THE SWIMMING PERFORMANCE AND POST-SWIM BODY ION CONCENTRATIONS OF JUVENILE PINK SALMON, AND THE EFFECT OF PARASITIC SEA LICE ON THESE PARAMETERS

by

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Abstract

Pink salmon (*Oncorhynchus gorbuscha* Walbaum) stocks in the Broughton Archipelago BC have seen a general decline in recent years. This is thought to be due to parasitism by sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837), on pink salmon during early marine life stage. To investigate this, I measured swimming performance, an integrated measure of fish health, and post-swim body ion concentrations, a secondary stress response, of control and sea lice infected juvenile pink salmon (mass < 3.0 g).

Using five different protocols (ranging in duration from 8 – 112 min), four constant acceleration tests (rates between 0.005 - 0.053 cm s^{-2}) and a repeated critical swim speed test, it was found that the final swimming speed of juvenile pink salmon (mass <5.0 g) at baseline was independent of the swim protocol ($P > 0.05$). Given this finding, estimates of swim performance in juvenile pink salmon can be accurately measured with an acceleration test lasting < 10 minutes.

Using a repeated, constant acceleration (0.05 cm s^{-2}) protocol, the effects of sea lice on swimming performance and post-swim body ion concentrations were measured in artificially infected river-caught (RC-fish, mean body mass 0.3 $\pm$ 0.05 g) and ocean-caught infected (OC-fish, mean body mass 1.1 $\pm$ 0.1 g) juvenile pink salmon. Infection levels ranged in intensity (1 - 4 sea lice per fish) and development stage (chalimus 1 - adult). Swimming performance of RC-fish was not affected by lice intensity ($P>0.05$) but was affected by lice stage with swimming performance decreasing at chalimus 3 stage (-20.4%) and even further at more advanced sea lice stages (chalimus 4, -26.5%; motile, -37.9%). Sea lice parasitism had no significant effect on the swimming performance of larger OC-fish when compared to control. The absence of an additive effect on swimming performance of 1 to 3+ sea lice per fish suggests drag forces induced by the

ectoparasite was not a major factor. In contrast, post-swim body Na^+ and Cl^- concentrations were typically elevated in infected compared to control RC-fish ($P < 0.05$), but not OC-fish ($P > 0.05$).

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Co-Authorship Statement

The majority of the experiments, including over 400 swim tests, and data analyses for this thesis were completed by me, L.J. Nendick. The following co-authors, whom assisted with the experiments, data analyses, and editing are listed on the manuscripts: Tony P. Farrell, Colin J. Brauner, Mike Sackville, Steven Tang, Manuela Gardner, and Amelia Grant.

A. Grant and M. Gardner analysed the whole body ion samples in the swim test protocol study. Additionally, A. Grant was integral in the fish husbandry at CAER, and M. Gardner performed body ion analyses on samples from the study on the effects of sea lice, as well as commented on editions of the swim test protocol manuscript.

M. Sackville, and S. Tang assisted extensively in field measures of swimming performance, as well as carrying out artificial infections and wild fish collection. Their input was also integral to experimental design of the study on the effects of sea lice.

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Chapter 1 : Introduction

The health and survival of fish is challenged by environmental stressors that can be natural or anthropogenic in origin (Brett and Groves, 1979). Parasites such as sea lice are considered a stressor. 'Sea lice' is a common name for a large number of species of marine ectoparasitic copepods, many of which are widespread and important disease causing agents that infect both wild and cultured fish (Pike and Wadsworth, 1999; Tully and Nolan, 2002; Johnson and Fast, 2004; Johnson *et al*., 2004; Costello, 2006). Of these, the salmon louse *Lepeophtheirus salmonis* has received a great deal of interest because of its economic impact on the salmonid aquaculture industry (Pike and Wadsworth, 1999) and its possible impacts on wild salmonid populations (White, 1940; Johnson *et al*., 1996; Whelan and Poole, 1996; Heuch *et al*., 2005).

While *L. salmonis* is a natural parasite of wild Pacific salmon (Kabata, 1979), Atlantic salmon (*Salmo salar*) farms are acting as incubators for this natural parasite and may be creating conditions where this parasite is transferred back to the surrounding ecosystem at sufficient levels to infect juvenile wild salmon at high, possibly lethal levels (Krkošek *et al*., 2007). During early marine life juvenile pink salmon may be particularly susceptible to ectoparasitic impacts, not only because of their small body size but also because of energy consuming activities that occur during this life stage, such as transition from freshwater (FW) to seawater (SW) and migration. If salmon farms are located near the migratory paths of juvenile Pacific salmon, then parasitism by *L. salmonis* may be more severe than experienced naturally. This anthropogenic stress to juvenile Pacific salmonids is a

relatively recent phenomenon on BC'sPacific coast (Morton and Williams, 2004) because aquaculture is a relatively new industry in Canada, and as such the effect of sea lice on Pacific salmonids has not been extensively examined.

Pink salmon (*Oncorhynchus gorbuscha* Walbaum) are the smallest of all Pacific salmonids when they first enter the marine environment, and are allegedly on track to be extirpated in the Broughton Archipelago, BC as a result of lethal sea lice loads transferred to them from farmed adult fish (Krkošek *et al*., 2007). Despite this claim, the effects of sea lice on pink salmon at this life stage are largely unknown. Studying the sub-lethal physiological effects of varying sea lice infections on pink salmon in this potentially sensitive juvenile life stage, as I do in this thesis, may provide environmental regulators with information that can be used to minimize the effect of aquaculture on wild salmon.

The challenge is then selecting a physiological indicator that can be readily measured in a controlled manner, that integrates the physiology of the whole fish, and has some ecological relevance. The swimming ability of fishes has received considerable attention in this regard (reviews: Beamish, 1978; Hammer, 1995; Keiffer, 2000), and has been used for close to half a century as an integrated indicator of fish health (Blazka, 1960). Salmon are particularly strong swimmers, migrating long distances to and from the ocean, as well as down and up rivers. Research focusing on the swimming performance of salmonids as small juveniles has been conducted (Griffiths and Alderdice, 1972; Brauner *et al*., 1994; McDonald *et al*., 1998; McFarlane and McDonald, 2002; Pon *et al*., 2007),

although very few studies measure swimming performance individually in juvenile fish and none have examined swimming performance of pink salmon.

This thesis will focus on quantifying swimming performance, and associated changes in ionic balance, of post-emergent pink salmon, and the effect that sea lice parasitism has on both of these parameters. The following five sections of the introduction now consider the general background, the significance of the topic and the relevant literature. The sections are:

1. Salmon and Canada – an overview of the significance of wild and farmed salmon in Canada.
2. The Broughton Archipelago – a brief description of this remote area of British Columbia and the current regional conflict between wild and farmed salmon.
3. Pink Salmon – the life history of this species of Pacific salmon.
4. Sea Lice – the life history of this parasite and a review of past findings and current research status of the effects of *L. salmonis* on its host.
5. Swimming Performance – a brief description of the merits for measuring swimming ability in fishes as well as the types of tests available to quantify swimming performance.

The introduction concludes with a summary of the central hypothesis, the research objectives and research predictions of the experiments conducted in this thesis.

Salmon and Canada

Salmon play an important role in the social and economic fabric of the Canadian Pacific coast. First Nations of British Columbia greatly value Pacific salmon because the fish hold a central place in the ceremonial, subsistence and commercial aspects of their lives. Along with a cultural and historical value intricately woven into the society, the economic value of the salmon has a tremendous impact on the quality of life.

Each year the commercial and recreational salmon fisheries are worth millions of dollars to the Canadian economy. In 2007, British Columbia's commercial fishery of Pacific salmon was valued at $40.7 million, a decline from 2006 which was valued at $60.1 million (BC Ministry of Environment). The value of wild commercial catch continues to decrease because of declining numbers caught from depleted stocks and a lower price due to increased availability of farmed salmon products. Indeed, salmon aquaculture in Canada has rapidly expanded over 25 years and was valued at $364.4 million in 2007 (Ministry of Environment). This juxtaposition of wild and cultured salmon presents challenges in striking a sustainable balance in resource management.

Not only are wild salmon valuable socially and economically to Canada, but also environmentally, as they can be used as sensitive indicators of ecosystem health. The abundance of wild runs of Pacific salmon is evidence of healthy ecosystems, particularly in more remote regions where direct human impact is minimal. With human impact, habitat loss and climate change, the status of Pacific salmon populations can serve as a valuable bio-indicator of ecosystem resilience.

The Broughton Archipelago

While the importance of salmon to all of Canada is enormous, some communities are particularly reliant on. The Broughton Archipelago is a remote area between mainland British Columbia and northern Vancouver Island (Fig. 1.2), where not only the people, but the entire ecosystem is heavily dependent on local salmon stocks. As a primary food source for multiple species such as bears, whales and eagles, wild salmon are an integral component of the ecology. Historically pink salmon returns to this region have fluctuated widely (Fig. 1.1), which is a natural occurrence for pink salmon coast wide; however, the continued decline in these stocks since a record return in 2001 has caused alarm. Using a mathematical model it was predicted that within four generations (yr: 2015) there would be a total collapse of the pink salmon stocks in the Broughton Archipelago (Krkošek *et al*., 2007). This "extinction hypothesis" was attributed to sea lice parasitism on juvenile fish, with the parasite having its origin in salmon farms.

The first aquaculture farms were introduced in British Columbia in the 1970's, and since then the industry has grown rapidly, with as many as 30 individual sites tenured in the Broughton Archipelago in 2008 (Ministry of Environment) rearing Atlantic salmon (*Salmo salar*) in open net pens (Fig. 1.2). The number of salmon farms reached its peak in 1995 as a result of a Provincial government moratorium on fish farm site development, although the volume of cultured salmon production has continued to increase (BC Ministry of Environment). The precipitous declines in wild stocks of pink salmon in the Broughton Archipelago in 2002 coincided with the peak production (at the time- this value had since been exceeded) of commercial fish farms that are situated

along wild salmon migration routes in the Broughton Archipelago (Fig. 1.2). Sea lice, primarily the species *L. salmonis*, parasitize farmed Atlantic salmon and can translocate to wild fish species in the local environment. Therefore, it is alleged that sea lice are provided to the natural ecosystem in such large numbers, that juvenile pink salmon migrating past the farms become fatally infested (Morton and Routledge, 2005).

Pink Salmon Life History

Pink salmon are found from the coast of central California to Alaska in North America and from Japan to the Arctic in Asia (Heard, 1991). In Canada, pink salmon are the most abundant species of Pacific salmon commercially captured, representing over half the annual catch. Pink salmon migrate to sea where they grow to maturity and return after two years to their natal stream, which has resulted in odd-year and even-year populations that do not interbreed. They produce small eggs, generally in the lower reaches of rivers, and die after spawning (Heard, 1991). The embryos hatch and over-winter in the gravel of river beds, emerging from the gravel in the spring as fry, and migrate directly to sea at a size of ca. 0.2 g (Heard, 1991). Given that most anadromous salmon require a period of preparation before entering seawater, it is remarkable that pink salmon are able to survive early marine life without a conventional period for smoltification (Houston, 1961), although this life history strategy is not without challenges. For example, in the first week of ocean residence, the concentration of body ions doubles (Grant *et al*., 2009), suggesting that the early stages of pink salmon are not fully prepared for life at sea; however, this physiological stress progressively declines with a concurrent

increase in gill Na^+-K^+-ATPase activity, an enzyme responsible for driving sodium and chloride removal from the fish. In the absence of sea lice parasitism, a steady ionic state is achieved after about 8 weeks in SW (Grant *et al.,* 2009).

During the first 3-4 months in SW, pink salmon migrate through the Broughton Archipelago to the open ocean, while at the same time growing rapidly, doubling in size every month (Ricker, 1964; Grant *et al.,* 2009). Consequently, the additional challenge of an ectoparasite that disrupts the skin (possibly causing further ionic challenges) and adding drag during locomotion are likely to be particularly taxing during this life stage.

Sea Lice

Life History

Lepeophtheirus salmonis and *Caligus clemensi* are species of sea lice (Copepoda) found naturally parasitizing salmonids in the Pacific Ocean. *Lepeophtheirus salmonis* is a SW specialist, predominantly parasitizing anadromous salmonids of the Northern Hemisphere, although alternative hosts are known (Bruno and Stone, 1990; Jones *et al.,* 2006; Pert *et al*., 2006). *Lepeophtheirus salmonis* undergoes direct development, which includes planktonic (2 nauplii), infective (1 copepodid), and parasitic (4 chalimus, 2 pre-adult, 1 adult) stages (Fig. 1.3) (Kabata, 1972). Of the parasitic stages, the 4 chalimus stages are attached to the host by a filament and, as such, are non-mobile, except during a moult when a new filament is formed. The 2 pre-adult and 1 adult stages are mobile. While the primary infectious stage is the copepodid, pre-adults and adults are known to transfer between hosts (Bruno and Stone, 1990). Factors governing

lice development include host species (Johnson, 1993), temperature and salinity (Johnson and Albright, 1991; Connors *et al*., 2008), among others (Genna *et al.,* 2005). At 10 ^{o}C, *L. salmonis* progress on Atlantic salmon from the first copepodid stage to the second pre-adult stage in about 35.3 days (Johnson and Albright, 1991). A salinity range of 25-30 ppt is suitable for copepodid development and survival (Johnson and Albright, 1991), but below this salinity development is retarded or halted. Parasitic stages range in size from < 1 mm in length for chalimus 1, to >10 mm in length for female adult. By comparison, a 0.2 g pink salmon is ca. 30 mm and a 1 g pink salmon is ca. 55 mm.

Sea Lice Effects on Fish

Lepeophtheirus salmonis has been extensively studied due to its common occurrence on farmed and wild salmon, a problem Canada shares with other nations such as Scotland (Tully and Whelan, 1993; Butler 2002; Butler and Watt, 2003), Ireland (Gargan *et al*., 2003) and Norway (Holst *et al*., 2003, Heuch *et al*., 2005), all of which farm fish in wild salmonid habitat.

Sea lice attach to the surface of fish and feed on the host's mucus, skin (Kabata, 1974; Bjørn and Finstad, 1998) and blood (Brandal *et al*., 1976). If severe, infestation may interfere with the fish's behaviour (Dawson *et al*., 1999; Webster *et al*., 2007), osmoregulatory ability (Grimnes and Jakabsen, 1996; Bjørn and Finstad, 1997) and swimming performance (Wagner *et al*., 2003, 2004; Wagner and McKinley, 2004).

A fish's ability to swim could be impaired by a number of mechanisms (Fig. 1.4). When in SW, fishes are hypo-osmotic to their environment, and so if the

barrier (the skin) protecting the fish from this environment is compromised, water will move down its concentration gradient, and out of the fish. This dehydrates the fish, and causes an increase in body ion concentrations. In an effort to regain water content, fish must increase their rate of drinking, and to obtain water they actively transport sodium and chloride across the gut, drawing water osmotically, and then expel excess sodium and chloride at the gills (Evans, 1982). Fish in SW may increase Na^+-K^+ ATPase activity at the gill to compensate for excess ions entering the body (Grant *et al*., 2009). These compensatory processes work to re-establish ionic homeostasis, but likely at an increased energetic cost to some other activity, such as locomotion. Alternatively, swimming performance could be directly impacted by an ionic imbalance disturbing muscle fibre activity. Muscle contractility and the neuromuscular apparatus (Houston, 1959) may be affected by elevated ion concentrations.

Another possible pathway for sea lice to impact fish swimming ability is mechanical in nature. Sea lice attach to the outer surface of fish, but the posterior portion of the louse is free to move in the current. It is possible that the drag forces from sea lice hanging off a fish may be sufficient to impair the swimming ability of the host. Of course, louse number, size and location relative to fish size are likely to be strong contributors to the magnitude of the drag produced. Thus, a simple prediction is that drag should increase with lice number and life stage.

Swimming Performance

Fish Health Indicator

Swimming ability varies between and within fish species. A good swim test, however, will produce repeatable results in healthy fish (Jain *et al.,* 1998). If the swimming ability of a fish in good health is known, i.e. baseline data are available, this information can be used as an integrated measure to evaluate the health of infected fish. Locomotion requires the contribution of multiple body systems, so if one of these systems is not functioning properly, this may manifest as a decreased ability to swim maximally. Thus, maximum swimming is generally considered to be an integrated measure of health, and a measure of a tertiary stress response. Additionally, since decreased swimming ability is likely to yield ecological repercussions, it might be a useful indirect measure of not only fish health, but also lifetime fitness.

Types of Swim Tests

Various methods have been employed to test swimming ability, the most popular of which is using a swim tunnel, which is analogous to an aquatic treadmill. Fish swim against measurable flows passed through the chamber in which they swim. Well-designed tunnels produce consistent and measurable flows, which is desirable when conducting a controlled laboratory experiment. Swimming performance of even the smallest fish (<0.5 g) can now be individually tested because of the advent of miniature swim tunnels (Loligo Systems, Denmark).

