cooke et al. 2004a), we were unable to calibrate the tags. We have limited our analysis of coded EMG values to coarse scale activity and have avoided grouping the data for all fish because of the technical limitation of using the transmitters.

Transmitters were manually tracked from shore at least twice a day using a telemetry receiver (SRX-600 or SRX-400, Lotek Engineering, Inc., Newmarket, Ontario) and a 3-element Yagi antenna. Transmitter locations (approximate rkm) were determined using zero point tracking, as the river is narrow and there are a minimum number of possible locations fish can inhabit, meaning transmitter locations are easily found with a high-degree of accuracy. Transmitters were tracked from June 30th to August 4th in 2007, June 30th to August 3rd in 2008 and June 2nd to August 7th in 2009. The range of days tracked encompassed the time period that summer-run Chinook salmon enter the river and the end of the period when pulse flows were administered. Fixed stations consisting of 1-3 Yagi antennas and telemetry receivers with external batteries were used at Stotan Falls, Nib Falls, and the Powerhouse Pool to determine time of ascent at the falls and the time that fish moved away from the Powerhouse Pool. In addition, EMG output values were recorded continuously at the key location fixed stations and all effort was made to maximize the number of fish being recorded during the control and pulse flow periods by using other single antenna fixed stations constructed along the river bank.

Pulse Flows

Pulse flows occurred throughout the month of July and the first week of August in 2007, 2008, and 2009 (Table 6.1). Mean river flow varied from 12.1 to 42.5 m³·s⁻¹ and was at least 6.1 m³·s⁻¹ above residual flows (approximately double). Ten of the 13 pulse flows had mean river flow between 12.1 and 13.2 m³·s⁻¹ and the pulse flows with higher river flow were due to increased run-off in the watershed. Also, all but one pulse flow lasted 48 hours. Pulse flow 5, 6, and 8 did not result in the flow at the Powerhouse Pool being

less than the flow in the mainstem of the river. Pulse flow 12 and 13 are not included in further analysis because tagged fish were no longer present in the impacted river reach.

Data Analysis

The migration and passage rates were not normally distributed and as such Kruskal-Wallis tests were used to compare migration per hour during the 11 pulse flows. Friedman tests were used to compare migration and passage rates for the three periods (control, pulse, and 12-h post-pulse flow). Wilcoxon tests were used to compare migration and passage rates for control and pulse, and control and 12-h post-pulse flow. Significance was tested at a Bonferroni corrected significance level ($\alpha = 0.005$) to account for the 11 pulse flows.

The time when fish passage occurred at Stotan and Nib Falls was determined by analyzing the recorded fixed station by assessing the antenna that the transmission was being received on and by using the power of the signal. Passage was also confirmed during subsequent manual tracking. Fish were considered to be present at each fall when fish were first located (using manual tracking) at the lower tier of the falls and when they were last located at the upper tier of the fall.

In general, because of constraints with using non-calibrated coded EMG transmitters, and the sampling rate of tag transmissions, we assessed gross scale relative changes in EMG outputs during pulse flow and non-pulse flow conditions for each fish tagged. Specifically, we measured whether mean EMG outputs were increasing or decreasing during non-pulse flow and pulse flow conditions. Data points were generated by calculating the modes of coded EMG values for each fish during the non-pulse flow

periods (control period) and the pulse flow periods (to account for the mean not being representative). Because fish holding in the Powerhouse Pool were not exposed to the same change in flow, we analyzed their coded EMG outputs separately. Fisher's Exact Tests were used to assess the relative changes of coded EMG output values during control and pulse flow periods.

Results

Migration Rates

Mean migration per hour during the 11 pulse flows did not differ statistically (Kruskal-Wallis; $\chi^2 = 15.573$; $P = 0.113$; Figure 6.2). In addition, there were no statistically significant differences between mean migration rate (per hour) during the pulse flow period, 12 hours after the pulse flow period, or during the control period (Table 6.2; Figure 6.2). However, pulse flow 5, 6, and 9 showed marginally significant differences in the mean migration rate during the three periods (Table 6.2). During pulse flow 9, mean migration rate was statistically higher during the pulse flow than during the control period, but not during the 12 hour post-pulse period (Table 6.3, 6.4; Figure 6.2). Pulse flow 5 resulted in fish moving at a greater rate during the control period than during the 12-hour post-pulse period, while fish moved marginally more during the pulse flow (Table 6.2, 6.4; Figure 6.2).