Tests performed using tunnels vary in time as well as the velocity regime. The most common and popular test used to date is the critical swimming (U_{crit}) test (Brett, 1964), whereby velocity is increased a set amount at set intervals (usually

between 20-60 min) until the fish fatigues. It is used to measure the maximum aerobic capability of fish. The major drawback with U_{crit} tests is that they take several hours to conduct, and as a result limits the number of fish that can be tested during a given study period. A ramped-U_{crit} test approximately halves the test time, by taking advantage of the fact that fish power locomotion aerobically, regardless of acceleration rate, when at speeds less than 60-80% of U_{crit} (Jain *et al*., 1997; Burgetz *et al*., 1998).

While U_{crit} tests are popular, incremental velocity tests employing step durations shorter (1-2 min duration) than those used in U_{crit} tests (> 20 min duration) are gaining popularity. These tests, often called constant acceleration tests, measure maximum swimming speed (U_{max}) in as little as 10 min (depending on the magnitude of the velocity step) and thus are an attractive alternative to U_{crit} tests when the study period is limited, as is the case with rapidly growing juvenile pink salmon.

U_{crit} and U_{max} tests likely do not produce the same fatigue velocity in adult fishes because of different metabolic processes utilized (Reidy *et al*., 2000; Farrell, 2008). In large fish, U_{max} is greater than U_{crit} because burst speeds can be powered by glycolytic processes, but only for short time periods. However, limited research in small juvenile salmonids (McFarlane and McDonald, 2002) suggests that fatigue velocity is not altered by test type, due to their limited anaerobic capacity. Therefore, an opportunity exists to measure the swimming performance of small juvenile fish, in a relatively short period of time using U_{max}, without compromising comparisons with literature values reported for U_{crit}.

Research Objectives and Hypothesis

This thesis has two main purposes. First, because limited information is available on the swimming ability of juvenile salmon <3.0 g, the size range at which juvenile pink salmon are thought to be most sensitive to sea lice parasitism, I wished to quantify the swimming performance of juvenile pink salmon of this size. I measured swimming performance using both a ramped repeated-U_{crit} test and four different rates of acceleration for a U_{max} test. I hypothesized that the velocity at which fish fatigued would not differ between types of swimming tests. During a phase of rapid growth, such as early marine life stage in anadromous salmonids, fish may have limited capacity for anaerobic metabolism, and therefore, unlike adult fishes, could power locomotion at all speeds aerobically. I predicted that this would yield similar final swimming speeds for all U_{max} tests, and ramped repeated-U_{crit} test protocols. This work represents Chapter 2 of my thesis.

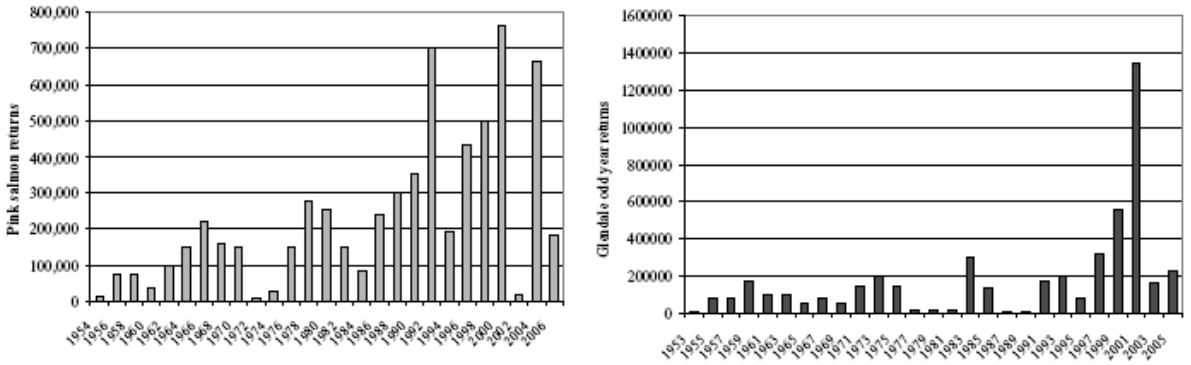
Second, because very little physiological data is available on the effects of sea lice parasitizing juvenile salmonids, and because swimming performance is a measure of overall fish health, I measured the swimming performance of fish infected with sea lice and compared this to control uninfected fish. Sea lice infection levels varied both in number, 1 – 4 sea lice per fish, and in maturity, chalimus 1 through to adult, with fish size ranging from 0.2 – 3.0 g. Swimming ability was measured in two groups of fish. One group was captured in FW in the Glendale river (river-caught, RC-fish) and were subsequently transferred to seawater in the laboratory and infected artificially with sea lice. The second group was captured in the ocean (ocean-caught, OC-fish) with and without existing sea lice infections. Two groups of fish were examined primarily to maximize the range

of fish sizes and sea lice infection levels that could be examined. This work represents Chapter 3 of my thesis.

Additionally, for both sets of experiments body ion concentrations were measured in fish tissue sampled post-swim. I hypothesized that body ions would be increased from sea lice infection, as a consequence of ionoregulatory disturbance caused by sea lice feeding on the skin and blood of fish. Within the size range tested, I also postulated that small fish, infected and control alike, would have an elevation in body Na^+ and Cl^- concentrations following exercise because they would not yet be in ionic homeostasis due to recent FW to SW transition.

Figures

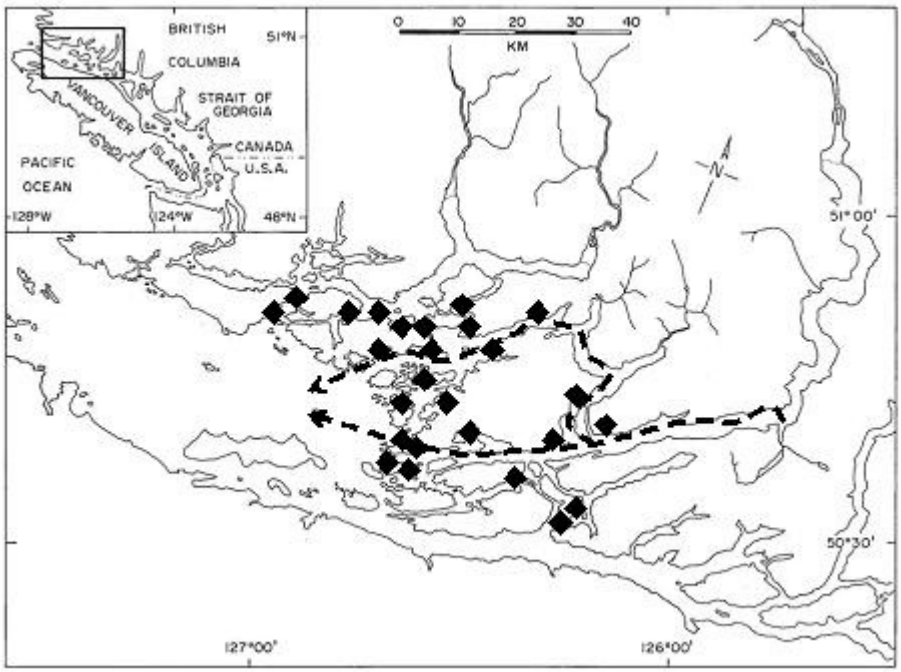
Figure 1.1 Pink salmon returns to Glendale River, the major salmon producing watershed in the Broughton Archipelago, during a) even and b) odd, years.



a) Even year b) Odd year

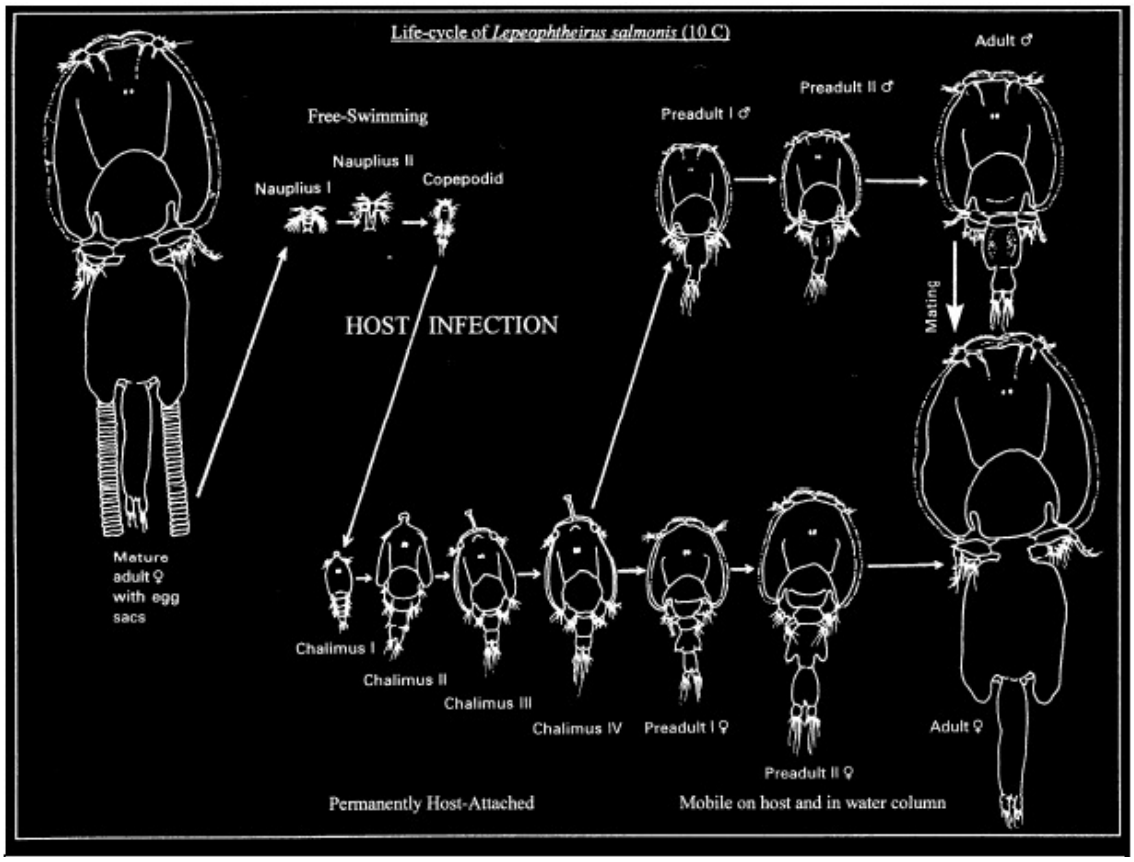
source: Department of Fisheries and Oceans Canada

Figure 1.2 Salmon farm tenures (black diamonds) and Glendale River salmon outward migration routes (dashed line) in the Broughton Archipelago.



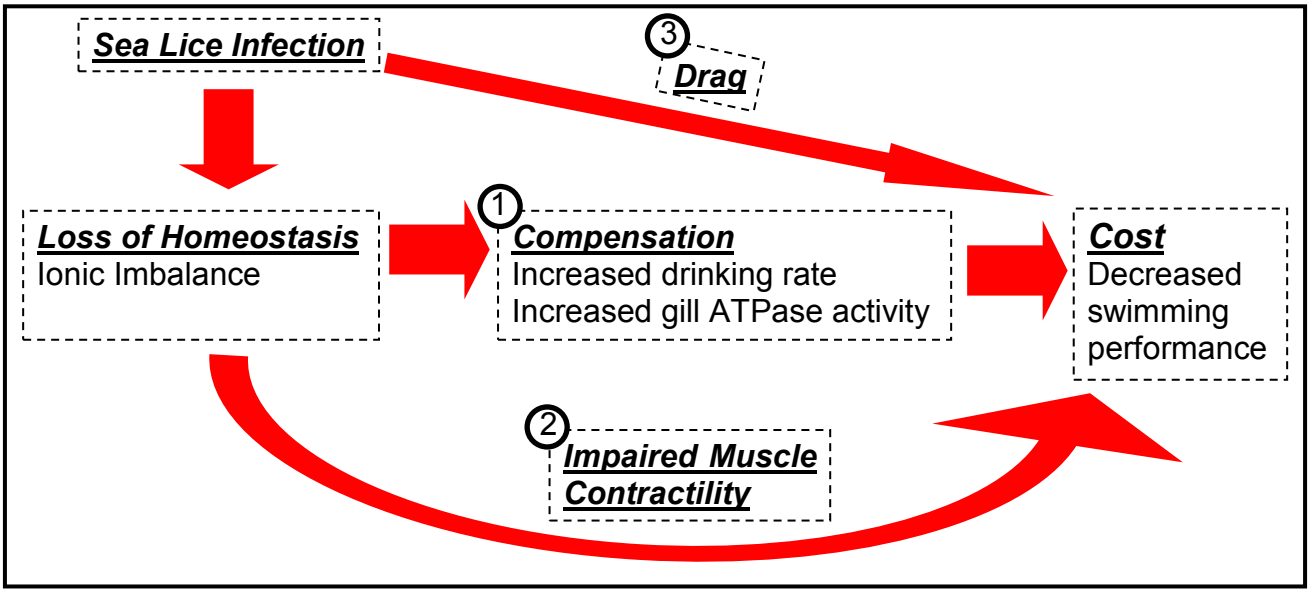
source: modified from Department of Fisheries and Oceans Canada

Figure 1.3 Life cycle of the parasitic sea louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae).



source: BC Pacific Salmon Forum, Protocols and Guidelines

Figure 1.4 Three potential mechanisms that sea lice parasitism could impact swimming performance in fish in seawater.



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Chapter 2 : Swimming performance and associated ionic disturbance of juvenile pink salmon (*Oncorhynchus gorbuscha* Walbaum) determined using different acceleration profiles[1]

Introduction

As the environment is altered by human impact, habitat loss and climate change the status of salmon populations serve as a valuable bio-indicator of ecosystem resilience. Pink salmon (*Oncorhynchus gorbuscha* Walbaum) is a Pacific salmonid currently being used as a keystone indicator of ecosystem health for the potential impacts of aquaculture (Morton *et al*., 2004; Krkošek *et al.,* 2006, 2007). It is alleged that recurrent louse infestations of wild juvenile pink salmon associated with salmon farms will result in the rapid local extinction of pink salmon in the area of the Broughton Archipelago (Krkošek *et al*., 2007). Hampering such assessments, however, is a general lack of information on the basic biology of pink salmon, especially the capacity of juveniles for exercise and to maintain ionic homeostasis. This baseline information is needed when evaluating sub-lethal disturbances of lice on fish.

The challenge of ionic homeostasis is particularly unique for pink salmon among the salmonids because they enter seawater (SW) almost immediately after emergence from the gravel in streams. Thus, they do not appear to progress through a recognisable smoltification period before migration and typically enter the marine environment as small as 0.2 g. Not surprisingly, an initial large increase in whole body ions is observed following transfer to SW (Grant *et al*., 2009); however, whole body ion levels recover in the ensuing 8-10 weeks in conjunction with a progressive increase in gill Na^+-K^+-ATPase activity (Grant *et al*., 2009).

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Swimming capacity is an important physiological measure because exercise is an integrated indicator of deleterious changes in ion regulation and muscle performance. Physiological limitations are revealed in premature fatigue and ionic disturbances (Randall & Brauner, 1991). Healthy salmonids can swim to maximum swimming velocity while maintaining normal plasma ion levels and osmolarity, and can repeat the same level of performance after only a short recovery period (40 min to 2 h) (Randall *et al*., 1987; Brauner *et al*., 1994; Jain *et al*., 1998). Conversely, unhealthy fish will exhibit ionoregulatory disturbance after a swim test and repeat swimming performance can be reduced by up to 33% (Brauner *et al*., 1994; Jain *et al*., 1998). Furthermore, ionoregulatory disturbance may impair swimming ability (Brauner *et al*., 1992, 1994). Ultimately such changes could affect the fish's ability to acquire food, swim within a school, avoid predation and successfully migrate to the open ocean.

While standardized tests of swimming performance have been used for close to half a century (Blazka, 1960; Brett, 1964), the difficult decision for the researcher is which swimming test to use as an index. The greatest information exists for critical swimming speed (U_{crit}), but these tests last many hours and therefore limit the number of fish that can be tested over a reasonable time period. Since juvenile pink salmon can double body mass in a month (Grant *et al*., 2009), a time limit exists over which swim trials can be conducted to look at a specific life stage. The ramped critical swimming speed (ramped-U_{crit}) offers a shorter test duration (Jain *et al*., 1998) and constant acceleration (U_{max}) tests shorten the duration even more. However, the fatigue velocity can vary with the test type. In 425 – 463 g rainbow trout (*Oncorhynchus mykiss* Walbaum; Farrell, 2008) and in 0.8 – 1.6 kg Atlantic cod (*Gadus morhua* L.; Reidy *et al.*, 2000) U_{max} or burst speeds were 30- 57% higher than U_{crit} speeds. In contrast, McFarlane and McDonald (2002) reported that sprint performance for 1.4 – 5.0 g juvenile rainbow trout was not statistically

different to U_{crit}, despite a large difference in test duration (3 min versus 180 min). These contradictory findings may be explained by the hypothesis that at an early life stage salmonids display the same fatigue velocity independent of the swimming test utilized. In this study, this hypothesis was tested. Pink salmon (mass = 1.0 – 5.0 g) swimming performance was tested using four U_{max} tests with acceleration rates that differed by an order of magnitude, as well as a repeat ramped-U_{crit} test. To characterise the ionic disturbance caused by swimming to fatigue, whole body and plasma [Na^+] and [Cl^-] ion concentrations were also measured following each swim test.

Materials and Methods

Fish Husbandry and Feeding

Pink salmon were captured by beach seine from the Broughton Archipelago, British Columbia (BC) on April 26, 2007. They were then transported to the Centre for Aquaculture and Environmental Research (CAER), West Vancouver BC, where they were housed in 60 L aquaria, outfitted with a flow-through SW system (100 % SW, 10.2 – 15.4 ^oC, 29.3 – 34.1 salinity, 5.0 – 9.1 mg L^{-1} dissolved oxygen). Fish were held under a simulated natural photoperiod to mimic natural daylight conditions and were fed a commercial mash daily (Bio-Vita starter feed; Bio-Oregon, Longview, WA), up to twice daily to satiation following a start up mixture (2 weeks of frozen bloodworms, krill and marine fish mixed with the commercial mash until mash alone was accepted).

Swim Trials

Swim trials were conducted in SW over a 24-day period starting July 25, 2007. Initially, fish were selected at random for tests; however, over the course of experiments, fish grew, to the point where body size became an issue (> 5.0 g) given the swim chamber dimensions. Therefore, there was a bias to select for smaller fish as the experiment progressed. A total of 50 fish (mean mass = 2.81 $\pm$ 0.29 g) were subjected to one of four U_{max} tests or a repeat ramped-U_{crit} test (n = 10). Duplicate Blazka-type (Blazka *et al.,* 1960) swim tunnels (mini swim tunnel, Loligo Systems, Denmark) allowed two fish to be individually tested at the same time. The swim chamber was 26.4 mm in diameter and 100.0 mm in length, and had plastic grids at either end to facilitate laminar flow and separate the fish from the propeller mechanism that produced the flow. Water velocities (cm s^{-1}) were calibrated using frame by frame video analysis (30 frames s^{-1}) of neutrally buoyant particles moving through the swim chamber. The blocking effect by fish accounted for > 10 % of the tunnel cross section area (mean 11.4 $\pm$ 1.6 %), and so correction for solid blocking was made for all fish in accordance with the equation described by Bell and Terhune (1970), using fish length, maximum height, and maximum width. Average water temperature was controlled to within 1.0 ^{o}C of the ambient temperature with a flow through of ambient water.

Prior to testing, fish were lightly anaesthetised with MS-222 (0.05 g L^{-1}) for rapid handling (measurement of mass, fork length, maximum depth and maximum width, and for transfer by hand to swim tunnel). Fish were never anaesthetized for more than 2 min and always recovered within 1 min upon transfer to the swim tunnel. Fish were left for ~ 5 - 10 min before water velocity was increased to 12.5 cm s^{-1}. At this speed fish oriented themselves toward the current and swam gently. A black cover was placed over the tunnel and fish were left for 30-60 min to habituate to the tunnel environment before swimming performance trials commenced. In both U_{max} and ramped-U_{crit} tests, fish were swum to fatigue, which was defined as the point that fish

rested the caudal fin on the posterior grid and did not move off the grid when stimulated mechanically. During the initial portion of the swim test, fish would occasionally refuse to move off the posterior grid. If fish would not swim within the first four velocity increments of a test, even when stimulated mechanically, the fish was removed from the tunnel and was not tested for U_{max} or ramped-U_{crit} swimming performance nor included in ion analyses (n = 16).

The experimental protocols were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council on Animal Care.

Ramped Critical Swimming Speed Test

A total of 10 fish were individually swam in ramped critical swimming speed tests (ramped-U_{crit}), which followed the protocols outlined by Farrell (2008) and Jain *et al*. (1997). Briefly, water velocity was increased at a rate of 1.83 cm s^{-1} every minute for 5 minutes, resulting in a ramp acceleration rate of 0.0304 cm s^{-2}. Following this procedure, water velocity was 21.7 cm s^{-1} which represented approximately 67 % of the measured U_{crit}. After 5 minutes at this velocity, the velocity increment was increased to 2.4 cm s^{-1} and step duration was increased to 20 min, yielding an acceleration rate of 0.002 cm s^{-2}. These 20-min velocity steps were continued until the fish fatigued (Fig. 2.1). At the conclusion of the swim test (swim one), velocity was decreased to 12.5 cm s^{-1} and fish were allowed to recover for 1 h, after which the ramped-U_{crit} performance test was repeated (swim two). Once a fish fatigued in the 2nd ramped-U_{crit} test, and the velocity brought to zero, the fish was quickly removed from the tunnel using a net, and euthanized with MS-222 (0.2 g L^{-1}) within 1 min of fatigue. U_{crit} was calculated according to Brett (1964) using the equation:

$$U_{crit} = U_i + [U_{ii}(T_i/T_{ii})],$$

with U_i the highest velocity maintained for the whole interval, U_{ii} the velocity increment (here: 2.2 cm s^{-1}), T_i the time elapsed at fatigue velocity, and T_{ii} the interval time (here: 20 min). Recovery ratio was determined according to Jain *et al*. (1998) by comparing 1st and 2nd swim U_{crit} values using the equation:

$$\text{Recovery ratio} = U_{crit}2/U_{crit}1$$

A value < 1 indicates that a fish swam more poorly on the 2nd swim compared to the 1st.

Maximum Swimming Speed Test

Maximum swimming speed (U_{max}) tests were performed with four differing acceleration rates (n = 10 fish). Fish were randomly assigned a test acceleration rate and all tests were spread out over the 24 days of experiments. The velocity steps were as follows: 0.32, 0.64, 1.27 and, 3.18 cm s^{-1}. The step duration was one min, which resulted in acceleration rates of 0.005, 0.011, 0.021, and 0.053 cm s^{-2}, respectively (Fig. 2.1). The water velocity that fish fatigued at was recorded as U_{max}. At fatigue, velocity was brought to zero, and the fish was quickly netted from the tunnel, to be euthanized with MS-222 (0.2 g L^{-1}) within 1 min of fatigue.

Fish Sampling Procedure

Fish euthanized post-swim were rinsed in freshwater (FW) to remove any SW on the skin surface, patted dry, and wrapped in foil before being flash frozen in liquid nitrogen and stored at -80 ^{o}C. For analysis, fish were weighed (frozen mass), defrosted, patted dry, cut into several pieces

(only for fish > 2 g) and placed in a pre-weighed falcon tube (15 or 50 mL). Samples were dried in an oven (65 ^{o}C) until a constant dry mass was obtained. Dry tissue was weighed (dehydrated mass) and then digested in 1 N nitric acid (with a maximum dilution factor of 60 for mass:volume) with daily degassing and vortexing to facilitate tissue digestion. The tissue digests were centrifuged and the clear supernatant was removed and stored at -4 ^{o}C until further analysis. Duplicate 10 μL supernatant samples were used to measure whole body [Cl^-] with a Digital Chloridometer (Haake Buchler Instruments Inc., Saddlebrook, NJ, U.S.A.) Whole body [Na^+] was measured in duplicate using a flame atomic absorption spectrometer (Spectra AA; Varian, Victoria, Australia). Whole body ions are reported relative to frozen mass and dehydrated mass of the fish. For U_{max} fish, frozen weight was consistently lower than live weight (mean 7.5 $\pm$ 0.4 %), which was not surprising considering the high surface area to volume ratio of these fish, but should be kept in mind when interpreting ion data relative to frozen mass.

Additionally, whole blood was collected from fish swum in ramped-U_{crit} tests following euthanization. Blood was collected by severing the caudal fin at the peduncle, and drawing blood into a heparinised haematocrit tubes. Haematocrit tubes were spun down, and plasma was collected and stored in 0.5 mL centrifuge tubes at -4 ^{o}C. Samples were diluted 1000 – 2000x with deionised water (5-10 μL plasma: 5 – 20 mL deionized water). Plasma [Cl^-] was measured using the colorimetric mercuric thiocyanate method (Zall *et al*., 1956). Plasma [Na^+] was measured using a flame atomic absorption spectrometer (Spectra AA; Varian, Victoria, Australia).

Statistical Analyses

All statistical analyses were performed using Sigmastat 3.05 (SPSS Inc., San Rafael, CA, U.S.A). Comparisons between swimming speeds and ion concentrations were performed with a

one-way ANOVA and pair-wise multiple comparison procedures (Holm-Sidak). Recovery ratios for repeated ramped-U_{crit} tests were calculated using paired t-test. Pearson product moment correlation was used to determine significance of plasma and whole body ion relationships. For statistical comparisons, $P < 0.05$ was used to establish significant differences. All values are presented as means $\pm$ S.E..

Results

Fish mass and length did not differ significantly between experimental groups (Table 2.1, $P > 0.05$), nor over the course of experiments (24 days, $P > 0.05$). U_{max} test durations ranged between 8 and 87 min depending on the acceleration rate used, and ramped-U_{crit} tests averaged 105 and 112 min for U_{crit1} and U_{crit2}, respectively (Fig. 2.1).

While test durations differed considerably, the mean swimming performance did not differ significantly among the different U_{max} acceleration groups (Table 2.1, $P > 0.05$), nor did they differ significantly from ramped-U_{crit1} and U_{crit2} (Table 2.1, $P > 0.05$). Consequently, the results for the 8, 21, 47 and 87 min U_{max} tests and the repeated ramped-U_{crit} test were statistically indistinguishable. Overall, the mean maximal swimming speed for each test ranged from 4.54 $\pm$ 0.12 to 5.2 $\pm$ 0.20 BL s^{-1}. The mean swimming speeds for each test are reported as both absolute (cm s^{-1}) and relative (body lengths per second; BL s^{-1}) in Table 2.1.

Fish tested with a repeated ramped-U_{crit} test performed two tests separated by 60 min. On average, U_{crit2} was not significantly different from U_{crit1} (Table 2.1, $P > 0.05$) and the recovery ratio was 1.05.

Ion concentration was evaluated in whole body tissues and plasma of fatigued fish, but only ramped-U_{crit} swum fish were sampled for plasma. Direct comparisons between the whole

body ion data of ramped-U_{crit} and U_{max} fish were not conducted because 2-3 gill arches as well as a small amount of blood were removed from ramped-U_{crit} fish but not from the U_{max} fish (Table 2.2). Comparison of whole body $[Na^+]$ and $[Cl^-]$ in U_{max} fatigued fish, as well as for a group of control, unswum fish (N = 8, mean live mass = 2.57 $\pm$ 0.42 g) are shown in figure 2.2, where ion levels are expressed relative to frozen mass (a) and dehydrated mass (b). $[Na^+]$ and $[Cl^-]$ were significantly higher in all U_{max} fish compared to unswum control fish when expressed as frozen mass (Fig. 2.2a, $P < 0.05$). Comparing among the different U_{max} tests, $[Na^+]$ was significantly elevated in the shortest (8 min) U_{max} test when compared to the 2 longest U_{max} tests (47 and 87 min), but no differences were observed for $[Cl^-]$. When expressed as dehydrated mass (Fig. 2.2b), no significant differences were seen between control and U_{max} tested fish for either $[Na^+]$ or $[Cl^-]$ ($P > 0.05$).

Whole body (minus blood and 2-3 gill arches) $[Na^+]$ and $[Cl^-]$ in repeat ramped-U_{crit} fish was elevated compared to control fish (n = 5, mean live mass 2.99 $\pm$ 0.17 g, minus blood and 2-3 gill arches), when expressed relative to frozen mass (Fig 2.3a, $P < 0.05$) and dehydrated mass (Fig. 2.3b, $P < 0.05$). Both $[Na^+]$ and $[Cl^-]$ in the plasma were significantly elevated in repeat ramped-U_{crit} swum fish compared to control fish (Fig. 2.3c, $P < 0.05$).

In ramped-U_{crit} fish, the inverse relationship between post-swim plasma and whole body $[Na^+]$ ions (Fig. 2.4) was not statistically significant ($m = -0.589$, $P > 0.05$), though a trend was observed ($P = 0.073$). This was not the case for unswum, control fish $[Na^+]$ ($m = 0.288$, $P > 0.05$), or for $[Cl^-]$ ions in either group (ramped- U_{crit} fish, $m = 0.06$, $P > 0.05$; control fish, $m = 0.387$, $P > 0.05$).

Discussion

The primary objective of this study was to establish and compare the ramped-U_{crit} and U_{max} swimming speeds in small juvenile pink salmon (*Oncorhynchus gorbuscha*). The results represent the first data on the swimming ability of individually swum pink salmon < 5.0 g and as such provide baseline data that can be used to assess the affect of environmental factors on swimming performance in pink salmon.

Other researchers have swum small salmonids either in groups (McFarlane & McDonald, 2002; McDonald *et al*., 1998) or using a short duration swim test, as explored in this study. McFarlane and McDonald (2002) swam 1.4 – 5.0 g juvenile rainbow trout in groups of 10. The present study with 1-5 g pink salmon is consistent with their result that swimming performance was independent of test duration (3 min – 180 min, McFarlane and McDonald, 2002; 8 min - 112 min, present study). Combined, these data support the hypothesis that early life stage fishes display the same fatigue velocity independent of the swimming test utilized. McFarlane and McDonald (2002) suggest that this is because early in development, fish depend predominantly upon glycolytic muscle force generation while the aerobic musculature continues to develop and this is discussed below.

In the present study, fish showed an excellent ability to recover rapidly from ramped- U_{crit} fatigue and swim to the same level of performance, or better (recovery ratio >1.0). This result is in agreement with previous studies on larger salmonids up to several kg (Jain *et al*., 1998; Farrell *et al*., 2003; Lee *et al*., 2003; Farrell, 2008), and suggests that fish were in good health.

The metabolic process utilized by a fish to power swimming is believed to be dependent on the type of swimming taking place. With step durations of one min, the U_{max} protocol employed here is essentially a protocol of constant acceleration test, while the ramped-U_{crit} test is essentially a step test of constant velocities. On the one hand, U_{max} tests are generally considered

to be measures largely of anaerobic swimming capacity because of the power required to accelerate at a high rate (Farrell, 2008). On the other hand, U_{crit} swimming tests are accepted to be good measures of aerobic performance, since anaerobic metabolism is recruited at speeds above 60 - 80 % of U_{crit} (Jain *et al.*, 1997; Burgetz *et al.*, 1998). However, juvenile salmonids may not fall in line with this concept of metabolic processes depending on the test employed, which has been based on fish about 100-times larger than those tested in this study. The lack of distinction between final swimming speeds in such different swim tests could be an indication that fish are utilizing similar metabolic processes, regardless of test protocol. In support of this, a study by McFarlane and McDonald (2002) designed to manipulate glycogen reserves, the primary fuel of anaerobic metabolism, found no effect on the sprint swimming speed. Sprint performance was identical for 2 g rainbow trout fed a restricted ration of 1 % body mass d^{-1}, and had glycogen levels that were reduced by more than 75 %, when compared to control (fed 4 % body mass d^{-1}).

Although not widely reported, whole body ions can be an effective means of revealing ionic disturbances. Houston (1959) found that the physical impairment following rapid SW transfer in chum salmon (*Oncorhynchus keta* Walbaum) was correlated with whole body $[Cl^-]$ and water levels. Here, whole body $[Na^+]$ and $[Cl^-]$ expressed relative to frozen body mass was significantly higher in fish sampled at fatigue when compared to control, regardless of test type. Additionally, plasma $[Na^+]$ and $[Cl^-]$ were significantly elevated in repeat ramped-U_{crit} fish at time of fatigue when compared to control. However, this was not the case when the U_{max} data were expressed as the dehydrated body mass. These results indicate that the ionoregulatory disturbance associated with the U_{max} test must be due to the water loss associated with exercise, rather than to a net ion gain. In contrast, ramped-U_{crit} data expressed as dehydrated body mass also increased, indicating that the ionoregulatory disturbance was associated with both water loss and a net gain

of ions. Brauner *et al*. (1994) reported when resting plasma ion values in larger SW-transferred coho smolts increased above an optimum level, subsequent U_{crit} performance decreased. Therefore, it would be interesting to see if the ionic disturbances that were observed either occurred after the first swim (and had no effect on the performance in the second swim), or were cumulative over the two swims (and may have affected performance in a third swim trial).

Wood and Randall (1973) found that water balance in rainbow trout tested for swim ability in FW exhibited an initial net gain (~ 30 min swimming) but a subsequent net loss (~ 30 – 70 min). In addition, the ion/gas exchange ratio decreases with activity in FW (Gonzalez and MacDonald, 1992), which may further reduce plasma and body ions. Thus, exercise in FW is associated with both disruption of water and ion balance. In the present study where fish were exercised to U_{max} in SW, a rapid increase in body $[Na^+]$ was observed within 8 minutes but the disturbance became significantly reduced with longer swim durations (U_{max} 47 and 87 min), although they were still significantly elevated over controls. Because body $[Na^+]$ levels increased following exercise when expressed as a function of wet weight but were not significantly different when expressed relative to dry weight, these changes must be associated with dehydration rather than ion gain. Body $[Cl^-]$ levels followed a qualitatively similar pattern. The partial recovery in body $[Na^+]$ levels with test duration may reflect partial compensation for the dehydration by increased drinking rate. Measurement of drinking rate, however, was not conducted here and would be interesting to measure in other studies.