Passage Rates

Passage rates at the natural barriers differed among pulse flows (Kruskal-Wallis; $\chi^2 = 32.075$; $P < 0.001$; Figure 6.3). During pulse flow 2 there was a difference between the rate at which radio-tagged Chinook salmon passed the two natural barriers (Table 6.2; Figure 6.3). During pulse flow 2, rate of passage past the natural barriers was statistically higher during the pulse flow than during the control period (Table 6.3; Figure 6.3). There were no differences in passage rates during the 12 hour post-pulse and control periods (Table 6.4).

To further understand the influence of flow on fish ascending Stotan and Nib Falls, we normalized the frequency of discharge at Stotan and Nib Falls when fish were present and when fish were ascending the barriers (Figure 6.4). In both cases, fish typically ascended the barriers during the flow that was most frequent. Some fish; however, ascended the barriers during “transitory” flows; that is flows that occur during the time when water was being released and drained prior to and after the stable flow periods (ramping-up and ramping-down periods).

Powerhouse Residency

Movements away from the powerhouse pool during pulse flows were observed. When all years and pulse flows were grouped, 45 % of fish were for some time period a resident of the Powerhouse Pool. Thirty-three percent of these fish ($n = 18$) moved to areas upstream of the Powerhouse Pool during pulse flows and 37 fish moved to upstream areas during non-pulse flows. Compared to a 50-50 likelihood of fish moving away from the pool during pulse flows, the observed difference was non-significant (Chi-squared test, $\chi^2 = 2.7081$, $df = 1$, $P = 0.1$).

Activity Levels

The difference in coded EMG output of fish in reach C during pulse flows was negligible when compared to control flow conditions (Figure 6.5). In addition there was no significant change to EMG output during pulse flows when compared to non-pulse flow conditions for fish holding in the Powerhouse Pool (Figure 6.5).

Discussion

In general, our results regarding the ability of pulse flows to enable upstream movement were equivocal. Upstream movement was only marginally significant during 3 of 11 pulse flows. Likewise, Thorstad et al. (1998) found no clear relationship between increased water flow and Atlantic salmon (*Salmo salar*) upstream migration. There are a number of reasons why the pulse flows in the Puntledge River did not stimulate upstream movement. The pulse flows may not have been administered at an appropriate flow rate, though previous work on the river suggests that this is not the case (Komori Wong Environmental and Bixby 2003). This preliminary study in 2003 found that 18 of 21 fish holding in the Powerhouse Pool moved upstream during a 48 hour pulse flow. Another possibility is the weather conditions at the time of the pulse flows may not have been favourable for upstream migration by salmonids, as Baxter (1961) suggested that precipitation in combination with pulse flows are more effective. In contrast, a similar study to the current one was done nearby on the Ash River and found no relationship between weather and cutthroat trout (*O. clarkii*) movement during pulse flows (Ecofish

Research Ltd. 2010). Furthermore, coarse scale telemetry studies completed in the American south-west have found no movements associated with pulse releases of water (Gido et al. 2000; Cocherell et al. 2010).

Another possibility is that upstream movement during the pulse flows by the tagged Chinook salmon in the Puntledge River was not detected because of inherent stock characteristics. The stock is considered to be summer-run and fish typically arrive in the river 4-5 months prior to the potential spawning date. There are two reproductive strategies fish may opt for when arriving early to the natal river: 1) Conserve energy by resting in habitat that minimizes energy use and then move to spawning grounds once sexual maturation has occurred; or 2) Move quickly upstream to spawning grounds and spawn when sexual maturation occurs. The trade-off for option one is that the fish may no longer have energy to expend on demanding movement through non-optimal flow conditions. The trade-off for option two is that sexual maturation may occur too early or too late if the spawning ground offers unfavourable habitat conditions (i.e., warm water temperatures). If fish are behaving according to option 1, upstream movement by tagged fish would not be observed because the fish are not moving, but resting in localized areas. In 2007 and 2008, fish were likely behaving in this manner, as fish were not found to move far during pulse flows. If fish are behaving according to option 2, upstream movement may be occurring at too fast of a rate for it to be attributed to the pulse flows. In 2009, fish moved relatively quickly to the upper section of the reach and large numbers of fish were only present in the reach during the first two pulse flows. Since the Puntledge River has been altered from its historic condition and a number of barriers have been exposed or constructed, fish should likely opt for option 2, as delaying

upstream movement likely would lead to energy deficits during the final upstream migration to the spawning ground. A recent study has also suggested that the river temperature is too high for fish that stay in the river during migration to successfully spawn during historic time frames because of accelerated depletion of somatic energy stores (Hasler et al., In Press [Chapter 4]).