In conclusion, U_{max} and ramped-U_{crit} protocols produce the same final swimming speed for juvenile pink salmon weighing less than 5.0 g. Whether this is a general characteristic of juvenile salmonids or other fishes requires further investigation. Given this result, U_{max} tests as short as 8 min can be used to accurately measure swimming performance in juvenile pink salmon. Such

abbreviated swim tests may prove very useful in future studies that necessarily must test large numbers of fish over a short time period to explore impacts of the environment, such as sea lice parasitism on juvenile pink salmon, or any other experiments involving juvenile fish swimming performance.

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Tables

Table 2.1 Juvenile pink salmon maximum or critical swimming speed. Body length, mass, rate of acceleration, maximum swimming speed (U_{max}) or critical swimming speed (U_{crit}) in relative and absolute terms, test duration and U_{crit} recovery ratio for each treatment group. All values are mean ± S.E. juvenile pink salmon tested with different rates of acceleration and compared with critical swimming speed (U_{crit}).

Test Group	n	Body Length (cm)	Mass (g)	Water Accel-eration ($cm\ s^{-2}$)	U_{max} or U_{crit} ($cm\ s^{-1}$)	U_{max} or U_{crit} ($BL\ s^{-1}$)	Test Length (min)	Recovery Ratio
U_{max}	8	7.15 ± 0.25	2.93 ± 0.29	0.0053	34.72 ± 2.14	4.84 ± 0.21	8	
	10	7.08 ± 0.28	2.97 ± 0.38	0.0110	37.12 ± 2.62	5.20 ± 0.20	21	
	10	6.82 ± 0.22	2.58 ± 0.27	0.0212	35.04 ± 2.18	5.11 ± 0.17	47	
	8	7.00 ± 0.16	2.90 ± 0.21	0.0531	34.97 ± 2.29	4.97 ± 0.26	87	
Ramped -U_{crit}	10	6.80 ± 0.25	2.55 ± 0.32	U_{crit1}	30.87 ± 0.83	4.54 ± 0.12	104	1.05 ± 0.05
				U_{crit2}	31.96 ± 0.71	4.70 ± 0.10	112	

Table 2.2 Juvenile pink salmon percent water content. Percent water content and percent evaporative loss (difference between live, and frozen mass) of whole body tissue (either complete, or blood and gills removed) measured in post-swim and control (not swim tested) juvenile pink salmon. All values are means ± S.E..

Test Group	Water Acceleration ($cm\ s^{-2}$)	Whole Body Tissue	n	% H_2O	% Evaporative Loss
Control		Complete	8	82.37 ± 0.72	11.04 ± 0.75
		blood and gills removed	5	81.81 ± 0.44	n/a
Ramped-U_{crit}		blood and gills removed	10	80.16 ± 0.90	n/a
U_{max}	0.0053	Complete	8	78.53 ± 1.44	7.94 ± 1.04
	0.0110	Complete	10	79.25 ± 2.02	7.81 ± 0.67
	0.0212	Complete	10	80.27 ± 1.20	6.39 ± 0.35
	0.0531	Complete	8	79.38 ± 1.48	8.23 ± 0.88

Figures

Figure 2.1 A schematic of the incremental changes in swimming speed that were used for the four acceleration tests and a ramped-U_{crit} test for juvenile pink salmon. Velocity at fatigue (mean $\pm$ S.E.) are indicated by filled (swim 1) and open (swim 2) circles. These circles also indicate the total duration of the test. No significant differences were observed between any fatigue velocities.

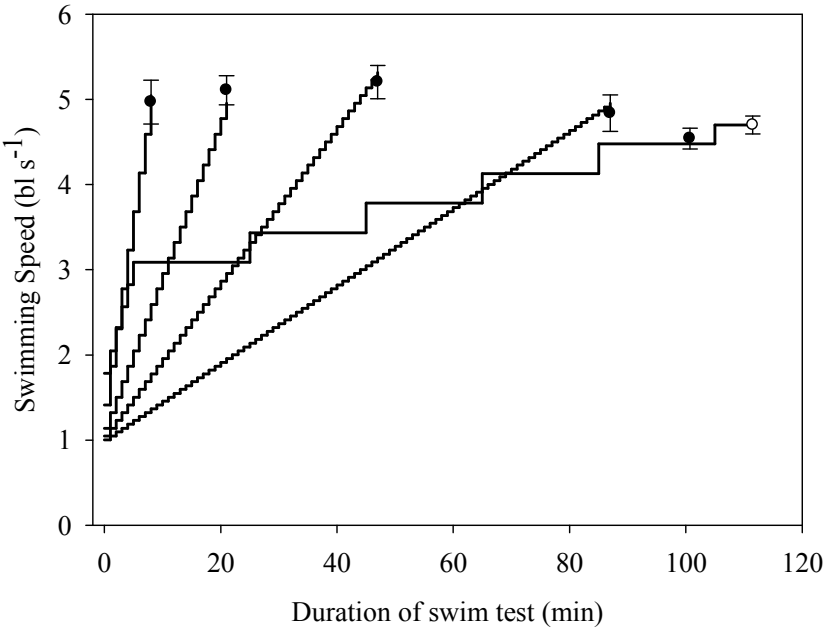


Figure 2.2 The effect of different U_{max} tests on whole body [Cl^-] (grey) and [Na^+] (white) at fatigue in juvenile pink salmon. The control group was sampled at rest. Whole body ion bars represent means $\pm$ S.E. (numbers within bars indicate sample sizes) and are presented as mmoles kg^{-1} of (a) frozen mass and (b) dehydrated mass. Different lower case (Cl^-) and upper case (Na^+) letters above bars indicate significant differences between test type ion concentrations.

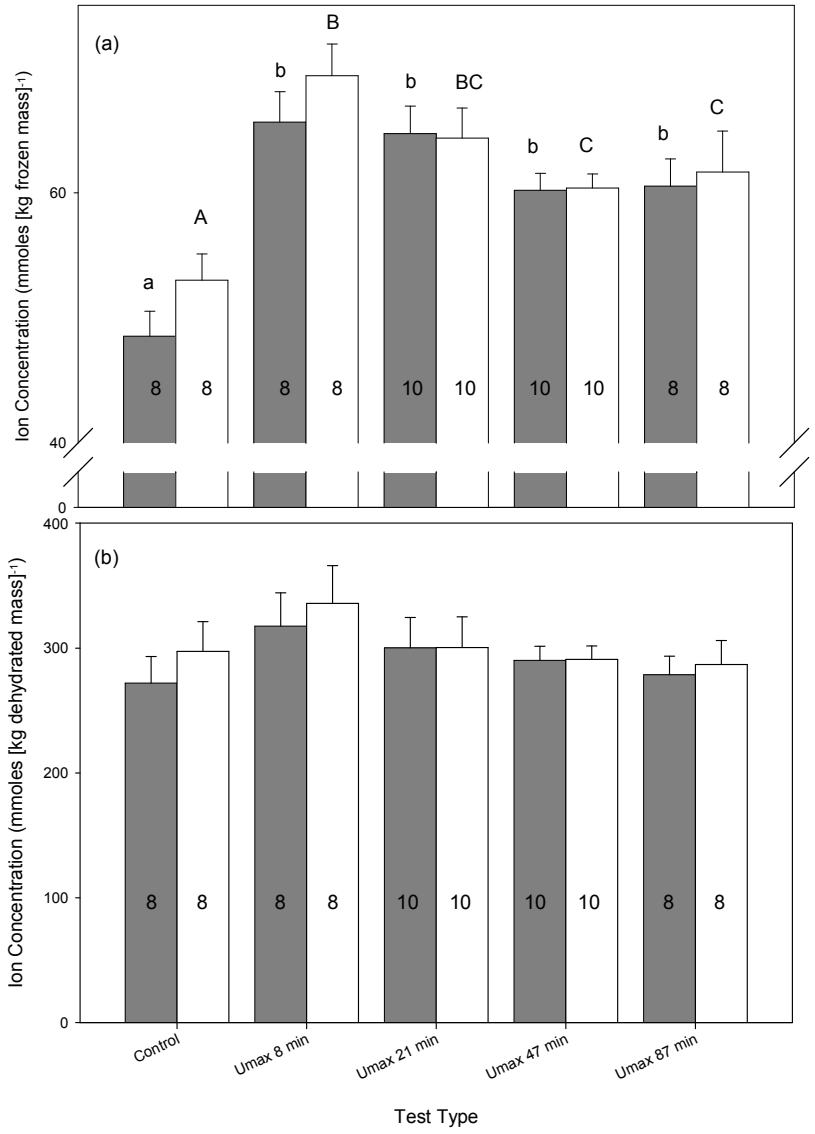


Figure 2.3 U_{crit} tests on plasma and whole body [Cl^-] (dark grey) and [Na^+] (light grey) at fatigue in juvenile pink salmon. The control group was sampled at rest. Whole body ions are presented as mmoles L^{-1} of (a) frozen mass, (b) dehydrated mass, while plasma ions (c) are presented as mequiv kg^{-1}. Bars represent means $\pm$ S.E. (numbers within bars indicate sample sizes).

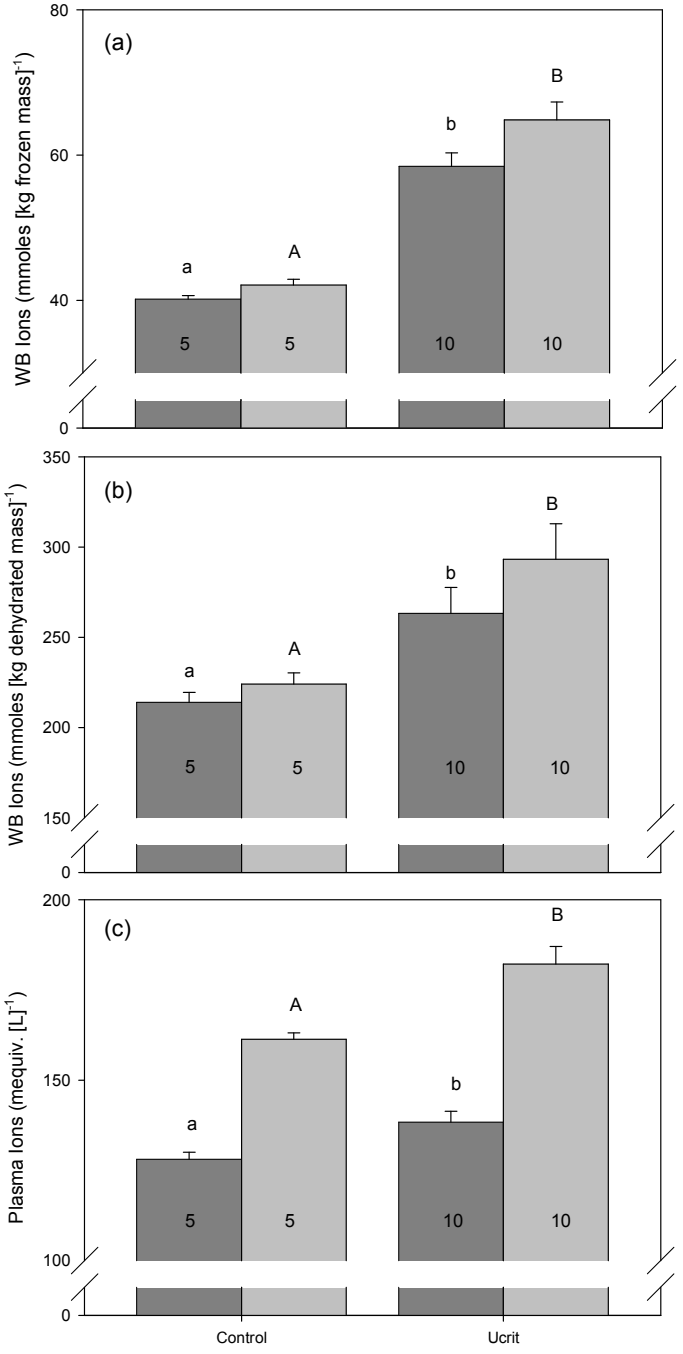
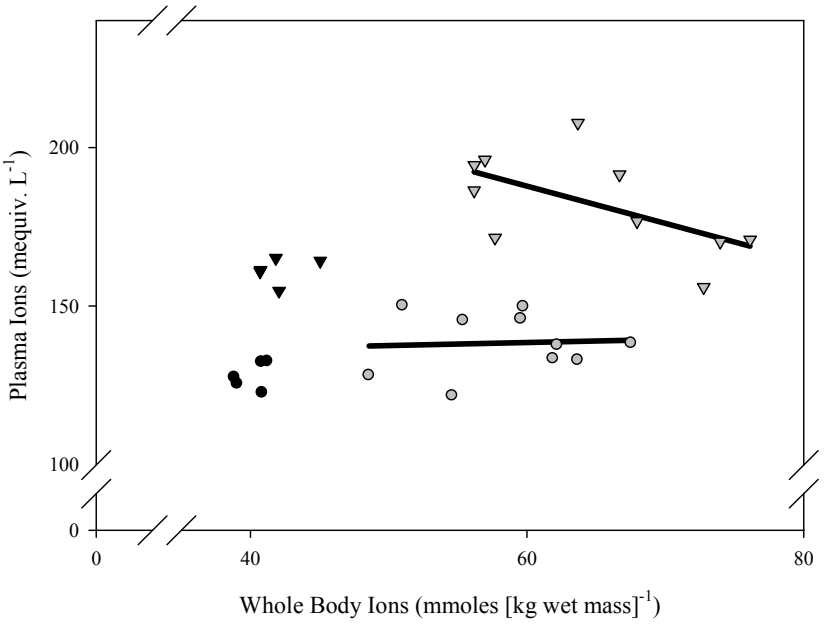


Figure 2.4 Relationship between plasma and whole body [Cl^-] (circles) and [Na^+] (inverted triangles) in control (resting, black) and ramped- U_{crit} swum fish at fatigue (grey). Data points indicate individual values.



Summary

1. Swimming performance was assessed in juvenile pink salmon (*Oncorhynchus gorbuscha* Walbaum; body mass < 5.0 g) using five different test protocols: four U_{max} tests each with a different acceleration and a repeated ramped-U_{crit} test. Regardless of the swim protocol, the final swimming speeds did not differ significantly ($P > 0.05$) among swim tests and ranged from 4.54 – 5.20 BL s^{-1}.

2. Given this finding for a small salmonid, estimates of swim performance can be accurately measured with acceleration tests lasting < 10 minutes, allowing a more rapid processing than is possible with a longer critical swim speed test.

3. Whole body and plasma [Na^+] and [Cl^-] measured at the conclusion of swim tests were significantly elevated when compared to control values ($P < 0.05$) and appear to be predominantly associated with dehydration rather than net ion gain.

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Chapter 3 : The effect of sea lice infection on swimming performance and post-exercise ion balance in juvenile pink salmon (*Oncorhynchus gorbuscha* Walbaum*)*

Introduction

For most, if not all fishes, the ability to swim is crucial for survival. This is especially true for Pacific salmonids, as they travel great distances migrating to, within, and from the ocean. Pink salmon (*Oncorhynchus gorbuscha*), a species of Pacific salmonid, start precocious outward ocean migration while they are especially small (ca. 0.2 g) (Houston, 1961). This life history contrasts with all other anadromous Pacific salmon, *Oncorhynchus* spp., (except chum salmon, *O. keta*), which reside in freshwater (FW) for 1-2 years prior to beginning their marine life phase often an order of magnitude larger.

Not only is it remarkable that pink salmon begin outward ocean migration at such a small size, but they do so without having spent appreciable time preparing physiologically for life in seawater (SW). As a result, juvenile pink salmon whole body ion concentrations virtually double following SW entry, and then decrease in the subsequent 8 to 10 weeks in association with a tripling of gill Na^+-K^+-ATPase activity (Grant *et al*., 2009). Given this life history and developmentally related ionic challenge, any further stressors are likely to be particularly problematic at this life stage. Therefore, the fact that juvenile pink salmon in the Broughton Archipelago, B.C., can acquire sea lice parasitism shortly after SW entry (Jones and Hargreaves, 2007) is a significant concern for fisheries management.

Sea lice (Copepoda: *Caligidae*) are ectoparasites, feeding on the mucus, skin (Kabata, 1974; Bjørn and Finstad, 1997) and blood (Brandal *et al.* 1976) of their host. Two sea lice species endemic to the Pacific Ocean, *Lepeophtheirus salmonis* and *Caligus clemensi*, are natural

parasites on salmon. *C. clemensi* is a generalist species, found on a wide variety of Pacific fish species, while *L. salmonis* is largely a Pacific salmonid specialist, although alternative hosts have been documented (Bruno and Stone, 1990; Jones *et al*., 2006a&b; Pert *et al*., 2006). The impact of *L. salmonis* parasitism can be behavioural, immunological and physiological in nature (reviews: Johnson and Fast, 2004; Wagner *et al*., 2008). It is alleged that wild pink salmon migrating within a certain distance of salmon farms become infected with sea lice, predominantly *L. salmonis*, resulting in levels of sea lice intensity and prevalence not seen historically in juvenile pink salmon (Morton *et al*., 2004; Brooks, 2005; Krkošek *et al*., 2007; Riddell *et al*, 2008). Within the last decade sea lice infection of juvenile wild pink salmon populations has been regularly monitored (Jones and Hargreaves, 2007; Krkošek *et al*., 2005), but estimates of sea lice prevalence on fish vary widely with year. A high in 2002 of 90% prevalence was recorded on juvenile pink salmon, but in the last four years both the prevalence and intensity of *L. salmonis* on juvenile pink salmon has seen a decline, and currently persists in the Broughton Archipelago at a prevalence of less than 10% and intensity of around 1.5 (Jones *et al*., 2009), which still may be higher than background levels (Gottesfeld *et al.,* 2009).

The number of sea lice and their developmental stage relative to the size of the fish are all likely to be important variables when considering the effects of this ectoparasite on salmonids. The sea lice intensity required to impact fish has received extensive attention, and is commonly reported in terms of lice density (sea lice g $fish^{-1}$) or load (sea lice $fish^{-1}$). Wagner *et al.* (2003) found that just 0.1 sea lice g $fish^{-1}$ caused reduced cardiac and exercise performance in adult Atlantic salmon (*Salmo salar*), while Wells *et al*. (2006) reported that 13 lice $fish^{-1}$ significantly increased plasma chloride, osmolality, glucose, lactate, and cortisol and significantly reduced haematocrit in 19-70 g brown trout (*Salmo trutta*). Comparatively little attention has been paid to

the effect of sea lice at various development stages. *Lepeophtheirus salmonis* grow from a pinhead sized infectious copepodid (<1 mm) to a mature adult (> 10 mm) in under 40 days at 10 ^{o}C (Johnson and Albright, 1991). Being the largest, it is assumed that adult lice are most harmful; however, when examining small fish, it is highly probable that sea lice could have significant impacts at less mature sea lice development stages. Regardless, a 0.2 g pink salmon that has just entered the ocean is likely at a greater risk from an ectoparasite rather than a month later when pink salmon have had time to double body mass (Grant *et al*., 2009).

In terms of the lethal effects of sea lice, just two motile sea lice have been reported to be lethal to juvenile pink salmon that inhabit the near shore environment (size not given, but likely < 3 g; Krkošek *et al*., 2006). Jones *et al*. (2008) report that sensitivity to sea lice is reduced in fish greater than 0.7 g in pink salmon. While data on lethal effects are important and have received much attention by the Canadian popular media, sub-lethal effects are likely to be of great significance in nature since they can incapacitate a fish's ability to survive. For example, depressed swimming performance may increase the likelihood of natural predation and reduce foraging efficiency. *L. salmonis* has already been shown to disturb ionic homeostasis (Wootten *et al*., 1982; Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1997) and swimming performance (Wagner *et al*. 2003, 2004; Wagner and McKinley, 2004) of salmon. Bjørn and Finstad (1997) reported that after the first appearance of pre-adult stage *L. salmonis*, artificially infected brown trout post-smolts developed severe osmoregulatory problems and anaemia; plasma chloride levels increased and haematocrit levels decreased significantly in the infected compared to the uninfected, control fish. Wagner *et al*. (2003) found that *L. salmonis* infected adult Atlantic salmon had altered cardiac performance and a 19-22% reduction in U_{crit} and elevated plasma chloride levels. No studies of ionic homeostasis or swimming performance, however, have used

juvenile pink salmon infected at their earliest life stage in SW, when they are likely to be more susceptible because of their small body size and their incomplete preparedness for ionoregulation in SW.

Therefore, the purpose of the present study was to determine for the first time the level of *L. salmonis* infection required to compromise the swimming ability and ionic homeostasis of 0.3-3.0 g pink salmon. This was achieved by measuring the maximum swimming (U_{max}) performance and the post-swim body [Na^+] and [Cl^-] of sea lice infected fish, and comparing these data with results for uninfected, control fish. Independent experiments were performed with different levels of lice intensity (1-4 lice per fish) and with different developmental life stages of sea lice (chalimus 1 - adult). A priori, my working hypothesis was that the tissue damage caused by the sea louse would cause an ionoregulatory disturbance as indicated by a change in whole body [Na^+] and [Cl^-]. Disruption to ionic homeostasis due to damage by sea lice may directly affect muscle contractility (Brauner *et al*., 1992, 1994) or force energy to be re-allocated to this system, reducing aerobic scope and limiting swimming performance. In addition, it was anticipated that the disruption to ionoregulation and swimming performance would be directly dependent on lice stage and on lice intensity, and inversely related to fish size. Ultimately such changes could affect the fish's ability to swim within a school, acquire food, avoid predation and successfully migrate to the open ocean.

Materials and Methods

All fish were collected in the Broughton Archipelago, British Columbia, Canada (Fig. 3.1) between March and July, 2008. Experiments were conducted at a field laboratory constructed in a float house located at Dr. Islets, along Knights Inlet, a major migratory corridor for numerous

salmonid species, including pink salmon (Fig. 3.1). Fish were captured both in FW (river-caught, RC-fish) and SW (ocean-caught, OC-fish) to maximize the use of resources and the amount of research that could be conducted during a limited time frame when fish were in the region and were < 3 g. The multiple factors that constrained the experimental design included:

1) Juvenile pink salmon double in mass every month (Grant *et al.,* 2009), requiring that experiments be conducted over a 3-month time span so as to focus on 0.3 g – 3.0 g fish.
2) A reliable source of sea lice infected OC-fish that were less than 1 g was impossible to obtain. Instead, experiments on the smallest salmon used controlled, artificial infections of RC-fish that were transferred into SW on-site. Consequently, the exact timing and extent of sea lice infection was known for the RC-fish.
3) Gravid sea lice taken from wild fish was not a reliable source to culture lice for the artificial infections because, during the spring of 2008, the prevalence of adult *L. salmonis* was extremely low (< 1%) among several thousand fish that were captured in the near shore locations up to 20 km from Dr. Islets. Instead, gravid sea lice were collected opportunistically from a harvest of adult Atlantic salmon at a nearby fish farm. Even so, the low abundance of gravid sea lice on these fish meant that there were only sufficient sea lice for 2 artificial infection trials early in the field season.
4) Juvenile salmon shed *L. salmonis* in a laboratory environment (Connors *et al*., 2008; Jones *et al.,* 2008). As a result, OC-fish were collected only when needed for experiments and were not held in the lab for more than 7 days before being used. The intensity and stage of sea lice at the time of the swimming experiment is reported. However, extent and duration of sea lice infection prior to capture was unknown and, as expected, the intensity of sea lice infection of OC-fish typically decreased from its initial level during

holding. The RC-fish were held for up to 28 days post-infection (DPI) to include the first motile louse stage (DPI 26-28). During this period, sea lice intensity and development were monitored, and the intensity of sea lice infection typically decreased from its initial level over time. Again, the level of sea lice infection at the time of the swimming experiment is reported.

5) The two sets of results for the OC- and RC-fish were analysed separately even though the size ranges overlapped. This approach was adopted primarily because the infection history of the RC-fish was known, whereas that of the OC-fish was not, i.e., a fish could have had a higher/lower infection intensity but shed/gained some lice before or during capture.

Captured fish were housed in 60 L aquaria, with flow-through, aerated, full-strength SW pumped from a depth of 30 m below the float house at the field site. SW temperature at this depth ranged from 7.0 ^{o}C in late March to 8.5 ^{o}C in late June. Fish were fed up to twice daily with a commercial mash (Bio-Vita starter feed; Bio-Oregon, Longview, WA).

River-Caught (RC) Fish

Approximately 2,000 fish were caught on March 27, 2008 using a screw trap in place for fish enumeration on the Glendale River which drains into Knights Inlet, the largest fjord in the Broughton Archipelago (Fig. 3.1). The Glendale stock of pink salmon, which comes from an enhanced rearing system, is the largest contributor to the Broughton Archipelago pink salmon population. In odd years Glendale contributes 89% of the total number of pink salmon, while in even years it contributes 39% (Brooks and Jones, 2008). The purpose of collecting fish from FW

rather than SW was two-fold. First, fish would be guaranteed to be free of *L. salmonis* at the outset of the study as this louse species cannot survive FW (Connors *et al*., 2008). This assured that artificial infection with *L. salmonis* would be a novel challenge and that the control fish had never been infected. Second, it provided a supply of earliest life stage fish (ca. 0.2 g) in SW. At time of capture, fish were actively migrating downstream, so it was assumed fish of this size could be found in SW at time of capture. These RC-fish were transported in FW by boat to the Dr. Islets field station where they were gradually introduced to SW over a 12 h period. Swimming ability was tested over a period of 36 days using a total of 146 RC-fish, of which 83 fish were infected and represented 3 – 29 DPI. The number of sea lice infecting a fish ranged from 1 to 4. Fish with 4 lice (n = 2) were pooled with fish with 3 lice (n = 24) to improve analytical power, and this group is collectively referred to as fish with 3+ lice. The reported number and development stage for the sea lice were those recorded at the time of the swim test and ranged from chalimus 1 (the first attached lice stage), to pre-adult 1 (the first motile lice stage). More advanced levels of sea lice maturity were not tested as motile lice infections could not be maintained in sufficient numbers in the laboratory (see also Connors *et al*., 2008)

Ocean-Caught (OC) Fish

Fish were collected, as needed, from the shoreline by beach or purse seine throughout April, May and June, 2008 (Fig. 3.1). Because of the low prevalence of *L. salmonis* on OC-fish, captured fish were graded and excess uninfected fish were released immediately. Of the over 10,000 OC-fish graded during these 3 months, infection prevalence averaged 10%, with an intensity of about 1. Captured fish were transported in aerated SW to the Dr. Islets field station, where they were held at least 16 h prior to further handling in an effort to minimize stress. Fish

were then sorted according to lice infection intensity and lice development stage and used for experiments within 7 days. The swimming performance experiments reported here were performed over a 49-day period using 214 OC-fish, of which 166 were infected with 1 – 4 sea lice. Again, the fish infected with 4 lice (n = 6) were pooled with fish infected with 3 lice (n = 14) and this group is collectively referred to as fish with 3+ lice. Lice stages were grouped as either early chalimus (chalimus 1 and 2), or late chalimus (chalimus 3 and 4) or motile (pre-adult and adult) because infections were not controlled on OC-fish, and different but close lice stages were common on individual fish.

Gravid Lice Collection

Adult gravid *L. salmonis* were collected from adult Atlantic salmon (*Salmo salar* L.) that were being harvested at Wicklow Point fish farm on March 24, 2008 (Fig. 3.1). Lice were transported in aerated SW to Dr. Islet's field site, where egg strings were removed from lice. Egg strings were split into two groups according to maturity. Each group was held in a 4 L closed container of full strength, aerated SW. Progression from hatching to naupliar stages to infectious copepodid stage was monitored daily and once the infectious copepodid stage became dominant, artificial infections were carried out.

Artificial Sea Lice Infection

Artificial infections were conducted at Dr. Islets field station on April 3 and 13, 2008. Naïve, SW-acclimated RC-fish were exposed to ca. 23 copepodids $fish^{-1}$ in a static, aerated 2 L SW infection bath for 4 h. Of 581 fish exposed to sea lice, 511 were successfully infected (prevalence = 88%). Infected fish were allowed to recover for 24 h before being sorted by

infection intensity (1, 2, 3, and 4+ lice) into holding bins outfitted with flow-through SW (as above). Lice development on the infected fish was monitored daily so that swim tests could be performed at pre-determined lice development stages.

Sham-treated (n = 150) and untreated control RC-fish (n = 30) were similarly maintained and tested. Sham fish were subjected to the same infection protocol as infected fish, but the infection bath contained no sea lice (SW filtered through 60 μm nitex mesh). Control fish were not exposed to any kind of infection protocol.

Swimming Tests

Swimming performance tests in SW were all performed during April, May and June, 2008. A total of 146 RC-fish (mean mass = 0.34 $\pm$ 0.02 g) and 214 OC-fish (mean mass = 1.14 $\pm$ 0.05 g) were subjected to a repeated maximum swimming (U_{max}) performance test.

Duplicate Blazka-type (Blazka, 1960) swim tunnels (Loligo Systems, mini swim tunnel) allowed two fish to be individually but simultaneously tested. The swim chamber was 26.4 mm in diameter and 100.0 mm in length, and had plastic grids at either end to facilitate laminar water flow and keep fish away from the propeller mechanism that produced the flow. Water velocities (cm s^{-1}) were calibrated using frame-by-frame video analysis (30 frames s^{-1}) of neutrally buoyant particles moving through the swim chamber. Correction for solid blocking was not required as fish solid blocking accounted for < 10% of the cross sectional area of the tunnel (Bell and Terhune, 1970). Average water temperature was controlled to within 1.0 ^{o}C of the ambient temperature. Fish were lightly anaesthetised with MS-222 (0.05 g L^{-1}) for transfer to the swim tunnel and for quantification of sea lice number and developmental stage.

The repeated maximum swimming speed (U_{max}) protocol followed that of Nendick *et al*. (2009, chapter 2), with minor modifications. Briefly, fish recovered immediately from the light anaesthesia (< 1 min) and were left for a further 5 - 10 min before water velocity was increased from 0 - 1.7 cm s^{-1}. At this speed, fish oriented themselves toward the current, swam with minimal effort and were left for 60 min to habituate to the tunnel environment before starting a repeated-U_{max} test. The water was then accelerated at a rate of 0.05 cm s^{-2} in 1.4 cm s^{-1} increments every 30 s until the fish fatigued. Fatigue was achieved when the fish could no longer maintain its position in the swim tunnel and drifted back so its caudal fin rested on the posterior grid and the fish did not move when lightly prodded. After fatigue, fish were left to recover for 30 min at a velocity of 1.7 cm s^{-1} before the swim test was repeated using an identical protocol. By swimming fish twice with only a short recovery period (a repeated swim test; Jain *et al*., 1998), the expectation was that any physiological disturbance accrued in the first swim would be exacerbated in the second one and hamper performance (Tierney and Farrell, 2004). Recovery ratio was determined according to Jain *et al*. (1998) by expressing the 2^{nd} U_{max} value as a ration of the 1^{st} value. Very occasionally (6 of 360 tests), fish rested on the grid without appearing fatigued and refused to move. These fish were excluded from the analysis.

The water velocity was stopped as soon as a fish fatigued during the second test to allow immediate fish removal and euthanization (an overdose of 0.2 g L^{-1} MS-222) prior to tissue sampling. Fish were then rinsed in FW to remove any SW on the skin surface, patted dry and fork length (BL) measured. Sea lice were removed, assessed for development stage using sizes reported by Johnson and Albright (1991), and preserved in 10% ethanol. Body mass was measured when weather permitted (the floating field laboratory prevented the electronic scale from being tared due to waves).

Body Ion Analyses

Blood and 2-3 gill arches were removed for analysis (data not presented). The remainder of the body was wrapped in tinfoil and flash frozen in liquid nitrogen. For tissue ion analysis, fish were weighed, defrosted and cut in half (from pelvic fin to dorsal fin), and the posterior portion was placed in a pre-weighed falcon tube (15 or 50 mL). By analysing only the posterior portion of the body, minor but variable tissue loss due to gill removal was controlled. Tissue samples were dried in an oven (65 ^{o}C) until a constant dry mass was obtained and then digested in 1 N nitric acid (with a maximum dilution factor of 60 for mass:volume) with daily degassing and vortexing to facilitate tissue digestion. The tissue digests were centrifuged and the clear supernatant was removed and stored at -4 ^{o}C until further analysis (Grant *et al*., 2009). Duplicate 10 μL supernatant samples were used to measure whole body [Cl^-] with a Digital Chloridometer (Haake Buchler Instruments Inc., Saddlebrook, NJ, U.S.A.). Whole body [Na^+] was measured in duplicate using a flame atomic absorption spectrometer (Spectra AA; Varian, Victoria, Australia).

The experimental protocols were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council on Animal Care.

Statistical Analyses

Body length and mass, body ions concentrations, and maximum swimming speed of control and infected RC-fish and OC-fish were analyzed for statistical differences using a one-way ANOVA with Holm-Sidak post-hoc test ($p < 0.05$). Recovery ratio was analysed for statistical significance using a t-test. Swimming speeds are reported in relative terms as body

lengths per second (BL s^{-1}). A linear model was designed using R software to examine the relative contributions of measured variables to U_{max} performance.

The progression of time, fish growth, DPI (RC-fish only), days post-SW transfer (RC-fish only) and sea lice development were known variables that occurred concurrently and therefore are somewhat interchangeable. Data of RC-fish are presented according to sea lice development stage (i.e. chalimus 1-4, or pre-adult) (Fig 3.4 and 3.5). Control fish, by definition, were not infected with sea lice but were time matched with infected fish which were grouped by sea lice development stage. This could be done because all RC-fish had a common date of capture, SW transfer, and artificial infection. This was not the case for OC-fish, because they were collected, as needed, over a period of about 3 months at varying locations. As such, only fish growth could be used as a common reference point for infected and control OC-fish. Consequently, OC-fish data are plotted as a function of fish fork length (Fig. 3.6 and 3.7). I acknowledge this method is not without flaws, but given that both infected and control OC-fish grew at the same rate (Fig. 3.2), I feel this is the best method for analyses without misleading the reader.