The second goal of the pulse flow was to facilitate fish ascending the two natural barriers (Stotan and Nib Falls). Passage rates did differ significantly across the pulse flows, meaning that some of the flows were successful in facilitating fish passage, while others were not. However, only pulse flow two had a significant increase in passage and it was this pulse flow that lasted an abnormally long time (360 h vs. 48 h). Thorstad et al. (1998) also found that not all pulse flows were able to stimulate fish to ascend barriers to migration. A number of studies have found that obstructions to migration are difficult for fish to move through under almost any flow conditions (Gowans et al. 2003; Thorstad et al. 2003). In particular, Thorstad et al. (2003) found that the stimulation of fish to ascend barriers during pulse flows was river-specific, and fishways that seemingly appeared to be easy for fish to migrate through were in fact difficult to pass.

When the river flow during the time fish were present at each barrier was compared to the river flow at the approximate time that fish passed, it was found that fish typically passed during the flows that occurred the most often (residual flows, 5-6 m³·s⁻¹). In addition, fish present at Stotan Falls also tended to ascend the barriers during pulse flows and during transition flows (i.e., river flows present when the pulse flows were either ramping up or ramping down). This tendency was not as prevalent at Nib Falls,

possibly due to structural differences between the sites, or the increased likelihood of finding appropriate spawning habitat in the Nib Falls area.

Limiting the residency times of fish to the turbine outflow at the Powerhouse Pool was a goal of the pulse flows. In general we found that over a third of fish that resided at the pool moved away from it during pulse flows. Though this was statistically non-significant, the Powerhouse Pool should be explored in more detail to understand the rheotactic stimuli at the site and determine if there are possible physical alterations that could be made to reduce residency times at the Powerhouse pool (or to eliminate attraction all together). In reality, the majority of fish were not attracted to the pool, and we suspect that these fish avoided being attracted to the pool by swimming on the opposite side of the river (without knowing what side the fish prefer, we would expect a 50-50 likelihood and in fact, we found that 55 % of fish avoided the pool). Managers may be able to decrease residence times at the pool by diverting fish to the opposite side of the river with in-river structures.

Though our analysis of the EMG output values was coarse, we should have been able to detect changes in coded EMG output values that would indicate biologically significant changes to activity patterns. We found no change in EMG output values during changes in flow, as fish will seek out habitat that optimizes their energy expenditure (Hinch and Rand 1998). Furthermore, in a similar study it was suggested that fish increase swimming speeds during initial increases in river discharge, but then found suitable habitat that resulted in reduced swimming speeds during periods of near peak flow conditions (Cocherell et al. 2011). It is possible that the sampling rate of the transmitters did not allow for brief and rare burst activity, as the coded EMG tags rectify

the muscle contraction activity over a 2 second period (the minimum setting; Cooke et al. 2004a). This technical consequence of using the coded EMG tags likely limited our ability to detect short and rapid changes in muscle EMG activity.

Conclusions and Management Applications

To summarize, no clear relationships were found between pulse flows administered in the Puntledge River and summer-run Chinook salmon migration. However, we did find some results that suggest that there may be some limited benefit of pulse flows. In addition, we found no evidence that fish holding in the reach during pulse flows were exhibiting increased activity suggesting that pulse flows do not have any significant negative consequences on summer-run Chinook. Previous studies on the river (and on other studied rivers in North American and Europe) have also shown equivocal findings and it may be appropriate to try other management options. These could include longer pulse flows, earlier timing (though timing is based on when fish are returning), and/or using different magnitudes of flows.

Table 6.1. Dates and mean flow (cm/s) of each pulse flow implemented in 2007, 2008, and 2009.