Results

Control Fish

Sham and control RC-fish did not differ significantly in U_{max} swimming performance (mean U_{max}: 5.38 $\pm$ 0.13 and 5.05 $\pm$ 0.11 BL s^{-1}, respectively) nor in repeat swim performance (mean recovery ratio: 0.96 $\pm$ 0.02 and 1.00 $\pm$ 0.03, respectively). As a result, sham and control fish were pooled to increase the statistical power for comparison to infected RC-fish.

Control RC- and OC-fish recovery ratios were not significantly different from 1.0 nor from each other ($P < 0.05$, mean recovery ratio: 0.98 ± 0.02 and 0.99 ± 0.02, respectively), indicating that all control fish were healthy (Jain *et al.,* 1998) and responded similarly to the testing protocol. Relative U_{max} swimming speed decreased with increasing body length (Fig. 3.3), in agreement with Brett (1964). Therefore, it was not surprising that the mean U_{max} performance of control OC-fish was significantly slower than that of control RC-fish ($P < 0.05$), given that OC-fish were significantly larger than RC-fish ($P < 0.05$).

River-Caught (RC) Fish

Following *L. salmonis* (sea lice) artificial infection, RC-fish (N = 146) were assessed for swimming performance between 3 and 29 DPI to determine the effect of a range of sea lice development stages. During this time, both sea lice and fish grew concurrently, with sea lice developing on fish from chalimus 1 to pre-adult 1 and fish growing significantly from 34 to 41 mm in length, (Table 3.1, $P < 0.05$). Mortality was observed in low numbers (< 5%) but was not quantified specifically.

The mean recovery ratio of control RC-fish did not differ from unity ($P < 0.05$). In contrast, RC-fish infected with sea lice had a mean recovery ratio significantly lower than control RC-fish and lower than unity (ratio = 0.91 ± 0.017, $P < 0.05$). U_{max} performance of control RC-fish did not change significantly over the course of experiments (Fig 3.4, $P > 0.05$). U_{max} performance of control RC-fish did not differ from that of RC-fish infected with sea lice of development stage chalimus 1 and 2, which were the youngest sea lice stages tested (3 DPI to 13 DPI) (Fig. 3.4, $P > 0.05$). Compared to control RC-fish (Fig 3.4, filled circles) U_{max} performance of infected fish (Fig 3.4, open symbols) was first significantly declined (20.4%) around 14 DPI,

when lice had developed to chalimus 3 (Fig 3.4). U_{max} remained depressed by 26.5% and 37.9% at subsequent sea lice stages of chalimus 4 and pre-adult 1, respectively, when compared to control ($P < 0.05$), but only at the pre-adult 1 stage was U_{max} performance significantly lower than the chalimus 3 stage performance. Therefore, U_{max} did not decrease step-wise with successive sea lice development stages and was insensitive up to chalimus 2 stage.

Analysis of sea lice number and sea lice development stage combined (Fig. 3.4, dashed lines) revealed no significant effect on RC-fish infected with chalimus 1 and 2 sea lice, independent of lice number. As expected, chalimus 3, 4 and preadult-1 sea lice significantly decreased U_{max} performance with all lice intensities, but surprisingly swimming performance at these development stages was independent of lice intensity, with the exception of the chalimus 4, where 3+ sea lice were significantly more detrimental to U_{max} performance than lesser intensities (Fig 3.4).

Concentrations of body Na^+ and Cl^- were measured in all control and sea lice infected RC-fish immediately after the completion of U_{max} tests. In all cases, infected RC-fish had elevated body [Na^+] and [Cl^-] when compared with time-matched control fish ($P < 0.05$), suggesting that a sea lice infection caused additional ionic loading post-swim (Fig. 3.5). However, the percentage increase in body ions of infected RC-fish was independent of lice stage; approximately 25% for [Na^+] (range: 23 – 28%) and 28% for [Cl^-] (range: 22 – 32%), with the exception of [Na^+] at the chalimus 2 stage (36%). Anticipated low statistical power prevented analysis of [Na^+] and [Cl^-] data by lice number and stage combined.

Based on data from resting juvenile pink salmon (Grant *et al.,* 2009), body [Na^+] and [Cl^-] were expected to decrease with fish growth/time (as indicated by lice stage in Fig. 3.5). While a trend was apparent within control and infected RC-fish, it was not statistically significant,

possibly either because tissues were sampled post-swim or because of the relatively narrow time window over which experiments were conducted relative to that of Grant *et al*. (2009).

The linear model considered fish mass and length, and post-swim body [Na^+] and [Cl^-], along with lice number and stage as factors influencing swimming performance. Fish size and post-swim ions had no significant effect on swimming performance, but lice number and stage did have a significant effect. Interestingly, preadult-1 was the only development stage that significantly impacted U_{max} independent of lice load ($P < 0.05$). Chalimus 1 was the only stage that had no effect on swimming performance, at any sea lice intensity. Chalimus 2, 3, and 4 stages all affected U_{max}, depending on the number of lice infecting the fish. Therefore, in terms of sea lice effects, the linear model is more conservative than the ANOVA in some, but not all respects.

Ocean-Caught (OC) Fish

For OC-fish, U_{max} performance was assessed during a 49-day period ($N = 214$). Fish growth (fork length) plotted against U_{max} revealed that both infected and control OC-fish U_{max} performance was affected by fish size (Fig. 3.6, $P < 0.05$).

The mean recovery ratio of control OC-fish did not differ from unity; however, the mean recovery ratio was significantly lower for infected OC-fish when compared to control (ratio = 0.94 ± 0.011, $P<0.05$). Interestingly, U_{max} was unchanged in fish infected with sea lice at all stages, when compared to control (Fig. 3.6).

Due to the rapid turnover of OC-fish in the laboratory, fish never spent more than a week in holding tanks. Consequently, mortality rates were low, like RC-fish, but mortality was not directly quantified.

OC-fish (mean mass of 1.1 g) were on average greater in size than that previously reported when ionic steady state is regained in SW (> 0.8 g, ca. 50 mm, Grant *et al.* 2009). As with swimming performance analyses, OC-fish [Na^+] and [Cl^-] data (Fig. 3.7) were plotted as a function of fish size (fork length). Contrary to expectations, post-swim [Na^+] and [Cl^-] for sea lice infected fish and post-swim [Na^+] for control fish declined with time ($P < 0.05$), suggesting that these fish were not yet in ionic homeostasis.

When lice number and stage, fish weight and length, and body [Na^+] and [Cl^-] were all held constant in a linear model examining the effect of these factors on swimming performance, the only factor significantly contributing to U_{max} was 3+ lice at motile stage ($P < 0.05$). Interestingly, in this model, fish length did not reach a state of significance as a contributor to U_{max} performance ($P = 0.09$).

Discussion

This study examined for the first time the sub-lethal physiological effects of *L. salmonis* infection on pink salmon less than 3.0 g, with parasitic intensities from 1-4 and sea lice developmental stages from chalimus 1 to adult. By using a large number of fish (n >350) the effects of lice intensity and developmental stage could be systematically teased out in terms of their effects on swimming performance and ion homeostasis. While only limited mortality was observed in this study, a large significant reduction in swimming performance was observed in artificially infected RC-fish that was independent of intensity, but affected by lice stage; up to a 38% reduction in U_{max} was observed in RC-fish infected with pre-adult sea lice. Whole body [Na^+] and [Cl^-] levels were also significantly elevated in infected RC-fish. In OC-fish, which were larger than RC-fish, no effect of sea lice on U_{max} or body ion levels was observed.

Logistically these studies were difficult to carry out in part because of the large number of fish required to control variables important to a parasite-host relationship, such as lice intensity, lice stage and fish size. Here, it was necessary to use two sources of fish to ensure sufficient numbers and even then, the ability of laboratory-held pink salmon to rid themselves of sea lice precluded examination of large numbers of fish with lice intensities greater than 3. Although the testing procedures were identical for RC-fish and OC-fish, the possibility that life history differed prevented the groups from being directly compared. While the natal stream of OC-fish is likely the same as RC-fish, since Glendale River is the largest producer of pink salmon in the area, I cannot say with certainty the origin of OC-fish, especially given that this study occurred in an even year (2008), when the Glendale River contributes 39% to the overall Broughton Archipelago population, rather than 89% in an odd year (Jones and Hargreaves, 2007). Furthermore, SW entry date, number of days infected with lice and number of occasions exposed to infectious sea lice were all controlled with RC-fish, but unknown for OC-fish because they were seined randomly, both spatially and temporally from the ocean. OC-fish were also on average larger than RC-fish.

Although I expected the strong relationship between fish size and U_{max} for the combined data set of control OC- and RC-fish (Fig. 3.3), the mean fish mass for RC-fish and OC-fish differed significantly and so it was not surprising that RC-fish swam relatively faster than OC-fish, given that small fish swim relatively faster than big fish, but absolutely slower (Brett and Glass, 1973). In spite of these differences, control RC- and OC-fish responded similarly to repeated-U_{max} tests. Both control groups had recovery ratios that were not different from 1.0, indicating that they fully recovered from the initial swim trial. However, this was not the case for infected RC- and OC-fish, which had significantly lower recovery ratios of 0.91 and 0.94,

respectively, when compared to control fish ($P<0.05$). In addition, infected RC-fish responded slightly differently to the swimming challenge compared with OC-fish, differences that are discussed below.

Given the reported lethal effects of sea lice, with just 2 motile sea lice killing juvenile pink salmon (Krkošek *et al.,* 2006), I was surprised at the low fish mortality rate during this study. Some of the initial lice infection densities were extreme in this study, with densities up to 30 lice g^{-1} being achieved via artificial infection. Less than 5 % mortality was observed for the duration of both RC and OC-fish experiments, much lower than the authors expected based upon previous studies (Krkošek *et al*., 2007; Morton and Routledge, 2005). In fact, rather than fish mortality, the major constraint in our study was the ability of juvenile pink salmon to shed sea lice. Here, pink salmon, even at ca. 0.2 g, were able rid themselves of sea lice in a laboratory environment, as reported in other studies (Jones *et al*., 2007; Connors *et al*., 2008). The method by which sea lice are shed is unclear and beyond the scope of this study. What is relevant, however, is that in the laboratory *L. salmonis* did not, for the most part, kill pink salmon, over periods of up to 28 DPI and with a lice intensity of up to 3 lice whether they were naïve RC-fish or infected OC-fish. This discrepancy relative to some earlier reports may be due to the very early developmental stages of the sea lice used in this study (pre-adult and early motile), the relatively short infection period used in this study (about 30-40 days for a motile *L. salmonis* to develop on a pink salmon), or the quality of our fish holding conditions that minimized additional stress to the fish which is of significance as stressors are often additive or synergistic.

River-Caught (RC) Fish

By artificially infecting naïve RC-fish with sea lice and subsequently testing swimming ability daily as sea lice and fish grew concurrently, I revealed that even one pre-adult sea louse could have a negative sub-lethal impact on the smallest of pink salmon in SW. RC-fish first decreased U_{max} with the chalimus 3 life stage. Surprisingly, there was no significant additive effect of increasing lice number within a specific development stage until fish were infected with 3+ lice at the chalimus 4 stage. Because fish shed lice, only two fish were tested with 3+ motile lice, thus I cannot say with certainty whether increasing lice intensity at the motile stage has an additive negative effect on U_{max}, and thus, further testing is required at this lice load. Even though the environmental relevance of 3+ lice intensities to the Broughton Archipelago may be questionable given that in recent field sampling the mode for lice intensity is now less than 2 sea lice $fish^{-1}$, data to confirm the necessity to maintain this relatively low level of intensity would be supremely useful and powerful to fisheries management.

The absence of a clear additive detrimental effect of sea lice on U_{max} suggests that additional drag by the louse on the body surface was not the principle problem since each additional louse would be expected to physically slow the fish to a progressively greater degree, perhaps exponentially. This does not exclude the possibility, however, that the impact on swimming performance was a result of the ectoparasite compromising ionoregulatory homeostasis by disrupting the skin epithelial barrier to salt entry and water loss. Indeed, it has been shown that in coho salmon parr, an elevation in resting plasma ion levels results in a subsequent impairment in swimming performance (Brauner *et al*., 1992) and that a reduced hypo-osmoregulatory ability in smolts transferred to seawater is associated with an impairment in swimming performance (Brauner *et al.,* 1994).

Sea lice infection significantly elevated whole body [Na^+] and [Cl^-], indicating that sea lice, independent of number and life stage, do have an impact on ionoregulatory capacity for the smallest juvenile pink salmon in SW, which has yet to develop scales (Jones *et al*., 2008). While these results collectively suggest that this ectoparasite likely compromises the protective external skin barrier, the level of ionic disturbance for both [Na^+] and [Cl^-] for the most part did not change with sea lice intensity or development. This result would contradict with the above suggestion unless the ionoregulatory challenge of swimming masks the additive effect of more and/or larger sea lice on routine ionic status. When fish swim in SW they take on ions and lose water as a result of the osmorespiratory compromise (Sardella and Brauner, 2007), independent of any disruption to the integrity of the skin. Even so, it must be remembered that fish with more developed and greater lice intensities tended to have a lower U_{max}. This result is then consistent with my suggestion that maintenance of ionic balance comes at a cost to swimming performance, with U_{max} diminishing in the face of increasing parasitic load in an effort to keep the concentration of body ions below some upper threshold.

Grant *et al*. (2009) found that immediately after SW transfer, Glendale River pink salmon had significantly elevated [Na^+] and [Cl^-], but ions decreased over time until at ~10 weeks post-SW transfer both [Na^+] and [Cl^-] levelled out at ~50mmol.kg wet $mass^{-1}$. Given that RC-fish had been transferred to SW for only 10 days, the absence of significant declines in post-swim body [Na^+] and [Cl^-] was surprising. Again, the challenge of swimming may have masked this effect. The lower body [Na^+] and [Cl^-] values obtained post-swim in this study compared with the routine values reported in Grant *et al*. (2009) for similarly sized fish probably reflects the partial sampling of body parts in the present study. Grant *et al.* (2009) used the entire fish, whereas only a posterior chunk, predominantly made up of muscle, was used here.

Ocean-Caught (OC) Fish

There was no control over the intensity and developmental stage of lice infecting OC-fish because the fish had been exposed to sea lice in the ocean for an unknown duration and often on multiple occasions, as indicated by different lice stages on a single fish. It is possible that fish may be better able to handle chronic low intensity exposures, where the parasite load increase is gradual, relative to a single "pulse" of infection, where the load is applied all at once. The "pulse" infection used with our artificial infections could be an additional explanation of why lice impacts were greater in RC-fish that OC-fish.

A more likely explanation for this difference is that OC-fish were considerably larger than RC-fish at the time of testing and so a priori the effects of a given sea lice intensity were expected to be diminished in the former for several reasons. If sea lice size is held constant, any drag effects on swimming would be reduced proportionally to fish size. The lower surface area to volume ratio of larger fish would proportionally reduce the effect of disruption of the skin barrier to ion and water exchange. Also, juvenile pink salmon develop scales on their skin when they reach 0.7 g (Jones *et al*., 2008), which might reduce the impact of parasitic feeding on the skin. While previous reports have indicated impacts of sea lice on pink salmon >1.0 g, here U_{max} performance for OC-fish (1.1 $\pm$ 0.3 g) was unchanged as a result of sea lice infection. The very nature of these fish being ocean-caught was somewhat problematic for data analyses in that the only common ground in which to compare control and infected OC-fish was fish size. Therefore, OC-fish could not be analysed as a function of sea lice development stage, as was done for RC-fish. This prevented direct comparisons to RC-fish. However, the linear model applied to both

data sets indicated that OC-fish U_{max} was not impacted by sea lice infection to the same degree as RC-fish.

The average OC-fish mass in this study was well over that at which Grant *et al.* (2009) reported ionic steady state in resting fish, thus the assumption was made that control OC-fish would have stable ion concentrations over time. This does not appear to be the case, however, as significant declines in post-swim body [Na^+], but not [Cl^-], were observed in control and infected fish when expressed relative to fish fork length. It is also somewhat surprising that sea lice infection was not associated with ion concentrations elevated over control, as was seen in RC-fish. This is possibly explained by the development of scales in pink salmon >0.7 g which may have helped defend against ionic disturbance.

In conclusion, swimming performance in artificially infected RC-fish was not significantly affected by sea lice intensity (range 1-4) but was affected by lice stage. RC-fish infected with chalimus 3 to pre-adult sea lice, exhibited a 20 to 38% reduction in U_{max}, respectively, which was associated with some degree of ionoregulatory disturbance. Similar infection intensity and lice stages in the larger OC-fish infected in the wild did not exhibit a reduction in U_{max} or disturbances to whole body ion levels. While the history of infection is unknown in OC-fish, the lack of effect of sea lice may in part be related to the size of these fish, which were on average close to 4-times larger than RC-fish. Although only limited mortality was observed in this study, swimming ability is likely an indirect indicator of lifetime fitness and here I reported that swimming performance was affected in the smaller RC-fish when infected with sea lice. I hope the results from this study will be useful in forthcoming management strategies of aquaculture and wild salmon in the area of the Broughton Archipelago.

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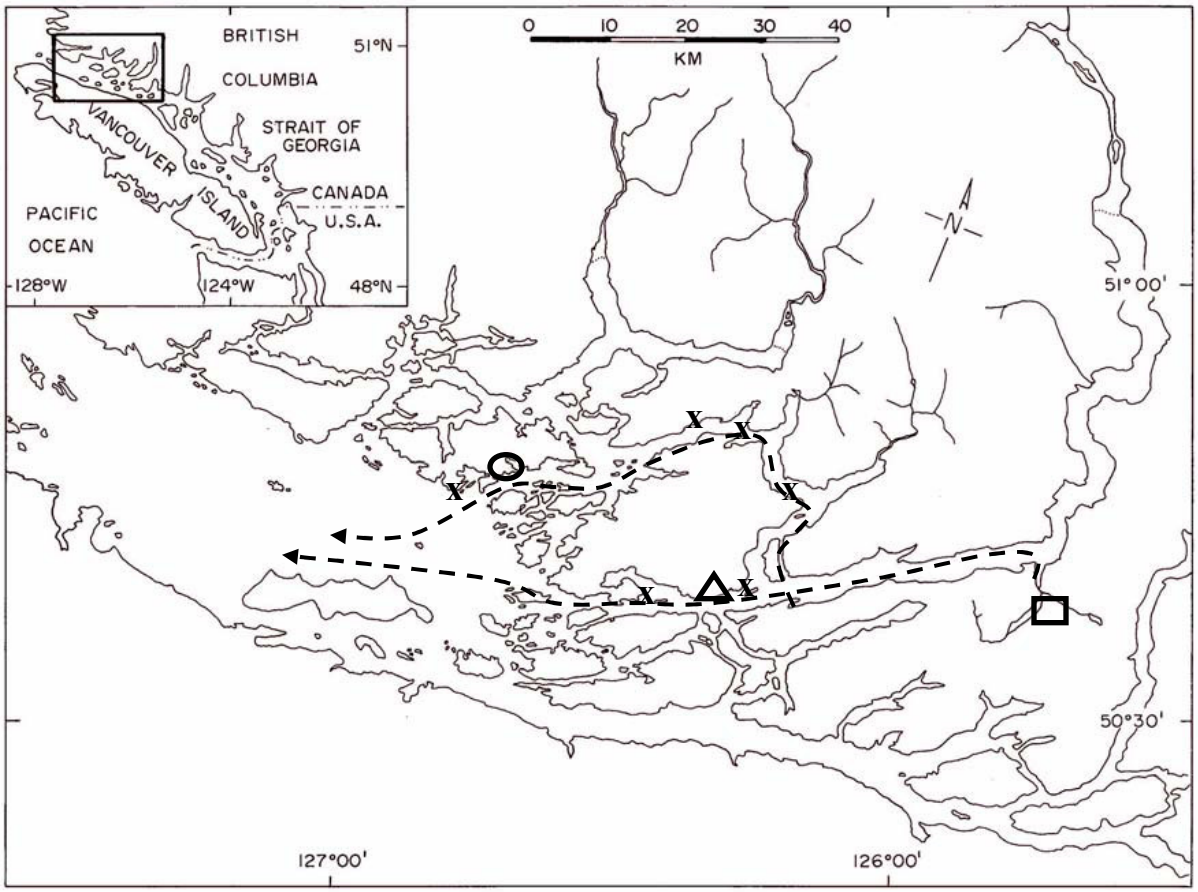
Tables

Table 3.1 Concurrent progression of development of sea lice and RC-fish following artificial infection. The time at which sea lice development stage was observed is reported as day post sea lice infection (DPI). Differing letters represent significant differences among fish lengths ($P<0.05$).

Lice Development Stage	DPI Stage Observed	n	Fork Length (mm + S.E.)
Chalimus 1	3	20	34.17 ± 0.64 a
Chalimus 2	7	32	35.03 ± 0.40 ab
Chalimus 3	14	29	36.44 ± 0.71 b
Chalimus 4	19	36	38.40 ± 0.60 c
pre-adult 1	26	20	40.82 ± 0.50 d

Figures

Figure 3.1 Broughton Archipelago showing potential outward migration routes of juvenile pink salmon (dashed line). Dr. Islets field station (triangle), lice harvest site (Wicklow Point fish farm, circle), freshwater fish collection site (Glendale River, square) and seawater fish collection sites (x).



source: modified from Department of Fisheries and Oceans Canada

Figure 3.2 Control and infected ocean-caught fish mass (g) individually plotted (small filled circles) as a function of fork length (mm). Mean size of infected (large open symbols) ocean-caught fish possessing early chalimus (circle), late chalimus (inverted triangle) and motile (square) sea lice stages. Control fish (large filled symbols) were size matched to infected fish. Error bars represent S.E..

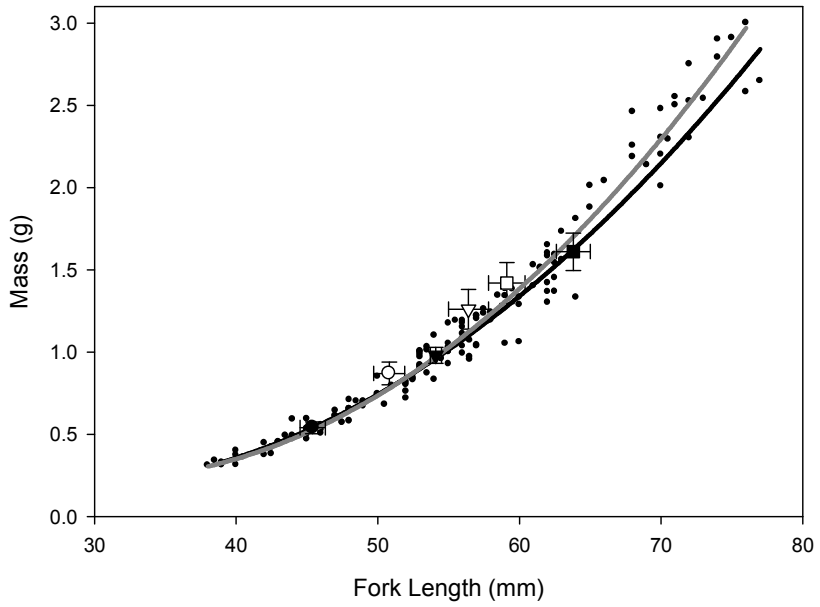


Figure 3.3 U_{max} swimming performance of river-caught fish (open circles) and ocean-caught fish (closed circles) not infected with sea lice (control) plotted versus fork length. The slope of the regression fit to both groups ($y = 0.036x + 6.55$, $r^2 = 0.26$)

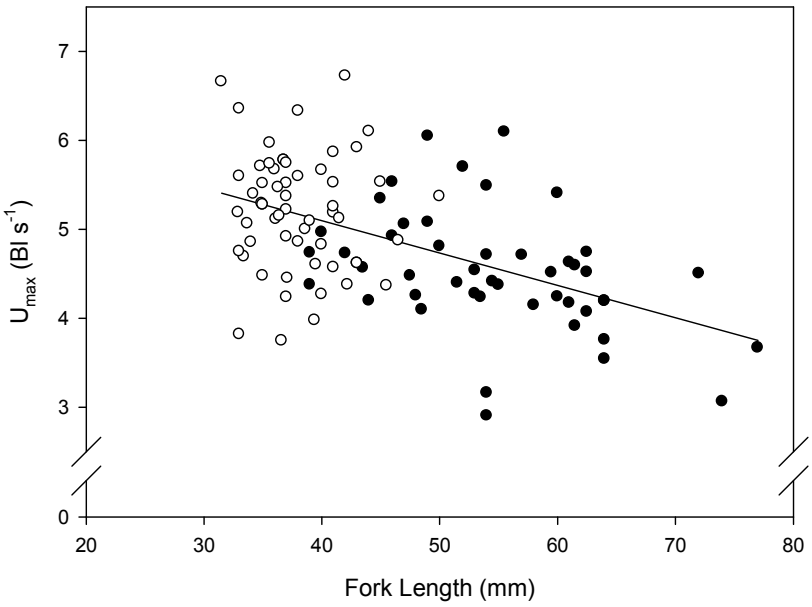


Figure 3.4 U_{max} performance of river-caught fish in the presence and absence of sea lice infection. Control fish (black solid line) and fish with sea lice infection intensity of 1 (light grey), 2 (grey), 3+ (dark grey), and all infection intensities pooled (black dashed line). Stars represent significant differences between control and infected (pooled or not) fish. Numbers within brackets are n values (reading left to right) for control, 1 louse, 2 lice and 3 lice per fish, respectively. Error bars represent S.E..

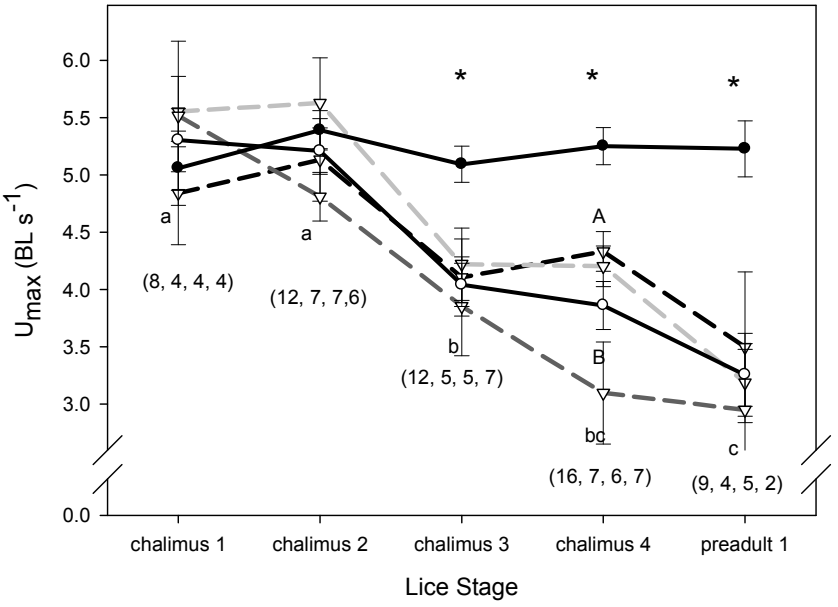


Figure 3.5 Post-swim body [Na^+] (triangle, left y-axis) and [Cl^-] (circle, right y-axis) of control (black) and infected (grey) river-caught fish, plotted as a function of lice development stage. Stars represent significant differences between control and infected fish. There was no significant difference in body [Na^+] or [Cl^-] among different lice development stages ($P > 0.05$). Error bars represent S.E..

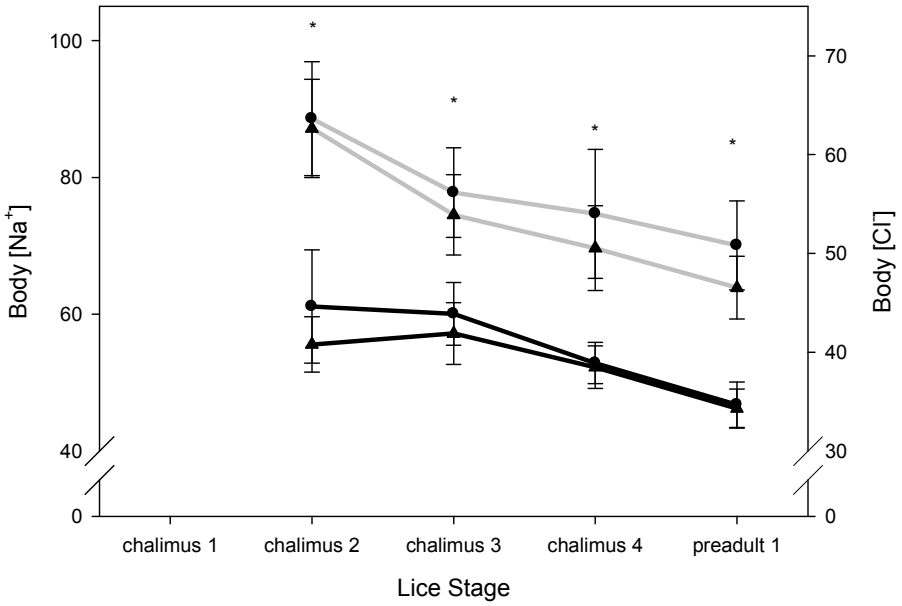


Figure 3.6 Individual control (n = 45, black circles) and sea lice infected (n = 165, inverted triangles) ocean-caught fish U_{max} performance plotted as a function of fish fork length. Sea lice infected fish are grouped by development stage: early chalimus (white), late chalimus (grey), and motile (dark grey). Regression lines of control (solid line, $U_{max} = -0.03L + 6.3$, $r^2 = 0.178$, $P < 0.001$) and infected (dashed line, $U_{max} = -0.04L + 6.5$, $r^2 = 0.261$, $P < 0.001$) OC-fish are not significantly different from one another.

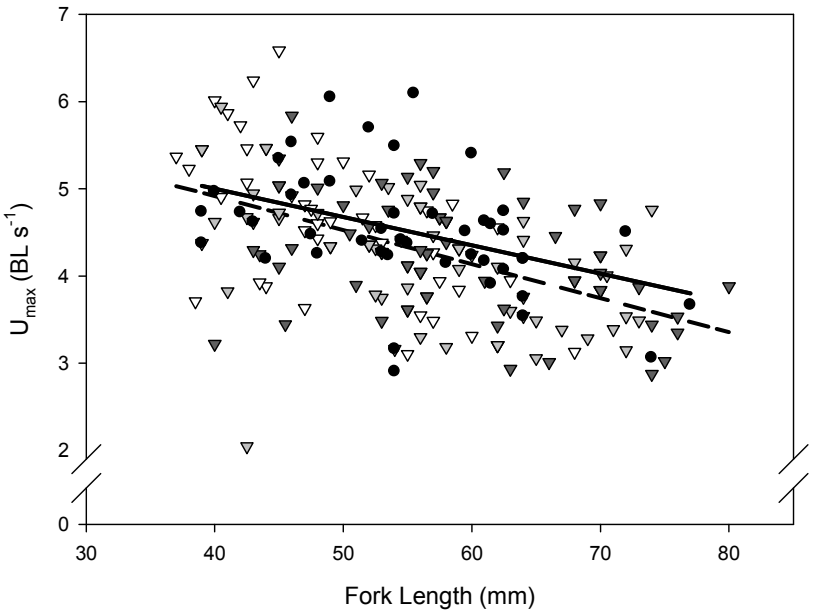
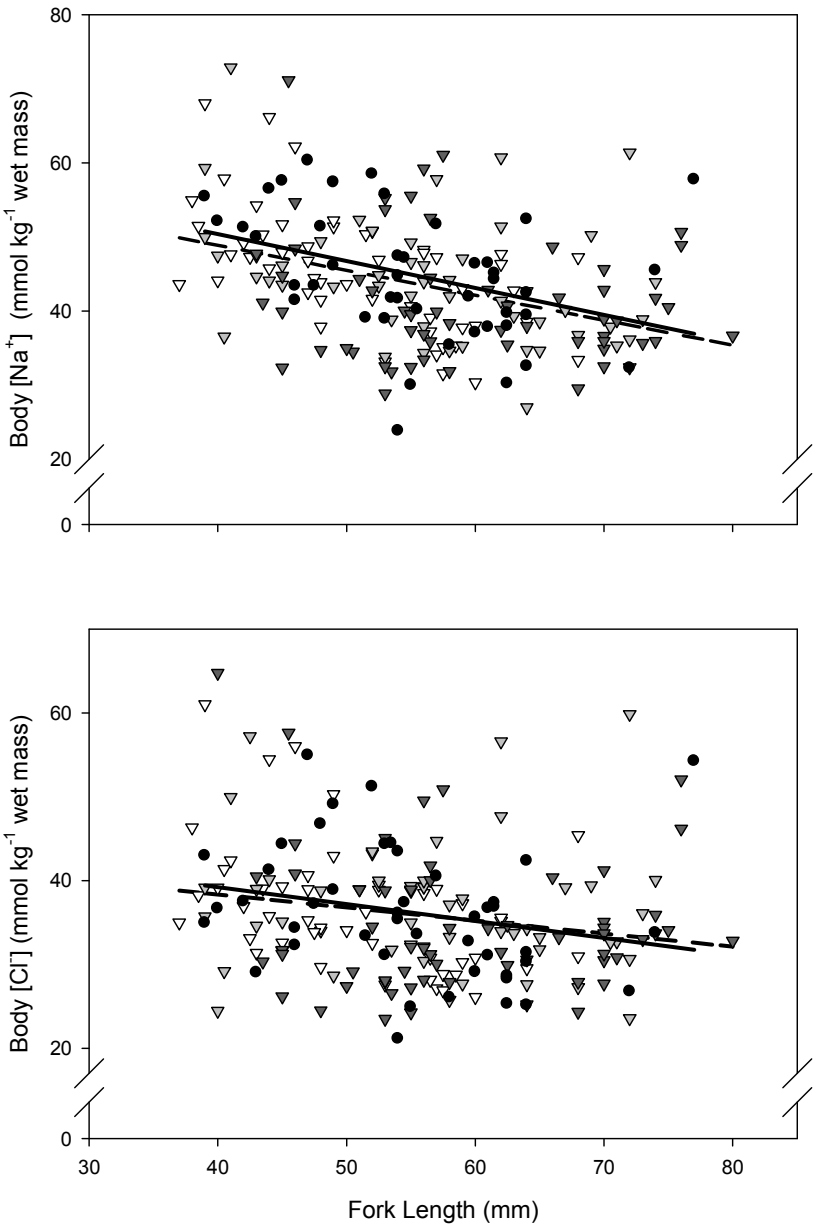


Figure 3.7 Post-swim body a) [Na^+] and b) [Cl^-] of control (n =44, black circles) and infected (n = 158, inverted triangles) ocean-caught fish, plotted as a function of fish fork length. Sea lice infected fish are grouped by development stage: early chalimus (white), late chalimus (grey), and motile (dark grey). Regression lines of control (solid line, $[Na^+] = -0.36L + 65.0$, $r^2 = 0.138$, $P < 0.05$; $[Cl^-] = -0.202L + 47.3$, $r^2 = 0.053$, $P = 0.128$) and infected (dashed line, $[Na^+] = -0.582L + 75.2$, $r^2 = 0.34$, $P < 0.05$; $[Cl^-] = -0.394L + 56.8$, $r^2 = 0.185$, $P < 0.05$) ocean-caught fish are not significantly different from one another.

a)

b)



Summary

1. The maximum swimming (U_{max}) performance of river-caught (RC) fish decreased in fish infected with sea lice, *Lepeophtheirus salmonis*, when compared to uninfected, control fish. The magnitude of this impact varied with sea lice development stage, but not infection intensity (number of sea lice $fish^{-1}$), with the greatest decrease (38%) observed in fish infected with pre-adult sea lice. U_{max} of ocean-caught (OC) fish was not affected by sea lice infection.