	Pulse Flow #	Date	Mean Flow (cm/s)
2007	1	4-5 Jul	18.1
	2	11-26 Jul	17.2
	3	1-2 Aug	13.1
2008	4	2-3 Jul	42.5
	5	9-10 Jul	13.0*
	6	16-17 Jul	13.1*
	7	23-24 Jul	13.2
	8	30-31 Jul	13.0*
2009	9	8-9 Jul	12.4
	10	15-16 Jul	12.8
	11	22-23 Jul	13.0
	12	29-30 Jul	12.1
	13	5-6 Aug	12.2

Table 6.2. Friedman's chi-squared and associated P value for the variables mean migration distance per hour and number of natural barriers ascended per hour among the three years and 11 pulse flows studied.

Year	Pulse Number	Number	Migration distance		natural barriers ascended	
			χ^2	P	χ^2	P
2007	1	6	0.429	0.807	n/a	
	2	11	1.724	0.422	12.286	0.002*
	3	21	5.134	0.077	8.857	0.012
2008	4	3	0.286	0.867	n/a	
	5	10	8.882	0.012	n/a	
	6	11	6.276	0.043	5.200	0.074
	7	19	3.250	0.197	2.00	0.368
	8	14	0.069	0.966	0.667	0.717
2009	9	15	7.837	0.020	1.600	0.449
	10	6	2.000	0.368	4.000	0.135
	11	2	2.000	0.368	2.000	0.368

Table 6.3 Mean migration distance per hour and the number of natural barriers ascended per hour during the pulse flow. Wilcoxon test compared pulse flow conditions to control conditions.

Year	Pulse Number	Number of radio-tagged Chinook salmon	Mean Migration distance per hour		Number of natural barriers ascended per hour	
			χ^2	<i>P</i>	χ^2	<i>P</i>
2007	1	6	0.287	0.592	n/a	
	2	11	3.965	0.047	9.456	0.002*
	3	21	4.110	0.043	6.173	0.013
2008	4	3	0.196	0.658	n/a	
	5	10	1.549	0.2121	n/a	
	6	11	5.539	0.019	2.224	0.136
	7	19	1.056	0.304	1.000	0.317
	8	14	0.245	0.620	n/a	
2009	9	15	8.540	0.004*	1.115	0.291
	10	6	1.307	0.253	2.200	0.138
	11	2	1.000	0.317	n/a	

Table 6.4. Mean migration distance per hour and the number of natural barriers ascended per hour during the 12 h post-pulse flow conditions. Wilcoxon test compared 12 h post-pulse flow conditions to control conditions.

Year	Pulse Number	Number of radio-tagged Chinook salmon	Mean Migration distance per hour		Number of natural barriers ascended per hour	
			χ^2	<i>P</i>	χ^2	<i>P</i>
2007	1	6	0.030	0.863		n/a
	2	11	0.201	0.654	1.000	0.317
	3	21	0.723	0.395	1.000	0.317
2008	4	3	0.088	0.767		n/a
	5	10	8.541	0.004*		n/a
	6	11	1.159	0.282	1.000	0.317
	7	19	0.216	0.642	0.352	0.553
2009	8	14	0.132	0.717	0.360	0.549
	9	15	0.845	0.358		n/a
	10	6	1.000	0.317		n/a
	11	2	1.000	0.317	1.00	0.317

Figure 6.1. Map of the location of the Puntledge River in the Pacific Northwest and a schematic drawing of the study reach.

Figure 6.2. Mean migration per hour of tagged fish during each of the pulse flows.

Whiskers represent standard deviation.

Figure 6.3. Mean passage per hour of tagged fish during each of the pulse flows.

Whiskers represent standard deviation.

Figure 6.4. Flows at Stotan Falls (SF) and Nib Falls (NF) when fish are present (solid line) and ascending (dotted line).

Figure 6.5. Paired mean EMG outputs for each fish during non-pulse flow and pulse flow conditions when fish were located in Reach C and when fish were located in the Powerhouse Pool.

Chapter 7: General discussion and future research directions

Broadly, the focus of this thesis was to understand how hydropower infrastructure and operational strategies influence the physiological ecology of migrating Pacific salmon (*Oncorhynchus* spp.). Specifically, the goal of this thesis was to identify possible biological mechanisms for the decline in stock size of the Puntledge River summer-run Chinook salmon (*O. tshawytscha*) using physiological techniques (Chapter 2).