2. Post-swim body [Na^+] and [Cl^-] of sea lice infected RC-fish was significantly higher than control fish. The magnitude of this ionic elevation did not change with sea lice development stage. Post-swim body [Na^+] and [Cl^-] of ocean-caught (OC) fish was unaffected by sea lice infection.

3. Possible explanations for the lack of impact of sea lice on OC-fish include, but are not limited too:
   - OC-fish were significantly larger than RC-fish.
   - OC-fish where chronically exposed to sea lice infection (RC-fish were acutely exposed) and had compensated over time.
   - OC-fish were developing or had developed scales, which acted as a potential barrier to sea lice feeding, whereas RC-fish had not yet developed scales.

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Chapter 4 : General Discussion and Conclusions

The overall objective of this thesis was to assess the swimming ability and associated ionoregulatory effects in juvenile pink salmon (*Oncorhynchus gorbuscha)* in relation to their level of sea lice (*Lepeophtheirus salmonis*) parasitism. This was achieved by first developing a protocol to characterise swimming performance, an integrated measure of fish health, and post-swim ionic disturbance, a secondary stress response, in uninfected pink salmon, and then using this protocol to compare fish infected with sea lice with uninfected fish in a field location. Sea lice infection levels ranged in number from 1 to 4 sea lice per fish and ranged in maturity from chalimus 1 to adult. Collectively, the studies provided the first comprehensive analysis of swimming performance in pink salmon during their early marine life stage and the physiological impact of sea lice parasitism on swimming performance and post-swim ion concentrations.

Swimming Ability of Juvenile Salmonids

The swimming performance results described in this thesis are, to date, the lone report of individual salmon swimming ability in fish < 3.0 g. The swimming performance of pink salmon was measured using five different tests lasting 8 to 112 min. This type of swimming can be generally categorized as prolonged, and so sprint and sustained swimming have yet to be characterized in pink salmon in early marine life stages. The capacity for short-term high performance (burst swimming) exercise is essential to the survival of juvenile pink salmon as it facilitates the capture of prey and avoidance of predators, not to mention assisting in downstream navigation (Beamish, 1978), and may have been indirectly measured in the U_{max} and U_{crit} tests by observation of gait transition (from steady-state swimming to burst-coast swimming) (Peake and Farrell, 2006), but was

not separated out for analysis. Sustained or cruising swimming is used during migrating as well as routine daily movements including foraging and station holding (Beamish, 1978). Given the ecological relevance of burst, sprint and sustained swimming during this life stage, their quantification is warranted in future studies.

Previous studies on juvenile salmonid swimming ability have shown that tests for sprint and prolonged performance produce repeatable results, using fixed and incremental velocity tests (McDonald *et al*., 1998; McFarlane and McDonald, 2002). U_{crit} tests are by far the most popularly reported of all standardized swim tests and are considered an accurate measure of aerobic capacity. U_{max} tests, a more rapid incremental velocity test than U_{crit}, are largely measures of anaerobic capacity (Farrell, 2008), at least in adult fishes. Direct comparisons of U_{max} and U_{crit} in adult rainbow trout, *Oncorhynchus mykiss,* (Farrell, 2008) and Atlantic cod, *Gadus morhua,* (Reidy *et al*., 2000) found significant differences between fatigue velocity produced by each test, as would be expected when differing metabolic pathways (aerobic vs. anaerobic) are being recruited.

In studies described in chapter 2, I quantified and compared U_{max} and U_{crit} for the first time in juvenile pink salmon uninfected with sea lice and found no difference in final velocity at fatigue between four U_{max} tests with different acceleration rates, and a single repeated ramped-U_{crit} test. Similarly, in a study examining the white muscle fuel depletion of juvenile rainbow trout in relation to fatigue, McFarlane and McDonald (2002) noted that velocity at fatigue did not differ between a sprint (fixed velocity) test lasting 3 min and U_{crit} (incremental velocity) test lasting 180 min. These results suggest that compared with adult fish, juvenile fish have a diminished anaerobic capacity, which has been shown to scale with body length (Goolish, 1989; McFarlane and McDonald, 1998), and as such U_{max}

tests may, like U_{crit}, be a measure of aerobic metabolism in juvenile salmonids. To test this hypothesis, muscle metabolites of juvenile salmonids could be measured before, during, and after swim tests classically categorized as aerobic, such as U_{crit} and fixed velocity tests of >200 min duration (measures sustained swimming), and anaerobic, such as U_{max} and burst tests. Using the change in resting to fatigued levels of lactate, ATP and PCr, anaerobic energy expenditure could be calculated according to McDonald *et al.* (1998) and would be a good indication of the metabolic pathway being employed to power swimming in the respective swim tests. Determining anaerobic energy expenditure during swim tests might also shed light on the timing of the switch over from wholly aerobic metabolism during exercise, to the addition of a significant anaerobic component, if this change occurs at all.

Scope for activity is another useful measure of fish health, which is derived from the difference between maximal and standard metabolic rate. Aerobic scope reflects the capacity to perform all oxygen-consuming functions above minimal metabolic requirements, and potentially, the ability to respond to environmental extremes or other challenges (Farrell, 2002; Pörtner and Knust, 2007). This parameter has not yet been characterized in juvenile pink salmon and could be used to evaluate the impact sea lice parasitism has on juvenile pink salmon.

Sea Lice Effects on Juvenile Salmonids

Regardless of the physiological process that powers fish swimming, a decrease in swimming performance likely has ecological repercussions. Studies in this thesis represent the first effort to measure impacts on swimming by sea lice parasitism on salmon at the earliest juvenile life stages in SW. Wagner *et al.* (2003) reported that a lice load of 0.13 sea

lice g^{-1} produced a 19 - 22% reduction in U_{crit} of adult Atlantic salmon, whereas I found that ca. 2 - 3 sea lice g^{-1} (reported in chapter 3 in terms of load, 1 - 3 lice per fish) caused a 20 - 38% decrease in U_{max} in artificially infected fish. It would be interesting to further investigate the scaling relationship of parasitic effects on swimming performance with fish size and perhaps compare different species of Pacific salmonids reported to host sea lice in juvenile life stage (*Oncorhynchus* spp., Beamish *et al*., 2007). These species have different life history strategies and may have different responses to *L. salmonis* parasitism (*O. kisutch* and *O. keta*, Jones *et al.,* 2007).

I found that a load of one louse per fish had no effect on swimming performance, except in small fish (RC-fish) and until the louse had matured to chalimus 3 and beyond. This could perhaps fit a stable model for *L. salmonis* parasitism. If a single louse parasitizes a fish, then it can successfully complete its life cycle. Of course it will need to find a mate, but if it kills its host before reaching sexual maturity, as may be the case if a potential mate is already located on the same host, then it jeopardizes its ability to survive and reproduce. As stated previously, *L. salmonis* are naturally occurring parasites of Pacific salmon, and perhaps this low lice intensity of 1 is an acceptable balance for both host and parasite. This of course does not hold for fish averaging 0.3 g, as RC-fish reported in chapter 3 were of this size, but suffered both swimming and ionic impacts at a lice intensity of 1. It could be argued, however, that sea lice parasitizing fish at this size is unnatural, and I think I would agree. In our collections of OC-fish in 2008, only 8 fish were less than 0.4 g and infected with sea lice. While fish farms are situated in the area, they had been fallowed. From a managerial perspective, I feel it would be wise to be particularly vigilant with fallowing practices of farm sites located proximate to natal

streams and rivers, so as to prevent sea lice infection in fish at this particularly vulnerable size and hopefully this would facilitate wild juvenile pink salmon growing to a size > 1 g before encountering sea lice parasitism above background levels.

In this thesis I measured the impact sea lice parasitism has on swimming performance of pink salmon <3.0 g and reported as much as a 38% decrease in U_{max} when compared to control (uninfected) fish. I did not, however, directly examine the mechanisms causing this decrease in swimming ability, though I did measure body ion concentrations, which may shed insight into physiological mechanisms. There are multiple pathways by which sea lice could affect the ability of a fish to swim, none of which have been looked at extensively to date (Fig. 1.4, introduction)

Houston (1959) theorized that the reduced locomotory activity in chum salmon rapidly transferred to seawater was a direct result of altered electrolyte concentration on the neuromuscular apparatus. Indeed, electrochemical gradients are integral components of the firing of muscle fibres, and so if this gradient is lost or altered by an ionic disturbance induced by sea lice, muscles may not be physically capable of powering swimming. Here I reported body ions were increased post-swim in sea lice infected fish when compared to control. Therefore, one possible mechanism by which sea lice could be affecting the ability of a fish to swim is via ionic disturbance impairing muscle contractility and directly impacting swimming. This is potentially supported by findings presented in chapter 3.

Drinking rate and Na^+-K^+ ATPase activity should increase in an effort to regain the post-swimming ionic imbalance in infected fish. The present study did not determine if these ionic disturbances were present before the swimming test. Regardless, compensatory activities, either before or as a result of swimming, would consume energy which may be

drawn from other systems such as locomotion, thus swimming performance is decreased in compensation. Therefore, another possible mechanism by which sea lice could be impairing fish swimming is via energy reallocation, away from locomotion.

A third potential pathway by which lice could create swimming impairment, is by the creation of drag forces as fish swim. Given the small size of post-emergent pink salmon (as small as 30 mm), and the large size of adult sea lice (as large as 13 mm), it is conceivable that lice hanging from the fish could produce sufficient drag forces to slow a fish. Of course this would be highly dependent on the location of the louse on the fish, with a louse attached to the caudal fin likely producing the most drag, and a louse tucked under the operculum, likely producing the least. Quantifying drag produced by lice may be particularly tough to measure for a number of reasons: 1. Sea lice are small so to quantify the force produced by their bodies may be a challenge. 2. It is difficult to categorize louse location, and even trickier to convincingly determine where and for how long an adult louse will settle on a fish. As such, drag measures are likely to be representative of a louse location that is static, rather than dynamic, which may be misleading given that lice can, and do, move on fish (Johnson and Fast, 2004). 3. It is close to impossible to control where infectious stage lice attach to a fish. The most common form of artificial infection is by "pulsing" fish with copepodid sea lice, which are less than 1 mm in length. Obviously to place these small lice on fish where attachment is desired would be a challenge, requiring much time, lice, fish and patience. All considered, drag may be a factor in the decreased swimming ability of fish infected with ectoparasites, but it is likely not easy to systematically test. The data presented in this thesis, however, may be evidence against this mechanism. The fact that increasing sea lice intensity or sequential sea lice

development did not cause additive impacts to fish swimming performance argues against the potential for significant effects of sea lice drag forces. Anecdotally, based on my observations while measuring the swimming performance of over 250 fish infected with sea lice, I would advise that drag does not appear to significantly impair swimming unless the louse is attached at a particularly precarious location, namely the tip of a pectoral or caudal fin.

Perspectives

When I first arrived in the Broughton Archipelago to conduct my research pertaining to the issue of sea lice parasitism on wild juvenile pink salmon, I witnessed first hand the geographic layout and location of farm sites within it. It seemed surprising to me that there would be an issue at all. The maze of large wide fjords was seemingly endless, with salmon farms tucked here and there along the shoreline. I was expecting farms to be situated on narrow channels, literally blocking the lone migratory path of wild fishes. Early in my stay I realized that the large wide channels and fjords were somewhat deceiving in that juvenile salmonids in early marine life stage generally stick close to the shoreline, feeding in kelp beds found in shallow waters, and therefore the useable habitat for these fish was in fact much less vast than I originally thought. Not only that, but this habitat coincided with the location of the fish farms. Even so, naupliar sea lice are at the mercy of the ocean currents, and thus given the shear volume of the water ways in the area, I reasoned that sea lice would be dispersed from their point source, allegedly fish farms, to such a degree that there was no way the extinction hypothesis (Krkošek *et al*., 2007) could possibly be correct.

Indeed, my doubts were reinforced by our group's struggle to catch parasitized fish. Hours upon hours were spent sorting through thousands of pink salmon <3.0 g looking for the apparently illusive parasitic sea louse on a pink salmon host. We did not numerate sea lice infection prevalence exactly but I guesstimated anywhere from 1-10% on average (levels seen in regions without fish farms, Gottesfeld *et al*., 2009). We were somewhat puzzled because we knew that not only had prevalence in previous years been well above this (in the region of 90%), but even that very year (2008) a colleague was reporting prevalences in Fife Sound, a location in the Broughton Archipelago about 30 km "downstream" from our field site, that were well above what we were finding. Not fully believing, we investigated ourselves, and sure enough, in the area of Fife Sound, the prevalence of sea lice on pink salmon was around 90% (again, this was not specifically quantified). While I do not have a definitive answer for these apparent discrepancies from year-to-year and location-to-location, I can offer an educated guess. I believe that the practice of fallowing and SLICE-ing fish farms during times for wild fish migration in the nearshore environment comes close to eradicating the occurrence of sea lice above background levels. Logical reasoning tells us that without farmed fish (fallowed site), there is no sea lice incubator which yields no translocation of sea lice to wild fish. The application of SLICE, a chemical administered to farmed fish through feed that disrupts the lifecycle of *L. salmonis*, is similar in final effect, no sea lice translocation to wild fish. In previous years when sea lice prevalences were particularly high, little or no effort was made to fallow farms during migration season. Also, the colleague who reported high infection prevalence during 2008 was primarily sampling in and around Fife Sound. Fish migrating through this region may be predominantly from the region of Kingcome Inlet,

rather than Knight Inlet (Glendale River), a region where fallowing was not being practiced during the migration season of 2008 at some farm sites located on migration routes. I recognize that my claims are not ground breaking. Bron *et al*. (1993) was the first to publish findings that the fallowing of farms was an effective means of controlling *L. salmonis* on farmed fish. Morton *et al*. (2005) reported that *L. salmonis* levels were significantly reduced ($P < 0.0001$) in wild juvenile fish collected in the Broughton Archipelago during fallowing but returned to the original level after fallowing. Beamish *et al*. (2006) suggested that the exceptional returns of adult pink salmon in 2004 along the eastern margin of Queen Charlotte Straight, might in part be explained by the establishment of a fallowed migration corridor for pink salmon. Such results are encouraging to those looking to find a solution to this issue. It is conceivable that with better understanding of the relationship between farmed and wild salmon, and sea lice transmission between the two, effective management strategies could eliminate the conflict altogether, allowing both aquaculture and wild salmon to exist together. Finding this responsible balance is something of a holy grail to environmentalists and fish farmers alike, but with continued scientific study, may not be too far out of reach.

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Appendices

Appendix A

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A07-0055

Investigator or Course Director: Colin Brauner

Department: Zoology

Animals:

Salmon Atlantic Salmon 40
Salmon chum salmon 1100
Salmon Pink salmon 1100

Start Date: March 1, 2007 **Approval Date:** May 15, 2009

Funding Sources:

Funding Agency: Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title: Juvenile pink salmon and the health of the Broughton Archipelago ecosystem

Funding Agency: British Columbia Pacific Salmon Forum
Funding Title: Effects of sea lice on the physiology and health of pink salmon

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

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