Radiotelemetry devices were coupled with non-lethal blood samples (Chapter 3), thermal histories (Chapter 4), and on-board activity sensors (Chapter 5) to identify possible biological mechanisms for the stock decline. In addition to understanding the basis for the decline, a management directive (i.e., pulsed flows) to increase the number of returning adults on spawning grounds was assessed for effectiveness using the above stated techniques (Chapter 6). Furthermore, each study was designed in a manner to build on previous scientific findings associated with migratory biology, fish movements in hydropower-impacted systems, and the physiological ecology of Pacific salmon.

The use of physiological techniques to study the suborganismal causes of conservation issues has been advocated for in the past (Wikelski and Cooke 2006); however, the use of physiological techniques to understand the impact of hydropower infrastructure and operational strategies on fish has been minimal (Chapter 2; Hasler et al. 2009). By using such techniques, the biological basis for the negative (and positive) consequences of hydropower systems can be assessed, and managers and policy makers will be better equipped to implement 'best practices' for dealing with hydropower associated issues. Management applications will be discussed later.

In Chapter 3 the Puntledge River summer-run Chinook salmon were assessed for physiological condition, that is, nutritional status, osmoregulatory status, and blood borne energy store indicators, to identify signs of stress and to detect potential causes for spawning migration failure. A principle component analysis was used to group highly correlated data and to relate physiological condition to migratory behaviours sampled using radiotelemetry-derived fish locations. It is worth noting that this is one of the first times that multivariate approaches have been used in the study of fish physiology data. A major finding was a failure of blood correlates to predict most behaviours, and the stock showed no signs of physiological stress at the time of sampling. Similar studies that have focused on Pacific salmon stocks and migration through hydropower-impacted systems have also found limited evidence that blood correlates could account for variation in behaviours at hydropower influenced sites (Pon et al. 2009a,b; Roscoe et al. 2010a). Furthermore, relative to the number of suborganismal physiology variables analyzed, few have predicted spawning migration success/failure in Pacific salmon (e.g., Cooke et al. 2005b, 2006, 2008b) despite a recent genomic study that suggests underlying gene expression can predict migratory failure in Fraser River sockeye (Miller et al. 2011). This current lack of individual variation in physiology to predict spawning outcome may bring into question the physiology/life history nexus (Ricklefs and Wikelski 2002) that suggests the functional responses of animals to their environment are limited by metabolism, nutrition, and thermal relationships. Likewise, using physiology techniques to mitigate conservation issues associated to hydropower-impacted systems, and Pacific salmon in general, may be futile if future research continues to find limited relationships between physiology and migratory success/failure (i.e., no ‘silver bullet’). Perhaps we

are simply measuring the wrong physiological metrics which could be clarified with genomics tools.

In Chapter 4 the thermal biology of the Puntledge River summer-run Chinook salmon was quantified in order to assess to what extent the stock was exposed to extreme temperatures and if cold-water refuge was available to the migrating salmon. Furthermore, available energy was modeled using variable temperature regimes to understand the effects of differences in temperature on available energy during migration, and thus spawning outcomes. Migrating salmon had no cold-water refuge available to them, thus negating the possibility to use behavioural thermoregulation to conserve energy (Berman and Quinn 1991; Brett 1995) and only fish that could access the cooler water of Comox Lake would have appropriate amounts of available energy to spawn during historical time frames. Hydropower systems likely result in more uniform river temperature profiles (Poff et al. 1997; Cassie 2006), which during warm periods may expose fish to non-optimal water temperatures (Keefer et al. 2008a,b, 2009). However, this phenomenon may be in fact due to other factors, such as climate change, as migrating salmon in non-hydropower impacted rivers have also been exposed to warming river temperatures (Lee et al. 2003; Farrell et al. 2008) and have limited opportunities to use behavioural thermoregulation (Donaldson et al. 2010). At the individual level, the locomotory capacity (i.e., scope for aerobic activity) of fish does evolve rapidly and may allow fish to cope with dynamic river temperatures (reviewed by Feder et al. 2010); however, changing environments will likely push aerobic activity beyond typical tolerance levels (reviewed by Hofmann and Todgham 2010). It is possible that rising

river temperatures and the lack of cool water refuge may lead to the extirpation of salmon from warming rivers (Gustafson et al. 2007; Waples et al. 2008).

In Chapter 5, the relative activity of tagged Puntledge River summer-run Chinook salmon was related to habitat and environmental characteristics but varied extensively among individuals. Variation in activity level was mostly accounted for by variation in individual behaviours and transmitters. The finding that variation between individuals was more important than habitat and environmental characteristics may indicate the importance of accounting for individual variation in analysis of activity. The somewhat surprising lack of relationship between environmental characteristics and activity given the importance of temperature on energy use (Brett 1995) and discharge on activity (Taylor et al. In Review) may have resulted because of the scale at which the data were collected. For example, if river flow at the nose of the fish were quantified, discharge may have been a more important variable for predicting activity. It is plausible that activity is an important contributor to spawning success/failure because of the nature of the salmon migration but further research is needed.

Holistically, the findings of this thesis indicate some consequences of Pacific salmon migrating through a hydropower system. In the Puntledge River, hydropower represents a permanent anthropogenic disturbance, and migratory rates of tagged fish were slower than in other systems and higher temperatures may be causing premature mortality. Prior to ~200 years ago, with the exception of exploitation from First Nation's peoples, Puntledge river salmon were only exposed to natural disturbances. The abrupt changes to river habitat caused by the introduction of hydropower (magnitude, frequency, duration, and predictability of flow regimes [Poff et al. 1997; Lytle and Poff 2004])

means that fish have to adapt to new and dynamic habitat features to avoid extirpation from the river (Waples et al. 2008). A possible adaptation could include arriving at the river later in the year to avoid low summer flows and high summer temperatures- essentially changing to fall-run salmon (a life history currently present in the river and summer-run fish are thought to be hybridizing with fall-run salmon). Other than life history adaptations fish may be able to use behavioural adaptations such as using areas of refugia to avoid hydropower operations and unfavourable environmental characteristic (Lytle and Poff 2004). These sites may be available above Stotan Falls in back channels and may be available in Brown's River should more river flow be present. In general, should behavioural adaptations fail to occur in the summer-run Chinook salmon, it is likely that the future of the stock is bleak. The rate at which Pacific salmon can adapt to anthropogenic disturbances is unclear, though Waples et al. (2008) suggest that salmon should be best able to adapt if disturbances mimic natural disturbances in space and time.

Management Applications

Though adaptations to disturbance are possible, the ecological time frame at which the Puntledge River summer-run Chinook salmon has been exposed to the hydropower disturbance is short, and evidence does not suggest that the population is adapting well. Thus, it is important that intervention by managers occur in order to ensure that the stock remains unique and present in the river. A hatchery is present on the river and this facility may serve as a way to increase the number of returning salmon to the mouth of the river. During the research period of the thesis, most fish were diverted into the hatchery facility to be used 3-4 months later as brood stock. Effort was made to transport

as many fish as possible (> 200 fish) to a cold water facility located approximately one hour from the hatchery to avoid warm temperatures in the hatchery raceways (rectangular concrete fish holding areas that are filled with river water). Chapter 4 supports this management action, as water temperature is increasing in the river and is likely contributing to failed spawning migrations. However, in river habitat features may also be inhibiting natural recruitment by preventing sufficient returns of spawning adults to spawning grounds. To attempt to increase the number of spawning adults returning to spawning ground, the power utility has introduced managed flows to induce upstream movement of fish.

The focus of Chapter 6 was on the effectiveness of introduced pulse flows on the migratory behaviours and upstream movement of the tagged summer-run Chinook salmon. Pulse flows were only found to result in significant upstream movement in one case; however, a number of methodological limitations likely impeded statistical significance. It is also important to note that no negative consequences of the pulse flows were found during the study. Moreover river flow only had minimal importance to variation in activity levels of tagged fish (Chapter 5) and river temperature should be manipulated in a similar manner as flows (Chapter 4). Managers could also further experiment with the pulse flows, and/or considering other means for moving fish upstream. Other options could include trap and truck, further altering the two fall complexes to ease passage, and/or attempting to manipulate hatchery fish by imprinting with specific cues from spawning grounds.

Other major findings of this thesis may help identify future management directives and support current initiatives. One finding of this thesis that will aid

managers is the knowledge that, for the most part, the nutritional condition, osmoregulatory status, and blood borne indicators of energy levels of the returning adults are within appropriate ranges for migrating Chinook salmon (Chapter 3). Because fish are in 'good' physiological condition when they arrive at the hatchery (sampling location), managers should be able to ensure that the brood stock used to produce the next generation of adults is of high quality. However, with this in mind, the migratory behaviours of the returning salmon were slow relative to similar stocks, and may be a direct effect of fish being imprinted at the hatchery site and not the historic spawning area (Quinn 1993).

Another finding of this thesis was that elevated water temperatures are a possible driving force for premature mortality and river conditions may be too warm for fish to hold into prior to spawning (Chapter 4). Hatchery management is aware of this issue; however, fish that are not captured at the hatchery and swim the river proper are exposed to the elevated temperatures. Possible management initiatives could focus on reducing the river temperature by using large chillers (Nickel et al. 2004), releasing cooler hypolimnetic water from Comox Lake dam, and/or capturing and trucking fish to Comox Lake where the water is cooler.

Using the findings of this thesis to make management decisions beyond the Puntledge River summer-run Chinook salmon may be unwise, especially if policy outcomes are needed. Species and populations vary greatly, even within local spatial scales, and positive management outcomes in one river may be negative outcomes in another. Little is known about the management of river systems at the ecosystem scale (Walters 1997) and rigorous studies that span watersheds, fish communities and

populations, and management outcomes need to occur before proper policy can be developed. Focus by Fisheries and Oceans Canada to understand altered flow regimes in the context of policy should make Canada a leader in this respect. Also, HydroNet (a recently funded strategic network by the Natural Science and Engineering Research Council) should increase our knowledge of how rivers can be managed to promote sustainable and healthy ecosystems (Smokorowski et al. In Review).

Future Research Directions

In summary, several research directions should be undertaken to further our basic understanding of migratory biology and Pacific salmon physiological ecology. These include:

- 1) Comparative studies across systems. Understanding how migratory behaviours with respect to environmental conditions are conserved across different stocks of salmon may increase our understanding of the basic mechanisms that control migration.
- 2) Using genomics to predict migratory behaviours. Recent work has highlighted genomics as a predictor of migratory behaviours. Future studies should aim to use genomics in a variety of taxa and systems to understand how the genome influences variation in migratory behaviours.
- 3) Interdisciplinary techniques in fish biology. Using a suite of physiological tools to explain the physiological ecology of fishes should lead to a more

thorough understanding of the mechanisms that affect fish behaviours and population outcomes. However, much work is still needed to field test physiological tools and to ensure accurate field measurements are being obtained (e.g., EMG transmitters, forensic techniques, stable isotopes).

- 4) Quantify the entire thermal history of Pacific salmon. With changing global climate, understanding the thermal history of Pacific salmon should allow managers and scientists to better place conservation efforts.
- 5) Quantifying the bioenergetics of an entire migration of a stock of Pacific salmon would identify habitats and environmental characteristics that cause increased energy use and possibly indicated areas where management should focus directives.

Research that improves the Puntledge River management of the summer-run Chinook salmon should also be explored:

- 1) Fish passage to Comox Lake should be a priority to managers. The cold-water refuge present in the lake is likely sufficient for reproductively maturing salmon, and may offer managers an inexpensive directive to ensure sustainable fish populations. Fish captured for brood stock could also be held in net pens in the lake to produce high quality gametes.

- 2) Understanding the fine scale flow dynamics at the Powerhouse Pool and at the fall complexes (i.e., Stotan and Nib Falls) may help to improve fish passage at the sites. Bioengineers could also use this knowledge to construct habitat that guides fish to spawning territory.
- 3) Other initiatives by the power utility to induce upstream movement of salmon should be explored. Closing the river to human recreational activity, and releasing water to mimic the natural flow regime may be appropriate alternatives to pulse flows.

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Appendices

Appendix 1: Co-author and journal permissions

Cooke, Steven J. (Co-author for Chapter 2, 3, 4, 5, and 6)

Thursday, May 12, 2011 8:47 PM

Subject: RE: Thesis permission
Date: Thursday, May 12, 2011 7:18 PM
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SJC

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Donaldson, Michael R. (Co-author for Chapter 3 and 4)

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2 March 2011

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Lucas Pon

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